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Morphometric Studies of Megakaryocytes in Human and Rat Fetal, Infantile and Adult Hematopoiesis
II. Observations on rat fetuses and adult rats

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ABSTRACT

In author morphological study of megakaryocytes of rats, a comparative study was made on the size and ultrastructure of megakaryocytes of rat fetuses and adult rats. Furthermore, in relation to megakaryocytes, a comparison was also made on platelet count of rats during the fetal stage and after birth.

The mean megakaryocyte size during the fetal stage was significantly smaller than that of the adult. Study on the ultrastructure revealed a slight difference in DMS between the fetus and the adult.

The electron density of granules in the megakaryocytes was higher in the adult than in the fetus. The platelet count was found to be significantly smaller during the fetal stage than after birth.

In the first report of this series, the author, have reported that the megakaryocyte size of the human fetus is smaller than that of the adult, but megakaryocytes increase in size from the late fetal stage to the newborn stage and at the age of one year the area shows no difference from that of the adult. From these findings it has been speculated that the pattern of platelet production may be different between megakaryocytes of the fetus and those of the adult. With the aim of quantitating the relationship between fetal age, megakaryocyte size, and platelet count, animal experiments were conducted in the present study with the use of rats.

As micromegakaryocytes which appear in human blood dyscrasias influence the number and other features of platelet production, it has been suggested that platelets produced from micromegakaryocytes during the fetal stage differ both morphologically and functionally from those of the adult. In order to elucidate the relation between megakaryocyte size and platelet production, it is also necessary to take into account the maturity of megakaryocytes. It has been reported in the study on the ultrastructure of megakaryocytes of the human fetus that the formation of demarcation membrane in these micromegakaryocytes is unsatisfactory and also that the distribution of specific granules is poor.

Observations have been made in rats on the ultrastructure of megakaryocytes of the fetus and adult, but there has been no report in the literature on studies focussed on whether there are differences in the ultrastructure of mature megakaryocytes. As a part of the basic studies to elucidate the platelet production mechanism of mature megakaryocytes in the adult and fetus, this study was made on the ultrastructure of both the adult and fetus.

MATERIALS AND METHODS

In the present study, Donryu strain rats (Nippon CLEA, Tokyo) about three months of age after birth and weighing 180~240 g were used. Male and female rats were placed in the same cage in the evening and on the following morning at ten those female rats with sperms detected on vaginal smear were designated day 0. The weight of the fetus on the 18th day of gestation was about 2 g and on the second day after birth it was about 6 g and on the 20th day, about 30 g.

Determination of platelet count

Fetuses at the fetal age of 18, 19, 20, and 21 days obtained from female rats on the 18th, 19th, 20th and 21st day of pregnancy, respectively, newborn rats 2, 7, 14, and 21 days after birth, and adult rats were employed. Blood was drawn from the heart using a disposable syringe (1 ml) whose internal surface was treated with heparin and platelet count was determined by Brecher Cronkite method.

Measurement of megakaryocyte size

Fetuses having a fetal age of 18, 19, and 21 days, newborn rats 2 and 7 days after birth, and adult rats were used. The liver and bone marrow of the fetuses and newborn rats and the bone marrow of the adult rats were fixed in 4% formalin and 4 μm paraffin specimens were prepared. After staining with hematoxylin and eosin (H.E), the mature megakaryocytes of each specimen were observed by a light microscope at 1,000 power magnification and photographed. The photographic negatives were enlarged at a fixed enlargement rate and the megakaryocyte size on the enlargement was measured by a mortion analyzer (manufactured by NAC Company).

Transmission electron microscopy

The liver of fetuses having a fetal age of 18 days and the bone marrow of adult rats were used. Specimens about several mm^3 in size obtained within one minute after death were minced to about 1 mm^3 in size in 2% glutaraldehyde, 0.1 M phosphate buffer and then prefixed for 4 hr. in the same solution at room temperature. Postfixing was made for 2 hr. in 5% OsO_4, 0.1 M phosphate buffer. The specimens after dehydration with ethanol were embedded in Queto 1812 (Nisshin EM). The ultrathin sections after double staining in uranyl acetate and lead acetate were observed with transmission electron microscope (JEM 100B).

RESULTS

Determination of platelet count

The results are shown in Fig. 1. No remarkable change in platelet count could be observed from the fetal age of 18 days to birth, but on the second day after birth a rapid increase was seen. During the neonatal stage after the seventh day following birth, there was no remarkable change in platelet count. A slightly increasing tendency was observed in the platelet count of mature rats when compared to rats of the neonatal stage.

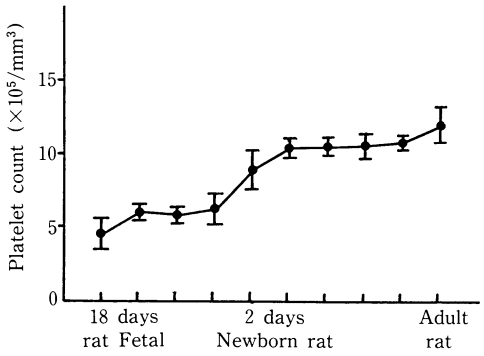

Fig. 1. Rat platelet count rat Fetal, Newborn rat, Adult rat

Table 1. Size of megakaryocytes in fetal liver and in bone marrow of rats

		Number of cases	Number of megakaryocytes	Cell size (Actual measurement cm^2)	(True cell size) μm^2
Feuts	18 days	10	266	23.0 ± 4.0	(368.0)
	19 days	5	90	24.6 ± 5.8	(393.6)
	21 days	6	96	24.8 ± 5.2	(396.8)
Newborn	2 days	11	175	26.2 ± 4.3	(419.2)
	7 days	11	198	26.8 ± 4.5	(428.8)
Adult rat		10	180	27.7 ± 4.2	(443.2)

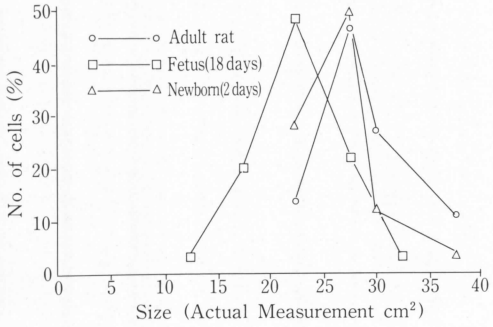

Fig. 2. Distribution of megakaryocyte size, in rat fetus (18 days)
New born rat and Adult rat

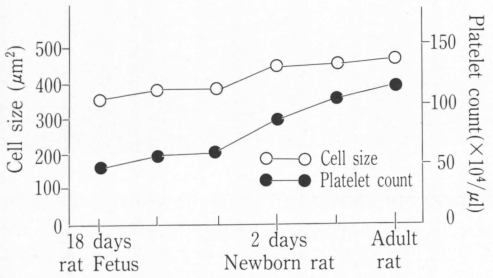

Fig. 3. Corelation between platelet count and cell size

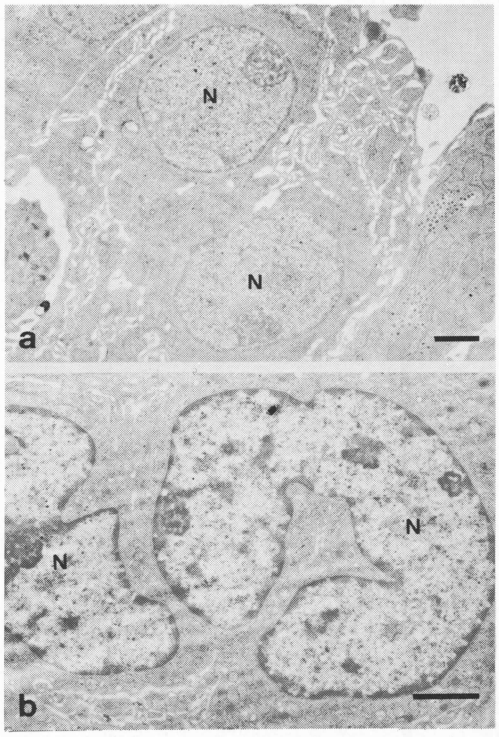

Fig. 4. Electron micrographs of the megakaryocytes in the 18-day rat fetal liver (a) and in the rat adult bone marrow (b). N: nucleus, bar = 2μm

Megakaryocyte size

Figure 1 and Table 1 show the changes in mean size of megakaryocytes from the fetal age of 18 days to mature rats. The mean size of megakaryocytes did not show any change from the fetal age of 18 days to 21 days, but an increase was observed immediately after birth. Figure 2 presents the distribution of megakaryocyte size in rat fetus 18 days after gestation, newborn rat 2 days after birth, and adult rat. Figure 3 compares the platelet count and mean megakaryocyte size.

Ultrastructure of megakaryocytes of the fetus at the fetal age of 18 days

In the liver of the rat fetus at the fetal age of 18 days, mature megakaryocytes could be observed by electron microscopy. The maturity was determined by the level of development of the demarcation membrane and distribution of granules in the megakaryocyte. Mature megakaryocytes were remarkably larger than the adjacent liver cells. The nuclei were almost relatively spherical in form (Fig. 4a). The demarcation membrane showed a very good development within the cytoplasm of mature megakaryocytes and in the transmission electron microscopic photograph it appeared like a network of discontinuous space (Fig. 4,5a). Many parts of the cytoplasm was demarcated into platelet zones (PZ) by demarcation membrane (Fig. 5a). Amorphous matrix-like material could be seen within the space of the demarcation membrane system (Fig. 6a). Within the cytoplasm of mature megakaryocytes, including mature platelets, formation of cell skeleton and microbubles seen in platelets could not be observed. A large number of mitochondria could be seen around the nucleus. These mitochondria were smaller than the mitochondria of liver cells (Fig. 5,6a). Granules were well developed in the cytoplasm (Fig. 5,6,7a), but only a few Golgi bodies which are considered to produce these granules could be

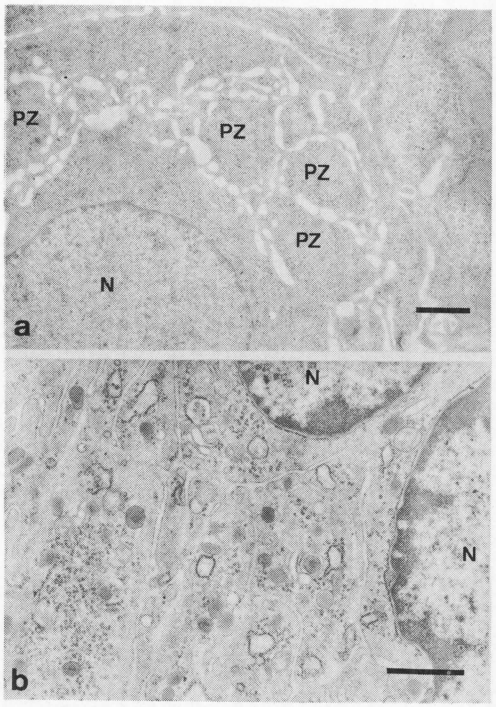

Fig. 5. Electron micrographs of the megakaryocytes in the 18-day rat fetal liver (a) and in the rat adult bone marrow (b). N: nucleus, PZ: platelet zone, bar = 1 μm

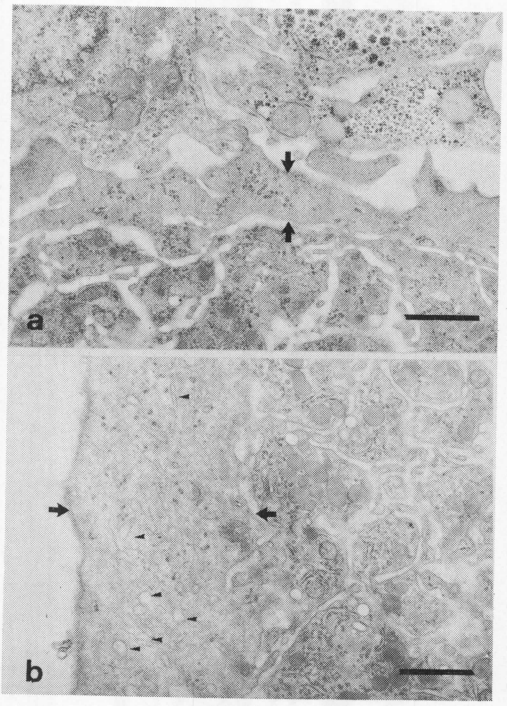

Fig. 7. The marginal zone arrows of the megakaryocyte in the rat fetus (a) and in the adult rat (b). The smooth endoplasmic reticulum-like structures are seen in the marginal zone of the megakaryocyte in the adult rat (arrowheads in b). bar = 1 μm.

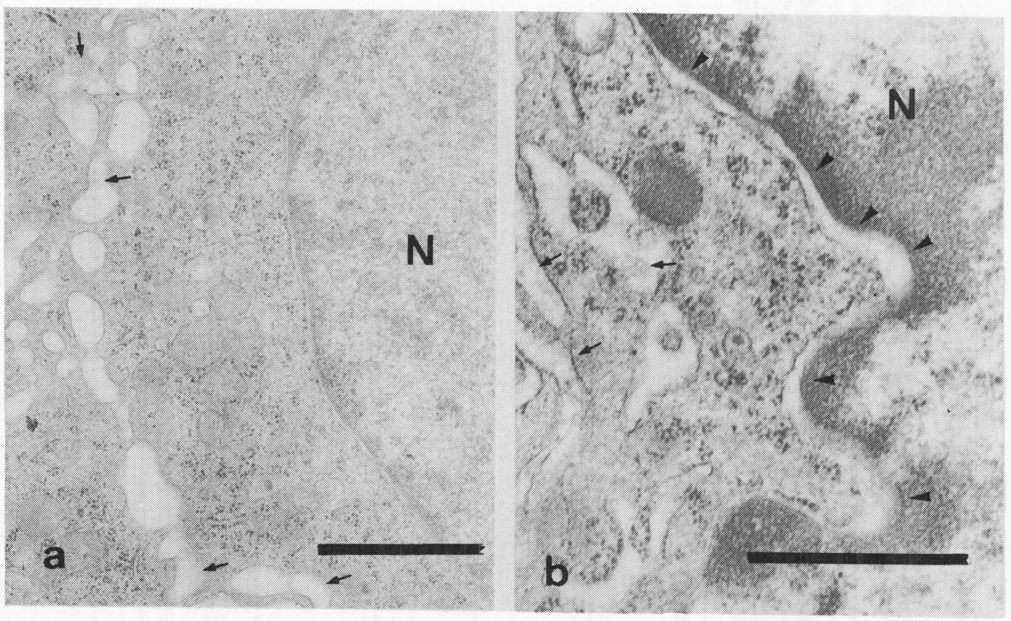

Fig. 6. High power electron micrographs of the megakaryocytes in the rat fetus (a) and in the adult rat (b). Arrows show amorphous matrix-likes materials in the space of the demarcation membrane system. Arrowheads show expanded nuclear membrane of the megakaryocyte in the adult rat. bar = 1 μm.

observed.

In the periphery of some mature megakaryocytes, marginal zones scanty of intracytoplasmic organelles could be observed (Fig. 7a).

Ultrastructure of mature megakaryocytes of adult rats

A large number of large mature megakaryocytes could be seen within the bone marrow of adult rats. the nuclei were very large and polymorphous (Fig. 4b).

The demarcation membrane was well developed. The demarcation membrane of megakaryocytes of adult rats showed little disruption as seen in mature megakaryocytes of the rat fetus and appeared as a long extended and narrow space (Fig. 5b). Matrix-like material was also observed within the demarcation membrane space of mature megakaryocytes of adult rats (Fig. 5,6b). The electron density of the granules was higher than that of mature megakaryocytes of the rat fetus (Fig. 5,7b). The number of Golgi bodies was small as in the case of mature megakaryocytes of the rat fetus. Also, development of microtubules could not be observed. The nuclear membrane was in two layers. Further, the nuclear membrane space was remarkably expanded when compared to that of mature megakaryocytes of the rat fetus (Fig. 6.a.b).

Marginal zones could also be observed in mature megakaryocytes of the adult rat, but smooth ER-like substance could be seen their cytoplasm (Fig. 7b).

DISCUSSION

Megakaryocyte size and platelet count

The results of the present study confirmed as in our previous study on megakaryocytes of the human fetus[5,6,9] that megakaryocytes of the rat fetus are smaller than those of the adult rat and that the megakaryocyte size increases from immediately after birth to the neonatal period. In the present study, megakaryocytes in the liver of the fetus and those in the bone marrow of the adult were used. The authors have reported previously that there is no significant difference in the size of megakaryocytes in the human liver and that of megakaryocytes in the human bone marrow[9].

The results of the present study have also demonstrated that there is a correlation between megakaryocyte size and platelet count from the fetal to the adult stage. Platelet count increases in proportion to megakaryocyte size from immediately after birth to the early neonatal stage[11]. This finding is in agreement with the result of a report on the changes of platelet count during the neonatal period.

The foregoing results suggest that the platelet count of human fetus is lower than that of adults.

In contrast to an increase of 17% in megakaryocyte size from an 18-day old fetus to an adult, platelet count makes a twofold increase. Though the count of mature megakaryocytes in adults shows an increase over that of the fetus, the foregoing results suggest that the platelet production rate per megakaryocyte is higher in adults than in fetuses.

It has been reported in man that the platelet production rate per megakaryocyte is adjusted by thrombopoietin[4,7,14], but it is considered that in rats a same or similar substance is at work from immediately after birth to the neonatal stage.

In blood dyscrasias such as CML, AML, and preleukemia, micromegakaryocytes appear, but as these micromegakaryocytes produce platelets having deficient function[12,15,16], it is assumed that micromegakaryocytes of the human fetus and the newborn produce platelets different in function and morphology from those of the adult. Majima has reported that by using human materials juvenile type platelets are predominant during the neonatal stage[11], but thereafter the juvenile type gradually decreases. Furthermore, it has been reported[6] that the formation of demarcation membrane is inadequate in micromegakaryocytes of the human fetus and that the number of specific granules is small[8,13], but there is a possibility that immature platelets having a small number of specific granules can be produced by such megakaryocytes. It is said that the aggregation function and release reaction are depressed in these platelets.

Electron microscopic findings

The noteworthy electron microscopic finding observed in the present study is that the morphology of the demarcation membrane of mature

megakaryocytes of the fetus is different from that of the adult. This difference in morphology of the demarcation membrane suggests that the pattern of demarcation is different between mature megakaryocytes of the fetus and mature megakaryocytes of the adult. However, it can be judged from these results that the development of the demarcation membrane of mature megakaryocytes of the fetus is not immature when compared to that of the adult.

Matrix-like material was observed in the space of the demarcation membrane system of mature megakaryocytes of the fetus similar to that of the adult.

The other different in the morphology of mature megakaryocytes of the rat fetus versus that of mature megakaryocytes of the adult is that the electron density of granules within the megakaryocytes differs. At least two types of granules can be detected in the platelet, one being specific granules nd the other being granules of high electron density. However, Benhnk and Pederson have reported[2,8,10] that is it is extremely rate of electron dense granules to be detected within megakaryocytes and therefore it can be concluded that these granules within megakaryocytes are specific granules. Though it has been demonstrated that these specific granules are produced in Golgi bodies, in the present study[1,17], difference in morphology and distribution of Golgi bodies could not be demonstrated between mature megakaryocytes of the fetus and those of the adult. It can be speculated from these findings that the difference in electron density of specific granules is either a difference in density of the contents of the granules or difference in the content itself or both. If it is assumed that the function originating from specific granules differs between platelets produced from mature megakaryocytes of the rat fetus and platelets produced from mature megakaryocytes of the adult, it can be suggested that this is attributable to the difference in the specific granules themselves and not because the number of specific granules of mature megakaryocytes of the fetus is lower than that of those of adults.

The findings of the present study indicate that the nuclear membrane of mature megakaryocytes of the adult has an expanded space as in the case of the demarcation membrane.

The nucleus of megakaryocytes of the human fetus during the hematopoietic stage is mature megakaryocytes and platelets. Based on the results of the present study, the authors consider that small size megakaryocytes of the rat fetus are mature enough to produce platelets, but the release function of platelets is inferior to that of mature megakaryocytes of the adult rat. Furthermore, it is assumed that there is no substantial difference in the major functions of platelets between the rat fetus and adult rat.

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REFERENCES

1. **Behnke, O.** 1968. An electron microscope study of the megakaryocyte of the rat bone marrow. I. The development of the demarkation membrane system and the platelet surface coat. J. Ultrastruct. Res. **24**: 412-433.
2. **Behnke, O.** 1969. An electron microscope study of the rat megakaryocyte. II. Some aspects of platelet release and microtubules. J. Ultrastruct. Res. **26**: 111-129.
3. **Behnke, O. and Pedersen, N.T.** 1974. Ultrastructural aspects of megakaryocyte maturation and platelet release, p. 21-31. *In* M.G. Baldini and S. Ebbe (ed.), Production, function, transfusion and storage. Grune and Stratton, New York.
4. **Ebbe, S., Stohlman, F., Jr., Donovan, J. and Overcash, J.** 1968. Megakaryocyte maturation rate in thrombocytopenic rats. Blood **32**: 787.
5. **Enzan, H. Takahashi, H., Kawakami, M. and Ohkita, T.** 1978. Light and electron observation of hepatic hematopoiesis of human fetuses. Acta Pathol. Jpn. **28**: 411-426.
6. **Enzan, H. Takahashi, H., Kawakami, M., Yamashita, S., Ohkita, T. and Yamamoto, M.** 1980. Light and electron microscopic observations of hepatic hematopoiesis of human fetuses. II. Megakaryocytopoiesis. Acta Pathol. Jpn. **30**: 937-954.
7. **Harker, L.A.** 1970. Regulation of thrombopoiesis. Am. J. Physiol. **218**: 1376.

8. **Hrodek, O.** 1966. Blood platelets in the newborn. Their function in haemostatsis and haemocoagulation. Acta Univ. Carolina Monogr. **22**.
9. **Izumi, T., Kawakami, M., Enzan, H. and Ohkita, T.** 1983. The size of megakaryocytes in human fetal, infantile, and adult hematopoiesis. Hiroshima J. Med. Sci. **32**: 257-260.
10. **MacPherson, G.G.** 1972. Origin and development of the demarkation system in megakaryocytes of rat bone marrow. J. Ultrastruct. Res. **40**: 167-177.
11. **Majima, T.** 1961. Studies on platelets in infancy and childhood II. Acta Paediatrica Japonica **65**: 143.
12. **Maldonado, J.E.** 1974. Dysplastic platelets and circulating megakaryocytes in chronic myeloproliferative diseases. Blood **43**: 811-820.
13. **Mull, M.M. and Hathaway, W.E.** 1970. Altered platelet function in newborns. Pediat. Res. **4**: 229.
14. **Schreiber, D.P. and Levin, J.** 1970. Detection of thrombocytopoeitic activity in plasma by stimulation of suppressed thrombopoiesis. J. Clin. Invest. **49**: 1709.
15. **Smith, W.B., Ablin, A. Goodman, J.R. and Brecher, G.** 1973. Athypical megakaryocytes iln preleukemic phase of acute myeloid leukemia. Blood **42**: 535-549.
16. **Wiesneth, M., Pfliezer, H., Kubanek, B. and Heimpel, H.** 1980. Micromegakaryocytes in human bone marrow. Acta Haemat. **64**: 65-71.
17. **Zamboni, L.** 1965. Electron microscopic studies of blood embyogenesis in humans. II. Hemapoietic activity in the fetal liver. J. Ultrastruct. Res. **12**: 524-541.