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Abstract

Red blood cell and plasma polyamines in umbilical and maternal blood at delivery were measured using high performance liquid chromatography. The concentration of each polyamine in red blood cells and plasma of umbilical blood was significantly higher than in maternal blood. Spermidine and spermine concentrations in fetal red blood cells decreased markedly with the progress of pregnancy. In addition, younger red blood cells contained more polyamines than older cells. Red blood cell polyamines are closely associated with the cell membrane. Plasma polyamine in umbilical blood reflect active fetal metabolism, whereas red blood cell polyamines mainly reflect alterations in erythropoiesis in bone marrow and may indicate the proliferation of the bone marrow.

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CONCENTRATIONS OF POLYAMINES IN UMBILICAL BLOOD

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Abstract. Red blood cell and plasma polyamines in umbilical and maternal blood at delivery were measured using high performance liquid chromatography. The concentration of each polyamine in red blood cells and plasma of umbilical blood was significantly higher than in maternal blood. Spermidine and spermine concentrations in fetal red blood cells decreased markedly with the progress of pregnancy. In addition, younger red blood cells contained more polyamines than older cells. Red blood cell polyamines are closely associated with the cell membrane. Plasma polyamine in umbilical blood reflect active fetal metabolism, whereas red blood cell polyamines mainly reflect alterations in erythropoiesis in bone marrow and may indicate the proliferation of the bone marrow.

Key words : polyamine, umbilical blood, red blood cell, plasma, fetal growth.

The naturally occurring polyamines spermidine and spermine and their precursor putrescine are low molecular weight aliphatic compouds. These amines are distributed ubiquitously in biological meterials, and are found in highest concentration in tissues that actively synthesize protein and have high RNA content (1, 2). Many studies suggest that polyamines play important roles in cell growth and proliferation (2, 3).

Clinically, considerable interest in polyamine as possible markers of malignancy has arisen following the initial report of increased amounts of polyamines in the urine of cancer patients (4). There are alterations in cell kinetics which reflect fetal growth during pregnancy, but there have been few reports concerning the functions of polyamines in fetal growth (5-7).

We have investigated the alterations in extracellular polyamines during pregnancy and the neonatal period in human beings and rats (5, 8). We have also studied the alterations in ornithine decarboxylase (ODC) and S-adenosylmethionine decarboxylase (SAMDC), which are the rate limiting enzymes in polyamine biosynthesis, during pregnancy and in experimentally induced intrauterine growth retardation (IUGR) rat fetuses (9).

The present study was undertaken to determine polyamine concentrations in the umbilical blood at various stages of pregnancy and to reveal the possible role of polyamines in fetal growth.

MATERIALS AND METHODS

Maternal blood was taken from the cubital vein with a heparinized syringe immediately after the birth of a baby from a normal pregnancy. Umbilical blood was collected separately from the umbilical artery and vein. Samples of umbilical blood in the early stage of gestation were obtained from patients who had premature delivery or abdominal hysterectomy because of uterine myoma with pregnancy. Plasma was separated and stored at −40℃ until analysis.

Preparations of red blood cell sample. About 0.1 ml of blood was inserted into a polyethylene capillary tube and centrifuged at 12,000 × g at 4℃ in Kubota KR/180B refrigerated centrifuge for 20 min. The packed red blood cell portion was transferred to a test tube and shaken with 10% trichloroacetic acid (TCA) containing 0.1% triton X-100 and an internal standard in order to lyse the cells and deproteinize. Then lysed cells were centrifuged at 2,000 × g, for 5 min at room temperature, and 20 μl of the supernatant was used for analysis.

As internal standard, we used artificially synthesized triethylene tetramine (supplied by Dr. Samejima, Josai University) which has the molecular structure: $NH_2(CH_2)_2NH(CH_2)_2NH(CH_2)_2NH_2 \cdot 4HCl$.

Preparation of plasma sample. One ml of plasma was treated with an equal volume of cold 10% TCA containing the internal standard, and held in ice for 30 min in order to precipitate the protein. The sample was centrifuged, and the supernatant fluid was transferred to an ampul. The pellet was resuspended in 0.5 ml of 10% TCA and recentrifuged. The supernatant fluid was added to the previous wash. This final sample was hydrolyzed for 24 h at 110℃ in 6 N HCl. After hydrolysis, the sample was evaporated using a centrifugal evaporator Model RD-21 (Yamato Scientific Co., Tokyo). The residue was reconstituted in 100 μl of 0.1 N HCl and centrifuged; then, 20 μl of this aliquot was loaded on an amino acid analyzer. The recovery rates of the polyamines were 92-97 percent.

Age-separation of red blood cells. To understand further the potential significance of red blood cell polyamine, red blood cells were age-separated. The method employed was that of Cooper *et al.* (10). The blood was centrifuged at 2,000 × g for 20 min at room temperature. The plasma and buffy coat was removed, and the cells were resuspended in the plasma. The hematocrit was adjusted to 90% (v/v), and the cells were placed in a polypropylene tube. Separation by age was performed by centrifugation for 1 h at 39,000 × g at 30℃ in a Kubota Model KR/180B centrifuge with an angle head rotor. The desired fractions were collected from the top 10% layer, middle 10% layer and bottom 10% layer by careful aspiration with a syringes. These fractions were individually resuspended in the original plasma at a hematocrit of 40-50%. The red blood cell count, white blood cell count, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated using a Coulter Counter Model S-Plus (Coulter Electronics, Inc., U.S.A.). Each age-separated red blood cell sample was prepared for analysis of polyamines as above.

Polyamine analysis. Polyamine concentrations were determined by high performance liquid chromatography (model HLC-805, Toyo Soda, Co., Tokyo) by the stepwise elution method, followed by fluorometric detection with O-phthalaldehyde (OPA). The detailed composition of buffers, analytical conditions and elution program were reported previously (5, 11).

In our system, putrescine, spermidine, the internal standard and spermine were separated well (Fig. 1). The red blood cell samples required 17 min for analysis, while the plasma samples 28 min, in order to elute peptides or unknown proteins before the polyamine separation. The coefficient of variation of each polyamine was under 4 percent. Polyamine concentrations were expressed as pmol/ml packed red blood cells and pmol/ml plasma. For statistical ana-

lysis, Student's t-test was used.

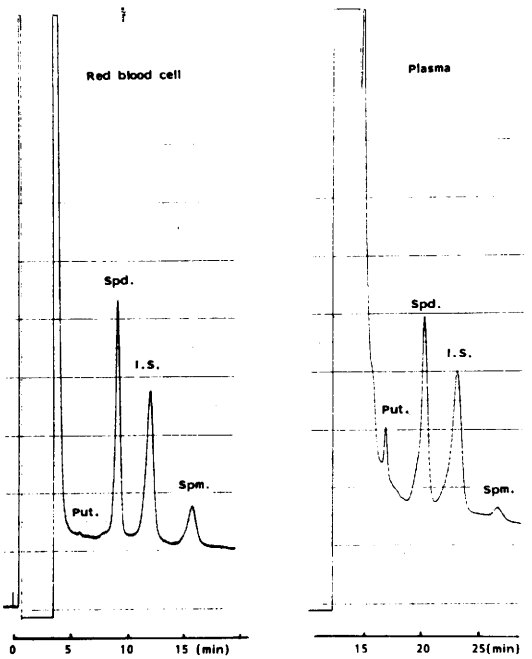

Fig. 1. Chromatograms of polyamines in red blood cells and plasma of the umbilical blood. Elution time of red blood cells was 17 min, and plasma, 28 min. Put.= putrescine, Spd.= spermidine, I.S.= internal standard, Spm.= spermine.

RESULTS

Red blood cell polyamine concentration at delivery. Forty-five samples were obtained after 37 to 41 weeks of gestation and analysed (Table 1). The putrescine concentrations in umbilical venous blood (UV) and umbilical arterial blood (UA) were significantly higher than in maternal venous blood (MV) ($p < 0.001$), but there were no significant differences between UV and UA. The spermidine concentrations in umbilical blood were approximately 2-fold higher than in MV, and spermine,

TABLE 1. CONCENTRATION OF RED BLOOD CELL POLYAMINES AT 37-41 WEEKS OF GESTATION.

	n	Putrescine	Spermidine	Spermine
MV	45	0.57 ± 0.22	19.27 ± 6.31	29.61 ± 13.33
UV	45	0.83 ± 0.27*	37.98 ± 10.54*	45.52 ± 16.42*
UA	45	0.79 ± 0.25*	38.68 ± 10.17*	43.34 ± 15.65*

Values are the mean ± S.D. (nmol/ml packed RBC). MV=maternal venous blood, UV=umbilical venous blood, UA=umbilacal arterial blood. * $p < 0.001$ compared with MV level.

which is the most abundant polyamine in red blood cells, was 1.5-fold higher. However, there were no significant differences between UV and UA.

Plasma polyamine concentration at delivery. Plasma polyamines were measured in 50 normal pregnant women who gave birth after 37 to 41 weeks of gestation (Table 2). All polyamines showed significantly higher concentrations in UV and UA than in MV ($p < 0.001$). There were no significant differences between UV and UA, but each polyamine in UA was slightly higher than in UV.

Alterations of red blood cell polyamines in umbilical blood. Polyamine concentrations in UV at various stages of gestation are shown in Fig. 2. Spermidine and spermine concentrations decreased dramatically with the progress of pregnancy. Especially,

TABLE 2. CONCENTRATIONS OF PLASMA POLYAMINES AT 37-41 WEEKS OF GESTATION

	n	Putrescine	Spermidine	Spermine
MV	50	87.69 ± 33.12	80.08 ± 42.49	141.95 ± 56.63
UV	50	198.63 ± 76.20*	251.96 ± 107.66*	319.20 ± 103.89*
UA	50	238.25 ± 92.44*	296.67 ± 120.28*	340.06 ± 125.25*

Values are the mean ± S.D. (pmol/ml). MV= maternal venous blood, UV= umbilical venous blood, UA= umbilical arterial blood. * $p < 0.001$ compared with MV level.

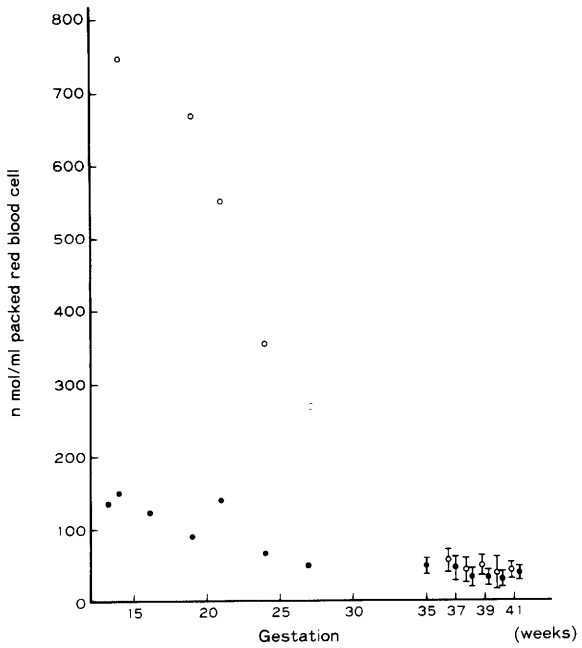

Fig. 2. Alterations of red blood cell polyamines in umbilical blood. (●): spermidine, (○): spermine. Prior to 30 weeks of gestation, each point represents the value of one sample. After 31 weeks of gestation, each point represents the mean ± S.D. of 7-10 samples.

the spermine concentrations at 14 weeks of gestation was 25 times higher than at near term. The putrescine level was very low compared with the other polyamines and showed no remarkable changes (data not shown).

Polyamine concentrations in red blood cells of various ages. Red blood cells from five patients at 38 to 40 weeks of gestation were separated according to age. Each polyamine concentration in UV and MV decreased markedly from the top to the bottom fractions as shown in Fig. 3. Furthermore, MCV decreased from top to

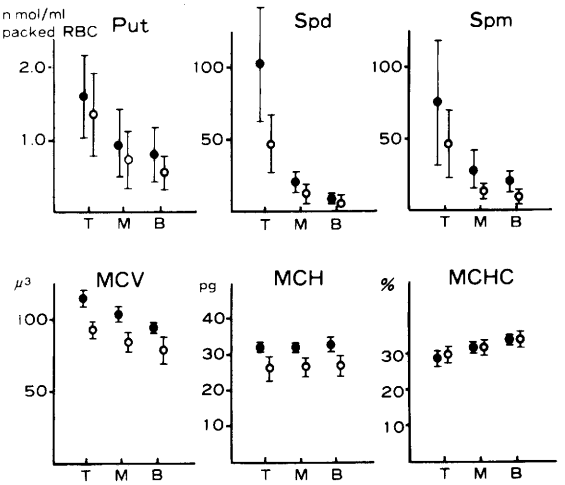

Fig. 3. Polyamine concentrations of age-separated red blood cells. Values are the mean ± S.D. of 6 patients at 38-40 weeks of gestation. (●): umbilical venous blood, (○): maternal venous blood, T = top 10 %, M = middle 10 %, B = bottom 10 %, Put = putrescine, Spd = spermidine, Spm = spermine, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration.

bottom, while MCHC increased, and MCH remained constant. These data were equivarently to the age-dependent changes described by others (10, 12). In the other words, older red blood cells decreased in size (MCV), presumably through membrane loss *in vivo* with age, but they did not lose hemoglobin (constant MCH), and as a result, MCHC increased. In addition, these data revealed that younger red blood cells contained more polyamines than older cells.

DISCUSSION

The relationship between increased polyamine synthesis and rapid proliferation is well established (2). Namely, in rapid growth, the increase in polyamine concentration parallels the rate of cellular proliferation. ODC, which is the rate limiting enzyme of polyamine biosynthesis, always shows a high peak in the early stage of proliferation (2).

A high polyamine concentration in physiological fluid, which is thought to reflect cell kinetics, is noted in various diseases (13). For example, in patients with advanced cancer, increased extracellular polyamines in plasma and urine related to tumor loss (14, 15). In pregnant women and rats, elevated polyamines in amniotic fluid, maternal plasma and urine reflect the active cell proliferation accompaning fetal growth (5, 6, 11).

We reported previously that changes in polyamines in maternal blood and urine during pregnancy were difficult to discuss (11), because the fetal metabolic changes first appear in umbilical blood and finally in maternal urine. Furthermore, maternal blood and urine reflect metabolic changes of the pregnants with gestation. However, polyamines in amniotic fluid may be useful as a biochemical marker of fetal growth (11).

In this study, we investigated the polyamines in umbilical blood which reflect fetal metabolic changes.

The concentrations of plasma polyamines in UV and UA at 37 to 41 weeks of gestation were significantly higher than in MV, which reflects the active metabolic changes in the fetus. In human liver, ODC and SAMDC activities, which control polyamine biosynthesis, are high during development, and the concentration of putrescine displays a distinct peak at 5 to 6 months of gestation (16). In addition, in rats, we found polyamine concentrations and ODC and SAMDC activities in the liver, brain and placenta to be significantly elevated during pregnancy and markedly decreased in experimentally induced IUGR rat fetuses (9).

The difference in plasma polyamine concentrations between umbilical blood and maternal blood agrees with the differences in polyamine oxidase. Illei and Morgan (17, 18) reported that polyamine oxidase activity in the serum of pregnant women increased as pregnancy advanced, however, no polyamine oxidase activity was found in fetal cord serum.

A significantly high polyamine concentration was also noted in umbilical erythrocytes at term. This phenomenon appears to reflect not only active fetal metabolism but also active erythropoiesis in fetal bone marrow. It is well known that fetal bone marrow is in a state of erythroid hyperplasia (19) and that umbilical blood at term contains 250-500/mm^3 of nucleated red blood cells and 2.5-6.5 % reticulocytes (20). Nucleated blood cells including nucleated red blood cells, mononuclear and polymorphonuclear leucocytes and reticulocytes contain significantly higher levels of polyamines than unnucleated blood cells such as red blood cells and platelets (10).

Furthermore, younger erythrocytes, which are abundant in umbilical blood, have a higher polyamine concentration than older erythrocytes (Fig. 1). This finding may be associated with the residual ribosome and RNA content of reticulocytes and were disposed of concomitantly with the ribosomes. This interpretation is consistent with the finding that polyamine concentrations in bone marrow are higher than in peripheral blood (21). Thus, umbilical blood contains more polyamine than maternal blood.

Presumably the alterations in spermidine and spermine in UV at various stages of gestation (Fig. 2) mainly reflect changes in erythrocyte compornents with the progress of pregnancy. Namely, umbilical blood contains many premature erythrocytes which contain much polyamine at the early stage of pregnancy, but fewer premature erythrocytes as pregnancy progress (22).

The date mentioned above suggest that red blood cell polyamine is related more closely to erythropoiesis in bone marrow than metabolic changes of the fetus, whereas plasma polyamine is related to active fetal metabolism.

In investigating the physiological function of red blood cell polyamines, we must consider the localization of polyamines in red blood cells. There are two theories concerning the localization of red blood cell polyamines. Shimizu *et al.* (23) reported that polyamines occur in and around the nucleus, but Chun *et al.* (24) reported that polyamines bind on the surface of red blood cells by zeta potential. The data presented in Fig. 3 indicate that the concentration of putrescine, spermidine and spermine decreased with age. Additionally, MCV decreased with age, while MCH did not, indicating that red blood cells lose cell membrane compornents and membrane assiciated enzymes as they age (12, 25, 26). These findings suggest that polyamines in red blood cells are highly associated with the cell membrane. Polyamines may indicate the proliferative responce of bone marrow in a variety of anemias of questionable etiology. Several investigators used choline esterase and glutamine oxaloacetic transferase (GOT) as indicators, and GOT has been shown to be the most sensitive indicators (26). However, our data suggest that polyamines may be more sensitive than either choline esterase or GOT, because the top to bottom ratio of spermidine: 10 and spermine: 5 are higher than that of GOT: 4.5 (26). In addition, our analytical method to determine red blood cell polyamine levels is very simple.

In conclusion, the present results suggest that polyamines play some important roles in fetal growth, although the precise physiological function is still uncertain. Metabolic changes with fetal growth are reflected in umbilical blood plasma, whereas red blood cell polyamine levels reflect mainly the condition of erythropoiesis in bone marrow. Studies are ongoing to determine polyamine concentrations in various anemias, and the action of many hormones on polyamine metabolism, because several hormones which increase during pregnancy have influences on polyamine biosynthesis.

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