Lipid composition and metabolism in megakaryocytes at different stages of maturation

Paul K. Schick, Kira Williams-Gartner, and Xiaoli He

Cardeza Foundation for Hematologic Research and Department of Medicine, Jefferson Medical College, Philadelphia, PA 19107

Abstract The lipid composition and metabolism of isolated guinea pig megakaryocyte subgroups at various stages of maturation were investigated. Three groups were studied: *1*) 67% of megakaryocytes in Group A were immature; *2*) Group B was heterogeneous and contained both immature and mature subgroups of megakaryocytes; *3*) 92% of megakaryocytes in Group C were mature. Lipid composition was determined by thin-layer chromatography, lipid-phosphorus, and gas-liquid chromatography. Cholesterol, ceramide, and de novo fatty acid synthesis were evaluated with [^{14}C]acetate. [^{14}C]Glycerol was used to assess de novo phospholipid synthesis. ^{14}C-Labeled fatty acids were used to evaluate fatty acid uptake. The phospholipid and cholesterol content was found to be four times greater in mature megakaryocytes than that in immature megakaryocytes, which paralleled the protein content and volume of mature and immature cells. The cholesterol-phospholipid ratio was similar and there were no differences in the phospholipid species in the three groups. Phospholipid and cholesterol synthesis were established in immature megakaryocytes and persisted at about the same level in mature megakaryocytes. The uptake of arachidonic and palmitic acids also occurred primarily in immature cells, while the de novo synthesis of palmitic acid occurs predominantly in mature megakaryocytes. There was an inverse relationship between the uptake of exogenous palmitic acid and fatty acid synthesis, but the uptake of palmitic acid primarily inhibited fatty acid synthesis in mature megakaryocytes. There were differences in the acylation of phospholipid species with arachidonic acid in megakaryocytes at different stages of maturation since the acylation of phosphatidylcholine occurred primarily in immature megakaryocytes. The observation that the lipid content per cell increases markedly as megakaryocytes mature indicates that extensive synthesis and uptake of lipids must occur in the course of megakaryocyte maturation. The study demonstrated that most lipids are synthesized primarily in immature megakaryocytes. —**Schick, P. K., K. Williams-Gartner, and X. He.** Lipid composition and metabolism in megakaryocytes at different stages of maturation. *J. Lipid Res.* 1990. **31:** 27-35.

Supplementary key words fatty acid synthesis • phospholipid • ceramide • cholesterol

Lipids and their metabolism are considered to have an important role in the maturation of cells and tissues. There is a developmental increase in fatty acid synthase in fetal lung (1), gangliosides can modulate the activity of growth factors (2), and dimethyl sulfoxide (DMSO) can induce changes in lipid metabolism in several cancer cell lines that precede and may influence the subsequent effects on cell differentiation and growth (3).

The megakaryocyte provides an opportunity to study the relation of lipid metabolism to maturation in a nonmalignant cell. Each megakaryocyte can synthesize from 1,000 to 4,000 platelets within a relatively short period of time. Thus, thrombopoiesis must be dependent on active membrane and lipid synthesis in megakaryocytes. There is evidence that megakaryocytes have a greater capacity for lipid metabolism than platelets and can, to some extent, determine the lipid composition of platelets (4–6). For example, only megakaryocytes but not platelets can synthesize cholesterol (5). In a study in which the uptake of [^{3}H]arachidonic acid in individual cells was studied by autoradiography, we determined that arachidonic acid was preferentially incorporated into immature megakaryocytes (7). Our laboratory has recently introduced a method for isolating subgroups of megakaryocytes at various stages of maturation that are suitable for the investigation of the biochemistry of maturation (8). We have studied the lipid content and metabolism in megakaryocyte subgroups isolated by this procedure. The lipid content was found to be considerably greater in mature than in immature cells. Cholesterol and phospholipid synthesis and the uptake of fatty acids occurred primarily in immature megakaryocytes. However, de novo fatty acid synthesis occurred predominantly in mature megakaryocytes. The implications of these observations for the maturation of megakaryocytes and the production of platelets are considered.

Abbreviations: HPTLC, high performance thin-layer chromatography; GLC, gas-liquid chromatography.

METHODS

The subgroups of megakaryocytes at different phases of maturation were prepared as recently described (8). Bone marrow cells were scraped from the long bones of guinea pigs and megakaryocytes were partially purified by albumin density gradient centrifugation as described by Levine and Fedorko (9) and Schick et al. (6, 7, 10). Thereafter, the cells were layered as a monolayer on an albumin gradient and separated by unit gravity in a Celsep Separation Chamber. Twenty two fractions were collected; the cells had been separated by size. Megakaryocytes were identified by morphologic criteria. The identity of small megakaryocytes was verified by using anti-vWF antibody, a megakaryocyte marker (8). As a result of these evaluations we determined that fractions 2–18 contained primarily megakaryocytes, in that 88% of the cells were megakaryocytes, and the remainder of cells were erythrocyte and leukocyte precursors. Fractions 19–22 contained primarily other bone marrow cells, erythrocyte and leukocyte precursors. Fractions 2–18 were pooled to form three groups that contained megakaryocytes at distinct phases of maturity as described recently (8).

Isolation of contaminating bone marrow cells

Megakaryocyte-depleted cell suspensions were obtained by combining fractions 19 and 20 collected during the Celsep procedure (8). These megakaryocyte-depleted fractions were used in control experiments.

Counting of cells, sizing of cells in suspension, and testing for viability

Megakaryocyte number and contamination were determined in triplicate in a hematocytometer under phase contrast microscopy. Viability was determined by trypan blue exclusion and acridine orange inclusion under fluorescence microscopy (7, 8, 10).

Protein analysis was performed by the method of Lowry et al. (11)

Analysis of megakaryocyte ploidy, morphological stage, and size (diameter)

Ploidy was determined by the estimation of cellular DNA using Chromomycin A3 and microdensitometry as previously described (7, 8, 10). The morphological stage of megakaryocytes was determined by the assessment of nuclear–cytoplasmic ratio and nuclear configuration. The diameter of megakaryocytes was measured with an optical micrometer. The mean of the shortest and longest diameters was used to determine the size of ellipsoid megakaryocytes. The data were analyzed by a computer-assisted program.

Metabolic studies

[^{3}H]Glycerol (11.5 Ci/mmol), [U-^{14}C]acetate (58 mCi/mmol), [^{14}C]palmitic acid (56 mCi/mmol), [^{3}H]palmitic acid (30 Ci/mmol), and [^{14}C]arachidonic acid (52 mCi/mmol) were obtained from the New England Nuclear Co., Boston, MA. Isolated megakaryocytes in Eagles medium with 0.1% fatty acid free albumin at a concentration of 100,000 cells per ml were incubated with radiolabeled lipid precursors for 18 h at 37°C. Incorporation of radioactivity into lipid species was determined by scintillation spectrometry with correction for quenching.

In order to determine the effect of fatty acid uptake on fatty acid synthesis, different concentrations of palmitic acid along with radiotracer amounts of [^{3}H]palmitic acid were complexed with fatty acid-free albumin as previously described (12) and incubated with megakaryocytes. The uptake of palmitic acid into megakaryocyte lipids was determined by scintillation spectrometry. The release of palmitic acid synthesized from [^{14}C]acetate into the incubation medium was determined by measuring radioactivity in lipids that had been extracted from the incubation medium and separated by HPTLC.

Lipids were extracted by the Bligh-Dyer method and lipid species were separated by high performance thin-layer chromatography (HPTLC). Neutral lipids were separated on HPTLC chromatoplates (Analtech, Newark, DE) developed in petroleum ether–diethyl ether–glacial acetic acid 70:20:1 (v/v/v). Phospholipids were separated on E. Merck HPTLC chromatoplates (#6541) E-M Sciences, Cherry Hill, NJ) developed in chloroform–methanol–glacial acetic acid–water 81:10:45:1 (v/v/v/v). In order to investigate the synthesis of ceramides, the lipid extract was purified by silicic acid chromatography and ceramides were separated by TLC (13).

Phospholipids were quantitated by the determination of lipid-phosphorus, and cholesterol was determined by GLC using a SP 2100 2-ft column (4). The fatty acid composition was studied by subjecting lipids to acid methanolysis and analyzing fatty acid methyl esters by GLC using a SP 2330 6-ft column (4).

Beta-oxidation and metabolism of lipids

The extent of beta-oxidation was determined by incubating the cells with [1-^{14}C]arachidonic acid and trapping and measuring the released [^{14}C]CO_2 in Center Wells (Kontes, Vineland, NJ). In order to determine whether arachidonic acid taken up by megakaryocytes had been elongated, desaturated, or metabolized, lipids were extracted from megakaryocytes and were subjected to acid methanolysis. Unsaturated fatty acids derived from megakaryocyte lipids along with authentic standards were separated according to unsaturation by argentation thin-layer chromatography (6) so that radioactivity in isolated arachidonic acid and potential arachidonic acid metabo-

lites could be assessed. In order to determine whether elongation of arachidonic acid had occurred, fatty acid methyl esters were separated according to carbon number by reverse phase HPTLC (7) using Merck RP-18 chromatoplates (E-M Science, Cherry Hill, NJ) with 90% acetonitrile as the solvent system.

Differences between the three groups of megakaryocytes were determined by using an unpaired Student's *t*-test. Data were accepted as significant if they differed with a P of less than 0.05.

RESULTS

Megakaryocytes were separated according to size by the Celsep procedure and 22 fractions were collected. Fractions 2–18 contained primarily megakaryocytes and the purity of megakaryocytes in these fractions was 88% by cell number. The assessment of purity by the determination of the number of megakaryocytes versus contaminating cells understates the purity of isolated megakaryocytes. Megakaryocytes are considerably larger than other bone marrow cells, and thus 88% purity by cell number is equivalent to greater than 98% purity by cell volume (8, 10). Purity based on volume rather than on cell number is more relevant when interpreting biochemical and metabolic data. Fractions 19–22 contained primarily erythrocyte and leukocyte precursors and virtually no megakaryocytes. About 90% of megakaryocytes in fractions 2–18 were viable based on trypan blue exclusion and acridine orange inclusion. The maturity of megakaryocytes was assessed by considering both morphologic stage and ploidy. Six subgroups of megakaryocytes were identified: mature megakaryocytes, stage III and IV cells, at 8N, 16N or 32N ploidy; immature megakaryocytes, stage I and II cells, at 8N, 16N or 32N ploidy. The fractions were pooled into three groups, which contained megakaryocytes at different phases of maturation: Group A, fractions 16–18, contained 67% immature megakaryocytes and was composed of primarily the 8N immature subgroup. Group B, fractions 12–15, contained 29% immature megakaryocytes and was heterogenous since it contained the 8N mature, 16N immature as well as the 16N mature subgroups. Group C, fractions 2–11, contained 8% immature megakaryocytes and the remainer of the megakaryocytes in this group were mature and consisted of only 16N mature and 32N mature subgroups (8).

The total lipid content of megakaryocytes in the three groups differed as shown in **Table 1**, and there were considerably greater amounts of lipids in mature than in immature cells. The relative amounts of cholesterol and phospholipids in Groups A, B, and C were approximately 1/2/4, whch paralleled that of the protein content of the three groups and the cell volume in the three groups (8). The data on the lipid-P and cholesterol were corrected for recovery by tracing the recovery of standards during the procedure. Recoveries were 96% for phospholipids and 97% for cholesterol.

The cholesterol/lipid-phosphorus ratio was similar in the three groups. There are five major fatty acids in guinea pig megakaryocytes: palmitic acid, stearic acid, oleic acid, linoleic acid, and arachidonic acid. The percent distribution of two of these fatty acids differed significantly in the three fractions. Palmitic acid represented 30.7 ± 1.2, 27.3 ± 1.7, and 23.3 ± 1.1% of total fatty acids in groups A, B, and C, respectively; linoleic acid represented 12.7 ± 0.5, 15.8 ± 1.6, and 17.8 ± 1.3% of total fatty acids in the three groups. The differences between groups A and C were significant at $P < 0.01$. Thus, there was a relative increase of palmitic acid and a relative decrease in linoleic acid in immature megakaryocytes. Five major phospholipids were detected in the three groups: phosphatidylcholine, phosphatidylethanolamine, sphingomyelin, phosphatidylserine, and phosphatidylinositol. The relative amounts of each of these lipid species were similar to that previously reported in isolated megakaryocytes (4), and there were no differences in the phospholipid composition in the three groups of megakaryocytes.

The results of metabolic studies that are described below are expressed as activity per 0.1 mg megakaryocyte protein rather than per a given number of cells. As noted above there were marked differences in the protein content and volume of cells in the three groups of megakaryocytes (8). It is more appropriate to assess biosynthetic activities per cell protein or volume than per cell.

Acetate had been primarily incorporated into cholester-

TABLE 1. Lipid composition of the three megakaryocyte groups

Group	Lipid-P per 10^6 Cells	Cholesterol per 10^6 Cells	Protein per 10^6 Cells	C/P Ratio
	μg	*μg*	*mg*	
A. Immature	46.0 ± 16	8.7 ± 0.9	0.30 ± 0.05	0.38 ± 0.05
B. Intermediate	129.8 ± 10	19.8 ± 0.8	0.65 ± 0.50	0.31 ± 0.04
C. Mature	203.8 ± 16	37.9 ± 1.5	1.29 ± 0.16	0.37 ± 0.04

Lipids and proteins in the three groups of megakaryocytes at different stages of maturation were extracted and analyzed. The mean ± SD of five experiments is shown.

ol and phospholipids. Following acid methanolysis of phospholipids, [^{14}C]acetate was shown to have been incorporated predominantly into palmitic acid rather than into the glycerol backbone of phospholipids. Thus, acetate that had been incorporated into phospholipids represented de novo fatty acid synthesis. The capacity of the three groups of megakaryocytes for cholesterol synthesis and de novo fatty acid synthesis from acetate is shown in **Fig. 1**. Cholesterol synthesis from acetate was active in immature megakaryocytes and persisted at approximately the same level in mature cells. However, de novo fatty acid synthesis occurred at a low level in immature megakaryocytes, and there was a fourfold increase in de novo fatty acid synthesis in mature megakaryocytes. About 2.3%, 2.1%, and 0.2% of the acetate was detected in ceramides, triglycerides, and cholesteryl esters, respectively. There were no significant differences in the incorporation of acetate into cholesteryl esters in megakaryocytes at different stages of maturation. However, there was a fivefold increase in the incorporation of acetate into triglycerides in mature versus immature megakaryocytes and, as was the case in phospholipids, the acetate was detected in triglyceride acyl groups and thus represented de novo fatty acid synthesis.

Glycerol was incorporated primarily into phospholipids and to a lesser extent into triglyceride. **Fig. 2** depicts the incorporation of glycerol into total lipids, phospholipids,

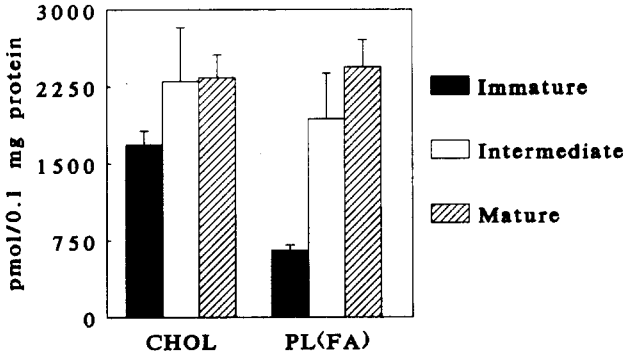

Fig. 1. Cholesterol and de novo fatty acid synthesis from [^{14}C]acetate. The three groups of megakaryocytes at various stages of maturation were prepared as described in Methods. The three groups were incubated with [^{14}C]acetate (0.1 mM) for 18 h, lipids were extracted, and neutral lipids and phospholipids were separated by thin-layer chromatography; the incorporation of radioactivity into cholesterol and other neutral lipids and into phospholipids was determined by scraping of bands and measuring radioactivity by liquid scintillation spectrometry; Chol, cholesterol; PL, phospholipids; FA, fatty acids. Following acid methanolysis of phospholipids, the radioactive acetate was detected only in fatty acids and not the glycerol backbone of phospholipids. Thus, the incorporation of acetate into phospholipids represented de novo fatty acid synthesis. The immature, intermediate, and mature groups consisted of megakaryocytes from groups A, B, and C, respectively. The mean ± SD of four experiments is shown. The difference in the synthesis of fatty acids between the immature and the mature groups was significant at $P < 0.01$. The differences in the cholesterol synthesis in the three groups were not significant.

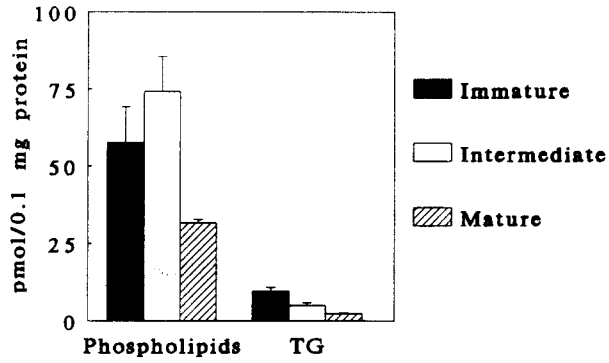

Fig. 2. De novo synthesis of phospholipids and triglycerides from [^{3}H]glycerol. The three groups of megakaryocytes at various stages of maturation, which were prepared as described in Methods, were incubated with [^{3}H]glycerol (0.04 mM) for 18 h, lipids were extracted and separated by thin-layer chromatography, and uptake of glycerol was assessed by scintillation spectrometry; TG, triglycerides. The mean ± SD of four experiments is shown. The difference in the synthesis of phospholipids between the immature and the mature groups was significant at $P < 0.01$. The differences in the synthesis of triglycerides between each of the groups of megakaryocytes were significant at $P < 0.01$.

and triglycerides per 0.1 mg megakaryocyte protein in the three groups. Glycerol, like palmitic acid, was primarily incorporated into phospholipids in immature megakaryocytes, particularly those in group B. The incorporation of glycerol into triglyceride occurred predominantly in immature megakaryocytes. Thus, the backbone of these glycerolipids is primarily synthesized in immature megakarocytes.

Palmitic acid was essentially incorporated only into phospholipids. About 5% of [^{14}C]palmitic acid had undergone beta-oxidation over an 18-h period. **Fig. 3** also depicts the uptake of palmitic acid by the three groups of megakaryocytes, and the incorporation of palmitic acid into mature megakaryocytes was significantly less than into immature cells ($P < 0.01$). The peak incorporation of the fatty acid occurred in group B megakaryocytes, which contain 8N mature megakaryocytes, 16N immature megakaryocytes, as well as 16N mature megakaryocytes. The data shown in Fig. 3 were derived from experiments in which the uptake of palmitic acid (0.018 mM) was determined. The results of the incubation of the three groups of megakaryocytes with palmitic acid (0.05 mM or 0.2 mM) were similar to the data shown in Fig. 3 and thus also indicated that more palmitic acid was taken up into immature than into mature megakaryocytes.

About 3% of arachidonic acid had undergone beta-oxidation in each of the three groups over 18-h incubations. The [^{14}C]arachidonic acid had not been degraded or oxygenated when assessed by argentation and reverse phase TLC (6, 7). Arachidonic acid had been incorporated primarily into phospholipids, and the peak incorporation of the fatty acid had occurred in immature megakaryocytes as shown in Fig. 3. However, there were differences in the

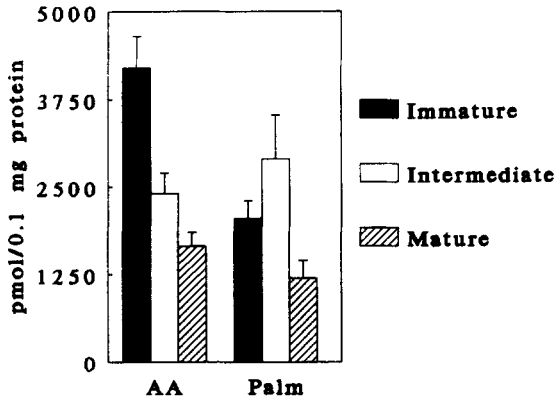

Fig. 3. The uptake of arachidonic or palmitic acids from ^{14}C-labeled fatty acids. The three groups of megakaryocytes, which were prepared as described in Methods were incubated with [^{14}C]arachidonic acid (17.8 μM) or [^{14}C]palmitic acid (17.8 μM) for 18 h, lipids were extracted and separated by thin-layer chromatography, and uptake was assessed by counting the bands by liquid scintillation spectrometry. The mean ± SD of four experiments is shown; AA, arachidonic acid; Palm, palmitic acid. The difference in the arachidonic acid uptake between the immature and the mature groups of megakaryocytes was significant at $P < 0.01$. The difference in the uptake of palmitic acid between the immature and the mature groups was significant at $P < 0.05$.

acylation of phospholipids in megakaryocytes at different stages of maturation. **Table 2** shows that most of the arachidonic acid was incorporated into phosphatidylcholine in immature cells. The observed decrease of total incorporation of arachidonic acid in mature megakaryocytes was primarily due to the decreased uptake into phosphatidylcholine. The majority of the arachidonic acid was incorporated into phosphatidylethanolamine in mature cells. In contrast, palmitic acid had been primarily utilized for the synthesis of phosphatidylcholine and there were no differences in the acylation of other phospholipids in megakaryocytes at different stages of maturation.

The influence of palmitic acid uptake on de novo synthesis of palmitic acid was studied. An inverse relationship between the amount of fatty acid uptake and fatty acid synthesis was detected in isolated megakaryocytes that had not been separated into subpopulations by the Celsep procedure in the dose-response studies. The data are shown in **Fig. 4**. A 50% reduction in the synthesis of palmitic acid occurred when megakaryocytes were incubated with 0.02 mM palmitic acid which resulted in the uptake of 13 nmol palmitic acid per 100,000 megakaryocytes. The incubation of megakaryocytes with comparable amounts of oleic and linoleic acids caused respective depressions of palmitic acid synthesis of 31% and 19%. There was a release of 22% of newly synthesized fatty acids into the incubation medium derived from radiolabeled acetate which was independent of the concentration of palmitic acid in the incubation medium. Thus, the observed decrease in the incorporation of radiolabeled acetate into megakaryocyte lipids was not due to an increase in the release of newly synthesized fatty acids into the incubation medium. This release of radiolabeled fatty acids was due to the presence of fatty acid-free albumin in the incubation medium and did not occur in incubation medium without albumin, which is consistent with the observation that platelet fatty acids are released when albumin is present in the incubation medium (14).

Incubation with 0.02 mM palmitic acid, a concentration that caused a 50% reduction in the synthesis of palmitic acid in isolated megakaryocytes that had not been separated by the Celsep procedure, was used to distinguish the effects of palmitic acid on the synthesis of palmitic acid in megakaryocyte subpopulations at different phases of maturation. The incubation of megakaryocytes with palmitic acid (0.02 mM) caused a 59 ± 4% reduction in the synthesis of palmitic acid in mature megakaryocytes but only a 12.1 ± 2.4% reduction of fatty acid synthesis in imma-

TABLE 2. Arachidonic acid uptake into phospholipid species in megakaryocytes at different phases of maturation

	Group A Immature	Group B Intermediate	Group C Mature
		%	
PC	46.5 ± 6.3 (1930)[a]	36.0 ± 3.1 (846)	20.3 ± 6.8 (335)
PI	24.0 ± 4.6 (996)	31.2 ± 4.8 (744)	35.3 ± 7.4 (582)
PS	7.5 ± 1.7 (311)	10.1 ± 0.8 (240)	10.0 ± 1.0 (165)
PE	21.1 ± 7.8 (872)	24.0 ± 1.9 (576)	35.7 ± 4.5 (598)

The three groups of megakaryocytes were incubated with [^{14}C]arachidonic acid (17.8 μM) for 18 h. Lipids were extracted; phospholipid species were separated by HPTLC; and uptake was assessed by scraping bands and by measuring radioactivity by scintillation spectrometry. The mean ± SD of four experiments is shown. The difference in the uptake of arachidonic acid into PC between immature and mature megakaryocytes was significant at $P < 0.01$. The difference in the uptake of arachidonic acid into PE between immature and mature megakaryocytes was also significant at $P < 0.01$. PC, phosphatidylcholine; PI, phosphatidylinositol; PS, phosphatidylserine; PE, phosphatidylethanolamine.

[a]The values in parentheses represent pmol arachidonic acid taken up per 0.1 mg megakaryocyte protein. See Fig. 3 for total amount of arachidonic acid taken up in each group of megakaryocytes.

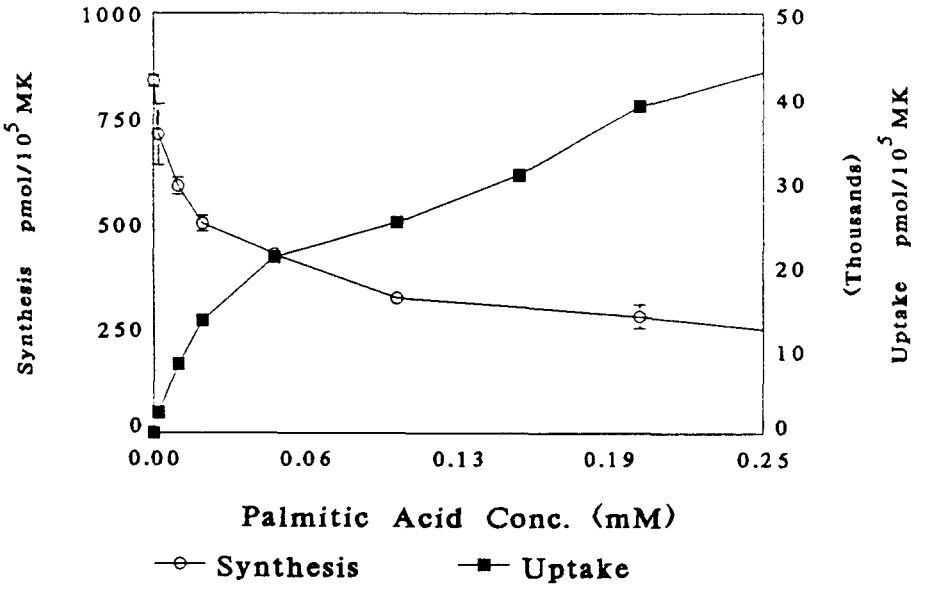

Fig. 4. The effect of the uptake of palmitic acid on de novo palmitic acid synthesis in megakaryocytes. Megakaryocytes, isolated by the method of Levine and Fedorko (9) but not separated into subpopulations by the Celsep procedure, were resuspended in incubation medium with different concentrations of palmitic acid. The concentration of palmitic acid in the incubation medium was assessed after lipid extraction and acid methanolysis by GLC. The uptake of palmitic acid by megakaryocytes was determined by using [^{3}H]palmatic acid. Synthesis of palmitic acid was determined by using [^{14}C]acetic acid (0.1 mM). Incubations were carried out for 18 h. The means ± SD of five experiments are shown.

ture megakaryocytes. The data represents the mean ± SD of four experiments.

As noted above, fractions 2–18 contained megakaryocytes at 88% purity by cell number and greater than 98% purity by cell volume. It was important to determine whether the contaminating bone marrow cells influenced the data. Therefore, the incorporation of palmitic acid, glycerol, acetate, and arachidonic acid was also studied in isolated Celsep fractions 19–20, which contained bone marrow cells but were depleted of megakaryocytes. Lipid synthesis and fatty acid uptake were determined per the number of contaminating cells that were present in each of the three groups of megakaryocytes. These control experiments indicated that less than 2% of these radiolabeled lipid precursors had been incorporated into contaminating bone marrow cells in the three groups of megakaryocytes. Therefore, nonmegakaryocyte bone marrow cells in the three groups of megakaryocyte suspensions did not significantly influence the interpretation of lipid synthesis in megakaryocytes.

DISCUSSION

The ability to isolate three groups of megakaryocytes that are at different phases of maturation has permitted the investigation of lipids and their metabolism in relation to megakaryocyte maturity. Cytoplasmic maturation was assessed by the determination of morphologic stage. Four morphologic stages were identified with stages I and II being immature cells and stages III and IV being mature megakaryocytes. Megakaryocytes collected from the Celsep apparatus were pooled into three groups as previously described (8). Megakaryocytes in the three groups were at distinct phases of cytoplasmic maturation, and the percent of immature megakaryocytes in Groups A, B, and C was 67%, 29%, and 8% respectively.

In order to comprehensively appraise the three groups of megakaryocytes, both ploidy and morphologic stage were considered. Megakaryocytes undergo endomitosis and are thus polyploid cells, and the majority of megakaryocytes in bone marrow are 8N, 16N, and 32N cells. The ploidy of megakaryocytes is thought to be established prior to the evolution of morphologic stage. Six subgroups of megakaryocytes were identified: mature cells at 8N, 16N, and 32N ploidy; and immature cells at 8N, 16N, and 32N ploidy. Group A contained primarily 8N immature and some 16N immature megakaryocytes; group B was more heterogeneous in that it contained 16N immature and 8N and 16N mature subgroups; and group C was homogenous in that it contained virtually only the mature 16N and 32N subgroups (8).

There were considerable differences in the total amount of phospholipids and cholesterol in the three groups of megakaryocytes when calculated as lipid per cell. On a per cell basis, the relative amounts of cholesterol and phospholipids in the groups A, B, and C were about 1/2/4, respectively, which parallels the differences in the volume

and protein content of the three groups. Thus, both the lipid and protein content were proportionate to the volume of cells in each of the groups. The cholesterol/phospholipid ratio was similar in the three groups. Each of the groups contained the five major phospholipids that have been previously detected in guinea pig megakaryocytes. The major phospholipid was phosphatidylcholine, and there were no differences in the phospholipid composition of megakaryocytes in the three groups. There was significantly less linoleic acid with a corresponding increase in palmitic acid in immature megakaryocytes, but the implications of this finding are not clear. Since there were no major differences in the lipid composition of megakaryocytes at the various stages of maturation, the study indicates that lipid composition is established in immature megakaryocytes. However, extensive synthesis of lipids must occur in megakaryocytes as they progress from stage I to IV or from 8N to 32N ploidy since our study has shown that there is considerably more lipid per cell in mature megakaryocytes and in cells of a higher ploidy class.

The synthesis of cholesterol and cholesteryl esters, monitored by acetate, was shown to be active in immature megakaryocytes and to persist at about the same level in mature cells. Megakaryocytes, unlike platelets, can synthesize cholesterol. Since both megakaryocytes and platelets have a limited capacity for cholesterol uptake, synthesis in megakaryocytes appears to be the primary mechanism for establishing the cholesterol composition of platelets. Human platelets contain a considerable amount of free ceramides, and can utilize acetate for the synthesis of these moieties (14). The current study indicates that active synthesis of ceramides is evident in guinea pig megakaryocytes.

In contrast, synthesis of phospholipids and triglycerides from acetate occurred primarily in groups B and C, which contain mature megakaryocytes, but was markedly decreased in group A, which contained the most immature megakaryocytes. The incorporation of acetate into these glycerolipids represents de novo fatty acid synthesis since acetate was shown to be utilized for synthesis of acyl groups but not the glycerol backbone of glycerolipids. Therefore, de novo synthesis of fatty acids was shown to occur primarily in mature megakaryocytes while the synthesis of cholesterol from acetate was established in immature megakaryocytes.

The key enzymes for de novo fatty acid synthesis are acetyl-CoA carboxylase and the fatty acid synthase complex. Fatty acid synthase is present in mature red blood cells (15) and leukocytes (16) but these cells are not capable of de novo fatty acid synthesis due to a lack of acetyl-CoA carboxylase. However, reticulocytes possess both enzyme systems and are capable of de novo fatty acid synthesis (17). Circulating platelets are the only peripheral blood cells that can synthesize fatty acids, and have been shown to contain both of these enzymes (18). Fatty acid synthase activity was increased in platelets after splenectomy, possibly due to increased megakaryopoiesis (18). Thus, these lipogenic enzymes are related to the maturation of bone marrow cells and the production of peripheral blood cells.

We have shown that de novo synthesis of fatty acids occurs primarily in mature megakaryocytes. The most likely basis for this observation is a decrease or absence of acetyl-CoA carboxylase and/or fatty acid synthase in immature megakaryocytes and the expression of these enzymes in mature megakaryocytes. Fatty acid synthase is detectable in high levels in differentiated cells but not in immature basal cells in the holocrine secretory tissue of the uropygial gland, and the signals for differentiation and fatty acid synthase accumulation appeared to be linked to one another and the cessation of cell division (19). In the investigation of developing fetal rat lung, there was a developmental increase in fatty acid synthase activity between 19 and 21 days gestation (1). Also, the developmental and hormone-induced changes in fetal lung de novo fatty acid synthesis were shown to be mediated by fatty acid synthase (1). Fatty acid synthase and/or acetyl-CoA carboxylase activity conceivably is involved in megakaryocyte maturation and platelet production.

The incorporation of glycerol, which represents de novo synthesis of the backbone of phospholipids, and palmitic acid occurred primarily in megakaryocytes in groups A and B, which contained immature megakaryocytes, with the peak being in group B. Significantly less of both of these lipid precursors were incorporated into megakaryocytes in group C which contained the most mature cells. This information indicates that de novo phospholipid synthesis occurs primarily in immature cells. Since the incorporation of palmitic acid paralleled that of glycerol, palmitic acid most likely was utilized for the synthesis of new phospholipids rather than remodelling of existing phospholipids.

Arachidonic acid has been shown to be preferentially incorporated into immature megakaryocytes in a cytological study that determined uptake by autoradiography (7). The current study confirmed that arachidonic acid is primarily taken up by immature megakaryocytes, but, in addition, showed that there are differences in the acylation of phospholipids with arachidonic acid in megakaryocytes at different stages of maturation. Arachidonic acid was primarily incorporated into phosphatidylcholine in immature megakaryocytes and into phosphatidylethanolamine in mature megakaryocytes. The observed decrease of the total uptake of arachidonic acid into mature cells is due primarily to a decrease of the incorporation of this fatty acid into phosphatidylcholine in mature megakaryocytes. Thus, the activities of arachidonyl acyltransferase, particularly those involved in the acylation of phosphatidylcholine, and possibly transacylases differ in mega-

karyocytes at various stages of maturation. Acyltransferases and transacylases have been detected in platelets (20, 21). The differences in acyltransferase activities appear to be unique to arachidonic acid since the incorporation of acetate and palmitic acid into phospholipid species was similar in megakaryocytes in the three groups of megakaryocytes. Most likely arachidonyl acyltransferase activity, like fatty acid synthase activity, plays an important role in megakaryocyte maturation and platelet production.

Another consideration is that the source of palmitic acid differs in megakaryocytes at different phases of maturation. De novo lipid synthesis of fatty acids occurs predominantly in mature cells while the uptake of palmitic acid occurs primarily in immature megakaryocytes. Conceivably, the palmitic acid content of immature megakaryocytes is more likely to be dependent on uptake of fatty acids from plasma and thus the fat content of diets. However, the saturated fatty acid content of mature megakaryocytes is more likely to be dependent on de novo fatty acid synthesis.

The uptake of palmitic, oleic, and linoleic acids can depress the synthesis of palmitic acid and this effect is greater in mature that in immature megakaryocytes. Thus, palmitic acid synthesis in mature megakaryocytes is regulated by both de novo fatty acid synthesis and dietary fat.

The study has provided new information about the biochemical characteristics of megakaryocytes at different stages of maturation. We have shown that serotonin uptake (22) and the synthesis of higher molecular weight proteoglycans (8, 23) occur primarily in immature megakaryocytes, while the synthesis of small proteoglycans (8, 23) and the expression of sialoglycoproteins occur primarily in mature megakaryocytes (24). The current study indicates that most aspects of lipid synthesis occur primarily in immature megakaryocytes but that there are differences in the acylation of phospholipids in megakaryocytes at different phases of development. De novo fatty acid synthesis, however, occurs primarily in more mature megakaryocytes. These differences in lipid metabolism may represent remodelling of membranes in the course of the maturation of megakaryocytes and during the final events leading to the production of platelets.

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