Human Platelets: Amino Acid Synthesis* (34018)

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Although great interest has been shown in the glycolytic pathway in human platelets, little attention has been given to the Krebs cycle. Furthermore, the synthesis of amino acids has not been previously demonstrated in platelets. Using U-^{14}C-D-glucose,[1] and U-^{14}C-acetate we have shown that glutamic acid, aspartic acid, glutamine, and asparagine are synthesized by the platelets.

Materials and Methods. Normal human blood was collected in acid–citrate–dextrose (ACD) anticoagulant (1). The platelets were isolated and concentrated in the homologous plasma by differential centrifugation at 4° as described elsewhere (2). The suspension was adjusted to contain a packed platelet volume of between 7 and 10% equivalent to between 0.97 and 1.29 $\times$ 10^{10} platelets/ml suspension. Contamination by erythrocytes, reticulocytes, and leukocytes was less than 1 cell/3000 platelets. The total leukocyte contamination per milliliter of platelet suspension is approximately 4 $\times$ 10^{6} cell. The volume occupied by the leukocytes is about 0.002 ml or 0.2% of the platelets' volume (3). Amino acid synthesis was studied by incubating platelet concentrates obtained from single donors with 10 μCi U-^{14}C-D-glucose (3 mCi/mmole) or 1 μCi of U-^{14}C-acetate (32.9 mCi/mmole). Aliquots of 2 ml were taken at intervals between 0 and 15 min and washed with 10 times their volume of cold Ringer's solution containing either 5 m*M* of unlabeled glucose or 5 m*M* of unlabeled acetate. The platelets were sedimented by centrifugation at 1465*g* and deproteinization carried out with 5% TCA. The resulting precipitate was separated by centrifugation and washed with 5% TCA. Chromatographic separations of glutamic acid, aspartic acid, glutamine, and asparagine in the clear and neutralized supernatants were performed on columns of AG 1-X4 (200–400 mesh chloride form) ion exchange resin[2] (4). The main fraction of each purified amino acid was dried at 60° under reduced pressure and each reconstituted in 2 ml of distilled water. One milliliter of solution was transferred into a glass vial containing 10 ml of scintillation counting solution (naphthalene 10%[3], PPO 0.4%,[4] bis-methyl styryl benzene 0.03%[5] and the radioactivity was measured in a Packard Tri-carb liquid scintillation spectrometer. The amino acid concentration in the remaining 1 ml was determined by the ninhydrin reaction using the Technicon autoanalyzer.

Results and Discussion. Amino acid content. The levels of glutamic acid, aspartic acid, glutamine, and asparagine found in platelets are given in Table I. The concentration of asparagine found in platelets in this study is 0.4 μmole/ml packed platelets. This value is significantly higher than that found in other tissues (5). Asparagine concentration has never before been measured in platelets. In view of the current interest in the origin of asparagine found in plasma, the possibility that platelets are the major source may deserve further investigation. The contribution of the amount of glutamic acid and glutamine by the leukocytes present in the

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[1] Nuclear-Chicago, Chicago, Illinois.

[2] Bio-Rad Laboratories, Los Angeles, California.

[3] J. T. Baker Chemical Co., Phillipsburg, N. J.

[4] Packard Instrument Co., Inc., Downers Grove, Illinois.

[5] New England Nuclear Corp., Boston, Massachusetts.

TABLE I. Amino Acid Levels in Human Platelets.

Amino acids	Packed platelets[a] (μmole/ml)	No. of experiments
Glutamic acid	2.19 ± 0.42	6
Aspartic acid	0.45 ± 0.15	6
Glutamine	0.81 ± 0.10	6
Asparagine	0.42 ± 0.05	6

[a] Average ± standard deviation.

platelet suspension is insignificant since McNemay (3) has determined 2.5 μmole/ml and 1.6 μmole/ml of leukocytes, respectively. Therefore, the amount of 4×10^6 leukocytes contributes only 0.005 μmole/ml and 0.0032 μmole/ml of glutamic acid and glutamine respectively.

Incorporation of U-^{14}C into the amino acids. In the experiments using U-^{14}C-D-glucose (Fig. 1) the specific radioactivities (S.A.) of glutamine and asparagine were higher than those of their precursors, glutamic acid and aspartic acid even at 2 min of incubation. The high S.A. ratio of product to its apparent precursor suggests that each amino acid is present in platelets in more than one homogeneous pool or compartment (6). Unless platelets can synthesize the amides by a pathway other than that from their own dicarboxylic acid precursors, the concept of compartmentation offers the most likely explanation of the data. Platelets are not unique in presenting compartmentalized amino acid

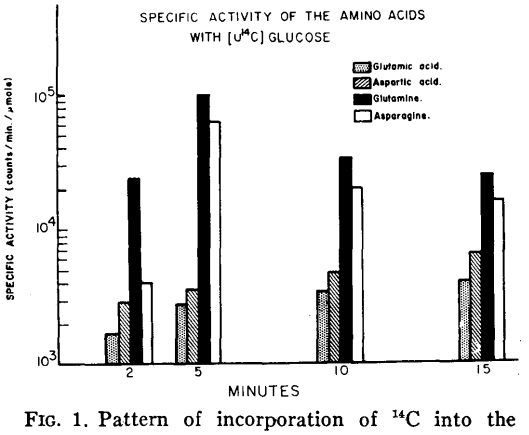

FIG. 1. Pattern of incorporation of ^{14}C into the amino acid after incubation of platelet suspension with U-^{14}C-glucose. The amides peaked at 5 min. (Number of experiments = 5).

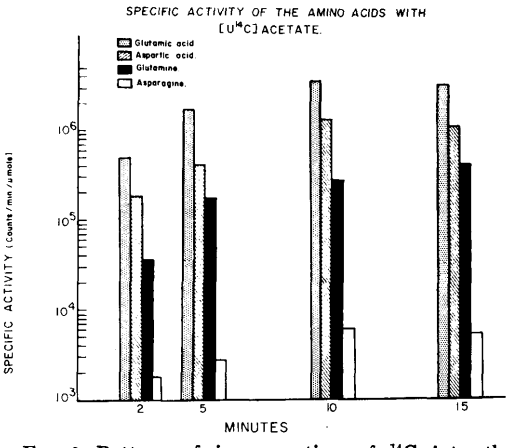

FIG. 2. Pattern of incorporation of ^{14}C into the amino acids after incubation of platelet suspension with U-^{14}C-acetate. The dicarboxylic acids peaked at 10 min. (Number of experiments = 5).

metabolism; brain amino acid compartmentation has been extensively studied (7–10).

Although each of the precursors must enter the Krebs cycle as acetyl-CoA, the result obtained by using U-^{14}C-acetate (Fig. 2) is in sharp contrast to the one obtained with U-^{14}C- D-glucose. The glutamic acid and glutamine pattern shows a more typical precursor-product relationship, since the S.A. of glutamic acid decreases as the S.A. of glutamine increases (6). Asparagine maintains a considerably lower S.A. than aspartic acid. It is suggested, therefore, that when U-^{14}C-acetate is the precursor, asparagine is formed from a less labeled compartment of aspartic acid and diluted by a larger pool of unlabeled asparagine. These data suggest that more than one pool of acetyl-CoA, and probably at least two separate pools of critic acid cycle intermediates exist. This possibility finds precedence in recent studies of brain tissue (11–14). There is increasing evidence that the mitochondrial population in brain and liver tissue may not be homogeneous in its enzyme content (12, 14, 15). This heterogeneity would support the hypothesis of more than one citric acid cycle, which shows selectivity for certain metabolites.

We cannot determine at this time whether the two citric acid cycles are operating within a single type of platelets or in different pop-

ulations of platelets which have different characteristics of substrate-absorption.

The results presented support the concept of amino acid metabolic compartementation and suggest mitochondrial heterogeneity in human platelets.

Summary. The rapid incorporation of labeled ^{14}C from U-^{14}C-acetate and U-^{14}C-D-glucose into free glutamic acid, glutamine, aspartic acid, and asparagine shows that human platelets are capable of synthesizing their own amino acids. Differences in the turnover rates of these amino acids indicate that the metabolic pools are localized in compartments. It is also suggested that more than one pool of acetyl CoA and probably at least two separate pools of citric acid cycle intermediates exist.

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