The Free Amino Acid Pool of Cultivated Human Skin Fibroblasts (36783)

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(Introduced by G. P. Youmans)
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The intracellular concentration of amino acids has been studied in Hela and L cells (1, 2), in transformed hamster fibroblasts (3), Ehrlich ascites tumor cells (4) and a number of specialized cell lines derived from human and animal tissues (5). In man, measurement of the intracellular content of cystine has been shown to be of value in detecting cystinosis in cell culture (6, 7). There has been, to our knowledge, no systematic study of the free intracellular amino acid pool of cultivated human skin fibroblasts. Since the majority of disorders of amino acid metabolism are presently not amenable to study in cell culture utilizing the approach of enzyme analysis, as the enzymatic deficiency in the specific disorder is either not detectable in culture or is unknown (8), measurement of the intracellular concentration of amino acids in human diploid fibroblasts under a variety of culture conditions could be of value in the investigation of these disorders. This study was undertaken to determine the composition of the free amino acid pool of normal human skin fibroblasts under commonly used tissue culture conditions.

Materials and Methods. Culture technique. Skin fibroblast cultures from ten normal individuals were established using the method of Nader *et al.* (9). The primary explants were grown in Ham's F-10 nutrient mixture (10) containing 15% fetal calf serum, penicillin (100 units/ml), streptomycin (100 μg/ml) and Fungizone (2.5 μg/ml) at 37° in a 5% CO_2 atmosphere. After 15 to 21 days the cells were trypsinized and transferred into 75 cm^2 Falcon plastic flasks. After three to four passages, the cultures were trypsinized and stored frozen in liquid nitrogen for a period of 2 to 10 mo. In preparation for amino acid analysis, replicate aliquots of each cell line were cultivated in either Ham's F-10 (Medium I) or Eagle's minimum essential medium (Medium II) (11). Each medium contained 15% fetal calf serum, 100 units/ml of penicillin, 100 μg/ml streptomycin and 2.5 μg/ml Fungizone. Confluent cultures were harvested four days after their last passage or 2 days after their last feeding and processed for amino acid analysis.

Amino acid analysis. At the time of harvest, the nutrient mixture was decanted and filtered. An aliquot of the cell free medium was then deproteinized with sulfosalicylic acid and frozen at −30° for further reference. The cell monolayers were washed twice with Puck's saline A, exposed to 0.25% trypsin solution for 1 min and incubated at 37° until detachment. The cells were collected and washed three times with cold isotonic saline and resuspended in 2 vol deionized distilled water. The cells were disrupted by freeze-thawing five times in a dry ice–acetone bath with intermittent agitation on a vortex mixer. After centrifugation at 100*g* for 10 min, an aliquot of the supernatant was taken for protein determination (12). The cell protein was precipitated with crystalline sulfosalicylic acid (1 mg/mg cell protein) and centrifuged at 4200*g* for 15 min in a Spinco model L preparative ultracentrifuge at 4° The aqueous cell extract was frozen in liquid nitrogen or assayed immediately with a modified version of a Beckman/Spinco model 120 B amino acid analyzer. Aliquots of cell homogenates containing 2 to 6 mg of protein or 0.5 ml deproteinized nutrient mixture were chromatographed using the method of Melancon and Tayco (13). The technique employed lithium citrate buffers and made

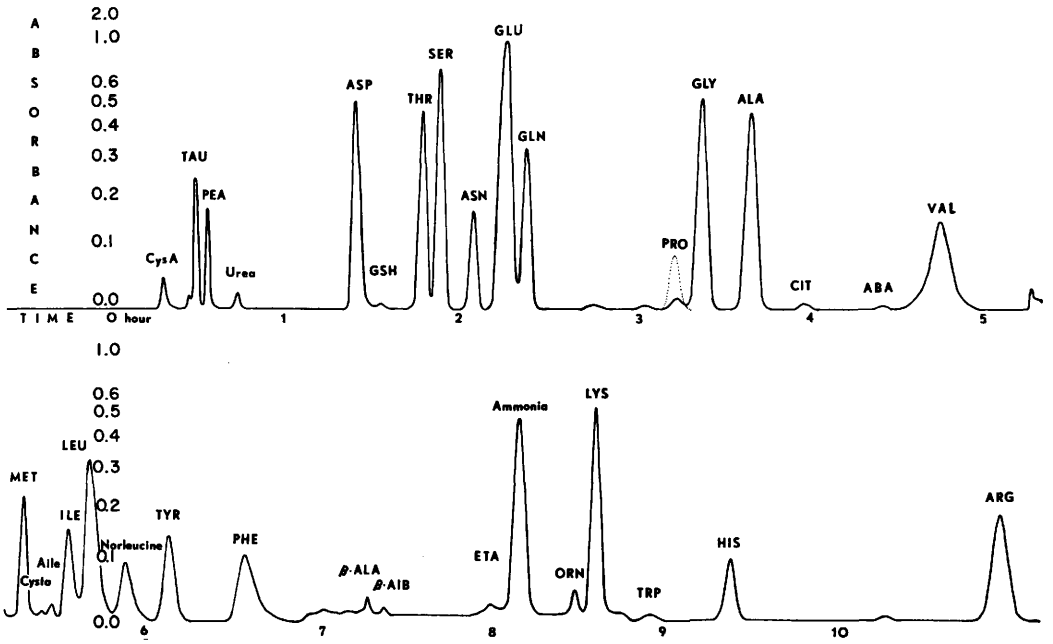

FIG. 1. Tracing of a chromatogram of the free amino acids and related compounds in an aqueous extract of normal cultivated skin fibroblasts equivalent to 5 mg cell protein. Abbreviations: CysA, cysteic acid; Cysta, cystathionine; Aile, alloisoleucine; Eta, ethanolamine. Other abbreviations are conventional ones.

possible accurate determination of the amides asparagine and glutamine independently from aspartic and glutamic acid.

Results. A typical analysis of the free amino acids and related compounds present in cultivated human skin fibroblasts is illustrated in Fig. 1. The mean intracellular concentrations of 26 amino acids found in 10 normal cell lines as compared with the amino acid concentration of each nutrient mixture at the time of harvest are summarized in Table I. Values are not shown for a number of amino acids or related compounds which were present in concentrations too low for accurate measurement. This includes glycerophosphoethanolamine, reduced glutathione, α-aminoadipic acid, α-amino-*N*-butyric acid, cystathionine, alloisoleucine, β-aminoisobutyric acid, γ-aminobutyric acid, ethanolamine and homocarnosine. Also, phosphoserine is eluted in almost the same bed volume as cysteic acid and the values listed for this latter amino acid may represent some contribution by phosphoserine. As judged by the values listed in Table I, there was no significant variation of the mean values of most amino acids in either growth medium, with the exception of glutamic acid and glutamine. Medium I contained a mean 3.4 mmoles of total amino acids/liter while Medium II contained 5.3 mmoles/liter. The total intracellular amino acid content was 605 μmoles/g protein for cells grown in Medium I and 780 μmoles/g protein in Medium II. There was no difference in the cell protein content of cultures grown in either media.

In order to account for the variation encountered when the amino acid concentrations are expressed as a function of cell protein, each amino acid was expressed as a function of total amino acid content of each chromatographic run. Table II illustrates the mean molar ratio of each measurable amino acid to the total amino acid pool of cultivated cells, growth media and normal plasma with the exception of urea and ammonia.

Discussion. The present study indicates that human skin fibroblast cultures exhibit a characteristic pattern of free amino acid content which differs in some way from the amino acid pool previously described in a number of other cultivated mammalian cells

TABLE I. Concentration of Amino Acids in Cultivated Human Skin Fibroblasts.

Amino acid	Medium I (Ham's F-10)		Medium II (Eagle's MEM)	
	Nutrient[a] mixture	Normal[b] fibroblasts	Nutrient[a] mixture	Normal[b] fibroblasts
Cysteic acid	7.2	3.03 ± 2.1	6.2	5.47 ± 1.7
Taurine	12.1	23.00 ± 6.0	18.0	27.49 ± 7.2
Phosphoethanolamine	—	14.34 ± 4.3	—	25.16 ± 6.1
Aspartic acid	113.8	18.87 ± 4.8	20.0	24.36 ± 4.1
Threonine	48.0	25.72 ± 12.6	355.3	32.73 ± 4.4
Serine	151.4	42.66 ± 21.9	45.5	54.03 ± 7.2
Asparagine	95.1	15.88 ± 7.3	—	20.57 ± 3.1
Glutamic acid	207.9	100.54 ± 28.9	169.0	162.53 ± 41.0
Glutamine	639.2	21.40 ± 10.8	1605.7	39.86 ± 6.6
Proline	115.1	37.12 ± 17.3	70.7	59.34 ± 11.7
Glycine	174.8	41.23 ± 13.9	98.5	53.56 ± 8.9
Alanine	210.9	45.28 ± 24.9	156.0	52.18 ± 8.5
Citrulline	7.3	Tr–11.5	12.9	Tr–14.7
Valine	65.5	26.34 ± 15.1	392.4	34.17 ± 4.8
Cystine	122.1	0–Tr	30.0	0–Tr
Methionine	22.0	13.90 ± 7.4	89.2	15.93 ± 2.4
Isoleucine	36.1	17.37 ± 10.6	361.0	17.78 ± 2.6
Leucine	105.7	39.33 ± 10.1	362.6	43.11 ± 6.0
Tyrosine	22.6	16.78 ± 9.3	185.6	19.13 ± 2.7
Phenylalanine	43.3	16.29 ± 9.1	181.8	20.31 ± 3.1
β-Alanine	—	Tr–16.8	—	Tr–31.6
Ornithine	229.2	2.46 ± 0.9	100.4	2.01 ± 0.5
Lysine	167.0	41.83 ± 24.4	313.5	34.08 ± 5.0
Tryptophan	—	Tr– 5.1	48.9	Tr– 3.0
Histidine	98.4	10.21 ± 5.1	166.5	10.50 ± 1.9
Arginine	715.5	33.24 ± 19.5	471.9	29.36 ± 4.4

[a] Expressed as μmoles/liter: mean of two determinations.
[b] Expressed as μmoles/g of cell protein: mean ± SEM.

(1–4). It is important to recognize that the amino acid content of cultivated fibroblasts as determined in this study may not represent the true intracellular amino acid pool at the time of harvest. Piez and Eagle (1) have shown that as much as 30% of the amino acid content of cultivated cells can be lost by strongly washing the cells with cold saline solution before harvesting. In contrast, the intracellular protein and DNA content does not change significantly during exposure to isotonic saline washes, thus accounting for the wide range of absolute values found when amino acid content is expressed as a function of cell protein. However, it is essential that nutient mixture be removed completely before homogenization and the dilution in the intracellular amino acids has been maintained constant by adhering to highly standardized and reproducible methods of processing. In addition, some of the more labile components, such as glutamine and asparagine, may undergo small quantitative changes during the preparation of the samples. Such a loss can be minimized by keeping the samples cold at all times after harvest.

In human skin fibroblast cultures as in most other cultivated mammalian cells and tissues, the major Ninhydrin-reactive components are serine, glutamic acid, proline, glycine, alanine, leucine, lysine and arginine. These eight compounds constitute about 62% of the total amino acid pool of cultivated human skin fibroblasts. This is in contrast with Hela and L cells where taurine gluta-

TABLE II. Molar Ratios of Amino Acids in Cultivated Skin Fibroblasts Compared with Nutrient Mixture at the Time of Harvest and Normal Plasma.[a]

Amino acid	Normal[b] plasma	Medium I (Ham's F-10)		Medium II (Eagle's MEM)	
		Nutrient[c] mixture	Normal fibroblasts	Nutrient[c] mixture	Normal fibroblasts
CysA	—	0.0021	0.0049	0.0012	0.0064
Tau	0.020	0.0035	0.0380	0.0034	0.0346
Pea	—	—	0.0231	—	0.0320
Asp	—	0.0333	0.0314	0.0038	0.0308
Thr	0.047	0.0140	0.0430	0.0676	0.0423
Ser	0.037	0.0443	0.0711	0.0086	0.0692
Asn	0.020	0.0275	0.0264	—	0.0256
Glu	0.008	0.0609	0.1670	0.0321	0.2076
Gln	0.223	0.1872	0.0347	0.3057	0.0512
Pro	0.063	0.0337	0.0611	0.0134	0.0756
Gly	0.082	0.0512	0.0677	0.0187	0.0679
Ala	0.124	0.0617	0.0744	0.0297	0.0666
Cit	0.012	0.0021	0.0031	0.0024	0.0024
Val	0.078	0.0192	0.0430	0.0747	0.0436
Met	0.008	0.0064	0.0231	0.0169	0.0205
Ile	0.021	0.0105	0.0281	0.0687	0.0230
Leu	0.041	0.0309	0.0645	0.0690	0.0551
Tyr	0.019	0.0066	0.0281	0.0353	0.0243
Phe	0.017	0.0126	0.0264	0.0346	0.0256
Orn	0.020	0.0671	0.0033	0.0191	0.0026
Lys	0.063	0.0489	0.0694	0.0597	0.0436
Trp	0.010	—	0.0038	0.0066	0.0015
His	0.031	0.0288	0.0165	0.0317	0.0128
Arg	0.029	0.2096	0.0545	0.0898	0.0371

[a] Values expressed as ratio of each amino acid/total amino acid content of sample analyzed.
[b] Values recalculated from Perry *et al.* (15) for 33 normal individuals.
[c] Mean of two determinations.

mine, glutathione and glycine were reported to be the major contributors to the amino acid pool. In transformed hamster fibroblasts and their primary cultures, there is a marked contribution of taurine, aspartic acid, proline, threonine, glycine and alanine. It is interesting to observe that cultivated human skin fibroblasts differ from these specialized cultures in that additional amino acids maintain a high intracellular concentration, *i.e.*, serine, leucine, lysine and arginine, while other compounds such as tryptophan, ornithine and cystine are found at low concentration in human skin fibroblasts as well as in specialized cell cultures.

A striking difference between cultivated skin fibroblasts and other mammalian cell cultures relates to taurine and arginine. Under comparable growth conditions and amino acid supply in the nutrient mixture, human skin fibroblasts maintain a high intracellular arginine concentration and moderately high level of taurine as opposed to a low arginine and high taurine level in other cultivated mammalian cells. Low intracellular levels of arginine are seen in cells infected with mycoplasma-like organisms (14) and are accompanied by an increased concentration of citrulline and ornithine. Our findings of a low intracellular citrulline and ornithine with a high arginine concentration suggest that serial measurement of these amino acids in skin fibroblast cultures might be a reliable biochemical means of detecting mycoplasma contamination.

Glutamine, which constitutes the bulk of

the amino acid pool in Hela cells, is relatively low in cultivated human skin fibroblasts even at high extracellular concentration. A possible explanation for this low intracellular concentration might be the rapid turnover of this amino acid in human fibroblasts or some limitation in its transport. Some amino acids, particularly tryptophan and cystine are barely measurable in cultivated skin fibroblasts as in other cultivated mammalian cells. The low normal cystine content of human fibroblasts and its accumulation in cystinosis has been used for the detection of both homozygotes and heterozygotes for this rare familial disease (6, 7).

The similarity of the amino acid pool and profile between cells grown in Mediums I and II deserves special mention. Although there was almost at twofold difference between the two media in the amount of amino acids available to the fibroblasts, the total intracellular amino acid pool was only slightly larger in cells grown in Medium II than in cells grown in Medium I. The major contribution to this difference comes from glutamic acid and the acidic amino acids. This observation is surprising in view of the composition of the respective media and suggests the presence of a highly effective metabolic system for synthesis of amino acids, or interconversion of readily available precursors into less available compounds.

The consistency with which the observed amino acid profile repeats itself within cultures grown from the same individual and between cell lines from different control individuals suggests that such an approach might be useful for the investigation of a number of inborn errors of amino acid metabolism and/or transport in man.

Summary. Skin fibroblasts from normal individuals were grown in Ham's F-10 and Eagle's minimum essential medium and the intracellular concentration of amino acids measured using an automatic amino acid analyzer. Cultivated human skin fibroblasts exhibited a characteristic pattern of free amino acid content which differs from the amino acid pool previously described in Hela and L cells as well as many mammalian tissues and biological fluids. The free amino acid pool of fibroblasts grown in Eagle's MEM nutrient mixture was generally larger than that found in cells grown in Ham's F-10 medium. However, the contribution of most amino acids to the pool was comparable using both media. These data suggest that the measurement of the free amino acid content of cultivated human skin fibroblasts may prove useful for the study of inborn errors of amino acid metabolism in cell culture.

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