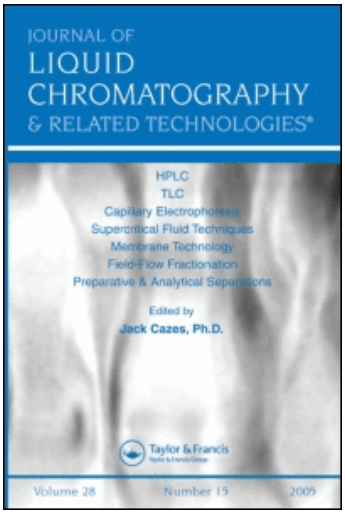

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HPLC Analysis of Amino Acids with Ion Exchange Chromatography and O-Phthalaldehyde Post-Column Derivatization: Applications to the Assay of Endogenous Free Amino Acids and Their Transport in Human Placental Villus

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HPLC ANALYSIS OF AMINO ACIDS WITH ION EXCHANGE CHROMATOGRAPHY AND O-PHTHALALDEHYDE POST-COLUMN DERIVATIZATION: APPPLICATIONS TO THE ASSAY OF ENDOGENOUS FREE AMINO ACIDS AND THEIR TRANSPORT IN HUMAN PLACENTAL VILLUS

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ABSTRACT

A method using high performance liquid chromatography (HPLC) for the analysis of primary amino acids in human placenta is described. This method involves separation of primary amino acids by high performance ion-exchange chromatography followed by post column derivatization using O-phlthalaldehyde (OPA) and 2-mercaptoethanol and fluorescence (excitation 340 nm and emission 410 nm) detection of derivatives. Waters 840 HPLC Amino Acid System was used for this purpose.

For analysis, villus tissue was extracted with acetonitrile, and the recovered amino acids were reconstituted in a sodium diluent (pH 2.2). The complete profile of the primary amino acids in the sample could be constructed in about 90 minutes. Up to 44 samples can be

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analyzed without special attention. Using this method, essential amino acids (threonine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine) and nonessential amino acids (aspartic acid, serine, glutamic acid, glycine, alanine, arginine) were detected and quantified in human placental villus in pmol quantities. Plots of peak heights (or areas) were linear for several amino acids. The same method was also used for (a) the assay of free primary amino acids in umbilical bloods, (b) the efflux of amino acids from isolated human placental villus, and (c) to study the uptake of α-aminoisobutyric acid (AIB), a non-metabolizable amino acid, by the isolated placental villus.

INTRODUCTION

Several diseases are associated with abnormalities of amino acid metabolism, which include (a) disorders of amino acid metabolism in liver disease (e.g., phenylketonuria), (b) specific disorders of amino acid transport (Hartnup Disease) and (c) non-specific disorders of amino acid transport (e.g., Wilson's disease) (1,2). In a majority of these diseases, one or more free amino acids are encountered in urine or plasma in larger amounts than usually occur among normal individuals. The extent and significance of such conditions can be assessed against a background of amino acid profiles of urine and plasma samples from normal healthy individuals.

In recent years, complete amino acid profiles have become very useful in at least two pharmacological situations. Final identification of pharmacologically active endogenous peptides like enkephalins and endorphins is by amino acid profiles (3,4). Amino acid disorders have been implicated in drug abuse including alcoholism (5), morphine or cocaine use (6,7), and tobacco smoking (8) especially maternal drug habits on the amino acid metabolism and utilization by the fetus.

Efficient transport of amino acids is essential for the normal growth of the fetus. The relationship between fetal intrauterine growth retardation and maternal drug abuse can be explained partially by fetal undernutrition during gestation. The uptake of amino acids by the human placental villus is an active process and is a critical step in the overall net transfer of amino acids from the maternal

blood to the fetal circulation. Therefore, determination of free placental concentrations of amino acids will provide information on the disturbances of amino acid transport under various physiological and pharmacological situations. For this purpose, a rapid convenient method for the determination of amino acid profiles in small tissue samples has become necessary.

For at least three decades, the measurement of amino acids has required the use of the Amino Acid Analyzer. The classical procedure developed by Stein and Moore (9) involving separation by ion-exchange chromatography followed by derivatization with ninhydrin forms the basis of amino acid analyzers. The classical amino acid analyzer is expensive and requires efficient technical help and long run times. It is dedicated and cannot be used for other purposes. Therefore, several recent reports emphasize the use of high performance liquid chromatography (HPLC) in amino acid analysis (10-13). The primary objective of most of these HPLC methods is to decrease analysis time, increase sensitivity and improve adaptability for use with less technical help (12). Two possible approaches have been used for this purpose: (a) separation of amino acids by high performance ion-exchange chromatography followed by post column derivatization and detection, and (b) precolumn derivatization of amino acids, separation of derivatives by high efficiency reverse-phase column and detection. The latter approach is a recent development, while conditions for the former one using solutions of amino acids have been established. Therefore, the former approach has been adapted for the analysis of amino acids in human placenta. The feasibility of this method was studied by investigating the (a) distribution of amino acids in different segments of human placenta, (b) uptake of non-metabolizable amino acid, α-aminoisobutyric acid (AIB), (c) efflux of amino acids from human placental villus, and (d) assay of amino acids in umbilical bloods. These techniques can easily be adapted to study metabolism of amino acids in the placentas of tobacco smoking, drug-addicted or alcoholic mothers.

MATERIALS AND METHODS

Chromatography system

Waters 840 HPLC Amino Acid System was used in all experiments. This system consists of (a) a stainless steel column (0.46 x 25 cm) containing a strong cation exchange resin, polystyrene divinylbenzine copolymer (sulfonated and equilibrated with Na^+ ions) which was maintained at 64° C, (b) Waters Intelligent Sample Processor (WISP) for sample injection; (c) two HPLC pumps for elution by gradient buffers (Na^+ buffers, pH 3.15 and 7.4); (d) a post-column reaction system for derivatization of primary amino acids using O-phthalaldehyde (OPA) and 2-mercaptoethanol to form fluorescence derivatives; (e) a fluorescence detector set for excitation at 340 nm and emission at 410 nm; (f) an interface module; and (g) a Digital Pro-350 computer and a printer.

For data acquisition, the Waters 840 system samples the analog output level from the detector. The analog signal is communicated to the Interface Module where the analog output level is measured in microvolts. The output level is converted to a digital number that corresponds to the magnitude of the signal. The digital number (raw data point) is stored in a file (Raw Data File) on the hard disk. The Raw Data File is used to produce an integrated Peak Data File. Peak width is estimated from the point of baseline liftoff to baseline touchdown.

The software makes necessary modifications for adjacent, fused or clustered peaks. It takes the retention times and areas stored in the system for all peaks detected during a run. The stored information is used to calculate component parts. The quantitation can be performed with an external standard or an internal standard using peak heights or areas. The instrument is calibrated with standards of known concentrations. For each amino acid, a response factor, RF (area or height of the peak/concentration of the amino acid) is calculated. Each amino acid has a different RF which is taken into consideration during calibration.

Standards

Amino Acid Standards were purchased from Pierce Chemical Co., Rockford, IL. They were diluted to 0.5 nmol injection size with Sodium Diluent (Na^+, 0.27 N, pH 2.2) from Pickering Laboratories, Mountain View, CA.

Reagents

Amino acids were derivatized with 6.3 mM o-Phthalaldehyde in borate buffer, pH 10.4, with 4% methanol and 2% 2-mercapto-ethanol.

Buffers

All separations of amino acids were made by gradient elution using two buffers (A and B) of different ionic strengths. Buffer A has a pH of 3.15 (0.2 N sodium content), and buffer B has a pH of 7.4 (1.0 N sodium). Both of these buffers were purchased from Pickering Laboratories, Inc., Mountain View, CA.

Placental tissues

Full term human placentas were obtained immediately after either vaginal or Caesarean section deliveries at Vanderbilt University Hospital. They were kept at 4° C and samples of placental tissues, chorionic plate, villus tissue and basal plate were dissected as described previously (14).

Each sample of chorionic plate, villus tissue and basal plate (0.4-1.0 g) was added to 6 ml of distilled water and homogenized with a glass tissue homogenizer for at least one minute. The homogenates were centrifuged at 4° C for 10 min and the supernatants were deproteinized as described below. The protein free extracts were analyzed for amino acids.

Human umbilical blood samples

Immediately after delivery, the cord was clamped and blood samples from both umbilical arteries and the umbilical vein were collected into heparinized tubes. Precautions were taken not to contaminate these umbilical blood samples with maternal blood. The tubes were stored at 4° C and brought to the laboratory. The samples were centrifuged and the plasma removed. The plasma sample could be stored at -20° for up to 6 hours without loss of amino acids (15). Therefore, the plasma samples were deproteinized within 6 hours by the method described below.

Protein removal procedures

Several techniques were described for deproteinizing samples prior to HPLC analysis (16,17). The following methods were found to be suitable for this purpose. (a) Precipitation using acetonitrile (or methanol), (b) precipitation using sulfosalicylic acid, and (c) ultrafiltration. All three methods were equally effective for deproteinizing tissue and plasma samples. In view of the simplicity of the method, acetonitrile precipitation was used for deproteinizing the samples. One ml of acetonitrile was added to 0.25 ml of plasma in a 5 ml glass test tube. Contents were mixed for 30 seconds using a vortex mixer, allowed to stand at room temperature for 15 min and centrifuged at 1650 g for 15 min. An aliquot of the supernatant was evaporated under a gentle stream of nitrogen, and the residue reconstituted in the medium necessary for HPLC analysis of amino acids or stored dry at -70° C. Acetonitrile extracts all components in plasma which are miscible with the buffer and can easily be removed. It precipitated more than 99.8% of the protein from each plasma sample.

Analysis of samples for amino acids

The dry acetonitrile extract was reconstituted in 1 ml of Sodium Diluent (Na^+, 0.27 N; pH, 2.2) and filtered through Millex-HA 0.45 μm

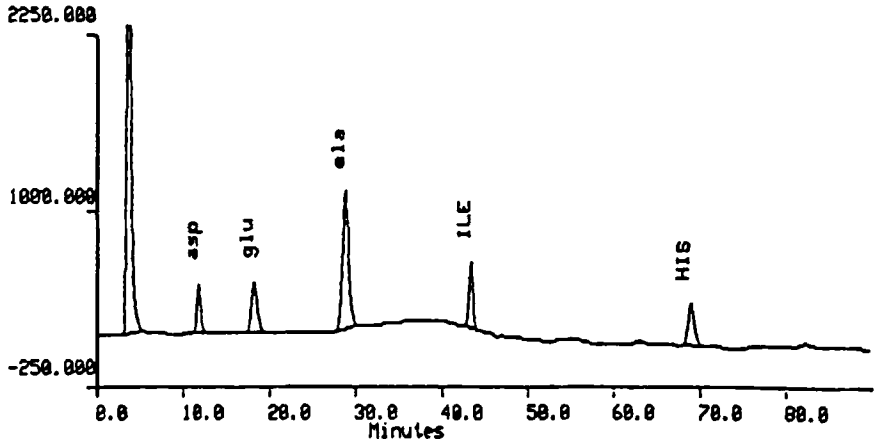

FIGURE 1. An amino-acid-gram using Waters HPLC 840 AAA system and selected standard amino acids. Each peak represents 400 pmoles of that particular amino acid.

filters (Millipore Corp., Bedford, MA). 20 µl was injected onto the column. Before an injection was made, the system was equilibrated with buffer B (pH, 7.4) for 30 min and buffer A (3.15) for 30 min (flow rate 0.5 ml/min). The injected sample was eluted by ionic strength gradient elution starting at pH 3.15 and increasing to pH 7.4 over the first 45 minutes. Total run time to elute all amino acids was 90 min/sample. After a run, the system was once again equilibrated (5 min, buffer B; 30 min, buffer A), and the next sample was automatically injected. As the separated amino acids emerge from the column, they react with OPA reagent which flows at a rate of 0.4 ml/min. The derivatives formed were detected by a fluorescence detector and the analog signals are communicated to the interface module.

RESULTS

The primary objective of the present investigation is to measure free amino acids in human placenta and to evaluate the feasibility

of the present method to (a) determine the placental concentration of amino acids, (b) distribution of amino acids in placenta, (c) uptake of nonmetabolizable amino acids like α-aminoisobutyric acid (AIB) by human placental villus and (d) efflux of amino acids from human placental villus.

Using Waters 840 Amino Acid Analyzer and OPA post-column derivatization, experimental protocols were developed for the analysis of amino acids. There is a satisfactory resolution of peaks for all standard primary amino acids (Figure 1). Plots of (a) peak heights and (b) areas under peaks as a function of concentration were linear for several amino acids (Figure 2). By this assay procedure, about 50 pmoles of amino acid can easily be assayed.

Primary amino acid concentrations in human placenta, and umbilical arterial and venous blood

A typical amino-acid-gram for the concentrations of amino acids in human placenta is shown in figure 3. The essential amino acids, threonine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, and the nonessential amino acids, aspartic acid, serine, glutamic acid, glycine, alanine and arginine were resolved clearly. Typical profiles of amino acids are shown in Table 1. The variations of each amino acid in each placenta are inherent in individual placentas but not in the method.

Typical amino-acid-grams for the concentrations of amino acids in human umbilical artery and umbilical vein are shown in Figure 4. Typical analytical profiles are shown in Table 1.

Distribution of primary amino acids in human placenta

The three primary regions of human placenta are villi, chorionic plate and basal plate. Since the villus transfers amino acids from maternal circulation to fetal circulation, analysis of the tissue

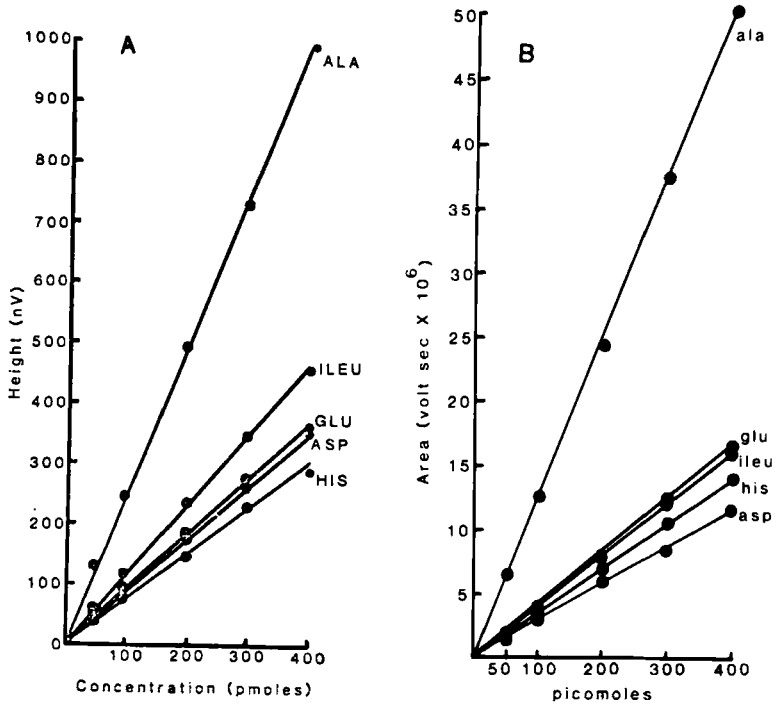

FIGURE 2. A: Linearity of the response of Waters 840 HPLC Amino Acid Analyzer as determined by the peak heights of selected primary amino acids, alanine (ala), isoleucine (ile), glutamic acid (glu), aspartic acid (asp), and histidine (his). B: Linearity of the response of Waters 840 HPLC Amino Acid Analyzer as determined by the area under the peak of selected primary amino acids. The first peak is due to phenol used as a preservative in the diluent (Na^+, 0.27 N, pH 2.2) used to suspend the amino acids for injection onto the analyzer.

samples from the villus region was undertaken separately from those of chorionic and basal plates (Table 2). As expected, concentrations of several amino acids were variable in the several regions of the placenta. If one were to analyze only placental tissues instead of its different anatomical regions, uniform sampling would be critical for analysis of amino acids.

TABLE 1

Concentrations of Amino Acids in Human Placenta, and Umbilical Arterial and Venous Blood

Amino Acid	Villus Tissue (nmol/g)			Arterial Blood (nmol/ml)			Venous Blood (nmol/ml)		
	#1	#2	#3	#1	#2	#3	#1	#2	#3
asp	119.4	73.8	335.6	--	--	--	--	--	7.2
thr	46.3	71.3	367.5	24.9	25.9	88.4	29.4	31.8	76.2
ser	100.0	113.8	271.9	51.1	17.3	26.2	35.4	19.9	38.4
glu	216.3	131.3	708.8	51.8	8.0	8.5	32.4	7.8	4.8
gly	260.6	237.5	518.1	198.8	83.1	108.7	183.0	102.6	103.2
ala	197.5	179.4	398.8	198.8	83.1	108.7	183.0	102.6	103.2
val	85.6	75.6	120.6	114.3	60.5	62.9	99.0	71.4	58.8
met	16.3	20.6	41.9	27.7	10.6	10.5	25.8	12.6	7.8
ile	33.8	35.6	45.0	32.7	21.9	20.1	28.8	26.4	15.0
leu	105.0	95.0	137.5	75.3	47.9	58.3	69.0	55.8	53.4
tyr	101.9	62.5	102.5	76.0	--	50.4	63.6	28.8	31.2
phe	95.6	62.5	82.5	83.1	35.2	46.5	72.0	41.4	42.0
lys	293.8	283.1	174.4	99.4	33.3	54.4	76.8	34.2	47.4
his	31.9	33.1	40.6	67.5	34.6	46.5	56.4	36.6	73.8
arg	53.1	67.5	132.5	40.5	29.3	36.0	34.2	27.6	39.0

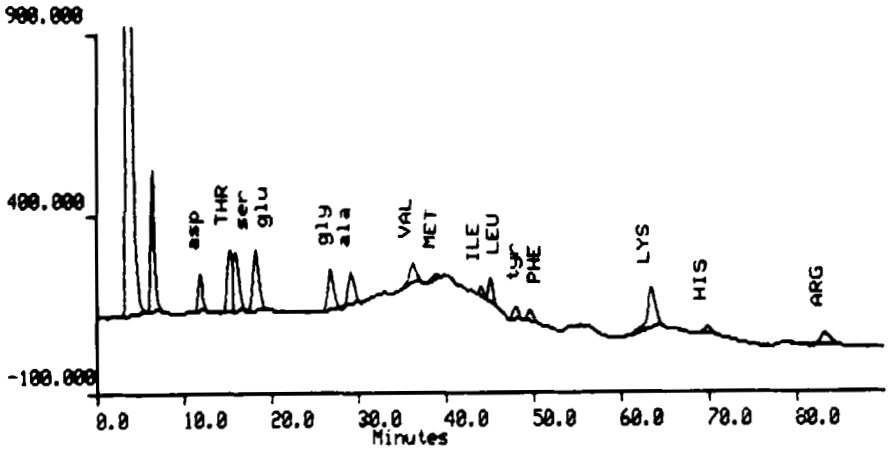

FIGURE 3. An amino-acid-gram of human placental extract using Waters HPLC 840 AAA systems. The peaks represent the amino acids: aspartic acid (asp, 108 pmol); threonine (thr, 247 pmol); serine (ser, 168 pmol); glutamic acid (glu, 238 pmol); glycine (gly, 197 pmol); alanine (ala, 149 pmol); valine (val, 103 pmol); methionine (met, 21 pmol); isoleucine (ile, 22 pmol); leucine (leu, 72 pmol), tyrosine (tyr, 44 pmol); phenylalanine (phe, 41 pmol); lysine (lys, 222 pmol); histidine (his, 20 pmol); and arginine (arg, 64 pmol).

TABLE 2

Concentration of Amino Acids in Different Regions of Human Placenta

Amino Acid	Villus (nmol/g)	Chorionic Plate (nmol/g)	Basal Plate (nmol/g)
asp	178.79	62.12	109.70
thr	249.70	80.30	235.87
ser	83.03	73.33	143.03
glu	544.85	210.30	420.61
gly	131.82	54.55	119.09
ala	140.00	49.39	159.09
val	45.76	12.42	42.73
met	8.48	7.88	15.76
ile	13.94	8.48	14.85
leu	31.52	16.97	46.67
tyr	22.12	15.15	36.06
phe	23.03	13.33	33.94
lys	341.82	104.55	222.12
his	21.21	14.55	28.48
arg	40.0	44.55	61.52

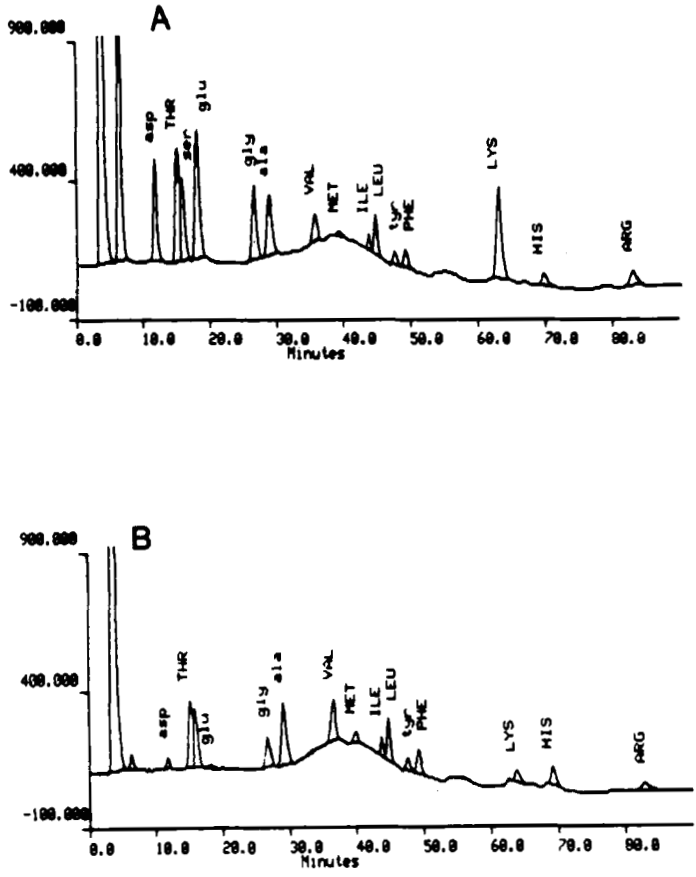

FIGURE 4. Amino-acid-grams of samples from human umbilical arterial plasma (A) and umbilical venous plasma (B). Peaks represent the amino acids: aspartic acid (asp, 316 pmol (A), 34 pmol (B)); threonine (thr, 408 pmol (A), 350 pmol (B)); serine (ser, 168 pmol (A), 219 pmol (B)); glutamic acid (glu, 413 pmol (A), 14 pmol (B)); glycine (gly, 303 pmol (A). 188 pmol (B)); alanine (ala, 257 pmol (A), 430 pmol (B)); valine (val, 228 pmol (A), 224 pmol (B)); methionine (met, 50 pmol (A), 42 pmol (B)); isoleucine (ile, 77 pmol (A), 68 pmol (B)); leucine (leu, 37 pmol (A), 166 pmol (B)); tyrosine (tyr, 69 pmol (A), 65 pmol (B)); phenylalanine (phe, 38 pmol (A), 115 pmol (B)); lysine (lys, 328 pmol (A), 73 pmol (B)); histidine (his, 14 pmol(A), 95 pmol (B)); and arginine (arg, 98 pmol (A), 67 pmol (B)).

Determination of α-aminoisobutyric acid

α-Aminoisobutyric acid (AIB) is a non-metabolizable amino acid. It participates in all neutral amino acid uptake systems (18), and its uptake by tissues gives a measure of the uptake of all neutral amino acids (6). Due to the availability of ^{14}C-AIB, it has been used extensively in transport studies (6,7,19). However, there are limitations to the use of ^{14}C-AIB in studies involving infusion of whole organs or whole animals in view of the amount of radioactivity required and the expense involved. Therefore, a sensitive method is necessary for the assay of AIB.

The Waters 840-HPLC Amino Acid analysis system is sensitive enough to detect and assay unlabeled AIB, which gave a very characteristic peak with a retention time of 32.78 min in the HPLC pattern (Figure 5A). It closely follows the elution of alanine and is clearly distinguishable at a concentration of 0.5 nmol. Its peak is not masked by the peaks of other endogenous amino acids (Figure 5B). Either the peak height or the area under the peak is suitable for the assay of AIB because there is linearity in response (Figure 6).

Uptake of AIB by the human placental villus

The details of measuring the uptake of AIB by the isolated placental villus have been described elsewhere (6,7). Unlabeled AIB uptake by isolated human placental villus was measured to validate the present method. The human placental villus was incubated in a physiological medium containing AIB. AIB was taken up by the human placental villus, and the I/O (I: intracellular concentration, O: extracellular concentration) ratio increased with time linearly ($r = 0.96$) for 90 minutes. The final ratio of 29.2 indicated active uptake of AIB by the placental villus.

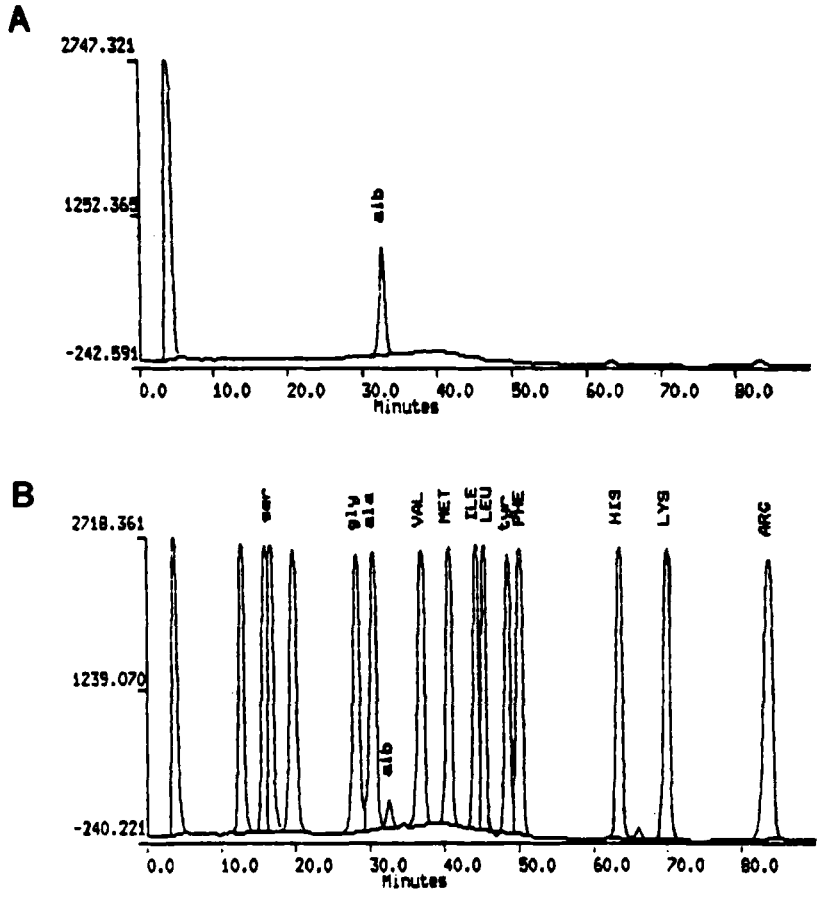

FIGURE 5. A: HPLC pattern of α-aminoisobutyric acid (AIB, 2 nmol) a common substrate for all neutral amino acid uptake systems. AIB is completely recovered. The estimated amount of AIB is 2.15 nmol. B: Non-interference of endogenous amino acids with the detection of α-aminoisobutyric acid (AIB, 0.5 nmol) which appears at 32.74 min.

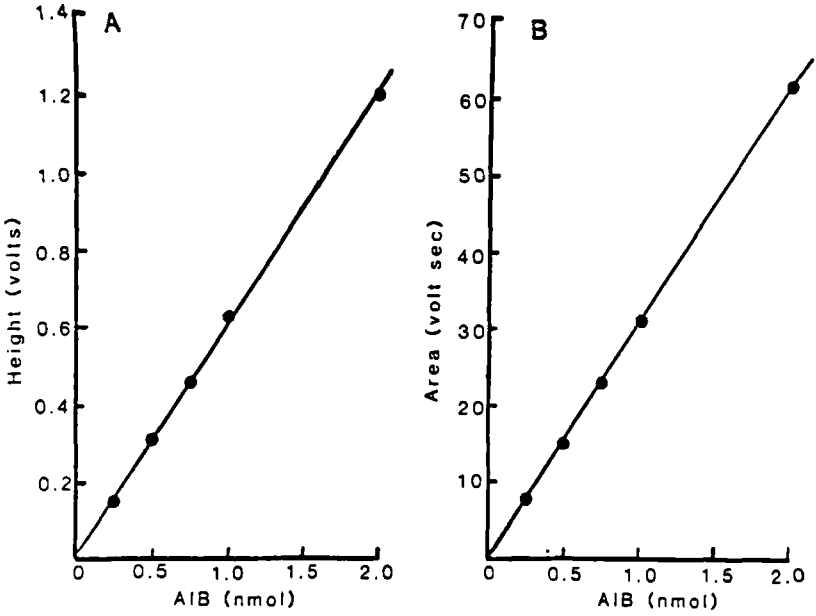

FIGURE 6. Linearity of response of Waters 840 HPLC Amino Acid Analyzer as determined by the height or area of the peaks of α-aminoisobutyric acid (AIB).

Efflux of amino acids from the isolated placental villus

There is a net unidirectional flow of AIB from maternal blood to the fetus inferred from in vivo studies using pregnant guinea pigs and radiolabeled AIB (19). In human placenta, the net uptake of AIB can easily be measured. However, it is difficult to measure the rate of efflux of endogenous amino acids from placental villus or other tissues because the rate of efflux is considerably lower than the rate of uptake. Therefore, the present HPLC method was used to measure the efflux from the isolated placental villus.

Placental villus was incubated for 90 minutes in Krebs bicarbonate buffer containing AIB as described previously (6,7). In Figure 7, the amino-acid-grams of the physiological medium at '0' time and at

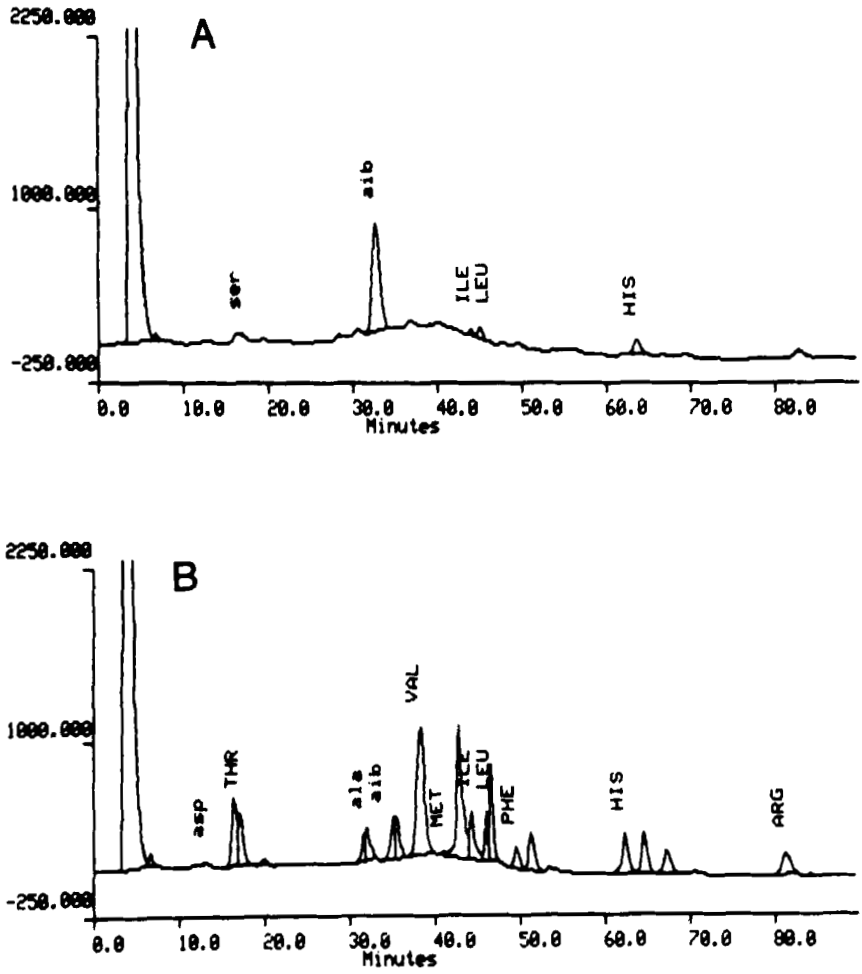

FIGURE 7. Efflux of amino acids from the isolated placental villus, which was suspended in Krebs bicarbonate buffer at pH 7.4 and 37° C, after 10 min of incubation (A) and after 1.5 hours of incubation (B). Exogenous AIB was added to the incubation bath. The peak at 32.85 min. represents 2.5 nmol AIB. A: serine (ser, 7 pmol); isoleucine (ILE, 33 pmol); leucine (LEU, 77 pmol); histidine (his, 127 pmol). B: aspartic acid (asp, 8 pmol); threonine (thr, 573 pmol); serine (ser, 392 pmol); alanine (ala, 142 pmol); valine (val, 1.357 nmol); methionine (met, 19 pmol); isoleucine (ile, 349 pmol); leucine (leu, 279 pmol); phenylalanine (phe, 157 pmol); lysine (lys, 332 pmol); and arginine (arg, 210 pmol).

TABLE 3

Efflux of Endogenous Amino Acids from Isolated Human Placental Villus

Amino Acid	nmoles Amino Acid Released/g wet tissue	
	60 minutes	90 minutes
Aspartic acid (asp)	0.0	16.89
Threonine (thr)	145.84	286.42
Serine (ser)	0.0	430.99
Glutamic acid (glu)	11.86	0.0
Glycine (gly)	207.25	388.43
Alanine (ala)	249.81	364.11
Valine (val)	175.15	246.57
Methionine (met)	55.13	70.93
Isoleucine (ile)	126.30	170.91
Leucine (leu)	285.40	374.92
Tyrosine (tyr)	120.02	147.26
Phenylalanine (phe)	145.14	187.12
Lysine (lys)	242.83	356.00
Histidine (his)	64.89	119.57
Arginine (arg)	126.30	136.46

90 minutes of incubation are shown. It is evident that efflux rate of several amino acids is within the limits of sensitivity of the present HPLC assay for amino acids (Table 3). Further, the concentration of AIB decreased in the medium due to the uptake by the tissue.

DISCUSSION

The primary goal of developing a sensitive HPLC method for detection of and analysis of amino acids is achieved as a part of this investigation. Waters 840 Amino Acid Analyzer, ion gradient elution, post-column derivatization and fluorescence detection were used. The post-column OPA derivatization has a long historical background and is done automatically on line. The reaction occurs after the chromatographic separation, and well established conditions are available for derivatization which are used in the present investi-

gation (13). However, consideration should be given to advantages and disadvantages of the method including (a) sensitivity, (b) adaptability for analysis and transport studies in placenta, (c) problems of ion gradient elution, (d) inferior separations, if any, (e) inferior detection of proline and hydroxyproline, and (f) problems in the analysis of aspartic acid and tryptophan. Some of the above problems were encountered by other investigators.

According to the present investigations, overall performance of Waters 840 HPLC Amino Acid Analysis system tailored to ion-exchange separation and post-column OPA reaction and fluorescence detection are excellent. There is a good linearity for all amino acids between 100-500 picomoles. The peaks of several essential amino acids (threonine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine and histidine) and non-essential amino acids (aspartic acid, serine, glutamic acid, glycine, alanine and arginine) can be detected and quantified in the above range. The peaks for the amino pairs, threonine and serine, leucine and isoleucine, tyrosine and phenylalanine are close to one another, but they are resolved adequately for computerized detection and analysis.

There are also certain advantages of using unlabeled non-metabolizable amino acids like AIB and HPLC analysis of the amino acid in the uptake studies by the human isolated placental villus and possibly other cell systems. The analytical purity of commercially available amino acids (e.g., AIB) is higher than the radiochemical purity. While the cells take up radioactive AIB or other amino acids from the physiological medium in which they are placed, the radiochemical impurity stays in the medium and introduces errors in the final concentrations. Analytically pure amino acids are commercially available, and their analysis by HPLC was found to give accurate results in uptake studies of amino acids. Normally efflux of amino acids from cells are not detected in view of difficulties involved in methodology. The present HPLC analytical method is sensitive enough to calculate the rate of efflux of amino acids from living cells (e.g., placental villus) into the culture medium.

In the present studies, gradient elution using two buffers of different ionic strength are used. As expected, acidic amino acids eluted first, followed by hydroxylated, neutral and basic acids. The method is sensitive at the 50 picomole level. The main disadvantage of the method is wear on pump seals due to high salt content. This problem is partially avoided by purging the system of the chloride containing buffers with water before shutdown and storage.

Inferior separations of glycine (20,21), threonine (20,21) serine (22) were reported in occasional reports. All these reports deal with pre-column derivatization and reverse phase separation. None of these problems were noticed in the present investigation.

Attempts have been made for the detection of proline and hydroxyproline by peroxidation of these compounds by hypochlorite prior to reaction with OPA. The method works well with standard samples. However, problems arose when the method was used for samples of placental tissue, umbilical venous plasma and umbilical arterial plasma. The concentrations of these amino acids are very low in the above tissues compared to the other primary amino acids, and, therefore, sensitivity should be below the 50 picomole level, while the optimum sensitivity for other endogenous primary amino acids is about 100-500 picomoles. There are two ways to solve the problem: (a) increase the injection volume, or (b) analyze a second sample separately for proline and hydroxyproline. The latter is preferable because analysis of all other essential amino acids will not be compromised for the detection of two minor components.

There are some problems in the detection and analysis of aspartic acid. The values obtained for aspartic acid are variable from sample to sample. Some investigators (23) attributed it to continuous degradation of OPA/2-ME/ amino acid (24,25). Aspartic acid is the first amino acid to be eluted from the column. A question arises as to whether this acid carries with it a trace of impurity from the column from a previous run. According to some investigators, aspartic acid may be overestimated as much as 20% after the first injection (23). In an automated unit with on line injection system, this can be detected or eliminated by incorporating a dummy run (diluent without

amino acids), a run of pooled standard amino acids and a second dummy run for analysis of every ten samples in a day.

Tryptophan was not detected in the placental tissue, umbilical arterial plasma nor umbilical venous plasma samples. Tryptophan is unique among the plasma amino acids in that most of the circulating tryptophan is bound to serum albumin (26,27). All procedures used for protein removal also remove tryptophan from samples. An independent method has to be developed for tryptophan detection.

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