Absorption kinetics of dietary hydrolysis products in conscious pigs given diets with different amounts of fish protein

2. Individual amino acids

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1. Concentrations of amino acids (AA) in blood obtained from arterial and portal permanent catheters were measured together with the portal hepatic blood flow-rate during a post-prandial period of 8 h, in nine conscious pigs (initial mean body-weight 49·3 (SEM 1·8) kg) receiving experimental meals (500, 600 or 1000 g) at 3–4 d intervals from 6–8 to 20 d after the surgical implantation of the catheters and electromagnetic flow probe. The semi-synthetic starch-based diets contained variable amounts of fish meal giving crude protein (nitrogen × 6·25; CP) concentrations (g/kg) of 80 (seven meals), 120 (seven meals), 160 (five meals) and 240 (three meals).

2. During the post-prandial period, variations in the individual blood AA concentrations were parallel to those of total amino-N (Rérat *et al.* 1988) to a greater or lesser extent according to the AA considered. Portal concentrations, which always exceeded arterial ones (except for glutamic acid and glutamine), increased quickly and, after a peak, returned slowly to reach initial values (small intake) or above (large intake) after 8 h.

3. Relations between amounts of each AA appearing during 8 h after the meal and amounts ingested were characterized by a highly significant linear regression (with the exceptions of glutamic acid and cystine). There were also close and significant relations between amounts of AA absorbed during the first 2 and 4 h after the meal and the amounts ingested.

4. For a mean intake of 90 g CP, aromatic AA showed the highest hourly absorption coefficients (about 0·10 /h), and sulphur-AA (0·07/h), lysine (0·07/h) and arginine (0·056/h) the lowest ones. Alanine was synthesized (amounts absorbed within 8 h exceeding those ingested) at the expense of glutamic acid (absorption coefficient 0·01/h).

5. For a given period of time, the AA absorption coefficients decreased with increasing intake, but not in the same proportions for all AA, resulting in an enrichment (lysine, arginine, serine, proline) or depletion (branched-chain AA, histidine) of the absorbed mixture.

6. Some substances of the urea cycle were synthesized in rather large amounts in the gut wall (for a mean level of intake of 90 g CP: citrulline 2·41 g/8 h, ornithine 1·09 g/8 h). Blood glutamine was taken up by the gut wall in larger amounts (4·28 g/8 h).

In our previous paper (Rérat *et al.* 1988), it was shown how the ingestion of variable amounts of fish protein by the pig led to the appearance of a large flux of amino-nitrogen in the portal blood which increased with the amount of protein eaten. It was also shown that amino-N appeared more rapidly in the efferent blood of the digestive tract than glucose derived from the carbohydrates in the same meals. However, the trends revealed by the estimation of amino-N cannot be extrapolated to each individual amino acid of fish protein. Their possible differential absorption and the potentially important metabolic consequences of this finding need to be determined more accurately. In the present report, values are presented from a study on the absorption kinetics of the individual amino acids (AA) of the fish meal used in the experiments described in the previous paper (Rérat *et al.* 1988).

Table 1. *Composition of the diet used before and between experimental meals and the protein-free diet (g/kg diet)*

Ingredients	Protein-free diet	Diet used before and between experimental meals*
Fish meal†	—	170
Peanut oil	50	41
Granulated sugar	50	41
Purified cellulose‡	120	100
Maize starch	640	532
Minerals§	30	25
Vitamins§	10	8
Vermiculite ‖	100	83

* Dry matter 890 g/kg fresh matter (FM).

† Composition: crude protein (nitrogen × 6·25; CP) 706 g/kg FM; dry matter 927·5 g/kg FM; amino acids (AA) (g/kg): lysine 52, histidine 15, phenylalanine 28, leucine 55, isoleucine 33, methionine 20, valine 39, threonine 30, serine 27, arginine 39, aspartic acid + asparagine 67, proline 31, glutamic acid + glutamine 100, glycine 44, alanine 46, cystine 5, tyrosine 17, total AA 649; N 139 g/kg total AA (R. Calmes, unpublished results).

‡ Purified wood cellulose.

§ Mineral and vitamin supplements described by Henry & Rérat (1964).

‖ Mica powder.

Table 2. *Number of experimental meals for each level of protein (i.e. diet) and amounts offered*

Fish-meal protein (g/kg diet)...	80	120	160	240
Amount of diet (g)				
500	4	2	2	1
600	—	1	2	—
1000	3	2	3	2
No. of animals receiving each diet	4	5	5	2

EXPERIMENTAL

Animals, diets, feeding conditions and measurements

Nine castrated Large White male pigs of initial mean body-weight 49·3 (SEM 1·8) kg were prepared as described in the previous paper (Rérat *et al.* 1988). In the initial experimental scheme four experimental meals were to be given to each animal at 3–4 d intervals, with recovery on a well-balanced diet for the intervening days between each measurement (Rérat *et al.* 1988). However, because of experimental difficulties (early obstruction of catheters, early dysfunction of probes) the number of actual experimental meals per animal varied between two and four, the same animal never receiving a given experimental diet at a given level more than once. The experimental diets included a protein-free feed and different amounts of fish meal to obtain crude protein (nitrogen × 6·25; CP) concentrations of 80, 120, 160 and 240 g/kg (Table 1). On the day of the measurement, after a fasting period of 18 h, the animal ingested at 9.00 hours, in less than 20 min, a meal including a given amount (500, 600 or 1000 g, Table 2) of one of the experimental diets. The meal-feeding pattern was devised so that each animal received diets with different CP levels or the same

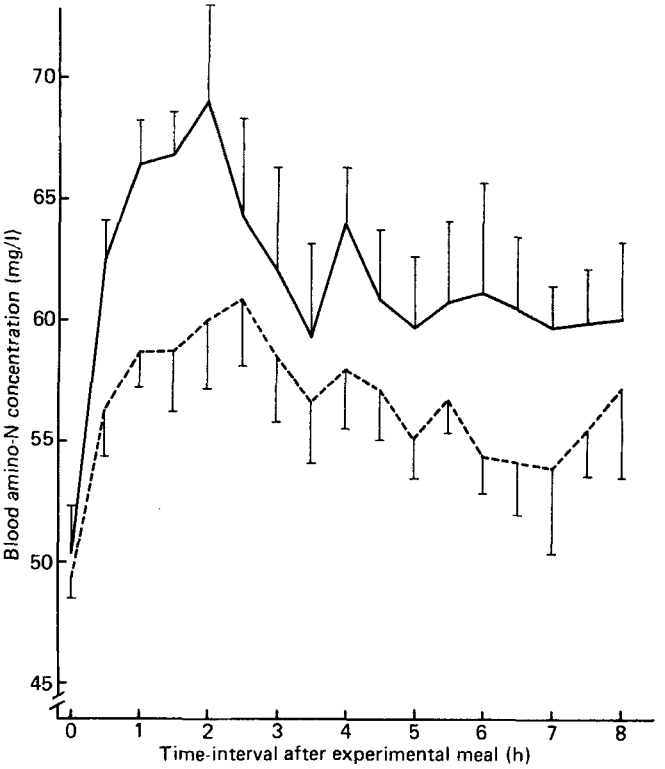

Fig. 1. Variations in portal (——) and arterial (– – –) blood concentrations (mg/l) of nitrogen of free amino acids after a meal of 80 g crude protein (N × 6·25; CP) from fish meal (for details, see Table 1) together with a protein-free diet to give 80 g CP/kg (no. of meals (*n*) 3) or 160 g CP/kg (*n* 2). Values are means with vertical bars representing the standard error of the mean.

diet at two different levels of intake, or both (Table 2). Blood was collected (10 ml/sample) every 15 min during the first post-prandial hour, every 30 min during the next 5 h and every 60 min during the last 2 h. Samples were recovered in cold (−20 °) pure ethanol (930 ml/l; 1:7, v/v) and placed in a deep-freeze (−20 °). Free AA, with the exception of tryptophan, were then analysed by column chromatography (Moore *et al.* 1958) according to the method of Pion & Rérat (1967).

Calculations and statistical analyses

The procedures used were those described in the previous paper (Rérat *et al.* 1988). Each meal was considered as an individual value because of the random distribution of the twenty-two meals without repetition of the same meal (protein concentrations × level of feeding) in the same animal. Because of the imbalance of the actual experimental scheme it was not possible to take into account between-animal and within-animal variation. But the validity of regressions obtained with all values was checked by comparison with regressions using only the first meal given to each animal.

RESULTS

Portal blood flow-rate

The mean blood flow-rate calculated for the whole experimental period on the basis of successive values (means per 5 min) varied from one animal to another and from day-to-

Table 3. *Free amino acid blood pattern after 18 h fasting* in the pig with a comparison of the amino acid pattern of fish protein*
(Mean values with their standard errors of the means)

Amino acid	Portal blood		Arterial blood		Fish protein
	Mean	SEM	Mean	SEM	
Histidine	2·28	0·20	2·30	0·18	2·3
Lysine	5·23	0·87	5·27	0·95	8·0
Phenylalanine	2·56	0·19	2·66	0·29	4·3
Leucine	4·84	0·24	4·91	0·28	8·5
Isoleucine	4·23	0·36	4·12	0·28	5·1
Methionine	1·92	0·20	1·93	0·21	3·1
Valine	6·79	0·44	6·85	0·48	6·0
Threonine	3·19	0·27	3·29	0·30	4·6
Arginine	1·90	0·26	1·80	0·27	6·0
Cystine	1·25	0·16	1·31	0·11	0·8
Tyrosine	3·62	0·31	3·53	0·24	2·6
Glutamic acid	5·94	0·43	6·28	0·48	15·4
Glutamine	7·28	0·59	8·95	0·96	—
Aspartic acid	1·08	0·07	1·05	0·08	10·3
Asparagine	2·49	1·09	2·42	1·03	—
Proline	6·83	0·69	6·65	0·61	4·8
Hydroxyproline	1·95	0·20	2·05	0·19	—
Serine	2·75	0·23	2·80	0·27	4·2
Glycine	17·35	1·28	17·19	1·20	6·8
Alanine	6·87	0·75	6·12	0·78	7·1
Citrulline	4·81	0·44	4·16	0·41	—
Ornithine	2·63	0·22	2·71	0·27	—
Σ TAA (mg/l)	353·3	8·2	346·6	8·3	—
Nitrogen (g/kg Σ TAA)	139·6	—	139·3	—	—

Σ TAA, sum of total amino acids.
* Mean of blood samples (time 0) collected before experimental meals, corresponding to 40 and 80 g crude protein (N × 6·25) intake (n 9, df 7).

day in the same animal. No relation was found between the calculated mean flow-rate and the amounts of ingested protein or carbohydrate. The mean value for the nine animals was 1893 (SEM 52) ml/min, i.e. 39·5 (SEM 1·7) ml/min per kg.

Post-prandial variation in blood levels of free AA

The blood level of free AA varied with the AA considered and the level of CP intake. An example of post-prandial variations in blood N concentration of total free AA (addition of individual AA: N content of each AA × concentration of the AA) (total AA (TAA)) for one level of intake is given in Fig. 1. After an intake of 80 g CP (i.e. 72·0 g TAA or 10·0 g N according to the chromatographic analysis), the concentration of N from free AA which was similar in the portal and arterial blood at time zero (t_0) (initial concentration (IC) 50·6 (SEM 1·9) and 49·4 (SEM 0·7) mg/ml respectively) increased rapidly, reaching a peak in portal blood (68·8 (SEM 3·9) mg/l, i.e. 136% of IC) 2 h after the meal. This peak was more marked (not significant) than in the arterial blood (60·8 (SEM 2·7) mg/l, i.e. 123% of IC) in which the peak concentration occurred later (150 min after the meal). The concentration thereafter progressively decreased, with fluctuations, to reach a concentration 8 h after the meal which exceeded IC (119 and 116% of IC respectively for portal and arterial blood). The concentration differences which reached a maximum of 8·8 mg/l, averaged 4·9 (SEM

Table 4. *Maximum increase in the concentration of amino acids (% of the initial concentration (IC)) and time of appearance after a meal (fish-meal protein in a semi-synthetic diet)**

Intake (g)†...	40				80			
	C_p		C_a		C_p		C_a	
	% IC	Time (min)	% IC	Time (min)	% IC	Time (min)	% IC	Time (min)
Total amino acids	129	30	120	30	136	120	118	150
Histidine	120	30	115	60	133	120	113	330
Lysine	132	30	119	30	174	120	150	150
Phenylalanine	159	30	129	30	174	120	139	120
Leucine	157	30	134	30	181	60	142	60
Isoleucine	150	30	127	30	138	60	131	150
Methionine	137	30	133	30	148	120	127	150
Valine	132	30	122	30	149	120	137	150
Threonine	138	30	121	30	174	120	138	180
Arginine	161	60	148	60	220	120	149	150
Aspartic acid	118	210	107	210	142	450	136	420
Asparagine	139	30	114	60	148	120	120	60
Serine	142	30	126	30	149	120	120	90
Glutamic acid	108	30	111	30	111	420	123	360
Glutamine	132	30	119	60	116	30	104	180
Glycine	113	300	103	60	108	90	107	30
Alanine	214	60	194	60	199	60	173	60
Proline	131	30	120	30	125	90	126	30
Cystine	108	90	103	30	125	450	134	480
Tyrosine	118	30	121	30	127	90	114	60
Citrulline	112	60	116	60	125	120	123	150
Ornithine	120	180	114	120	137	240	144	180

C_p, portal blood concentration; C_a, arterial blood concentration.
* For details, see Table 1.
† Crude protein intake (nitrogen × 6·25).

0·5) mg/l (*n* 17) and were still perceptible (about 2·8 mg/l) at the end of the observation period (8 h).

The free AA pattern in the blood after 18 h of fasting (Table 3) was characterized by a very high glycine concentration and rather high concentrations of lysine, leucine, valine, glutamic acid, proline, alanine and glutamine. The portal blood tended to contain more alanine and citrulline and less glutamic acid and glutamine than the arterial blood, but differences were not significant.

During the post-prandial period, variations in the individual AA concentrations were parallel to those of TAA, although to a more or less marked extent. The portal concentrations, which always exceeded the arterial concentrations (with the exception of glutamic acid and glutamine) generally increased more. The maximum concentration appeared earlier when the intake was low than when it was high, generally 0·5 h after ingestion of 40 g CP and 2 h after that of 80 g CP (Table 4), but this time-interval was variable according to the level of intake and the particular AA. The relative peak values (Table 4) varied according to the AA and generally increased with the level of intake. Whatever this level they were higher than those of the TAA for some AA (phenylalanine, leucine, arginine and alanine) and lower for others (cystine, glutamic acid and glycine). At 8 h after the meal, the portal and arterial concentrations always exceeded the initial ones, the porto-arterial differences remaining marked for most amino acids.

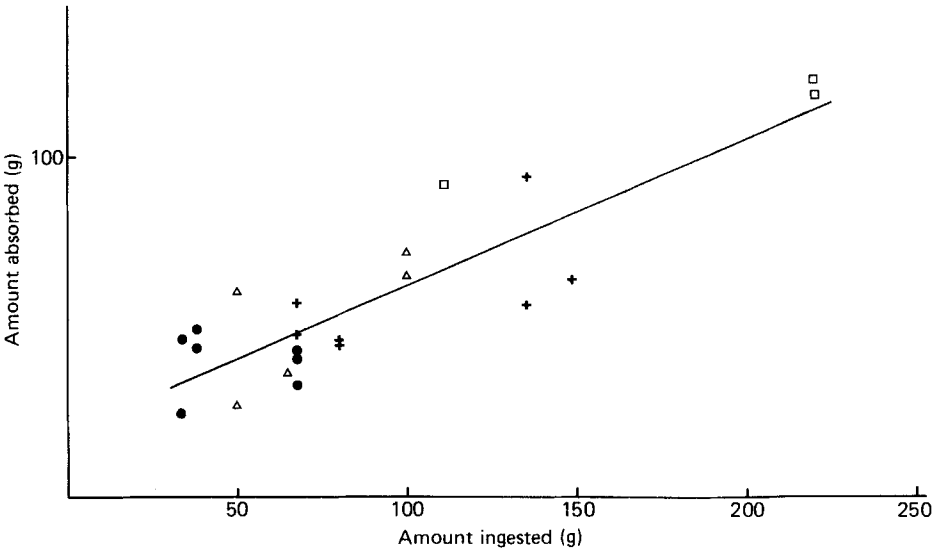

Fig. 2. Relations between the amounts of total amino acids absorbed (Y; g) during 8 h after a meal, and the amounts ingested (X; g).

$$Y = 0{\cdot}433\ X + 19{\cdot}593,\ r\ 0{\cdot}867\ (P < 0{\cdot}01)$$

(Standard error of regression coefficient 0·055, df 20; no. of experimental meals 22; crude protein (nitrogen × 6·25) (g/kg) provided by fish meal (for details, see Table 1): (●), 80; (△), 120; (+), 160; (□), 240.

Table 5. *Relations between amounts of total amino acids absorbed* (Y; *g*) *after a meal* (*fish meal in a semi-synthetic diet*)† *and the intake of total amino acids* (X; *g*) *relative to time-interval after the meal* (n *22*, *df 20*)

Time-interval after the meal (h)	$Y =$	r	X	Y	S_b	Statistical significance
2	$0{\cdot}132X + 3{\cdot}651$	0·862	89·405	15·474	0·017	**
4	$0{\cdot}232X + 11{\cdot}030$	0·826	89·405	31·816	0·035	**
6	$0{\cdot}348X + 15{\cdot}405$	0·860	89·405	46·544	0·046	**
8	$0{\cdot}433X + 19{\cdot}593$	0·867	89·405	58·291	0·055	**

S_b, standard error of regression coefficient.
** $P < 0{\cdot}01$.
† For details, see Table 1. For intake levels, see Fig. 2.

Variation in the amount of free AA appearing in the portal blood at different time-intervals after the meal

Relations between the TAA absorbed at different time-intervals after the meal (Y; g) and the AA ingested (X; g) are shown in Fig. 2 and Table 5. They are characterized by linear regressions with a high and very significant correlation coefficient; the slopes and intercepts of the regressions were greater the longer the period of cumulative absorption after the meal. The equations obtained for free TAA were very close to those calculated from blood amino-N relative to N ingested (Rérat *et al.* 1988), in terms of both slope and intercept.

They were not modified when only the values of the first meals given to the nine animals were taken into account (for 8 h: $Y = 0{\cdot}458X + 16{\cdot}823$, r 0·86, n 9).

Regressions of the amount of each AA appearing in the portal vein during 8 h after the meal relative to the amount ingested are shown in Figs. 3 and 4. The relation between these variables was linear, very close and significant for all essential AA and for almost all non-essential AA with the exceptions of glutamic acid and glutamine (r 0·08) and cystine (r 0·10). The correlation coefficients were generally very high for the essential AA (r ranging from 0·793 to 0·886) with the exception of histidine (r 0·690); they were substantially lower for hydroxyproline + proline (r 0·723) and glycine (r 0·692).

After an intake of 89·4 g CP (mean of all experiments) the hourly absorption coefficient (Table 6) calculated from regression equations for 8 h of absorption ranged from 0·079 (essential AA) to 0·083 (non-essential AA) with an average value of 0·081 for TAA. However, among the essential AA the aromatic AA showed hourly absorption coefficients of about 0·010, the sulphur-AA and lysine about 0·07, and arginine 0·05–0·06. Among the non-essential AA, alanine was synthesized (with the amounts absorbed greatly exceeding those ingested 8 h after the meal) at the expense of glutamic acid, which had an hourly coefficient of about 0·01, and probably aspartic acid (hourly coefficient of about 0·04).

The same general differences between AA were found when regressions of amounts absorbed relative to amounts ingested during the first 2 or 4 h were calculated. These relations were also linear and significant for all essential AA and almost all non-essential AA (except cystine, 2 and 4 h after the meal and glutamic acid + glutamine 4 h after the meal). The slopes of the regression equations decreased with shorter cumulative absorption periods after the meal and this was generally also the case for the intercepts. This showed that the cumulative amounts of absorbed AA increased with the time-interval after the meal.

The absorption coefficient for a given period of time also varied with the quantities ingested (Table 6). It was very high for the low levels of intake, more than 80 % of each AA ingested appearing in the portal vein within 8 h after ingestion of 50 g TAA. There were, however, some exceptions, e.g. coefficients were low for lysine and methionine, very low for arginine and dicarboxylic acids, high for aromatic AA and very high for alanine. After ingestion of 150 g TAA, the absorption coefficient greatly decreased, little more than half the amount ingested appearing in the portal vein within 8 h. With increasing intake the differences recorded for some AA relative to the mean of TAA were either reduced (lysine, arginine), maintained (aromatic AA) or reversed (histidine). Thus, with low levels of intake, the absorbed mixture (Tables 7 and 8) showed a lower content of some AA (lysine, arginine, serine and proline) and a higher content of others (branched-chain amino acids, histidine) than with high levels of intake.

Of special interest are AA involved in the urea cycle, i.e. arginine, ornithine and citrulline, the latter two being absent from the diet. The cumulative amounts of these AA appearing in the portal vein (Fig. 4) were closely related to the protein intake. For a mean intake of 89·4 g CP, the amounts of citrulline and ornithine synthesized in the gut wall and taken up into portal blood increased with increasing time-interval after the meal to 2·41 (SEM 0·23) g and 1·09 (SEM 0·15) g respectively after 8 h; citrulline thus represented 69 % of the sum of these two AA.

The arterial concentrations of glutamine and glutamic acid often exceeded those in portal blood; the uptake of these compounds from the blood by the intestinal wall may be evaluated from the arterio-portal difference ($C_a - C_p$). For a mean intake of 89·4 g CP, the amounts taken up within 8 h were 4·28 (SEM 0·47) g for glutamine and 2·14 (SEM 0·20) g for glutamic acid.

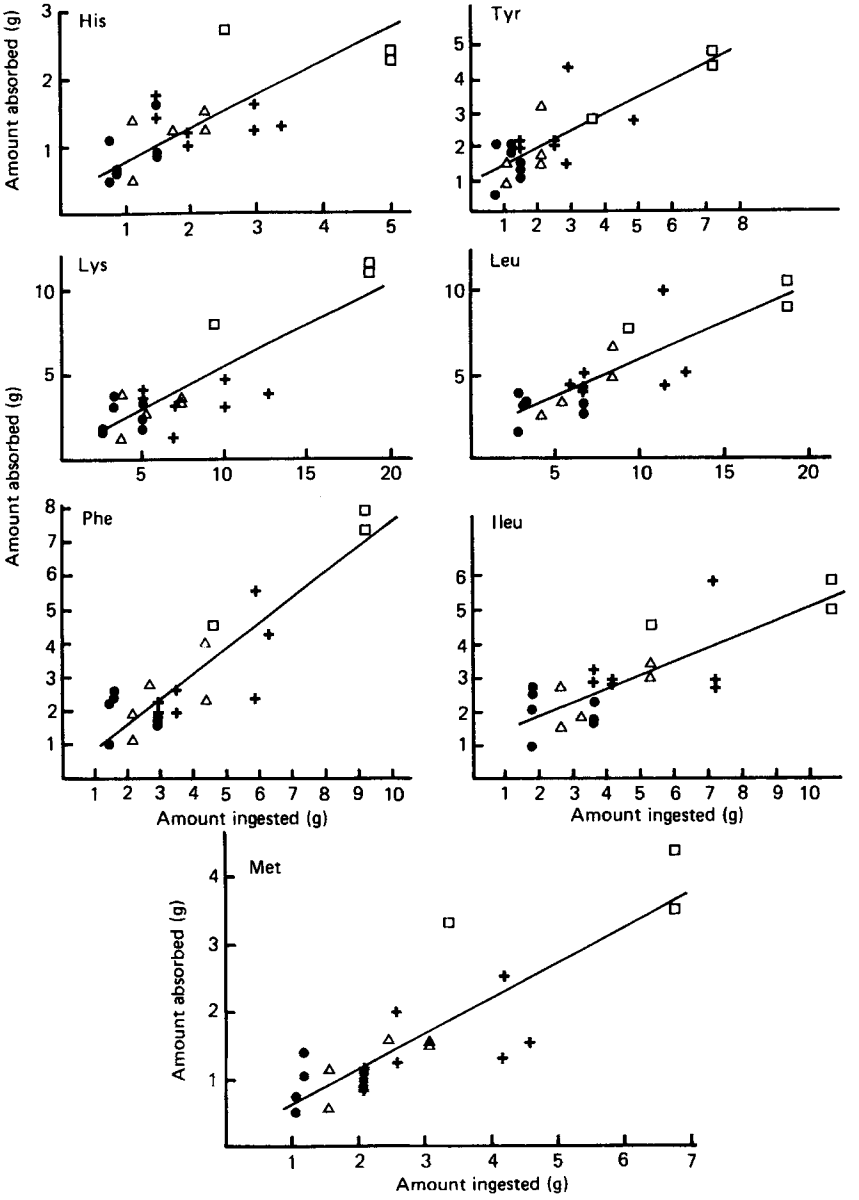

Fig. 3. Relations between the amounts of each essential amino acid (EAA; Y; g), and sum of EAA, absorbed during 8 h after a meal and the amounts ingested (X; g). No. of experimental meals 22, df 20; crude protein (nitrogen × 6·25) (g/kg) provided by fish meal (for details, see Table 1): (●), 80; (△), 120; (+), 160; (□), 240.

	$Y =$	r	S_b
Histidine	$0{\cdot}344X + 0{\cdot}613$	0·690 ($P < 0{\cdot}01$)	0·041
Lysine	$0{\cdot}508X + 0{\cdot}348$	0·842 ($P < 0{\cdot}01$)	0·039
Phenylalanine	$0{\cdot}749X + 0{\cdot}096$	0·891 ($P < 0{\cdot}01$)	0·042
Tyrosine	$0{\cdot}514X + 0{\cdot}954$	0·809 ($P < 0{\cdot}01$)	0·050
Leucine	$0{\cdot}444X + 1{\cdot}658$	0·842 ($P < 0{\cdot}01$)	0·036
Isoleucine	$0{\cdot}402X + 1{\cdot}136$	0·793 ($P < 0{\cdot}01$)	0·033
Methionine	$0{\cdot}531X + 0{\cdot}120$	0·849 ($P < 0{\cdot}01$)	0·037
Cystine	$0{\cdot}072X + 0{\cdot}423$	0·105 (NS)	0·081

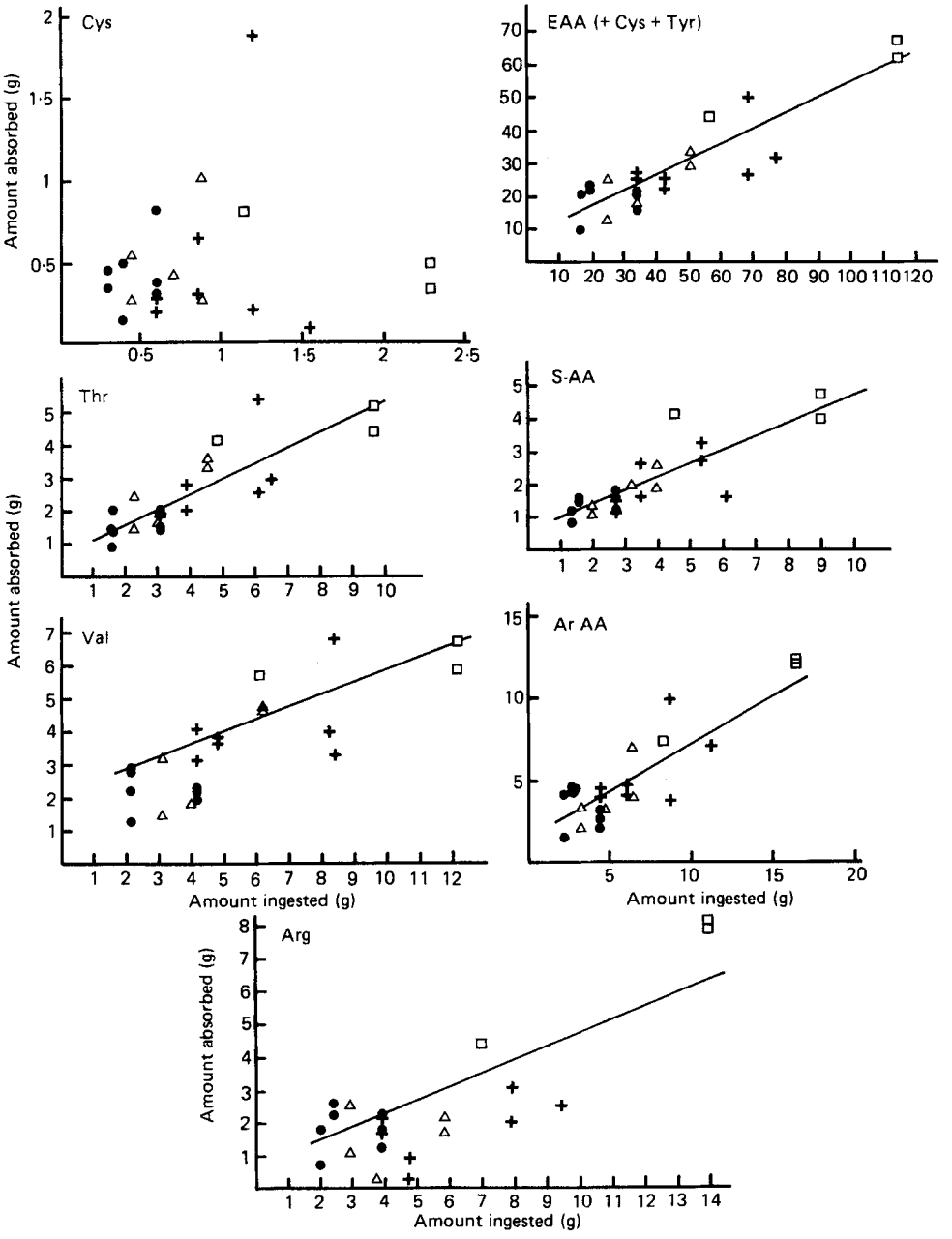

Fig. 3. *Contd.*

	$Y =$	r	S_b
Threonine	$0{\cdot}458X+0{\cdot}725$	0·837 ($P < 0{\cdot}01$)	0·033
Valine	$0{\cdot}438X+1{\cdot}207$	0·796 ($P < 0{\cdot}01$)	0·035
Arginine	$0{\cdot}506X-0{\cdot}254$	0·836 ($P < 0{\cdot}01$)	0·039
Sulphur-AA	$0{\cdot}423X+0{\cdot}538$	0·841 ($P < 0{\cdot}01$)	0·035
Aromatic AA (ArAA)	$0{\cdot}662X+1{\cdot}017$	0·886 ($P < 0{\cdot}01$)	0·041
EAA†	$0{\cdot}480X+7{\cdot}051$	0·885 ($P < 0{\cdot}01$)	0·029

NS, not significant; AA, amino acids; S_b, standard error of regression coefficient. † Sum of essential amino acids including tyrosine and cystine.

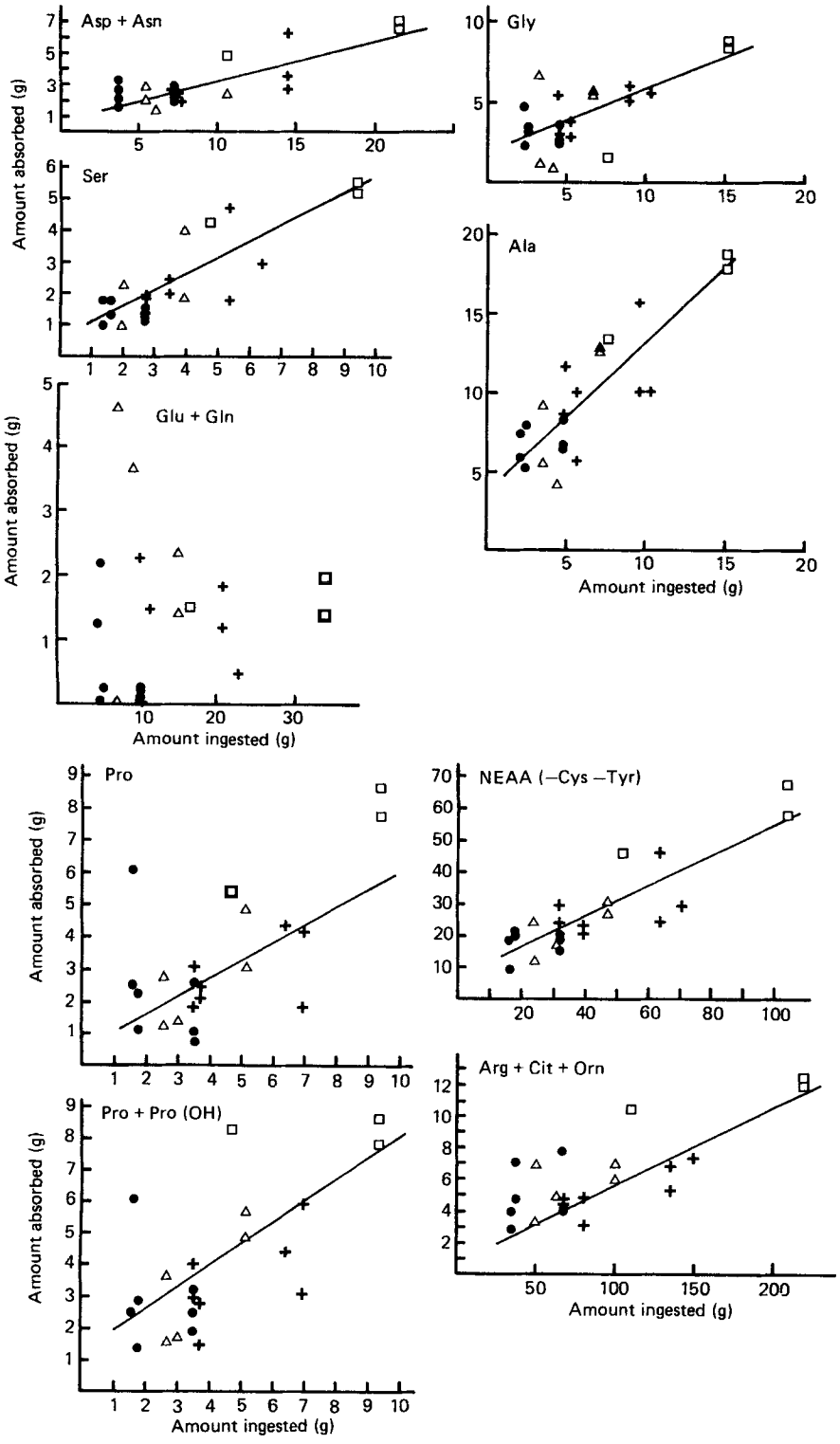

Fig. 4.

Table 6. *Changes in hourly absorption coefficients* of amino acids appearing in the portal vein (amount appearing in the portal vein/amount ingested) during a period of 8 h after a meal (fish-meal protein in a semi-synthetic diet)† according to the amount of total amino acids ingested*

Amount of total AA ingested (g)...	50	89·4	150
Total amino acids	0·103	0·081	0·070
Essential amino acids (EAA)‡	0·094	0·079	0·071
Histidine	0·111	0·081	0·066
Lysine	0·074	0·069	0·067
Phenylalanine	0·099	0·097	0·095
Leucine	0·104	0·083	0·072
Isoleucine	0·105	0·081	0·069
Methionine	0·076	0·072	0·070
Valine	0·105	0·083	0·072
Threonine	0·097	0·079	0·070
Arginine	0·053	0·057	0·060
Cystine	0·117	0·069	0·045
Tyrosine	0·153	0·113	0·094
Sulphur-amino acids	0·086	0·071	0·064
Aromatic amino acids	0·118	0·103	0·095
Non-essential amino acids (NEAA)§	0·112	0·083	0·069
Aspartic acid + asparagine	0·051	0·042	0·038
Serine	0·095	0·081	0·074
Glutamic acid + glutamine	0·021	0·012	0·008
Glycine	0·114	0·086	0·071
Alanine	0·253	0·193	0·162
Proline	0·098	0·084	0·077
Proline + hydroxyproline	0·145	0·117	0·102

AA, amino acids.
* Calculated from the regression equations (Figs. 3 and 4).
† For details, see Table 1.
‡ Sum of EAA including tyrosine and cystine.
§ Sum of NEAA excluding tyrosine and cystine.

Fig. 4. Relations between the amounts of each non-essential amino acid (NEAA; Y; g), and sum of NEAA, absorbed during 8 h after the meal and the amounts ingested (X; g); no. of experimental meals 22, df 20; crude protein (nitrogen × 6·25; CP) (g/kg) provided by fish meal (for details see Table 1): (●), 80; (△), 120; (+), 160; (□), 240.

	$Y =$	r	S_b
Aspartic acid + asparagine	$0{\cdot}249X + 0{\cdot}811$	0·819 ($P < 0{\cdot}01$)	0·019
Serine	$0{\cdot}513X + 0{\cdot}518$	0·847 ($P < 0{\cdot}01$)	0·037
Glutamic acid + glutamine	$0{\cdot}012X + 1{\cdot}151$	0·080 (NS)	0·019
Glycine	$0{\cdot}395X + 1{\cdot}763$	0·692 ($P < 0{\cdot}01$)	0·048
Alanine	$0{\cdot}935X + 3{\cdot}828$	0·846 ($P < 0{\cdot}01$)	0·075
Proline	$0{\cdot}535X + 0{\cdot}601$	0·723 ($P < 0{\cdot}01$)	0·053
Proline + hydroxyproline	$0{\cdot}646X + 1{\cdot}231$	0·682 ($P < 0{\cdot}01$)	0·072
NEAA†	$0{\cdot}384X + 12{\cdot}357$	0·829 ($P < 0{\cdot}01$)	0·029
Arginine + ornithine + citrulline‡	$0{\cdot}039X + 2{\cdot}490$	0·773 ($P < 0{\cdot}01$)	0·004

NS, not significant; S_b, standard error of regression coefficient. †Sum of NEAA excluding cystine and tyrosine. ‡Relations with total CP intake.

Table 7. *Pattern of essential amino acids (EAA) absorbed relative to that of ingested mixture of EAA* from fish meal and variations with intake*

Amount of total AA ingested (g)...	50	89·4	150
EAA†	0·916	0·969	1·013
Histidine	1·173	1·021	0·918
Lysine	0·787	0·877	0·940
Phenylalanine	1·053	1·217	1·338
Leucine	1·105	1·044	1·004
Isoleucine	1·120	1·022	0·961
Methionine	0·807	0·904	0·977
Valine	1·117	1·047	1·004
Threonine	1·028	1·001	0·986
Arginine	0·558	0·722	0·836
Cystine	1·254	0·868	0·635
Tyrosine	1·622	1·441	1·311
Sulphur-AA	0·913	0·899	0·896
Aromatic AA	1·252	1·300	1·329

AA, amino acids.

* From Fig. 3, $\frac{\text{AA \% mixture EAA absorbed}}{\text{AA \% mixture EAA ingested}}$.

† Relative to total AA.

Table 8. *Pattern of non-essential amino acids (NEAA) absorbed relative to that of ingested mixture of NEAA* from fish meal and variations with intake*

Amount of total AA ingested (g)...	50	89·4	150
NEAA†	1·077	1·023	0·982
Aspartic acid + asparagine	0·459	0·506	0·545
Serine	0·862	0·981	1·081
Glutamic acid + glutamine	0·180	0·142	0·111
Glycine	1·024	1·023	1·025
Alanine	2·264	2·307	2·331
Proline	0·885	1·012	1·122
Proline + hydroxyproline	1·308	1·403	1·482

AA, amino acids.

* From Fig. 4: $\frac{\text{AA \% mixture NEAA absorbed}}{\text{AA \% mixture NEAA ingested}}$.

† Relative to total AA.

DISCUSSION

The advantages and limitations of the methodology used to quantify the kinetic appearance of AA in the portal vein have been discussed elsewhere (Rérat *et al.* 1980; Rérat, 1982). The blood concentrations of N contained in the free AA assessed by chromatography in the present work and those of amino-N obtained by the trinitrobenzene sulphonate (TNBS) method in the previous paper (Rérat *et al.* 1988) cannot easily be compared for various reasons. First, in the TNBS method amino-N not only from AA, but also from primary amines (seldom found in blood) is measured; it is only partly sensitive to basic AA (75%) and dicarboxylic AA (50%) and not at all to imino acids (Waring & Bolton, 1967). On the

other hand, free tryptophan was not assessed with the chromatographic method used in the present work. Thus, TNBS amino-N did not exactly correspond to the sum of N of the various free AA determined by chromatography. Second, the curves representing the blood concentration of N obtained with either of these two methods were derived from different animals with different N intakes. However, it should be pointed out that despite the differences between these two methods, the pattern of the porto-arterial differences was similar in both cases and absorption calculated by the two methods was the same for animals given the same diets (Rérat *et al.* 1987). This result was also found in the present experiment in which the absorption efficiency of total free AA measured by chromatography was analogous to that of amino-N measured previously by the TNBS method (Rérat *et al.* 1988) whatever the level of fish-protein intake.

Because of the imbalance of the actual experimental scheme, due to experimental difficulties, it was not possible to proceed to a classical analysis of variance taking into account within-animal and between-animal variation. However, the regression equations involving only the first meal given to each animal were similar to those involving all the values with a variable number of meals given to each animal.

The pattern of free AA in the blood of our fasting animals was rather different from that obtained by some authors (Richardson *et al.* 1965; Nordstrom *et al.* 1970; Typpo *et al.* 1970) but similar to that recorded by others (Boomgaardt & McDonald, 1969). The differences may be due to the previous nutritional status of the animals, affected by the composition of the diets that they usually receive and which may be highly variable from one trial to another. However, in the present experiment a comparative analysis of ingesta and blood of fasting animals (Table 3) did not reveal any relation between the composition of fish proteins in the experimental diets and those used between experiments and that of the free AA in the blood. Moreover, the pattern of free AA in the blood of fasting animals (Table 3) was similar in the portal and arterial blood except that the contents of glutamic acid and glutamine were higher in arterial blood, and those of alanine and citrulline lower than in portal blood. This means that glutamine and glutamic acid of the arterial blood were steadily taken up by the cells of the intestinal wall, while alanine and citrulline constituted metabolism products of the gut wall. This is in agreement with observations made in the rat concerning the metabolism of blood glutamine by the gut cell wall to ammonia, citrulline, alanine and proline (Windmueller & Spaeth, 1980). The uptake of blood glutamine by the intestinal wall was also found after administration of protein-free diets (Rérat *et al.* 1976).

The post-prandial increase in aminoacidaemia, more marked in the portal than in the arterial blood, has already been established (Dent & Schilling, 1949; Denton & Elvehjem, 1954; Peraino & Harper, 1963; Pion *et al.* 1963, 1964; Nordstrom *et al.* 1970). The porto-arterial difference is mainly due to the uptake of AA by the liver during the post-prandial period (Elwyn *et al.* 1968; Ostrowski, 1969; Typpo *et al.* 1970; Bloxam, 1972). The variable rate of increase in the blood AA concentration has also been demonstrated (Pion *et al.* 1963; Dawson & Holdsworth, 1962) and, like the intensity and duration, it depends on the quantity (McLaughlan *et al.* 1963; Weller *et al.* 1969) and composition of proteins ingested (Krysciak *et al.* 1966; Whitaker & Patrick, 1971).

For most AA the relations between absorbed and ingested amounts were very close and increased with increasing time-interval after the meal. However, for some AA the influence of the level of protein intake did not predominate, the correlation coefficient of the regression equations being lower than 0·7 signifying that more than half the variation in amounts absorbed was due to other factors. This was the case for glutamic acid + glutamine and cystine and to a lesser extent for histidine, glycine, and proline + hydroxyproline.

Each increase in protein intake caused an increase in the hourly amount of TAA

appearing in the portal vein and a relative decrease in the absorption coefficient of TAA and individual AA. In other words, the digestive tract responded to the enhanced protein supply by accelerating the digestive processes, but this acceleration was inadequate to cope fully with the additional amount of food material to be broken down. This partial regulation may occur at various steps of the digestive process. For example, it has been well established that increasing the level of protein intake leads to a relative decrease in gastric emptying rate which is only perceptible 4 h after the meal (Cuber & Laplace, 1979). In the present case, the decrease in the absorption coefficient could be detected after 2 h; thus other factors were involved such that larger amounts of nutrients could not be hydrolysed in the small intestine during that period or their degradation products could not be absorbed. It may therefore be assumed that during the first period after the meal, the secretion of proteolytic enzymes or the hydrolysis conditions (especially pH) were not optimum to cope with the larger flow of substrates within the time available. It may also be assumed that competition for absorption occurred between AA and carbohydrates, thus reducing the transport rate of the former (Murer *et al.* 1975). It should also be emphasized that even when there was a relative slowing of gastric emptying 4 h after increasing the protein supply, the mass of protein present in the gut was always much larger than the TAA absorbed within the same period of time. Thus, 7 h after ingestion of 160 g proteins, the difference between the mass of proteins emptied from the stomach (138 g; Laplace *et al.* 1981) and AA absorbed (about 80 g) was 58 g, which can only be explained by the cumulative effect of a series of phenomena, e.g. time taken for hydrolysis and absorption and uptake of nutrients in the cells of the gut wall during their transport (which may reach 20–25% of the amounts ingested in the case of cereals such as barley and wheat, Rérat, 1981).

It would be interesting to analyse the reason for the differences between AA as regards their appearance in the portal vein and how this changed with protein intake. For a constant intake, the enrichment of portal blood with one AA may correspond to a higher absorption rate or to interconversion from other AA during transport. The former is probably the case for the aromatic AA and the latter for proline and alanine synthesized from glutamine and glutamic acid (Pion *et al.* 1963; Windmueller & Spaeth, 1980) or for histidine which can be released from dipeptides such as carnosine. Thus, the apparent extraction of blood histidine could be relatively greater with low levels of protein intake. Conversely, for a given intake, the decrease in the proportion of one AA in the mixture during absorption may either be due to catabolism, as evidenced by the transamination of dicarboxylic acids (Neame & Wiseman, 1957; Pion *et al.* 1963) or by a lower absorption rate as might be the case for lysine, arginine and the sulphur-AA. The modification of the composition of the absorbed mixture when the protein intake increased may be due to a change in the ratio, amounts of AA actually absorbed: amounts of AA taken up by the gut wall for protein synthesis in the enterocyte and secretion of endogenous N. For example, it has been established that after administration of protein-free diets the ileal contents are very rich in proline, serine, threonine, glycine and dicarboxylic acids (Holmes *et al.* 1974). Thus, for serine, proline, glycine and aspartic acid, the enrichment of the absorbed AA mixture when the dietary protein supply was increased might be due to a reduction in the relative importance of endogenous secretion of these AA.

Amino acids such as ornithine and citrulline appear in rather large amounts in the portal blood during digestion. Ornithine seems to be derived from dietary arginine, one-third of which is metabolized in the gut wall in the rat, and from aspartic acid (Windmueller & Spaeth, 1976). Most of the synthetized ornithine seems in turn to be catabolized to citrulline, proline, glutamine, organic acids and carbon dioxide. Citrulline seems to originate from the metabolism of blood glutamine, producing simultaneously ammonia, alanine and proline (Windmueller & Spaeth, 1980).

In contrast to the results of Elwyn (1970), our findings showed that the proportions of exogenous AA were somewhat changed by the digestive wall during their transport. It should however be pointed out that Elwyn (1970) fed his experimental animals (dogs) almost exclusively with protein-rich diets (horse meat) whereas rather small amounts of protein were used in the present experiment together with large amounts of carbohydrate. Modifications of the ingested essential AA mixture during absorption, which were less marked with increasing protein intake, mainly involved an increase in aromatic AA and a decrease in basic and S-AA. On the other hand, in the absorbed mixture of non-essential AA the content of glutamic acid declined and that of alanine rose.

Are these events in agreement with the kinetics of disappearance of individual AA in the digestive tract? According to Zebrowska (1981) methionine, isoleucine, leucine and arginine are the first AA to disappear from the gut after administration of various protein hydrolysates and threonine, histidine and lysine the last ones. These observations, confirmed by ileal digestibility values for fish meal AA (Darcy *et al.* 1982), are thus in disagreement with the kinetic hierarchy of appearance in the portal vein. The measurements are of two complementary physiological phenomena, separated by a living membrane. This discrepancy therefore results mainly from uptake and transformations by the gut wall, the extent of which has already been emphasized. Study of the disappearance of AA from the gut lumen reveals the absorption efficiency of AA from various proteins; study of the appearance of AA in the portal blood leads to a better approach to liver and tissue metabolism. The link between these two events is the metabolism in the gut wall; further knowledge is required on this topic for a better understanding of total protein metabolism in the body.

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