Intestinal and hepatic nitrogen balance in the rat after the administration of an oral protein load

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The fate of a small oral dose of protein given to overnight-starved rats was studied. After 3 h, 62% of the protein amino acids had been absorbed. Most of the absorbed N went into the bloodstream through the portal in the form of amino acids, but urea and ammonia were also present. About one-quarter of all absorbed N was carried as lymph amino acids. The liver was able to take all portal free ammonia and a large proportion of portal amino acids, releasing urea. The hepatic N balance was negative, indicating active proteolysis and net loss of liver protein.

Liver: Intestine: Protein load: N balance: Rat

After a meal the products of protein breakdown, essentially amino acids, are absorbed through the intestine (Wiseman, 1968), and sifted and partly retained by the liver (Ohnen *et al.* 1956; Schimassek & Gerok, 1965; Demigné & Rémésy, 1977). A proportion of the amino acids taken up is converted into other amino acids (Dent & Schilling, 1949; Goldberg & Guggenheim, 1962), part is used for an increased synthesis of protein (Omstedt & van der Decken, 1974) and the rest is deaminated and the hydrocarbon skeletons are used in the metabolism for energy (Krebs, 1969) or gluconeogenesis (Felig, 1973). The fine modulation of the fate outlined for ingested N is essentially dependent on the amino acid composition of the meal (Fafournoux *et al.* 1990) as well as on the actual immediate needs of the organism, for both energy and N (Young, 1987).

There has been much research on the actual role of splanchnic organs in the retention, transformation and eventual oxidation of the ingested amino acids (Ohnen *et al.* 1956; Schimassek & Gerok, 1965; Marliss *et al.* 1971; Demigné & Rémésy, 1977). However, the numerous methodological problems caused by the long digestion periods needed for protein breakdown and absorption (Darcy, 1984) have made it difficult to apply quantitative criteria when establishing the fate of ingested protein.

We present here the N balance of intestine and liver measured under controlled conditions for 3 h after the administration of a standard protein load.

MATERIALS AND METHODS

Male Wistar rats weighing 200–220 g were used. The animals were kept under standard conditions (temperature 22–23°; lights on from 08.00 to 20.00 hours; humidity 65–75%) and were fed on type A02 chow pellets (Panlab, Barcelona, Spain) and tap water. The animals were kept without food for 24 h before the administration of the protein load

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Table 1. *Mean values of the metabolic variables measured in rats in 180 min period after receiving an oral load of protein*

(Values are means of groups of five to six animals used for each measurement)

Variable	Site	Range*		Mean†	Statistical significance of effect of time (one-way ANOVA)‡:	
		Minimum	Maximum		*F*	*P*
α-NH_2-N (mM)	PV	$5{\cdot}0^{120}$§	$6{\cdot}9^{150}$	6·2	2·67	0·03
	SHV	$4{\cdot}8^{90}$	$6{\cdot}8^{0}$	6·1	2·99	0·02
	AA	$4{\cdot}4^{90}$	$7{\cdot}5^{0}$	6·0	2·21	0·07
	lymph	$5{\cdot}6^{180}$	$7{\cdot}0^{90}$	6·3	0·58	0·74
Urea (mM)	PV	$5{\cdot}7^{30,120}$	$8{\cdot}1^{60}$	6·6	9·24	0·00
	SHV	$6{\cdot}0^{0}$	$7{\cdot}9^{90}$	6·8	4·39	0·00
	AA	$5{\cdot}8^{120}$	$7{\cdot}7^{60}$	6·6	3·01	0·02
Ammonia (μM)	PV	84^{60}	141^{150}	112	0·96	0·51
	SHV	13^{120}	49^{30}	26	2·76	0·03
	AA	13^{120}	34^{180}	23	1·24	0·32
Glucose (mM)	PV	$4{\cdot}5^{0,30}$	$5{\cdot}5^{180}$	5·0	1·21	0·33
	SHV	$4{\cdot}9^{0}$	$5{\cdot}6^{180}$	5·2	0·45	0·84
	AA	$4{\cdot}9^{0}$	$5{\cdot}9^{150}$	5·3	1·76	0·14
Lactate (mM)	PV	$1{\cdot}00^{90}$	$1{\cdot}55^{0}$	1·27	2·29	0·06
	SHV	$0{\cdot}68^{90}$	$1{\cdot}08^{0}$	0·89	1·91	0·12
	AA	$0{\cdot}72^{60}$	$1{\cdot}25^{0}$	0·99	1·52	0·21
Glycogen (μmol)	liver	60^{30}	87^{180}	74	0·67	0·67
Plasma IR insulin (mU/l)	PV	28^{180}	83^{60}	52	17·62	0·00
	AA	$7{\cdot}5^{120}$	$19{\cdot}6^{60}$	9·4	4·01	0·01
Blood flow (μl/s)	PV	152^{60}	275^{120}	208	2·42	0·05
	SHV	267^{60}	582^{120}	370	11·43	0·00
	HA	77^{150}	313^{120}	162	—	—

PV, portal vein; SHV, suprahepatic vein; HA, hepatic artery; AA, aorta.

* Maximum and minimum values (for time-group means) found in the 180 min studied after oral load.

† Mean for the 180 min time interval.

‡ 27–29 df.

§ Superscript numbers represent the time-interval after load (at 30 min intervals) when these maximum and minimum values were attained.

(between 10.00 and 12.00 hours). The protein load was given by means of a stomach cannula, and contained 300 mg protein (a widely used dietary protein supplement, Top Protein 90; Eurodietética, Madrid, Spain) of known amino acid composition (equivalent to 2·04 mmol amino acids; the mixture represented a mean 1·298 atoms N per amino acid molecule), suspended in 2 ml water. The rats were killed at 30 min intervals after the administration of the protein, up to 180 min. At 5 min before killing the rats were anaesthetized with an intraperitoneal injection of sodium pentobarbital (60 mg/kg). At the stated times samples of portal and aortic blood were obtained with heparinized 1 ml syringes. Portions of blood were immediately centrifuged and their plasma used for the estimation of ammonia (DaFonseca-Wollheim, 1973*a*, *b*). The remainder was stored at $-30°$ until processing for analyses.

Blood samples were deproteinized with acetone and α-NH_2-N was measured using a fluorescamine method (Klein & Standaert, 1976). Glucose (Trinder, 1969), lactate (Gutmann & Wahlefeld, 1974) and urea (Fawcett & Scott, 1960) were measured by enzymic methods in blood deproteinized with perchloric acid (removed with K_2CO_3). Immunoreactive insulin was measured in plasma with a radioimmunoassay method using rat

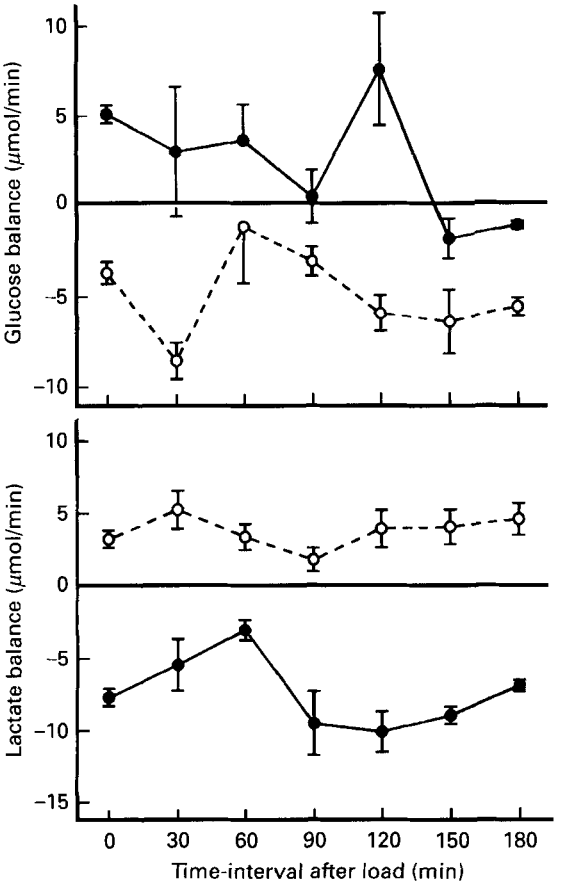

Fig. 1. Intestinal (○--○) and hepatic (●–●) glucose and lactate balances in rats for 3 h after the administration of an oral load of protein. The points represent the means with their standard errors for five to six animals. Positive balance (for both liver and intestine) represents a net release, and a negative balance represents a net uptake. Statistical analysis (ANOVA) of the effect of time-interval after load

Variable	df	*F*	*P*
Glucose balance:			
Intestinal	6, 27	2·61	0·0398
Hepatic	6, 27	1·93	0·1115
Lactate balance:			
Intestinal	6, 27	0·62	0·7117
Hepatic	6, 27	1·11	0·3800

insulin as standard (Heding, 1972; Iglesias *et al.* 1985). Liver glycogen was estimated after alkaline digestion (Good *et al.* 1933).

Another series of rats was treated as described previously, but a mesenteric vein was cannulated and infused with *p*-aminohippurate. The dilution pattern of the marker was then used for the estimation of portal and suprahepatic vein as well hepatic artery blood flow (Katz & Bergman, 1969; Casado *et al.* 1987).

A third series of rats was used for the extraction (under sodium pentobarbital anaesthesia) of intestinal lymph samples at the stated times after oral dosing, using plastic syringes and fine gauge hypodermic needles. These lymph samples were used for the

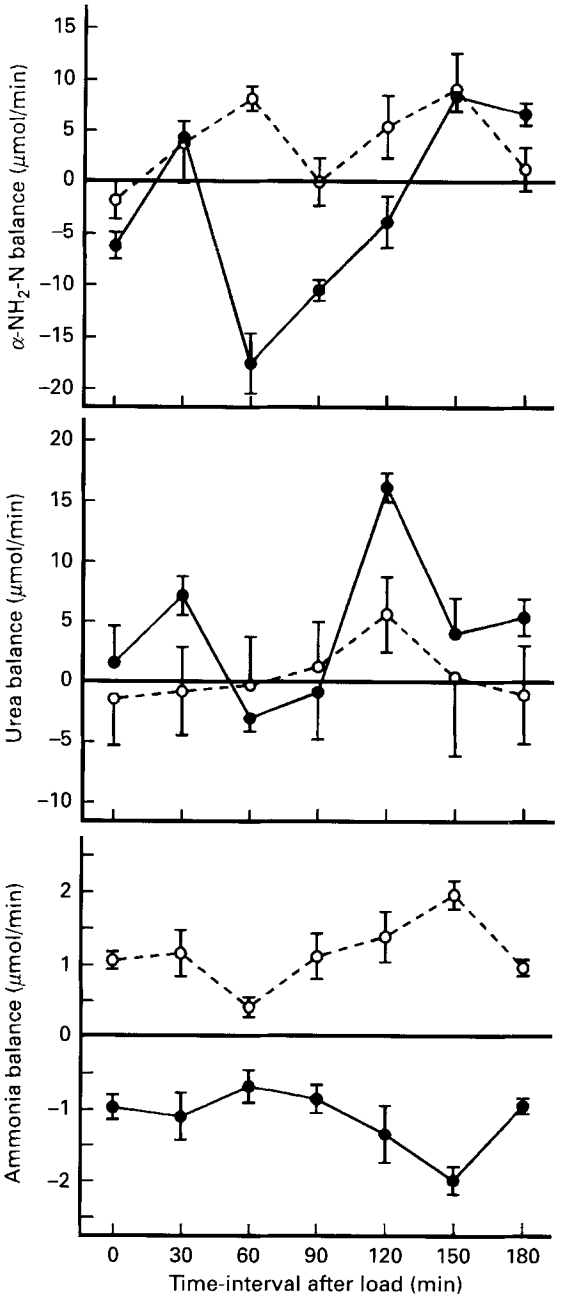

Fig. 2. Intestinal (○--○) and hepatic (●–●) α-NH_2-N, urea and ammonia balances in rats for 3 h after the administration of an oral load of protein. The points represent the means with their standard errors for five to six animals. Positive balance (for both liver and intestine) represents a net release, and a negative balance represents a net uptake. Statistical analysis (ANOVA) of the effect of time-interval after load

Variable	df	*F*	*P*
α-NH_2-N balance:			
Intestinal	6, 28	2·75	0·0316
Hepatic	6, 28	8·30	0·0000

estimation of total α-NH_2-N with fluorescamine (Klein & Standaert, 1976) only, because of their small volume.

The portal and arterial concentrations and blood flows were used to calculate net intestinal amino acid, urea, and ammonia balances (IB) across the intestine, by using the formula:

$$IB = \Phi_P[C_P - C_A],$$

where Φ_P is the portal blood flow at a given time; C_P and C_A are the concentrations at a given time in portal and arterial blood respectively. Likewise, for hepatic balances:

$$HB = \Phi_H C_H - [\Phi_P C_P - \Phi_A C_A]$$

where C_H is the suprahepatic vein concentration, and Φ_H and Φ_A are the suprahepatic vein and hepatic artery blood flows respectively.

The combined portal (small and large intestine, spleen, stomach, etc.) blood flow of conscious (fed and 24 h starved) rats was measured with a radioactive microsphere distribution method (Ahokas *et al.* 1984), and compared with the values obtained in rats anaesthetized (5 min earlier), using the *p*-aminohippuric acid dilution method.

Statistical significance of the differences between means was established with a standard one-way analysis of variance program, with the limit of significance set at $P < 0{\cdot}05$.

RESULTS

The amount of label derived from the oral protein load and present in the intestine and its contents diminished progressively with time. At 180 min, a mean 38% of the label remained in the intestine, the rest was to be found in other body compartments or was lost as excretion products. This 62% of the protein was considered the amount of α-NH_2-N actually taken up by the intestine and released into the bloodstream.

The portal blood flow of conscious fed rats estimated with a radioactive microspheres method was 315 (SEM 42) μl/s, and that obtained with the *p*-aminohippuric acid dilution method 297 (SEM 17) μl/s; for 24 h starved rats, the results were respectively 169 (SEM 32) μl/s and 183 (SEM 26) μl/s (n 5 for all four groups). No significant differences in the results were observed attributable to the method of measurement of blood flow. Both methods showed significantly lower ($P < 0{\cdot}05$) values for starved *v.* fed values.

Table 1 shows the means of the metabolic variables measured in rats that received an oral load of protein. The circulating levels of α-NH_2-N, urea, ammonia, glucose and lactate showed little variation during the 180 min study. No changes were appreciated either in liver glycogen content. Plasma insulin practically halved after the first hour. The widest variation was observed in portal blood flow (by almost a factor of six).

From the data summarized in Table 1, the hepatic and intestinal balances for glucose and lactate are presented in Fig. 1, and those for α-NH_2-N, urea and ammonia are shown in Fig. 2. The intestine showed a constant uptake of glucose from the bloodstream and a continued output of lactate, whereas the liver took up more lactate than that produced by the intestine and released limited amounts of glucose during the first hour and 2 h after the oral load.

The intestine showed a continued net positive balance of α-NH_2-N for the 3 h studied.

Urea balance:			
Intestinal	6, 28	0·35	0·9055
Hepatic	6, 28	6·04	0·0004
Ammonia balance:			
Intestinal	6, 28	6·20	0·0006
Hepatic	6, 28	4·63	0·0022

The liver changed from a net initial negative balance to a transient net release at 30 min followed by a longer period of net accumulation of amino acids. The last part of the period studied again showed a net positive balance for liver amino acids.

There was a small production of urea by the intestine, peaking at 120 min. The liver produced larger amounts of urea at 30 min and again from 120 min onwards; the maximum rate of production was attained at 120 min. The pattern of release of ammonia from the intestine changed little with time, showing a minimum at 60 min and a maximum at 150 min. The net liver uptake of ammonia was practically a mirror image of this pattern (Fig. 2).

A very high proportion of the digestion of the protein load was completed within 3 h, since about two-thirds were already absorbed as amino acids (i.e. more than 90% of the peptide bonds must have been split for such an amount of amino acids to be released). Coinciding with the absorption, several changes took place in a short time; the intestinal glucose consumption increased, probably a reflection of the higher energy needed to process the protein load. The intestinal release of amino acids peaked in about 60 min, coinciding with the highest hepatic uptake and lowest glucose consumption and lactate and ammonia production by the intestine. This coincided also with the lowest hepatic glucose output, maintaining the glycaemia, however, and lactate uptake. After that phase the liver and intestine increased their amino acid efflux; urea production was significant for both liver and intestine and liver glucose output and ammonia intake also increased. At 180 min, most variables tended to return to the pre-load levels, except for hepatic glucose and amino acid balances.

DISCUSSION

Anaesthesia is known to affect organ blood flow and glycaemia in the rat (Arola *et al.* 1981; Lang *et al.* 1986; Tuma *et al.* 1986; Pénicaud *et al.* 1987). The strategy used here, of minimizing the effects of anaesthesia by limiting its duration, has little effect on blood flows, since the data obtained on conscious animals with a suitable radioactive microsphere method gave values very close to those obtained with the *p*-aminohippuric acid dilution method under the limited-anaesthesia conditions outlined. Since the portal data flows for conscious animals were also in the range of values in the literature obtained under similar conditions in fed rats (Ishishe *et al.* 1980), it can safely be assumed that the effects of our anaesthesia protocol on the blood flows of rats receiving the oral protein load are minimal.

Food deprivation in the rat for 24 h affects its levels of reserves, especially glycogen (Newgard *et al.* 1983; Katz *et al.* 1986); the levels observed were very low, in agreement with those expected after overnight fasting (Palou *et al.* 1981). Glycaemia, however, was well maintained, in part because of liver gluconeogenesis from lactate and amino acids (Felig *et al.* 1970; Exton, 1972); the liver showed an initial (time 0) net, albeit small, glucose production. The uptake of lactate and α-NH_2-N and net urea release agree with this interpretation. The administration of a relatively small oral protein load (about 5 kJ of energy) had little effect. Since a rat that size has a mean energy output of about 2 W (Rafecas *et al.* 1989), the energy of the whole load was in a range of about one-quarter of that actually required for maintenance during the 3 h span studied.

Intestinal lactate production was in part a consequence of high intestinal glucose consumption (Nicholls *et al.* 1983), but the lactate produced was less than one-third of the glucose consumed by the intestine.

The contribution of lymph to the transport of amino acids from the intestinal lumen to the bloodstream is only an estimate, based on indirect lymph flow measurements (Fernández-López *et al.* 1992), but it represents a substantial proportion (about one-quarter of all absorbed N) with respect to the net amino acid efflux through the portal. The

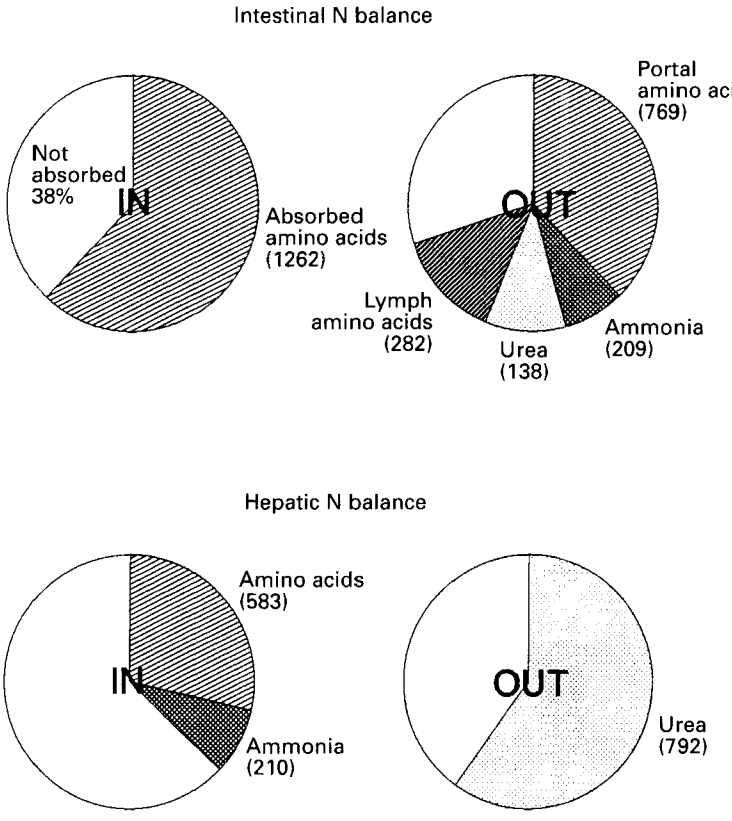

Fig. 3. Cumulative 3 h intestinal and hepatic N balance in rats after an oral load of protein. Values in parentheses represent N in each component (μmol). Since there are no values available for the actual composition of lymph and portal amino acid balances, the value has been obtained by applying the same value for N atoms/amino acid molecule as that in the initial protein load.

intestine profoundly alters the amino acid composition of the load in coordination with the uptake abilities of liver and other organs (Demigné & Rémésy, 1977). Lymph amino acids would circumvent the splanchnic bed organs and thus, would be directly available to peripheral organs.

Fig. 3 shows a synopsis of the fate of the protein load in 180 min, presented as N balances of intestine and liver. The intestinal N balance after 3 h was practically zero, with most of the output in the form of α-NH_2-N, in agreement with data in the literature (Goldberg & Guggenheim, 1962; Demigné & Rémésy, 1977; Fafournoux *et al.* 1990). A small but significant part of the overall N balance was due to a net intestinal urea balance, probably as a consequence of its arginase activity (Konarska & Tomaszewski, 1975), and a similar proportion of ammonia (Van Leeuwen *et al.* 1984; Weber *et al.* 1988). Lymph flow contributed a sizeable proportion of intestinal amino acid output. The results presented suggest that intestinal urea production, under the conditions studied, can over-compensate its known hydrolysis by the flora (Gibson *et al.* 1976). The net synthesis observed is very probably limited in time to conditions of high energy demand and high luminal amino acid availability.

The intestine produces ammonia mainly as a result of glutamine deamidation (Weber *et al.* 1988; Hartmann & Plauth, 1989), a process acting essentially on systemic glutamine synthesized in peripheral organs. The liver is able to retain practically all portal ammonia

(Rémésy *et al.* 1986; Cooper *et al.* 1987), i.e. it recovers all the ammonia released by the intestine. The fate of this ammonia is split between glutamine production and, especially, urea synthesis (Häussinger, 1987, 1989). The large liver urea output cannot be explained only as a need to detoxify portal ammonia, since this can only justify about one-third of that needed for the synthesis of the urea released by the liver. Consequently, under the conditions stated, the liver had a high ammoniogenic potential from amino acids (Shepartz, 1973). This is in agreement with its gluconeogenic role during food deprivation (Felig *et al.* 1970; Aikawa *et al.* 1973) and its ability to sustain its metabolism with amino-acid-derived energy.

The net hepatic N balance in 3 h was clearly negative, since the urea output was much larger than the combined influx of ammonia and amino acids. The balance could only come from liver protein made available as an energy source through proteolysis (Mortimore & Pösö, 1984). The balance depicted in Fig. 3 could be justified with about 4% of liver protein, a value not far from the changes in protein content the liver undergoes during starvation (Mortimore & Pösö, 1984).

The results presented suggest an efficient and very active disposal of an oral load of amino acid-N by the intestine and liver which, thus, seem to make their energy-rich hydrocarbon skeletons available for metabolic maintenance.

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