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Control of alanine metabolism in rat liver by transport processes or cellular metabolism

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1. Factors governing hepatic utilization of alanine were studied *in vivo* and *in vitro* in rats adapted to increasing dietary protein. 2. Hepatic alanine utilization was enhanced 5-fold with a 90%-casein diet, compared with a 13%-casein diet. The increased uptake resulted from enhanced fractional extraction in the presence of high concentrations of alanine in the portal vein. 3. The increase in alanine metabolism on high-protein diets was associated with an increase in alanine aminotransferase and in pyruvate utilization for gluconeogenesis. 4. The emergence of a high-affinity component appeared to be responsible for the enhanced transport of alanine with high-protein diets. 5. High extracellular concentrations after alanine loads resulted in a maximal rate of utilization and of accumulation of alanine by liver cells *in vivo* and *in vitro*. Alanine accumulation was particularly active with high-protein diets. 6. In starved rats, alanine transport was also increased, but low concentrations of alanine in afferent blood contributed to make transport limiting for alanine utilization. 7. In fed rats, the rates of transport and catabolism of alanine generally appear to undergo parallel changes; both processes thus play a fundamental role in the control of alanine utilization by the liver.

Alanine is the main glucogenic amino acid taken up by the liver (Yamamoto *et al.*, 1974; McDonald *et al.*, 1976; Rémésy *et al.*, 1978) and, besides lactate, alanine may be considered as a major source of cytosolic pyruvate. The first step in the metabolism of alanine is its transport across the plasma membrane, essentially mediated by a Na^+-dependent carrier, which is responsive to hormonal (Le Cam & Freychet, 1976; Fehlmann *et al.*, 1979; Canivet *et al.*, 1980) or dietary (Fafournoux *et al.*, 1982) factors. Alanine catabolism in liver cells may be affected by dietary protein content or by starvation, owing to adaptation of alanine aminotransferase (Krebs, 1964; Swick *et al.*, 1965) and, less specifically, to changes in the rate of pyruvate utilization (McDaniel, 1979; Halestrap *et al.*, 1980).

The identification of a rate-limiting step for alanine catabolism has been the subject of previous reports debating the respective importance of transport and cellular metabolism (Sips *et al.*, 1980; McGivan *et al.*, 1981; Groen *et al.*, 1982). These studies support the view that the transport of alanine across the plasma membrane is the only rate-controlling step in alanine metabolism, but with some disagreement about the concentration of alanine, if any, at which transport ceases to be rate-limiting. In fact, most investigations on the control of alanine metabolism have been carried out *in vitro*, on isolated hepatocytes, and it is of interest to ascertain whether similar conclusions will apply *in vivo*. Modifications of dietary protein content may bring about considerable changes in the concentrations of alanine in the portal vein and alterations in the rate of alanine metabolism (Rémésy & Demigné, 1982*a*). Under such conditions, the rate-limiting step in alanine metabolism might vary according to the nutritional or metabolic status.

To investigate this possibility, we have compared the rate of hepatic utilization of alanine in rats adapted to increasing dietary protein, either *in vivo* in anaesthetized rats or *in vitro* on isolated hepatocytes. We suggest that alanine transport *in vivo* may actually be rate-limiting in situations of stimulated uptake by the liver, such as occur with high-protein diets or during starvation. However, factors controlling alanine concentrations in blood entering the liver and the concentrations of other amino acids sharing the same carrier system might also play an important role *in vivo*.

Materials and methods

Chemicals

L-[U-^{14}C]Alanine (150 Ci/mol) was purchased from The Radiochemical Centre (Amersham,

0306-3283/83/030645-08$2.00

Bucks., U.K.); ^3H_2O (1 mCi/ml) and [^{14}C]inulin (8.5 Ci/mol) were obtained from the Commissariat à l'Energie Atomique (Gif sur Yvette, France). Amino-oxyacetic acid and albumin were from Sigma (St. Louis, MO, U.S.A.) and collagenase was from Boehringer (Meylan, France).

Animals and diets

Male Wistar rats weighing 180–200 g were adapted for 21 days on semi-purified diets providing various proportions of dietary protein. The compositions of the four diets (%, w/w) were: 5% casein, 85% wheat starch (5%-casein diet); 13% casein, 77% wheat starch (13%-casein diet); 50% casein, 40% wheat starch (50%-casein diet); 90% casein (90%-casein diet). The remainder of the diet contained 9% of energy as corn oil, 5% salt mixture and 1% vitamin. The salt and vitamin mixtures were purchased from UAR (Villemoisson sur Orge, France). Animals were fed from 09:00 to 17:00 h. The lighting schedule was: light 21:00–09:00 h, dark 09:00–21:00 h.

Sampling procedures and measurement of alanine utilization in vivo

Fed rats were sampled 4–5 h after the beginning of food intake, and those designated as starved (adapted to 13%-casein diet) were sampled 48 h after food removal. The rats were anaesthetized with sodium pentobarbital (40 mg/kg) and maintained at 37°C on a hot-plate. After laparotomy, 1 ml of blood was withdrawn in heparinized syringes either from the portal vein and then the aorta, or from the hepatic vein (in the left lobe) and then the portal vein. Liver sampling was performed as previously described (Rémésy *et al.*, 1978). Hepatic blood flow was measured by an indicator-dilution method, using *p*-aminohippurate (Rémésy & Demigné, 1982*b*). Briefly, the indicator (0.2 mg/ml in 0.9% NaCl) was infused into a mesenteric vein, with a 0.4 mm needle, at a rate of 0.10 ml/min during 10 min, after administration of a priming dose of 0.1 mg. Blood was slowly sampled from the hepatic vein, then from the aorta (hepatic blood flow), or from the portal vein and then the aorta (portal blood flow). If x (%) is the contribution of the portal vein to liver blood supply, the afferent concentration is (x)[portal-vein alanine] + $(100-x)$[arterial alanine]; the hepatic balance is the difference [hepatic-vein alanine] − [afferent alanine], and the fractional extraction is the ratio ([hepatic-vein alanine] − [afferent alanine])/[afferent alanine].

Analytical procedures

Standard enzymic methods were used for determination of alanine (Williamson, 1974), glutamate (Bernt & Bergmeyer, 1974), pyruvate (Czok & Lamprecht, 1974) and 2-oxoglutarate (Bergmeyer & Bernt, 1974).

For the determination of hepatic activity of alanine aminotransferase (EC 2.6.1.2), rats were killed by decapitation and liver was disrupted in 5 vol. of cold medium (140 mM-KCl, 5 mM-Tris/HCl and 5 mM-mercaptoethanol, pH 7.4), with a glass Potter–Evehjem homogenizer. The homogenate was centrifuged at 20000 ***g*** for 30 min at 4°C and the supernatant was used for assays of enzyme activity as described by Swick *et al.* (1965).

Preparation of isolated hepatocytes

Hepatocytes were isolated from fed rats adapted to the 13%- or 90%-casein diets, 5 h after the beginning of food intake, or from 48 h-starved rats. Collagenase dissociation was performed by the method of Berry & Friend (1969) as modified by Krebs *et al.* (1974). The integrity of the hepatocytes (90–95%) was estimated by cell-membrane refractoriness in phase-contrast microscopy. Incubations were performed in Krebs–Henseleit (1932) bicarbonate buffer containing 3% (w/v) dialysed albumin at pH 7.40.

Transport experiments

For measurement of alanine transport, hepatocytes were preincubated for 10 min at 37°C in the presence of 1 mM-amino-oxyacetic acid, which was assumed to inhibit completely the metabolism of alanine (McGivan *et al.*, 1981). Transport was initiated in 2 ml Eppendorf Microfuge tubes containing L-[^{14}C]alanine (0.1 μCi) by the addition of 400 μl of hepatocyte suspension (about 3×10^6 cells/ml) in Krebs albumin buffer (Krebs *et al.*, 1974). The resulting suspension was incubated at 37°C for 2 min with appropriate agitation. For the measurement of the time-course of alanine uptake, 2 ml of hepatocyte suspension was incubated at 37°C with radioactive alanine (1 μCi) over a period of 15 min in 10 ml flasks, with agitation, and gassed with CO_2/O_2 (1:19). Samples (500 μl) of cell suspension were taken at the required times. The reaction was stopped by addition of 2 ml of ice-cold Krebs albumin buffer to the Microfuge tubes, which were immediately centrifuged for 5 s at about 1000 ***g***. The supernatant was discarded and the cells were resuspended in 2 ml of chilled buffer. The same procedure was used to collect the cell pellets, which were transferred to counting vials with 3 ml of scintillation liquid (Instagel; Packard, Paris, France).

The residual extracellular activity that was still associated with the cells after the washing procedure did not exceed 3% of the total uptake. By determination of the time-course of radioactive alanine efflux at 0°C, it was checked that there was minor loss of radioactivity by the cells with this procedure (1% in 1 min); as the time of washing and isolation did not exceed 1 min, this factor was therefore not critical.

To measure alanine distribution during its metabolism, cells were incubated with increasing concentrations of alanine (0.6–6.0mM) with ^3H_2O and [^{14}C]inulin for 10min at 37°C. The incubation was then stopped by centrifuging the cell suspension (800μl) in Eppendorf Microfuge tubes through 300μl of silicone oil (AR 200/AR 20, 1:3, v/v; Wacker Chemie, Munich, Germany) layered above 200μl of 1.2M-$HClO_4$. The supernatant was immediately deproteinized by 2vol. of 0.6M-$HClO_4$.

Results

Changes in availability of alanine in afferent blood and hepatic utilization in vivo

In fed rats, arterial concentrations of alanine were practically unaffected by large changes in dietary protein (Fig. 1), whereas alanine concentrations in portal vein (hence in afferent blood) underwent large changes. However, the rise in portal-vein alanine was not entirely proportional to dietary casein, because of intestinal production, which accounts for a large part of the alanine appearing in portal blood. In starved rats, arterial alanine and afferent concentrations fell, as a result of the drastic decrease in dietary amino acid supply.

The relative constancy of arterial alanine with varying protein in the diet may be ascribed to hepatic utilization, which efficiently kept pace with the considerable increase in alanine supply (Table 1). Alanine utilization in the liver was 5 times greater after adaptation to a 90%-casein diet, compared with a normal diet. This adaptation was reflected in large changes in fractional extraction of alanine, from less than 20% with a protein-deficient diet to about 70% with a 90%-casein diet. With high-protein diets, the increase in portal concentrations

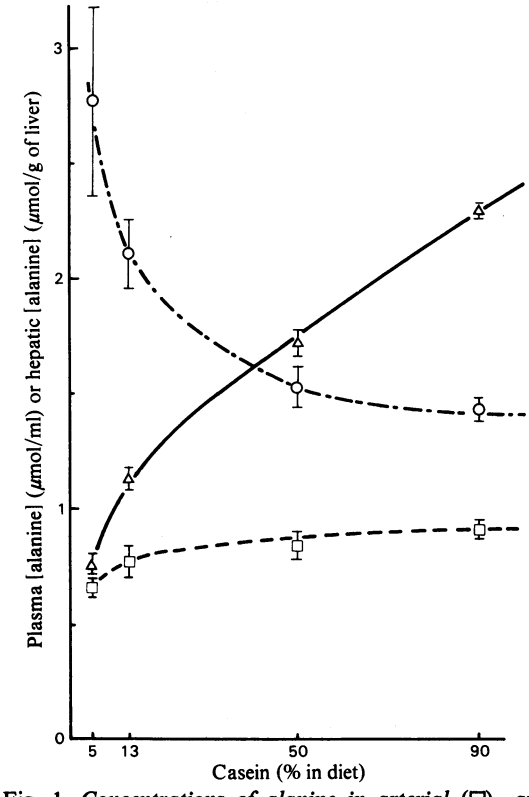

Fig. 1. *Concentrations of alanine in arterial* (□) *and hepatic afferent* (△) *plasma, and in the liver* (○)
From measurements of the contribution of portal vein to hepatic blood flow (71–74%), afferent concentrations were calculated by the procedure described in the Materials and methods section. Values are means ± S.E.M. for ten rats; the values for starved rats were: artery 0.25 ± 0.02mM, afferent plasma 0.32 ± 0.03mM, liver 0.41 ± 0.04mM.

Table 1. *Effects of changes in dietary protein and of starvation on hepatic utilization of alanine and activity of alanine aminotransferase*

The hepatic utilization was calculated from the difference in alanine concentration between hepatic vein and afferent plasma, and from hepatic blood flow (2.4–2.8ml/min per g of liver for fed rats, and 2.1ml/min per g for starved rats). The rate of alanine utilization is expressed as μmol/min per 200g body wt.; for calculation of the fractional extraction see the text. Activity of alanine aminotransferase is expressed as μmol of substrate/min per g of liver and the hepatic concentrations of the reactants for alanine transamination are expressed as μmol/g of liver. Values are means ± S.E.M. for eight rats in average. The statistical significance ($P < 0.05$) of the difference, compared with the 13%-casein diet, is indicated by * (change in dietary protein) or by † (starvation).

	Casein in diet (%)				13%-Casein diet, 48h starvation
	5%	13%	50%	90%	
Rate of alanine utilization	1.99 ± 0.21*	4.64 ± 0.52	14.45 ± 1.54*	21.16 ± 3.46*	1.94 ± 0.22†
Fractional extraction	20%	25%	49%	68%	55%
Alanine aminotransferase activity	19.6 ± 2.8	25.5 ± 2.1	60.8 ± 5.2*	78.5 ± 6.2*	35.8 ± 3.7†
Hepatic [alanine]	2.77 ± 0.41	2.11 ± 0.15	1.53 ± 0.11*	1.43 ± 0.05*	0.41 ± 0.04†
Hepatic [2-oxoglutarate]	0.22 ± 0.03	0.30 ± 0.03	0.24 ± 0.04	0.20 ± 0.02*	0.20 ± 0.02†
Hepatic [glutamate]	2.10 ± 0.20	1.90 ± 0.11	2.71 ± 0.12*	3.90 ± 0.32*	2.40 ± 0.30
Hepatic [pyruvate]	0.51 ± 0.06*	0.30 ± 0.01	0.13 ± 0.01*	0.07 ± 0.01*	0.05 ± 0.01†
$\frac{[\text{Alanine}][\text{2-oxoglutarate}]}{[\text{Glutamate}][\text{pyruvate}]}$ ratio	0.60	1.11	1.04	1.05	0.68

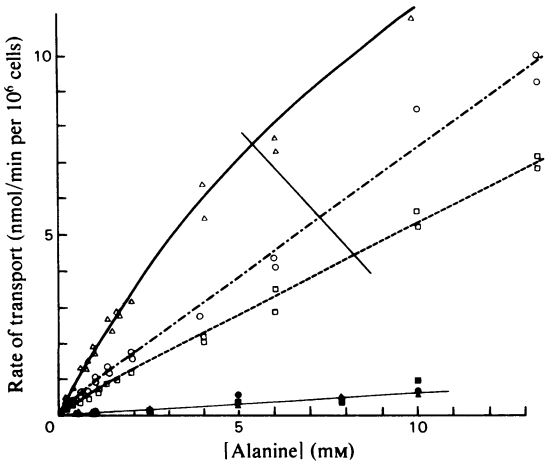

Fig. 2. *Effect of external concentrations on alanine transport in vitro*

The Figure shows the effects of increasing concentrations of alanine in the medium on the rate of alanine transport in rats fed on a (□) 13%- or (△) 90%-casein diet, and in starved rats (○). Cells were incubated over 2 min with alanine concentrations ranging from 0.2 to 13 mM. Choline was substituted for Na^+ when Na^+-free medium was required for transport assays in fed rats on a (■) 13%- or (▲) 90%-casein diet, or starved rats (●). The values are the means of two determinations from the same hepatocyte preparation.

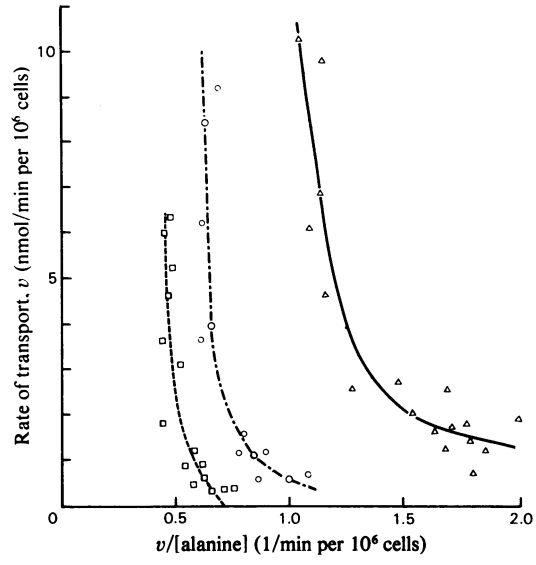

Fig. 3. *Woolf–Augustinson–Hofstee representation of Na^+-dependent alanine transport*

Values are means of two determinations from the same hepatocyte preparation: from fed rats on a (□) 13%- or (△) 90%-casein diet, or starved rats (○).

was concomitant with a fall in hepatic concentrations, thus giving rise to more favourable blood/liver gradients for hepatic uptake. In starved rats, alanine extraction was very efficient, but the overall uptake of alanine was limited by the low afferent concentrations.

As shown in Table 1, the activity of alanine aminotransferase was markedly enhanced with high-protein diets, but this rise was not entirely proportional to the hepatic utilization of alanine. The activity of the enzyme with the protein-deficient diet was little different from that with the normal diet, whereas it was slightly enhanced in starved rats adapted to a normal diet. The mass-action ratio of the reactants of alanine aminotransferase was near 1 in crude hepatic extracts from rats on a 13%-casein diet. This ratio was not changed by increasing dietary protein, in spite of a dramatic rise in alanine utilization. In contrast, the ratio was depressed in rats on a protein-deficient diet, in keeping with the fall in alanine utilization, and in starved rats, when alanine supply was decreased.

Alanine transport and accumulation in isolated hepatocytes

It was checked that the initial rate of alanine transport was linear with time over the first 3 or

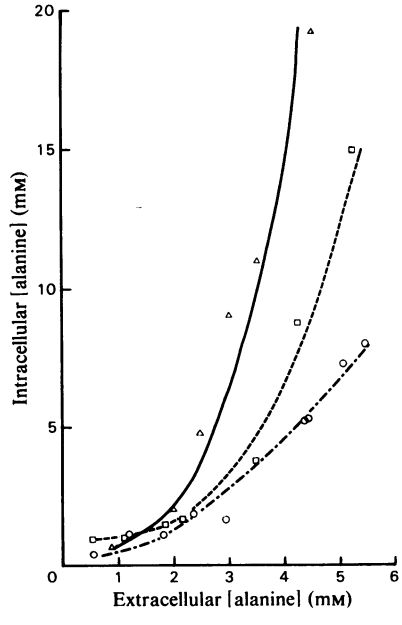

Fig. 4. *Effects of external concentrations on intracellular alanine in vitro*

Hepatocytes were incubated for 10 min with alanine concentrations ranging from 0.6 to 6 mM: from fed rats on a (□) 13%- or (△) 90%-casein diet, or starved rats (○). The values are means of duplicate determinations for two hepatocyte preparations.

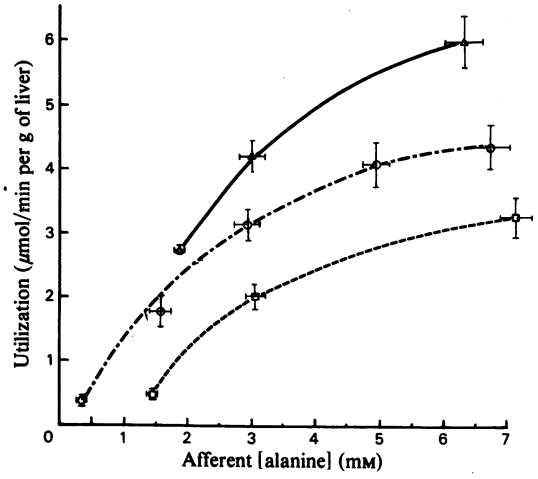

Fig. 5. *Changes in the hepatic utilization in vivo after alanine infusion into the portal vein*

For experimental details, see the text. Alanine was infused into the portal vein, via a mesenteric vein, by the procedure described for blood-flow measurements in the Materials and methods section. The rate of alanine infusion was 6 or 8 μmol/min/100 g body wt. for rats on a (□) 13%- or (△) 90%-casein diet, and 3, 6, 12 or 18 μmol/min per 100 g body wt. for starved (○) rats. Values are means ± S.E.M. for eight rats on average for each point.

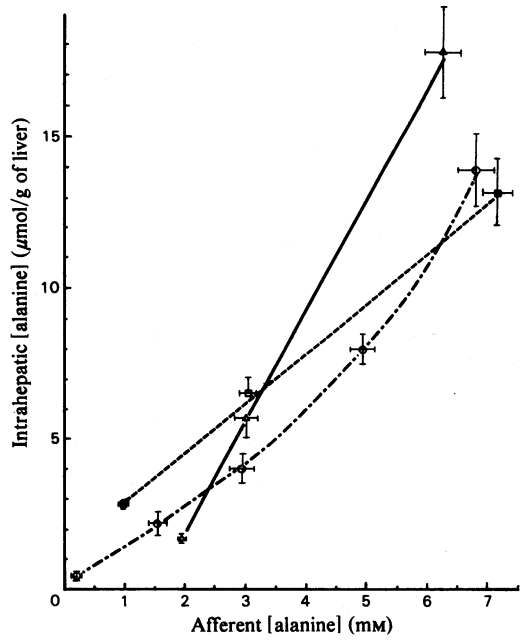

Fig. 6. *Changes in hepatic concentrations of alanine in vivo after alanine infusion into the portal vein*

For experimental details and significance of the symbols, see the text and Fig. 5. Values are means ± S.E.M. for eight rats on average for each point. The concentrations of the reactants of alanine aminotransferase were determined for alanine infusion at a rate of 18 μmol/min per 100 g body wt., corresponding to afferent [alanine] of about 6–7 mM. The concentrations of pyruvate, 2-oxoglutarate and glutamate were respectively (μmol/g fresh wt.): 0.26, 0.19 and 3.92 (13%-casein diet, fed), 0.29, 0.19 and 5.49 (13%-casein diet, starved) and 0.20, 0.32 and 4.48 (90%-casein diet, fed). The mass-action ratios were: 2.2 (13%-casein diet, fed), 1.5 (13%-casein diet, starved) and 6.0 (90%-casein diet, fed).

4 min of incubation (in keeping with data from McGivan, 1979); transport assays were thus performed within 2 min. In addition, Na^+-independent fluxes were not significantly different between the batches of cells, and alanine uptake was considered as only affected by active processes. As shown in Fig. 2, over the entire range of concentrations, there was a markedly higher uptake of alanine by hepatocytes from rats on a 90%-casein diet. Starvation elicited a lesser rise in the rate of transport. The plot of the reciprocal of the transport rate ($1/v$) against 1/[alanine] indicates that, considering the lowest concentrations of substrate, the K_m of the high-affinity component for alanine transport could be around 4 mM, in agreement with results of Joseph *et al.* (1978) and Sips *et al.* (1980). The plot of v against v/[alanine] deviated slightly from linearity with the 13%-casein diet (Fig. 3), in contrast with previous results with 2-aminoisobutyrate as substrate (Fafournoux *et al.*, 1982). By contrast, the plot was definitely curvilinear with hepatocytes from rats on a 90%-casein diet. This feature could be compatible with the fact that the high-affinity component performs a large part of alanine transport in these conditions.

The intracellular concentrations of alanine with increasing concentrations of external alanine, over a period of 10 min (to limit disappearance of alanine at the lowest concentrations), are shown in Fig. 4. Alanine accumulation was maximal after adaptation to a high-protein diet; with this diet, intracellular alanine remained relatively low up to about 2 mM external alanine, but higher external concentrations readily elicited a dramatic accumulation in the cells. This threshold could correspond to the saturation of alanine catabolism; it must be pointed out that the transport systems enable the cell to accumulate alanine, even with a very high cell/medium gradient. In rats on a normal diet accumulation was also evident, but there was a more progressive increase in intracellular alanine. This fact suggests that the rate of alanine transport is low, all the more since the metabolism is limited by transaminase activity. In hepatocytes from starved rats, alanine concentrations in the cells were always lower than in the

fed state. In spite of the induction of the A system during starvation, accumulation of intracellular alanine with high concentrations of external alanine was less striking than in rats on high-protein diets.

Alanine uptake and accumulation by the liver with increasing concentration of portal alanine

As shown in Fig. 5, alanine utilization was markedly enhanced with increasing afferent concentrations. In rats adapted to a 13%-casein diet, the hepatic utilization was 4–5-fold enhanced when the afferent concentration reached 3 mM, but alanine utilization was more responsive to alanine loads during starvation, particularly for moderate loads. Maximal utilization was observed with rats adapted to a 90%-casein diet (more than 5 μmol/min per g of liver). The plot of intracellular alanine against afferent alanine shows that the capacities for alanine accumulation were substantially higher with the high-protein diet (Fig. 6), reaching very high values (17 μmol/g of liver). The increase in hepatic alanine was relatively linear in fed rats on a normal diet; during starvation, the increase was moderate for afferent alanine concentrations of 1–3 mM, suggesting that cellular metabolism could efficiently keep pace with the amounts of transported alanine. However, with higher concentrations in portal vein, alanine was extensively accumulated in the liver. The concentrations of the reactants of alanine aminotransferase have been determined for the highest afferent alanine concentration (6–7 mM): glutamate was about 2-fold enhanced in rats adapted to a 13%-casein diet, particularly during starvation, whereas pyruvate was increased with the high-protein diet (3-fold) and in starved rats (6-fold). Owing to alanine accumulation, the mass-action ratio was elevated in favour of alanine utilization, particularly with the high-protein diet.

Discussion

Results on alanine metabolism have frequently been obtained in poorly defined nutritional conditions (chow diets), and disregarding the fact that alanine concentrations in afferent blood are much higher than arterial concentrations, particularly with high-protein diets. The K_m for alanine transport, essentially reflecting the activity of the A system for low concentrations, appears to be close to 4 mM, thus markedly higher than the K_m reported for 2-aminoisobutyrate (approx. 1 mM; Le Cam & Freychet, 1977). However, the efficiency of the A system for alanine is probably higher, thus allowing detection of a noticeable activity of the A system in rats on a 13%-casein diet. As alanine in portal vein is generally lower than the K_m of the carrier for alanine, the rate of transport into liver cells might closely depend on alanine concentrations in blood entering the liver. In fact, alanine arising from the digestive tract in fed rats is almost quantitatively removed by the liver, whatever the proportion of dietary protein (Rémésy & Demigné, 1982*a*,*b*), showing that hepatic uptake may efficiently keep pace with large changes in alanine production and absorption from the digestive tract.

In fed rats, the mass-action ratio for alanine aminotransferase was slightly lower than the equilibrium-constant value of 1.52 reported by Krebs & Veech (1969). This might suggest that the flux through the enzyme is in the direction of alanine formation, which is in contrast with the observation that alanine is actually utilized in all conditions. In fact, the measured values in crude hepatic extracts for the reactants of the enzyme may not exactly reflect the cytosolic concentrations, owing to extracellular contaminations and subcellular compartmentation (Siess *et al.*, 1982). In spite of these limitations, the comparison of the mass-action ratio provides comparative information: it appears that equilibrium of the reactants may be affected when the availability of alanine is either very low (protein deficiency, starvation) or extremely high. After alanine infusion, hepatic utilization is likely to be affected by pyruvate metabolism with high-protein diets, or by both the activity of ureogenesis and pyruvate utilization with low-protein diets.

Reports favouring the importance of transport as a rate-limiting step for alanine catabolism have appeared, particularly for alanine concentrations lower than 1 mM (Sips *et al.*, 1980; McGivan *et al.*, 1981). However, Groen *et al.* (1982) failed to observe any accumulation of hepatic alanine, even after infusion of a high concentration (6 mM). Contrary to these results, we obtained a dramatic accumulation of alanine in liver cells, up to external alanine of about 3 mM, even after adaptation to a normal diet. Consequently, most investigations *in vitro* with 5–10 mM-alanine as substrate are probably performed with unphysiological concentrations of intracellular alanine. In fact, alanine accumulation might inhibit ureogenesis (Hensgens & Meijer, 1980) and the activity of pyruvate kinase (Friedrichs & Schoner, 1974). The fact that alanine accumulates in liver cells at extracellular concentrations higher than 3 mM may reflect the concentrative capacity of the A system when the rate of alanine metabolism reached a plateau. The discrepancy between our results and those of Groen *et al.* (1982) could possibly stem from their procedure for determination of cellular metabolites (digitonin fractionation). According to Groen *et al.* (1982), the transport rate appears too low for alanine accumulation, which is in disagreement with our data *in vivo* and *in vitro* and results of McGivan *et al.* (1981). In fact, the plot of hepatic alanine against external alanine affords a possibility to identify extracellular concentrations at which the

saturation of alanine catabolism appears. As expected, this concentration is markedly higher with the high-protein diet *in vitro*, but *in vivo* the rise in hepatic alanine in parallel to external alanine was much more progressive, thus hampering any evaluation of a concentration threshold for alanine utilization. In fact, alanine accumulation may be still very active in the presence of very high cellular concentrations, even with a normal diet. However, excess alanine accumulation in the cells could bring about disturbances in the cell/medium ionic gradients. *In vivo*, responses to alanine loads might also be complicated by the stimulation of glucagon secretion consequent on hyperalaninaemia (Muller *et al.*, 1971); however, comparable responses have been obtained for alanine accumulation *in vivo* and with isolated hepatocytes.

In fed rats, hepatic utilization of alanine *in vivo* was enhanced 5-fold after adaptation to a 90%-casein diet, whereas transport capacities *in vitro* were increased practically 8-fold, compared with a normal diet. However, alanine transport may be affected by competition for the A system by other amino acids (Joseph *et al.*, 1978; Kilberg *et al.*, 1981). For instance, portal concentrations of various neutral glucogenic amino acids were enhanced 2-fold in rats fed on a 50%-casein diet, compared with a 13%-casein diet (Rémésy *et al.*, 1978). In starved rats, alanine utilization by the liver was markedly decreased in spite of a more efficient extraction of available alanine. The induction of alanine aminotransferase appeared more responsive to dietary protein than to starvation, supporting the view that the main specific adaptation of alanine metabolism in starved rats affects transport. Joseph *et al.* (1978) reported that inhibition of transport by ouabain did not affect the rate of metabolism of alanine and concluded that transport was not rate-limiting. The present results support the opposite view in starved rats: hepatic concentrations are very low, in the range of afferent concentrations, in spite of the induction of a concentrative system; furthermore, the rate of alanine metabolism appears readily enhanced, even with small increments of afferent alanine. However, it must be kept in mind that alanine transport *in vivo*, taken as a whole, reflects both the activity of the translocator (increased, owing to induction of the A system) and afferent concentrations of alanine (markedly depressed). During starvation, alanine utilization is thus strongly dependent on alanine availability, itself closely related to the inter-organ relationship for glutamine and branched-chain amino acids (Felig & Koivisto, 1979).

The statement that alanine metabolism could be affected by transport processes has thus to be qualified. The rates of alanine transport and catabolism frequently exhibit parallel changes. With normal diets comprising moderate contents of protein, the rates of both alanine transport and intracellular metabolism are low: transport processes do not appear to constitute a limiting step. Furthermore, the capacities of alanine transport seem sufficiently high for removal of large amounts of endogenous alanine produced in acute situations such as shock, hypoxia or exercise (Felig, 1973; Leclerc *et al.*, 1976; Wannemacher *et al.*, 1980). In contrast, there are situations for which transport actually appears limiting, frequently despite the induction of a high-affinity system, such as during starvation or with high-protein diets. However, alanine transport in starved rats seems closely dependent on afferent alanine itself, whereas high concentrations of various glucogenic amino acids with high-protein diets might competitively affect alanine transport.

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