Biochem. J. (1975) **150**, 379–387
Printed in Great Britain

Factors Regulating Amino Acid Release from Extrasplanchnic Tissues in the Rat

INTERACTIONS OF ALANINE AND GLUTAMINE

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(*Received* 27 *March* 1975)

1. Factors regulating the release of alanine and glutamine *in vivo* were investigated in starved rats by removing the liver from the circulation and monitoring blood metabolite changes for 30min. 2. Alanine and glutamine were the predominant amino acids released into the circulation in this preparation. 3. Dichloroacetate, an activator of pyruvate dehydrogenase, inhibited net alanine release: it also interfered with the metabolism of the branched-chain amino acids valine, leucine and isoleucine. 4. L-Cycloserine, an inhibitor of alanine aminotransferase, decreased alanine accumulation by 80% after functional hepatectomy, whereas methionine sulphoximine, an inhibitor of glutamine synthetase, decreased glutamine accumulation by the same amount. 5. It was concluded that: (*a*) the alanine aminotransferase and the glutamine synthetase pathways respectively were responsible for 80% of the alanine and glutamine released into the circulation by the extrasplanchnic tissues, and extrahepatic proteolysis could account for a maximum of 20%; (*b*) alanine formation by the peripheral tissues was dependent on availability of pyruvate and not of glutamate; (*c*) glutamate availability could influence glutamine formation subject, possibly, to renal control.

Alanine and glutamine have been shown to be the predominant amino acids released from rat skeletal muscle (Ruderman & Lund, 1972; Ishikawa *et al.*, 1972). Recent studies in this laboratory have indicated that the relative rates of release of both alanine and glutamine from the extrasplanchnic tissues in the rat are affected by nutritional and hormonal status (Blackshear *et al.*, 1974*a*) and by pyruvate dehydrogenase (EC 1.2.4.1) activity (Blackshear *et al.*, 1974*b*, 1975). Further experiments are described in this paper which clarify the pathways and interactions of alanine and glutamine formation by these tissues *in vivo*.

Materials and Methods

Animals

Male Ash/Wistar rats weighing 185–215g were used. They were allowed free access to water and a standard laboratory rat diet (diet 41*b*; Oxoid Ltd., London SE1 9HF, U.K.) at all times except where stated in the text.

Experimental design

Fed rats were anaesthetized with sodium pentobarbitone (60mg/kg body wt., intraperitoneally) and polythene cannulae (no. 161R, Bardic 1-Catheter, C. R. Bard International Ltd., Clacton-on-Sea, Essex, U.K.; no. 2FG Intravenous Cannula, Portex Ltd., Hythe, Kent, U.K.) were inserted into the left femoral artery and vein. The rats were then placed in restraining cages and allowed free access to water for the next 24h. The experiments were begun when the animals had been deprived of food for 24h.

The functional hepatectomy preparation described previously (Blackshear *et al.*, 1974*a*) was used for these experiments. This involves the ligation of the coeliac axis and superior mesenteric arteries and the hepatic portal vein, thus removing the liver and the rest of the splanchnic bed from the circulation.

In the first series of experiments ten animals received 2h intravenous infusions of either 0.9% (w/v) NaCl (1.2ml/h) or sodium dichloroacetate (5g/100ml, pH7.4; 1.2ml/h = 300mg/kg per h). Dichloroacetate has been shown to activate pyruvate dehydrogenase in several tissues (Whitehouse *et al.*, 1974). After 2h of infusion, functional hepatectomies were performed. In this experiment, blood samples of 0.5ml were drawn from the arterial cannulae before the infusion, immediately before functional hepatectomy, and 30min after functional hepatectomy. These samples were used for whole-blood amino acid analysis as described below.

In a second series of experiments, control animals

received a single intravenous injection of NaCl (0.5ml) 1h before functional hepatectomy. They were then anaesthetized with intravenous sodium pentobarbitone (60mg/kg), functionally hepatectomized and received an additional intravenous bolus of NaCl (0.5ml) immediately after functional hepatectomy. Test animals were given L-cycloserine (10mg in 0.5ml of NaCl, pH7.4), an inhibitor of alanine aminotransferase (EC 2.6.1.2) (Barbieri *et al.*, 1960; Otto, 1965), as a single intravenous injection either 1h before or immediately after functional hepatectomy. Methionine sulphoximine (30mg in 0.5ml of NaCl, pH7.4), an inhibitor of glutamine synthetase (EC 6.3.1.2) (Pace & McDermott, 1952), or amino-oxyacetate (4.5mg in 0.5ml of NaCl, pH7.4), an inhibitor of both alanine aminotransferase and aspartate aminotransferase (EC 2.6.1.1) (Hopper & Segal, 1962, 1964), was injected into other animals 1h before functional hepatectomy.

In the last series of experiments, animals cannulated in the usual way were infused with dichloroacetate (300mg/kg per h) or NaCl (0.9%; 1.2ml/h). Both functional hepatectomies and nephrectomies were then performed. Animals which had been pre-infused with dichloroacetate received 0.5ml of NaCl as an intravenous bolus, and animals pre-infused with NaCl received either 0.5ml of NaCl or 10mg of L-cycloserine in 0.5ml of NaCl immediately after the operation.

Blood sampling

Unless otherwise specified, blood samples (0.3ml, in duplicate) were drawn from the arterial cannula into heparinized syringes immediately before functional hepatectomy; further samples (0.3ml) were drawn 5, 10, 20 and 30min after functional hepatectomy. A portion of this blood (0.2ml) was immediately deproteinized in 2.0ml of ice-cold 3% (v/v) $HClO_4$; these samples were prepared for enzymic analyses as described previously (Schein *et al.*, 1971).

Assays

Enzymic assays were performed for: glucose (Slein, 1963), glycerol (Eggstein & Kreutz, 1966), lactate (Hohorst *et al.*, 1959), pyruvate (Bücher *et al.*, 1963), L-alanine (Williamson *et al.*, 1967), L-glutamine (Lund, 1970) and L-glutamate (Bernt & Bergmeyer, 1963). Pyruvate was determined immediately after neutralization of the acid extracts; all other metabolites were determined within 48h.

The automated amino acid analyses were performed on a model JLC-6AH Amino Acid Analyser (Japan Electron Optics Laboratory Co., Tokyo, Japan). Glutathione interferes with the whole-blood analyses of both threonine and serine in this system, so values for these amino acids are not included.

Results are expressed as means ± S.E.M.; significant differences were determined by using Student's *t* test.

Special chemicals

Enzymes and coenzymes were supplied by Boehringer Corp. (London) Ltd., London W5 2TZ, U.K., except for glutaminase, supplied by Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey, U. K., as were methionine sulphoximine and amino-oxyacetic acid. Pentobarbitone sodium (Nembutal) was from Abbott Laboratories, Queensborough, Kent, U.K. Dichloroacetic acid was from BDH Chemicals, Poole, Dorset, U.K. L-Cycloserine was a gift from Dr. E. Lorch, Hoffmann-La Roche and Co. A.G., Basel, Switzerland.

Results

(a) Functional hepatectomy studies

(1) *Changes in blood amino acids in normal animals.* Table 1 lists the changes in all amino acids measured after functional hepatectomy. The concentrations of most amino acids measured increased after the splanchnic bed had been removed from

Table 1. *Whole-blood amino acid concentrations before and 30min after functional hepatectomy*

Normal rats ($n = 6$) starved for 24h were infused with NaCl for 2h, after which time a functional hepatectomy was performed. Amino acids were measured in blood samples taken immediately before and 30min after each functional hepatectomy; their concentrations are expressed below as means ± S.E.M. For other details, see the text.

		Concentration (μM)		
Amino acid	Time ...	0min	30min	Change
Taurine		52 ± 4	45 ± 3	−7
Aspartate		33 ± 5	33 ± 7	0
Glutamate		207 ± 53	172 ± 11	−35
Glutamine		628 ± 56	1146 ± 55	+518
Proline		62 ± 9	275 ± 28	+213
Glycine		368 ± 11	598 ± 22	+230
Alanine		135 ± 8	506 ± 31	+371
Valine		207 ± 11	353 ± 16	+146
Methionine		15 ± 1	46 ± 12	+31
Isoleucine		108 ± 4	176 ± 9	+68
Leucine		194 ± 9	346 ± 14	+152
Tyrosine		43 ± 2	135 ± 6	+92
Phenylalanine		55 ± 6	155 ± 9	+100
Ornithine		52 ± 6	57 ± 5	+5
Lysine		331 ± 30	657 ± 50	+326
Histidine		42 ± 1	101 ± 22	+59
Tryptophan		38 ± 3	43 ± 3	+5
Arginine		123 ± 9	317 ± 18	+194
Asparagine		108 ± 6	169 ± 14	+61

the circulation. Alanine and glutamine showed the greatest changes, and there were also notable increases in the branched-chain amino acids, the basic amino acids, and glycine and proline.

(2) *Effects of dichloroacetate pretreatment on blood amino acids.* Dichloroacetate pretreatment resulted in significant changes only in the concentrations of the branched-chain amino acids and alanine. The concentrations of all three branched-chain amino acids rose significantly after dichloroacetate infusion, but with a subsequent functional hepatectomy these amino acids accumulated to a lesser extent than those from NaCl-treated control animals (Fig. 1). However, the final values for valine and leucine were still significantly higher than control values 30min after functional hepatectomy. In contrast, [alanine] decreased from $119\pm18\,\mu$M to $81\pm6\,\mu$M after 2h of dichloroacetate infusion, then rose to $168\pm8\,\mu$M after functional hepatectomy; the corresponding control values were 157 ± 10, 135 ± 8 and $506\pm31\,\mu$M ($P<0.01$) respectively.

(3) L-*Cycloserine pretreatment.* Pretreatment with L-cycloserine resulted in significantly increased blood [alanine] before functional hepatectomy, owing presumably to inhibition of alanine transamination both in liver and in extrahepatic tissues. Fig. 2 shows the changes in blood [glucose], [lactate] and [pyruvate] in response to L-cycloserine given either 1h before or immediately after functional hepatectomy. There were no significant differences in glucose disappearance in the three groups; however, pretreatment with L-cycloserine significantly stimulated accumulation of lactate (31%) and pyruvate (47%) after functional hepatectomy.

Fig. 3 shows the responses of three amino acids to L-cycloserine treatment of the animals. Treatment both before and immediately after functional hepatectomy resulted in marked decreases in the rate of alanine accumulation, by 82% and 62% respectively. Neither treatment had any significant effect on blood [glutamate]. However, pretreatment with L-cycloserine resulted in a significant increase in glutamine accumulation at 30min of almost 50%.

(4) *Pretreatment with methionine sulphoximine or amino-oxyacetate.* Table 2 shows the blood concentrations of metabolites measured 1h after the injection of methionine sulphoximine or amino-oxyacetate and then 30min after functional hepatectomy. Methionine sulphoximine infusion caused significant increases in blood [glucose] and [alanine] and a marked decrease in blood [glutamine] before functional hepatectomy; amino-oxyacetate caused significant increases in blood [lactate], [pyruvate] and [alanine]. At 30min after functional hepatectomy, both drugs slightly inhibited glucose disappearance, and amino-oxyacetate caused significantly increased accumulation of lactate and pyruvate (Table 2). The responses of the amino acids

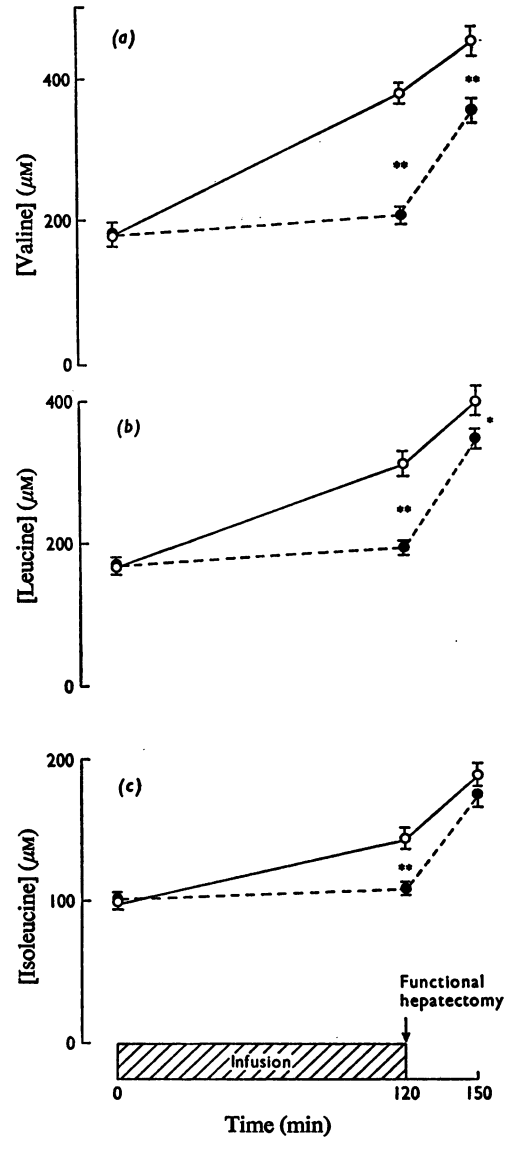

Fig. 1. *Changes in branched-chain amino acid concentrations* [(*a*), *valine*; (*b*), *leucine*; (*c*), *isoleucine*] *before and after dichloroacetate infusion and after functional hepatectomy*

Starved normal rats were infused with NaCl (●; $n=6$) or dichloroacetate (○; $n=6$) as described in the text, after which time a functional hepatectomy was performed. $*P<0.05$, $**P<0.01$, when values from dichloroacetate-treated animals are compared with those from NaCl-treated rats, by using Student's t test. For other details, see the text.

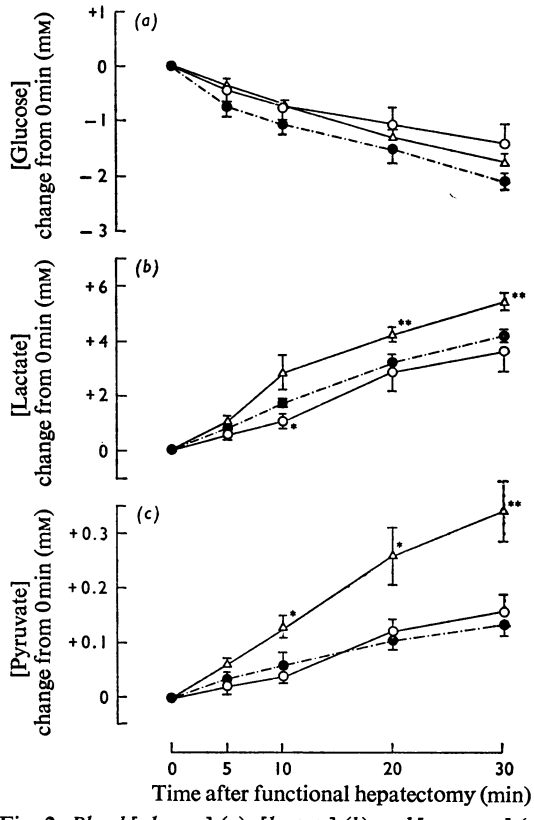

Fig. 2. *Blood [glucose] (a), [lactate] (b) and [pyruvate] (c) changes after functional hepatectomy and cycloserine treatment*

Normal starved animals were treated with NaCl (●; $n = 5$), cycloserine immediately after functional hepatectomy (○; $n = 5$) or cycloserine 1h before functional hepatectomy (△; $n = 5$) as described in the text. $*P<0.05$, $**P<0.01$, when values from cycloserine-treated animals are compared with those from NaCl-treated animals at each time-point, by using Student's t test. Metabolite concentrations immediately before functional hepatectomy were as follows: the numbers represent means±S.E.M. from animals treated with NaCl, cycloserine after functional hepatectomy, or cycloserine 1h before functional hepatectomy respectively (mM): [glucose]: 3.53±0.25, 3.70±0.32, 3.32±0.53; [lactate]: 0.55±0.03, 0.88±0.26, 0.76±0.12; [pyruvate]: 0.06±0.01, 0.07±0.01, 0.05±0.00. For other details, see the text.

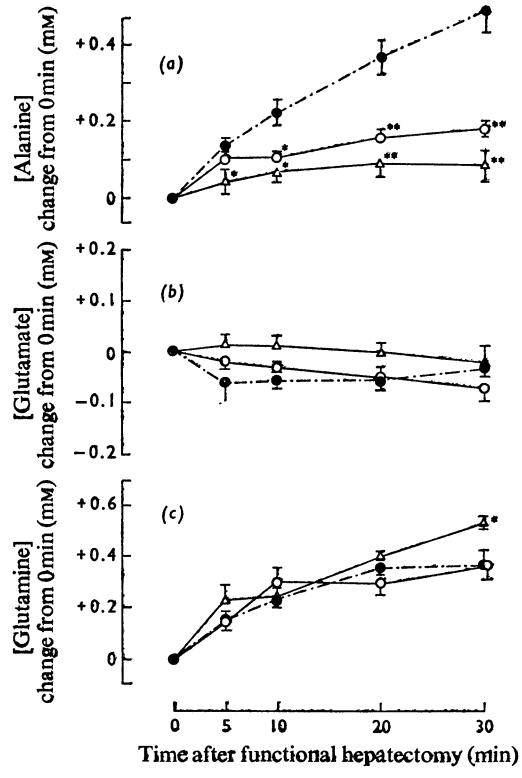

Fig. 3. *Blood [alanine] (a), [glutamate] (b) and [glutamine] (c) changes after functional hepatectomy and cycloserine treatment*

Symbols and other details are as in Fig. 2. Metabolite concentrations before functional hepatectomy, in the order of NaCl-treated, cycloserine-treated after functional hepatectomy, and cycloserine pretreatment, are as follows (mM): [alanine]: 0.16±0.01, 0.16±0.03, 0.24±0.02 ($P<0.01$ when values from cycloserine-pretreated animals are compared with those from NaCl-treated animals); [glutamate]: 0.17±0.01, 0.16±0.02, 0.13±0.01; [glutamine]: 0.48±0.04, 0.52±0.03, 0.49±0.02. For other details, see the text.

to these agents are shown in Fig. 4. Amino-oxyacetate decreased alanine accumulation by 42%, but alanine accumulation was relatively unaffected by pretreatment with methionine sulphoximine. Both compounds caused significantly higher blood glutamate concentrations. Amino-oxyacetate slightly inhibited glutamine accumulation, whereas methionine sulphoximine strikingly inhibited glutamine accumulation by 82% after functional hepatectomy.

(b) Functional hepatectomy and nephrectomy studies

These studies were undertaken to assess the role of the kidney in removing lactate, pyruvate and glutamine after functional hepatectomy. As shown in Fig. 5, glucose disappeared in the control animals in a manner identical with that after functional hepatectomy alone (Fig. 2), implying that the kidney did not contribute significantly to blood [glucose] in this situation. Treatment either with dichloroacetate or with L-cycloserine significantly decreased the rate of glucose disappearance in these animals.

The control lactate accumulation was also very similar in the functionally hepatectomized and

Table 2. *Blood metabolite and amino acid concentrations before and 30min after functional hepatectomy and treatment with NaCl, methionine sulphoximine or amino-oxyacetate*

Normal starved rats were treated with NaCl, methionine sulphoximine or amino-oxyacetate 60min before functional hepatectomy as described in the text. Values are means±S.E.M.; there were five animals in each group. $*P<0.05$, $**P<0.01$, when comparing values from rats treated with methionine sulphoximine or amino-oxyacetate with values from NaCl-treated animals at each time. For other details, see the text.

	Concentration (μM)					
	Zero time			After 30min		
Treatment ... Metabolite	NaCl	Methionine sulphoximine	Amino-oxyacetate	NaCl	Methionine sulphoximine	Amino-oxyacetate
Glucose	3.53±0.25	4.20±0.10*	3.85±0.26	1.43±0.22	2.60±0.31	2.07±0.17*
Lactate	0.55±0.03	0.51±0.04	1.52±0.21**	4.66±0.20	4.34±0.27	6.34±0.51*
Pyruvate	0.06±0.01	0.08±0.01	0.09±0.01*	0.20±0.02	0.22±0.01	0.30±0.02**
Alanine	0.16±0.01	0.21±0.02*	0.21±0.02*	0.64±0.06	0.75±0.03	0.49±0.01*
Glutamate	0.17±0.01	0.14±0.01	0.18±0.04	0.13±0.01	0.13±0.01	0.20±0.01**
Glutamine	0.48±0.04	0.19±0.02**	0.56±0.04	0.84±0.08	0.26±0.02**	0.86±0.04

nephrectomized animals (Fig. 5) to its accumulation in the functionally hepatectomized animals (Fig. 2). Dichloroacetate significantly decreased the rate of lactate accumulation by 46%; L-cycloserine, however, stimulated the accumulation of lactate by 50%.

The control elevation in [pyruvate] was significantly higher than that seen after functional hepatectomy alone, implying that the kidney may have been extracting a substantial amount of pyruvate from the circulation (Fig. 5). L-Cycloserine treatment caused no change in pyruvate accumulation, whereas dichloroacetate markedly inhibited it by 88%.

Finally, functional hepatectomy and nephrectomy caused no marked change in the rate of accumulation of alanine or glutamine (Fig. 6) when compared with the functionally hepatectomized animals (Fig. 3). In the former group of animals, both L-cycloserine and dichloroacetate caused significant decreases in alanine accumulation of 35% and 61% respectively (Fig. 6). Neither compound caused a marked change in blood [glutamate]. Both agents, however, caused increases in blood glutamine accumulation of 40% after dichloroacetate and 68% with L-cycloserine; only the change with L-cycloserine was significant.

Discussion

(a) *Control of alanine release*

Alanine is known o be one of the quantitatively most important amino acids released from skeletal muscle both in the rat (Ruderman & Lund, 1972) and in man (Felig & Wahren, 1974). This fact, and the importance of alanine as a gluconeogenic substrate, have led Mallette *et al.* (1969) and Felig (1972) to postulate a glucose–alanine cycle (for review, see Felig, 1973). In this cycle, alanine would be formed in the extrahepatic tissues, largely by transamination of pyruvate derived from glycolysis; alanine released into the circulation would then serve both as a precursor of glucose and as a means of transport of amino groups from the periphery to the liver for ureogenesis. Although this theory has been recently questioned (Ozand *et al.*, 1973), Felig (1973) cites a number of physiological and pathological situations in which the glucose–alanine cycle seems to apply.

The studies *in vivo* described here support the proposition that extrasplanchnic alanine production is dependent on adequate pyruvate availability. The evidence for this conclusion is as follows. When muscle pyruvate dehydrogenase was activated in either starved or severely ketotic diabetic rats by dichloroacetate (Blackshear *et al.*, 1974*b*, 1975), accumulation of both lactate and pyruvate was markedly decreased in the blood of functionally hepatectomized rats. In this situation, one would expect decreased pyruvate availability, and consequently the inhibition of alanine formation, which indeed occurred. This was confirmed in the present study. Secondly, L-cycloserine, an inhibitor of alanine aminotransferase, caused a marked decrease in alanine accumulation when administered either before or after functional hepatectomy (Fig. 3). These results are supported by the finding of Ruderman & Berger (1974) that perfusion of the rat hindquarter with leucine and L-cycloserine decreased net alanine release compared with leucine infusion alone. Thirdly, the administration of amino-oxyacetate, an inhibitor of both alanine aminotransferase and aspartate aminotransferase, also decreased alanine accumulation after functional

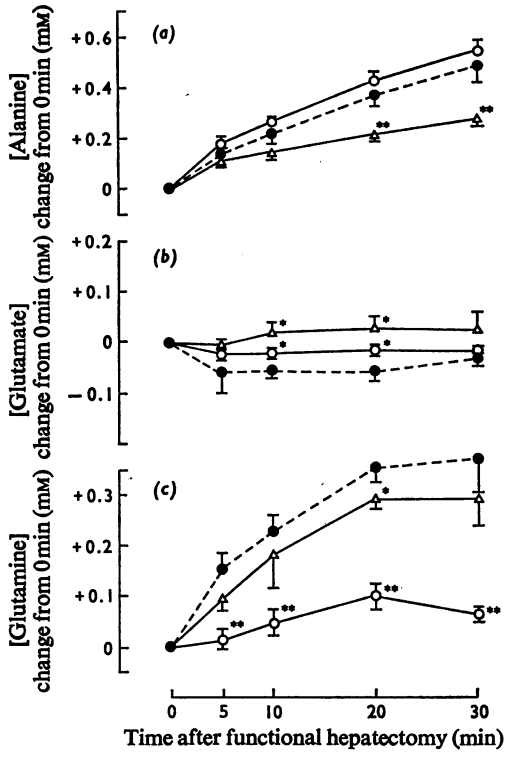

Fig. 4. *Blood [alanine] (a), [glutamate] (b) and [glutamine] (c) changes after functional hepatectomy and treatment with methionine sulphoximine or amino-oxyacetate*

Starved normal rats were injected with NaCl (●; $n = 5$), methionine sulphoximine (○; $n = 5$) or amino-oxyacetate (△; $n = 5$) 1 h before functional hepatectomy as described in the text. $*P<0.05$, $**P<0.01$, when values from animals treated with methionine sulphoximine or amino-oxyacetate are compared with those from NaCl-treated rats at each time-point, by using Student's t test. The blood metabolite concentrations before functional hepatectomy in this experiment are given in Table 2. For other details, see the text.

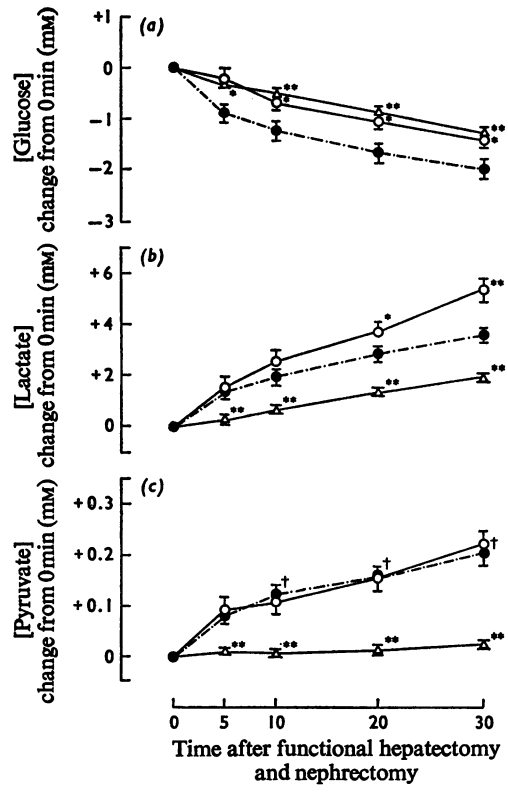

Fig. 5. *Blood [glucose] (a), [lactate] (b) and [pyruvate] (c) changes after functional hepatectomy and nephrectomy and treatment with dichloroacetate or cycloserine*

Starved normal rats were treated with NaCl (●; $n = 6$), dichloroacetate (△; $n = 5$) or cycloserine (○; $n = 5$) before functional hepatectomy and nephrectomy as described in the text. $*P<0.05$, $**P<0.01$, when values from animals treated with dichloroacetate or cycloserine are compared with those from NaCl-treated animals, by using Student's t test. $\dagger P<0.05$, $\dagger\dagger P<0.01$, when values from NaCl-treated animals after functional hepatectomy and nephrectomy are compared with those from NaCl-treated rats after functional hepatectomy alone (Fig. 2). Blood metabolite concentrations before functional hepatectomy, in the order NaCl-treated, dichloroacetate-treated, cycloserine-treated animals, were as follows (mM): [glucose]: 3.73 ± 0.18, 2.26 ± 0.05 ($P<0.01$ compared with value from NaCl-treated animals), 3.67 ± 0.29; [lactate]: 0.55 ± 0.08, 0.16 ± 0.06 ($P<0.01$ compared with value from NaCl-treated animals), 0.61 ± 0.07; [pyruvate]: 0.07 ± 0.00, 0.02 ± 0.00 ($P<0.01$ compared with value from NaCl-treated animals), 0.08 ± 0.00. For other details, see the text.

hepatectomy (Fig. 4). Both L-cycloserine and amino-oxyacetate caused increases in the rates of accumulation of lactate and pyruvate after functional hepatectomy; this would be expected after the inhibition of a pathway of pyruvate metabolism.

These results indicate that most of the carbon skeletons needed *in vivo* for alanine synthesis *de novo* derive from pyruvate. When animals were pretreated with L-cycloserine, the net alanine release seen after functional hepatectomy was decreased by 82%. Thus in this situation, the maximum amount of net alanine release derived from extrasplanchnic proteolysis was only 18% of the total amount. These values are similar to those of Odessey *et al.* (1974), who studied alanine formation from glucose in starved-rat diaphragms.

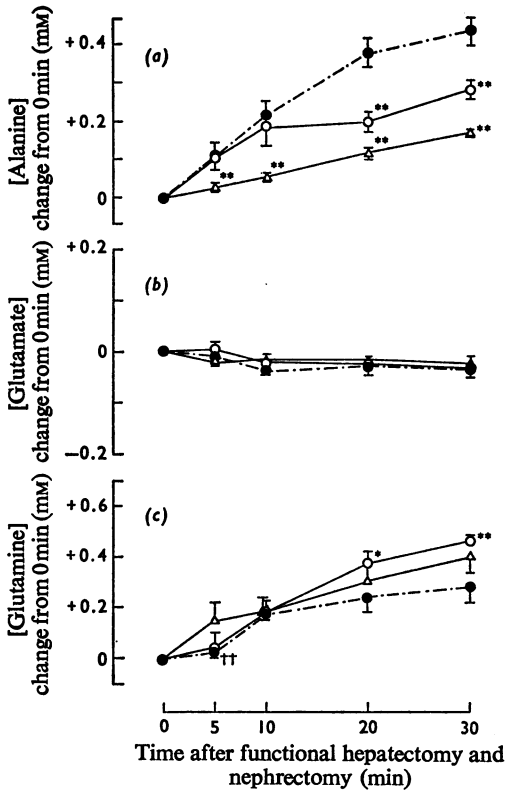

Fig. 6. *Blood [alanine] (a), [glutamate] (b) and [glutamine] (c) changes after functional hepatectomy and nephrectomy and treatment with dichloroacetate or cycloserine*

Symbols and other details are as in Fig. 5. Blood metabolite concentrations before functional hepatectomy, in the order NaCl-treated, dichloroacetate-treated, cycloserine-treated animals, were as follows (mM): [alanine]: 0.16±0.01, 0.06±0.01 ($P<0.01$ compared with value from NaCl-treated animals), 0.19±0.03; [glutamate]: 0.42±0.07, 0.36±0.05, 0.31±0.05. For other details, see the text.

(b) Control of glutamine release

Glutamine is also released from the human forearm (Marliss *et al.*, 1971) and the perfused rat hindquarter (Ruderman & Lund, 1972) in amounts higher than could be accounted for by muscle protein breakdown alone. In the present study its accumulation in the blood after functional hepatectomy was greater than that for any other amino acid (Table 1). Glutamine synthetase is present in rat skeletal muscle (Iqbal & Ottoway, 1970) and could catalyse the synthesis of glutamine *de novo* from glutamate. Methionine sulphoximine, a specific inhibitor of glutamine synthetase (Pace & McDermott, 1952), markedly inhibited glutamine accumulation after functional hepatectomy, indicating that the glutamine synthetase pathway is the source of most of the glutamine released by the extrasplanchnic tissues. At most, tissue proteolysis could account for 18% of total glutamine release. Ruderman & Berger (1974) also found that methionine sulphoximine plus leucine decreased glutamine release by the perfused rat hindquarter.

Dichloroacetate decreased alanine accumulation and increased glutamine accumulation in the blood of functionally hepatectomized normal and severely ketotic rats, suggesting a reciprocal mechanism controlling the relative release of these amino acids (Blackshear *et al.*, 1974*b*, 1975). This possibility was investigated *in vivo* by injecting L-cycloserine immediately after functional hepatectomy. As shown in Fig. 3, no change in glutamine accumulation occurred in this situation. The possibility was investigated that the kidney was removing glutamine from the circulation in increased amounts after cycloserine injection. In rats subjected to a nephrectomy as well as a hepatectomy, glutamine accumulation was significantly increased after cycloserine administration, lending support to the 'reciprocal' hypothesis (Fig. 6). In addition it was possible that a time-lag after inhibition of alanine formation was necessary for a build-up of glutamate to occur and stimulate glutamine release. This was explored by injecting the cycloserine 1 h before functional hepatectomy, and again this resulted in a significant stimulation of glutamine accumulation (Fig. 3). Only in the functional-nephrectomy studies was the increase in glutamine accumulation sufficient to account for the amino groups ordinarily transferred to alanine, although other amino acids may also have been involved. Further, amino-oxyacetate, which markedly inhibited alanine accumulation, did not increase that of glutamine; nor did methionine sulphoximine, which severely decreased glutamine accumulation, significantly stimulate the accumulation of alanine. These results suggest that when alanine formation is inhibited *in vivo*, glutamine may assume part, but not all, of its amino-group-transporting role, subject perhaps to renal regulation. However, the reverse is probably not the case.

The source and the fate of alanine have been shown to be related to glycolysis and gluconeogenesis respectively (Felig, 1973). However, neither the source of carbon skeletons for glutamine synthesis in the extrasplanchnic tissues, nor their ultimate fate, has been conclusively demonstrated. Iqbal & Ottoway (1970) found that the concentrations of glutamate and ATP found *in vivo* for glutamine synthesis were effectively saturating, and concluded that the amount of free NH_3 present in muscle must control the rate of glutamine synthesis. In addition, Ruderman & Berger (1974) and Hills *et al.* (1972) found that perfusion of the rat hindquarter or dog

hind leg with NH_4Cl resulted in increased net glutamine release, with no change in alanine release. These authors concluded that the carbon needed for glutamine synthesis was derived from glutamate via the catabolism of other amino acids present in muscle, and that the free NH_3 concentration was the primary determinant of the rate of glutamine formation. The present results indicate that when alanine formation was inhibited by cycloserine or dichloroacetate, net glutamine release was increased, suggesting that increased tissue glutamate concentrations may be responsible for an increase in glutamine release in some situations. However, since net alanine release was not increased after methionine sulphoximine treatment, the rate of alanine formation appears to be primarily dependent on pyruvate availability and not on that of amino groups from glutamate.

We have recently shown that the accumulation of both glutamine and alanine after functional hepatectomy in starved rats was decreased by the administration of insulin or an antilipolytic agent or by refeeding (Blackshear *et al.*, 1974*a*). The mechanism of this inhibition of glutamine release may thus be related to inhibition of intracellular proteolysis by insulin (Morgan *et al.*, 1972), which would decrease the amount of glutamate formed from the metabolism of other amino acids and thus inhibit glutamine formation and release.

(c) Effects of dichloroacetate on branched-chain amino acid metabolism

Because of the marked effects of dichloroacetate on alanine and glutamine metabolism, its influence on the blood concentrations of other amino acids was investigated. Dichloroacetate infusion markedly increased the blood concentrations of all three branched-chain amino acids (Fig. 1), when compared with NaCl-infused controls; this response could result from decreased uptake of these amino acids by one or more tissues. After functional hepatectomy there was a marked increase in the concentrations of these amino acids in the blood of the control animals, contrary to the prevailing opinion that branched-chain amino acids are not taken up by the liver to any significant extent (Mortimore & Mondon, 1970). The present results are supported by those of Aikawa *et al.* (1973), who found that valine, leucine and isoleucine accumulated in the plasma after evisceration and nephrectomy in 24h-starved rats. In addition, the liver has the necessary enzymes to transaminate the branched-chain amino acids and to oxidize the resulting branched-chain α-oxo acids (Dawson & Hird, 1967; Connelly *et al.*, 1968). However, a variety of other tissues can oxidize leucine (Dawson & Hird, 1967) and the possibility remains that the gut is responsible for removing the branched-chain amino acids from the circulation in this situation. Dichloroacetate treatment resulted in increased concentrations of these amino acids before functional hepatectomy, but decreased rates of accumulation afterwards. This pattern may reflect decreased uptake of these amino acids by some tissues, with the resulting high blood concentrations further inhibiting continued release. It is also possible that discontinuation of the dichloroacetate infusion after functional hepatectomy was responsible for the decreased rates of accumulation, although effects on several other metabolites were still obvious.

The enzyme complex responsible for the oxidative carboxylation of the branched-chain α-oxo acids is closely related to the pyruvate dehydrogenase system (Dancis & Levitz, 1972). Activation of the pyruvate dehydrogenase complex *in vivo* with dichloroacetate (Whitehouse *et al.*, 1974) may account for the apparent inhibition of uptake and oxidation of the branched-chain amino acids. As has been postulated for ketone-body oxidation by McAllister *et al.* (1973), this effect of dichloroacetate may be due to competition for CoA between activated pyruvate dehydrogenase and the oxidative enzymes for the branched-chain α-oxo acids, which also require CoA (Dancis & Levitz, 1972).

In summary, the results of these studies indicate that about 80% of the alanine and glutamine released by the extrasplanchnic tissues is derived from the alanine aminotransferase and glutamine synthetase pathways respectively, rather than from tissue proteolysis. When alanine formation was decreased, by either activation of pyruvate dehydrogenase by dichloroacetate or inhibition of alanine aminotransferase by L-cycloserine, glutamine formation was enhanced, probably by the direction of glutamate through the glutamine synthetase pathway. There was some evidence that the kidney regulated the amount of glutamine accumulation in this situation. However, when glutamine synthetase was inhibited by methionine sulphoximine, alanine accumulation did not increase, suggesting that pyruvate availability is the main determinant for the rate of alanine formation in the peripheral tissues. Finally, dichloroacetate increased the blood concentrations of the branched-chain amino acids in the starved rat, suggesting an interaction between the oxidative enzymes of the branched-chain α-oxo acids and pyruvate dehydrogenase, possibly through competition for CoA.

We thank Mrs. Sandra Slade and Mrs. Patricia Badham for excellent technical assistance, Dr. D. H. Williamson for constant advice and encouragement, Professor H. A. Krebs and Professor P. J. Randle for critical comments and helpful discussion, and Professor P. B. Beeson for his constant encouragement. We gratefully acknowledge financial aid from the Rhodes Trust, the Wellcome Trust and the Medical Research Council.

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