Nutritional aspects of amino acid metabolism

2.* The effects of starvation on hepatic portal–venous differences in plasma amino acid concentration and on liver amino acid concentrations in the rat

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(*Received* 6 *October* 1970 – *Accepted* 3 *September* 1971)

1. Concentrations of the amino acids in the plasma of blood from the portal vein and hepatic vein and in the liver of fed rats and rats starved for 1 d or 3 d were measured. The 1 d values were compared with the equilibrium concentrations of the amino acids found in the perfusion medium during perfusion of livers from rats starved for 1 d.

2. The measurements of portal–venous differences in amino acid concentrations confirmed the idea that postprandially and during starvation most of the amino acids flow from extrahepatic tissues to the liver but also showed that during starvation tryptophan, cystine, ornithine, valine, leucine and isoleucine flow in the opposite direction, from liver to extrahepatic tissues.

3. The blood levels of the non-essential amino acids fell markedly during starvation while those of the essential ones tended to be maintained. This contrasts with the pattern of changes known to take place in rats and man given low-protein diets. In the liver, changes in amino acid concentrations were generally related to those in the blood but not strictly parallel. The relative changes in amino acid concentrations in blood and liver indicate that as starvation progresses the concentrative ability of the liver is enhanced for most of the amino acids which are taken up and that the increased output of those which are released is also due to changed membrane transport.

4. The changes in plasma amino acid concentrations in the blood passing through livers of rats starved for 1 d were, except for tryptophan and perhaps cystine, consistent with the extracellular changes found during perfusion of livers from rats starved for 1 d, indicating that the perfused liver influences concentrations of extracellular amino acids substantially as it does in vivo.

5. The results suggest a mechanism whereby the liver may control the maintenance of the essential amino acids during starvation.

The purpose of the work described in this paper was threefold: firstly, to find whether the liver in a new perfusion system (Bloxam, 1971*a*) behaves in the same way towards amino acids as it does in vivo; secondly, to provide essential preliminary information for a study of nutritional aspects of amino acid metabolism; and thirdly, to give information about the control of amino acid availability to tissues in the rat. The latter topic is of nutritional importance, particularly in view of recent suggestions that for liver, and perhaps also for other tissues, concentrations of particular amino acids individually (Munro, 1968, 1969; Wurtman, Shoemaker & Larin, 1968) or in groups (Kirsch, Saunders, Frith, Wicht & Brock, 1969; Jefferson & Korner, 1969) positively control protein synthesis. The immediate availability of the amino acids, particularly of the essential ones, to most tissues for their metabolic requirements,

* Paper no. 1: *Br. J. Nutr.* (1971), **26**, 393.

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including protein synthesis, depends on their plasma concentrations, which are markedly influenced by the liver (Miller, 1962; McMenamy, Shoemaker, Richmond & Elwyn, 1962; Elwyn, Parikh & Shoemaker, 1968). The results given here confirm this view and suggest a mechanism by which the essential amino acids may be maintained during starvation.

Preliminary accounts of some of the results given in this paper have been presented elsewhere (Bloxam 1971*b*, 1972*b*).

METHODS

Animals. The animals used in this work were male Wistar rats of the University College London substrain or male Wistars from Oxford Laboratory Animal Colonies (Blackthorn, Bicester, Oxfordshire), fed on diet 86 (E. Dixon and Sons, Ware, Hertfordshire) comprising (parts by weight): wheat 50; barley 25; pure white fish meal (66% protein) 7; meat meal (60% protein) 6; dried yeast, unextracted 5; grass meal (18% protein) 5; salt 1; molasses 5. Groups of rats were fed, starved for 1 d (18–22 h) or for 3 d (66–70 h); water was provided *ad lib.* The fed animals weighed 250–290 g and the starved animals were of the same weight range when food was withdrawn.

Blood sampling method. Blood from the portal vein and hepatic vein was taken under halothane (Fluothane; ICI) anaesthesia, which was induced and maintained as described previously (Bloxam, 1971*a*). Halothane has been found to be less likely to affect the results than the anaesthetics usually employed for this kind of work (Bloxam, 1967*a*, *b*, 1971*a*). The abdominal cavity was opened and, to reduce contamination of the hepatic venous blood by that from the inferior vena cava to a minimum, the inferior vena cava was clamped by haemostat between the right renal vein and the hepatic vein immediately before withdrawal of the hepatic vein sample. The portal vein sample was then taken as quickly as possible after clamping this vessel close to the liver to avoid withdrawing blood from the liver. A sample of 1·5–2 ml of blood was taken from each vessel into a (separate) heparinized syringe and centrifuged at 4° to remove cells. The plasma samples were stored at −30° until required for assay.

The blood samples were all taken between 10.00 and 14.00 hours to avoid as much as possible errors due to diurnal variations of amino acid concentration.

Liver sampling method. The rat was anaesthetized as described above, the abdominal cavity was opened and the left lobe was frozen *in situ* with Wollenberger clamps (Wollenberger, Ristau & Schoffa, 1960) cooled in liquid nitrogen. The samples were taken between 10.00 and 14.00 hours.

Liver perfusion method. This was as described previously (Bloxam, 1971*a*).

Measurement of amino acids in perfusate, blood and liver. Amino acid concentrations were measured with a Technicon automatic amino acid analyser 20 h run system (Technicon Instruments Co., Amino Acid Analyzer Handbook, 2nd issue). Perfusate samples were analysed as described previously (Bloxam, 1971*a*). The blood plasma samples, after addition of norleucine as internal standard, were deproteinized by adding 3 vol of 3% sulphosalicylic acid, and the supernatant fractions were applied to the column immediately. Each frozen liver sample, immediately after removal from

the rat, was dropped into 5 vol of ice-cold 3% sulphosalicylic acid containing the norleucine standard and homogenized in a plastic homogenizer with a glass pestle cooled in solid CO_2. After centrifugation the supernatant fractions were stored for up to 2 weeks at $-30°$ until required for assay as before.

Glutamine cannot adequately be measured from the automated amino acid analysis because it breaks down to a considerable and variable extent during both the sample loading procedure and the chromatographic elution. This decomposition leads to the formation of glutamate – via 5-oxo-2-pyrrolidonecarboxylate which is not detected – and therefore breakdown of glutamine during loading also leads to high values for glutamate, though breakdown during elution does not, once these two substances are separated. For example, with the usual procedure for physiological samples, and precaution against glutamine breakdown before loading, the analyser gives values for glutamate in normal blood plasma about 60% too high. To overcome this problem, the plasma samples were deproteinized and centrifuged at 0–4° and the supernatant fraction was loaded on to the column at room temperature instead of at the normal temperature for physiological samples (45°). After elution was started the temperature of the column was gradually increased from room temperature to 45° over the first 10 min of the run.

During manipulation of the samples, cysteine is converted into cystine. Therefore, by 'cystine' is meant 'cysteine plus cystine' of the perfusate, plasma or tissue.

Measurement of DNA in liver. The DNA was extracted from the tissue with 0·2 M-perchloric acid as described by Munro & Fleck (1966) and treated with 0·5 M-perchloric acid at 70° before assay by the diphenylamine method as described by Burton (1956).

Results are expressed as the mean values with their standard errors.

RESULTS

Effects of starvation on plasma amino acid concentrations. Fig. 1 shows that the amino acids may be divided into four groups according to the manner in which their plasma concentrations behaved during starvation; i.e. those which (1) fell significantly as starvation progressed, (2) were raised after 1 day's starvation before falling, (3) tended not to fall although taken up by the liver and (4) were maintained by output from the liver. For a statistical comparison of the blood levels in the different conditions, see Table 1.

Those of the first group, whose concentrations fell markedly during starvation, are the non-essential, glucogenic (Meister, 1965) amino acids – aspartate, arginine, proline, serine, glycine and alanine. The amino acids in group 2 were lysine and threonine, which rose after 1 day's starvation (the rise of threonine was not statistically significant, see Table 1) before falling. Of the amino acids whose plasma concentrations did not fall appreciably during starvation (group 3), the ketogenic amino acids tyrosine and phenylalanine and the glucogenic glutamate were particularly well maintained although continually taken up by the liver. The concentration of methionine, also, did not fall much and those of histidine and citrulline, after a substantial fall during the 1st day of starvation, did not change appreciably during the next 2 d. The only non-essential

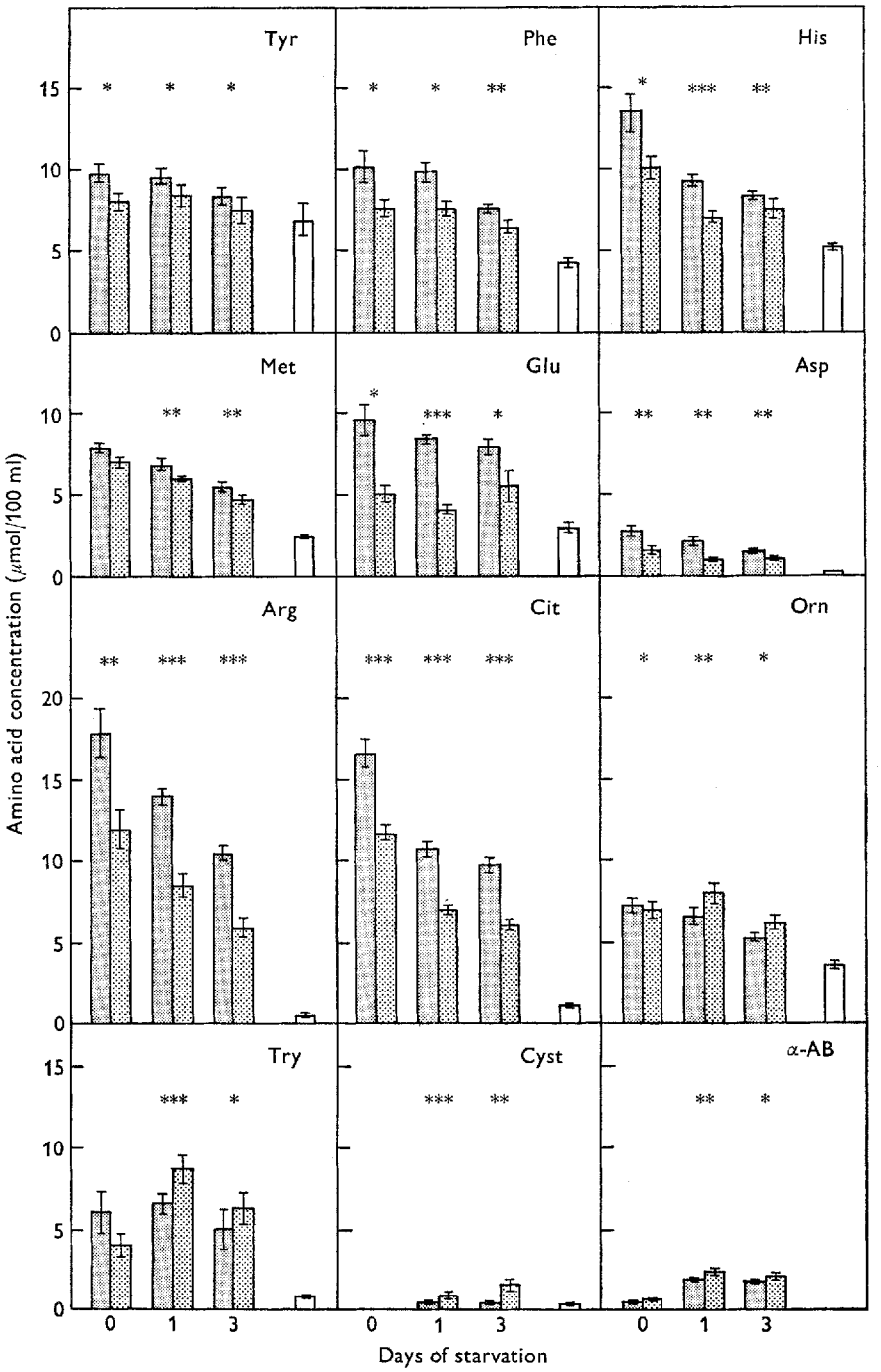

Fig. 1. Concentrations of the amino acids in the plasma of portal (shaded) and hepatic (stippled) venous blood from fed rats and from rats starved for 1 d and 3 d compared with perfusate equilibrium concentrations (plain) from perfusion of livers from rats starved for 1 d. The values are for six, eight, seven animals and five perfusions respectively and the bars indicate SEM. (The values for glutamate and tryptophan in fed rats are from three determinations only because of erroneous values and inadequate chromatographic separation of these respectively. The conditions of sample preparation and loading, and the composition of the eluting buffers were altered after three of the six samples from fed rats had been measured to obtain correct values for glutamate (see p. 235) and to separate tryptophan.) The *P* values give the statistical significance of uptake or output by the method of paired comparisons: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. A statistical comparison between the different groups of animals is given in Table 1.

https://doi.org/10.1079/BJN19720090 Published online by Cambridge University Press

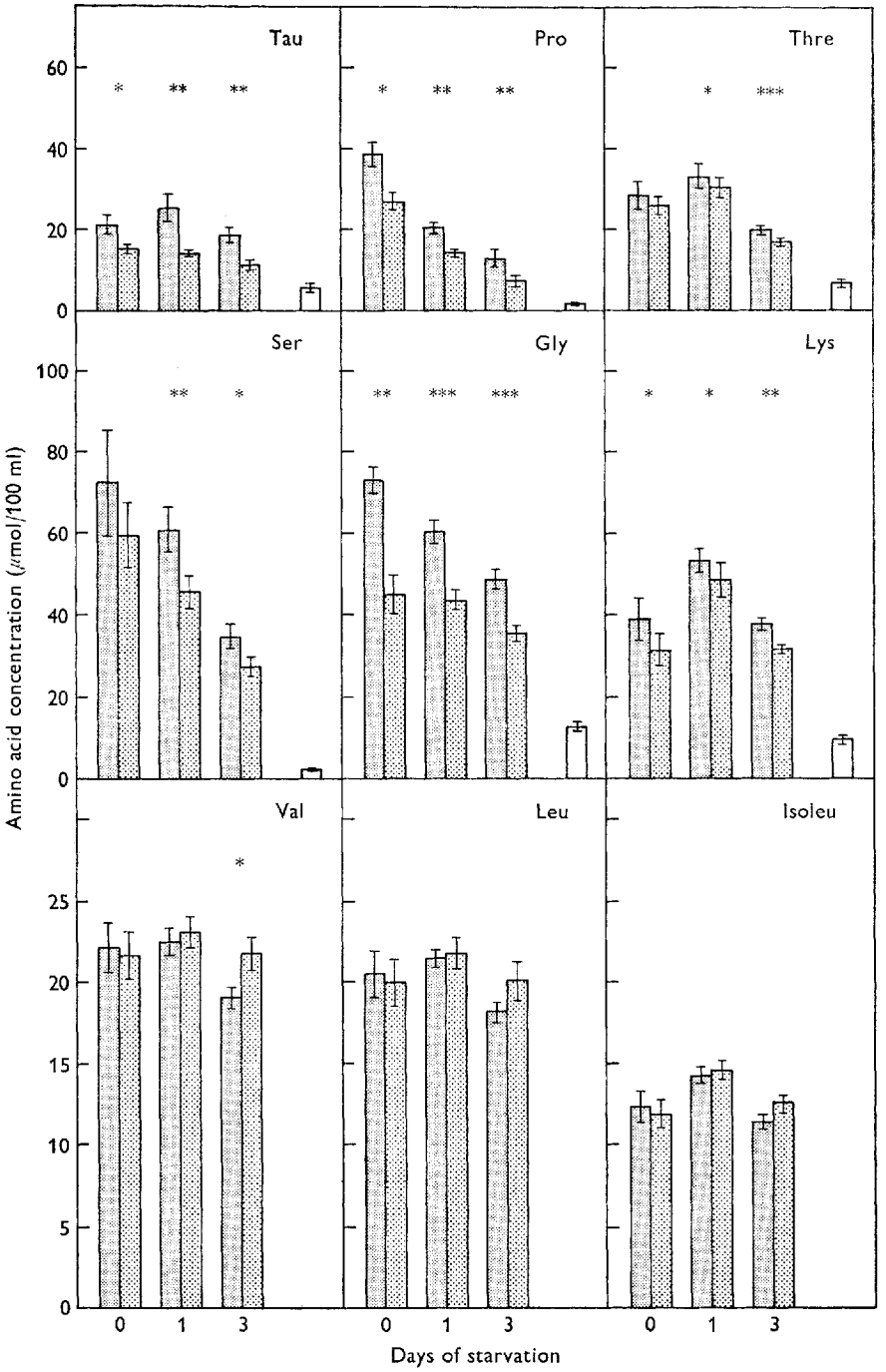

Fig. 1 (continued). For legend see opposite.

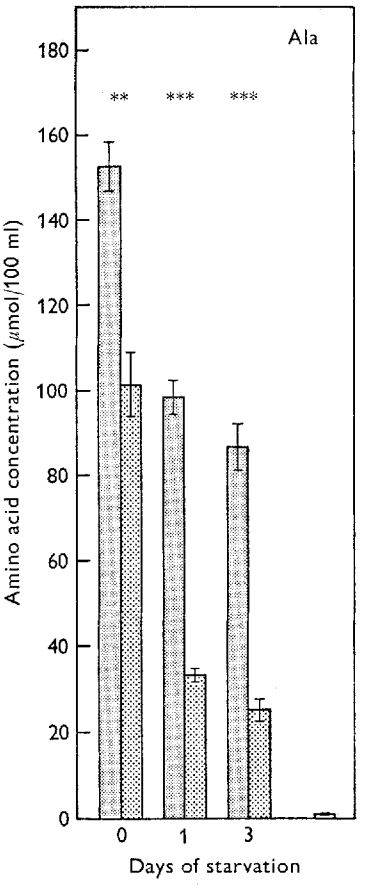

Fig. 1 (continued). For legend see page 236.

amino acids whose plasma concentrations on starvation did not fall much were glutamate and taurine.

The concentrations in the blood of all the amino acids which are given out by the liver – ornithine, tryptophan, cystine, α-aminobutyrate, valine, leucine and isoleucine – were maintained during starvation or actually increased (see Fig. 1 and Table 1). The concentrations of ornithine did not change, while those of α-aminobutyrate rose significantly during the 1st day of starvation ($P < 0{\cdot}001$ for both portal and venous blood plasma) but were thereafter not changed at 3 d. The fed levels of cystine were too low to measure (< 0·2 μmol/100 ml plasma) in both portal and venous blood plasma but they were markedly increased after 1 d of starvation. Between 1 and 3 d of starvation the portal blood levels were not changed but the portal–venous difference appears to have become greater as starvation progressed, though, perhaps because of analytical errors near the limit of measurement, the venous level at 3 d was not significantly greater than at 1 d ($P = 0{\cdot}2$). This pattern of changes – a fall in the levels of the non-essential amino acids and a conservation of the essential amino acids – is in sharp contrast to the pattern of changes from feeding low-protein diets

in the rat and in man (Swendseid, Villalobos & Friedrich, 1963; Swendseid, Griffith & Tuttle, 1963; Holt, Snyderman, Norton, Roitman & Finch, 1963), where the essential amino acids are lowered in concentration and the non-essential ones are maintained.

Amino acid movements during starvation. It can be seen from the portal–venous differences shown in Fig. 1 that most of the amino acids were taken up by the liver in the fed rats and in both degrees of starvation. Nearly all the values for these uptakes were statistically significant (see Fig. 1). Thus, in the postabsorptive period these amino

Table 1. *Statistical comparison of results in Fig. 1*

	t test probability value (*P*)					
	Fed *v.* starved for 1 d		Starved for 1 d *v.* starved for 3 d		Fed *v.* starved for 3 d	
Amino acid	HP	HV	HP	HV	HP	HV
Taurine	NS	NS	NS	NS	NS	< 0·10
Aspartate	NS	< 0·05	NS	NS	< 0·01	< 0·05
Threonine	NS	NS	< 0·01	< 0·001	0·05	< 0·01
Serine	NS	NS	< 0·01	< 0·01	< 0·01	< 0·01
Glutamate	< 0·05	NS	NS	NS	NS	NS
Proline	< 0·001	< 0·001	< 0·05	< 0·01	< 0·001	< 0·001
Citrulline	< 0·001	< 0·001	NS	NS	< 0·001	< 0·001
Glycine	< 0·05	NS	< 0·02	< 0·05	< 0·001	NS
Alanine	< 0·001	< 0·001	NS	0·02	< 0·001	< 0·001
α-Aminobutyrate	< 0·001	< 0·001	NS	NS	< 0·001	< 0·01
Cystine	—	—	NS	NS	—	—
Methionine	< 0·10	0·05	< 0·05	0·01	< 0·001	< 0·01
Tyrosine	NS	NS	NS	NS	NS	NS
Phenylalanine	NS	NS	< 0·05	0·10	< 0·10	< 0·10
Ornithine	NS	NS	< 0·02	0·05	< 0·01	NS
Lysine	< 0·05	0·02	< 0·01	0·01	NS	NS
Histidine	< 0·01	< 0·01	< 0·10	NS	< 0·01	< 0·05
Tryptophan	NS	0·02	NS	NS	NS	NS
Arginine	< 0·05	< 0·05	< 0·001	0·05	< 0·01	< 0·01
Valine	NS	NS	< 0·02	NS	NS	NS
Leucine	NS	NS	< 0·01	NS	NS	NS
Isoleucine	NS	< 0·05	< 0·01	NS	NS	NS

HP, comparison of amino acid concentrations in hepatic portal blood.
HV, comparison of amino acid concentrations in hepatic venous blood.
NS, $P > 0{\cdot}10$.

acids were continually flowing from extrahepatic tissues to the liver in the normal rat. On the other hand, ornithine, tryptophan, cystine, α-aminobutyrate, valine, leucine and isoleucine were each, in some of the conditions, given out by the liver. None of these appeared to be released in the fed rat, in fact, ornithine was taken up to a very small but statistically significant extent, and the uptake of tryptophan was undoubtedly real, although the portal–venous difference was not statistically significant ($t = 1{\cdot}99$; $0{\cdot}1 < P < 0{\cdot}2$) because of the small number of determinations in this group (see legend to Fig. 1).

The hepatic portal–venous differences of cystine, valine, leucine and isoleucine became more pronounced as starvation progressed, in the case of the branched-chain

group becoming marginally significant in the animals starved for 3 d (valine, $P = 0{\cdot}02$; leucine, $0{\cdot}10 < P < 0{\cdot}20$; isoleucine, $P < 0{\cdot}10$). For reasons discussed later, the results for these branched-chain acids probably represent real outputs by the liver in the rats starved for 1 d and 3 d. The significance of these results, and the question whether the increased portal–venous differences indicate greater rates of output by the liver in the experiments are discussed later.

Comparison of extracellular amino acid changes in vivo and during liver perfusions. It has been shown in the previous paper (Bloxam, 1971*a*) that the liver of the rat starved for 1 d, when perfused, exerts an apparently homoeostatic type of control over the amino acid concentrations in the perfusion medium except for valine, leucine and isoleucine. In Fig. 1 the changes in amino acid concentrations in portal and hepatic venous blood plasma are compared with the perfusion medium equilibrium values found in five blank 1·5 or 2 h perfusions of livers from rats starved for 1 d (see also Bloxam, 1971*a*, Figs. 5 and 6). No perfusate values for the branched-chain acids are given because, being continually given out by the liver, they do not reach equilibrium values, or for α-aminobutyrate, which was not chromatographically separated during analysis of the perfusate samples.

Comparing (Fig. 1) the changes in the rats starved for 1 d with the perfusate equilibrium concentrations, it is seen that for nearly every amino acid the plasma concentration was adjusted towards the perfusion equilibrium level for that acid. In most instances the equilibrium level was not actually reached and this is to be expected in view of the short time during which the blood is in contact with the liver in the intact animal and because of the influx of amino acids from the periphery. In addition, re-equilibration of amino acids between the red cells and plasma and leakage of amino acids from red cells before centrifugation may have led to somewhat elevated plasma values. However, the behaviour of tryptophan, and also possibly of cystine and ornithine, does not appear to be consistent with the perfusion results. Tryptophan was given out ($P < 0{\cdot}001$) by the liver of the rat starved for 1 d although its concentration in the portal blood plasma was significantly higher than in the perfusion equilibrium ($P < 0{\cdot}001$), so that uptake might have been expected. Cystine was given out ($P < 0{\cdot}001$) although the mean portal plasma level was no different from the equilibrium level ($P > 0{\cdot}5$). Again, from the value of the perfusate concentration shown in Fig. 1, ornithine might have been expected to be taken up, but in this case the value, like that of citrulline, is almost certainly an underestimate since the final equilibrium levels of these had not been reached at the end of the 1·5 or 2 h of perfusion (see, for instance, Bloxam, 1971*a*, Fig. 5). The apparently anomolous behaviour of tryptophan and cystine is discussed later. (See also Bloxam, 1972*a*.)

A notable feature of the results from the liver perfusions, the continuous output of the branched-chain amino acids, is in agreement with the results found here in vivo, although in the rats starved for 1 d the values for the hepatic portal–venous differences were not statistically significant. Judged by the rates of output of these amino acids from the perfused liver, the portal–venous differences would be expected to be barely detectable even in ideal sampling conditions. In fact, contamination of

Table 2. *Concentrations (μmol/100 g wet weight) of amino acids in livers of fed rats and of rats starved for 1 d and 3 d*

(Mean values with their standard errors)

Amino acid	Fed	Starved for 1 d	Starved for 3 d
Taurine	292·8 ± 36·2	509·2 ± 39·2	660·9 ± 130·5
Aspartate	158·5 ± 11·1	120·0 ± 5·8	165·8 ± 13·6
Threonine	36·2 ± 3·0	44·1 ± 2·7	56·8 ± 9·3
Serine	106·6 ± 6·7	86·3 ± 9·3	96·3 ± 11·7
Glutamate	149·6 ± 9·2	134·3 ± 8·0	198·6 ± 24·5
Proline	27·0 ± 1·6	18·0 ± 2·3	18·4 ± 1·3
Citrulline	9·9 ± 0·8	7·0 ± 0·4	7·7 ± 0·8
Glycine	237·1 ± 12·4	249·0 ± 18·1	215·9 ± 19·2
Alanine	191·1 ± 16·0	42·7 ± 7·8	47·8 ± 6·3
α-Aminobutyrate	1·2 ± 0·3	2·5 ± 0·3	5·6 ± 0·9
Cystine	< 0·8	< 0·7	< 0·7
Methionine	12·7 ± 0·6	13·1 ± 1·0	12·0 ± 0·6
Tyrosine	7·3 ± 0·8	8·9 ± 0·5	11·1 ± 0·6
Phenylalanine	6·8 ± 0·5	5·4 ± 0·5	8·8 ± 1·1
Ornithine	21·0 ± 1·7	15·3 ± 1·3	21·4 ± 1·2
Lysine	44·8 ± 3·2	58·8 ± 3·8	58·4 ± 3·0
Histidine	55·9 ± 2·7	47·2 ± 2·0	44·3 ± 2·7
Tryptophan	1·9 ± 0·1	2·7 ± 0·3	2·2 ± 0·3
Arginine	9·7 ± 0·8	5·8 ± 0·3	6·9 ± 0·4
Valine	16·3 ± 0·8	15·9 ± 1·2	17·0 ± 0·9
Leucine	15·6 ± 1·0	14·2 ± 1·1	15·0 ± 0·7
Isoleucine	8·4 ± 0·4	9·0 ± 0·6	9·3 ± 0·4

The values for livers of fed rats and rats starved for 1 d and 3 d were for eight, nine and seven animals, respectively.

Table 3. *Statistical comparison of the amino acid concentrations in Table 2*

Amino acid	*t* test probability value (*P*): Fed *v.* starved for 1 d	Starved for 1 d *v.* starved for 3 d	Fed *v.* starved for 3 d
Taurine	< 0·01	NS	< 0·02
Aspartate	< 0·01	< 0·01	NS
Threonine	< 0·10	NS	< 0·10
Serine	NS	NS	NS
Glutamate	NS	< 0·05	< 0·10
Proline	< 0·01	NS	< 0·01
Citrulline	< 0·01	NS	< 0·10
Glycine	NS	NS	NS
Alanine	< 0·001	NS	< 0·001
α-Aminobutyrate	< 0·02	< 0·01	< 0·01
Cystine	—	—	—
Methionine	NS	NS	NS
Tyrosine	NS	< 0·02	< 0·01
Phenylalanine	NS	< 0·02	NS
Ornithine	< 0·05	< 0·01	NS
Lysine	< 0·02	NS	< 0·02
Histidine	< 0·05	NS	< 0·02
Tryptophan	< 0·02	NS	NS
Arginine	< 0·001	< 0·10	< 0·02
Valine	NS	NS	NS
Leucine	NS	NS	NS
Isoleucine	NS	NS	NS

NS, $P > 0·10$.

hepatic venous blood by blood from the inferior vena cava and the various phrenic veins probably tends further to mask the differences.

Changes in liver amino acid concentrations during starvation. Table 2 shows amino acid concentrations expressed per 100 g wet weight in livers of fed rats, and of rats starved for 1 d and 3 d. This probably gives a reasonable comparative indication of average intracellular concentration for most of the amino acids. Their amounts relative to DNA may be calculated from the values in Table 4, as an estimate of their pool sizes in the different conditions. Table 4 shows how wet weight changes relative to DNA during starvation.

Table 4. *Effect of starvation on liver weight relative to DNA in rats*

(Mean values with their standard errors)

	Fed	Starved for 1 d	Starved for 3 d
Wet wt/DNA (g/100 mg)	47·8 ± 0·95***	31·2 ± 0·99	27·0 ± 0·28***
No. of rats	12	8	10

*** Significantly different from the value for the liver of the rat starved for 1 d ($P < 0.001$).

Generally, the changes in amino acid concentrations during 3 d of starvation were not great. The most pronounced decreases in concentration included most of the glucogenic amino acids, notably alanine. Interestingly, there were increases in the liver concentrations of taurine, threonine, tyrosine, lysine and tryptophan after 1 d of starvation, of aspartate and glutamate after 3 d of starvation, and of α-aminobutyrate during both periods of starvation. The changes were on the whole similar to but less pronounced than those found by Wu (1954) in livers of rats after 9 d of starvation, except for the branched-chain amino acids which Wu found to be raised after 9 d. They are also similar to the changes found for aspartate, glutamate, alanine and serine plus threonine in rats starved for 48 h by Williamson, Lopes-Vieira & Walker (1967). Comparison of Fig. 1 with Table 2 reveals that the pattern of amino acid changes in the liver during starvation was related to, but not strictly parallel with, those in the blood. The concentrations in plasma fell relative to those in the liver as starvation progressed (see p. 241).

The changes calculated relative to DNA indicate that, in contrast to their concentrations, the pool sizes of most of the amino acids fell substantially during the 1st day of starvation, with little further change 2 d later.

DISCUSSION

Before discussing the results, consideration must be given to some of the limitations of the blood sampling method used in this work. A small degree of dilution of the hepatic venous blood sample by inferior vena cava blood was inevitable in spite of the precaution of stopping the flow in the vena cava immediately before taking the sample (see p. 234). The blood from the phrenic veins which drains directly into the hepatic vein (Beck & Baxter, 1960) will also have contaminated the sample, though again

not greatly. This, together with the likelihood that the act of withdrawing the blood may to some extent have altered the flow-rate of the blood through the liver, and the fact that the hepatic artery was not sampled, limits the degree to which quantitative conclusions can be drawn from the results. The values of the portal–venous differences depend on the rates of flow of the blood through the livers. Thus, whether the relative size of the portal–venous differences, in addition to giving the direction of movement of the amino acids into or out of the liver, give an indication of their relative rates of flow in the different groups of animals, depends on whether the rate of flow of blood through the liver is appreciably altered by starvation. What little evidence there is suggests that the rate is not changed much. It is well known that total hepatic blood flow increases in rate during digestion of food in the rat (Reininger & Sapirstein, 1957), dog (Shoemaker, Yanof, Turk & Wison, 1963; Elwyn *et al.* 1968) and man (Brandt, Castleman, Ruskin, Greenwald & Kelly, 1955), but it falls back to the original level in 1·5–2 h in man (Brandt *et al.* 1955) and also in the dog (Shoemaker *et al.* 1963; Elwyn *et al.* 1968), remaining constant throughout 24 h except during digestion of meals (Elwyn *et al.* 1968). In the present work food was withdrawn from the 'fed' animals at least 1 h before taking samples. The figures of Barcroft & Shore (1913) show no significant difference in blood flow-rate in dogs, fasted for 18 h and 36 h, through the portal vein ($P > 0{\cdot}5$), hepatic artery ($P > 0{\cdot}1$) or hepatic vein ($P > 0{\cdot}1$, the present author's statistics). Also, Reininger & Sapirstein (1957) state that their values for starved controls for blood flow-rates were taken from rats fasted for between 24 and 72 h, suggesting that they found no substantial difference during this period of starvation. Therefore, marked changes in portal–venous difference in the present work are cautiously taken to suggest altered rates of amino acid movement.

There is surprisingly little information in the literature about the effects of total starvation on blood amino acid concentrations. The results of Henderson, Schurr & Elvehjem (1949), using a paper chromatographic technique for estimation of the amino acids, were inconclusive, but Wu (1954), using microbiological methods, found in rats starved for 9 d falls in the concentrations of alanine, glycine, serine and threonine in plasma of blood taken from the heart, as was observed in the present work with rats starved for up to 3 d, but also falls of tyrosine and glutamate, and increased levels of valine, leucine and isoleucine – a pattern of changes more similar to those found for diabetic rats starved for 1 d (Bloxam, 1972*a*).

Amino acid movements during starvation. The results from Fig. 1 show that in the postabsorptive period and as starvation progresses most of the amino acids continuously flow from extrahepatic tissues to the liver, confirming suggestions of Munro (1964), Christensen (1964), McMenamy *et al.* (1962) and Bloxam (1971*a*, *b*), but that during starvation ornithine, α-aminobutyrate, tryptophan, cystine, valine, leucine and isoleucine flow in the opposite direction, from the liver to extrahepatic tissues. For cystine and the branched-chain acids this outflow seems to become greater as starvation progresses (bearing in mind the proviso given above), apparently providing for increased (net) utilization of these amino acids by extrahepatic tissues during starvation. The uptake of tryptophan in the fed rat reverts to an output in the starved animal and this may perhaps be related to the importance of this acid in brain metabolism (see,

for instance, Costa, 1960; Page, 1958; Rodnight, 1961) and the possible consequent special need for its blood concentration to be maintained. The net output of ornithine, α-aminobutyrate, tryptophan, cystine, valine, leucine and isoleucine is presumably a result of the flow of these particular amino acids into the intracellular pool from synthesis or protein breakdown being more rapid than their removal from the pool by catabolism, conversion into glucose, or other paths, subject, of course, to the limitations of transport processes. For instance, the release of valine, leucine and isoleucine from the livers of rats starved for 1 d and 3 d is probably derived from the net protein breakdown which is taking place in this period (Munro, 1964; Addis, Poo & Lew, 1936), since it is known that they are not very rapidly oxidized in liver (Miller, 1962; see also the discussion in Bloxam, 1971*a*). Presumably there is net uptake of these branched-chain amino acids on feeding when there is overall protein synthesis taking place (Munro, 1964; Addis *et al.* 1936; Potter, Baril, Watanabe & Whittle, 1968). It is true that there was no significant uptake of these amino acids in the fed rats, but the blood samples were taken at least 1 h after withdrawal of food. During this interval, the protein synthesis in the liver which immediately follows feeding would be changing over to breakdown.

The reduced portal–venous differences in the concentrations of most of the amino acids which are taken up by the liver in the starved compared with the fed rat suggest that the uptake of these amino acids is reduced in starvation (assuming again that the hepatic blood flow-rate is not altered much). This, and the overall picture of amino acid flow, are in general agreement with the findings of Cahill and his co-workers (Felig, Marliss, Pozefsky & Cahill, 1970) in obese humans starved for 5–6 weeks. Notable exceptions in the present work are alanine and taurine, whose uptake, if anything, seems to be increased on starvation for 1 d. The apparently increased uptake of alanine is interesting in view of its special importance in hepatic gluconeogenesis (Felig, Pozefsky, Marliss & Cahill, 1970; Felig, Marliss *et al.* 1970), and is consistent with the increased gluconeogenic capacity of the liver during this period of starvation in the rat (Exton, Jefferson, Butcher & Park, 1966; Wimhurst & Manchester, 1970).

Comparison of the concentrations of the amino acids in the liver (Table 2) during starvation with those in the hepatic venous blood plasma (Fig. 1) shows that the ratio of liver concentration to venous plasma concentration increased as starvation progressed. This suggests that the concentrative power of the liver increases as starvation progresses in spite of the indication of reduced degree of uptake of many of the amino acids, mentioned above, which is probably a result of the reduced blood concentrations and perhaps also of reduced liver pool sizes. This is consistent with the observations that the glucocorticoids whose concentrations in blood are raised in starvation (Boulouard, 1963; Slater, 1962) cause an increased uptake of amino acids by the liver (Christensen, 1964; Noall, Riggs, Walker & Christensen, 1957). It is also possible that glucagon, acting relatively unopposed by insulin during starvation (Jefferson, Exton, Butcher, Sutherland & Park, 1968; Exton *et al.* 1966) exerts a similar effect on the liver (Mallette, Exton & Park, 1969).

Comparison of extracellular amino acid changes in vivo and during liver perfusions. The measurements of portal–venous differences in rats starved for 1 d were, except for

tryptophan and probably cystine, consistent with the changes in perfusate amino acid concentrations during the perfusion of livers from such rats (Bloxam, 1971*a*), suggesting that they were behaving in substantially the same manner towards amino acids in vivo as in vitro. The in vivo results are also generally consistent with the changes in plasma amino acid concentrations when livers of rats starved for 1 d were perfused with blood (Fisher & Kerly, 1964; Spruyt, 1962), but perhaps rather less so with the results of Schimassek & Gerok (1965) and of Mondon & Mortimore (1967) from perfused livers of fed rats. A feature of the results of both these groups of workers is a large output of glutamate by the liver, leading to perfusate concentrations well above normal concentrations in blood plasma. On the other hand, Hems, Stubbs & Krebs (1968), using the perfused livers of rats starved for 48 h and liver slices, suggest that for glutamate and perhaps also aspartate there is a permeability barrier to and from liver cells. The results shown in Fig. 1, in agreement with the previous perfusion results (Fig. 5 of Bloxam, 1971*a*), show that in vivo glutamate and aspartate are not normally given out by the liver, as the results of the former two groups of workers indicate, but neither is there a permeability barrier for these acids, as Hems *et al.* suggest, since they are taken up relatively rapidly by the liver in both the fed and the starved rat, when at physiological levels.

The unexpected behaviour of tryptophan and cystine may be due to the absence of hormones in the liver perfusions compared with in vivo, and results obtained in vivo with diabetic rats shed some light on this question (Bloxam, 1972*a*).

Does the liver control the availability of amino acids in vivo? The concentrations of the amino acids in the blood are influenced and perhaps regulated by the dynamic interchange between the blood and all the other tissues. The liver is clearly not unique in this. But the liver is unique in being the tissue in which urea, the major end-product of nitrogen metabolism, is synthesized and as such must in some way discriminate between the amino acids finally broken down so that the organism does not lose undue amounts of essential amino acids or those in short supply. In addition, portal blood carrying amino acids from digestion of food must pass through the liver before entering the general circulation, so that the proportion of the absorbed amino acids reaching the extrahepatic tissues depends on the behaviour of the liver towards them. Thus, teleologically, the liver may be expected to be particularly involved in the control of the availability of amino acids to the rest of the organism.

If a homoeostatic mechanism for the amino acids is operative, it may not be concerned with the rigorous minute-to-minute control of blood amino acid concentrations in the sense perhaps that blood glucose concentrations are controlled, but it may be involved in more long-term control. This is not to say, as is suggested by Mallette *et al.* (1969), that the liver does not therefore exert a decisive influence on blood amino acid concentrations in the short term.

In the longer term, it is seen from Fig. 1 that in vivo the concentrations of the non-essential amino acids in the main fall substantially during starvation while most of the essential ones are maintained, some apparently by reduced uptake and others by actual output by the liver. This discrimination in uptake between the essential and non-essential amino acids may be explained in part as follows. If, as seems likely (Felig,

Marliss *et al.* 1970), the rate of release of amino acids from extrahepatic tissues decreases as starvation progresses, their steady-state concentrations may be expected to tend towards the perfusion equilibrium values. In the fed animals, the portal vein concentrations of the non-essential amino acids are much greater than their perfusion equilibrium concentrations, so there is room for a large fall in the plasma concentrations of these during starvation. On the other hand, the portal vein concentrations of the essential amino acids plus taurine and glutamate in the fed rat are already close to their equilibrium values so that there can be little fall in the plasma concentrations of these. In simple terms, the transport characteristics may be such as to act as a kind of barrier to net entry of the amino acids when their blood concentrations fall to certain values. If, on the other hand, the portal plasma concentration of an amino acid falls below the equilibrium value, perhaps through nutritional insufficiency, the perfusion results suggest that it may be released from the liver. It has yet to be ascertained whether the equilibrium values themselves are altered during starvation.

It is probable, as is indicated by a comparison of the changes in concentrations of the amino acids in the liver and in the blood during starvation, that the rates of metabolic disposal of individual amino acids are also important in the regulation of blood amino acid concentrations and the relations between transport and intracellular metabolism have yet to be investigated.

However, the results given here, together with results from the liver perfusion experiments suggest that the liver has a distinct role in the control of blood amino acid concentrations and therefore of amino acid availability to extrahepatic tissues.

I would like to express my thanks to M. I. Khan, who carried out some of the amino acid analyses.

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Printed in Great Britain