Biochem. J. (1971) **124**, 653–660
Printed in Great Britain

Control of Glutamine Synthesis in Rat Liver

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(*Received* 1 *June* 1971)

1. On perfusion of livers from fed rats with a semi-synthetic medium containing no added amino acids there is a rapid release of glutamine during the first 15min (15.6 ± 0.8 μmol/h per g wet wt.; mean ± S.E.M. of 35 experiments), followed by a low linear rate of production (3.6 ± 0.3 μmol/h per g wet wt.; mean ± S.E.M. of three experiments). The rapid initial release can be accounted for as wash-out of pre-existing intracellular glutamine. 2. Addition of readily permeating substrates, or substrate combinations, giving rise to intracellular glutamate or ammonia, or both, did not appreciably increase the rate of glutamine production over the endogenous rate. The maximum rate obtained was from proline plus alanine; even then the rate represented less than one-fortieth of the capacity of glutamine synthetase measured *in vitro*. 3. Complete inhibition of respiration in the perfusions [no erythrocytes in the medium; 1mM-cyanide; N_2+CO_2 (95:5) in the gas phase] or perfusion with glutamine synthetase inhibitors [L-methionine DL-sulphoximine; DL-(+)-allo-δ-hydroxylysine] abolishes the low linear rate of glutamine synthesis, but not the initial rapid release of glutamine. 4. In livers from 48h-starved rats initial release (0–15min) of glutamine was decreased (10.6 ± 1.1 μmol/h per g wet wt.; mean ± S.E.M. of 11 experiments) and the subsequent rate of glutamine production was lower than in livers from fed rats. Again proline plus alanine was the only substrate combination giving an increase significantly above the endogenous rate. 5. The rate of glutamine synthesis *de novo* by the liver is apparently unrelated to the tissue content of glutamate or ammonia. 6. The blood glutamine concentration is increased by 50% within 1h of elimination of the liver from the circulation of rats *in vivo*. 7. There is an output of glutamine by the brain under normal conditions; the mean arterio-venous difference for six rats was 0.023 μmol/ml. 8. The high potential activity of liver glutamine synthetase appears to be inhibited by some unknown mechanism: the function of the liver under normal conditions is probably the disposal of glutamine produced by extrahepatic tissues.

According to Schimassek & Gerok (1965) the liver plays a major role in the regulation of the amino acid concentration of blood plasma. In their experiments on the isolated rat liver with a medium containing no amino acids, they found that the liver adjusts the concentrations of plasma amino acids within 1h to approximately the physiological values. Exceptions were the branched-chain amino acids, which reached higher concentrations. Because the intracellular content of free amino acids was not diminished at the end of the perfusions, Schimassek & Gerok (1965) suggested that a labile protein is the source of the amino acids appearing in the medium.

The object of the present work was to study the role of the liver in the control of the synthesis and plasma concentration of glutamine in the presence of an abundant supply of precursor. Glutamine was chosen because, although much work has been done on glutamine synthesis in the liver, mainly in the intact animal (Schoenheimer, 1942; Duda & Handler, 1958; du Ruisseau, Greenstein, Winitz & Birnbaum, 1957; Addae & Lotspeich, 1968), the function of the liver in the maintenance and regulation of the plasma glutamine concentration is still uncertain. Glutamine is of particular interest for this type of study because its concentration in plasma is higher than that of any other amino acid and because the liver contains high activities of both glutamine synthetase (EC 6.3.1.2) and glutaminase (EC 3.5.1.2).

The experiments reported in the present paper are mainly concerned with glutamine formation by the isolated perfused rat liver. Several experiments

designed to show the contribution of extrahepatic tissues to the plasma glutamine concentration are included.

MATERIALS AND METHODS

Liver perfusion. The basic perfusion method was that described by Hems, Ross, Berry & Krebs (1966). The perfusion medium was also as described by Hems *et al.* (1966), except that albumin was a product of Pentex Biochemicals (Miles Laboratories Inc., Kankakee, Ill., U.S.A.). Female Wistar rats weighing 200–240g were used. Substrates were added to the medium as 0.2M neutral solutions. Metabolic rates (μmol/h per g wet wt.) were calculated from the gradient of the plot of total metabolite in the medium versus time. Samples of medium (2ml) were taken at 15min or 30min intervals and added to 0.1ml of 60% (w/v) $HClO_4$. A measured volume of supernatant was neutralized with a known volume of KOH to precipitate the $KClO_4$. Perfusions of livers from fed rats were always carried out in the mornings, when the stomach was full.

Metabolite concentrations were determined in the tissue at the end of some perfusion experiments. A piece of liver was quickly excised and pressed between metal clamps previously cooled in liquid N_2 (Wollenberger, Ristau & Schoffa, 1960). Further treatment of the tissue was as described by Williamson, Lund & Krebs (1967). For all samples exposure to acid conditions was kept to a minimum to prevent hydrolysis of glutamine.

Anaerobic perfusions. Anaerobic conditions were produced by omitting erythrocytes from the basic medium, by using N_2+CO_2 (95:5) in the gas phase and adding 1mM-NaCN to the medium at 30min intervals.

Reagents. Glutaminase (grade IV) was obtained from Sigma (London) Chemical Co. Ltd., London S.W.6, U.K.; other purified enzymes were from Boehringer Corp., London W.5, U.K. L-Methionine DL-sulphoximine and DL-(+)-allo-δ-hydroxylysine hydrochloride were obtained from Sigma (London) Chemical Co. Ltd. and Mann Research Laboratories, New York, N.Y., U.S.A., respectively. Norite SX2 (activated charcoal) was obtained from Harrington Bros Ltd., London W.12, U.K.

L-[U-^{14}C]Glutamine was a product of The Radiochemical Centre, Amersham, Bucks., U.K.

Determination of metabolites. ATP, ADP, AMP and α-oxo acids were determined immediately in the neutralized tissue extracts. Other metabolites could be measured after storing the extracts at −15°C. Lactate and pyruvate were determined by the method of Hohorst, Kreutz & Bücher (1959) and α-oxoglutarate was determined by the method of Bergmeyer & Bernt (1963). Pyruvate and α-oxoglutarate were determined in the same cuvette by successive additions of lactate dehydrogenase and glutamate dehydrogenase. L-Glutamate was assayed by the method of Bernt & Bergmeyer (1963) with the addition of 1μmol of ADP to speed up the reaction by activation of glutamate dehydrogenase (Tomkins, Yielding, Talal & Curran, 1963). The ATP assay method used was that described by Lamprecht & Trautschold (1963), and those for ADP and AMP were described by Adam (1963). Ammonia was determined with glutamate dehydrogenase (Kirsten, Gerez & Kirsten, 1963) after treatment of the neutralized extract with Norite SX2 activated charcoal (50mg/ml of extract), which minimizes non-enzymic 'creep' in the assay (Brosnan, 1968). The ammonia assay time is also shortened by the inclusion of 1μmol of ADP/cuvette. There was no 'creep' in ammonia assays of the perfusion medium, and these

Table 1. *Rates of glutamine and glutamate output from various precursors by perfused livers of fed rats*

The livers were perfused as described in the Materials and Methods section. Substrates were added to the medium at 30min to a final concentration of 5mM unless stated otherwise. Rates are expressed as μmol/h per g wet wt. of tissue. Averages of two experiments are given except where there are three or more experiments in the group when means±S.E.M. are given with the numbers of observations in parentheses. The rates of glutamine output during 0–15min and 15–30min periods were 15.6±0.8μmol/h per g wet wt. (35) and 2.3±0.5μmol/h per g wet wt. (35) respectively. The corresponding rates of glutamate output were 1.5±0.1μmol/h per g wet wt. (35) and 0.6±0.1μmol/h per g wet wt. (35) respectively.

	Rate of glutamine output (μmol/h per g wet wt.)		Rate of glutamate output (μmol/h per g wet wt.)	
Substrate added at 30min	30–90min	90–120min	30–90min	90–120min
None (3)	3.5±0.3	3.6±0.3	1.2±0.4	1.4±0.6
L-Alanine (5)	8.7±1.7	3.2±1.8	1.3±0.5	0.8±0.4
L-Proline (3)	4.1±0.6	3.4±0.8	0.9±0.2	0.3± 0.5
L-Histidine	4.3	5.0	8.6	18.3
L-Asparagine	6.0	4.0	3.5	0.8
L-Ornithine	5.5	3.8	2.1	0.5
NH_4Cl	3.5	4.2	1.5	−0.1
L-Proline+L-alanine (4)	10.8±0.9	6.7±1.3	2.3±0.7	1.8±0.5
10mM-L-Proline+10mM-L-alanine (3)	9.9±1.5	2.9±1.3	4.2±1.6	2.9±0.9
L-Alanine+L-histidine	7.5	−0.3	4.5	24.1
L-Alanine+2mM-L-ornithine	6.9	4.0	1.5	1.1
L-Alanine+L-proline+2mM-L-ornithine	6.9	4.1	1.5	1.2
L-Alanine+L-proline+NH_4Cl	3.8	5.7	2.1	1.2
L-Proline+NH_4Cl	5.0	3.9	1.5	1.0

extracts were untreated. Glutamine was determined after enzymic hydrolysis as described by Lund (1970*b*), with glutaminase IV (0.05mg/assay) from Sigma (London) Chemical Co. Ltd.

RESULTS

Glutamine and glutamate production by perfused livers from fed rats. Glutamate, the direct precursor of glutamine, is not a satisfactory substrate for the study of glutamine synthesis in the perfusions because of the limited permeability of rat liver cells to glutamate (Schwerin, Bessman & Waelsch, 1950; Hems, Stubbs & Krebs 1968) and to other di- and tri-carboxylic acids (Ross, Hems & Krebs, 1967). Therefore more readily penetrating and rapidly metabolized amino acids, such as L-proline, L-alanine and L-histidine, which give glutamate or ammonia or both as metabolic products, were used as precursors of glutamine.

In all perfusions of livers from fed rats there was a rapid output of glutamine into the medium during the 30min before the addition of substrate. The rate during the first 15min was most rapid (15.6 ± 0.8 μmol/h per g wet wt. of liver; mean ± S.E.M. of 35 perfusions), then decreased to 2.25 ± 0.47 μmol/h per g wet wt. (mean ± S.E.M. of 35 perfusions) during the 15–30min period. After the addition of substrate glutamine output was variable, but in no case did the rate reach the 0–15min value (Table 1). Throughout the perfusion glutamate appeared slowly; the rate for 0–15min was 1.5 ± 0.1 μmol/h per g wet wt. (35 experiments) and was subsequently less than 2.0 μmol/h per g wet wt. except when histidine or proline plus alanine were added to the medium (Table 1).

In the absence of any added substrate, glutamine output was approximately linear throughout the 150min perfusion period (3.6 ± 0.3 μmol/h per g wet wt.; mean ± S.E.M. of three experiments) after the rapid initial output at 0–15min. Most substrates tested gave no appreciable increase in this rate. Alanine gave inconsistent results; the rates were non-linear, and varied widely, as shown by the high S.E.M. (Table 1). Proline alone did not stimulate glutamine release. By contrast, the combination of alanine plus proline gave consistently greater rates of glutamine production than the unsupplemented control. Even then, the maximum rate obtained was only about 12 μmol/h per g wet wt., which is approximately one-fortieth of the capacity of rat liver glutamine synthetase measured in crude extracts at pH 7.4 with 75mM-L-glutamate, 20mM-ammonium chloride and an ATP-regenerating system (Lund, 1970*a*). Since histidine gave rise to an accumulation of glutamate and ammonia in the tissue (in one experiment 17.7 and 3.1 μmol/g wet wt. respectively, 60min after the addition of 5mM-histidine) with no increase in glutamine production, it appears that the capacity of the perfused liver to synthesize glutamine is unrelated to the intracellular glutamate or ammonia content.

Despite the presence of potential precursors of glutamine the rate of glutamine production decreased with time. This was not due to limitation of substrate because it also occurred when the concentration of alanine plus proline was doubled (Table 1). With every precursor tested the final concentration of glutamine in the medium was less than 1.5mM, and except where proline plus alanine were substrates it was less than 1.0mM. The plasma glutamine concentration of normal fed rats is 0.74 μmol/ml.

Source of the glutamine appearing in the medium in the early stages of the perfusion. Because intracellular free amino acid concentrations, including those of essential amino acids, were unchanged at the end of the perfusions carried out by Schimassek & Gerok (1965), these authors concluded that a labile protein is the source of the amino acids appearing in the medium. For the essential amino acids the existence of a labile protein or peptide must indeed be postulated, but the following experiments show that the initial shedding of glutamine and glutamate is largely due to wash-out of the free amino acids from the tissue.

Livers from fed rats were perfused for 10 or 15min. The total glutamine and glutamate contents of the medium and of the tissue were then determined. The concentration in the medium was corrected for the glutamine and glutamate initially present in the erythrocytes. After 10min or 15min approx. 30% of the glutamine remains in the tissue (Table 2); the rest, within the limits of error, can be accounted for by that in the medium. Of the glutamate appearing in the medium approx. 50% could be accounted for by the decrease of tissue glutamate.

Thus wash-out accounts for a major part of the non-essential amino acids appearing in the medium in the early stages of perfusion, but the tissue glutamine is replenished to some extent during the later period because at the end of the perfusion (150min) 3–4 μmol/g wet wt. is found.

Effect of varying the volume of perfusion medium. To test for feedback control of hepatic glutamine production by the concentration of glutamine in the medium, the volume of the medium was varied from 75ml to 300ml. With half the standard volume, the rate of synthesis was somewhat lower (2.6 and 2.9 μmol/h per g wet wt. in two experiments) than the control (3.6 ± 0.3 μmol/h per g wet wt.; mean ± S.E.M. of three experiments), and with twice the volume it was somewhat higher (6.2 and 4.6 μmol/h per g wet wt. in two experiments; Table 3). These results suggest that the glutamine

Table 2. *Glutamine and glutamate contents of tissue and medium after a short period of perfusion*

Livers of fed rats were perfused as described in the Materials and Methods section. A small piece of tissue was freeze-clamped at the end of the 10 min or 15 min perfusion. 'Total expected (μmol)' values were calculated on the basis of glutamine and glutamate contents of liver of normal fed rats of 6.0 and 3.7 μmol/g fresh wt. respectively.

Duration of perfusion (min)	Liver wt. (g)	Total in medium (μmol)		Total in tissue (μmol)		Total found (μmol)		Total expected (μmol)	
		Glutamine	Glutamate	Glutamine	Glutamate	Glutamine	Glutamate	Glutamine	Glutamate
10	6.6	22.7	1.7	18.5	8.9	41.2	10.6	39.6	24.4
10	6.65	27.4	2.3	12.0	8.7	39.4	11.0	39.9	24.6
15	7.3	25.0	3.1	13.1	11.7	38.1	14.8	43.8	27.0
15	7.1	24.0	3.1	13.2	12.8	37.2	15.9	42.6	26.2

concentration in the medium may be a controlling factor.

Effect of the presence of glutamine in the medium on glutamine release. If the above conclusion is correct, the presence of 1 mM-glutamine should decrease the rate of any further synthesis. When approx. 1 mM- glutamine was added at zero time the net result was an uptake rather than a release of glutamine during the first 30 min. Then the concentration began to increase at a rate slightly lower than that in the perfusions without substrate (Table 4).

On the other hand, when proline plus alanine were added at 30 min, 1 mM-glutamine having been present from the start, the rate of glutamine production was decreased to about half that found from this substrate combination in the absence of added glutamine.

Experiments with ^{14}C-labelled glutamine showed that uptake and release occur simultaneously in the early stages of perfusion. Livers were perfused with a medium containing 2.5 μCi of L-[U-^{14}C]glutamine in a final concentration of 0.7 mM- or 1 mM-glutamine. The specific radioactivity of the glutamine in the medium was determined initially and at intervals. The calculation of specific radioactivity is based on total radioactivity in the deproteinized extracts of medium, because during the relevant period of perfusion (0–15 min) there was no radioactivity in glutamate as determined by chromatography on Polygram Cel 300 (see Lund, 1970*a*). The specific radioactivity fell by approx. 20% during the first 5 min of perfusion, with little further change during 5–15 min. Calculations made from the decrease in specific radioactivity and the decrease in glutamine concentration represented rates of glutamine output of 16.2 and 17.6 μmol/h per g wet wt. calculated over the 0–15 min period. Thus the presence of glutamine in the medium has no inhibitory effect on the initial rapid release of glutamine from the tissue, even though the net result, in terms of concentration, is an apparent uptake.

Effect of 48 h *starvation on glutamine output.* The initial release of glutamine (0–15 min period) by livers of 48 h-starved rats was lower (10.6±1.1 μmol/h per g wet wt.; mean±S.E.M. of 11 experiments) than by livers of fed rats (15.6±0.8 μmol/h per g wet wt.; mean±S.E.M. of 35 experiments). The subsequent rates, with or without added substrates, were about the same as those obtained with livers from fed rats (Table 5) except that glutamine output decreased at an earlier stage than in the livers from fed rats, even though the concentration of glutamine in the medium was lower. As in the livers from fed rats, proline plus alanine was the most effective glutamine precursor.

Measurement of metabolites in livers freeze-

Table 3. *Effect of the volume of medium on the rate of appearance of glutamine on perfusion of liver from fed rats*

The livers were perfused as described in the Materials and Methods section. The volumes of perfusion medium were either half (75ml) or twice (300ml) the normal volume. No substrate was added. The rate of appearance of glutamine in the medium is expressed as μmol/h per g wet wt. of liver. Values for the control (150ml) are means±S.E.M. of three experiments; other values are the averages of two experiments.

Vol. of medium (ml)	Period ...	Rate of glutamine production (μmol/h per g wet wt.) 0–15min	15–30min	30–90min	90–150min
Control		13.5±3.3	3.6± 0.3	3.5±0.3	3.6±0.3
75		11.1	3.7	2.8	2.4
300		21.6	5.8	5.4	5.2

clamped 60min after addition of substrate showed a very high concentration of glutamate (11.7± 3.5μmol/g wet wt.; mean±S.E.M. of four experiments) on perfusion with alanine plus proline, but normal values for α-oxoglutarate, ATP, ADP, AMP and glutamine. The intracellular ammonia concentration was slightly raised. It follows that the cessation of glutamine synthesis cannot have been due to a lack of intracellular glutamate, which conforms with the results obtained for histidine in the livers from fed rats. Proline or alanine alone increased the glutamate content from the control value of 1.6±0.2 (mean±S.E.M. of four experiments) to 4.2.±0.3 (mean±S.E.M. of three experiments) and 4.9±1.0 (mean±S.E.M. of five experiments) μmol/g wet wt. respectively, but the combined effect of proline plus alanine was greater than the sum of the two separately. On perfusion with alanine alone, the tissue glutamine content was lower than in the perfusions with no added substrate.

Effect of inhibitors of glutamine synthetase. Perfusion with a glutamine synthetase inhibitor was expected to make it possible to distinguish between synthesis *de novo* and breakdown of a labile protein (Schimassek & Gerok, 1965) as the source of glutamine appearing in the control perfusions after the initial wash-out of tissue glutamine. Methionine sulphoximine causes irreversible specific inhibition of glutamine synthetase (Meister, 1969), and allo-hydroxylysine is a less specific non-competitive inhibitor of the enzyme (Wu, 1963). Livers from fed rats were perfused with the inhibitor present in the medium from the start at a concentration of 1.85mM-L-methionine DL-sulphoximine or 4mM-DL-allo-hydroxylysine. No other substrate was added. In the presence of either inhibitor the glutamine output during the first 30min was only slightly lower than that of the controls. Thereafter there was very little increase, and in some cases there was an uptake of glutamine towards the end of the perfusion period. After 120min glutamine was barely detectable in the tissue (less than 0.6μmol/g), and the ATP content was not significantly different from the control value.

Glutamine output under anaerobic conditions. During anaerobic perfusion of livers from fed rats glutamine release during the first 30min was only slightly lower than in the aerobic controls and then the concentration remained virtually constant. The concentration of ammonia in the medium increased. The experiments confirm that the glutamine release after the first 30min of perfusion is energy-dependent, and supports the view that it results from synthesis *de novo*.

Perfusion with L-*glutamine as substrate.* Glutamine (5mM) was rapidly taken up by the liver (up to 100μmol/h per g wet wt.). The rate decreased markedly at approx. 1mM, and below 1mM the concentration began to increase slightly with time.

Role of the liver in glutamine production in vivo. Further evidence that the liver is not involved in a major way in any net production of glutamine was provided by experiments *in vivo* in which the liver was eliminated from the circulation and blood glutamine and glutamate concentrations were determined at intervals.

Rats were lightly anaesthetized with ether and a small amount of Nembutal (6mg in 0.1ml). The animals were artificially ventilated by O_2+CO_2 (95:5) through a tube inserted in the trachea. The liver was eliminated from the circulation by ligating the hepatic artery and the portal vein. Blood samples from the vena cava (approx. 0.5ml). were added to 0.5ml of 20% (w/v) perchloric acid in a weighed centrifuge tube, and the tube was reweighed to obtain the weight of blood. Glutamine and glutamate were determined in the neutralized extracts. In fed rats, both glutamate and glutamine increased in concentration within 30min of elimination of the liver from the circulation, whereas in 48h-starved rats only that of glutamine increased (Table 6). Although the initial glutamine concentrations were different, the increase in concentration during the first 60min was approximately the same in all cases (0.32, 0.28 and 0.26μmol/ml).

Table 4. *Effect of including a low concentration of glutamine in the perfusion medium on the subsequent rate of glutamine production by livers of fed rats*

The livers were perfused as described in the Materials and Methods section. Glutamine was present in the medium from the start of the perfusion at the concentration stated. A minus sign (−) indicates uptake of glutamine by the liver. Rates are expressed as μmol/h per g wet wt. of tissue.

Initial concn. of glutamine (mM)	Substrate added at 30 min	Period	Rate of glutamine production (μmol/h per g wet wt.)			Rate of glutamate production (μmol/h per g wet wt.)			
			0–15 min	15–30 min	30–90 min	90–150 min	0–15 min	15–30 min	30–90 min	90–105 min
1.09	None	...	−10.5	−0.5	1.8	3.1	1.6	1.2	0.7	0.7
1.00	None		−24.5	−13.2	1.7	2.3	1.7	1.7	1.2	1.1
0.76	None		−10.6	−1.6	2.3	1.9	2.7	0.6	2.5	2.3
1.1	Alanine+proline		−12.4	−1.6	4.7	3.3	2.4	1.9	2.0	1.7
1.1	Alanine+proline		−17.0	−4.2	7.2	3.2	1.0	0.7	2.0	1.3

In these experiments no attempt was made to follow the fate of the gut constituents that could normally appear in the portal vein. Amino acids and ammonia are most relevant in this context, and 48h-starved rats were included to minimize the possibility that the increases found were simply due to gut constituents that would have normally been taken up by the liver. The small blood samples available made it impossible to determine ammonia at the same time as glutamine and glutamate, and removal of even 0.5ml of blood at 30min intervals soon depletes the total blood volume of the rat. However, all experiments were stopped before the heart action became weak, and, in spite of uncertainties about the technique, it is certain that the blood glutamine concentration does increase after a short time when the liver is absent.

Arterio-venous difference in glutamate and glutamine concentrations across the brain. The venous-blood sample was taken from the confluence of the sagittal and transverse sinuses at the same time as an arterial sample, as described by Hawkins, Williamson & Krebs (1971). Fed rats were used. Glutamine and glutamate concentrations were determined in the neutralized perchloric acid extracts. The results are given in Table 7. In all six rats the glutamine concentration in the venous blood was higher than that in the arterial samples. The arterio-venous difference for glutamine was 0.023 ± 0.007 μmol/ml (mean ± S.E.M. of six animals), which indicates that a net output of glutamine by the brain occurs under normal conditions.

DISCUSSION

Regulation of glutamine synthesis in rat liver. The low rates of glutamine synthesis by the perfused liver, despite the high capacity of glutamine synthetase, cannot be explained by non-availability or inaccessibility of substrate. After 60min perfusion with histidine, or with proline plus alanine, the intracellular glutamate and ammonia contents were as high as 17 and 2–3 μmol/g wet wt. respectively, with, especially in the case of histidine, no significant increase in glutamine production above the control rate. Nor can the low rates be explained by a low affinity of enzyme for the substrate: the kinetic properties of highly purified rat liver enzyme are similar to those of the brain enzyme (Tate & Meister, 1971) in which the Michaelis constants for L-glutamate, ATP and ammonia are 2.5×10^{-3} M, 2.3×10^{-3} M and 1.8×10^{-4} M respectively (Pamiljans, Krishnaswamy, Dumville & Meister, 1962). It is true that a higher K_m for L-glutamate is found for the enzyme in crude extracts [1.1×10^{-2} M by Wu (1963); 5.5×10^{-2} M by Richterich van Baerle, Goldstein & Dearborn (1957); 1.5×10^{-2} M by Lund (1970*a*)], possibly because of the presence of an

Table 5. *Rates of glutamine and glutamate output from perfused livers of 48h-starved rats*

The experimental conditions were as described for Table 1. Rates of glutamine output were 10.6±1.1 (mean±S.E.M. of 11 experiments) and 0.9±0.6 (mean±S.E.M. of 11 experiments) μmol/h per g wet wt. during the 0–15min and 15–30min periods respectively. The corresponding rates of glutamate output were 1.9±0.3 and 0.5±0.1 μmol/h per g wet wt. respectively.

		Rate of glutamine output (μmol/h per g wet wt.)		Rate of glutamate output (μmol/h per g wet wt.)	
Substrate added	Period ...	30–60min	60–90min	30–60min	60–90min
None (3)		2.7±0.2	3.3±0.6	0.5±0.1	0.1±0.1
L-Proline (3)		2.7±0.5	3.2±0.2	0.4±0.1	0.4±0.2
L-Alanine (5)		6.2±0.9	−1.5±1.2	1.8±0.5	1.3±0.6
L-Proline+L-alanine (4)		11.9±1.7	4.0±1.8	4.4±0.9	5.5±1.0

Table 6. *Blood glutamine and glutamate concentrations* in vivo *after elimination of the liver from the circulation*

For details of the technique see the text. Blood samples were taken at the times stated after ligation of the hepatic artery and portal vein.

Expt. no.	Physiological state	Time (min)	Concn. in blood (μmol/ml) Glutamine	Glutamate
1	Fed	0	0.55	0.12
		60	0.87	0.28
2	Fed	0	0.64	0.18
		30	0.80	0.29
		60	0.92	0.39
		120	1.30	0.32
3	48h-starved	0	0.40	0.16
		30	0.56	0.12
4	48h-starved	0	0.44	0.18
		30	0.59	0.17
		60	0.70	0.17

Table 7. *Arterio-venous differences in concentration of glutamate and glutamine across the brain of the rat*

Blood samples were taken from the femoral artery and the confluence of the sagittal and transverse sinuses of the brain of fed rats. The concentrations of glutamate and glutamine are expressed as means±S.E.M. for six animals.

	Concn. in whole blood (μmol/ml) Glutamate	Glutamine
Arterial blood	0.181±0.014	0.573±0.011
Brain venous blood	0.173±0.010	0.596±0.006

inhibitor accompanying the enzyme in crude extracts. In contrast the isolated perfused rat kidney synthesizes glutamine from glutamate at a constant rate of 30 μmol/h per g wet wt. (calculated from the results of Nishiitsutsuji-Uwo, Ross & Krebs, 1967), even though activity of the kidney enzyme *in vitro* is approximately fourfold lower than that of the liver enzyme (Wu, 1963; Lund, 1970*a*). It is not clear why rat kidney, in which the intracellular content of the reactants of glutamine synthetase is not very different from liver, should have the capacity for a continuous rate of synthesis whereas the liver does not. If availability of substrate is not the rate-limiting factor in liver, it must be that the enzyme is largely inactivated *in vivo*. In this context it is noteworthy that L-alanine inhibits the activity of highly purified rat liver glutamine synthetase by approx. 50% when Mn^{2+} replaces Mg^{2+} in the assay system. No explanation can be offered for the fact that alanine plus proline should be the most effective glutamine precursors in the present experiments.

To what extent the steady-state concentration of glutamine in the liver depends on the balance between glutamine synthetase activity and glutaminase activity is still uncertain (see Lueck & Miller, 1970). The apparent inhibition of glutamine synthetase by glutamine in some of the present perfusion experiments could be due either to feedback inhibition of glutamine synthetase or to an activation of glutaminase. Some observations favour the second possibility. Glutamine synthetase is not inhibited by glutamine, but glutamine at rather high concentration is an allosteric activator of liver phosphate-dependent glutamine amidohydrolase *in vitro* (Katunuma, Katsunuma, Tomino & Matsuda, 1968; Pestana, Marco & Sols, 1968).

Increase in blood glutamine concentration after elimination of the liver from the circulation. The increases in blood glutamine concentration that occur after elimination of the liver from the circulation are consistent with the data of Flock, Mann & Bollman (1951) and of McMenamy, Vang & Drapanas (1965), who found increased concentrations of amino acids in general, including glutamine, in the blood of dogs several hours after hepatectomy. Under these conditions some of the glutamine appearing in the circulation is formed in the brain

(Flock, Block, Grindlay, Mann & Bollman, 1953). The kidney can make a contribution to the circulating glutamine in the rat, but not in the dog (Krebs, 1935). Skeletal muscle is a major source of glutamine in humans (Marliss, Aoki, Pozefsky, Most & Cahill, 1971), in the dog (A. G. Hills, personal communication) and in the rat (N. B. Ruderman & P. Lund, unpublished work).

The experiments *in vivo* thus show that extrahepatic tissues can make a considerable contribution to the amount of circulating glutamine. They support the conclusion that rat liver is concerned with regulation of plasma glutamine concentrations and that the net production of glutamine is not a function of the liver under normal conditions. Further support for this view is provided by the results of du Ruisseau *et al.* (1957) and Brosnan (1968), who found increases in alanine and aspartate amounts after administration of an ammonia load to rats *in vivo* with little or no increase in the amount of glutamine in livers from fed rats and a decrease in the amount in starved rats.

I am very grateful to Sir Hans Krebs, F.R.S., for his helpful advice, and to Mr R. Hems, Dr J. F. Biebuyck and Dr R. Hawkins for help with the surgical procedures. The work was supported by grants to Sir Hans Krebs from the Medical Research Council and the U.S. Public Health Services (Grant no. AM 11748). The author is a member of the External Staff of the Medical Research Council.

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