Substrate and pH effects on glutamine synthesis in rat liver

Consequences for acid–base regulation

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Switching in acidosis of hepatic nitrogen disposal from urea synthesis to NH_4^+ and net glutamine production was demonstrated in the isolated perfused livers of starved male Wistar rats. Lactate was preferred to glucose as the substrate for the carbon skeleton of glutamine synthesized over the pH range 6.9–7.5. This is necessary if the switch away from a proton-producing process (ureagenesis) in acidosis is to constitute an acid–base regulating system intrinsic to the liver. Glutamine balance shifted with pH from marked net uptake to small net output under acidotic conditions (pH 7.5–6.9), an effect due solely to a decrease in glutamine uptake. NH_4^+ uptake by the liver had a linear relationship with pH, being markedly decreased in acidosis because glutamine synthesis was insufficient to compensate for the decreased incorporation into urea. Animals rendered chronically acidotic showed a lower central venous plasma urea concentration and a raised NH_4^+ concentration, but their livers synthesized no more glutamine when perfused at an acidotic pH than did normal livers. We conclude that perivenous hepatocytes may not be efficient scavengers of NH_4^+ ions, which must be partly disposed of elsewhere by non-proton-generating pathways if inhibition of ureagenesis is to represent a hepatic acid–base regulating system.

INTRODUCTION

Urea synthesis from CO_2 and NH_4^+ ions in the liver generates protons according to the following overall reaction:

and takes place predominantly in the periportal zone of the hepatic lobule, where the majority of gluconeogenesis also occurs [1,2].

A decrease in urea synthesis is readily demonstrated during the perfusion of isolated livers under acidotic conditions [3,4], and a proton-producing process is thus inhibited. This mechanism has been proposed as a component of pH homeostasis [2,5], but poses a problem as to the possible fate of potentially toxic NH_4^+ ions which have been either generated peripherally or derived from the action of glutaminase within the liver, and which would have been converted into urea under normal acid–base conditions. It has been proposed [2,5] that the NH_4^+ derived in this way is 'scavenged' by glutamine synthesis, which is confined to the cells immediately adjacent to the central vein of the hepatic lobule [3,6,7].

It is shown in the Appendix that if glucose is the preferred carbon-containing substrate for glutamine synthesis, then H^+ ions are generated and the acid–base advantage of inhibiting urea synthesis is negated. In contrast, if lactate is the preferred substrate, the advantage is preserved. Little attention has previously been paid to the source of carbon for glutamine synthesis, or to the potential consequences of the source for acid–base regulation. One purpose of the present study was therefore to determine the preferred substrate for the synthesis of the carbon skeleton of glutamine under normal and acidotic conditions.

A second purpose was to examine whether glutamine synthesis increased as proposed [2,5] under acidotic conditions and to record the effect on net NH_4^+ balance. The relative contributions of glutamine production and consumption to net glutamine

balance can be determined by using L-methionine *S*-sulphoximine, an irreversible inhibitor of glutamine synthetase [6,8]. Changes in hepatic glutamine synthesis in chronic metabolic acidosis were compared with those in acute simulated acidosis, and the possibility of an adaptive system was thus explored.

MATERIALS AND METHODS

Perfusions

Isolated liver perfusions were established from male 48 h-starved Wistar rats (approx. weight 175 g) as previously described [9], except that a non-recirculating erythrocyte- and albumin-free perfusion medium was used and flow rates were correspondingly increased (1 ml/10 g rat wt. per min). Starved rats were used to ensure that minimal endogenous glucose was available for glutamine synthesis. The basic perfusion medium was Krebs bicarbonate buffer, equilibrated with O_2/CO_2 (19:1) [10]. Hepatic-venous effluent $p\text{O}_2$ was always > 10 kPa. pH was varied by lowering the bicarbonate concentration, with a corresponding increase in chloride. NH_4Cl , glutamine, lactate, pyruvate, glucose and ornithine were added as indicated in the text. When inhibition of glutamine synthetase was required, L-methionine *S*-sulphoximine (LMS, 0.2 mM) was added, the animals also having in this case been pre-treated by administration of LMS (5 mg/100 g rat wt.) or saline vehicle (control) intraperitoneally 2 h before perfusion. This regime has been demonstrated [6] to inhibit glutamine synthetase completely in the isolated perfused rat liver, and we have confirmed this finding. Perfusions were equilibrated for 30–35 min before paired timed samples (see the text) of portal-vein media and hepatic-venous effluent were obtained for estimation of pH, $p\text{CO}_2$, $p\text{O}_2$, urea and substrate concentrations. Uptake or output was calculated by the Fick principle and expressed as nmol/g rat wt. per min.

Abbreviation used: LMS, L-methionine *S*-sulphoximine.

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Induction of chronic acidosis

An indwelling right-atrial cannula was positioned under ether anaesthesia for monitoring of pH and $p\text{CO}_2$. Metabolic acidosis was then induced by mixing 30 ml of 0.8 mM-HCl with 30 g of Rat Chow (Dixon's, Ware, Herts., U.K.). This diet was given for 4 days, and right-atrial blood sampling was then carried out in the unanaesthetized state after the animal had settled in a restraining cage.

Determination of venous concentration of substrates *in vivo*

Under ether anaesthesia heparinized blood samples were obtained from the inferior vena cava, above the renal veins, of seven normal fed rats and seven rats rendered acidotic. Blood was similarly taken from the hepatic portal vein of six 48 h-starved rats. Samples were deproteinized in 0.6 M-HClO₄, and the concentrations of NH₄⁺, lactate and urea were measured.

Radioisotope tracer experiments

In paired perfused-liver experiments in which glutamine synthesis took place from near-physiological mixtures of substrates (0.6 mM-NH₄Cl, 10 mM-glucose, 2 mM-lactate, 0.1 mM-ornithine, 0.3 mM-pyruvate), either 100 μCi of [U-¹⁴C]glucose (to produce a perfusate glucose specific radioactivity of 2 mCi/mol) or 100 μCi of [U-¹⁴C]lactate (to produce a perfusate lactate specific radioactivity of 10 mCi/mol) was added and the rate of appearance of radioactivity in glutamine was observed. Because of cross-over in the oxaloacetate pool [11], absolute rates of incorporation of lactate and glucose into glutamine cannot be validly calculated. However, the ratio of ¹⁴C incorporation from lactate to that from glucose should give a valid comparison of relative lactate and glucose contributions to glutamine synthesis, assuming that under identical conditions of study percentage errors from cross-over for the two labelled substrates and loss in CO₂ are also identical and therefore cancel out. Samples (10 ml) of portal and hepatic-venous effluent were collected into tubes containing 0.6 ml of 2 M-HClO₄ 40 min after the start of the perfusion for radioactivity counting and glutamine determination.

Isolation and counting of [¹⁴C]glutamine

Neutralized perfusate (10 ml) was chromatographed on a 1 cm × 17 cm Dowex 50WX8 column (H⁺ form, equilibrated with water). Organic acids were eluted with 20 ml of water. Amino acids were eluted with 10 ml of 4 M-NH₃ and freeze-dried before being resuspended in 2 ml of water, followed by re-chromatography on an anion-exchanger (Dowex 2X8, acetate form, equilibrated with water; 1 cm × 17 cm column). Glutamate bound to the resin, whereas glutamine was eluted with 10 ml of water. The glutamine fraction was freeze-dried and concentrated by resuspension in 0.25 ml of water. Of the concentrate 0.1 ml was kept for glutamine assay, and 0.03 ml samples were subjected to t.l.c. (Polyester Sigmacell, type 100 cellulose plate) at room temperature with butan-1-ol/acetic acid/acetone/water (7:2:7:4, by vol.) as solvent. The glutamine spot was identified by ninhydrin staining of a standard run in an adjacent strip and transferred to scintillant (Packard Pico-Fluor 40). The vials were then counted for radioactivity for 4 h. The specific radioactivity of the glutamine was calculated from the known concentration of glutamine in the 0.03 ml spot and was corrected for the specific radioactivity of the substrates. The correction assumes that both [U-¹⁴C]glucose and [U-¹⁴C]lactate form glutamine through pyruvate.

Assays

Samples (10 ml) of portal and hepatic-venous perfusate were collected into 0.6 ml of ice-cold 2 M-HClO₄ and kept frozen. Concentrations of glutamine, glutamate, NH₄⁺, urea, glucose and lactate in the neutralized perfusate or plasma were measured by standard enzymic techniques [12]. pH and $p\text{CO}_2$ were measured on samples of blood and perfusate with an IL 1304 pH/blood-gas analyser.

Chemicals

All reagents were of analytical grade and obtained from Sigma. Enzymes were obtained from BCL or Boehringer Mannheim, and [U-¹⁴C]glucose and [U-¹⁴C]lactate were from Amersham International.

Statistical methods

Results are expressed as means ± S.E.M. For normally distributed data, the unpaired Student's *t* test was used, and for paired non-normally distributed data, the Wilcoxon signed-rank test. Standard linear-regression analysis was used, and the slopes/intercepts of pairs of regression lines were compared by analysis of covariance. Tests of significance are two-tailed.

RESULTS

Hepatic portal-vein substrate concentrations

In six 48 h-starved rats the mean hepatic portal-vein plasma concentrations of NH₄⁺ and lactate were 0.12 ± 0.02 mM and 1.65 ± 0.15 mM respectively.

Nitrogen switching

Fig. 1 shows urea production and net glutamine balance rates in 14 livers perfused with media containing a near-physiological mixture of NH₄⁺ (0.6 mM), glutamine (0.6 mM), pyruvate (0.3 mM), ornithine (0.1 mM) and lactate (2 mM) in Krebs buffer over a pathophysiological pH range (7.0–7.5). The rate of production was established from the mean value of three assays

Fig. 1. Urea production (○) and glutamine balance (●) in rat livers perfused at different pH values

pH was varied as described in the Materials and methods section. The perfusing media contained: glutamine (0.6 mM), NH₄⁺ (0.6 mM), lactate (2 mM), ornithine (0.1 mM) and pyruvate (0.3 mM). Each point represents a separate perfusion. Rates are expressed as nmol/min per g body wt.: + represents net output.

Fig. 2. Net NH_4^+ uptake in rat livers perfused at different pH values

Rates are expressed as nmol/min per g body wt. These data were obtained in the same experiments as shown in Fig. 1. (Regression line: NH_4^+ uptake = $-164.6 \pm 27.29 \text{ pH}$, P value for slope < 0.01 .)

Fig. 3. NH_4^+ concentrations in the hepatic-venous effluent from livers perfused under conditions as described in Fig. 1 (●; regression line: $[\text{NH}_4^+] = 2.241 - 0.280 \text{ pH}$, P value for slope < 0.01) compared with those obtained in the presence of LMS (○; regression line: $[\text{NH}_4^+] = 1.436 - 0.159 \text{ pH}$, P value for slope < 0.01)

Each point represents a separate perfusion.

performed in triplicate on three paired samples taken 5 min apart after an equilibration period of 35 min. There is a decrease in ureagenesis with pH which is linear, at least in the upper part of the pH range. It is matched by a shift in glutamine balance from net consumption to net production. Fig. 2 demonstrates that net NH_4^+ consumption decreases under these conditions as pH falls. NH_4^+ not consumed in ureagenesis is inadequately scavenged by the perivenous cells responsible for glutamine synthesis, thus permitting increasing quantities of NH_4^+ to escape from the liver as the pH falls (Fig. 3). Fig. 3 also shows data from studies in which LMS was present to inhibit glutamine synthesis; the effluent concentration of NH_4^+ is higher in the presence than in the absence of LMS, especially at the normal end of the pH scale. The cells responsible for glutamine synthesis are few in number and confined to the immediate vicinity of the centrilobular vein.

Fig. 4. Glutamine production from rat livers perfused at normal pH (7.4) with NH_4^+ (0.6 mM) and various concentrations of (a) lactate and (b) glucose

Rates are expressed as nmol/min per g body wt. Each point represents a separate perfusion. See the text for details.

Table 1. Glutamine production from perfused livers with lactate or glucose as substrate

Glutamine production (nmol/min per g body wt.) from livers perfused at pH 7.4 or 7.0 with NH_4^+ (0.6 mM) and either lactate (2 mM) or glucose (10 mM) as substrate in bicarbonate-buffered Krebs medium. Results are means $\pm$ S.E.M.

Substrate	Perfusion at...	Glutamine synthesis	
		pH 7.4	pH 7.0
Lactate (2 mM)		2.62 ± 0.07 ($n = 5$)	1.88 ± 0.10 ($n = 5$)
Glucose (10 mM)		1.13 ± 0.03 ($n = 4$)	0.80 ± 0.22 ($n = 5$)
		$P < 0.01$	$P < 0.01$

If it is assumed, as is likely, that glutamine synthesis constitutes the major pathway of NH_4^+ disposal by these cells, then the concentration of NH_4^+ in the effluent from LMS-treated livers will represent that reaching those centrilobular cells in untreated livers. Fig. 3 therefore shows that in acidotic conditions the concentration of NH_4^+ reaching the glutamine-synthesizing cells is increased.

Derivation of the carbon skeleton of glutamine

Figs. 4(a) and 4(b) demonstrate the relationship of glutamine production to either lactate or glucose concentration over a physiological range of portal-vein concentrations of these substrates in the presence of NH_4Cl (0.6 mM) at pH 7.4. In the absence of glucose or lactate there is a basal glutamine production of approx. 2 nmol/min per g body wt., presumably from endogenous substrates. Glutamine production was somewhat lower than in the experiments of Häussinger [6]; this finding is possibly the result of our use of starved rather than fed rats [13]. Increasing the concentration of glucose was not accompanied by an increased glutamine production. In contrast, raising the concentration of lactate resulted in a rise in the rate of production of glutamine, reaching a near-maximal rate at 1.5 mM. This greater rate of production of glutamine from lactate was seen to hold true at both normal pH (pH 7.4) ($P < 0.01$) and under acidotic conditions (pH 7.0) ($P < 0.01$) (Table 1).

When livers were perfused with $[\text{U}-^{14}\text{C}]\text{lactate}$ ($n = 8$), or $[\text{U}-^{14}\text{C}]\text{glucose}$ ($n = 8$), in the context of a near-physiological substrate mixture (see the Materials and methods section), the specific radioactivity of the resulting glutamine (after separation, and correction for the original specific radioactivity of the substrates) was significantly higher from $[\text{U}-^{14}\text{C}]\text{lactate}$ (mean

Fig. 5. Net glutamine balance from livers perfused under conditions described in Fig. 1 in the absence (●) and in the presence (○) of LMS

Rates are expressed as nmol/min per g body wt. Each point represents a separate perfusion; + represents output. For regression equations see the text.

Fig. 6. Glutamine production from livers perfused at different pH values with lactate (2 mM), ornithine (0.1 mM), NH_4^+ (0.6 mM) and pyruvate (0.3 mM)

Rates are expressed as nmol/min per g body wt. Each point represents a separate perfusion. There is no significant regression.

8.7 d.p.m./mmol; range 4.5–12.6) than $[\text{U-}^{14}\text{C}]$ glucose (mean 3.4 d.p.m./mmol; range 0–14.7; $P < 0.05$). Because radioactivity was detectable in glutamine in only two of the eight experiments

with $[\text{U-}^{14}\text{C}]$ glucose, but in all experiments with $[\text{U-}^{14}\text{C}]$ lactate, the Wilcoxon signed-rank test was used for comparison. These results suggest that when glucose and lactate are simultaneously presented at physiological concentrations lactate is the preferred substrate for glutamine synthesis.

Glutamine balance

Fig. 5 shows the effect of varying the perfusate pH on glutamine balance across the liver. The perfusate contained lactate (2 mM), glutamine (0.6 mM), NH_4Cl (0.6 mM), pyruvate (0.3 mM) and ornithine (0.1 mM). In one group of animals (control) no LMS was used (results shown in Fig. 1); in the second group the animals were pre-treated with LMS, and LMS was added to the perfusate to inhibit glutamine synthesis. In the control group there is a shift from a marked net uptake of glutamine at normal pH to a modest net output in acidosis. Similar changes in glutamine balance occur with pH in the LMS group, but the data points are displaced downwards and net output is never achieved. If it is assumed that the data represent a linear regression, the regression lines for the two groups ($y = 180 - 25.036x$ without LMS, and $y = 152.65 - 22.267x$ with LMS, P values for both slopes < 0.01) are almost parallel over this pH range. From analysis of covariance, there is no evidence that the two lines are not parallel ($P = 0.69$); there is, however, a highly significant difference in intercept ($P < 0.001$). The vertical difference between the two lines represents glutamine synthesis, which is therefore constant over this pH range under these conditions. This conclusion is supported by observations recorded in Fig. 6, in which glutamine and LMS have been excluded from the perfusate, so that the only contribution to glutamine balance is synthesis; again, no change in the rate of glutamine synthesis is seen over this pH range. Although it could be argued that the data in Fig. 5 could represent a biphasic response, with a positive shift of glutamine balance linear with pH in the upper half of the pH range studied, but little change at the lower pH values, this would not alter the conclusion that glutamine synthesis is unaffected by pH changes, since if curves were fitted to the data of Fig. 5 they would remain essentially 'parallel'.

Chronic acidosis

All the above results were obtained from experiments in which acute metabolic acidosis was simulated by adjusting the composition of the perfusate. Table 2 shows results obtained from animals exposed to a period of chronic acidosis compared with seven controls. Venous blood was obtained on day 4 immediately before perfusion. A right-atrial blood pH < 7.3 was achieved within 24 h of starting the acidic feed and was maintained in the acidotic range for 4 days. The control animals had the same initial right-atrial blood pH, the same initial weight and the same

Table 2. Central venous blood NH_4^+ and urea concentrations and glutamine production in livers from chronically acidotic rats

NH_4^+ and urea concentrations in central venous blood obtained from chronically acidotic rats (ingestion of HCl over 4 days), immediately before perfusion at pH 7.0. Glutamine production is the average of three observations made over the period 35–40 min of perfusion, and is the mean of seven studies and expressed as nmol/min per g body wt. Results are means $\pm$ S.E.M.; NS, not significant.

Rats	Central-venous pH		Central-venous [urea] (mmol) Day 4	Central-venous $[\text{NH}_4^+]$ (mmol) Day 4	Glutamine production (nmol/min per g)
	Day 0	Day 4			
Control	7.374 (0.011)	7.387 (0.018)	3.73 (0.29)	0.11 (0.03)	145.3 (16.6)
Acidotic	7.394 (0.012)	7.214 (0.021)	2.30 (0.31)	0.23 (0.05)	153.4 (14.7)
	NS	$P < 0.01$	$P < 0.01$	$P < 0.05$	NS

weight of food was eaten, although weight gain was less in the experimental group. Adaptation to the acidosis was seen to have occurred, central venous blood from the acidotic animals having a lower mean urea concentration ($P < 0.01$) and a higher mean NH_4^+ concentration than controls ($P < 0.05$; Table 2). Isolated liver perfusions were then established by using 0.6 mM- NH_4Cl and 2 mM-lactate as substrates at pH 7.0 in Krebs buffer with pyruvate (0.3 mM) and ornithine (0.1 mM). As shown in Table 2, there was no significant increase in the rate of glutamine synthesis from the livers obtained from chronically acidotic animals.

DISCUSSION

Recent models of acid-base regulation have drawn attention to systems intrinsic to the liver that have potential homeostatic roles [5,14]. One of these is the control of ureagenesis by pH. The synthesis of one molecule of urea from CO_2 and NH_4^+ is accompanied by the production of two protons, which are normally mainly consumed in the neutralization of bicarbonate generated by oxidation of the carbon skeletons of amino acids [15]. Acidosis has long been known to depress urea synthesis [14,16] by inhibition both of hepatic glutaminase [17] and of incorporation of NH_4^+ into urea [18]. In this way proton production and subsequent neutralization of bicarbonate would be decreased, leading to amelioration of the acidosis.

Häussinger *et al.* [19] have drawn attention to the possible role of hepatic acinar organization, ureagenesis being principally located in the periportal zone. It was proposed that in acidosis increased quantities of NH_4^+ , which had not been utilized periportally for ureagenesis, passed to a small number of specialized cells in the immediate perivenous region, for incorporation into glutamine by a high-affinity pathway; glutamine synthesis, unlike ureagenesis, does not result in proton generation. This 'switch' of the route of nitrogen disposal therefore constituted both an acid-base homeostatic system and a potentially effective method of NH_4^+ detoxification.

The present studies were directed at two aspects of this proposed intrinsic hepatic system. Firstly, no attention has previously been paid to the acid-base consequences of the source of the carbon skeleton of glutamine synthesized in the liver. As shown in the Appendix, the acid-base advantage of the switch from urea to glutamine synthesis occurs if the glutamine carbon skeleton is derived from lactate, but not if it is derived from glucose. These two carbohydrates are the most obvious possible candidates as substrates for glutamine synthesis, because of their relatively high concentrations. The most obvious interpretation of the 3-fold increase in glutamine synthesis observed (Fig. 4) at perfusate lactate concentrations > 0.5 mM (compared with the rate in the absence of lactate) is that lactate is being used for the carbon skeleton of glutamine. Other interpretations are, however, possible; for instance, a change in redox status induced by lactate might stimulate reductive amination of 2-oxoglutarate. However, we have shown (M. K. Almond, R. D. Cohen & A. Smith, unpublished work) that neither addition of 3-hydroxybutyrate or acetoacetate in high concentrations to the perfusate, nor omission of pyruvate, affects the rate of glutamine synthesis; an explanation based on changes of mitochondrial or cytosolic redox is therefore unlikely. Support for the hypothesis that lactate contributes directly to the carbon skeleton of glutamine comes from the observation of incorporation of label from [^{14}C]lactate into glutamine; the absolute contribution of lactate cannot, however, be calculated from the isotope data (see the Materials and methods section).

Other substrates may contribute in the physiological situation. Although glutamate derived from periportal hydrolysis of glut-

amine is not incorporated into newly synthesized glutamine [20], portal-vein glutamate may be. It can be shown that portal-vein glutamate and 2-oxoglutarate may both make small contributions to the carbon skeleton of new glutamine under near-physiological conditions [21,22].

The above arguments suggest that at physiological concentrations lactate may be a major contributor to the carbon skeleton of glutamine, and, in view of the lack of contribution from glucose, one necessary condition for operation of the scheme proposed by Häussinger [2] is therefore satisfied. The data show that lactate is preferred over glucose at both pH 7.4 and 7.0. It should be noted that new glutamine synthesis from glutamate or 2-oxoglutarate would also be unaccompanied by net proton generation. We also showed that urea synthesis is inhibited linearly by decrease in pH, at least in the upper part of the pH range 7.4–7.0, thereby demonstrating the responsiveness of the system even to modest falls in perfusate pH.

The second purpose of the study was to demonstrate whether the previously reported [16] switch in acidosis from net glutamine uptake to net output resulted from a decrease in glutamine disposal, increase in glutamine synthesis, or, as favoured by Häussinger [6], a combination of both elements. By inhibiting glutamine synthesis we demonstrated that the switch from negative to positive glutamine balance across the liver in acidosis was entirely due to inhibition of glutamine disposal. Glutamine synthesis did not vary with pH, despite the higher concentration of NH_4^+ presented to the perivenous cells in acidosis (Fig. 3); this contrasts with the marked variation of glutamine synthesis observed at pH 7.4 with change in lactate concentration (Fig. 4a) and in response to changes in NH_4^+ concentration over the same range, 0–0.8 mM (results not shown). Since the NH_4^+ concentration was higher in the hepatic venous effluent in acidosis than at normal pH, this suggests failure of perivenous cells to scavenge efficiently (in the way previously suggested [2,5]) the NH_4^+ rejected by the periportal cells. It may be calculated from the slopes of the regression lines in Figs. 1 (not shown) and 2 that the extra NH_4^+ appearing in the effluent is only about 60% of that expected from the inhibition of urea synthesis. Part of this shortfall may be attributed to the inhibition of periportally located glutaminase in acidosis, thereby decreasing the amount of NH_4^+ presented to the ureagenesis pathway, which is itself inhibited by acidosis [18]. It is also possible that alternative pathways of NH_4^+ disposal, e.g. alanine formation, may also contribute to the shortfall. It may be seen from Figs. 1 and 3 that under the conditions of these experiments glutamine synthesis only accounts for about 10% of the disposal of nitrogen (urea + glutamine) at pH 7.4; at pH 7.0 the fraction rises to 38% (although glutamine synthesis is unchanged in absolute terms).

Though these acute studies give little support to the proposed role of hepatic glutamine synthesis as part of an acid-base regulatory system, it was possible that the situation in chronic acidosis might be different, for example, because of induction of enzymes concerned in glutamine synthesis. However, this suggestion is not supported by the present observation that, in livers taken from chronically acidotic rats and perfused at pH 7.0, no increase in glutamine synthesis could be demonstrated over that in livers from control animals similarly perfused. These observations are a functional counterpart in intact liver to the finding that maximum hepatic glutamine synthetase activity *in vitro* is not elevated in chronic acidosis [23]. It is noteworthy that these chronically acidotic animals showed raised plasma NH_4^+ and decreased plasma urea concentrations; though with the data available it is not possible definitely to attribute these changes to inhibition of urea synthesis by acidosis and failure of efficient perivenous scavenging of NH_4^+ , they are compatible with such effects.