Interrelations between Ureogenesis and Gluconeogenesis in Isolated Hepatocytes

THE ROLE OF ANION TRANSPORT AND THE COMPETITION FOR ENERGY

By ANNA B. WOJTCZAK, ELŻBIETA I. WAŁAJTYS-RODE and MATH J. H. GEELLEN*
Department of Cellular Biochemistry, Nencki Institute of Experimental Biology, 02-093 Warsaw, Poland

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1. Glucose synthesis from lactate plus pyruvate and from lactate plus alanine was measured in the presence or absence of 1 mM-oleate or 2 mM-octanoate at low (2 mM) or high (8 mM) concentrations of NH_4Cl . 2. Both fatty acids alone or with 2 mM- NH_4Cl doubled glucose production from lactate plus pyruvate. Glucose synthesis from lactate plus alanine, in the presence of oleate, was decreased 16% by 2 mM- NH_4Cl . 3. In the presence of fatty acids, 8 mM- NH_4Cl decreased gluconeogenesis by 60–65% from both lactate plus pyruvate and lactate plus alanine. This inhibition was correlated with a high accumulation of aspartate and a drastic decrease in 2-oxoglutarate and malate in the cells. 4. In the presence of 2 mM- or 8 mM- NH_4Cl , oleate and glucogenic precursors, the addition of 2.5 mM-ornithine stimulated urea synthesis. 5. This was paralleled by a decrease of 16% in glucose synthesis from lactate plus pyruvate in the presence of 2 mM- NH_4Cl and had no effect at 8 mM- NH_4Cl . In the system producing glucose from lactate plus alanine, ornithine completely reversed the inhibition caused by 2 mM- NH_4Cl and only partly that by 8 mM- NH_4Cl . 6. Gluconeogenesis from pyruvate was also inhibited by 2 mM- NH_4Cl in the presence of oleate or ethanol. This was due to the decrease of malate, which is the C_4 precursor of glucose in this system. 7. The limitation of gluconeogenesis by 2-oxoglutarate and malate concentrations in the liver cell and the competition for energy between glucose and urea synthesis is discussed.

In the last few years it has been established that the transport of anions between the cytosol and mitochondria can limit gluconeogenesis and ureogenesis. This was demonstrated in perfused liver (Williamson *et al.*, 1970) and in isolated hepatocytes (Stubbs & Krebs, 1975; Meijer *et al.*, 1975). Stubbs & Krebs (1975) observed that the massive accumulation of aspartate in the presence of ethanol and ammonia caused a very strong inhibition of glucose synthesis from lactate. This inhibition was correlated with a drastic diminution of 2-oxoglutarate content in the cells, and was explained as a disturbance of the transamination of aspartate in the cytosol. The addition of ornithine accelerated the urea cycle and could prevent aspartate accumulation, partly releasing the inhibition of gluconeogenesis. A similar observation has been made by Meijer *et al.* (1978), who found a large accumulation of aspartate and glutamate in the presence of lactate, oleate and ammonia. This was also accompanied by a strong depression of glucose synthesis. It should be noted, however, that both Stubbs & Krebs (1975) and Meijer *et al.* (1978) used only a high (10 mM), non-physiological, concentration of ammonia.

* Permanent address: Laboratory of Veterinary Biochemistry, State University of Utrecht, Utrecht, The Netherlands.

In the present study we wanted to establish (a) how much the transport of oxoglutarate and malate from mitochondria to the cytosol can affect gluconeogenesis from various precursors in the presence of low and high concentrations of ammonia (as NH_4Cl), and (b) whether the competition for ATP between urea and glucose syntheses can limit the rate of either pathway.

Methods and Materials

Liver cells from male rats deprived of food for 24 h were prepared by the method of Berry & Friend (1969) as modified by Geelen & Gibson (1976). Cells (20–30 mg wet wt./ml) were incubated in Krebs & Henseleit (1932) bicarbonate buffer containing 0.84 mM- Ca^{2+} , which was one-third of the concentration originally described, and 1% defatted and dialysed bovine serum albumin (Chen, 1967). The concentrations of substrates and other details are described in the legends to Figures and Tables. Incubations were conducted in 25 ml Erlenmeyer flasks at 37°C with shaking (90 oscillations/min). During the incubation, vessels were continuously gassed with O_2/CO_2 (19:1). Incubation was terminated by addition of HClO_4 (final concn. 0.4 M).

Metabolites were determined in the neutralized extracts by use of enzymic assays.

For the determination of cell constituents and their distribution between cells and the medium, hepatocytes were separated by rapid centrifugation at 8000g for 1min (Unipan Microfuge, model 320; Unipan, Warsaw, Poland) through silicone oil AR 100 (Wacker-Chemie G.m.b.H., Munich, Germany). Cell suspension (0.5–0.8ml, representing 10–15mg wet wt.) was layered on 0.6ml of the silicone oil and 0.2ml of 8% (w/v) HClO_4 . The amounts of metabolites estimated in the cells were not corrected for adherent medium.

Glucose was determined spectrophotometrically (Unicam SP.700 recording spectrophotometer) with glucose oxidase (Bergmeyer & Bernt, 1974), urea with glutamate dehydrogenase followed by urease (Gutmann & Bergmeyer, 1974), and ketone bodies with 3-hydroxybutyrate dehydrogenase (Williamson & Mellanby, 1974). Aspartate, glutamate, 2-oxoglutarate, malate and lactate were assayed fluorimetrically (Perkin-Elmer fluorescence spectrophotometer MPF-3L) with the use of glutamate-oxaloacetate transaminase, glutamate dehydrogenase, malate dehydrogenase and lactate dehydrogenase respectively and by standard methods (Williamson & Corkey, 1969).

Enzymes, coenzymes and intermediary metabolites were obtained from Sigma Chemical Co. (St. Louis, MO., U.S.A.) or Boehringer Mannheim G.m.b.H. (Mannheim, Germany). Bovine serum albumin (fraction V) was obtained from Sigma. Collagenase

from *Clostridium histolyticum* (grade II) was the product of Boehringer, and amino-oxycetate was from Eastman-Kodak (Rochester, N.Y., U.S.A.).

Results

In Table 1 the effects of 2mM- and 8mM- NH_4Cl and fatty acids on glucose formation from lactate plus pyruvate were compared. Although the aspartate content of the cells was doubled, glucose synthesis was stimulated only slightly or not at all by 2mM- NH_4Cl . Oleate or octanoate increased both gluconeogenesis and the aspartate content by 100%. Fatty acids plus 2mM- NH_4Cl had additive effects on aspartate formation without further increasing glucose synthesis. 2-Oxoglutarate decreased significantly in these conditions. In the presence of fatty acids, 8mM- NH_4Cl caused about 60% decrease of glucose synthesis, a tenfold rise in aspartate content, a further decrease of 2-oxoglutarate and a decrease of malate. This is in agreement with the previous observations by Stubbs & Krebs (1975) and Meijer *et al.* (1978). The high rate of ketone-body production with oleate and octanoate showed that added fatty acids were oxidized in the cells very rapidly (Table 1). NH_4Cl significantly lowered the 3-hydroxybutyrate/ acetoacetate ratio, indicating a shift in the glutamate dehydrogenase reaction in the direction of glutamate formation. This shift was greatly dependent on NH_4Cl concentration; the subsequent transamination of glutamate with oxaloacetate led to an accumulation of aspartate that was especially high at 8mM-

Table 1. *Effects of NH_4Cl and fatty acids on glucose production and the content of aspartate, 2-oxoglutarate and malate in the cells in the presence of lactate plus pyruvate*

Cells were incubated for 30min as described in the Methods and Materials section. The initial concentrations of the substrates were: 10mM-lactate, 1.5mM-pyruvate, 1mM-oleate and 2mM-octanoate. Glucose and ketone bodies were determined in the suspension of the cells. The results are expressed as μmol of the product formed/h per g wet wt. The rate of the formation of these products was linear during this time. The amounts of metabolites represent their content in the cells after 30min of incubation. The values are the means $\pm$ S.E.M. for four to eight experiments or for two experiments where S.E.M. values are not indicated. Statistical difference was measured by the paired Student's *t* test against the control without NH_4Cl and fatty acids: * $P < 0.01$; ** $P < 0.02$; *** $P < 0.05$.

Additions	Production of:		3-Hydroxy- butyrate acetoacetate ratio	Content		
	Glucose ($\mu\text{mol/h}$ per g wet wt.)	Ketone bodies ($\mu\text{mol/h}$ per g wet wt.)		Aspartate (nmol/g wet wt.)	2-Oxoglutarate (nmol/g wet wt.)	Malate (nmol/g wet wt.)
None	53.4 $\pm$ 2.2	20.4 $\pm$ 2.1	0.27 $\pm$ 0.01	256 $\pm$ 20	215 $\pm$ 20	306 $\pm$ 29
NH_4Cl (2mM)	61.4 $\pm$ 4.7	19.6 $\pm$ 2.8	0.15 $\pm$ 0.01	526 $\pm$ 51*	132 $\pm$ 25***	199 $\pm$ 22***
Oleate	100.7 $\pm$ 5.2*	55.2 $\pm$ 4.1*	0.89 $\pm$ 0.04*	536 $\pm$ 30*	124 $\pm$ 4*	792 $\pm$ 28*
Oleate + NH_4Cl (2mM)	96.0 $\pm$ 4.8*	44.4 $\pm$ 4.5*	0.60 $\pm$ 0.06*	1086 $\pm$ 50*	68 $\pm$ 4*	705 $\pm$ 66*
Oleate + NH_4Cl (8mM)	33.9 $\pm$ 2.4*	46.0	0.32	11958 $\pm$ 1343*	36 $\pm$ 4*	142 $\pm$ 16*
Octanoate	103.5 $\pm$ 7.7*	110.5 $\pm$ 8.1*	1.43 $\pm$ 0.12*	696 $\pm$ 73*	235 $\pm$ 17	1128 $\pm$ 32*
Octanoate + NH_4Cl (2mM)	97.4 $\pm$ 7.4*	107.5 $\pm$ 3.0*	0.87 $\pm$ 0.18**	991 $\pm$ 15*	223 $\pm$ 11	909 $\pm$ 38*
Octanoate + NH_4Cl (8mM)	41.3	91.4	0.46	14816 $\pm$ 2450*	28 $\pm$ 5*	254 $\pm$ 10

NH_4Cl . It is noteworthy that with 2mM- NH_4Cl the amount of 2-oxoglutarate was higher in the presence of octanoate than in the presence of oleate, but with 8mM- NH_4Cl the amount of 2-oxoglutarate was very low with both fatty acids.

The question then arises as to whether the strong inhibition of gluconeogenesis by 8mM- NH_4Cl and fatty acids is caused mainly through the lack of 2-oxoglutarate or by the utilization of energy to permit the massive accumulation of aspartate. To examine the first possibility the experiments were performed with alanine as glucogenic precursor. To eliminate the dependence of glucose synthesis on the supply of reducing equivalents, a mixture of lactate and alanine was used. Fig. 1 shows that the maximum rate of gluconeogenesis was attained at an alanine/lactate ratio of 1:5. This system is more sensitive to NH_4Cl than that with lactate plus pyruvate; 2mM- NH_4Cl plus oleate caused a significant decrease of glucose synthesis that is also correlated with the decrease in 2-oxoglutarate. The inhibition by NH_4Cl was increased by increasing the alanine/lactate ratio. Glucose production of 50 $\mu\text{mol/h}$ per g wet wt. corresponds to an oxoglutarate content in the cells of about 30 nmol/g wet wt., which occurs in the presence both of 8mM- NH_4Cl with lactate plus pyruvate (Table 1) and of 2mM- NH_4Cl with lactate plus

alanine (Fig. 1). This points to a crucial role of oxoglutarate concentration in the inhibition of gluconeogenesis by NH_4Cl .

The dependence of gluconeogenesis on anion transport and energy requirement was further studied in a system producing glucose and urea simultaneously. In Table 2 the rates of glucose and urea syntheses from two systems of glucogenic precursors, under various metabolic conditions, are shown. In this set of experiments the alanine/lactate ratio of 1 was chosen, which is close to that found in the blood, but the absolute concentrations of these substances were 10 times higher than in the serum. The addition of NH_4Cl significantly increased urea production in both systems, and this was doubled by a further addition of 2.5mM-ornithine. The latter effect confirms the conclusion of Stubbs & Krebs (1975), Meijer *et al.* (1975) and Briggs & Freedland (1976) that the endogenous ornithine in isolated hepatocytes is insufficient for the maximum rate of urea synthesis. In agreement with the data of Williamson *et al.* (1976), 2mM- NH_4Cl appeared to be sufficient for the maximum rate of ureogenesis. In accordance with the experiments shown in Table 1 and Fig. 1, glucose formation was not affected by a low concentration of NH_4Cl in the system with lactate plus pyruvate (a), but was decreased in the system with lactate plus alanine (b). In the system (a) plus 2mM- NH_4Cl , ornithine decreased glucose production by 20%, but in the system (b) plus NH_4Cl it increased gluconeogenesis to the value for the control without NH_4Cl . The stimulating effect of ornithine can be explained by an increase in urea synthesis connected with an enhanced production of malate in the cytosol. In fact ornithine caused an increase of the total amount of malate and decreased the amount of aspartate in both systems. Fig. 2 shows the sequence of changes in the metabolite content in the cells occurring after addition of ornithine to the system (b). The amount of malate in the cells increased almost immediately after addition of ornithine. Subsequently an increase of 2-oxoglutarate was observed, which was probably caused by the acceleration of the exchange of cytosolic malate against mitochondrial 2-oxoglutarate. An enhancement of glucose synthesis appeared to be the last in this sequence of events. The difference in the amount of malate between the systems with and without ornithine is more marked when measured in the cells after separation (Fig. 2) than in the total suspension (Table 2). Fig. 2(a) shows that urea production is linear with time up to 30 min.

Although ornithine was able to reverse the inhibitory effect of 2mM- NH_4Cl on gluconeogenesis from alanine, this releasing mechanism was apparently not efficient enough at a high NH_4Cl concentration (Table 2). In both systems gluconeogenesis was strongly decreased, the amount of malate

Fig. 1. Effect of NH_4Cl on gluconeogenesis from lactate plus various concentrations of alanine

Cells were incubated for 30 min in standard conditions as described in the Methods and Materials section. The values show the results of a representative experiment; 5mM-lactate and 0.5mM-oleate were present in all incubations. ●, No other additions; ▲, plus 2mM- NH_4Cl . Numbers in parentheses represent the content of 2-oxoglutarate (nmol/g wet wt.) in the cells after 30 min of incubation.

Table 2. Comparison of the effects of NH_4Cl and ornithine on glucose and urea formation from: (a) 10 mM-lactate plus 1.5 mM-pyruvate and (b) 5 mM-lactate plus 5 mM-alanine

Cells were incubated for 30 min as described in the Methods and Materials section. Oleate (0.5 mM) was present in all incubations. The concentration of ornithine was 2.5 mM. The results are expressed as μmol of the product formed/h of incubation per g wet wt. The rates of glucose and urea formation were linear with time during this period, except for the urea production with 2 mM- NH_4Cl . In this case linearity was observed only up to 30 min and the rate for 1 h was extrapolated. The values for metabolites represent the total amounts accumulated in the cell suspension after 30 min of incubation. The results are the means $\pm$ S.E.M. for three to six experiments, and for glutamate are the means for two experiments. Values that are statistically different by paired Student's *t* test are indicated by * $P < 0.01$; ** $P < 0.05$; *** $P < 0.1$. For glucose the comparison is made against the control without NH_4Cl . For urea and the metabolites *P* was calculated against the samples with 2 mM- NH_4Cl . † $P < 0.05$; †† $P < 0.1$ (paired difference) against 2 mM- NH_4Cl , ornithine and oleate as a control.

Additions	Glucose ($\mu\text{mol/h}$ per g wet wt.)	Urea ($\mu\text{mol/h}$ per g wet wt.)	Aspartate ($\mu\text{mol/g}$ wet wt.)	Malate ($\mu\text{mol/g}$ wet wt.)	Glutamate ($\mu\text{mol/g}$ wet wt.)
(a)					
None	95.2 $\pm$ 7.3	16.8 $\pm$ 1.7	1.09 $\pm$ 0.32	0.99 $\pm$ 0.08	1.34
NH_4Cl (2 mM)	94.8 $\pm$ 8.4	55.7 $\pm$ 3.7	1.86 $\pm$ 0.42	0.89 $\pm$ 0.01	2.36
NH_4Cl (2 mM) + ornithine	80.2 $\pm$ 10.5*	97.8 $\pm$ 4.1*	0.89 $\pm$ 0.14***	1.16 $\pm$ 0.21	2.56
NH_4Cl (8 mM)	55.2 $\pm$ 5.6*	42.6 $\pm$ 2.4*	13.24 $\pm$ 1.96*	0.40 $\pm$ 0.08*	5.91
NH_4Cl (8 mM) + ornithine	55.7 $\pm$ 4.8*	99.1 $\pm$ 6.3*	6.63 $\pm$ 1.42**	0.46 $\pm$ 0.04*	4.89
(b)					
None	83.6 $\pm$ 3.9	28.6 $\pm$ 6.0	2.66 $\pm$ 0.34	0.44 $\pm$ 0.04	4.77
NH_4Cl (2 mM)	59.4 $\pm$ 0.8*	42.9 $\pm$ 2.9	7.36 $\pm$ 0.88	0.39 $\pm$ 0.02	6.13
NH_4Cl (2 mM) + ornithine	79.4 $\pm$ 4.3	143.2 $\pm$ 11.5*	2.71 $\pm$ 0.48*	0.51 $\pm$ 0.08	4.51
NH_4Cl (2 mM) + ornithine (no oleate)	43.1 $\pm$ 0.7†	147.2 $\pm$ 14.9	1.33 $\pm$ 0.28††	0.27 $\pm$ 0.03†	1.89
NH_4Cl (8 mM)	25.2 $\pm$ 2.1*	35.4 $\pm$ 2.6***	17.81 $\pm$ 0.92*	0.21 $\pm$ 0.02*	5.40
NH_4Cl (8 mM) + ornithine	36.0 $\pm$ 2.3*	147.1 $\pm$ 6.3*	4.55 $\pm$ 0.25**	0.20 $\pm$ 0.01*	4.81

remained very low and the accumulation of aspartate was only partly prevented by ornithine.

An energy limitation of gluconeogenesis at 8 mM- NH_4Cl can be excluded, since ornithine doubled urea production without changing the rate of glucose formation. However, the competition for energy was observed in the system (a) with 2 mM- NH_4Cl (Table 2), where a stimulation by ornithine of urea synthesis was accompanied by 20% decrease in glucose production. The total pool of the carboxylated products (aspartate, malate and glutamate) was also decreased in this case. Furthermore, in the absence of oleate, gluconeogenesis from lactate plus alanine and the amount of carboxylated compounds were decreased by 50%, whereas urea formation was not altered (Table 2b).

The rate of glucose formation in the presence of ornithine with 2 mM- NH_4Cl and oleate is equal in both systems (Table 2) and probably represents the maximum rate of gluconeogenesis when the urea cycle is fully operating.

With pyruvate as the only substrate for glucose synthesis, malate is the main C_4 precursor as well as the source of reducing equivalents (Krebs *et al.*, 1967; Rognstad & Katz, 1970). It might therefore be expected that the addition of ethanol would provide reducing equivalents in the cytosol, thus enabling aspartate to be used as a precursor of glucose. How-

ever, Fig. 3(a) shows that this appears not to be the case. Although glucose synthesis was enhanced by ethanol and oleate, this process was, up to 30 min of incubation, completely insensitive to 0.5 mM-amino-oxyacetate. At the same time a linear production of lactate was observed (Fig. 3b). Since the cells are unable to maintain a gradient of lactate, it appears that reducing equivalents coming from ethanol and oleate oxidation were preferentially used for pyruvate reduction. After 30 min of incubation the concentration of lactate in the medium reached 2.8 mM, and, after that time, glucose synthesis started to be partly sensitive to amino-oxyacetate (Fig. 3a). On the other hand, an almost constant amount of malate was established after 10 min of incubation. This was because malate, in contrast with lactate, is confined mainly inside the cells. The changes in the content of metabolites after addition of NH_4Cl , oleate and ethanol confirm that glucose synthesis during the first 30 min was correlated with the content of malate in the cells (Table 3). Oleate and ethanol exhibited additive effects in this respect. Addition of NH_4Cl caused an increase of aspartate and alanine (results not shown) concomitant with a decrease of malate, resulting in a decrease of glucose synthesis. In no case was gluconeogenesis correlated with oxoglutarate content in the cells. The cell/medium gradients of malate and aspartate measured after 30 min of

Fig. 2. Effects of ornithine on glucose and urea formation and on the content of malate and 2-oxoglutarate in the cells, in the presence of lactate plus alanine plus 2 mM- NH_4Cl

Cells were preincubated under standard conditions in the presence of 5 mM-lactate, 5 mM-alanine, 0.5 mM-oleate and 2 mM- NH_4Cl . After 15 min, ornithine was added (2.5 mM, final concentration). $\circ$, Δ , Control; $\bullet$, $\blacktriangle$, plus 2.5 mM-ornithine. The samples were removed at the times indicated. Glucose and urea were determined in the whole suspension. For determination of malate and 2-oxoglutarate, the cells were rapidly centrifuged through oil (details are described in the Methods and Materials section): (a) production of glucose ($\circ$, $\bullet$) and urea (Δ , $\blacktriangle$); (b) the content of malate ($\text{---}\circ\text{---}$, $\text{---}\bullet\text{---}$) and 2-oxoglutarate ($\text{---}\circ\text{---}$, $\text{---}\bullet\text{---}$) in the cells. The data show the results of a typical experiment.

Fig. 3. Gluconeogenesis from pyruvate

(a) Effect of ethanol (10 mM), oleate (0.5 mM) and amino-oxyacetate (0.5 mM) on glucose production from pyruvate (10 mM), $\circ$, Control; Δ , ethanol and oleate; $\bullet$, ethanol, oleate and amino-oxyacetate. (b) Time course of lactate production (total amounts were measured in the suspension) and malate content in the cells. $\circ$, Lactate formed from pyruvate; Δ , lactate formed in the presence of pyruvate, ethanol and oleate; $\bullet$, malate content in the presence of pyruvate; $\blacktriangle$, malate content in the presence of pyruvate, ethanol and oleate. Numbers in parentheses indicate the concentration of lactate in the suspension of the cells.

incubation were about 100, confirming that these metabolites are mainly intracellular.

Discussion

The present study illustrates that the effect of NH_4Cl on gluconeogenesis depends on its concentration and on glucogenic precursors. These effects can be summarized as follows.

(1) NH_4Cl at low concentration (2 mM) causes an increase of aspartate in the cells and activates gluconeogenesis in the systems utilizing aspartate as

Table 3. *Gluconeogenesis from pyruvate: changes of metabolites in the presence of oleate, NH₄Cl and ethanol*

Cells were incubated for 30 min as described in the Methods and Materials section. The concentrations of the substrates were: pyruvate, 10 mM; oleate, 0.5 mM; NH₄Cl, 2 mM; ethanol, 5 mM. Glucose was measured in the cell suspension. Metabolites were determined in the cells after rapid centrifugation of the suspension. The values represent the means $\pm$ S.E.M. for four experiments or are the means for two experiments.

Additions	Glucose (μ mol/h per g wet wt.)	Aspartate (nmol/g wet wt.)	2-Oxoglutarate (nmol/g wet wt.)	Malate (nmol/g wet wt.)	Cell/medium gradient	
					Aspartate	Malate
None	32.2 $\pm$ 2.8	279	484	362	88	80
Oleate	45.1 $\pm$ 6.4	388	484	453	100	73
Oleate + NH ₄ Cl	26.6	450	—	275	—	—
Ethanol	61.4	234	—	725	—	116
Ethanol + NH ₄ Cl	47.8	468	278	480	—	—
Ethanol + oleate	70.7 $\pm$ 8.6	468	494	906	106	91

C₄ precursor. The activation of gluconeogenesis from lactate by 2 mM-NH₄Cl has been observed by Zahlten *et al.* (1974). A similar activation by lysine was described by Tolbert & Fain (1974), but the effect of lysine could be most likely ascribed to the increased ammonia and glutamate production (Cornell *et al.*, 1974).

(2) On the other hand, NH₄Cl suppressed gluconeogenesis in a system utilizing malate as C₄ precursor. This was observed in our experiments with pyruvate and by Zahlten *et al.* (1974).

(3) NH₄Cl in the presence of ethanol or fatty acids had an inhibitory effect on gluconeogenesis when used at 8–10 mM with lactate plus pyruvate and at 2 mM with lactate plus alanine. This was explained by Stubbs & Krebs (1975) and Meijer *et al.* (1978) as being due to the lack of 2-oxoglutarate required for the transamination involved in the transport of the C₄ skeleton. The extent of this inhibition, as found in the present study, was not strictly dependent on the concentration of NH₄Cl. However, the rate of glucose synthesis was correlated with the amount of 2-oxoglutarate and malate in the cells. This is in line with the evidence presented by Parrilla & Ayuso-Parrilla (1976) that the content of 2-oxoglutarate limits gluconeogenesis from alanine in perfused liver.

Stubbs & Krebs (1975) observed an apparent activating effect of ornithine on gluconeogenesis in the presence of lactate, ethanol and 10 mM-NH₄Cl. Gluconeogenesis from lactate is, however, strongly decreased by ethanol (Krebs *et al.*, 1969), in contrast with fatty acids, which are potent activators. Therefore, the effect of ornithine may be considered as activatory or not, depending on the system used for comparison. Actually, there is no discrepancy between the results of Stubbs & Krebs (1975), Meijer *et al.* (1978) and our own because the rates of glucose synthesis from lactate (plus pyruvate) plus 10 mM-NH₄Cl and ornithine in the presence of

ethanol or fatty acids are reasonably comparable.

In agreement with the results of Krebs *et al.* (1976), we observed no inhibition of urea synthesis due to the competition for ATP by gluconeogenesis. However, in contrast with these authors, we were able to find conditions under which gluconeogenesis was inhibited by high urea synthesis. This occurred at low NH₄Cl concentrations (Table 2a) and could not be explained by the shortage of 2-oxoglutarate. Similar inhibition was also observed by van den Meer & Tager (1976) in perfused rat hepatocytes. These authors found the rates of urea and glucose production comparable with those measured in perfused liver (Exton & Park, 1967), but the concentrations of the substrates used in perfusion experiments were much lower, close to those found in the blood. These observations may therefore indicate that the competition for energy between glucose and urea formation can also occur *in vivo*. However, as shown in Table 2(a), ureogenesis can depress glucose formation only when the latter process proceeds at high rate, but not at its half-maximum rate.

Although the present results are in many respects similar to those of Krebs *et al.* (1976), maximum values of urea synthesis were substantially lower in our experiments. Neither were we able to find any additional stimulation by oleate of ureogenesis in the presence of lactate in hepatocytes from rats that were deprived of food. The reason of these discrepancies is not known.

There is also an observation that we are unable to explain. Urea production at 8 mM-NH₄Cl is lower by 20% than at 2 mM-NH₄Cl. This difference occurs only in the absence of ornithine. This may be related to the observation by Stubbs & Krebs (1975), who found that ethanol inhibited urea synthesis in the presence of lactate plus 10 mM-NH₄Cl, and this inhibition was fully reversed by ornithine. In both cases the inhibition of urea synthesis was accompanied by a massive accumulation of aspartate.

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