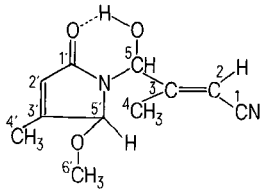
J = ~ 1 Hz, 2 $-CH_3$), 3.26 (s, 3H, $-OCH_3$), 5.23 (d, J = 9 Hz, 1H, $-CH(OH)-$), 5.56 (m, 1H, $-(CH_3O)CH-N=$), 5.76 (m, 1H, =CH−), 5.93 (m, 1H, =CH−), and 6.25 (d, J = 9 Hz, 1H, D_2O exch.); ^{13}C-NMR (20.1 MHz, in DMSO-d_6, ppm) 171.0 (C-1′), 161.5 and 160.8 (C-3 and C-3′), 120.2 (C-2′), 117.3 (C-1), 96.1 (C-2), 81.0 (C-5, C-5′), 55.6 (C-6′), 17.6 (C-4) and 13.3 (C-4′) [see Chen et al.[19] for the ^{13}C-NMR of structure **5a**].



17 The possibility that the acetal **3b** undergoes acid catalized isomerization to the thermodynamically more stable **3a** prior to hydrolysis has been ruled out by an independent experiment, in which hydrolysis of Z-isomer of pyruvaldehyde diisopropylacetal did not give aldehyde **4**.
18 Attempt to convert the unsaturated aldehyde **4** by baker's yeast fermentation (F.G. Fisher and O. Wiedermann, Annalen *513*, 265 (1934)) to (3S)-4-hydroxy-3-methylbutyronitrile, which has a chiral carbon corresponding to the natural dihydrozeatin, gave the unsaturated alcohol **5a** instead.
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Effect of glucagon on ethanol oxidation in isolated rat liver cells[1]

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Summary. Addition of glucagon 5 min after ethanol was found to stimulate the rate of ethanol oxidation in hepatocytes isolated from starved rats. This stimulation is of the same order of magnitude as that mediated by asparagine. The glucagon effect is suppressed by antiproteolytic agents such as insulin or NH_4Cl. The stimulating effect of glucagon on ethanol oxidation is probably linked to enhanced proteolysis and an elevated glutamate level in the hepatocytes.

In fasting pigs[2], in rats[3] and in man[4], acute ethanol ingestion has been shown to elevate the blood glucagon level. Conversely, glucagon administration has been observed to result, under defined experimental conditions, in a significantly increased rate of alcohol metabolism[5,6].

Thus, the existence of a relationship between glucagon concentration and ethanol oxidation rate is conceivable. Freshly isolated hepatocytes responsive to hormone stimuli seem to be a useful model to investigate such a connection at the cellular level.

This paper presents the action of glucagon on ethanol oxidation in liver cells isolated from starved rats and incubated either without energetic substrates other than ethanol or in the presence of asparagine, an amino acid which by itself favours ethanol oxidation[7]. For purpose of comparison, the action of insulin and of cortisol have also been investigated.

Materials and methods. Isolated hepatocytes were prepared as previously described[7] from overnight fasted female Wistar rats (180–200 g b.wt), the viability of the hepatocytes being evaluated according to Beaugé et al.[8]. Suspensions of hepatocytes (equivalent to 50 mg liver wet wt/ml) were incubated at 37 °C in Krebs-Ringer bicarbonate buffer pH 7.4 gassed with 95% O_2–5% CO_2. Cortisol 21-phosphate (10 μM) was added 60 min before ethanol (8 mM), NH_4Cl (4 mM) and asparagine (4 mM) 5 min before ethanol, whereas glucagon (0.1 μM) and insulin (0.1 μM) were added 5 min after ethanol. 40 min after ethanol addition, perchloric acid deproteinization was carried out. Ethanol, glucose, urea, aspartate and glutamate were assayed enzymatically in the supernatant according to Bergmeyer and Gawehn[9].

Cortisol 21-phosphate, glucagon and insulin were purchased from Serva (Heidelberg, Germany), L-asparagine from Sigma (Saint-Louis, Mo, USA), enzymes and cofactors from Boehringer (Mannheim, Germany). The purity of

Effect of glucagon insulin, NH_4Cl and asparagine on the rates of ethanol oxidation and on metabolite accumulation by isolated liver cells from starved rats

Additions	Glucagon	Ethanol oxidation	Glucose	Urea	Aspartate	Glutamate
None	−	0.75 ± 0.10 (6)	< 1 (6)	11.4 ± 1.4 (6)	< 0.2 (6)	1.3 ± 0.3 (6)
None	+	1.66 ± 0.22 (6)[a]	1.08 ± 0.22 (6)	18.6 ± 2.2 (6)[a]	1.60 ± 0.25 (6)	2.2 ± 0.6 (6)[a]
Insulin	−	0.86 ± 0.08 (3)	< 1 (3)	11.9 ± 2.8 (3)	< 0.2 (3)	1.5 ± 0.3 (3)
Insulin	+	0.93 ± 0.09 (3)	< 1 (3)	9.1 ± 2.4 (3)	< 0.2 (3)	1.2 ± 0.2 (3)
NH_4Cl	−	0.92 ± 0.20 (3)	< 1 (3)	47.4 ± 3.5 (3)[a]	< 0.2 (3)	0.8 ± 0.2 (3)
NH_4Cl	+	0.70 ± 0.13 (3)	< 1 (3)	44.9 ± 2.7 (3)[a]	< 0.2 (3)	0.9 ± 0.2 (3)
Asparagine	−	1.70 ± 0.10 (6)[a]	13.6 ± 0.9 (6)	36.8 ± 1.9 (6)[a]	10.7 ± 1.6 (6)	2.90 ± 0.25 (6)[a]
Asparagine	+	1.84 ± 0.10 (6)[a]	15.6 ± 0.5 (6)[b]	47.5 ± 10.0 (5)[a,b]	10.1 ± 2.0 (6)	2.78 ± 0.40 (6)[a]
Insuline + asparagine	−	1.95 ± 0.24 (3)	12.4 ± 1.8 (3)	40.6 ± 10.2 (3)[a]	12.8 ± 0.9 (3)	2.4 ± 0.4 (3)[a]

Glucagon (0.1 μM) and/or insulin (0.1 μM) were added 5 min after ethanol (8 mM) whereas NH_4Cl (4 mM) or asparagine (4 mM) were added 5 min before ethanol. The substrate determinations were performed 40 min after ethanol addition. The rates of ethanol oxidation are expressed as μmoles/min per g wet wt and the other substrates as μmoles/g wet wt. Each value is the mean ± SEM of the number of experiments with different cell preparations indicated in parentheses; within each experiment 3 determinations were performed.

Statistical significance: [a] $p < 0.01$ compared to results obtained without any addition except ethanol; [b] $p < 0.01$ compared to results obtained with the same additions except glucagon.

ethanol (Prolabo, France) was checked by gas liquid chromatography. Statistical analysis was performed using Student's t-test.

Results. Conforming previous studies[10], we observed that hepatocytes isolated from starved rats and incubated without substrates other than ethanol oxidize ethanol (8 mM) at a rate of 0.7–0.8 μmoles/min per g wet wt (table). Whereas insulin does not significantly affect this oxidation rate, addition of glucagon leads to a substantial stimulation of this rate (98%), induces an increase in the cellular level of aspartate and especially glutamate (+71%) and stimulates ureogenesis (+69%) and gluconeogenesis (table). Insulin, which does not by itself affect the ethanol oxidation rate, prevents the effects of glucagon on ethanol oxidation, urea formation, aspartate and glutamate concentrations (table). Likewise, NH_4Cl, which enhances strongly urea production, has by itself no apparent effect on ethanol oxidation but inhibits the stimulating effect of glucagon on this oxidation as well as on glutamate accumulation (table). On the contrary, preincubation with cortisol does not influence the effects of glucagon on ethanol oxidation and on urea and glutamate levels although it significantly enhances glucose production (results not shown).

As shown in the table, addition of asparagine has a stimulating effect on ethanol oxidation, the magnitude of which is similar to that exerted by glucagon alone. When glucagon and asparagine are added together no statistically significant additive effects on ethanol oxidation or on the glutamate and aspartate cellular levels are observed, although glucose and urea productions are enhanced. Preincubation with cortisol, which under some conditions exerts a permissive effect on the stimulating action of glucagon on amino acid transport and/or metabolism[11,12], is without apparent effect on ethanol oxidation in the presence of asparagine and glucagon (results not shown). Insulin is also devoid of any effect on ethanol oxidation in the presence of asparagine (table).

Discussion. It has previously been established that glucagon exerts a large number of rapid effects on the liver: enhancement of glycogenolysis, amino acid uptake, gluconeogenesis and ureogenesis[13,14]. The glucagon effects on gluconeogenesis and ureogenesis have been found to be reproducible in isolated hepatocytes and to be related to increased mitochondrial ATP production[15,16]. The present data indicate that glucagon also stimulates ethanol oxidation when hepatocytes from starved rats are incubated without energetic substrates other than ethanol. This effect is furthermore accompanied by increased urea and glucose production and glutamate concentration. Since, under these particular experimental conditions, liver protein is the only possible source of carbon, it seems likely that the stimulating effect of glucagon on ethanol oxidation is linked to the well-known proteolytic action of this hormone[17,18].

The fact that stimulation of ethanol oxidation and increase in the glutamate level induced by glucagon are both prevented by NH_4Cl or by insulin, two well-contrasted proteolysis inhibitors[18–20], adds support to the mechanism suggested.

It is well established that intermediates of the shuttles which promote the transfer of NADH from cytosol to mitochondria, such as glutamate or aspartate, are depleted during the isolation and washing procedures involved in the preparation of isolated hepatocytes from starved rats[21]. The concentration of these metabolites thus becomes the major factor limiting the rate of ethanol oxidation in such cells[22,23]. Therefore, glucagon, through its proteolytic action, may restore aspartate and glutamate levels and consequently may favour the shuttle activity as does asparagine, which also stimulates ethanol metabolism[7,24].

The finding that the ethanol oxidation rate is similar when the cells are incubated with asparagine or glucagon, added either alone or in combination, is surprising, because the increased glucose and urea production occurring when asparagine is added together with glucagon is likely to reflect a stimulation of ATP consumption, which might influence the mitochondrial reoxidation of NADH[7,25]. The lack of further increment in ethanol oxidation when asparagine is added together with glucagon can be related to the finding that it does not influence the cellular glutamate level. Whereas Crow et al.[23] have shown that the ethanol oxidation rate is correlated with the cellular glutamate level during lactate stimulated ethanol oxidation, we have previously observed[7] that such a correlation does not occur when hepatocytes from starved rats are incubated with L-glutamine or L-proline. However, the present results show a positive correlation ($r = 0.917$) between the rate of ethanol oxidation and glutamate concentration in the presence of glucagon and/or asparagine.

Further experiments are required to establish the physiological relevance of the present data to in vivo situations.

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