mucopolysaccharidosis type I, the other component being dermatan sulfate[15]. In addition to that, Gordon et al.[16] have found that GAG were almost exclusively heparan sulfate in the mucopolysaccharidosis type III (Sanfilippo syndrome) and a mixture of heparitin and dermatan sulfate in the type I and II (Hurler and Hurler syndromes). The total GAG concentration was significantly increased (about 25fold) in our Hurler patients. In control livers, heparitin sulfate accounts for 81% of the total GAG content, the other component being dermatan sulfate (22%). In our Hurler patients, liver heparitin sulfate accounts for 82% of the total GAG, but dermatan sulfate content is about 12%. Compared to the uronic acid-containing compounds, changes in the glycoproteinic sugars are small. The most marked effects were obtained for total sialic acid (3fold increase) and total hexoses (50% increase). The above results confirm and extend those of Gordon[16] and Roukema[17] on the content and distribution of acid glycosaminoglycans and glycoproteins in the Hurler syndrome.

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Depression by ethionine of phosphorylating oxidation in hepatic mitochondria

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Summary. Induction of hepatic steatosis and suppression of hepatic ATP levels, protein synthesis and gluconeogenesis subsequent to administration of ethionine may be consequences of interference by this compound with mitochondrial phosphorylation of ADP. The mitochondrial dysfunction is not a direct action of ethionine on the organelle.

Administration of ethionine results in a variety of morphological and biochemical lesions in the liver and other organs of the rat. Ethylation (presumably via S-adenosylethionine) of hepatic nucleic acids occurs[1] and a high incidence of hepatocellular carcinomas result[2]. The aminoacyl-tRNA synthetase does not descriminate between methionine and ethionine, and when the latter is fed it is incorporated into proteins in positions normally occupied by methionine[3]. Therefore, reduction of protein biosynthesis[4,5] is probably indirect and may be due to an energy deficit consequent to an effect on oxidative phosphorylation. It has been proposed that depression of protein biosynthesis occurs when ethionine, replacing methionine in S-adenosylmethionine, traps available adenosine and thereby prevents synthesis of ATP[6].

The ethionine induced fatty liver is preceded by a marked decrease in hepatic ATP levels[7], is prevented by administration of ATP[8], and is thought to be associated with, or a consequence of, inhibition of hepatic protein synthesis via a block in the synthesis of lipoprotein apoproteins for subsequent transport[9]. Ethionine injection also suppresses gluconeogenesis and hepatic ATP levels and results in increased free fatty acids in liver, plasma and adipose. These parameters return to normal simultaneously with recovery of the hepatic ATP concentration[10]. Moreover, ethionine itself is capable of inhibiting induction of fatty livers due to choline deficiency or orotic acid feeding[11] and this may be another reflection of depressed ATP availability and its requirement in triglyceride synthesis. Livers of rats treated with ethionine were found to have an altered ratio and

Table 1. In vitro effect of ethionine on mitochondrial respiratory activities

DL-ethionine (mM)	Glutamate respiration State 3	State 4	RCR	Succinate respiration State 3	State 4	RCR
0	52.2±4.9	10.7±0.3	4.87±0.28	132.9±7.5	24.7±0.7	5.36±0.18
10	52.3±4.1	11.2±0.4	4.68±0.34	129.8±9.3	26.9±1.0[f]	4.81±0.20[f]
20	51.0±4.1	12.4±0.7[e]	4.16±0.42	-	-	-
30	51.0±4.2	13.0±0.4[a]	3.99±0.45	135.2±7.6	28.4±0.7[b]	4.74±0.17[d]
100	54.1±3.4	14.0±0.4[a]	3.85±0.22[c]	139.8±5.6	31.4±0.6[a]	4.45±0.16[b]

Respiratory rates (ng atoms of oxygen consumed/min/mg mitochondrial protein) were determined polarographically at 30 °C with a Clark fixed voltage electrode. The 3 ml reaction mixture (pH 7.4) contained the indicated concentration of DL-ethionine plus 0.33 M mannitol, 5 mM $MgCl_2$, 3.5 mM potassium phosphate, 3.5 mM KCl, 0.33 mM EDTA, 4 mg dialyzed crystalline bovine serum albumin, 1.4 mM L-glutamate or succinate, and mitochondria corresponding to 2.5 mg of mitochondrial protein. RCR is the ratio of the respiratory velocity in presence of 0.4 µmoles ADP (added in 30 µl) (state 3) to the velocity after exhaustion of ADP (state 4). Means ± SE for 5 mitochondrial preparations at each ethionine concentration and 10 control preparations. [a]$p<0.001$; [b]$p<0.005$; [c]$p<0.02$; [d]$p<0.025$; [e]$p<0.05$; [f]$p<0.1$.

depressed turnover of adenine nucleotides as well as a decreased total adenine nucleotide content and these were attributed to a possible reduction in the rate of ATP production which would result from a diminution of oxidative phosphorylation[12].

Since so many of the actions of ethionine have been related directly or indirectly to a possible interference with cellular energy transactions, we examined the immediate effect of this compound on the ATP generating capacity of hepatic mitochondria both in vivo and in vitro.

Materials and methods. For in vitro experiments hepatic mitochondria were prepared from male Sprague-Dawley rats[13] and their respiratory rates and respiratory control ratios (RCR) were determined[13] in media containing DL-ethionine in the concentration range 0–100 mM. For in vivo experiments the rats were injected i.p. with 2 successive doses, separated by 1 h, of 1 mg DL-ethionine per g b.wt in saline. Controls received 0.9% NaCl only. Hepatic mitochondria were prepared and assayed 6 h after the 1st injection. A 2-tailed Student's t-test was used to ascertain levels of significance[14].

Results and discussion. The respiratory control ratio (RCR) is the most sensitive measure of the functional intactness, and hence phosphorylating capacity, of mitochondria and it is defined as the ratio of the ADP stimulated respiratory velocity (active state, state 3) to the velocity obtaining on exhaustion of ADP (resting state, state 4)[15]. Table 1 shows that ethionine had no effect on the active state respiration of hepatic mitochondria oxidizing pyridine nucleotide linked (glutamate) or flavin linked (succinate) substrates. Concentrations of 30 mM or more were required to depress respiratory control. The latter was a consequence of minor stimulation of the resting state respiration and hence enthionine may be considered a very weak uncoupling agent in vitro.

The depression of respiratory control by ethionine was much more pronounced when the compound was administered in vivo (table 2) and it produced, in the treated animals, a 50% reduction in the active state respiration of the organelles. Ethionine itself is not an inhibitor of phosphorylating oxidation since state 3 respiration was not depressed in vitro (table 1). Thus its effect is more complex in the intact animal and results from a more general disturbance of metabolism, e.g. by trapping adenine nucleotides by way of S-adenosylethionine formation[6]. It is not uncommon for metabolically active agents (e.g. ethanol[16,17] and hydrazine[18]) to have different influences on mitochondria when the animal is treated in vivo and when the isolated organelles are exposed to the compound in vitro.

Ethionine-induced depression of respiratory control in hepatic mitochondria of treated animals is therefore another example of a biochemical lesion produced by a lethal synthesis mechanism, as defined by Peters[19]. It is reasonable to assume that depression by ethionine administration of the diverse endergonic processes of protein synthesis[4,5], gluconeogenesis[10] and experimental induction of fatty livers[11] are all at least in part consequences of decreased phosphorylating oxidation in hepatic mitochondria.

Table 2. Effect of ethionine treatment in vivo on mitochondrial respiratory activities

	ng Atoms oxygen/min/mg		
	State 3	State 4	RCR
Control	77.2 ± 9.0	15.4 ± 0.7	4.89 ± 0.35
Ethionine-treated	38.4 ± 6.1*	11.4 ± 0.7*	3.25 ± 0.33*

Ethionine treatment described in 'materials and methods'. Assay conditions as in table 1; substrate: L-glutamate. Means ± SE for 8 rats per group. *$p < 0.005$.

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Bacterial utilization of cyclo(glycyl-L-prolyl)[1]

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Summary. 3 strains of soil bacteria (2 *Achromobacter*, 1 *Flavobacterium*) were isolated by growth on cyclo(Gly-L-Pro) as carbon/nitrogen source. Good growth required yeast extract supplements. Utilization of cyclo(Gly-L-Pro) was inducible. Many efforts failed to obtain active cell-free preparations. Injected radioactive cyclo(Gly-L-Pro) was excreted intact by the albino rat; in contrast, injected radioactive H-Gly-L-Pro-OH was extensively metabolized.

Cyclic dipeptides (diketopiperazines) are found in fungi, yeast and bacteria[2] but little is known concerning their enzymatic synthesis or degradation. Older studies of compounds of this class, as earlier reviewed[3,4], suggested only questionable metabolism by mammals and no cleavage by well-known proteases. Intact *Claviceps* mycelium was reported[5] to degrade cyclo(L-Leu-[U-^{14}C]Pro) and cyclo(L-Val-L-[U-^{14}C]Pro). We are not aware of definitive studies in bacteria of the metabolism of naturally-occurring diketopiperazines.