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]

E. Adams, D.A. Blass, J.B. Harford and J.A. Adams

Department of Biological Chemistry, University of Maryland School of Medicine, Baltimore (Maryland 21201, USA), 6 October 1977

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.

This note reports the isolation from soil of 3 bacterial strains selected by growth on cyclo(Gly-Pro) as carbon-nitrogen source. The 3 strains (DKP-1,2,3) have the respective American Type Culture Collection numbers 27965, 27966, 27967, and were classified as *Achromobacter* (DKP-1,2) or *Flavobacterium* (DKP-3). Cyclo(Gly-Pro) was made on a large scale by hydrogenolysis[6] of Z-Gly-L-Pro-NH_2, prepared as described earlier[6] except that Z-Gly-OH was coupled with H-Pro-OBzl,HCl by use of dicyclohexylcarbodiimide rather than via Z-Gly-Cl. In small-scale radioactive syntheses, H-L-Pro-[U-^{14}C]Gly-OH or H-Gly-L-[3,4-^{3}H]-Pro-OH was cyclized by heating in phenol[7]. Cells were grown aerobically at room temperature with vigorous stirring in liquid mineral media[8] with the addition of 0.2% cyclo(Gly-Pro), sterilized by filtration. For good growth (up to 300 Klett units[9]), each strain required the addition of small supplements of yeast extract (Difco), routinely added at 0.05%. At full growth we observed disappearance from the medium of cyclo(Gly-Pro) but could identify no specific product. Stock cultures were stored on trypticase soy broth (BBL) slants at 5 °C.

Utilization of radioactive cyclo(Gly-Pro), usually cyclo([U-^{14}C]Gly-Pro), by cells or extracts was assayed by spotting aliquots of the supernatant of incubation mixtures on thin-layer silica gel sheets. Development in n-propanol-methanol-water (7:2:3) for several hours separated residual cyclo(Gly-Pro) (R_f about 0.7) from more polar compounds such as H-Gly-Pro-OH and H-Pro-Gly-OH (R_f about 0.25). Dried sheets were cut into small squares which were placed directly in scintillation fluid for counting. Recovery of expected radioactivity in blank incubations (no cells or boiled cells) was close to 100%.

After growth on cyclo(Gly-Pro), washed whole-cell suspensions (50 mg wet cell weight/ml) of each strain utilized the radioactive substrate (final concentration, 4.4 µmoles/ml; 3.4×10^5 cpm/ml) at a rate of about 2–3 µmoles/ml/h. No radioactive peptide products were detected in these incubations. Most data were obtained with DKP-3. After growth of cells on H-Gly-L-Pro-OH, H-L-Pro-Gly-OH, cyclo(Gly-Gly), glucose-NH_3, or yeast extract, there was no consistent utilization of cyclo(Gly-Pro) by washed cells. In 1 experiment, apparent induction by growth on H-Gly-L-Pro-OH was observed, but could not be repeated; contamination of the open peptide substrate by spontaneous formation of the cyclic peptide on storage, is a possibility. After growth on cyclo(Gly-Pro), washed cells stored at 5 °C (1 week) or at −15 °C (4 weeks) completely lost ability to utilize the substrate.

Many attempts failed to obtain cell-free preparations of DKP-3 capable of utilizing cyclo(Gly-Pro). These included long or short periods of sonication (Branson Sonifier), extraction of acetone-dried cells, grinding with alumina, treatment in the Braun homogenizer or the French press, and exposure to lysozyme. Recombination of centrifugation fractions after various methods of cell breakage, or addition of yeast extract or boiled whole cells of DKP-3, failed to stimulate utilization of the cyclic dipeptide. More limited efforts to extract active enzyme from the other 2 cell strains, by sonication or alumina grinding, were also unsuccessful.

It is of parenthetic interest that s.c. injection of cyclo(Gly-L-[3,4-^{3}H]Pro) (5.8×10^5 cpm) in a 200-g albino rat led to 80% recovery of radioactivity in the urine (24 h), essentially all in the intact cyclic peptide, by chromatography. In contrast, injection of 6.5×10^5 cpm of H-Gly-L-[3,4-^{3}H]Pro-OH in a similar rat resulted in only 10% recovery of radioactivity in urine, of which half appeared to be $^{3}H_2O$, by distillation.

Our difficulty in obtaining active cell-free preparations from bacteria which metabolize cyclo(Gly-Pro), together with the dependence on added yeast extract for cell growth on this substrate, suggest the possibility of an unidentified cofactor requirement and/or a more complex initial reaction than hydrolysis to a free dipeptide. A rat experiment with cyclized and open-chain radioactive Gly-L-Pro was consistent with earlier data (with unlabeled compounds) indicating that a number of cyclic dipeptides are metabolically inert in mammals.

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Effect of relative humidity on the free-living stages of *Strongyloides papillosus* (Rhabdiasoidea: Nematoda)

S. Nath[1]

Department of Zoology, Gaya College, Gaya, Bihar (India), 23 September 1977

Summary. The eggs and larvae are affected by the changes in the humidity level around them. The eggs do not develop below 87% relative humidity at 30 °C and 25 °C. At sub-developmental rel. hum. they remain viable for 30 h at 81%, but at 73% rel. hum. level they do not survive beyond 18 h. Survival of larvae in 100% rel. hum. is longer at 30 °C than at 25 °C and 35 °C, and also they exhibit a poor resistance to desiccation.

The effect of this environmental condition on the free-living stages of various species of *Strongyloides*, including *S. papillosus*, has been studied by a number of workers[2–6] in countries having a temperate climate. Among the environmental parameters that affect the biology of living organisms, rel. hum. plays a significant role in governing the rate of growth, survival, fecundity, and geographical distribution. The present communication is a report on the effect of rel. hum. on the free-living stages of *Strongyloides papillosus*, under tropical conditions.

Materials and methods. In order to get pure infection, young rabbits were infected with 8000–10,000 infective larvae of *S. papillosus*, obtained from cultures of sheep faecal pellets, by placing them on the clean-shaven abdominal skin for 1 h. The animals were kept in wire-bottomed cages separately. The desired level of rel. hum. was obtained in