investigators do not give experimental details such as strain, sex and weight of the rats, and the number of experiments performed, thus making more difficult an evaluation and comparison of their results.

The experiments with thymectomized rats exclude further any analogy between enzyme induction and capacity of immune response, the latter being greatly impaired in neonatally-thymectomized rats[6,7].

Riassunto. La somministrazione di triptofano per via endoperitoneale nei primi giorni di vita non influenza l'induzione della triptofano pirrolasi nel fegato di ratto ad opera del triptofano o del cortisone somministrato a 20–25 giorni di età. L'induzione dell'enzima é normale in ratti di 30 giorni timectomizzati alla nascita. Questi risultati non confermano l'esistenza di un fenomeno simile alla tolleranza immunitaria nella sintesi adattativa di questo enzima.

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Istituto di Patologia Generale dell'Università di Bologna (Italy), 22nd December 1966.

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[7] We thank Sig. E. Lorenzoni for skilled technical assistance and Merck Sharp & Dohme Italia for a generous gift of Cortone. The work was supported by a grant from Consiglio Nazionale delle Ricerche, Rome.

Effect of D,L-Glyceraldehyde on Bacterial Cells Metabolism

It is only recently that experimental investigations have been directed to the problem of the relationship between energy metabolism and protein synthesis processes. Research on animals and neoplastic cells has been especially concerned with glycolysis, and the oxidation of glucose in relation to the incorporation of labelled aminoacids, the latter being taken as an index of protein synthesis. D-L-glyceraldehyde has proved considerably useful in these studies[1,2] as it inhibits glycolysis at levels which do not affect oxidation. L-glyceraldehyde, in fact, binds the di-hydroxyacetonphosphate with the formation of L-sorbosium-1-phosphate, which is a hexokinase inhibitor[3].

It seemed of interest to extend this study to bacterial cells. In previous investigations we have observed that D-L-glyceraldehyde inhibits the growth of several germs[4]: *E. coli* 817 ISI, *E. coli* B. 806 ISI, *Salmonella typhi*, *Paratyphi C*, *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Brucella melitensis*, *Serratia marcescens*. This inhibition is shown by a decrease in the growth rate, up to a complete absence of development, which is related to the concentration of D-L-glyceraldehyde and to the type of medium used. The results obtained in studies with *E. coli* in culture are summarized in Table I.

The effect of D-L-glyceraldehyde on the glycolysis and oxidation as well as on the leucine-C^{14} incorporation has been studied with *E. coli* 806 ISI cells cultivated in 1‰ glucose Nutrient Broth, collected in log phase by centrifugation and washed 3 times in H_2O. The Warburg procedure was employed for the glycolysis and oxidation. The leucine-C^{14} incorporation was studied by collecting the cells on Millipore filters and measuring C^{14} content in counts/min with a Nuclear Chicago D47 flowmeter. The results are summarized in Tables II and III.

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Table I. Effects of D-L-glyceraldehyde on growth rate of E. coli at 37 °C without shaking

D-L-glyceraldehyde	Media					
	Nutrient broth[a]		G.G.Y.[b]		Glucose minimal medium[c]	
	Growth rate in h^{-1}	%	Growth rate in h^{-1}	%	Growth rate in h^{-1}	%
Control	0.015	100.0	0.021	100.0	0.015	100.0
1.1 m*M*	0.015	100.0	0.022	100.0	0.008	53.3
2.2 m*M*	0.015	100.0	0.019	86.0	0.000	0.0
3.3 m*M*	0.013	86.3	0.006	27.2	–	–
4.4 m*M*	0.010	50.0	0.000	0.0	–	–
5.5 m*M*	0.007	46.6	–	–	–	–

The results are mean values of 5 experiments. [a] Nutrient broth 8‰ + NaCl 5‰. [b] Glucose 10 g ‰, glycine 20 g ‰, extract yeast g 1 ‰. [c] Medium as described by Monod. The growth rate is calculated from Monod: $\mu = \frac{\log x - \log x_0}{t \log 2}$, μ = growth rate; x = density of cells at 't' time; x_0 = density at zero time.

Weak concentrations of D-L-glyceraldehyde (0.05–0.1 mM) increase both the respiration and the leucine-C^{14} incorporation considerably. When only glucose is present there is a marked increase in respiration, and, to a lesser extent, of leucine-C^{14} incorporation. When both glucose and a weak concentration of D-L-glyceraldehyde are present, the respiration and the leucine-C^{14} incorporation increase in the same extent as when only D-L-glyceraldehyde is present.

Middle concentrations of D-L-glyceraldehyde (0.66 mM) inhibit the endogenous respiration, the glucose oxidation, and, to a lesser extent, the leucine-C^{14} incorporation. At higher concentrations (1.32 mM) D-L-glyceraldehyde causes a considerable reduction of the endogenous respiration and only slight inhibition of leucine-C^{14} incorporation. When glucose is added the respiration is slightly inhibited (570–423%, taking the basic respiration as 100) and the leucine-C^{14} incorporation is slightly

Table II. Effect of D-L-glyceraldehyde on respiration and aerobic incorporation of Leucine-C^{14}

	Cells	Cells + D-L-glyceraldehyde 0.05–0.1 mM	Cells + glucose 10 mM	Cells + glucose + D-L-glyceraldehyde 0.05–0.1 mM
CO_2 (μl/h/mg cells dry weight)	− 6.0 (100%)	− 22.4 (373%)	− 15.12 (252%)	− 25.5 (425%)
Leucine-C^{14} (cpm/mg cells dry weight)	435 (100%)	1675 (385.5%)	455 (104.5%)	1630 (374.7%)
	Cells	Cells + D-L-glyceraldehyde 0.66 mM	Cells + glucose 10 mM	Cells + glucose + D-L-glyceraldehyde 0.05–0.1 mM
CO_2 (μl/h/mg cells dry weight	− 5.62 (100%)	− 3.96 (70.4%)	− 32.5 (578.2%)	− 28.05 (499.1%)
Leucine-C^{14} (cpm/mg cells dry weight)	460 (100%)	423 (92.0%)	660 (143.4%)	638 (138.6%)
	Cells	Cells + D-L-glyceraldehyde 1.32 mM	Cells + glucose 10 mM	Cells + glucose + D-L-glyceraldehyde 0.05–0.1 mM
CO_2 (μl/h/mg cells dry weight)	− 5.7 (100%)	− 2.3 (40%)	− 32.5 (570%)	− 24.15 (423%)
Leucine-C^{14} (cpm/mg cells dry weight)	488 (100%)	353 (72.3%)	660 (135.2%)	587 (120.2%)

Cells of *E. coli* (10–15 mg, dry weight) were incubated at 37 °C for 60 min in Krebs-Ringer phosphate containing 0.5 μmoles of D-L-leucine-C^{14} (s.a., 3.8 mc/mmole). Medium volume, 3 ml. Gas phase: air. The results are mean values of 3 experiments.

Table III. Effects of D-L-glyceraldehyde on glycolysis and anaerobic incorporation of Leucine-C^{14}

	Cells + glucose 10 mM	Cells + glucose 10 mM + D-L-glyceraldehyde 0.33 mM	
$QCON_2$ (μl/h/mg cells dry weight)	+ 54.5 (100%)	+ 55.5	102 %
Leucine-C^{14} (cpm/mg cells dry weight)	577 (100%)	653	113.2%
	Cells + glucose 10 mM	Cells + glucose 10 mM + D-L-glyceraldehyde 0.66 mM	
$QCON_2$ (μl/h/mg cells dry weight)	+ 55.9	+ 39.6	70.9 %
Leucine-C^{14} (cpm/mg cells dry weight)	535	447	82.3 %
	Cells + glucose 10 mM	Cells + glucose 10 mM + D-L-glyceraldehyde 1.32 mM	
$QCON_2$ (μl/h/mg cells dry weight)	+ 56	+ 32.3	57.7 %
Leucine-C^{14} (cpm/mg cells dry weight)	630	210	31.8 %

Cells of *E. coli* (10–15 mg, dry weight) were incubated at 37 °C for 60 min in Krebs-Ringer bicarbonate containing 0.5 μmoles of D-L-leucine-C^{14} (s.a., 3.8 mc/mmoles). Medium volume, 3 ml. Gas phase: 95% N_2: 5% CO_2. The results are mean values of 3 experiments.

decreased (135–120%) (Table II). It may be concluded that under aerobic conditions the inhibiting effect of D-L-glyceraldehyde, both on the respiration and the leucine-C^{14} incorporation, is very weak. Under anaerobic conditions, however, the picture is quite different (Table III). The addition of D-L-glyceraldehyde only results in absence of fermentation, with no CO_2 being produced.

The addition of D-L-glyceraldehyde in weak concentrations (0.33 mM) does not result in a reduction of glycolysis or of leucine-C^{14} incorporation, but may even produce a slight increase.

Slight inhibition of glycolysis and aminoacid incorporation begins at concentrations of 0.66 mM. At higher concentrations (1.32 mM) of D-L-glyceraldehyde glycolysis is reduced to about a half, and the leucine-C^{14} incorporation to a third. The results obtained in developing cells and in resting cells are in agreement.

Investigations are currently in progress on the influence of D-L-glyceraldehyde on RNA cellular synthesis to establish at what level the inhibition of protein synthesis begins[5].

Riassunto. In aerobiosi la D-L-gliceraldeide a notevoli concentrazioni ha una lievissima azione inibente sulla ossidazione del glucosio e sulla incorporazione di leucina-C^{14}. In condizioni di anaerobiosi invece la D-L-gliceraldeide in concentrazioni medie inibisce notevolmente sia la glicolisi che la incorporazione di leucina-C^{14}.

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[5] This work was carried out with the help of 'Consiglio Nazionale delle Ricerche' (CNR).

The Presence of a L-Leucyl-β-Napthylamide Hydrolyzing Enzyme in Snake Venoms

Although the hydrolysis of proteins by snake venoms is well recognized, no extensive study has been carried out on venom exopeptidases. The inability of *Crotalus atrox* (Western Diamondback rattlesnake) venom to hydrolyze *N*-carbobenzoxyglycyl-L-phenylalanine, hippuryl-L-arginine, and hippuryl-L-lysine, lead BROWN[1] to the conclusion that carboxypeptidases A and B were absent in this venom. Similar conclusions were drawn by WAGNER and PRESCOTT[2] when *N*-carbobenzoxyglycyl-L-phenylalanine and hippuryl-β-DL-phenyllactate were not hydrolyzed by 4 rattlesnake venoms. In this same investigation, WAGNER and PRESCOTT found no evidence for a true amino peptidase.

Recently, TU et al.[3] studied the di- and tripeptide hydrolyzing ability of 12 species of rattlesnake and 8 cobra species. From this study, it was concluded that many of the peptides hydrolyzed by these venoms were those which would also be hydrolyzed by leucine amino peptidase. Therefore, the synthetic substrate L-leucyl-β-napthylamide, commonly used as a test for leucine amino peptidase, was employed in determining the hydrolyzing ability of a number of species from each family of poisonous snakes.

Enzymatic activity was determined by a modified method of GOLDBARG and RUTENBURG[4]. A solution consisting of 1 ml venom (1 mg/ml) was incubated at 37 °C with 1 ml of 0.02% substrate in 0.01 M phosphate buffer pH 7.1 for 1 h. The reaction was stopped by the addition of 1 ml of 25% trichloroacetic acid. Following

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