cytoplasmic membrane vesicles whose content in PHB is noticeably higher. Thus, cytoplasmic membrane can be considered as a concentration site for PHB. The amount of PHB incorporated in cells is approximately proportional to the applied concentration. The incorporation kinetics show, in the first 2 min of exposure, a very rapid fixation, following which, the speed decrease rapidly. 80 to 100% of PHB incorporated in 30 min is already in the cells after the first 5 min. PHB binds directly to proteins or other SH- containing compounds without involvement of an active transport mechanism; moreover localization does not differ in cells exposed for a short time (5 min) or for a longer time (> 30 min). By comparing with an inorganic mercury, such as Hg $(NO_3)_2$, PHB appears to be more rapidly incorporated, but localization seems to be the same. The inhibitory effect of PHB on protein synthesis was demonstrated in an exponentially growing culture, whose L-leucine incorporation into cells was immediately blocked after addition of PHB.

## Effect of Colimycin on the Decarboxylation of Pyruvate from Serine in *Escherichia coli* and *Citrobacter intermedius*

*Jocelyne Cornu-Favet and Elisabeth Schorer*
*Laboratoire de Microbiologie générale, Département de Biologie végétale, Université de Genève, 3, place de l'Université, CH-1211 Genève 4*

The inhibition of pyruvate decarboxylase by colimycin in *E. coli* and *C. intermedius* has been assayed by enzymatic titration of pyruvate. The bacteria were maintained in undivided conditions since they were deprived of elements necessary for growth. The catabolism of serine passes through pyruvate when they receive the latter only in the form of either D, L or DL. In normal conditions one finds, for example, 0.17% (in *E. coli*) and 0.12% (in *C. intermedius*) of the initially given DL-serine as pyruvate in the medium after 4 h. After treatment with colimycin, pyruvate has a tendency to accumulate in the medium and the values are 1.47% for *E. coli* (about 9 times more) and 9.46% for *C. intermedius* (about 79 times more). However, between 2 and 4 h, the pyruvate in excess represents only 5 to 30% of the amount of serine degraded, no matter the type of serine provided. The decarboxylases producing pyruvate in the bacteria are only partially inhibited.

In conclusions, it seems that there is an inhibition of the pyruvate decarboxylase in *E. coli* and *C. intermedius* by colimycin, although the decarboxylation is not entirely inhibited. This suggests that not all decarboxylases are accessible to the antibiotic. (GOUDA, SCHORER and CHODAT, Archs Sci., Genève *18*, 591–598, 1965; MARTIN-HERNANDEZ, FUENTE-SANCHEZ and SANTOS-RUIZ, Rev. espan. Fisiol. *12*, 143–152, 1956.)

## Antigens Extracted from Oral Microorganisms in Lymphocyte Stimulation

*A. Hefti, J. J. Burckhardt and B. Guggenheim*
*Abteilung für orale Mikrobiologie und allgemeine Immunologie, Zahnärztliches Institut der Universität, Plattenstrasse 11, CH-8028 Zürich*

Periodontal disease can be induced in a gnotobiotic rat model (Infect. Immun. *10*, 565, 1974). Rats monoassociated with *Actinomyces viscosus Ny 1* and fed with a high sucrose containing diet developed large amounts of dental plaque, gingival inflammation and severe bone loss. Antigen dependent lymphocyte reaction was studied in a miniculture lymphocyte stimulation assay. Lymphocytes were collected from either germ-free or conventional Sprague-Dawley rats by canulation of the thoracic duct. These cells were incubated with bacterial antigens for 24 h in serum-free RPMI 1640 medium. After cell harvesting $^3$H-uridine incorporation was measured in a liquid scintillation counter. Lymphocytes from germ-free animals were activated by the extracellular heteroglycan of *A. viscosus*. No stimulation resulted when cells from conventional animals were used. Rantz-Randall extracts prepared from 10 different microorganisms were tested in the same culture system. An increased $^3$H-uridine incorporation was measured only in cells from conventional animals incubated with the extract from *Pseudomonas aeruginosa* and *Proteus mirabilis*. This effect might be due to bacterial endotoxin. A comparison between the lymphocyte activation of germ-free animals and conventional animals suggests the presence of a suppressor factor in cultures with cells from conventional animals. The immune reaction of conventional animals as evaluated by this test system may be compared to that of human peripheral leucocytes obtained from healthy subjects.

## Regulation of the Glyoxylate Cycle in *Acetobacter aceti*

*S. Brunner and L. Ettlinger*
*Mikrobiologisches Institut, ETH-Zentrum, CH-8092 Zürich*

Growth of *Acetobacter aceti* LBG B 4114 on ethanol was inhibited for several hours by glyoxylate but not growth on pyruvate. During growth of the organism on pyruvate, no measureable activity of isocitrate lyase, the key enzyme of the glyoxylate cycle, could be detected. The glyoxylate cycle and especially the enzyme isocitrate lyase appeared therefore as a possible site for the growth inhibiting action of glyoxylate. Either the activity or the synthesis of the enzyme or both could be controlled by glyoxylate. Isocitrate lyase was strongly inhibited by succinate ($I_{0.5(V)} \leq 1.7$ m$M$), inhibited to a lesser extent by phosphoenolpyruvate (2.5 m$M$), malate (5.5 m$M$), oxaloacetate (8.7 m$M$), fumarate (18.2 m$M$) and not at all inhibited by glyoxylate. This is in contrast to observations with *Acinetobacter calcoaceticus* (KLEBER, Acta biol. med. germ. *34*, 723, 1975), where not only the other compounds but also glyoxylate were inhibitors of isocitrate lyase. The time course of growth and specific activity of isocitrate lyase was studied in shake flask experiments with and without glyoxylate in the growth medium. Without glyoxylate, there was a rapid rise of specific activity at the onset of growth, a maximum in the middle of the log phase (7 h) and a sharp decline towards the end of the log phase (10 h). With small amounts of glyoxylate in the growth medium (4 m$M$), there was practically no isocitrate lyase activity as long as growth inhibition by glyoxylate lasted (about 10 h). As soon as about one half of the glyoxylate had disappeared from the medium and growth was resumed, there was an increase in specific activity of the enzyme up to less than half the maximal value of growth without glyoxylate. To our knowledge, repression of isocitrate lyase by glyoxylate has not been observed before.

Supported by SNSF, grant 3.154–0.73