

metabolism is partially modified, but this cannot be ascribed to a decrease of AICR transformylase.

These results seem to demonstrate that riboflavin deficiency determines a decrease of storage of folate coenzymes. This could be considered the common biochemical lesion responsible in teratogenicity observed either in riboflavin or in folate deficiency.

Résumé. Des rats carencés en riboflavine, comparés aux contrôles, ont présenté une remarquable augmentation de la quantité de FIGlu éliminé. Une augmentation

moins évidente a été observée dans l'élimination de AIC. Aucune variation, au contraire, en ce qui concerne les enzymes FIGlu transferase et AICR transformylase.

P. PASQUALI, C. BOVINA,
L. LANDI and M. MARCHETTI

*Istituto di Chimica Biologica e di
Biochimica Applicata dell'Università,
40126 Bologna (Italy), 19 May 1969*

Microbial Degradation of Aliphatic Branched Compounds: Isobutyric, 2,2-Dimethylmalonic and 2,2-Dimethylsuccinic Acids¹

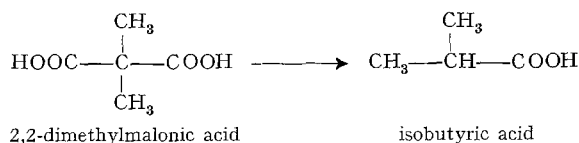
In order to improve research on the microbial degradation of synthetic chemicals in soil, we have been trying to isolate microorganisms able to grow on aliphatic branched compounds supplied as only carbon source. These compounds are very resistant to bacterial oxidation, especially those having a quaternary carbon atom². Concerning this, only KERSTIN³ reports the isolation of microorganisms capable of growing on 2,2,4-dimethylpentane and MOHANRAO and MCKINNEY⁴ the degradation of some quaternary carbon acids by activated sludges.

Results. The enrichment cultures were effected by direct incubation of river waters, added with the mineral salts of TAUSSON medium⁵ and the required carbon source. At present, 3 microorganisms able to oxidize isobutyric, 2,2-dimethylmalonic and 2,2-dimethylsuccinic acids have been isolated.

From enrichments with isobutyric acid a motile, Gram-negative short rod, which produces a fluorescent green-yellow pigment on King B medium, but not phenazine pigment on King A medium⁶, has been isolated. It does not produce gelatinase, and, by the criterion proposed by STANIER et al.⁷, we may consider this organism to be a strain of *Pseudomonas putida*. This microorganism is unable to grow on 2,2-dimethylmalonic and 2,2-dimethylsuccinic acids.

After a long incubation time from an enrichment culture with 2,2-dimethylmalonic acid, a motile, Gram-negative short rod has been isolated which does not produce fluorescent and phenazine pigment, gelatinase and gas from carbohydrates; it may be tentatively assigned to the genus *Achromobacter*. First the growth on 2,2-dimethylmalonic acid was slight but very abundant after several subcultures. This strain is able to grow also on isobutyric acid as only carbon source, not on 2,2-dimethylsuccinic acid. Different items prove that the growth on 2,2-dimethylmalonic acid is really supported by this compound and not by isobutyric acid, which can be formed by non-enzymatic decarboxylation from 2,2-dimethylmalonic acid: 2,2-dimethylmalonic acid, whose purity had been tested after vacuum sublimation at 100°C, was always supplied to the sterile media by amicrobial filtration. *Pseudomonas putida* isolated from isobutyrate enrichment cultures was unable to grow on 2,2-dimethylmalonic acid; washed cells of *Achromobacter* sp. grown on asparagine were not sequentially induced to oxidize 2,2-dimethylmalonic and isobutyric acids, while cells grown on 2,2-dimethylmalonic acid were sequentially induced to oxidize 2,2-dimethylmalonic and isobutyric acids; 2,2-dimethylmalonic acid was oxidized with the production of 2 moles of CO₂ per mole of substrate, while isobutyric acid with the production of 1 mole of CO₂ per mole of substrate. The same

cells, heated at 100°C for 2 min, do not give oxygen uptake and CO₂ release with the above compounds. From this it can be assumed that the first step of the degradation of 2,2-dimethylmalonic acid is the formation of isobutyric acid by enzymatic decarboxylation.



From enrichment cultures with 2,2-dimethylsuccinic acid a motile, Gram-negative short rod has been isolated, different from the above strain, which does not produce fluorescent and phenazine pigment, gelatinase and gas from carbohydrates: it may be tentatively ascribed to the genus *Achromobacter*. This microorganism is able to grow on 2,2-dimethylsuccinic, 2,2-dimethylmalonic and isobutyric acids. Washed cells of this strain grown on 2,2-dimethylsuccinic acid immediately oxidize 2,2-dimethylsuccinic, not 2,2-dimethylmalonic acid. Research is in progress to isolate microorganisms able to grow on hydrocarbons with quaternary carbon atom.

Riassunto. Da colture di arricchimento si è isolato un ceppo di *Pseudomonas putida* capace di crescere in presenza di acido isobutirrico, un *Achromobacter* sp. capace di crescere in presenza degli acidi 2,2-dimetilmalonico e isobutirrico e un altro *Achromobacter* sp. capace di crescere in presenza degli acidi 2,2-dimetilsuccinico, 2,2-dimetilmalonico ed isobutirrico. Esperienze di induzione sequenziale hanno dimostrato che l'acido 2,2-dimetilmalonico viene degradato ad acido isobutirrico per decarbossilazione enzimatica.

C. SORLINI and V. TRECCANI

*Cattedra di Microbiologia del Terreno,
Università di Milano (Italy), 18 April 1969.*

¹ This work was supported by a grant of C.N.R. for the International Biological Program.

² E. J. MCKENNA and R. E. KALLIO, Proc. Rudolph Res. Conf. Rutgers University. *Principles and Applications in Aquatic Microbiology* (Ed. John Wiley & Sons, New York 1964).

³ F. M. KERSTIN, Nature 209, 1047 (1966).

⁴ G. J. MOHANRAO and R. E. MCKINNEY, Proc. 2nd Int. Conf. Wat. Pollution Res. Tokyo 2, 245 (1964) (Pergamon Press).

⁵ W. O. TAUSSON, Planta 4, 214 (1927).

⁶ E. O. KING, M. K. WAND and D. E. RANEY, J. Lab. clin. Med. 44, 301 (1954).

⁷ R. Y. STANIER, N. J. PALLERONI and M. DOUDOROFF, J. gen. Microbiol. 43, 159 (1966).