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EDMOND R. COLE
J. L. KOPPEL
JOHN H. OLWIN

Coagulation Research Laboratory,
Department of Surgery,
Presbyterian-St. Luke's Hospital,
Chicago, Illinois.

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HISTOLOGY

Activity of D(-)- β -Hydroxybutyric Dehydrogenase in Scurvy

LITTLE is known of the mechanism underlying the marked alterations in the structure and metabolism of bones and teeth in scurvy. Various reports indicate that protein synthesis (particularly collagen)¹, carbohydrate metabolism^{2,3} and fatty acid metabolism⁴ are impaired. Since these processes require energy, we have investigated, with histochemical methods, the activity of a number of dehydrogenases involved in metabolism.

Male Hartley guinea pigs weighing about 200 g were fed a scorbutogenic diet⁵ (purchased from Nutritional Biochemicals Corporation, Cleveland, Ohio). Twenty-two test animals received the scorbutogenic diet alone and nine control animals received the diet and a daily supplement of 5 mg ascorbic acid. Four separate experiments were done. The animals were killed after 21 days on the diet. The test animals were found to be markedly scorbutic and resembled the animals described earlier⁶. A right or left side of a mandible and the distal end of a femur were removed from each animal and demineralized with cold buffered ethylenediamine tetraacetic acid⁷. The specimens were then frozen on a block of carbon dioxide ice and sectioned in a cryostat at 12 μ . Sections were placed in appropriate media for the demonstrations of succinic, malic, isocitric, glucose-6-phosphate, 6-phosphogluconate, glutamic, α -glycerophosphate, lactic and β -hydroxybutyrate dehydrogenases and DPN- and TPN-diaphorases according to methods previously described⁸.

Only in sections incubated for the demonstration of D(-)- β -hydroxybutyric dehydrogenase was a distinct and reproducible effect of scurvy observed. A profound depression of D(-)- β -hydroxybutyric dehydrogenase activity in osteoblasts and odontoblasts was invariably seen (Figs. 1 and 2). Levels of D(-)- β -hydroxybutyric dehydrogenase activity were assessed by determining the minimum time required for the deposition of a detectable quantity of formazan in sections from scorbutic and normal animals incubated simultaneously in the same media with D(-)- β -hydroxybutyrate, DPN and either 'Nitro-BT' or 'Tetranitro-BT' (purchased from Nutritional Biochemicals Corporation, Cleveland, Ohio). Whereas observable amounts of reduced tetrazolium (formazan) were deposited in osteoblasts and odontoblasts within 15-20 min in sections from control animals, a 2-h incubation was required for the deposition of the same amount of formazan in these cells in sections from scorbutic animals. On the other hand, osteoclasts did not appear to be affected by the scorbutic state. Apparently equal quan-

Fig. 1. Demineralized section near the apex of a lower molar from a normal guinea pig stained for D(-)- β -hydroxybutyric dehydrogenase. 30 min incubation. Note intense (dark) staining of odontoblasts (O). D, Dentin; P, pulp. ($\times 167$)

Fig. 2. Demineralized section near the apex of a lower molar from a guinea pig on a scorbutogenic diet for 21 days stained for D(-)- β -hydroxybutyric dehydrogenase. 30 min incubation. Note the deficiency of enzymatic activity in odontoblasts (O). A comparable deficiency in osteoclasts, but not in osteoblasts, was also observed. D, Dentin; P, pulp. ($\times 167$)

ties of formazan were deposited in osteoclasts in sections from both scorbutic and control animals after 5 min incubation. Thus, cells concerned with the formation and resorption of mineralized connective tissues are affected disparately by vitamin C deficiency. This finding is in accord with the progressive bone resorption and lack of bone and dentin formation characteristically associated with the scorbutic state. Moreover, this appears to be a specific result of vitamin C deficiency since only D(-)- β -hydroxybutyric dehydrogenase of the several dehydrogenases tested was invariably affected. It is possible that altered activity of β -hydroxybutyric dehydrogenase is an important consequence of vitamin C deficiency since diminished activity of this enzyme would be expected to alter metabolism through various pathways. Further experiments are in progress to determine whether or not this selective suppression D(-)- β -hydroxybutyrate dehydrogenase activity in osteogenic and dentinogenic cells is a primary result of vitamin C deficiency.

HAROLD M. FULLMER
G. R. MARTIN

National Institute of Dental Research,
Laboratory of Histology and Pathology
and Laboratory of Biochemistry,
National Institutes of Health,
Bethesda 14, Md.

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