Ultrastructure and Ion Distribution of the Intestinal Cell during Experimental Vitamin-D Deficiency Rickets in Rats*

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Summary. Rats with an initial weight of 40–50 g developed significant rickets, when fed on a vitamin-D free, low-phosphorus diet continuously for 4 weeks. In contrast, a vitamin-D free, normal-phosphorus diet had no rachitic effect. The rachitic animals showed an elevation of alkaline phosphatase and a decrease of inorganic phosphorus in serum, a reduction of growth and body weight and marked rachitic alterations in bone. Surprisingly, the serum calcium showed a significant elevation, the cause of which will be discussed. Light and electron microscopy revealed only nonspecific signs of metabolic damage, such as swelling of mitochondria, endoplasmic reticulum and Golgi complex in the duodenal cell. A sensitive precipitation method, using potassium pyroantimonate for ultrastructural demonstration of calcium as well as sodium ions, however, showed a characteristic difference in distribution pattern of the precipitates normally found at the surface of the microvilli, in the endoplasmic reticulum, in Golgi apparatus, mitochondria and extracellular space. In rickets, there were additional deposits within the inner space of microvilli and a marked reduction of mitochondrial granules. After vitamin-D substitution these deposits within microvilli disappeared, and mitochondria were temporarily overloaded with ions. If the precipitates in microvilli and mitochondria mainly represent calcium and not sodium, which seems probable but cannot be proved at present, these findings support the concept that in rickets at least a part of the calcium is able to pass the microvillar membrane and that the following step of transcellular calcium transport is impaired. After vitamin-D substitution the carrier system for ion transport at the top of the cell seems to recover earlier than the ion-exclusion mechanism in the basal portion of the enterocyte.


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Mitochondrien vorwiegend Calcium und nicht Natrium darstellen, was wahrscheinlich ist aber gegenwärtig nicht bewiesen werden kann, sprechen die Befunde für die Vorstellung, daß bei der Rachitis wenigstens ein Teil des Calciums die Mikrovillusmembran passieren kann und daß der darauffolgende Schritt des transeellulären Calciumtransports gestört ist. Nach Vitamin D-Substitution scheint sich im Enterozyten der apikale Ionenzustrom schneller zu erholen als der basale Ionenausschleusungsmechanismus.

In recent years many important data concerning the mechanism of normal and rachitic calcium absorption in intestine were obtained. There are for instance the elucidation of vitamin-D metabolism, the demonstration of calcium transporting enzymes and the detection of calcium binding proteins (CaBP). At the cellular level, however, the exact localization of these factors, associated with the intracellular calcium homeostasis, is still lacking. Furthermore the role of the different cell organelles in physiology and pathophysiology of calcium absorption remains still obscure. The literature in this subject gives only few morphologic details about rachitic intestine (Nordio et al., 1968; Sampson et al., 1970; Matthews et al., 1971; Sampson et al., 1972). Therefore the purpose of this paper is to study the morphological changes of the duodenal enterocyte during rickets by light and electron microscopy. In addition it is attempted to get some information about the site of the defective mechanism of calcium transport within the cell by comparing the intracellular ion distribution in normal and rachitic state. For ion detection we used a method, which differs from that used by Sampson et al. (1970) in an in situ precipitation of calcium. Artificial ion translocation during tissue preparation is thereby avoided.

Materials and Methods

27 female Wistar albino rats (initial weight 40-50 g) were divided in four groups. The rats were kept in total darkness. Demineralized water was given ad libitum.

Group 1. 4 rats were given a vitamin-D free, calcium phosphorus normal diet (Altromin® Nr. C 1000 without vitamin D) for 4 weeks.

Group 2. 5 rats were given a vitamin-D free, low phosphate (0.2%), normal calcium (0.8%) diet (Altromin® rachitogenic diet Nr. C 1420) for 4 weeks.

Group 3. 9 rats were kept as described in group 2. After 4 weeks the rats were given 200 IU vitamin D₃ (Vigantol®) diluted in corn oil by oral tube and killed 15, 22, and 36 hours after vitamin-D substitution.

Group 4. 9 rats as controls were given a normal standard diet (Altromin®) for 4 weeks.

At the end of the period the rats were killed by ether anaesthesia. 24 hours prior and just before death blood was collected from the retroorbital plexus for chemical analysis. The duodenum of the groups 2, 3, and 4 was taken and immediately fixed for light and electron microscopic observation.

For light microscopy tissue was fixed in buffered formalin and embedded in paraffine. Sections were stained with H.E., PAS, Astrablue and the v. Kossa reaction. Kryostat sections were stained with Fettrot 7B for demonstration of fat and with an azo method for demonstration of alkaline phosphatase (Pearse, 1968).

For electron microscopy tissue was fixed by immersion fixation in an ice-cold Caulfield-solution (Veronal buffered OsO₄), dehydrated in ethanol and embedded in Epon 812 in the usual way.

For electron microscopic ion detection small tissue samples were fixed for 2 hours in a solution of ice-cold 2 % potassium-pyroantimonate in 1 % OsO₄ (Komnick and Komnick, 1963). The fixative was adjusted to pH 7.3 by 0.01n acetic acid. After a short rinsing in 8 % sucrose the tissue was dehydrated in ethanol and embedded in Epon.