A UNIQUE HISTOLOGICAL FEATURE OF VITAMIN D RESISTANT RICKETS OBSERVED IN FOUR CASES[1]

By

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INTRODUCTION

In 1958, the writer reported a unique finding in a single case of so-called vitamin D resistant rickets observed in fresh, undecalcified, undehydrated bone sections made from tibias and fibulas of a boy who required corrective osteotomies for the deformities accompanying the disease (2). Since that time, three additional cases have been examined, two by us and one reported elsewhere by *Engfeldt, Zetterström & Winberg* (1). All four cases reveal the same phenomenon, suggesting that it is a unique feature of the disease and worthy of wider attention.

The method of preparing the sections made from the material submitted from the Operating Room are reported in detail elsewhere and need be discussed only briefly here (3, 4). In essence, perfectly fresh, hydrated, unfixed, unembedded sections are prepared by hand grinding under water or physiological saline on special carborundum surfaced sandpaper. The cells in such sections are still living at the conclusion of the section making process if they were living at the beginning of it (3). The material is then stained in a basic fuchsin reagent, the surface stain ground off both sides (thus effectively "clearing" the sections), the section dehydrated and mounted in one of the standard resins as a permanent mount.

Sections prepared in this manner reveal cytology poorly. However,

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[3] Material from our three cases was generously submitted courtesy of Dr. *Joseph Fleming.*

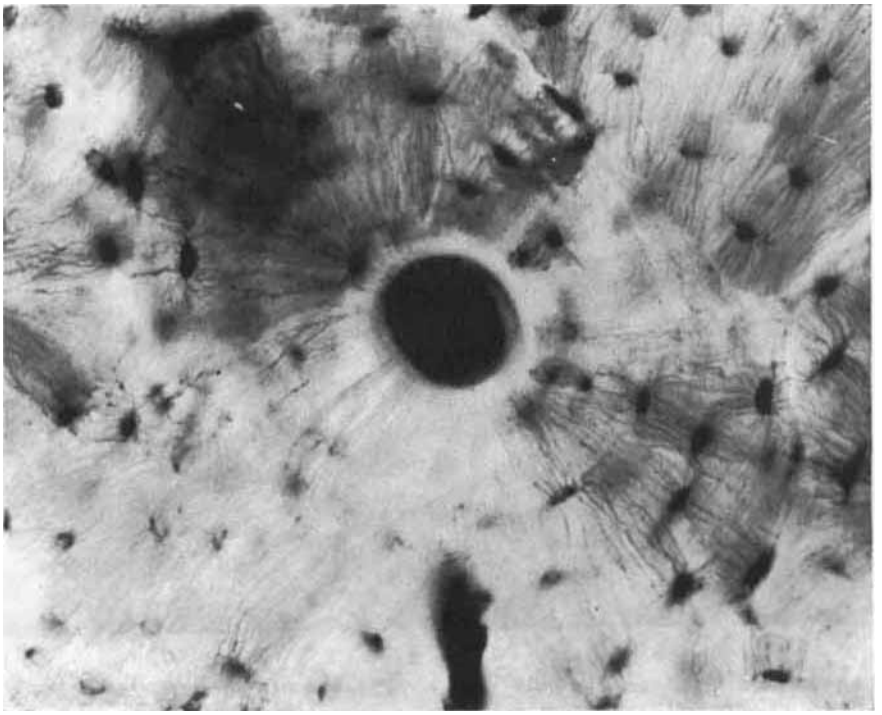

Fig. 1.

Undecalcified cross-section rib, 22 year old woman. 600 ×, basic fuchsin, Wratten 58. The large circular density in the center of the figure is the Haversian canal seen parallel to its longitudinal axis. The stained contents of the canal plus the filter used to obtain adequate contrast on the negative make the canal appear opaque. The bone around the canal is for the most part transparent because it is unstained. Around the Haversian canal are numerous osteocyte lacunae, appearing as small oval densities. Numerous canaliculae may be seen extending from the lacunae. These canaliculae are 0.35 microns in diameter and connect the lacunae to the Haversian canal. Note that the unstained bone extends right up to the lacunar walls. The apparent diffuse darkening in the neighbourhood of groups of canaliculae and lacunae is due to the presence of out-of-focus structures of similar nature above and below the optical cut which comprises the illustration.—At 10: 00 o'clock of the Haversian canal, there is a patch of diffusely staining bone which is somewhat wedge shaped and slightly larger than the Haversian canal. This is low-density bone, is a mineralization aberration and is termed feathering (5).

they reveal the structure of the bony substance containing the cells beautifully. One of the peculiarities of such sections is that diffuse permeation of the mineralized bony substance occurs only when the mineralization is about 0.85 of the maximum observed in a unit volume

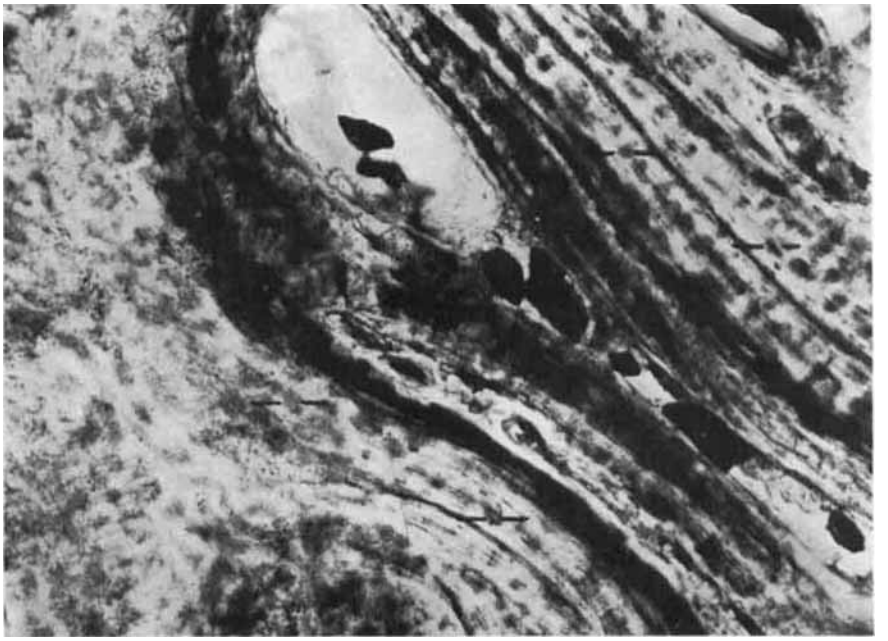

Fig. 2.

Undecalcified cross-section, fibula, of I. K. referred to in the text. About 100 ×. At this magnification individual canaliculae cannot be seen. There are innumerable fuchsin halos surrounding osteocyte lacunae. These halos are artificially darkened with the Wratten 58 filter. In addition to the perilacunar mineralization disturbance there are three broad streaks of low density bone which are also stained diffusely with fuchsin. This latter pattern is typical of rickets or osteomalacia in general; the perilacunar disturbance has been observed only in vitamin D resistant rickets.

on the microscopic scale. The reasons for this peculiarity need not concern us here. The diffusion peculiarity referred to leads to ready visual separation of low density areas from high density areas. These somewhat general statements have recently been confirmed by *Vose* (8), using quantitative microradiography, both on his own and on some of our material.

Fuchsin stained sections prepared by the methods noted therefore reveal, among other things, the *pattern* and *loci* of low density regions in undecalcified bone sections. The histologist accustomed to hematoxylin and eosin stained, decalcified material must adjust this thinking processes accordingly when inspecting this material.

In sections of material from over 700 patients, the sections being prepared as noted above, there is rarely any perilacunar pattern of low

density bone. The few instances in which peculiar perilacunar patterns of fuchsin-demonstrable low density mineralization have appeared have been reported elsewhere (7). It is noteworthy that the pattern illustrated here has been observed *only* in material from cases of vitamin D resistant rickets. See Fig. 1.

The case material will now be described briefly.

CASE MATERIAL

1) I. K., a nine year old boy with vitamin D resistant rickets which is also present in the paternal background. There was typical bowing of tibias, fibulas and femurs, typical widening and irregularity of the epiphyseal plates. Typical, untreated serum calcium 8.0 mg%, serum

Fig. 3.

Undecalcified section from the femur of A. S. referred to in the text. About 300 ×. Exactly the same pattern is seen as in Fig. 2. At this higher magnification it is more readily appreciated that there is a perilacunar halo due to diffuse fuchsin staining. Contrast the appearance of these halos with the sharply defined lacunae in Fig. 1. At the right edge of the figure lies a Haversian Canal. Lining the wall of this canal is an osteoid seam. The seam appears in this figure somewhat like a layer of glass *applied* to the Haversion canal wall.—The India ink bars in all of the figures illustrate exemplary fuchsin-stained perilacunar mineralization defects.

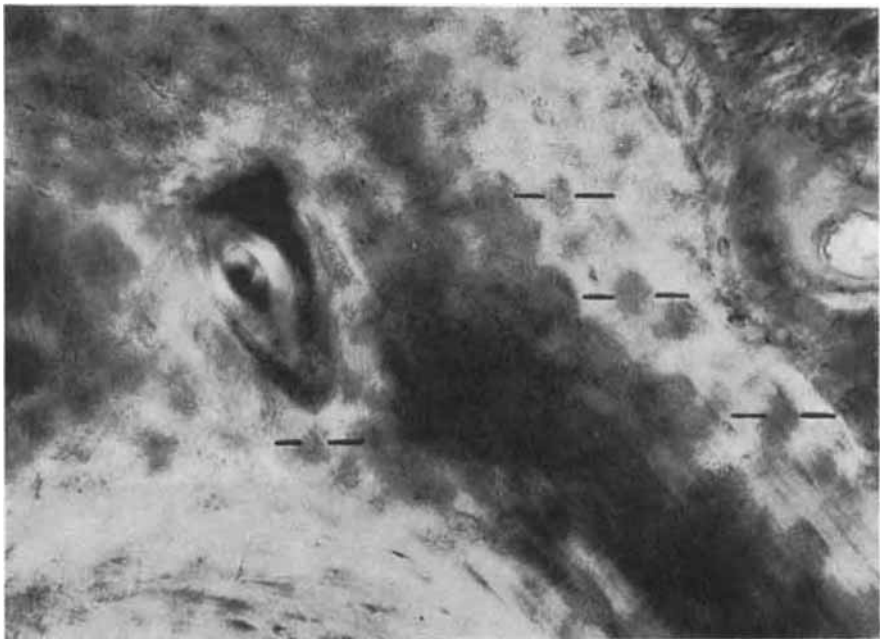

Fig. 4.
Section from R. J. referred to in the text. The similarity of all three figures is striking. In all three cases, only about 30% of the lacunae exhibit the feature described, the other 70% appearing normal with the techniques described.

phosphate 1.5 mg.%, alkaline phosphatase 16 Bodansky units. Received from 20,000 to 100,000 units of vitamin D per day. No treatment for three months prior to surgery. See Fig. 2.

2) A. S., an eight year old girl with a clinical and laboratory picture the same as in Case 1. There was no family history of the disease, and all treatment was withheld for three months prior to surgery. Treated with massive doses of vitamin D in the past. See Fig. 3.

3) R. J., a five year old boy whose two siblings had the disease. Clinical deformities, serum calcium, phosphate and alkaline phosphatase were similar to those in Case 1 and 2. All treatment was withheld for three months prior to surgery. Treated with massive doses of vitamin D in the past, and also with supplementary dietary phosphate. See Fig. 4.

4) The fourth case is one published by *Engfeldt, Zetterström & Winberg* (1). The microradiograph accompanying these authors' article clearly reveals the perilacunar mineralization deficit already referred to.

DISCUSSION

The peculiar morphological feature of these four cases is incomplete mineralization in the perilacunar bone, while inbetween lacunae mineralization density is higher.

This peculiar feature, and its consistent and unique pattern, suggest to the writer that there is some metabolic abnormality of the osteocyte residing in an affected lacuna. In theory, the result of this abnormality is that the osteocyte bathes itself in an extracellular fluid whose composition prevents full mineralization of the lacunar wall. Note that if the cause of the morphological feature being discussed were solely the abnormal serum concentrations of inorganic ions, *all* parts of the bone should be equally exposed to their effects, therefore equally affected and a perilacunar locus would be incomprehensible.

In view of the interval of three months between last treatment with vitamin D and osteotomies in our three cases, it is unlikely that the feature discussed is caused by vitamin D.

A dozen cases of rickets and osteomalacia of other types in the files of the laboratory do not reveal the morphological phenomenon illustrated here, indicating that it is not merely a peculiarity of the rachitic or osteomalacic state in general.

It seems reasonable to propose that the histological feature described is characteristic only of so-called vitamin D resistant rickets, and might conceivably be of diagnostic significance and use.

SUMMARY

A perilacunar "halo" of low density bone has been observed around the osteocyte lacunae in undecalcified sections obtained from four cases of vitamin D resistant rickets, three being from Henry Ford Hospital Orthopaedic Research Laboratory and one previously reported by *Engfeldt, Zetterström & Winberg*. This feature and its pattern appear to be characteristic of and unique to this disease.

RESUME

Un halo périlacunaire de basse densité osseuse a été observé autour de l'ostéocyte lacunaire dans une section non dé calcifiée obtenue de quatre cas de rachitisme résistant aux vitamines D, trois venant du Laboratoire de recherches orthopédiques de l'Hôpital Henry Ford et un rapporté antérieurement par *Engfeldt, Zetterström & Winberg*. Ce phé-

nomène et la forme sous laquelle il se manifeste paraît être caractéristique de cette maladie.

ZUSAMMENFASSUNG

Eine perilakunäre Aufhellung von Knochen mit herabgesetzer Dichte wurde um die Osteocyten Lacunæ in nichtentkalkten Schnitten von vier Fällen von Vitamin D resitenter Rachitis beobachtet. Drei wurden am Henry Ford Hospital orthopädischen Forschungslaboratorium gesehen und einer wurde vorher von *Engfeldt, Zetterström & Wiberg* veröffentlicht. Diese Erscheinung und ihre Anordnung scheint charakteristisch und einzigartig für diese Erkrankung zu sein.

ACKNOWLEDGMENT

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