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A REPORT OF SOME EXAMPLES OF ABNORMAL HALO VOLUME*

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INTRODUCTION

H. V. (halo volume) is the designation given to a peculiar, normal state of selective permeability in the intact bony wall of osteocyte lacunae and the parts of canaliculae close to these lacunae. The permeable zone lies around the lacunae and canaliculae as a three-dimensional halo or cloud.[6,7,11] (See Fig. 1) The selective permeability under normal circumstances is such that simple, inorganic ions can diffuse in limited quantities 2-4 microns into the bony wall of lacunae and canaliculae but larger ions and molecules — on the order of the coal-tar dyes — cannot.[9,11] H. V. permeability disappears both *in vivo* and *in vitro* upon death of the osteocyte normally residing in the lacuna.[9,11] H. V. are stained in perfectly fresh, wet, undecalcified sections properly prepared.[11] Under certain circumstances *in vivo* and *in vitro* enlargement of H. V. is encountered and variations in the morphology of H. V. may be seen.

It has been shown *in vitro* that enlargement in H. V., due to increase in the depth of permeability of the bony wall of the lacunae and canaliculae, occurs in the presence of lowered pH[10] or/and increasing citrate ion concentration.[21] A chemical peculiarity of the organic matrix in the H. V. part of bone has also been shown.[22]

What does this zone of selective permeability mean?

For years it has been suspected that osteocyte metabolism exerts some effect on the exchange of inorganic substances between bone and blood.[9,16,17,19] Some work indicates that the path of pyruvate and Kreb's cycle metabolism is abnormal in bone.[1,2,13,17,20,22] This work also suggests that parathormone and vitamin D or vitamin D analogs are in some way involved in the abnormality. The presence of an abnormality in osteocyte metabolism should be detectable if the proper methods for detection were available. It may be that H. V. abnormalities reflect the disturbances in pyruvate and Kreb's cycle biochemistry already referred to. If so, H. V. study is one route open for studying these biochemical activities of osteocytes.

Even if H. V. abnormality is eventually found to be related to other aspects of metabolism than those referred to,[15] a report of cases in which detectable abnormality occurred is warranted. Such a report focuses attention on a basic problem — osteocyte metabolism — and indicates the need for more work and thought on this problem.

In this paper we report some instances of H. V. abnormality detected in several human cases either on biopsy material or an autopsy material.

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METHODS

Fresh, undecalcified sections were made.[5] Fuchsin stained sections were made from every case.[6] Special H. V. stains by a boiling permanganate technique were done in 3 cases.[6,11]

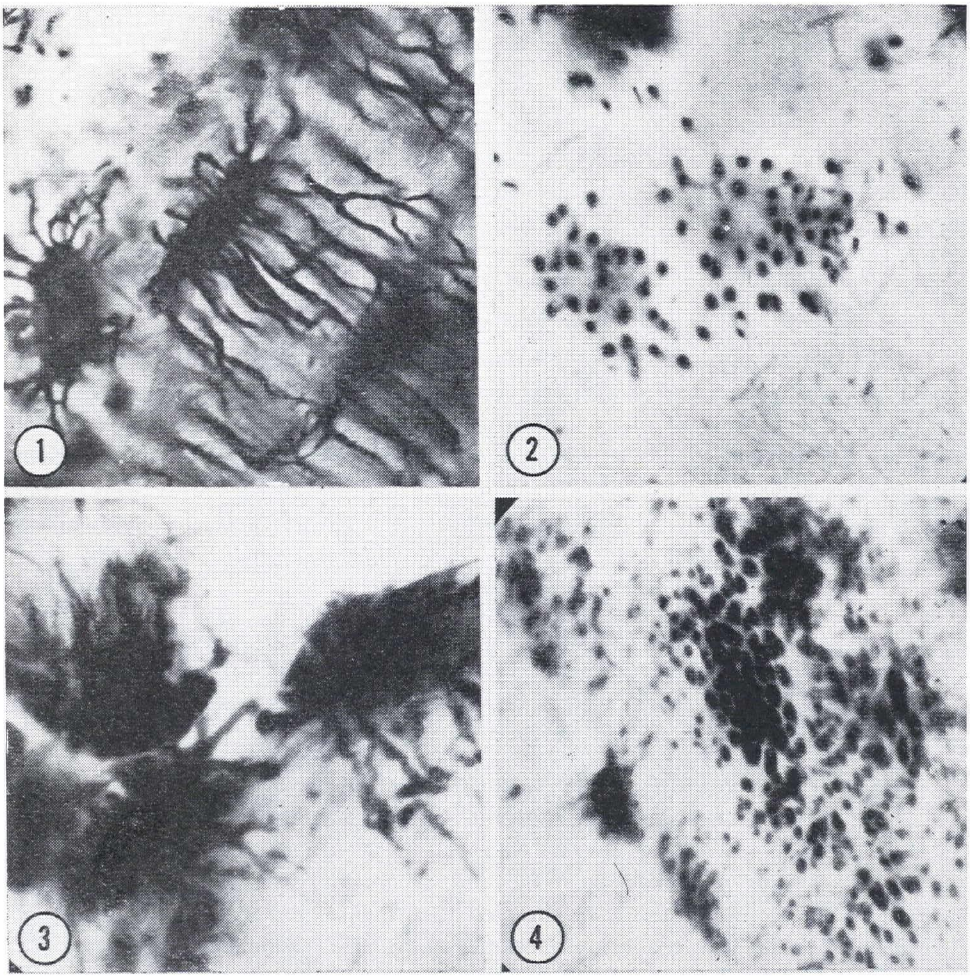

Figure 1

Undecalcified, fresh, undehydrated sections human bone.

(1) and (2) are oil immersion views of a lacuna and its canaliculae in longitudinal and cross section respectively. Stained with boiling basic fuchsin. The apparent size of the structures closely resembles the real size. About 2000 X.

(3) and (4) are similar views of lacunae and canaliculae in another section of the same bone, but stained with boiling permanganate. The magnification is the same as in (1) and (2). The apparent increased thickness of lacunae and canaliculae is the result of penetration of permanganate into the walls of the lacunar and canalicular lumens.

The walls are thus permeable to small, but not large, ions. This permeability and its selectivity constitute halo volume.

It must be understood that there are two degrees of abnormally large H. V. In the case of mild enlargement, detection may be achieved only with simple inorganic ions and such enlargement is not accompanied by microradiographic abnormality. In the case of severe enlargement, detection may be made by staining even with very large molecules, and in these cases there is a corresponding and obvious decrease in density on microradiographs provided the resolving power of the microradiographic equipment is adequate (better than about 3 microns), and sections are not too thick (under 15 microns).

Normal H. V. are illustrated in Fig. 1.

It is understood that the size of the selective permeability demonstrated by the various staining procedures is limited by *in vitro* factors such as staining time, temperature and so on. The actual, "biological" size of the readily permeable zone during life may be quite different from that shown on the various illustrations accompanying this article. The demarcation between H. V. bone and non-H. V. bone may also be artificially sharp on these illustrations. It is most likely that permeability varies gradually rather than abruptly in a single moiety of bone.

The 4 cases presented here are part of the 515 cases (as of this writing) that have contributed over 1400 bones to the Henry Ford Hospital Orthopaedic Research Laboratory. Fresh, undecalcified, fuchsin-stained sections have been made from all of these cases. Permanganate H. V. stains have been done on about 100 of them. These figures are quoted first, to indicate that the laboratory's experience with human bone is more than casual and second, to indicate the frequency of the abnormalities which will be presented.

CASE REPORTS

(1) C. M.,* 11 yr. WM. Diagnosis of Albers-Schonberg's disease by X-ray. Two biopsies of the femoral cortex obtained at intervals of two months. The bone biopsied was the normal, circumferential lamellar collar surrounding the chondro-osseous complex. (See Fig. 2)

This patient came under observation during routine chest X-ray screening.

The following values were normal in this case: urea nitrogen, total serum protein and A/G ratio, serum uric acid, serum calcium and phosphorus, cephalin flocculation, thymol turbidity, urine Sulkowich, WBC and differential. The alkaline phosphatase was 10.2 Bodansky units, the acid phosphatase 1.5 G.G. units, the P.S.P. excretion after 2 hours varied between complete excretion to 67% excretion. Hemoglobin was 10.2 gm% - 12.5 gm%. Hematocrit 40%.

As Fig. 2 reveals, there are large numbers of fuchsin permeable and enlarged halo volumes in the bipsy specimens. This feature is seen on both biopsies but is most prominent in the first one.

(2) J.R., 14 WM. Congenital ureteral obstruction with hydroureter and hydronephrosis. Repeated, prolonged urinary tract infections. Died 1960 in uremia. Specimens: distal third of tibia, one rib, obtained at postmortem. Kept in formalin for 24 hours before sectioning.

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*From the House of St. Giles the Cripple, Brooklyn, N. Y.

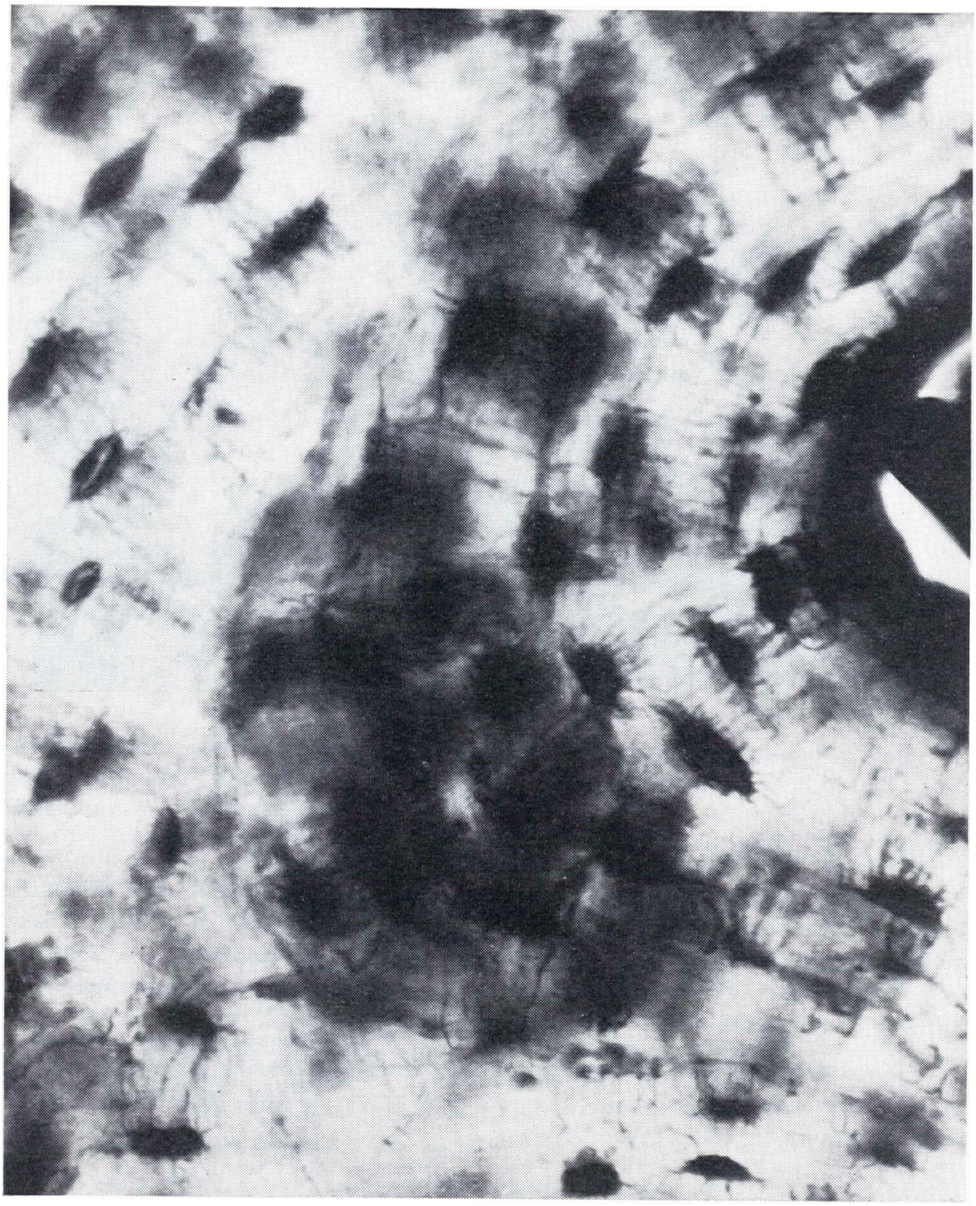

Figure 2

Cross section, undecalcified, basic fuchsin, about 1500 X, 11 year old boy with osteopetrosis. The specimen is a biopsy from the lateral femoral cortex, M/3. Radiographically this was an area of lamellar bone; histologically it was also lamellar bone.

Near the center of the plate are a group of osteocyte lacunae that are surrounded by halos of fuchsin-stained bone. To have a fuchsin halo the bone around the lacunae must be incompletely mineralized.

No permanganate stain done on this case. Biopsy specimen prepared and stained fresh.

Note that the halo affects the perilacunar bone more than the pericanalicular bone.

Laboratory data: serum Calcium 8.5 mg%; serum PO_4 11.2 mg%; NPN 100; urea nitrogen 60-70 mg%. Alkaline phosphatase 12.8 Bodansky. Creatinine 4.2 mg%. The boy was dwarfed, had secondary rickets, was pale, underweight, had a protruberant abdomen, duplication of the epiphyses and a rachitic rosary. Fig. 3 reveals the rachitic changes in the distal tibial and fibular epiphyses.

Figure 3

X-rays of Case (2) revealing rachitic changes in distal tibial and fibular epiphyses. Widening and irregularity of the plate are present.

In Fig. 4 photomicrographs of this patient's tibia may be seen. The numerous and enlarged, fuchsin-permeable halo volumes are well displayed. This patient had parathyroid hyperplasia, the parathyroid glands being enlarged and massive osteoclastic activity being present in the bone sections.

(3) I.K.; 9 WM[8] - so-called vitamin D resistant rickets, actually renal phosphate diabetes. Dwarfed, severe clinical and radiologic manifestations of clinical rickets; positive family history. Typical laboratory data: alkaline phosphatase 16 Bodansky units; serum calcium 9 mg%; serum PO_4 2.0 mg%; 24 hour urinary amino acid nitrogen 73 mg; renal tubular phosphate reabsorption 73%.

The patient was free of all vitamin D or vitamin D analog treatment 8 weeks prior to operation for correction of severe bowing of the legs. The resected pieces of bone comprised the specimens examined.

In Fig. 5 the large, fuchsin permeable halo volumes present in this case may be seen. It should be noted that microradiographs of other cases of this disease reveal the same defect in mineralization in the shell of bone enveloping the osteocytes that is revealed here. In other words, this finding is not unique in this case but appears to be a typical feature of the disease. Of incidental note is that the sections revealed evidence of severe rickets: numerous, thick osteoid seams, much low density bone.

(4) J.K.; 58 WM;[4] Patient has an unusual and bizarre trabecular pattern in the bones of the axial skeleton. (See Fig. 6). Extensive clinical laboratory studies were normal and include fecal calcium, renal tubular phosphate reabsorption, renal

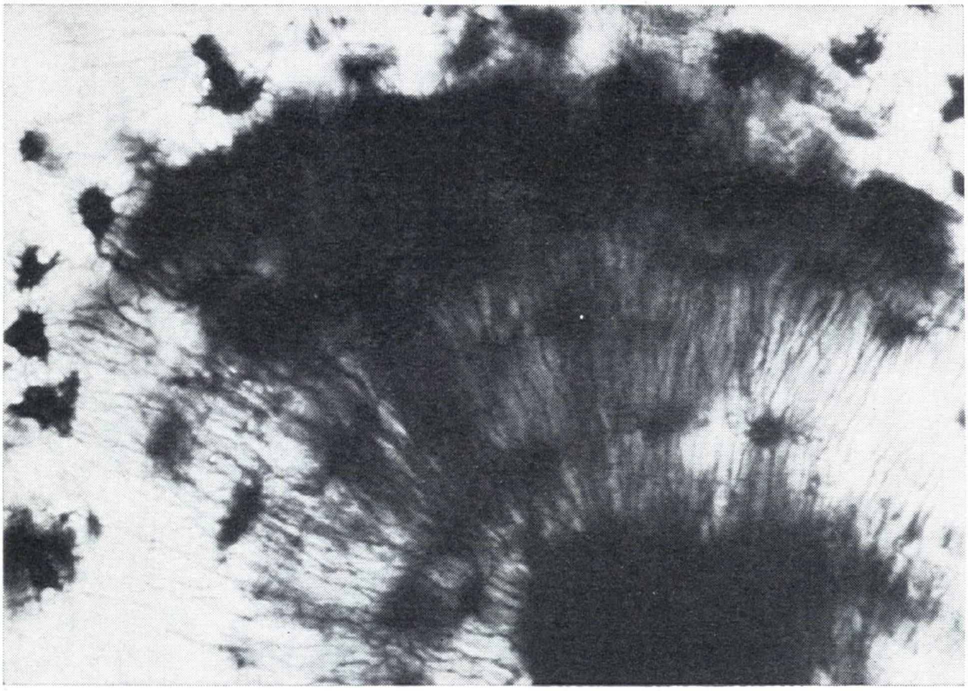

Figure 4

Undecalcified cross section D/3 tibia, basic fuchsin of boy with Uremia, secondary rickets. At the lower right is an Haversian canal in cross section. Near the top are a group of lacunae surrounded by fuchsin halos. The halos are accentuated in all these photomicrographs through use of appropriate filters when taking the original photos.

Above the group of affected lacunae is a narrow strip of additional undecalcified bone containing normal lacunae. This "spotty" distribution of abnormal H. V. seems to be the rule in pathological material. Material obtained at autopsy.

function, CBC, serum calcium and phosphorus serum acid and alkaline phosphatase, serum bilirubin, FBS and urea nitrogen. An iliac crest biopsy reveals severe feathering and an excess of osteoid seams. A permanganate halo volume stain done on a part of the fresh biopsy specimen reveals enlarged halo volumes. (See Fig. 7). This is one of three other similar cases reported to the American College of Physicians, Miami, May, 1961 by Frame, Ormond, Frost and Hunter. This patient was initially seen and X-rayed for a cervical radiculitis, and in this respect is similar to the other 3 cases.

DISCUSSION

It has been suggested elsewhere that the abnormal halo volume seen in vitamin D resistant rickets (or renal phosphate diabetes) is a characteristic feature of this disease.[8] In the present case there was no treatment with vitamin D or its analogs for at least 8 weeks prior to the osteotomies from which the biopsy material was obtained. This seems to rule out an effect of vitamin D on osteocyte metabolism as a cause of the observed abnormality. We are left with the suggestion that a disturbance in osteocyte metabolism is one of the features of this disease. It has been pointed out

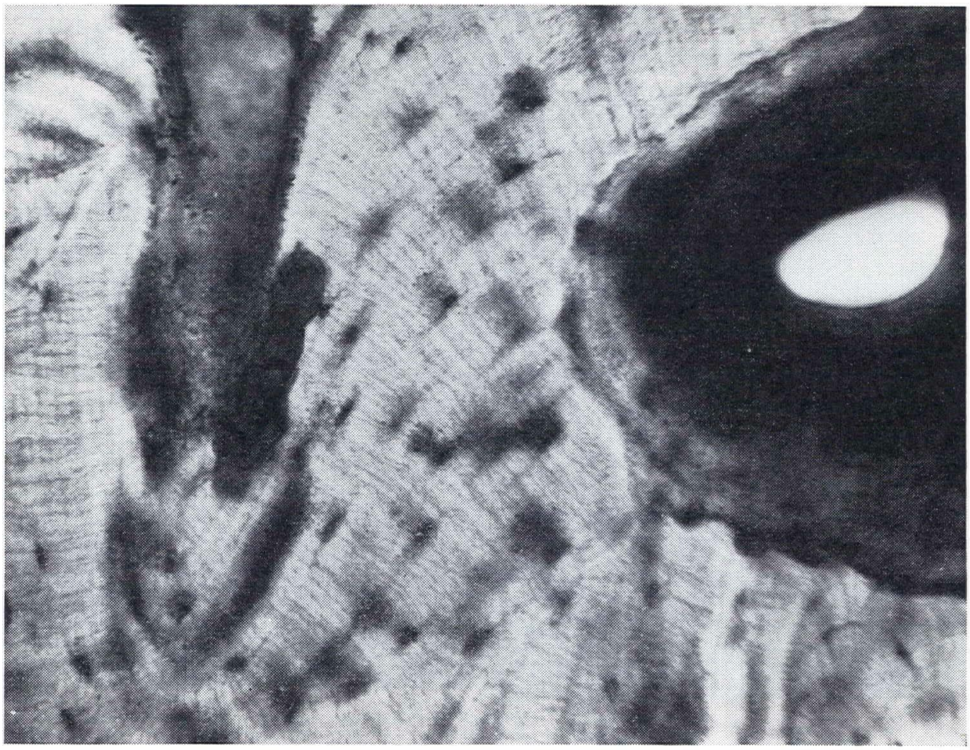

Figure 5

Undecalcified, fuchsin-stained cross section fibula from 9 year old boy with vitamin D refractory rickets. Part of an Haversian system is seen at the right; the Haversian canal is lined with an osteoid seam.
In the center of the figure osteocyte lacunae may be seen. Some of these lacunae are accompanied by a fuchsin halo. The halo is unusual, in that it seems to be always on one and the same side of the lacunae. This is the side containing the majority of the canaliculae and indicates that here there is an abnormal canalicular rather than lacunar halation.
Material obtained fresh from operating room.

Figure 6A

A lateral view of cervical spine of 58 year old man with bizarre, anarchic trabecular pattern in most of the bones of the axial skeleton and in none of the bones of the appendicular skeleton or skull.

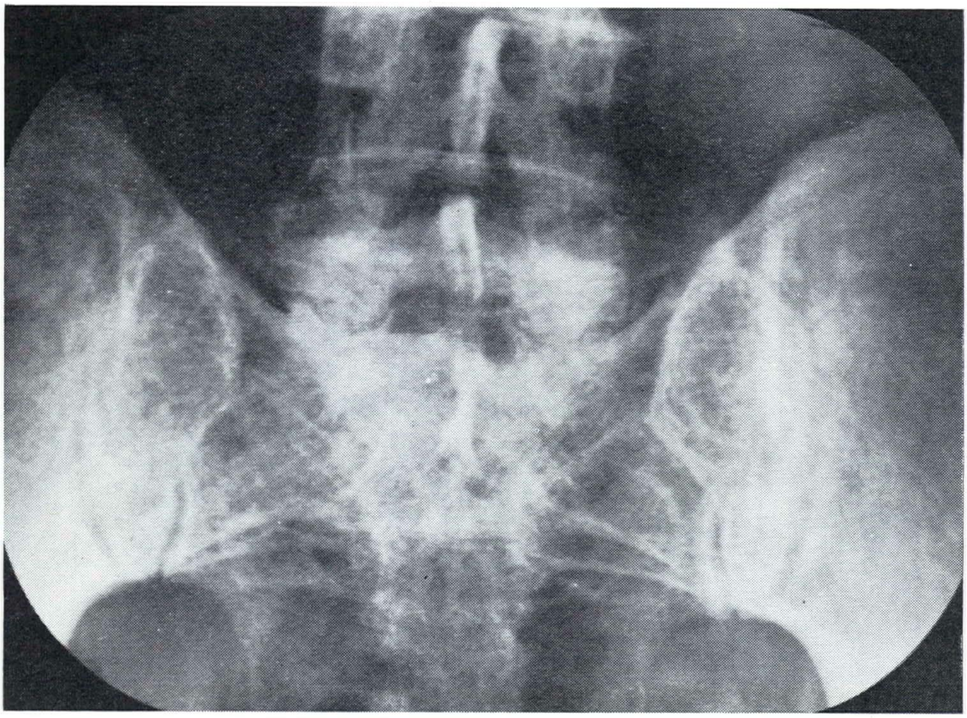

Figure 6B

A-P view of L-S junction region in same case as in 6A. There is less anarchy in trabecular orientation here but the trabeculae are irregularly thickened.
Four of these cases are being separately reported.

elsewhere why the observed abnormality cannot be explained on the basis of disturbed serum chemical values.[8]

On the basis of morphology it would appear that the halo volume disturbance in the case of vitamin D resistant rickets is the result of a different metabolic abnormality in the osteocyte than is the case in the other three patients presented. In other words, our illustrations are compatible with the presence of at least two different osteocyte metabolic disturbances. It is understood that while this is suggested, it is not established.

Various theories of parathormone action have been proposed in which the central idea is that parathormone in some manner stimulates the formation of large amounts of citrate by osteocytes.[17,20] If such were the case, it would logically be possible that removal of some of the calcium from the bony wall around the osteocytes would occur and lead on undecalcified sections to an enlarged halo volume.[21] This attractive theory, proposed by the Neumanns[17] and Nordin,[20] is unproved. At first glance our boy with parathyroid hyperplasia would seem to be a confirmatory case. This is brought up, however, to discourage the idea. There are three other cases of hyperparathyroidism in the Research Laboratory's files and none of them exhibit a similar disturbance. All are adults, however, and as Jaffe has observed,[12] the age of the

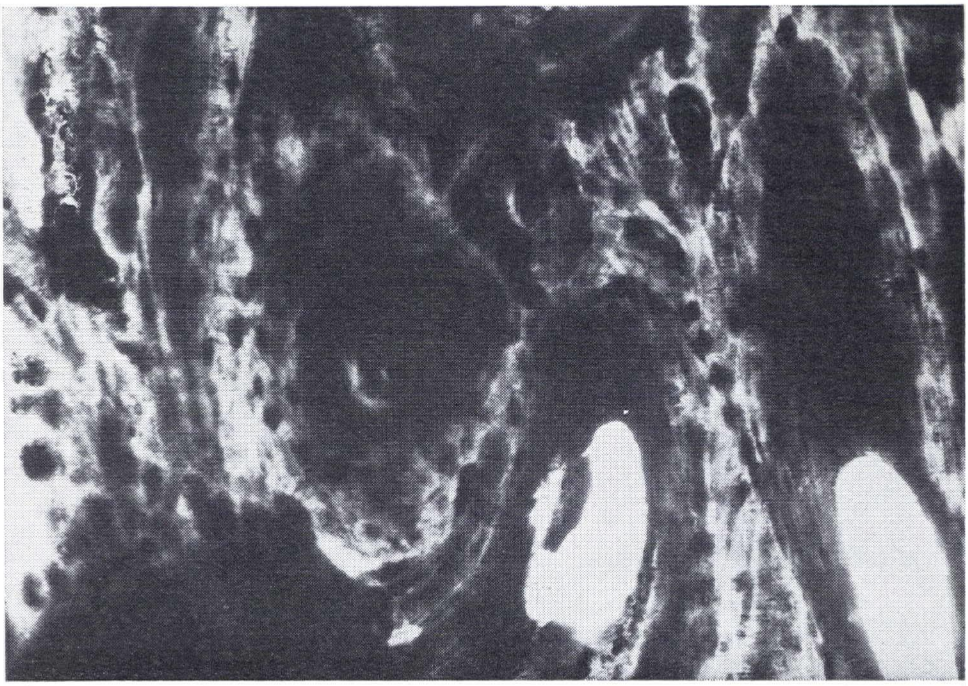

Figure 7

Permanganate stain of fresh, undecalcified iliac crest biopsy specimen obtained from case (4) of test; same patient as in Figures 6A, 6B.

The numerous small dark blotches represent permanganate stained, enlarged H. V.; the much larger, darkened areas are permanganate-stained feathered bone.

In this man it is necessary to make the provocative observation that he (and the 3 similar ones being reported with it) has no clinical illness to relate with the x-ray and histologic abnormalities.

individual affects the manner in which he responds to excess parathormone. Accordingly, either the case in the present study is an example of parathormone effect in a child, or the abnormal H. V. are the result of some other biochemical disturbance than one in the Kreb's cycle.

At present there are no grounds which would permit us to attempt identification of the metabolic disturbance in the osteocytes in the four cases reported, although there are some possibilities to explore. We cannot even postulate why only some of these cells display the abnormality rather than all of them. There obviously is room for a great deal of work in this area.

SUMMARY

Four cases are presented, drawn from among 515 in the files of the Henry Ford Hospital Orthopaedic Research Laboratory, in which a morphological disturbance in halo volume is present. Halo volume is a state of selective permeability in the bony wall of lacunae containing living osteocytes. Enlargement of halo volume to an abnormal degree is considered suggestive of a disturbance in the metabolism of the osteocyte. The exact nature of this disturbance is unknown.

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