Halo Volume - Part III: Existence of a Pattern in the Matrix

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INTRODUCTION

In previous reports concerning the halo volume feature the peculiarities in the mineral fraction of bone have been emphasized. These peculiarities, in brief, consist of a quasipermeability which is maintained in some fashion only by the living osteocyte. It has been assumed previously that no difference in the organic portion of bone, or matrix, accompanied the difference in the mineral fraction.

In this paper a peculiar halo volume staining pattern present in the matrix under certain conditions is described.

MATERIALS

Perfectly fresh, wet, unfixed ribs from 6 patients have been subjected to the manipulations described next. In addition to this material, various ribs, clavicles, femurs, and tibiae have been subjected to the manipulations referred to, these bones having been kept in bulk in storage in 40% ethanol for periods varying from one to eight months.

METHODS

1) Thin (30 u) undecalcified sections are made. Sections were then washed in distilled water or in 4% formalin; there is no preference for the present purpose. Time: 5 minutes.

2) Sections are decalcified in Versene for one hour. Varying times were experimented with to determine the effect on the final staining pattern. Prolonging Versene decalcification to 24 hours nearly eradicated the halo volume pattern, while 48 hours completely eradicated it. Versene concentration was 1%, pH buffered to 7.0 with sodium carbonate.

3) Sections are again washed in a large volume of distilled water for 5 minutes. The polychrome methylene blue stain used for staining frozen sections in routine clinical pathological work is then applied to the sections for 5 minutes. Sections are again washed in distilled water, 2 changes.

4) Sections are dehydrated in ascending alcohol concentrations, cleared in xylol and mounted in Harleco Synthetic Resin. Sections are handled with tissue forceps during the manipulations described. Sections do not shrink appreciably during the dehydration and clearing. Cross sections, longitudinal sections or both may be used; both were used in the present work.

5) In some instances sections were placed 1 minute in 2% nitric acid after versene decalcification, and then stained. Others, after the exposure to acid just noted, were soaked overnight in 0.1 molar solutions of disodium monohydrogen phosphate, and then stained.
6) In addition, after Versene decalcification some sections were stained with silver nitrate\(^7\) to detect the presence of phosphates and/or carbonates.

7) In some cases, after the recommended Versene decalcification, sections were stained in 0.1\% alizarin red \(S\)\(^7\) to detect the presence of unremoved calcium.

**OBSERVATIONS**

A halo volume pattern consistently appears in stained sections decalcified in Versene for an hour. The pattern is faint after 24 hours, and absent after 48 hours in Versene. The pattern is totally absent after a minute's soaking in 2\% nitric acid, but is faintly restored in such sections by afterwards soaking for 12 hours in 0.1 molar \(\text{Na}_2 \text{HPO}_4\).

The silver nitrate stain reveals that after an hour in Versene the only remaining ions which form insoluble compounds with silver ion are in the halo volume portion. Staining with alizarin reveals that after an hour in Versene there is an alizarin demonstrable halo volume pattern left in the sections.

The halo volume stain demonstrated with the above method does not depend on the existence of a demonstrable osteocyte nucleus in the lacuna and is not sensitive to drying, dehydration, and aging as is the mineral halo volume feature reported

![Figure 1](image-url)

*Figure 1*

Longitudinal section, decalcified in Versene 1 hour, then stained with polychrome methylene blue. There is more stain present in the halo volume part of the matrix. In the lower half of the figure canaliculae are seen in cross section. In the upper half of the figure some lacunae are seen in longitudinal section. The dark masses in the lacunae are osteocyte nuclei.
Halo Volume

previously. Storage of bulk bone for up to 8 months did not affect the halo pattern stain visible with the present method. See Figure 1.

DISCUSSION

The consistency with which the halo volume pattern revealed itself with the present technique is proof that it is the result of some difference in the matrix in the stained and unstained parts. The reasoning is simple.

First, there must be some difference in composition of the halo volume part of the sections to account for its consistency denser staining.

Second, the prompt disappearance of this staining pattern in strong acid, its slow disappearance in Versene over longer decalcification times, the presence of silver precipitable ions in the halo volume part of the section and of alizarin demonstrable calcium ions in the same location all mean that the halo volume staining illustrated in Figure 1 is the result of persistent ions in the decalcified matrix. (Recall that Versene may chelate cations in bone but has relatively little effect on anions.)

Third, in order for ions to resist the decalcifying fluid consistently in the halo volume part of the bone there must be “stronger bonds” tying them to the matrix than elsewhere in the bone.

Fourth, stronger bonds mean some difference in the chemistry, stericism or both in the halo volume part of the matrix.

In addition to the above conclusion, several additional things may be deduced from the facts so briefly stated.

First, the chemical peculiarity of the matrix dealt with in this paper is a stable one in comparison to the permeability peculiarity characterizing the mineral halo volume.

Second, being stable, death of the osteocyte residing in the lacuna has little effect upon the existence of a matrix halo volume pattern.

Third, the physical location of the matrix halo volume pattern necessitates the inference that it is caused by some chemical product elaborated by the osteocyte early in its existence.

Fourth, the matrix halo should prove to contain characteristically charged side chain groups.

Fifth, it is necessary to revise our present terminology so that no confusion need occur in the future about which halo volume is being referred to. The following is recommended:

A) Mineral halo volume

B) Matrix halo volume

This terminology is as simple as it is lucid.
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SUMMARY

By appropriate methods a halo volume in the matrix of human bone may be demonstrated. Available facts indicate that, in contrast to the mineral halo volume, the matrix halo volume is stable and is demonstrated when mineral ions remain in the matrix halo volume after decalcification but before complete demineralization of the matrix.