Halo Volume - Part II: The Effect of Citrate Ion Concentration on Size

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INTRODUCTION

Good but not conclusive evidence exists to support the inference that the live osteocytes in bone produce citrate ion in large amounts under certain conditions. If this is true then the citrate ion concentration must be considerably higher within the lacuna than it is in the blood, for reasons discussed later in this series of publications. Accordingly it seems reasonable to determine in vitro what effect varying citrate ion concentration would have on halo volume.

MATERIALS

Five fresh human ribs were obtained from the operating room. The ribs were removed at thoractomy for varying reasons but not for any local or systemic bone disease. The ribs were kept moist between the operating room and the laboratory. Since the ribs were obtained on different days, the experiments reported were duplicated with each rib.

METHODS

Fresh, thin, undehydrated, undecalcified, unfixed sections were made by Frost's method. These sections were incubated in the test solutions for 48 hours, sections being placed within the solutions within half an hour of removal of the rib from the patient. After 48 hours permanganate halo volume stains were done as described elsewhere, the sections dried and mounted for observation and photography.

Control sections were incubated in distilled water but otherwise similarly treated. Longitudinal sections were incubated in each case, cross sections in some cases. No information was obtained from cross sections that was not evident on the more easily prepared longitudinal sections.

A Beckman pH meter was used to control pH to the nearest 0.03 pH units. The pH of the citrate solutions tested was 6.5, 7.4, and 8.0.

Citrate concentrations of 0.01, 0.1, and 1.0 molar were tested. The solutions were made up by mixing separate solutions of citric acid and sodium citrate of the desired molarity. In the pH 8.0 solutions small amounts of dilute carbonate solution were added to obtain the desired pH. Sections were incubated in 30 cc. aliquots of the buffer solution at 37 C.

OBSERVATIONS

The criterion of significant change adopted was a readily evident change in halo volume size and density of stain under 100 x magnification. For a number of reasons this is a harsh criterion and change according to it is drastic change.

In all pH ranges with the exception of pH 8.0, increasing citrate ion concentration resulted in larger size and denser stains of halo volume. See figures 1 and 2.
Halo Volume

Figure 1
Longitudinal, undecalcified rib section incubated in 0.01 normal citrate 48 hours at pH 7.4. Small halo volumes. About 80x.

Figure 2
A section incubated in 0.1 normal citrate 48 hours at pH 7.4. Considerable enlargement of the halo volume. The canaliculae are seen in cross section. About 150x; apologies for not using identical magnifications!
DISCUSSION

It may be inferred that in vivo a possible means of varying the size and permeability of halo volume would be by varying the concentration of citrate ion in the fluid between the osteocyte membrane and the bony wall of the lacuna. An abnormally high concentration of citrate ion in the lacuna would result in diffusion of citrate out of the lacunae, through the canaliculæ and into the blood. It would appear inevitable that large quantities of citrate produced and secreted into the blood in this manner would carry with the citrate considerable quantities of calcium ion and some magnesium. The reason is the complex formed between citrate and calcium and, to a lesser degree, magnesium.

It is tempting to assume that at least some of the hypercalcemia encountered in hyperparathyroidism and in vitamin D intoxication is the result of calcium removed from the halo volume portion of the skeleton in the manner postulated above. This postulate, while reasonable, is not proved, and other mechanisms beside the citrate — dependent one above, and resorption of bone by osteoclasts, must be sought for.

SUMMARY

Halo volume increases in size with increasing concentration of citrate ion in the lacunar fluid.