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Compact Bone Deficiency in Protein-Calorie Malnutrition

Abstract. *Children hospitalized for acute protein-calorie malnutrition in Guatemala City were not delayed in ossification status as compared with Guatemalan Indian children on their customary low-protein diets, but were markedly and often dramatically deficient in cortical bone.*

In reviewing radiographs made on admission to the clinic of 95 infants and children hospitalized for protein-calorie malnutrition in Guatemala City, we were greatly impressed by the radiolucent quality of both round and tubular bones. Compared with the degree of skeletal maturity attained, these malnourished patients appeared to show the kind of bony rarefaction reported in experimental protein-calorie malnutrition in animals (*1*), and they presented a clinical picture of juvenile osteoporosis.

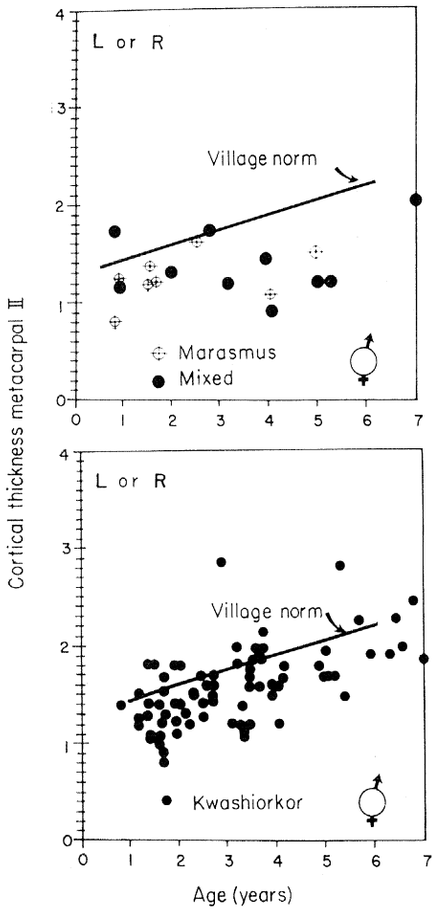

Fig. 1. Reduced thickness of cortical bone in the second metacarpal in 95 children with protein-calorie malnutrition.

For more critical comparison, we studied the children living in the two Guatemalan Indian villages of Santa Cruz Balanyá and Santa María Cauqué, where the diet was characterized by low average intake of animal protein (*2*). We studied these children simultaneously with the hospitalized patients during 1959–1963. Ossification status in both patients and controls was measured objectively as the number of postnatal ossification centers visible in postero-anterior hand-wrist radiographs (*3*). Compact bone was measured simply as the total thickness of cortical bone at midshaft on the second metacarpal (*4*). Radiographic enlargement approximated 1 percent throughout, and therefore no correction was used. Replicability of both ossification status and thickness of cortical bone exceeded 0.98.

Ossification status in the 95 boys and girls with protein-calorie malnutrition was not retarded compared with that in the 694 children from the two Indian control villages. By the Chi-squared (χ^2) test the number of hospitalized children above and below the sex-specific village trend lines did not differ significantly from chance.

In contrast to ossification status, the thickness of cortical bone was greatly diminished in the children hospitalized for protein-calorie malnutrition. As shown in Fig. 1, 75 percent of the hospitalized patients fell below the village trend for both sexes for cortical thickness. Deficiency of compact bone was observed for all three of the clinically-defined subgroups of protein-calorie malnutrition (kwashiorkor, kwashiorkor-marasmus, and marasmus) as defined by Scrimshaw and Béhar (*5*). The more marasmatic the child, the greater the degree of compact bone deficiency.

The fact that some of the hospitalized patients had no more compact bone at 4 to 6 years than might be expected for a 1-year-old Guatemalan Indian child, despite comparable ossification status, led us to consider that the cause might be an actual bone loss rather than simple failure to gain bone. While this supposition could not be tested retrospectively, it was confirmed by study of serial, longitudinal radiographs of the patients, taken during the period of hospitalization. Approximately 10 percent exhibited a concomitant bilateral increase in measurable bone length and decrease in cortical thickness during the recovery period, thus giving metrical support to the hypothesis of cortical dumping in acute protein-calorie malnutrition.

Guatemalan Indian children suffering from extreme protein-calorie malnutrition are therefore no further delayed in ossification status than children on low-protein diets in the control villages, though the latter are obviously retarded in comparison with middle-class Ohio-born American white children (*6*). The fact that in cases of acute protein-calorie malnutrition at comparable "bone age" there is a marked deficiency in compact bone, especially in the children with marasmus, suggests actual bone loss as well as simple failure to gain (*7*).

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Strontium and Calcium Uptake by the Green Alga, Oocystis eremosphaeria

Abstract. *The uptake of calcium and strontium by a green unicellular alga cultured in media containing these elements in concentrations found in nature is directly proportional to the concentration in the media. Variation in the concentration of either element has a slight inverse influence on the uptake of the other. The increase of strontium uptake when the calcium concentration is very low indicates that strontium is substituted for calcium when calcium is limited and suggests that the alga requires either element.*

The fact that ^{90}Sr is a potentially dangerous contaminant in an environment has resulted in many investigations of both its biogeochemistry and its metabolism by plants and animals (*1*, *2*). Many of these studies have centered on the relation and similarity between Sr and Ca metabolism. As a result, ^{90}Sr-Ca ratios have been used widely to predict or interpret the behavior of ^{90}Sr in the environment. However, in many of the otherwise controlled experiments the Ca and Sr compounds used in media or diet preparations probably contained significant amounts of the complementary element as an impurity (*3*). Thus study in the lower range of Sr concentrations in the environment has been difficult because Sr-free calcium compounds are not readily available.

The object of this investigation was to study, under rigorously controlled chemical conditions, the uptake of Sr and Ca by an alga, in order to obtain information regarding the fate of Sr, especially ^{90}Sr, in an aquatic environment, and to attempt to interpret Sr uptake with regard to environmental Sr concentrations rather than Sr-Ca ratios. A green, unicellular alga, *Oocystis eremosphaeria*, was used. Concentrations of Ca and Sr in the media were selected to cover the usual range found in freshwater environments. Stock algae were cultured in modified Hoagland's solution, and cells were transferred, after centrifugation, to a series of 250-ml erlenmeyer flasks. The conditions in the flasks—fluorescent light (4400 lu/m^2, 12-hour photoperiod), temperature (29°C), solution volume (150 ml), and nutrients other than Sr and Ca—were kept constant. The solutions were aerated and agitated by a filtered stream of air bubbles. The series of flasks was established according to a factorial design (3 Sr × 3 Ca) with two replications, thus giving nine combinations of Sr and Ca in the media. Strontium concentrations were 1.5×10^{13}, 15×10^{13}, and 150×10^{13} atoms per milliliter, and Ca concentrations were 1.5×10^{16}, 15×10^{16}, and 150×10^{16} atoms per milliliter. All chemicals used for media preparation were cross-checked for Ca and Sr impurities by the Analytical Chemistry Division of Oak Ridge National Laboratory, and certain Ca compounds were specially purified to eliminate Sr impurities; the Sr impurity of the $CaCO_3$ used in the study was reduced to 1.8 μg of Sr per gram of $CaCO_3$.

Immediately after the flasks had been inoculated with algae, 0.116 μc of carrier-free ^{85}Sr and 0.124 μc of ^{45}Ca, with negligible carrier (1.5×10^{13} atoms of stable Ca), were added to each flask as tracers. Samples of algae were taken at 1, 4, 24, 48, 72, 144, and 215 hours during the first run of the study and at 1, 4, 24, 48, 96, 168, 240, 360, 552, and 840 hours during the second run. Samples were collected by pipette, and the algae were removed with membrane filters (filter area, 2.54 cm^2; pore diameter, 0.45 μ) and then rinsed on the filters with tracer-free medium. The filters bearing the algae were oven-dried at 35°C and weighed; the tare weight of the filters was subtracted to obtain the dry weight of the algae. The filters were glued to counting planchets and counted for ^{45}Ca and ^{85}Sr, respectively, in gas-flow and gamma scintillation counters. The volume of the algal samples was maintained at a dry weight below 1.3 mg (0.5 mg/cm^2) to keep self-absorption of beta particles at negligible levels. After correcting the radioisotope counts for background and counter efficiencies, and on the assumption of no discrimination between radioisotope and stable isotope by the algae, the uptake of stable Ca and Sr was calculated from specific activities; for example,

$$\text{Stable Sr}_{al} = \frac{(\text{Sr}^{85}{}_{al})\ (\text{stable Sr}_{med})}{\text{Sr}^{85}{}_{med}}$$

where the subscripts *al* and *med* represent algae and media.

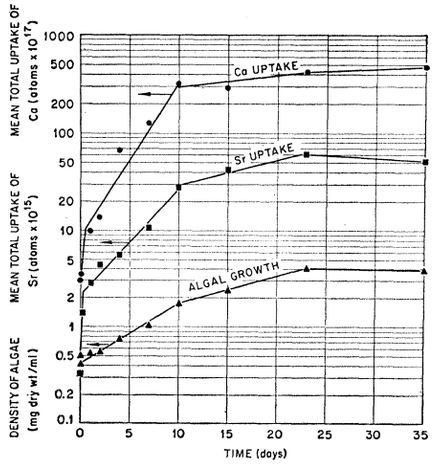

Fig. 1. Mean accumulated uptake of Sr and Ca by algae, and mean population growth of algal cultures (arrows indicate proper legend on ordinate for each curve).

Figure 1 shows the uptake of the two elements and the growth of algal populations during the second run of the study. Results for the first run were similar but less complete. Each point in Fig. 1 is the average of results from samples from all 18 flasks. Since the uptake of either element was dependent on its concentration in the medium, the points are not true averages, but are only intended to demonstrate the relation of the uptake to the growth. The algae demonstrated, at all concentrations of media, a rapid uptake of both elements during the first 24 hours, indicating a large initial uptake, probably exchange of both elements between cells and media. Uptake of both elements continued at a rate slightly greater than the population growth rate during the period from 24 to 240 hours. After 240 hours, the uptake paralleled the growth rate of the algae, indicating equilibrium between cellular and media concentrations. Three-dimensional graphs of the uptake of Sr and Ca by *O. eremosphaeria* appear in Figs. 2 and 3. The plotted points are the averages of samples from each pair of flasks from near equilibrium until termination of both runs of the study. Thus each point is the average of duplicate samples taken at 144 and 216 hours during the first run, and at 168, 240, 360, 552, and 840 hours during the second run. The response plane for Sr (Fig. 2) reveals that uptake is directly proportional to Sr in the medium, with enhanced