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Chelation of Calcium by Lactose: Its Role in Transport Mechanisms

Abstract. *Lactose, like other sugars and polyols, prevents or delays the precipitation of calcium ion in the presence of bicarbonate buffer. That an uncharged calcium-lactose complex is formed was demonstrated by the use of paper electrophoresis and of calcium-45 as a label for the complex. Enhancement of the intestinal absorption of calcium ion by sugars could result from a complex of low molecular weight.*

The effect of dietary factors, especially sugars and amino acids, on the gastrointestinal absorption of calcium and other alkaline earth metals is well documented (*1*). Of the sugars tested, lactose has been found to be most effective (*2*). Despite extensive experimental study of intestinal microflora, influence on the *p*H of the intestinal tract, and the higher osmotic or hydrostatic pressures induced by the hypertonic solutions of sugars, no adequate theory of the mechanism of this absorption has been proposed. Lactose must therefore operate directly in the lumen of the intestine since it must be present simultaneously with the calcium in order to manifest its activity. Lactose, injected intravenously or present in another segment of the intestine, has no effect on the absorption of Ca^{++} (*3*).

Polyhydroxy compounds, particularly reducing sugars, form stable, soluble complexes of low molecular weight with metal ions (*4*). Furthermore, one of these compounds, the iron-fructose chelate, rapidly induces the deposition of iron in spleen and liver when administered orally to rats (*5*).

It is assumed that, within the intestinal lumen, calcium enters from the acid environment of the stomach as a free ion. As the *p*H is raised, a small portion of the metal is directly bound and transported by sites on or in the mucosal membrane. A major fraction is precipitated as an insoluble salt or complex, such as the carbonate, phosphate, oxalate, acylates of fatty acids, and so forth. We propose that the mechanism by which the sugars promote the absorption of Ca is by the formation of a complex with the metal whereby a soluble form of Ca^{++} is readily available for transport.

The ability of various sugars to prevent or delay precipitation of calcium as the carbonate was studied, since bicarbonate is the principal anion in the intestinal lumen (*6*). Measurement of the rate of formation of $CaCO_3$ by turbidimetric methods yielded inconsistent results because of the difficulty in obtaining precipitates with uniform characteristics and dispersion. However, qualitatively, it was easily shown that a molar ratio of 40:1 of lactose to Ca^{++} delays, if it does not prevent, the appearance of precipitate for extended periods of time.

The rate of precipitation of $CaCO_3$ was measured quantitatively in the following manner. Stock solutions of sugars (0.4*M*), $CaCl_2$ (0.2*M*), and $NaHCO_3$ (1.0*M*), were prepared from analytical grade reagents. With continuous stirring, 20 ml of the sugar solution and 1.0 ml of the $CaCl_2$ were mixed, and the *p*H was monitored with a *p*H meter. Initial *p*H of the solution was 6.50. The reaction was started by adding 0.5 ml of the $NaCHO_3$ solution. There was an immediate rise of *p*H to 7.65. The rate of reaction was followed by the fall of *p*H according to the equation

$$Ca^{++} + HCO_3^- \rightleftharpoons CaCO_3 + H^+.$$

As a control, 20 ml of H_2O was used. The results of a typical experiment, comparing the activity of lactose and fructose, are presented in Fig. 1. Initial rates of preciptation can be obtained by extrapolating the curves to zero time and measuring their slopes. The sugars and polyols, in order of decreasing effectiveness in delaying precipitation are: lactose > ribose > fructose = mannose > glucose > sorbitol = mannitol = sucrose.

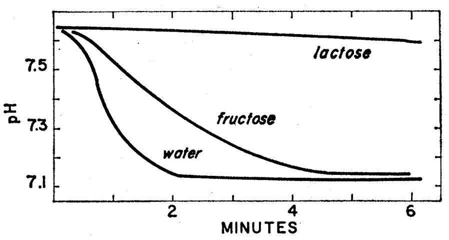

Fig. 1. Rate of H^+ liberation during formation of $CaCO_3$ by Ca^{++} in the presence of HCO_3^- buffer, without sugar and with fructose and lactose.

The calcium-lactose complexes were studied by paper electrophoresis. To 1.0 ml of $CaCl_2$ (0.01*M*) was added 10 μl of $Ca^{45}Cl_2$, whose activity was 1.5 mc/ml (obtained from Oak Ridge National Laboratory). A two-chambered electrophoresis apparatus was used; in one compartment the buffer solution was 0.05*M* $NaHCO_3$ at *p*H 7.0 and, in the other, 0.05*M* $NaHCO_3$ plus 0.4*M* lactose, at *p*H 7.0. The paper strips were soaked in their respective buffer solutions, and a 0.1 aliquot of radioactive $CaCl_2$ solution, approximately 600 count/min, was applied to the origin of each. The strips were subjected to electrophoresis for 18 hours at 450 volts, 5 ma, 2°C. They were removed, dried, and analyzed for radioactivity with a Forro strip scanner and recorder. The migration patterns of the Ca^{45}, with and without lactose present, are shown in Fig. 2. Lactose, in the absence of Ca^{++}, migrates to the same position as

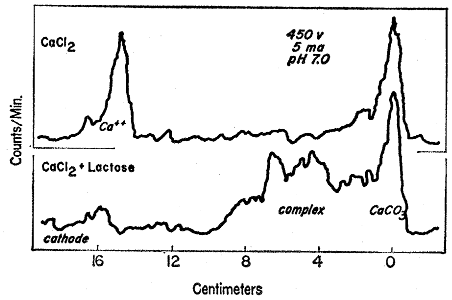

Fig. 2. Paper-electrophoretic migration of Ca^{45} in the presence and absence of lactose. The complex has no net charge and migrates at the same rates as lactose because of endosmotic flow.

the complex because of endosmotic flow. The profound change in the mobility of the isotope when lactose is present is indicative of the formation of a complex.

The crystalline compound, $CaCl_2 \cdot$ lactose $\cdot 7H_2O$, was isolated and identified by Herrington (*7*), but no data were reported on the nature of the binding of Ca^{++} to the sugar, or on the characteristics of chelation. Our electrophoresis experiments conclusively demonstrate that the Ca^{++} forms a soluble, uncharged complex, similar to that observed for iron (*4*), but different from that of Herrington. Mills (*8*), in a series of paper-electrophoresis and chromatographic studies, showed that Ca^{++} and other divalent metals alter the migration patterns of polyols and suggested that complexes were formed. Wasserman and Lengemann (*3*) were not able to effect the solution of $CaHPO_4$ with lactose because of the unfavorable rate of the reverse reaction and the relationship between the stability constant of the Ca-lactose complex and the solubility product of the insoluble salt.

Several aspects of the regulation and absorption of Ca^{++} and other divalent metal ions, as influenced by sugars, are important to emphasize. There must be a high ratio of sugar to metal ion in order to have effective complex formation (*4*).

The rapidity of absorption of the sugar is extremely important. For this reason, it is difficult to correlate the stability of a Ca-sugar complex in vitro with its effectiveness in vivo, particularly where the literature indicates little agreement (*9*), and where quite different rates of intestinal transport for each particular sugar have been observed.

Our evidence for chelation of Ca^{++} by sugars offers a chemical explanation for some previous results of nutrition studies. Whether the complex is transported through the biological membranes or merely serves as a metal-ion buffer to maintain Ca^{++} in a form readily available for transport is yet to be determined (*10*).

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Calcium Carbonate: Factors Affecting Saturation in Ocean Waters off Bermuda

Abstract. *Bermuda marine waters are not in equilibrium with the bulk carbonate sediments with which they are in contact, and they are supersaturated with respect to pure calcite. This apparent supersaturation seems to result from a metastable equilibrium between sea water and the most soluble solid phase available locally in excess.*

The near-shore regions of Bermuda offer a wide variety of environments in which marine waters of differing temperatures and chemical compositions are in contact with relatively pure carbonate sediments. The island thus offers an ideal setting in which to study carbonate sediment–sea-water systems. We have made a preliminary saturometer-type investigation (*1*) to establish whether the waters are in equilibrium with the subjacent sediment. The results were unexpected.

Samples of sea water and bottom sediment were collected by skin-diving and bottom-sampler techniques from five localities: an open coast environment at Tobacco Bay on the northeast end of the island; a shallow semi-restricted environment in Ferry Reach near the Bermuda Biological Station; and from three locations at depths of 3, 15, and 78 feet in Harrington Sound, an enclosed lagoon. The *p*H of each water sample was measured immediately upon collection (*p*H, Table 1). An excess of a solid carbonate phase was then added, and a glass electrode was inserted into the sediment. The indicated *p*H was then recorded at 5-minute intervals until no further change could be observed (generally about 30 minutes). The difference between the observed final *p*H in the presence of excess solid and the original *p*H of the water sample was then recorded (ΔpH, Table 1). All measurements were made with a Beckman model G *p*H meter, which has a nominal precision of ± 0.01 *p*H unit and an accuracy of ± 0.02 *p*H unit.

The interpretation of the data presented in Table 1 is not unambiguous. Using pure calcium carbonate as the solid phase and solutions of both distilled water and artificial sea water, Weyl (*1*) has shown that saturometer measurements made as described will show positive values of ΔpH when the solution is initially undersaturated, negative values when the solution is initially oversaturated, and no change when the solution is in equilibrium with the added solid phase. Weyl's results show further that the magnitude of the observed *p*H change will be proportional to the degree of over- or undersaturation. When the solid phase used is a solid solution of variable composition, however, and the fluid is a phase as complex as sea water, the interpretation becomes less straightforward. Differential complexing of the carbonate-mineral cations by *p*H-sensitive ions in sea water or peritectic exchange of the solid solution cations may occasion *p*H changes not directly related to the saturation state of the water sample. Such changes would be small, however, and in view of the large *p*H changes observed in the present study the data in Table 1 are interpreted, qualitatively, as though they relate to a simple binary system.

So interpreted, the data in Table 1 appear to indicate that only the deepest water sample from Harrington Sound is in equilibrium with the subjacent bulk sediment. Even this sample is in equilibrium only with a 3-inch surface layer of ooze containing more than 90 percent aragonite. All of the remaining samples appear to be oversaturated with respect to the sediment with which they