Non-specificity of the Divalent Cation Capable of Restoring Normal Intestinal Permeability after Chelation Depletion.* (31877)

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(Introduced by Martin G. Goldner)
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The role of the divalent cation, calcium, in regulating the permeability of epithelial tissues has been studied in a number of different preparations such as frog skin(1), intestinal mucosa of both dogs and rats(2,3), toad bladder(4), and rat corneal lens(5). In all of these tissues it has been shown that calcium removal, either by the use of calcium-free buffer or by chelating agents, causes a generalized increase in permeability which affects both ionized and non-ionized substances. Reports by both Tidball(6) and Hoffman(7) suggest that this alteration in permeability may not be completely attributable to calcium and that magnesium may also be involved. From the experimental work of both Nakas *et al*(8) and Cassidy and Tidball(9) it has become apparent that chelating agents such as disodiumdihydrogenethylenediaminetetraacetate (Na_2H_2EDTA) remove large quantities of both cations from the experimental tissue. On purely physicochemical grounds it would be expected that Na_2H_2EDTA should remove 8-fold more calcium than magnesium since the preferential affinity of chelation is 8:1 in favor of calcium. Chelation depletion experiments in the rat have revealed that approximately half as much magnesium as calcium is surrendered by the mucosal epithelium(9) despite the fact that this tissue contains twice as much magnesium as calcium (10).

Tidball(6) and Forte *et al*(11) have shown that permeability can be restored to normal by the application of calcium to the membrane; in addition, Tidball(6) has established that magnesium is also capable of reinstating normal permeability. These results suggested to us that the alteration in permeability evoked by cationic depletion of the tissue might be a non-specific phenomenon related to the physical characteristics of the alkaline earth series of metals. The present report deals with the differing abilities of the ions within this series to restore normal permeability after divalent cation depletion by Na_2H_2EDTA.

Methods. The methodology was similar to that described previously(6). Acute experiments were performed *in situ* on fasted male rats (Sprague-Dawley). The intestine was divided into 3 segments containing an equal number of vascular arcades. Control and experimental segments were randomized. All solutions placed in contact with the intestinal mucosa were adjusted to pH 7.0 and 300 mOsm/l. The control solution was isotonic saline. The absorption of 1 mM phenolsulfonphthalein (phenol red) was used as an index of passive aqueous permeability. Analysis of phenol red was determined by optical density measurements at 560 mμ in a Beckman DU spectrophotometer. The test solutions of 1 mM phenol red, 25 mM Na_2H_2 EDTA and various concentrations of the chloride salts of the alkaline earth elements, except radium which was not tested, were made isotonic with sodium chloride. All of the metals are soluble in saline at pH 7.0 with the exception of beryllium chloride which was used as a suspension. A range of concentrations between 2 and 50 mEq/l was tested for each element, except beryllium which proved to be toxic at concentrations above 20 mEq/l.

Results. The most striking effect of chelation depletion as reported by Cassidy and Tidball(9) was a 5-fold increase in intestinal permeability to phenol red when the mucosal epithelium was exposed to the chelating agent. In this study, although a somewhat smaller increase in intestinal permeability was observed, a highly significant chelation depletion effect was confirmed. The phenol red absorp-

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TABLE I. Permeability of Rat Intestine *in vitro* After the Following Sequence: a) A period of chelation depletion, and b) replacement rinse with various concentrations of alkaline earth metals.

Concentration of salt (mEq/l)	Salt rinse				
	$BeCl_2$	$MgCl_2$	$CaCl_2$	$SrCl_2$	$BaCl_2$
2	90 ± 29 (4)				
4	31 ± 18 (3)				
5		−144 ± 81 (4)			
8		92 ± 20 (8)			
10		54 ± 11 (4)	68 ± 21 (4)	41 ± 4 (3)	71 ± 29 (4)
20	45 ± 36 (7)	83 ± 22 (4)	−121 ± 41 (5)	82 ± 10 (6)	40 ± 17 (5)
35			70 ± 41 (4)	52 ± 18 (4)	132 ± 32 (6)
50		95 ± 18 (5)	87 ± 24 (4)	9 ± 53 (5)	−35 ± 112 (4)

Values are expressed as a percentage of normal control values for absorption of 1 mM phenol red. Underlined values are those referred to as the minimal restoration concentration for each ion.

tion, expressed as per cent of phenol red initially placed in the rat intestinal segments, showed a 2.7-fold increase in the segments treated with Na_2H_2EDTA. Under control conditions the phenol red absorption in 63 randomized experimental segments from 63 animals ranged from 0.0 to 17.6% per 40 minute absorption period. The mean of these determinations was 4.5% with a standard error of ± 0.5%. The 113 intestinal segments from 63 animals which were treated with Na_2H_2EDTA showed a range of phenol red absorption from 3.1 to 29.0% per 40 minute period. The mean of these observations was 12.5% with a standard error of ± 0.5%. These two sets of observations differ significantly from one another at the 99% confidence level.

In a series of control experiments it was found that, when the variance alkaline earth metals were placed in contact with intestinal mucosa not previously treated with Na_2H_2 EDTA, they did not affect the permeability of the intestine to phenol red.

Each of the alkaline earth metals was tested at concentrations between 2 mEq/l and 50 mEq/l for its ability to restore normal permeability of the *in situ* rat intestine following periods of chelation depletion. Table I presents the means and standard errors of the per cent recovery achieved with certain selected concentrations of the alkaline earth salt solutions. The basis for this selection was as follows: after the effect of the alkaline earth metals had been examined over the range of concentrations previously indicated it was possible to choose a minimal concentration which was effective in restoring permeability to at least 70% of the control value. For each ionic species this particular concentration was termed the minimal restoration concentration. Table I contains the permeability data obtained for those concentrations which were in the region of the minimal restoration concentration for each ion. In a few instances the results are expressed with a minus sign indicating that, rather than restore the normal permeability, this concentration of cation increased the permeability even further. In a few other cases the phenol red absorption following cation replacement actually reverted to a value which was less than that of the control value for that experiment. The values for minimal restoration concentrations are expressed in the form of a histogram (Fig. 1) which represents the means and standard errors of 5 separate experiments. It is apparent that the minimal restoration concentration for each alkaline earth metal may be correlated closely with the position of the metal in the periodic series. It may also be inferred that the concentration of each ion capable of reinstating normal permeability is characteristic and specific for each metal.

Table II compares some of the physical properties of the alkaline earth metals which could be related to their ability to reverse

TABLE II. Physical Properties of Alkaline Earth Elements.

Symbol	Atomic No.	Atomic wt	Ionic radius*	Electro-negativity	Charge density†	Minimum recovery conc‡
Be	4	9.13	.31 Å	1.91 ev	15.87	2 mEq/l
Mg	12	24.32	.65 Å	1.56 ev	1.73	8 mEq/l
Ca	20	40.08	.99 Å	1.22 ev	.49	10 mEq/l
Sr	38	87.63	1.13 Å	1.10 ev	.33	20 mEq/l
Ba	56	137.36	1.35 Å	1.02 ev	.19	35 mEq/l

* From Pauling, L. The Nature of the Chemical Bond, 3rd Ed. Cornell Univ Press, Ithaca, N. Y., 1960.

† Charge Density = charge (valence)/ionic volume ($Å^3$).

‡ Minimum recovery concentration—lowest concentration at which recovery of normal permeability was at least 70%.

permeability alterations in tissue depleted of cations. The ionic volumes were used to calculate charge density since the hydrated volumes were not available in the literature; implicit in the calculation is the assumption that the ions are spherical. The last column lists the minimal replacement concentration as determined experimentally. The charge density values are plotted against those for minimum replacement concentrations and the atomic numbers of the elements in Fig. 2: this mode of expression seeks to relate possible biochemical or physiological significance with a physico-chemical property of a group or series within the periodic table.

Discussion. Restoration of normal permeability following chelation depletion of mucosal epithelial tissue was found to be possible with all of the alkaline earth metals at concentrations below 50 mEq/l. The minimal concentration for each cation at which reversal of the chelation effect could be achieved was found to be characteristic for each ion. Well defined peaks of restorative ability for each alkaline earth metal were positioned along the concentration axis in their periodic order: $Be^{++} < Mg^{++} < Ca^{++} < Sr^{++} < Ba^{++}$. The variance in charge density relative to permeability restoration concentrations for this series of ions shown in Table II is comparable to that which exists for the variance of electronegativity within the series, or for the coagulative ability as in the Hofmeister Series. The ability of the alkaline earth elements to restore normal permeability appears to be related to some physical parameter which may well be charge density. This biological specificity pattern suggests a diffusion barrier concept in terms of charge fluctuation interactions based on coulombic forces or those of the London-Van der Waal's type. These interactions depend on the ionic constitution of the medium which, for instance, determines the balance between the highly specific attractive London forces and the non-specific repulsion

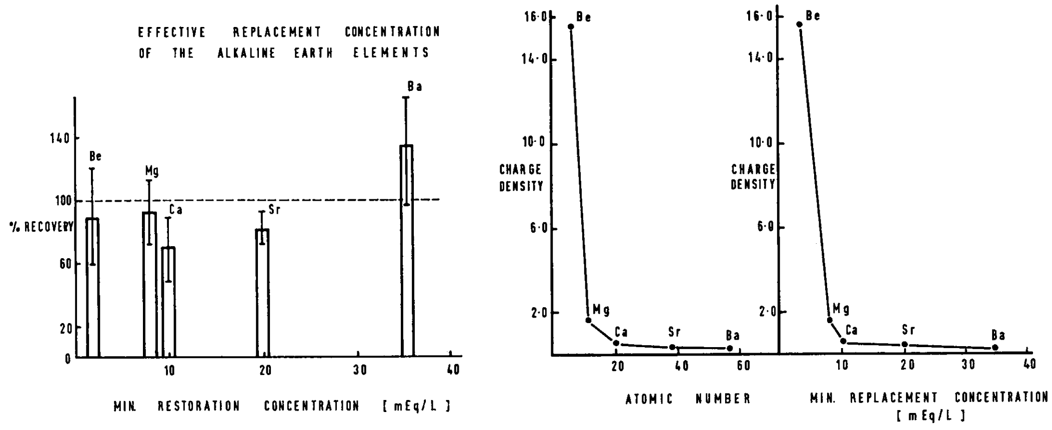

FIG. 1 and 2.

between identical molecules(12). Charge density decreases with the size of the ion; it might therefore be expected that a higher concentration of the larger ions is required to exert a specific force. An example of this type of force exerted by divalent cations is the coiling and uncoiling observable in a synapsed pair of chromosomes(13). The calcium ion has been specifically implicated in the crossing-over phenomenon(14).

Although the role of calcium and magnesium in biological adhesive phenomenon is well known, the importance to epithelial tissue permeability of intercellular diffusion channels and cell to cell attachments has not been rigidly evaluated. Coman's studies on calcium-deficient neoplastic tissue, and the ultrastructural appearance of chelated liver tissue, reveal wide separation of cell membranes which are closely apposed in normal tissue(15,16). Similar electron microscopic evidence, obtained from the preparation used in this work, appears to implicate the divalent cations in the maintenance of the junctional complex and lateral cell to cell attachments between epithelial cells(9). It has been suggested that the junctional elements in epithelial tissue may play a passive role in the maintenance of chemical and electrochemical gradients in epithelial tissue. However, the integrity of the tight junctional components of the junctional complex, bordering on the intestinal lumen, is retained with chelation treatment(9). Based on this fact and other kinetic evidence from passive permeability studies in the small intestine, we now consider that the primary locus of the chelation depletion effect is at the luminal membrane surface, and that the fenestration of the intercellular channels with separation of the lateral cell membranes is a secondary effect, invoked by movement of water into these areas.

Regardless of the precise site of action of the chelating agent, the results presented here suggest that the maintenance both of diffusion barriers and cell adhesive forces depends upon the presence of charged divalent cations to provide charge fluctuation interactions. Furthermore, the inauguration of these interactions is a property characteristic of the alkaline earth metals related to their charge density, rather than a specific dynamic force attributable to any particular ion.

Summary and conclusions. 1. All of the alkaline earth cations are able to restore normal aqueous permeability of the mucosal epithelial membrane following chelation depletion by Na_2H_2EDTA. 2. Each alkaline earth metal is capable of permeability reinstatement at a minimal concentration. These minimum levels of replacement for each ion are in accord with the position of the elements in the periodic table. 3. The relationship between minimal replacement concentration and charge density appears to indicate that a physical property of these ions is involved in the restoration of permeability, as opposed to a specific biochemical characteristic of a particular ionic species. 4. The particular alkaline earth metal, or combinations thereof, physiologically responsible for regulation of permeability and cell adhesion in epithelial tissue cannot be specified with any degree of certainty.

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