

Influence of Calcium and Magnesium on Eugenol-Induced Desquamation of Mucus Epithelium in Gastric Pouches*†

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In order to find a satisfactory topical stimulus for the study of the physiology of gastric mucus secretion, numerous agents have been investigated in this laboratory (9, 10, 11). Gentle massage, aqueous ether (saturated), 5 per cent aqueous clove oil emulsion, 50 per cent ethyl alcohol, distilled water, isotonic and hypertonic NaCl, and $\frac{1}{2}$ to 5 per cent aqueous eugenol emulsions were all found to induce desquamation of the surface epithelium in addition to acting as mucigogues. The most effective of these mucus stimuli was 5 per cent eugenol, and this also produced huge amounts of desquamation. Since there is reason to believe that pure gastric mucus is cell-free (9), this associated phenomenon of decreased cellular cohesion is of interest in relation to the general problem of gastric mucus function, but particularly with those aspects of the problem concerned with the gastric mucous barrier as a protective mechanism against chemical and physical irritants. The latter considerations arise especially in any investigation which is concerned with an exogenous, topical agent as an etiological factor in adenocarcinoma of the stomach. Furthermore, the loosening of the cement substance which results in this diminished cohesion may well be related to the difference between normal and cancerous tissues, which makes for the invasive character of the latter.

Some of the attempts to discover a common denominator for such decreases in cellular cohesion have resolved themselves around the ionic constituents of the intercellular cement. Overton (14) stressed the importance of an easily dissociating calcium salt as the basis of the cohesive material which binds cellular membranes. A partial explanation for the decreased cohesiveness of malignant cells has been related to a calcium deficiency of the tissues by Brunschwig *et al.* (1) and by

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Coman (5). Further support for the concept that calcium plays a role in the maintenance of cellular cohesion has been fostered by the observations that calcium-free solutions decrease the cohesiveness of normal epithelial cells (6), capillary endothelium (3), ciliated gill cells of *Mytilus* (13), and blastomeres of developing sea urchins (8). Methylcholanthrene, which is known to decrease the cohesiveness of squamous epithelial cells, likewise decreases the calcium content of the mouse epidermis (2).

Since both calcium and magnesium are constituents of cell membranes (16), both ions may be involved in the maintenance of cellular cohesion in general. Some foundation for this hypothesis is rooted in the fact that the reunion of sponge cells does not occur in the absence of either ion (15), and recently Zeidman (18) reported that the absence of calcium or magnesium or both decreases the cohesiveness of human squamous epithelium.

The present work was designed to determine whether calcium, alone or in the presence of magnesium, will diminish or prevent the reduction in cohesiveness of the gastric epithelium induced by the mucigogue, eugenol.

PROCEDURE

The study was conducted on five Heidenhain pouch dogs, using the technique previously described (9). One per cent eugenol emulsion was made in distilled water, or in CaCl₂ and MgCl₂ solutions at the several concentrations indicated in Table 1. Tergitol-Penetrant (1/40 per cent) was used to stabilize the emulsions, as heretofore. Since an acid pH also decreases the cohesiveness of cells (3), all the eugenol emulsions were buffered with NaHCO₃ (about 0.1 per cent in final concentration). Determinations of pH of the emulsion before and after its application to the pouch revealed no significant deviation from neutrality in the course of an experiment. Following addition of the bicarbonate to the CaCl₂ solutions of highest concentrations, there was a slow precipitation of CaCO₃, but

this was barely perceptible within the first 15 minutes. Hence, the salt-containing eugenol emulsion was not prepared until immediately before administration to the pouch. Each test experiment was preceded or followed on the same day by a control experiment with eugenol emulsion in distilled water.

Volume, viscosity, and opacity of half-hour samples of mucus were recorded over a period of 2 hours following each application of stimulus. Viscosity and opacity were evaluated on a scale from 1 to 5, using the standards described by Sober *et al.* (17). High viscosity and opacity are generally considered indicative of high columnar cell content, since these three factors are statistically correlated in significant degree (9). However, the presence of such cells was confirmed by microscopic examination of smears stained with toluidine blue. This was considered essential because coagulated mucin may also contribute to the opacity of mucus in considerable measure.

Our finding may be interpreted in any one of three ways: (1) the reaction between eugenol and the cement substance may involve calcium and magnesium in an irreversible (possibly non-ionic) manner, so that the presence of these cations at the surface of action of the desquamatory agent exercises no effect on this chemical process; or (2) this chemical reaction may be reversible, but eugenol is so powerful a desquamatory agent that its mass law effect cannot be offset by even the highest concentrations of these alkaline earth ions, which can be maintained at the surface of the mucosa under the conditions of these experiments; or (3) the desquamatory action of eugenol may be entirely independent of calcium and magnesium, *i.e.*, the mucigogue may act on some part of the cement substance which does not contain these elements. Apropos of the second of these possible interpretations, it should be noted that the concentration of eugenol used in these experiments was only 1 per cent, rather than 5 per cent as is being em-

TABLE 1

CHARACTERISTICS OF GASTRIC MUCUS SECRETED IN RESPONSE TO 1 PER CENT AQUEOUS EUGENOL EMULSIONS CONTAINING ABOUT 0.1 PER CENT NaHCO_3 AND OTHER SALTS

Eugenol emulsion made in:	No. of expts.	No. of samples	Average viscosity	Average opacity	Average volume	Columnar Cell content
Dist. H_2O	12	45	3.4	2.8	5.6	Considerable
0.02% CaCl_2	5	18	3.1	3.1	5.6	"
0.04% CaCl_2	3	10	4.6	2.6	2.6	"
0.06% CaCl_2	2	8	3.8	2.4	5.3	"
0.01% MgCl_2 , 0.02% CaCl_2	2	8	3.2	2.9	6.4	"
0.03% MgCl_2 , 0.06% CaCl_2	2	8	4.4	3.4	4.1	"

RESULTS

At none of the ionic concentrations here employed did the salts significantly affect the viscosity, opacity, and columnar cell content of the mucus produced by topical application of eugenol emulsion. The mean viscosity and opacity values (Table 1) are not appreciably different from those for the control experiments, and the smears invariably demonstrate the presence of a high columnar cell content.

DISCUSSION

The desquamating action of 1 per cent aqueous eugenol emulsion is not prevented by adding calcium, alone or with magnesium, to the emulsion. This observation is in accord with the work of Zeidman (18) in that he was unable to reverse the decreased cohesiveness of human squamous epithelium, induced by a calcium-free solution, by restoring the ion to the medium. On the other hand, Chambers (4) found that the diminished cohesiveness of capillary endothelium, induced by a calcium-free perfusate, is reversed by changing to normal Ringer's solution.

ployed in most of our other studies with this mucigogue.

The calcium deficiency found in malignant tissues, and which has been associated with their ability to invade adjacent tissues, may reflect some alteration of a calcium-binding complex in the cement substance (12). The chemical character of this complex is uncertain, but a protein structure has been suggested (12), and it may even be a lipoprotein (7). The desquamating ability of eugenol might be attributed to its lipid solvent ability, but as yet this does not seem feasible because hypertonic NaCl likewise produces desquamation in considerable degree (9). Although there is good reason to believe that mucigogue action is not necessarily accompanied by desquamatory action, the problem of how to stimulate large quantities of a cell-free gastric mucous secretion still remains unsolved.

SUMMARY

1. The object of these experiments was to determine whether calcium and magnesium can reduce eugenol-induced desquamation of gastric columnar epithelium. This was studied in dogs by the topical

application to Heidenhain pouches of 1 per cent buffered (NaHCO_3) eugenol emulsions containing these ions at several different concentration levels.

2. Such eugenol emulsions exercise the same mucigogue and desquamatory actions as do control emulsions containing none of the added electrolytes.

3. This finding fails to give support to the idea of a possible relation between the desquamatory action of a gastric mucigogue and the process of invasion by cancerous tissue.

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