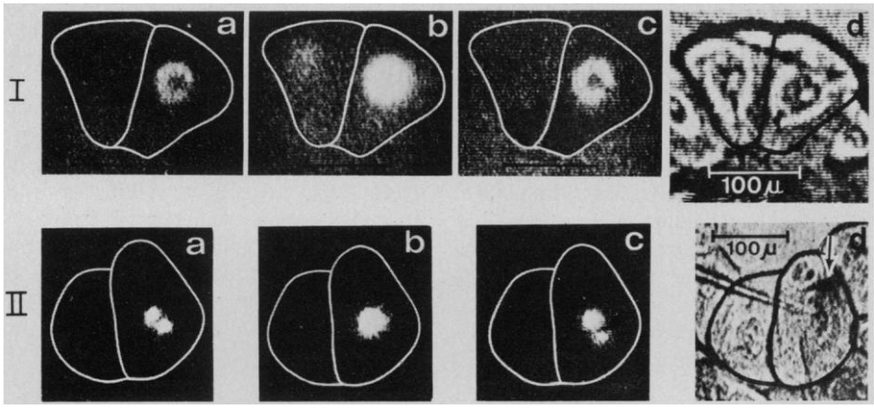

Fig. 4. Complex, energy-dependent Ca^{2+} distributions in the cytosol. Two examples (I and II) of aequorin glow figures produced by standard Ca^{2+} test pulses. (I) Before (a) and after (b) application of 5 m*M* CN (at this CN concentration there is also intracellular Ca^{2+} release); (c) after CN washout. (II) Before (a) and after (b) injection of 0.1 m*M* ruthenium red into cell; (c) several minutes later. The arrow in the bright-field picture (d) points to the cytoplasm stained by ruthenium red. Series I and II are from different preparations; cells are in Ca-free medium.

In considering the possibility of a mitochondrial role, it is interesting that some of our Ca^{2+} injections into the apical half of the cell (which contains fewer mitochondria) produced glows in the form of rings, bridged multiple spheres, and so forth, rather than the simple single spherical figures of most of our injections (Fig. 4). After CN treatment, these complex shapes transformed reversibly into simple single spherical ones and spread thus through the cytoplasm (Fig. 4, I). Ruthenium red injection produced a similar transformation, but here reversibility was rare (Fig. 4, II). Perhaps the complex figures reflect space with more sparse sequestering mechanisms. Fittingly also, the restoration of the complex forms and, in general, the return to constrained diffusion is faster after CN washout (1.5 to 2.5 minutes) than the lifting of the constraints after CN application (4 to 10 minutes) (Figs. 1 and 4). Such an asymmetry may be expected from the known mitochondrial property that in vitro Ca uptake stops only after exogenous ATP is sufficiently depleted, but resumes nearly as soon as electron transport (*1*, *9*).

What may be the functional adaptations of this restricted cytoplasmic Ca^{2+} diffusion? In a variety of physiological processes, information about a cell membrane event is thought to be communicated to the cell interior by Ca. For example, Ca is thought to mediate between membrane excitation and myofilament contraction in muscle cells (*10*); between nerve impulse and transmitter release at synaptic nerve terminals (*11*); and between membrane receptor mechanisms and the release of secretory products in gland cells (*12*); and to be involved in the excitation of visual cells (*13*). Thus, on the basis of the present results, we suggest that in some intracellular communications the Ca^{2+} messages may be effectively segregated in the cytosol; that is, the functional domains of Ca^{2+} are so small that several domains may coexist in a cell without message interference; or, stated differently, in cells with fast Ca^{2+} sequestering equipment, cellular constituents may be connected by discrete Ca^{2+} lines of communication. The number of possible lines will depend on the local density of the sequestering machinery. With a mitochondria population as dense as in the basal cell regions here, we suspect that this number is high.

Another corollary concerns the physiology of the cell junctions instrumental in intercellular communication by diffusible molecules (*14*). The permeability of these junctions falls drastically when the $[Ca^{2+}]_i$ at a junction rises above 5×10^{-5} to 8×10^{-5} *M* (*6*, *15*). Nonetheless, the junctional permeability may not be significantly perturbed by segregated cytoplasmic Ca^{2+} signals, as, in fact, has just been shown for the salivary gland cell (*6*). Thus, given the sufficient sequestering capacity, intracellular communication by Ca^{2+} signals and junctional intercellular communication are compatible. Heart and smooth muscle come most readily to mind.

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13 August 1975

Minor Salivary Glands as a Major Source of Secretory Immunoglobulin A in the Human Oral Cavity

Abstract. *Secretory immunoglobulin A is the predominant immunoglobulin in labial minor salivary gland secretions. Its mean concentration is four times higher in these secretions than in parotid gland secretion. The minor salivary glands can produce 30 to 35 percent of the immunoglobin A that enters the oral cavity. This, together with the potential accessibility of these glands to antigenic stimulation, suggests that they may be an important source of the immune factors that are involved in the regulation of the microorganisms in the oral environment.*

Saliva may function to regulate microorganisms in the oral environment (*1*). Experiments with human salivary secretions (*2*) and with animal models (*3*) suggest that certain aspects of this control can be mediated by the secretory immune system. Secretory immunoglobulin A (IgA) is the principal functional component of this system and is the predominant immunoglobulin of both whole saliva and individual secretions from the major salivary glands (*4*, *5*). In addition to these major glands, minor salivary glands (MG) are present in all soft tissues of the oral cavity except for the

anterior portion of the hard palate and the gingivae (*6*). However, there have been no reports that describe immune components of MG secretions. We thought that the MG's could be a major source of functional immunoglobulin in the oral cavity for the following reasons: (i) human labial minor salivary gland (LMG) biopsies are capable of incorporating isotopically labeled amino acids into IgA, IgG, and IgM (*7*); (ii) MG secretions constitute 7 to 8 percent of the volume of whole saliva (*8*); and (iii) there appears to be a substantial percentage of IgA in an individual whole saliva (approximately 45 percent) that cannot be accounted for by the sum of the IgA contributions from the major gland secretions and gingival crevice fluid (*5*).

Initially we investigated the concentrations of IgG, IgA, and IgM in LMG and parotid gland secretions of four subjects (Table 1). Midmorning samples of parotid and LMG secretions were collected with the use of sour lemon drops (Tootsie Roll Industries) for stimulation. The lower lip was isolated with cotton rolls in the maxillary and mandibular mucobuccal folds and wiped with moist cotton. LMG secretions (10 to 100 μl) were then collected into 10-μl micropipettes (Drummond Scientific). Contamination with major salivary secretions was prevented by use of a saliva ejector. Simultaneously, parotid secretions were obtained from the right Stenson's duct with a Carlson-Crittendon cup. After discarding the initial 0.2 to 0.3 ml, we collected these secretions for 3 to 5 minutes into test tubes on ice. Both LMG and parotid secretions were then stored at 4°C and assayed by single radial immunodiffusion (*9*) within 6 hours. The viscosity of the LMG secretions made consistently accurate application to the immunodiffusion plates difficult. Therefore, LMG secretions were diluted 1 : 4 into phosphate-buffered saline; these dilutions were later accounted for in the final calculations. The sensitivity (*9*) of the assay precluded the use of diluted parotid secretion. Table 1 shows that IgA is the major immunoglobulin in LMG secretions, as well as its previously established predominance in parotid secretion (*10*). Immunoglobulin G was detected in two of four of the LMG secretions (5 and 12 μg/ml) but was not detected in the parotid secretion of the same subjects. Immunoglobulin M was not detected in any LMG or parotid secretion even though LMG secretions were applied undiluted in the assays for this immunoglobulin as well as for IgG. Since it was clear that IgA was the major immunoglobulin in LMG secretions, this immunoglobulin was chosen for further study.

Table 1. Immunoglobulin levels in labial minor salivary gland (LMG) and parotid (P) secretions.

Immunoglobulins	Secretions (μg/ml) of subjects							
	M.T.		J.C.		V.I.		C.K.	
	LMG	P	LMG	P	LMG	P	LMG	P
IgA	228	87	320	72	393	94	256	35
IgG*	5	< 3	< 3	< 3	12	< 3	< 3	< 3
IgM*	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1

*LMG secretion was measured undiluted.

To characterize the nature of LMG IgA and to determine the validity of using a secretory IgA standard, 1 ml of pooled LMG secretion that contained approximately 480 μg of IgA was chromatographed on a column of Sephadex G-200. The resulting fractions were tested in single radial immunodiffusion analysis with antiserums directed to human IgA and to human secretory component (SC). The peaks of both IgA and SC antigenic activities were found only near the column void volume, well in advance of the elution position of IgG which was run as a marker protein on the same column. Immunoglobulin A and SC peaks were coincident, thus suggesting that IgA in LMG secretions contains bound SC and is, therefore, secretory in nature. No peak of free SC was detected.

The mean IgA concentrations in LMG and parotid secretions for 17 different subjects are shown in Fig. 1a. Of 17 subjects, 14 had higher secretory IgA concentrations in their LMG secretion than in their parotid secretion. Despite the wide variation of IgA levels in both secretions, the mean IgA concentration of 194 μg/ml in LMG secretions was significantly higher than the mean IgA concentration of 62 μg/ml in parotid secretions ($P < .005$). The IgA concentration in this latter secretion is in agreement with recorded values (*11*). In order to study this relation between IgA concentrations in LMG and parotid secretion among subjects, these data were expressed as ratios of the concentrations of LMG secretion IgA to parotid secretion IgA. The

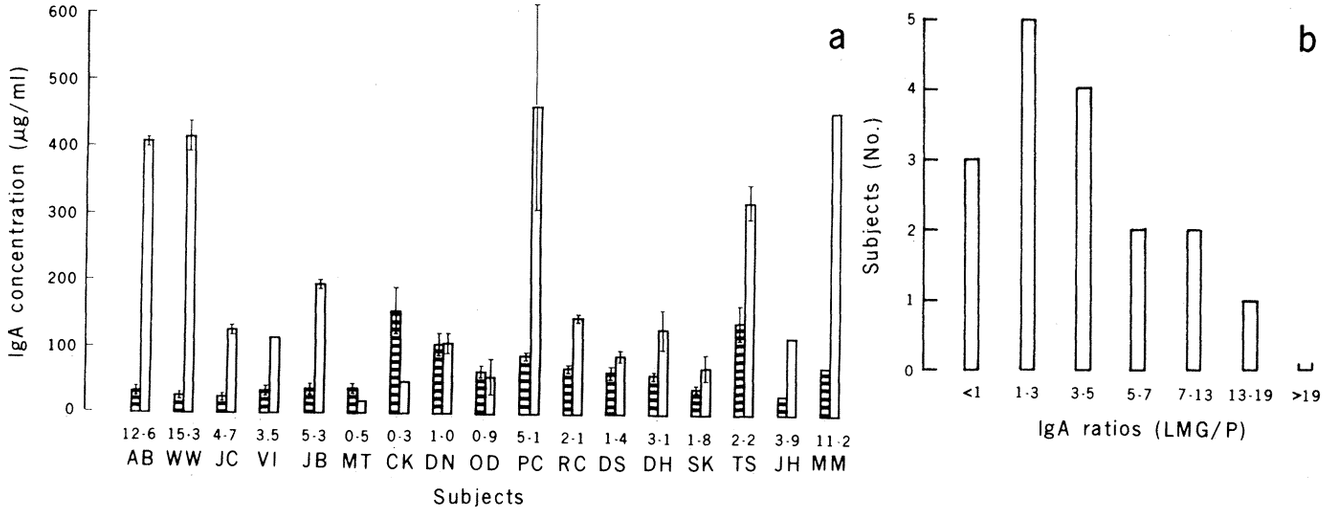

Fig. 1. (a) IgA concentrations (micrograms per milliliter) were determined in LMG (open bars) and parotid (striped bars) secretions of 17 male subjects from 24 to 41 years old. Bars indicate arithmetic means of duplicate or triplicate recordings ± 2 standard deviations. Only one determination each was made for subjects J.H. and M.M. Secretions were collected and assayed by single radial immunodiffusion (*9*). Ratios of IgA concentration in individual LMG secretions to that in individual parotid secretions are shown under the paired bars for each subject. (b) Distribution of the ratios of IgA concentration in LMG secretion to IgA concentration in parotid (P) secretions of the 17 subjects shown in (a). The subjects were grouped according to the numerical value of their ratios.

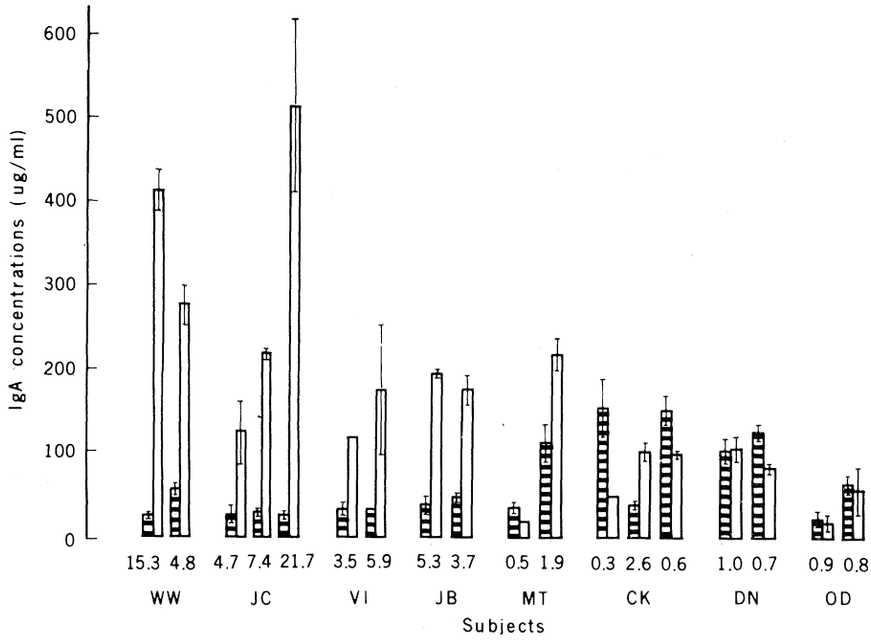

Fig. 2. Variation of IgA concentration (micrograms per milliliter) in LMG (open bars) and parotid (striped bars) secretions of eight male subjects sampled at 6- to 10-day intervals. Samples were collected and assayed by single radial immunodiffusion (*9*). Ratios of the IgA concentration in individual LMG secretions to that in individual parotid secretions at each time interval are indicated under the paired bars for all subjects.

mean ratio for the subjects studied was 4.4 with a range of 0.3 to 15.3. The numerical distribution of this range of ratios is shown in Fig. 1b. A normal distribution of values was obtained in which the majority of subjects had ratios from 1 to 5. Experiments on three subjects for whom IgA levels in undiluted LMG secretions could be examined yielded ratios for IgA in LMG secretions to IgA in parotid secretion of 2.1, 3.8, and 3.9. These values are within the midrange of the distribution, thus confirming that the IgA concentrations in LMG secretions are considerably higher than in parotid secretions.

In order to investigate the physiological constancy of the relation between LMG secretion IgA and parotid secretion IgA, the IgA concentrations in the two secretions of eight individuals, four with high ratios and four with low ratios, were studied after collection at 6- to 10-day intervals (Fig. 2). Some subjects showed considerable variation in IgA levels in the two secretions, probably attributable to variations in flow rate. However, it is apparent that subjects in this group tended to continue to have either high (3.7 to 21.7) or low (0.3 to 2.6) ratios of LMG secretion IgA to parotid secretion IgA and that these ratios remained remarkably consistent over a period of several weeks.

Our results show that the mean IgA concentration of LMG secretions is approximately four times that found in parotid secretion. Labial minor salivary gland secretions also contain at least six times as much blood group substance as is found in submandibular gland secretion (*12*). When taken with our data, these results suggest that the MG's may be a major source of certain salivary constituents. If the assumption is made that all MG secretions contain similar quantities of IgA, and since MG secretions constitute 7 to 8 percent of the volume of whole saliva (*8*), then the contribution of MG secretion IgA to the total salivary secretion can be determined. The resulting mean contribution is, therefore, about 30 to 35 percent of the total salivary IgA. This establishes the MG as a major source of potentially protective immunoglobulin.

Studies in vitro have demonstrated that salivary IgA antibody can interfere with the adherence of oral streptococci to oral mucosal cells (*2*) and have suggested that antibody can also interfere with attachment of these organisms to hard surfaces (*13*), such as teeth. Immunization experiments in animal models have implicated salivary IgA antibody in the reduced ability of *Streptococcus mutans* to colonize the tooth surface (*14*) and in the reduction of dental disease in immune animals (*3*). These effects may be the result of inhibition of glucosyltransferase (GTF), an enzyme that is important in adherence of oral *S. mutans* (*15*). Such inhibition of GTF by salivary IgA antibody has been demonstrated (*16*). The presence of IgA in dental plaque (*17*) may also represent immunological function in the oral cavity. The consistently high concentrations of IgA in MG secretions, as well as the proximity of these glands to dental surfaces, would seem to emphasize their importance in potential immunological effector functions of the secretory immune system in the oral cavity.

We have demonstrated SC that is apparently bound to IgA in secretions from MG. This finding does not appear to resolve the controversy that exists as to the type of cell responsible for the synthesis of SC. Mucous-type alveoli are predominant among the minor glands, including those in the labial region of the oral cavity (*6*). Tourville *et al.* (*18*) demonstrated immunofluorescence with antiserum to SC in mucous-type acinar epithelial cells of submandibular glands, but detected no staining of serous-type cells. On the other hand, others have been unable to demonstrate SC in mucous acini of bronchial and salivary glands, or in intestinal acinar or goblet cells (*19*).

Another important function of the MG may be to provide a pathway for the presentation of oral antigens to cells that are involved in the immune response. Synthesis of IgA antibody can be stimulated in parotid glands by artificial intraductal administration of antigen (*20*). However, it is difficult to conceive of a natural mechanism by which antigen could traverse the entire length of major salivary gland ducts. In contrast, most MG's are located superficially throughout the lamina propria of the oral cavity (*6*). They have short ducts that, at least in the case of the palatine glands, may have a sinusoidal structure (*6*). Therefore, we contend that these topographical and anatomical features could make the cellular elements in these minor glands more accessible to oral antigens, thus resulting in a greater antigenic challenge. It seems reasonable to assume that such a challenge would result in a greater secretory immune response. In fact, we have been able to demonstrate considerable increases in the IgA of LMG secretions relative to parotid secretion. The ease of presentation and maintenance of antigen in close proximity to the orifices of MG ducts for extended periods of time may prove to be an important natural method of oral immunization.

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17 July 1975; revised 8 September 1975

Vitamin B_6–Responsive and –Unresponsive Cystathioninuria: Two Variant Molecular Forms

Abstract. *Cystathionase activity in a lymphoid cell line extract from a vitamin B_6–responsive patient with cystathioninuria was increased strikingly by pyridoxal phosphate. Immunodiffusion with antiserum to human hepatic cystathionase showed identity between this cystathionase protein and cystathionase from an extract of normal lymphoid cells. Neither an increase in cystathionase activity nor immunochemical identity was found using extract of cells from a B_6-unresponsive patient.*

Primary cystathioninuria (*1*) is the result of an inherited deficiency of cystathionase [L-cystathionine cysteine-lyase (deaminating), E.C. 4.4.1.1] (*2*), which cleaves cystathionine to cysteine and α-ketobutyrate, the last step on the pathway of conversion of methionine to cysteine. Of the approximately 25 cases of cystathioninuria that have been reported, all but two (*3, 4*) have demonstrated a diminution of the excretion of cystathionine after administration of massive doses of vitamin B_6. This report presents definitive in vitro evidence that in B_6-responsive cystathioninuria there is an increase in the requirement for vitamin B_6 and that the coenzyme acts directly by stimulation of the cystathionase protein. Evidence also is presented that B_6-responsive and B_6-unresponsive cystathionase deficiencies arise from different mutations.

The defect was investigated in long-term lymphoid cell lines because cystathionase activity in cultured skin fibroblasts is, as we and others (*5*) have found, either absent or very low and liver is less readily available. Lymphoid cell lines from previously reported B_6-responsive (*6*) and B_6-unresponsive (*3*) patients and from one B_6-responsive obligate heterozygote were established by utilizing phytohemagglutinin stimulation of leukocytes and incubation with Epstein-Barr virus (*7*). Cystathionase was assayed in cell lysates prepared by freezing and thawing, under conditions established as optimal for normal lymphoid lines. The cysteine formed was measured directly, either colorimetrically with acid ninhydrin (*8*) or radioactively with L-[^{35}S]-cystathionine as substrate (*9*). The product formed by extracts both of normal cells and of cells from a B_6-responsive patient was confirmed to be cysteine by oxidation with performic acid and separation by high-voltage electrophoresis of the radioactive cysteic acid formed (*10*). The production of cysteine was linear with protein concentration up to 1.0 mg per tube and with time up to 5 hours.

In the absence of added pyridoxal phosphate (PALP) an extract of normal cells showed considerable cystathionase activity (Fig. 1). In contrast, extracts of cells from patients with either B_6-responsive or B_6-unresponsive cystathioninuria exhibited no measurable cystathionase activity when assayed without added coenzyme. The addition of PALP at a concentration of 0.25 m*M* increased the cystathionase activity of the extract of normal cells slightly. A total of 15 normal cell lines was examined in the presence of 0.25 m*M* PALP and found to have a mean specific activity of 29.5 ± 1.95 (standard error) nmole of cysteine per milligram of protein per hour.

In the presence of 0.25 m*M* PALP: Extract of cells from the patient with B_6-responsive cystathioninuria had a specific activity of 4.6 nmole mg^{-1} hour^{-1} (approximately 15 percent of the mean control value). Extract of cells from this patient's father had a specific activity of 17.7 nmole mg^{-1} hour^{-1}, which is intermediate between the mean control value and that of the affected line and gives evidence for an autosomal recessive manner of inheritance. Extract of cells from the patient with B_6-unresponsive cystathioninuria had no discernible activity.

In the presence of increasing concentrations of PALP: The extract from normal cells showed little change in cystathionase activity to 0.75 m*M* and a slight decrease at 1.0 m*M* (Fig. 1). Extract of cells from the B_6-responsive patient had a steady increase in cystathionase activity; at 1.0 m*M* the specific activity was 9.3 nmole mg^{-1} hour^{-1} (31 percent of the mean normal value). Extract of cells from the B_6-unresponsive patient had no discernible cystathionase activity, even at 1.0 m*M* coenzyme.

The activities of *S*-adenosylmethionine

Table 1. Blocking of antibody-binding sites for cystathionase by cell extract from a patient with B_6-responsive cystathioninuria. See text for experimental details. The data below represent one of two experiments. The values are averages of duplicate determinations.

γ-Globulin	Extract		Inhibition of cystathionase activity in normal cell extract (%)
	First preincubation	Second preincubation	
None (buffered saline)		Normal cells	0
Preimmune globulin		Normal cells	3.0
Anticystathionase globulin		Normal cells	54.2
Anticystathionase globulin	B_6-responsive cells	Normal cells	39.8
Anticystathionase globulin	B_6-unresponsive cells	Normal cells	54.8