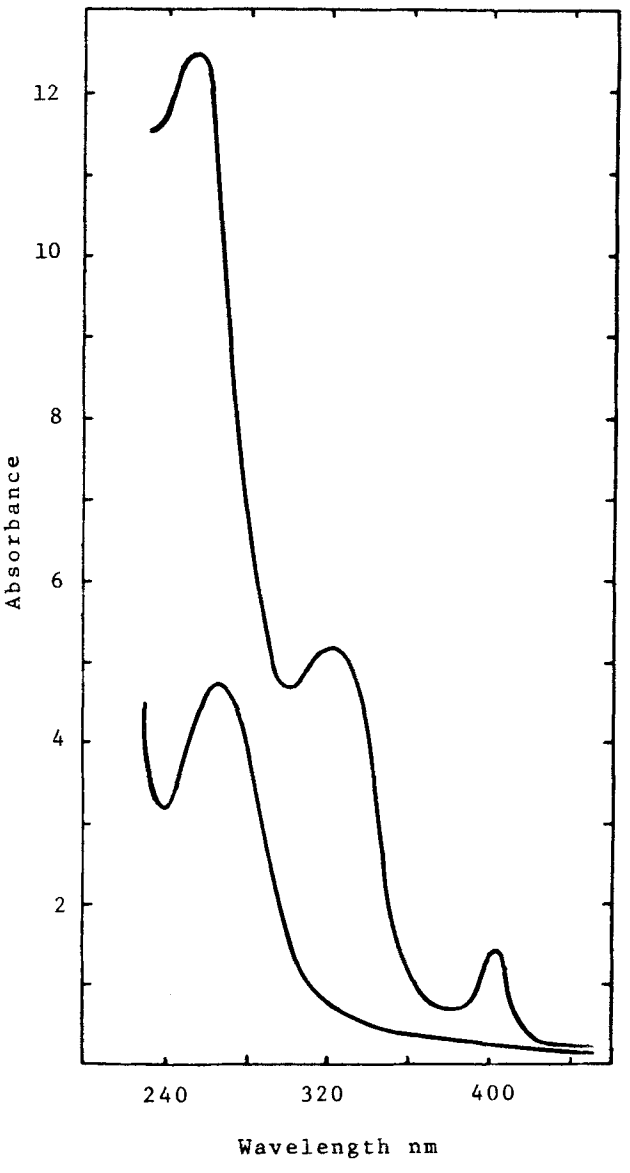

Spectra of the 3-day-old culture supernatants of *Bacillus subtilis* grown in FeDGM (upper) and in FeDGM plus 100 µg iron ($FeCl_3$)/l (lower).

soil (10084.151), collected during the Apollo 11 mission[9], the Zagami meteorite, postulated to be of Mars origin[10], and the Gorda Ridge basalt[11], a representative of the most abundant volcanic rock on earth, all completely inhibited excretion of itoic acid and coproporphyrin III. No inhibition of coproporphyrin III excretion and slight inhibition of itoic acid excretion were observed with the synthetic ilmenite. The Quebec ilmenite inhibited excretion of both, but not to the same extent as the other three natural samples (table). The result indicated that iron was released from the extraterrestrial and terrestrial materials, and transported into the organism through the 'low affinity'[2] itoic acid-independent transport system. However, the iron must have come from such sources as pyroxenes and olivines, but not from ilmenite, all of which are abundant in the lunar soil, the Zagami meteorite and the ocean ridge basalt. Failure of the synthetic ilmenite to release iron for utilization by the organism reflected the tight crystalline structure of ilmenite. The inhibition of excretion of these two compounds by the Quebec ilmenite indicated that this ilmenite sample was somewhat contaminated with other iron sources. It is interesting to note that ash from Mt St. Helen inhibited biosynthesis of rhodotorulic acid, a siderophore of *Rhodotorula pilimanae*[12].

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Differential effects of prostaglandins and isoproterenol on cAMP content and Na, K pump activity in rat submandibular acini

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Summary. The β-adrenergic agonist isoproterenol and prostaglandins E_1 and E_2 (but not $F_{2\alpha}$) increased the cAMP content of rat submandibular acini in vitro, but only isoproterenol enhanced ouabain-sensitive ^{86}Rb (K) uptake. These findings suggest that cAMP is not involved in the activation of the Na, K pump in salivary cells.
Key words. Submandibular acini; isoproterenol; prostaglandins; cAMP; K uptake.

Several lines of evidence indicate that activation of a Na, K pump localized in the basolateral cell membrane[1] is an important consequence of autonomic stimulation in the salivary glands[2–4]. Enhanced pump activity is observed with both cholinergic[5] and β-adrenergic[2] stimuli, but the specific mechanism in each case is not entirely clear, as the two types

of stimuli cause different ion fluxes and thus electrophysiological changes[6] and their respective responses are mediated by different intracellular mediators[7]. Stimulation of β-receptors causes an increase in the cyclic AMP (cAMP) content of salivarys glands[7] and it is possible that this mediator is involved in the significant activation of the cation pump observed upon exposure to β-agonists. Prostaglandins also increase cAMP formation in several tissues[8], but recent evidence indicates that they do not enhance ouabain-sensitive K uptake (i.e., pump activity) in isolated salivary acini[9]. This finding throws doubt, therefore, on the involvement of the cyclic nucleotide in pump activation. In this study we compared, therefore, the effects of prostaglandins and of the specific β-agonist isoproterenol on cAMP content and Na, K pump activation in dispersed acini isolated from the rat submandibular gland. The latter was assessed by the uptake of ^{86}Rb, an isotopic tracer which has been used to monitor K movements in salivary glands[10].

Methods. Salivary acini were isolated from submandibular glands of adult, male rats of the Sprague-Dawley strain by the method previously described[11]. Tissue fragments were incubated at 37 °C for 60 min in a balanced salt solution containing purified collagenase and hyaluronidase and were then mechanically dispersed, centrifuged, filtered, washed and resuspended in fresh medium containing 2.7 mM $CaCl_2$[11]. For cAMP measurements, acinar suspensions (20 ml) were incubated for 30 min with 60 µCi of ^{3}H-adenine, washed twice and divided into 1-ml aliquots in polypropylene tubes. Drugs were added to some tubes and the suspensions were centrifuged 2, 5 or 10 min later and the supernatant removed. The pellet was dissolved in 1 ml of 10% trichloroacetic acid and the mixture was poured over a Dowex column and eluted with 2 and 3 ml of water through alumina, followed by elution with 3 ml of 50 mM Tris buffer. The % conversion of ^{3}H-ATP into cAMP was calculated from the counts in the eluates (sample over total counts) as suggested by Shimizu et al.[12]. For measurement of K uptake, 0.1 µCi/ml of ^{86}Rb (New England Nuclear, specific activity 2.21–8.59 mCi/mg) was added to the suspensions with or without test substance and samples were subsequently removed in duplicate at timed intervals. The acini were separated from the medium by suction filtration through nucleopore filters (25 mm diameter, 3 µm pore size), followed by a wash with 10 ml of ice-cold, isotope-free medium. The radioactivity remaining in the filters was measured by liquid scintillation spectrometry. Protein was measured in aliquots of the acinar suspensions by the Lowry method.

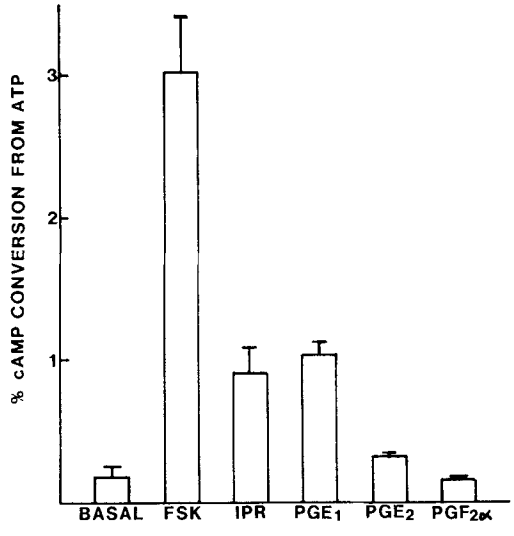

Figure 1. Percent conversion of ATP into cAMP in acini incubated in the absence of added test substance (basal) or in the presence of 10^{-5} M forskolin (FSK), 10^{-5} M isoproterenol (IPR) and 10^{-6} M prostaglandins E_1, E_2, and $F_{2\alpha}$. Data are the means ± SE from 12–16 measurements.

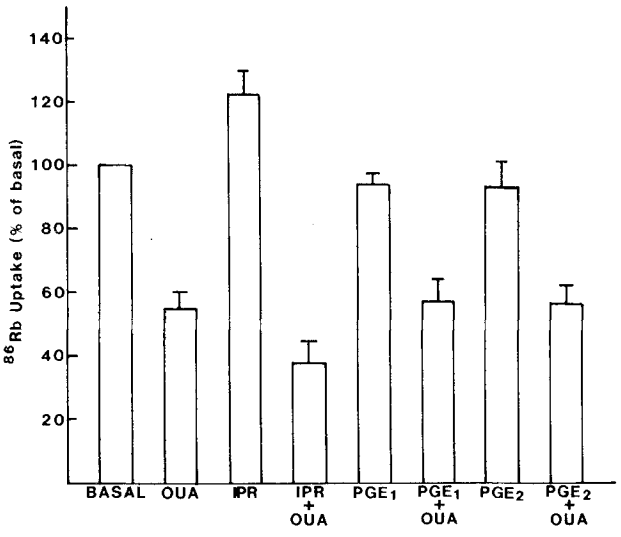

Figure 2. ^{86}Rb uptake in submandibular acini after 10 min of incubation in the absence of added test substance (basal) and in the presence of 10^{-3} M ouabain alone and in combination with isoproterenol (IPR) and prostaglandins E_1 and E_2. Drugs were used in the concentrations indicated in figure 1. Data are the means ± SE from 3–6 experiments and are expressed as percentages (basal content = 100%). Basal ^{86}Rb content after 10 min of incubation was between 380 and 417 nmol/mg protein in the various preparations.

Results. Figure 1 shows the effect of different test substances on the cAMP content of the acini. Basal activity (as % conversion of ^{3}H-ATP) was 0.17% and this activity was enhanced almost 18-fold by 10^{-5} M forskolin (% conversion = 3.00), 4.5-fold by 10^{-5} M isoproterenol (% conversion = 0.78), 6.0-fold by 10^{-6} M PGE_1 (% conversion = 1.03), 2-fold by 10^{-6} M PGE_2 (% conversion = 0.33), but was not affected by 10^{-6} M $PGF_{2\alpha}$ (% conversion = 0.16). Although the phosphodiesterase inhibitor 3-methyl-isobutylxanthine (MIX) did not have an effect on acinar cAMP content by itself, it enhanced the effect of isoproterenol and of PGE_1 and PGE_2 when the PDE inhibitor was added to the cell suspensions in combination with these agents (not shown). The effect was more marked in the case of isoproterenol, in such a way that the combined effect was almost twice as much as with isoproterenol alone (% conversion with the two drugs was 1.37%). Preliminary observations indicate that PGE_1 and PGE_2 have more than additive effects with isoproterenol, as the combined addition causes a greater effect than the sum of the individual ones (not shown).

Figure 2 shows the effect of isoproterenol and the prostaglandins on ouabain-sensitive K (^{86}Rb) uptake. Results have been expressed as percentages of the ^{86}Rb uptake observed after 10 min of incubation during basal conditions (in the absence of added test substance). The findings indicate that ouabain (10^{-3} M) reduces ^{86}Rb uptake to 54±5% of the basal level. Thus, 46% of the K uptake (accumulation) in the acini (as measured with the isotopic tracer ^{86}Rb) occurred by an ouabain-sensitive mechanism, i.e., by the Na, K pump. Accumulation of ^{86}Rb was enchanced 22±7% by 10^{-5} M isoproterenol (fig. 2) and this enhancement was blocked by ouabain, which reduced ^{86}Rb uptake to 37±7% (fig. 2). By contrast, none of the prostaglandins modified ^{86}Rb uptake by themselves, nor did they alter the effect of ouabain on this parameter (fig. 2). ^{86}Rb uptake in the presence of PGE_1 or

PGE_2 alone was, respectively, 94±4% and 93±7% of the uptake observed in the absence of added test substance. When ouabain was also present in the incubation medium, ^{86}Rb uptake decreased to 57±7% and to 57±6% with PGE_1 and PGE_2 (fig. 2).

Discussion. The results indicate that although both isoproterenol and prostaglandins E_1 and E_2 increase cAMP formation in rat submandibular acini, only the former substance increases ouabain-sensitive K (^{86}Rb) uptake by the Na, K pump. Thus, our findings indicate that cAMP is not the mediator in the enhancement of pump activity induced by β-adrenergic agonists in salivary cells. Such an effect is likely related to other effects, distinct from the formation of cAMP. Preliminary experiments suggest that this may involve differential effects on Na entry into the cells, which is enhanced by isoproterenol but not by the prostaglandins.

As cAMP does not mediate either ouabain-sensitive K uptake (this study) or Cl efflux in salivary acini[13], the question arises which role, if any, does this nucleotide fulfill in fluid secretion induced by β-receptor stimulation in salivary glands[2]. Both activation of the Na, K pump and of Cl efflux into the lumen through channels presumably localized in the apical cell membrane are believed to be important elements of the ionic mechanism underlying saliva secretion[2, 3, 11]. A recent finding that cAMP enhances tight junctional permeability[14] may provide an answer to this question. An important difference between isoproterenol and the prostaglandins, both of which increase cAMP formation in salivary acini, is that the former also increases Na accumulation in the intercellular space as a result of increased pump activity. This should provide a supply of osmotically active ions to move across the more permeable tight junction to provide a driving force for fluid secretion. In the case of the prostaglandins, tight junctional permeability may be enhanced by the increased formation of cAMP, but the failure to activate the Na, K pump would not provide a source of Na in the intercellular spaces for movement across the tight junction.

The effect of PG on cAMP in submandibular acini suggest that these cells have functional receptors for these substances. It remains to be seen if these receptors are directly coupled to adenylate cyclase or to some other aspect of cAMP metabolism, such as inhibition of phophodiesterase. Although rat submandibular cells may thus have PG receptors coupled to cAMP, the inhibitory effects of PG on fluid secretion in vivo[15] do not seem to be related to effects on ion transport systems[9]. Thus, these receptors may fulfill other cAMP-mediated roles in salivary acini, such as those related to protein synthesis and secretion. However, the inhibitory effects of PG on fluid secretion may still be related to other effects shared with β-adrenergic agonists, as previous exposure to isoproterenol also inhibits cholinergically-induced salivation in vivo[16].

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Casein and lactose concentrations in milk of 31 species are negatively correlated

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Summary. Casein and lactose concentrations in milks of various species vary by at least 1–2 orders of magnitude, yet no single species secretes both components at either the high or the low end of the ranges. This pattern of variation could result from evolutionary adaptations in a single secretory mechanism.

Key words. Casein; lactose; milk composition; milk secretion.

The mechanisms of milk secretion have been studied in detail in only a few species[1–3] most of which are eutherians. Consequently generalizations about the formation of milk in the approximately 4000 extant species must necessarily be circumspect. Nevertheless, the means by which proteins for export are synthesized and secreted by the secretory cells of the mammary gland appears to have much in common with the mechanism used in the export of proteins by other eukaryotic cells fulfilling many diverse functions[4–7]. Milk proteins are packaged for export in vesicles derived from the Golgi apparatus of the mammary secretory cell. The vesicles move to the apical membrane and pass their contents into the alveolar lumen by an exocytotic mechanism. Secretion of milk fat is by a separate process and mixing of the fat and aqueous phases occurs only in the alveolar lumen. The principal difference between the mammary gland and other protein-exporting cells is the synthesis of low molecular-weight carbohydrates such as lactose[8] in the Golgi vesicles of the former. This is thought to influence the volume of aqueous fluid secreted per unit time[9].

Taylor and Husband[10] were the first to recognise clearly the role of lactose synthesis in determining the daily yield of milk from cows. As originally envisaged, synthesis of lactose draws in water osmotically to increase the volume of the aqueous secretion. Since the synthesis of lactose could be accompanied by a compensating loss of an osmotically equi-