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Salivary gland carbonic anhydrases

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Summary. Carbonic anhydrases (CA) are zinc metalloenzymes that catalyze the reversible conversion of carbon dioxide and water to bicarbonate. For most mammalian cells, this reaction is essential to maintaining intracellular physiological pH. For salivary glands, it also has an important role in the regulation of the pH and buffering capacity of their secretory product, saliva, which is central to the activity of salivary digestive enzymes. This review is a chronological narrative of the discovery and distribution of CA and its isoenzymes in mammalian salivary glands and saliva and the role of CA in regulation of salivary pH via secretion of the salivary CA isoenzyme and the secretion and reabsorption of bicarbonate from the ductal lumen. The interaction of sodium/bicarbonate co-transporters and other factors in these processes is briefly described. The distribution of CA among mammalian species, salivary glands, and gland cells as determined by CA activity in saliva and gland extracts, enzyme histochemistry, and immunohistochemistry is presented in tables. The importance of salivary CA to oral health is underscored by the reduction in salivary gland and salivary CA activity by nutritional zinc deficiency and genetic variants of two of the CA isoenzymes, which cause dysgeusia and hyposalivation and are associated with increased dental caries.

Key words: Bicarbonate, Carbonic anhydrase, Enzyme histochemistry, Immunohistochemistry, Saliva, Salivary glands, Zinc metalloenzymes

Introduction

Carbonic anhydrases (CA; carbonate anhydratase; E.C.4.2.1.1) are enzymes that catalyze the reversible hydration of carbon dioxide to bicarbonate and a proton in two steps: $CO_2 + H_2O \leftrightarrows H_2CO_3 \leftrightarrows H_2CO_3 \leftrightarrows H^+ + HCO_3^-$ (Sand, 1949; Yoshimura et al., 1959). These reactions are essential to maintaining a physiologic pH of cells and the extracellular environment (Sly and Hu, 1995). In particular, they moderate the acidity of the H_2CO_3 that results from the cellular production of energy via oxidation of fuel to H_2O and CO_2. Salivary glands secrete a fluid (saliva) containing enzymes, mucins and electrolytes into the oral cavity. Salivary functions include moistening and cleansing the oral mucosa and teeth, lubricating solid food morsels, and initiating digestion of ingested food (Young and van Lennep, 1979). When stimulated, salivary glands secrete the resulting CO_2 as HCO_3^-, which together with K^+, Na^+ and Cl^-, provides the pH and other favorable conditions for the functioning of mucins, digestive enzymes, and other constituents of the saliva.

The genes that encode CAs are divided into eight familie (α, ß, γ, etc.), with mammalian CAs belonging to the α-CA families (Hewett-Emmett and Tashian, 1996; Garcia-Lorca et al., 2024). CA VI, the only secretory CA isoenzyme, is secreted in milk and saliva (Kivelä et al., 1999a), CAs I, II, III, VII and XIII reside in the cytosol, CAs IX, XII and XIV are membrane bound (Garcia-Lorca et al., 2024) and CA IV is tethered to the cell membrane (Brown et al., 1990; Wistrand et al., 1999). This review focuses on the discovery, distribution, secretion and functions of isoenzymes of CA in mammalian salivary glands and saliva.

Discovery

Knowledge of CA in saliva and salivary glands progressed as methods of assay and identification developed. Two methods for identifying the presence of CA in liquids were devised in the 1930s. The method of Meldrum and Roughton, (1933) measured the CO_2 escaping from buffered bicarbonate solutions. A white substance that they extracted from ox blood, which they named *carbonic anhydrase*, accelerated this process when diluted up to 1,000,000 times. Brinkman (1933) added a colorimetric step to this method. The method of Keilin and Mann, (1940) measured the conversion of sodium carbonate to bicarbonate when adding CO_2. The former measures the *liberation* and the latter the *uptake* of CO_2 in the respective reactions. Using both of these methods, Rapp (1946) was the first to show CA activity in human saliva. He demonstrated both uptake and

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liberation of CO_2 from stimulated, dialyzed human whole saliva, reactions that were almost completely inhibited when the dialysate was boiled or an enzyme inhibitor such as KCN or sulfanilamide was added. Van Goor and Bruens, (1948) found a range of 0 to 1.25 and an average of 0.54 units per 50 mm^2 in samples of human whole saliva. They also found that CA activity was highest, approaching that of blood, in the parotid glands, and 12 times lower in the submandibular and sublingual glands, in extracts of dog salivary glands ground with sand and corrected for blood content. Hoshi (1950) demonstrated CA activity in saline extracts of parotid glands from dog and ox.

Note that further variations in these methods by various workers that are in some of the following reports will not be cited in this review.

Distribution

By CA activity In saliva and salivary glands

Established methods of measuring CA activity in liquids having demonstrated the presence of CA in human saliva and in dog saliva and salivary glands, attention turned to finding and comparing CA activity in the saliva and salivary glands of other animals. To these ends, exploration of CA activity in whole saliva (WS), saliva collected from ducts of parotid (PS) and submandibular/sublingual (SMS) salivary glands, or in gland extracts (PG, SLG, SMG) was undertaken. Yoshimura et al. (1959) found a hierarchy of PG > SMG > SLG in dog and that rabbit PG was > dog SLG. Draus et al. (1962) showed that Cow SMG had very high CA activity (20 units/mg of extract). Szabó et al. (1966) established important guidelines for assays of CA activity in human saliva. They demonstrated that the CA activity in human stimulated PS increased in a straight line until a rate of 0.075 mL/min., then plateaued. When collected at 0.80 mL/min., there were no differences in CA activity among WS, PS, or SMS, nor among samples collected or not under oil, nor up to 48 hrs after collection, whether stored at room temperature or at 0°C (range 1.77-2.14 K/mL). They also noted that the CA activity transitioned from less than to greater than that of blood as the flow rate increased from resting (unstimulated) to stimulated. Pinheiro et al. (1972a) centrifuged the homogenates of salivary glands and measured CA activity in the supernatant. They reported that CA activity/g of tissue in PG was markedly decreased and in SMG markedly increased from controls in alloxan diabetic, pancreatectomized and partly pancreatectomized male rats. They also reported that CA activity/mg tissue in SMG was increased from controls in surgically and chemically (potassium perchlorate or propylthiouracil) thyroidectomized male mice (Pinhiero et al., 1972b). Taga and Pinheiro, (1972) examined the CA activity (units/g of tissue) of PMG and SMG of 11 mammals (Table 1). The range was more than 100 times from lowest in SMG of goat to highest in the SMG of ox, and it was higher in both PG and SMG of females in most species. Ten years later, Matsumoto et al. (1982) found that CA activity was higher in PG but not SMG of ruminant compared to non-ruminant animals (Table 2). The sex of the animals was not provided and their use of units/mg instead of units/g tissue must be considered in comparing their results to those of Taga and Pinheiro (1972).

Murakami and Sly, (1987) purified and characterized human salivary CA and named it CA VI. The DNA sequence of CA VI was determined from tissues or saliva from cow, dog, human, mouse and sheep by Fernley et al. (1989). By Western blot they found that the enzymes from cow, dog and human, but not mouse, cross-reacted with the sheep enyzme. Of eighteen tissues from sheep, CA VI was detected only in the parotid and submandibular salivary glands. By hybrid histochemistry, CA VI was localized to the acini and duct contents, confirming that it is a secretory enzyme. CA VI was cloned, and the cDNA was characterized by

Table 1. Carbonic anhydrase activity of mammalian salivary glands.

Animal	Number		Enzyme Units/g. of Tissue			
			Submandibular		Parotid	
	Female	Male	Female	Male	Female	Male
Mouse	5	6	950.3	543.4	973.3	831.5
Rat	5	6	1,338.6	1,112.4	822.6	773.6
Hamster	3	3	881.1	1,006.8	822.6	773.6
Guinea Pig	5	5	2,489.0	2,299.6	212.9	244.5
Cat	4	3	443.1	236.8	2,772.5	1,418.8
Rabbit	4	4	3,030.8	2,314.0	1,887.6	1,922.4
Dog	3	2	275.1	208.2	1,308.7	1491.0
Goat	1	1	50.7	58.7	1,283.0	2,013.8
Sheep	2	1	325.8	24.2	2,172.8	1,600.4
Hog	1	1	235.8	120.4	3,949.6	3,669.9
Ox	1	2	11,435.6	4,722.0	3,353.7	3,735.9

Reproduced from Table 1 in Taga and Pinheiro (1972).

Aldred et al. (1991). The development of fluorometric (Parkkila et al., 1993) and radioimmunoassay (Fernley et al., 1995) assays for CA VI enabled the accurate measurement of CA VI in human saliva.

In a number of studies, it had been determined that some patients with hypogeusia had low levels of salivary and serum zinc and atrophic taste buds, and that their taste acuity and taste buds returned to normal with administration of zinc (reviewed by Henkin et al., 1975). A zinc protein that they purified from human parotid saliva rapidly incorporated intravenously administrated ^{45}Zn. Assuming a relationship to taste, they named it *gustin*. Shatzman and Henkin, (1981) reported that decreased WS levels of zinc and gustin corresponded with decreased taste in humans. Chaudrey et al. (1981), noting that Meldrum and Roughton, (1933) and Keilin and Mann, (1940) had shown that zinc is required for CA activity, found that male weanling rats fed a zinc deficient diet for 4 weeks developed a 26% reduction in CA activity/g of tissue in SMG but that there was no effect on CA in PG. Interestingly, the activity of alkaline phosphatase, another zinc-requiring enzyme, was markedly reduced in both salivary glands. Gandor et al. (1983) found that the body weights and the CA and alkaline phosphatase activities (units/g of tissue) of SMG failed to increase in zinc deficient rats while all three increased in pair-fed rats. In human studies, Thatcher at al. (1998) determined that gustin is CA VI, and Henkin et al. (1999) showed that oral zinc administration restored CA VI level in CA VI- deficient patients. Goto et al. (2000) showed that the CA activity/mg tissue of the SMG of very and moderately zinc deficient rats was 36.4 and 66.3%, respectively, of SMG of pair-fed rats (zinc deficient rats consumed less food). Alkaline phosphatase activity was similarly reduced in the zinc-deficient rats. Subsequently, Goto et al. (2008) reported that it was CA II, not VI, that was reduced in the SMG of the zinc-deficient rats. In this regard, Mau et al. (2010) noted that CA II was secreted from bovine salivary glands.

Parkkila et al. (1995) found that CA VI (mg/mL) and α-amylase (units/mL) concentrations in the saliva of six male human beings were dramatically lower during sleep than while awake. Becerra et al. (2003) collected unstimulated SL/SM saliva from six human subjects at three 2 min. intervals and stimulated saliva at a constant rate for ten 1 min intervals. Bands on acrylamide gels were identified using antibodies against mucins, amylase and carbonic anhydrase and a parallel set was stained with the periodic-acid (PAS) reaction. Image analysis of intensity of the PAS reaction indicated that the concentration of CA (identified as CA VI) increased significantly with stimulation and was stable within the unstimulated and stimulated periods.

These reports on biochemical assays have provided information about the distribution of CA activity of salivary glands by gland and species of animal but not by intracellular structures, except that salivary CA activity suggests a secretory enzyme in the acini. The development of enzyme- and immunohistochemical methods to localize CA remedied this lack of knowledge.

Table 2. Carbonic anhydrase activity of salivary glands.

Animal	Number	Mean Enzyme Units/mg. Tissue_± SE	
		Parotid	Submandibular
Cow	5	78.08±2.26	0.76±0.00
Sheep	2	66.28±2.96	ND
Goat	4	52.02±4.70	2.91±0.00
Rabbit	4	1.00±0.19	24.72±2.49
Rat	5	8.19±1.77	22.30±1.02
Dog	4	45.21±5.49	6.67±0.89
Monkey	2	7.99±2.82	22.50±0.30

SE, Standard Error; ND, Not Done. Excerpted from Table 1 in Matsumoto et al. (1982), with permission.

By enzyme histochemistry

Kurata (1953) fixed slices of guinea pig gastric mucosa in acetone followd by incubating in a solution of $MnCl_2$ and $NaHCO_3$, embedding in paraffin, and staining the resulting manganese carbonate precipitate in de-paraffinized sections with potassium periodate. The parietal cells and red blood cells (rbcs) were intensely positive. Häusler (1958) developed and Waldeyer and Häusler (1959) modified another histochemical technique to demonstrate CA activity in tissues. Frozen sections were floated on an acid solution of $CoSO_4$, H_2SO_4 and Na_2SO_4 mixed immediately before use with $NaHCO_3$. The reaction results in precipitation of $CoCO_3$, which is visualized by reaction with $(NH_4)_2S$ as deposits of CoS. The long incubation time of 90 to 120 min. risked diffusion of the enzyme and reaction product. Further modifications by Kornhonen and Kornhonen, (1965) and Leder and Tritschler, (1966) reduced the reaction time to 15-20 min. but this was still too long. Hansson (1967) made major improvements by adding KH_2PO_4 to the solution and rinsing the tissues in saline before developing the stain in $(NH_4)_2S$. Sections were attached to slides by air-drying and then fixed in Carnoy's solution or other fixatives, counterstained with hematoxylin or nuclear fast red, and dehydrated with graded ethanol and xylenes. Cover slips were mounted with Canada balsam. The reaction was more sensitive, and the incubation time was cut to 1 to 3 min. Others, e.g., Gandor and Meyer, (1985) and Sugimoto et al. (1988) added further modifications. Morris and Swayne, (1964) were the first to apply enzyme histochemistry of CA to salivary glands, but they reported only "blackening of acini and ducts". Leder and Tritschler, (1966) used salivary glands of rats, mice, cats and rabbits to develop their method. Initial skepticism regarding the specificity of the Hansson technique was contested by Lönnerholm et al. (1980).

The distribution of CA by species, salivary gland

and epithelial cell type as determined by enzyme histochemistry is compiled in Table 3. No appreciable activity was detected in mucous acini. Fixation of the tissues with aldehydes tended to diminish CA reactivity in the serous acini and demilunes, but not the striated and excretory ducts, which had strong CA reactivity in all species and glands (Leder and Trechsler 1966; Carpentier et al., 1980, 1985; Ikejima and Ito, 1984; Menghi et al., 1983; Peagler et al., 1998). Not shown in Table 3 is that reactions also were localized to the luminal and basal areas of the serous acini and the striated and excretory ducts in all glands and to the basal area of the granular convoluted tubules of the submandibular glands of rodents. These CA reactions supported what had long been surmised, that the deeply folded and interdigitated basolateral membranes and large, elongated mitochondria which make up the *striations* in the striated and excretory ducts of human and rodent major salivary glands (Tandler 1963; Tamarin and Sreebny 1964), provide the energy and surface area that facilitate the trans-epithelial transport of HCO_3^- and other electrolytes such as Na^+, Cl^-.

Carpentier et al. (1984) demonstrated Ca activity in the secretory granules of rat parotid acini by enzyme histochemistry. They further showed that the number of secretory granules in gland slices was diminished proportionate to the release of CA into the medium by stimulation with isoproterenol. They concluded that the results validated the enzyme histochemical method of Hansson and showed that salivary CA was produced, stored and secreted in the secretory granules. Brown et al. (1984), displayed CA activity in the taste buds associated with the circumvallate papilla of the rat tongue. It was noted that in the same sections there was abundant CA activity in the acini and ducts of the serous lingual glands of von Ebner (VEG) and in residual rbcs. Although VEG do not have striated ducts, numerous cells in rat and human VEG main excretory ducts have abundant, large mitochondria (Hand, 1970; Testa Riva et al., 1985; Azzali et al., 1989). This together with strong luminal immuno-localization of CAs I and II (Redman, 2025) suggest that these ducts may engage in transepithelial bicarbonate transport to manage the pH of their saliva.

By immunohistochemistry

Purification and characterization by methods such as

Table 3. Enzyme histochemical localization of carbonic anhydrase in salivary glands.

Species	Fixative	Ac	ID	SD	ED	GCT	Reference
Parotid Gland							
Cat	Acetone	+	-	+	+		Leder and Tritschler (1966)
Mouse	Acetone	+	-	+	+		Leder and Tritschler (1966)
Mouse	Form/Glut*	+	-	+/-	+/-		Ikejima and Ito (1984)
Rabbit	Acetone	+	-	+	+		Leder and Tritschler (1966)
Rat	Acetone	+	-	+	+		Leder and Tritschler (1966)
Rat	Glut	+	-	+	+		Carpentier et al. (1985)
Rat	Acetone	+	+/-	+	+		Peagler et al. (1998)
Sublingual Gland							
Cat	Acetone	+	-	+	+		Leder and Tritschler (1966)
Mouse	Acetone	+	-	+	+		Leder and Tritschler (1966)
Mouse	Form/Glut	-	-	+	+		Menghi et al. (1983)
Rabbit	Acetone	+	-	+	+		Leder and Tritschler (1966)
Rabbit	Form/Glut	+	-	+	+		Menghi et al. (1983)
Rat	Acetone	+	-	+	+		Leder and Tritschler (1966)
Rat	Formol	-	-	+	+		Carpentier et al. (1980)
Rat	Form/Glut	+	-	+	+		Menghi et al. (1983)
Rat	Glut	+	-	+	+		Carpentier et al. (1985)
Submandibular Gland							
Cat	Acetone	+	-	+	+		Leder and Tritschler (1966)
Mouse	Acetone	+	-	+	+	+	Leder and Tritschler (1966)
Mouse	Form/Glut	+	-	+	+	+	Menghi et al. (1983)
Rabbit	Acetone	+	-	+	+		Leder and Tritschler (1966)
Rabbit	Form/Glut	+	-	+	+		Menghi et al. (1983)
Rat	Acetone	+	-	+	+	+	Leder and Tritschler (1966)
Rat	Formol	+	-	+	+	+	Carpentier et al. (1980)
Rat	Form/Glut	+	-	+	+	+/-	Menghi et al. (1983)
Rat	Glut	+	-	+	+	+	Carpentier et al. (1985)
Lingual von Ebner's Glands							
Rat	Glut	+	+		+		Brown et al. (1984)

*4% Formaldehyde + 0.5% Glutaraldehyde. Ac, Serous Acini and Demilunes; ID, SD, ED, Intercalated, Striated and Excretory Ducts; GCT, Granular Convoluted Tubules; +/-, some cells +, others – stain, OR weak stain. Mucous Acini -.

affinity chromatography and acrylamide gel electrophoresis afforded evidence of CA isoenzymes in salivary glands and saliva of species such as pig (Fernley et al., 1979) and rat (Feldstein and Silverman, 1984). These methods also provided samples to prepare antibodies of high specificity for immunohistochemistry. The development of immunohistochemical techniques included attaching a fluorescent molecule (reviewed by Coons, 1958), or an enzyme such as peroxidase to the primary antibody, followed by reaction with a chromogenic substrate of the enzyme (Nakane and Pierce, 1966). Further development involved attaching a peroxidase label to an antibody to the primary antibody ("Bridge" technique, Mason et al., 1969) and an avidin-biotin-peroxidsase complex ("ABC" technique, Hsu et al., 1981).

The distribution of CA immunohistochemical reactions by source of antibody, gland, species, and epithelial cell type is summarized in Tables 4-7.

Spicer et al. (1982) did a pioneer experiment in which rabbit antihuman rbc (combined CA I and II) immunolocalized to (*labeled*) the acini but not the ducts of human submandibular glands. Hennigar et al. (1983) found variations in labeling of anti-human CA I and II antibodies among acini and ducts in mouse and rat major salivary glands and between Bouin's and Carnoy's fixatives. In a *tour de force* of complementary methods, Ikejima and Ito, (1984) studied CA activity and presence in mouse parotid glands. With enzyme histochemistry (method of Hansson, 1967), strong reactions occurred in the luminal contents of the acini and ducts and in the cytoplasm but not the secretory granules of the acinar cells, and weak reactions occurred in the cytoplasm of the intercalated and striated ducts. Rabbit anti-mouse antibodies were prepared from CA purified from a homogenate of all three major salivary glands. By direct and indirect immunofluorescence, the secretory granules were strongly labeled. However, by an immunoperoxidase method (both light and electron microscopy), the label was strong in the rough endoplasmic reticulum but was limited to the secretory granule membranes of the acini. Saliva had strong CA activity whether

Table 4. Immunohistochemical localization of carbonic anhydrase I in salivary glands.

Antibody Host Anti-	Species	Fixative	Ac	ID	SD	ED	GCT	Reference
Parotid Gland								
Rabbit Dog rbc	Dog	Bouin's	-	-	-	-		Asari et al., 2000)
Rabbit Human rbc	Mouse	Bouin's	+	-	+/-	+/-		Hennigar et al. (1983)
Goat Human	Rat	Form*	-	+	+	+		Cho et al. (2009)
Rabbit Human rbc	Rat	Bouin's	+/-	-	-	-		Hennigar et al. (1983)
Goat Human rbc	Rat	Helly's	-	+	+	+		Peagler et al. (1998)
Goat Human rbc	Human	Carnoy's	+/-	-	+	+		Noda et al. (1986c)
Rabbit Human rbc	Human	Carnoy's	-	-	-	-		Parkkila et al. (1990)
Sublingual Gland								
Rabbit Dog rbc	Dog	Bouin's	-	+	+	+		Asari et al. (2000)
Rabbit Human rbc	Mouse	Bouin's	-	-	+/-	+		Hennigar et al. (1983)
Goat Human	Rat	Form*	-	+	+	+		Cho et al. (2009)
Rabbit Human rbc	Rat	Bouin's	-	-	-	+		Hennigar et al. (1983)
Goat Human rbc	Rat	Helly's	+	+	+	+		Redman et al. (2000)
Goat Human rbc	Human	Carnoy's	+	-	+	+		Noda et al. (1986c)
Submandibular Gland								
Rabbit Dog rbc	Dog	Bouin's	-	+	+	+		Asari et al. (2000)
Rabbit Human rbc	Hamster	Bouin's	-	-	+	+	+	Mori (1991)
Rabbit Human rbc	Mouse	Bouin's	+	+/-	+/-	+/-		Hennigar et al. (1983)
Rabbit Human rbc	Mouse	Bouin's	-	-	+	+	+	Mori (1991)
Rabbit Human rbc	Mouse	Carnoy's	-	-	+	+	+	Mori (1991)
Goat Human rbc	Mouse	Bouin's	-	+/-	+	+		Noda et al. (1986b)
Goat Human	Rat	Formal*	-	-	+	+	+/-	Cho et al. (2009)
Rabbit Human rbc	Rat	Bouin's	-	-	+/-	+/-		Hennigar et al. (1983)
Rabbit Human rbc	Rat	Carnoy's	+	-	+	+	-	Hennigar et al. (1983)
Rabbit Human rbc	Rat	Bouins	+/-	+	+	+	+	Mori (1991)
Rabbit Human rbc	Mouse	Carnoy's	-	+	+	+	+	Mori (1991)
Goat Human rbc	Rat	Helly's	-	+	+	+	+	Redman et al. (2000)
Goat Human rbc	Rat	Bouin's	-	-	+	+	+/-	Noda et al. (1986a)
Goat Human rbc	Human	Carnoy's	-	-	+	+		Noda et al. (1986c)
Rabbit Human rbc	Human	P-L-Form**	+	+/-	+/-	-		Ogawa et al. (1993)
Rabbit Human rbc	Human	Carnoy's	-	-	-	-		Parkkila et al. (1990)
Lingual von Ebner's Gland								
Goat Human rbc	Rat	Helly's	+/-	+		+		Redman (2025)

*Formaldehyde solution with no methanol. **Periodate-lysine formaldehyde. Ac, Serous Acini and Demilunes; ID, SD, ED, Intercalated, Striated and Excretory Ducts; GCT, Granular Convoluted Tubules; rbc, red blood cells; +/-, some cells +, others – stain, OR weak stain.

stimulated by phenylephrine or pilocarpine. CA activity in gland homogenates did not differ between samples taken before and after secretory stimulation. They concluded that the presence of CA in the secretory granules was "enigmatic". In subsequent studies, CA VI labeled the cytoplasm, but not distinctly the secretory granules, of serous acini in human parotid and submandibular glands (Kadoya et al., 1987; Ogawa et al., 1992, 1993; Peagler et al., 1998) and rat parotid and submandibular glands (Ogawa et al., 1998). However, CA VI was conclusively localized to the secretory granules of serous acini of human parotid (Parkkila et al., 1990; Piras et al., 2012) and submandibular glands (Parkkila et al., 1990).

In most studies, CA I and II antibodies labeled the larger ducts and variably the acini and intercalated ducts (Hennigar et al., 1983; Noda et al., 1986c; Spicer et al., 1990; Asari et al., 1991, 1993, 2000; Mori, 1991; Redman et al., 2000; Cho et al., 2009). Notably, the luminal aspect of the cytoplasm of the larger ducts was more strongly labeled with CA I, II and VI antibodies in studies on rat major and lingual serous (von Ebner's) glands (Peagler et al., 1998; Redman et al., 2000; Redman, 2025). CAs I and II labels were very strong in scattered columnar cells in the striated and excretory ducts and granular convoluted tubules of hamster (Noda

Table 5. Immunohistochemical localization of carbonic anhydrase II in salivary glands.

Antibody Host Anti-	Species	Fixative	Ac	ID	SD	ED	GCT	Reference
Parotid Gland								
Rabbit Human	Cow	Methanol	+	-	-	+		Mau et al. (2010)
Rabbit Dog rbc	Dog	Bouin's	+	+	+	+		Asari et al. (2000)
Rabbit Human rbc	G. Pig	Carnoy's	-	+	+	+/-		Spicer et al. (1990)
Rabbit Human rbc	Mouse	Bouin's	+/-	-	-	-		Hennigar et al. (1983)
Rabbit Human rbc	Mouse	Carnoy's	+/-	+/-	+/-	+/-		Spicer et al. (1990)
Goat Human	Rat	Formal*	+	+	+	+		Cho et al. (2009)
Rabbit Human rbc	Rat	Bouin's	+/-	+/-	+/-	+/-		Hennigar et al. (1983)
Rabbit Human rbc	Rat	PLP*	-	-	+	+		Ogawa et al. (1992)
Goat Human rbc	Rat	Helly's	-	+	+	+		Peagler et al. (1998)
Goat Human rbc	Human	Carnoy's	+	+	+	+		Noda et al. (1986c)
Rabbit Human rbc	Human	Carnoy's	+	-	-	-		Parkkila et al. (1990)
Sublingual Gland								
Rabbit Dog rbc	Dog	Bouin's	-	+	+	+		Asari et al. (2000)
Rabbit Human rbc	G. Pig	Carnoy's			+	+/-		Spicer et al. (1990)
Rabbit Human rbc	Mouse	Bouin's	-	-	+/-	+/-		Hennigar et al. (1983)
Rabbit Human rbc	Mouse	Carnoy's			+	+		Spicer et al. (1990)
Goat Human	Rat	Formal*	+	+	+	+		Cho et al. (2009)
Rabbit Human rbc	Rat	Bouin's	-	-	+/-	-		Hennigar et al. (1983)
Rabbit Human rbc	Mouse	Bouin's	-	-	+	+		Mori (1991)
Goat Human rbc	Rat	Helly's	+	+	+	+		Redman et al. (2000)
Goat Human rbc	Human	Carnoy's	+	+	+	+		Noda et al. (1986c)
Submandibular Gland								
Rabbit Dog rbc	Dog	Bouin's	-	+	+	+		Asari et al. (2000)
Rabbit Human rbc	G. Pig	Carnoy's	-	+	+	+/-		Spicer et al. (1990)
Rabbit Human rbc	Hamster	Bouin's	-	-	+	+	+	Mori (1991)
Rabbit Human rbc	Hamster	Carnoy's	-	-	+	+	+/-	Mori (1991)
Rabbit Human rbc	Mouse	Bouin's	-	-	+/-	+/-		Hennigar et al. (1983)
Goat Human rbc	Mouse	Bouin's	-	-	+	+	+	Noda et al. (1986a)
Rabbit Human rbc	Mouse	Bouins	-	-	+	+	+	Mori (1991)
Rabbit Human rbc	Mouse	Carnoy's	-	+	+	+	+	Mori (1991)
Rabbit Human rbc	Mouse	Carnoy's	+/-	+/-	+/-	-		Spicer et al. (1990)
Goat Human	Rat	Formal*	+	+	+	+	+/-	Cho et al. (2009)
Rabbit Human rbc	Rat	Bouin's	-	-	+/-	-		Hennigar et al. (1983)
Rabbit Human rbc	Rat	Bouin's	-	-	+	+	+	Mori (1991)
Rabbit Human rbc	Rat	Carnoy's	-	-	+	+	+	Mori (1991)
Rabbit Human rbc	Rat	PLP**	-	-	+	+	+	Ogawa et al. (1992)
Goat Human rbc	Rat	Helly's	-	+	+	+	+	Redman et al. (2000)
Goat Human rbc	Rat	Bouin's	-	-	+	+	+/-	Noda et al. (1986a)
Goat Human rbc	Human	Carnoy's	+	+	+	+		Noda et al. (1986c)
Rabbit Human rbc	Human	PLP*	+	+/-	+/-	+/-		Ogawa et al. (1993)
Rabbit Human rbc	Human	Carnoy's	+	-	-	-		Parkkila et al. (19900
Lingual von Ebner's Gland								
Goat Human rbc	Rat	Helly's	+	+		+		Redman. (2025)

*Formaldehyde solution with no methanol, **Periodate-lysine-formaldehyde. Ac, Serous Acini and Demilunes; ID, SD, ED, Intercalated, Striated and Excretory Ducts; GCT, Granular Convoluted Tubules; rbc, red blood cells; +/-, some cells +, others – stain, OR weak stain; No entry, not determined (sublingual gland) or gland (von Ebner's) has no SD.

et al., 1986a) and mouse (Noda et al., 1986a; Mori, 1991) and rat (Hennigar et al., 1983; Noda et al., 1986a) submandibular glands. It would be interesting to see if these are the dark cells that were shown to engage in H^+ secretion or HCO_3^- reabsorption in rat submandibular gland excretory ducts (Knauf et al., 1983).

CA IV mRNA was identified in homogenates of unspecified human salivary glands by real-time PCR/Northern and Southern blots (Fujikawa-Adachi et al., 1999). CAs III, IV, and IX labeled ducts almost exclusively except that CA III labeled only myoepithelium in a study on a human submandibular gland (Shamsutdinov et al., 1991). CA IV is anchored to the plasmalemma of (Zhu and Sly, 1990; Waheed et al., 1992), and has been immunolocalized to the luminal and basolateral cell membranes of endothelial and epithelial cells of lung and kidney (Brown et al., 1990, Fleming et al., 1995). CA IV has labeled the cytoplasm as well as the cell membranes of rat (Cho et al., 2009) and human (Redman and Bandyopadhyay, 2021; Redman, 2023) salivary glands.

Ca VI labeled serous acini and variably the striated and excretory ducts in most studies (Ikejima and Ito, 1984; Ogawa et al., 1992,1993; Peagler et al., 1998; Redman et al., 2000; Leinonen et al., 2001; Kaseda et al., 2006; Piras et al., 2012; Hsieh et al., 2016). The orifices of the main excretory ducts of VEG are located in the troughs of the vallate papillae and foliate furrows of many species, including human (Testa Riva et al., 1985; Azzali et al., 1989) and rat (Hand, 1970). These are thought to contribute to taste sensation by washing the taste buds in the troughs when their salivary secretion is stimulated during feeding activity. This notion was supported by the CA activity localized by the previously cited enzyme histochemistry in the taste buds and VEG (Brown et al., 1984) and by the immunolocalization of CA VI in the VEG acini and ductal contents (Leionen et al., 2002; Redman, 2025). It was also supported by the observations of Patrikainen et al. (2014) on perception of bitter taste in CA VI mutant and non-mutant mice.

Acini and ducts undergo atrophy and apoptosis, leaving clusters of small groups of cells without distinctive acinar or ductal characteristics in rat salivary glands after ligation of the main excretory duct and in human salivary glands after exposure to therapeutic

Table 6. Immunohistochemical localization of carbonic anhydrase VI in salivary glands.

Antibody Host Anti-	Species	Fixative	Ac	ID	SD	ED	GCT	Reference
Parotid Gland								
Rabbit Cow Saliva	Cow	Bouin's	+	-	+	+		Kaseda et al. (2006)
Rabbit Dog Saliva	Dog	Bouin's	+	-	+	+		Kasuya et al. (2007)
Rabbit Mouse Sal Gld	Mouse	F-P*	+	-	+/-	-		Ikejima and Ito (1984)
Rabbit Human Saliva	Rat	Carnoy's	+	-	-	-		Leinonen et al. (2001)
Mouse Rat Saliva	Rat	PLP**	+	-	-	-		Ogawa et al. (1992)
Goat Human Saliva	Rat	Helly's	-	-	+	+		Peagler et al. (1998)
Not provided	Human	Formalin	+	-	-	-		Hsieh et al. (2016)
Rabbit Human Saliva	Human	Carnoy's	+	-	-	-		Parkkila et al. (1990)
Goat Human Saliva	Human	Formalin	+/-	-	+/-	+/-		Peagler et al. (1998)
Rabbit Human Saliva	Human	F-G***	+	-	-	-		Piras et al. (2012)
Sublingual Gland								
Rabbit Cow Saliva	Cow	Bouin's	+	-	+	+		Kaseda et al. (2006)
Rabbit Dog Saliva	Dog	Bouin's	+	-	+	+		Kasuya et al. (2007)
Goat Human Saliva	Rat	Helly's	-	-	+	+		Redman et al. (2000)
Submandibular Gland								
Rabbit Cow Saliva	Cow	Bouin's	+	-	+	+		Kaseda et al. (2006)
Rabbit Dog Saliva	Dog	Bouin's	+	-	+	+		Kasuya et al. (2007)
Rabbit Human Saliva	Rat	Carnoy's	+	-	-	-		Leinonen et al. (2001)
Mouse Rat Saliva	Rat	PLP**	+	-	-	-		Ogawa et al. (1992)
Goat Human Saliva	Rat	Helly's	-	-	+	+	+	Redman et al. (2000)
Rabbit Human Saliva	Human	PLP**	+	-	-	-		Ogawa et al. (1993)
Rabbit Human Saliva	Human	Carnoy's	+	-	-	-		Parkkila et al. (1990)
Goat Human Saliva	Human	Formalin	+	-	+/-	+/-		Peagler et al. (1998)
Lingual von Ebner's Gland								
Rabbit Human Saliva	Rat	Carnoy's	+	+		+		Leinonen et al. (2001)
Goat Human Saliva	Rat	Helly's	+/-	+		+		Redman (2025)
Rabbit Human Saliva	Human	Formalin	+	-	-	-		Leinonen et al. (2001)
Lingual Mucous Glands								
Rabbit Human Saliva	Rat	Carnoy's	+	-		-		Leinonen et al. (2001)

*6% Formaldehyde and 0.1% Picric acid; ***1% Formaldehyde + 1.25% Glutaraldehyde.; **Periodate-lysine-formaldehyde. Ac, Serous Acini and Demilunes; ID, SD, ED, Intercalated, Striated and Excretory Ducts; GCT, Granular Convoluted Tubules; rbc, red blood cells; Sal Gld, Salivary Glands; +/-, some cells +, others – stain, OR weak stain.

irradiation for cancer of the head and neck (reviewed by Redman, 2008). In many of these duct-like structures, CA II labeling was moderate in ligated rat salivary glands (Noda et al., 1986c) and CA IV labeling was strong in an irradiated human submandibular gland (Redman, 2023), indicating that these were ducts. Some of the cell clusters that did not react with the CA IV antibody in the human gland were labeled by antibodies to α-amylase, indicating that these were atrophic acini.

One would think that antibodies directed against the target antigen of the subject species would be more specific than anti-other species antibodies, but anti-human rbc antibodies were used in all but one of the immunohistochemical studies on CA I and II. On one hand, variations in localization among acini and ducts and among fixatives occurred as much among human specimens as among specimens from other species. On the other hand, studies by Peagler et al. (1998), Redman et al. (2000), Redman (2025) exemplify a problem with using primary antibodies (anti-human) against other than the species being investigated (rat). These workers chose primary and secondary antibodies and a fixative that provided stronger or exclusive CA reactions in the striated and excretory ducts to facilitate a study on CA I,

Table 7. Immunohistochemical localization of Ither carbonic anhydrases in salivary glands.

Antibody Host Anti-	Species	Fixative	Ac	ID	SD	ED	GCT	Reference
			Carbonic Anhydrase III					
Parotid Gland								
Rabbit Dog rbc	Dog	Bouin's	-	+	-	-		Asari et al. (2000)
Rabbit Human muscle	G. Pig	Carnoy's	-	+	+	+		Spicer et al. (1990)
Rabbit Human muscle	Mouse	Carnoy's	-	-	-	+		Spicer et al. (1990)
Rabbit Horse	Rat	Bouin's	-	-	+/-	+/-		Asari et al. (1991)
Sublingual Gland								
Rabbit Dog rbc	Dog	Bouin's	-	+	-	-		Asari et al. (2000)
Rabbit Human muscle	G. Pig	Carnoy's			+	+/-		Spicer et al. (1990)
Rabbit Human muscle	Mouse	Carnoy's			+/-	+		Spicer et al. (1990)
Submandibular Gland								
Rabbit Dog rbc	Dog	Bouin's	-	+	-	-		Asari et al. (2000)
Rabbit Horse	Cow	Bouin's	-	+	+/-	+		Asari et al. (1993)
Rabbit Human muscle	G. Pig	Carnoy's	-	+	+/-	+/-		Spicer et al. (1990)
Rabbit Horse muscle	Hamster	Bouin's	-	-	+/-	-	-	Nishita et al. (1989)
Rabbit Human muscle	Mouse	Carnoy's	-	-	-	+		Spicer et al. (1990)
Rabbit Horse muscle	Rat	Bouin's	-	+	+	+	-	Nishita et al. (1989)
Rabbit Human	Human	Frozen Myoepithelial cells on Ac, ID, SD						Shamsutdinov et al. (1991)
			Carbonic Anhydrase IV					
Parotid Gland								
Goat Human	Rat	Form*	-	+	+	+		Cho et al. (2009)
Goat Human	Human	Formalin	-	+	+	+		Redman and Bandy-opadhyay (2021)
Sublingual Gland								
Goat Human	Rat	Form*	-	+	+	+		Cho et al. (2009)
Submandibular Gland								
Goat Human	Rat	Form*	-	+	+	+	+	Cho et al. (2009)
Goat Human	Human	Formalin	-	+	+	+		Redman (2023)
			Carbonic Anhydrase IX					
Parotid Gland								
Goat Human	Rat	Form*	-	+	+	+		Cho et al. (2009)
Sublingual Gland								
Goat Human	Rat	Form*	-	+	+	+		Cho et al. (2009)
Submandibular Gland								
Goat Human	Rat	Form*	-	+	+	+	+/-	Cho et al. (2009)
Carbonic Anhydrase XII								
Parotid Gland								
Rabbit Mouse	Mouse	Methanol	+		+	+		Hong et al. (2015)

*Formaldehyde solution with no methanol; Ac, Serous Acini and Demilunes; ID, SD, ED, Intercalated, Striated and Excretory Ducts; GCT, Granular Convoluted Tubule; rbc, red blood cells; +/-, some cells +, others – stain, OR weak stain.

II and VI as markers of striated and excretory duct differentiation in the major salivary glands and VEG of rats during postnatal development. The results were as expected (Fig. 1) except that the serous demilunes of the sublingual gland (Fig. 1a,b) and the acini of von Ebner's glands also stained moderately with CAs I and II in the

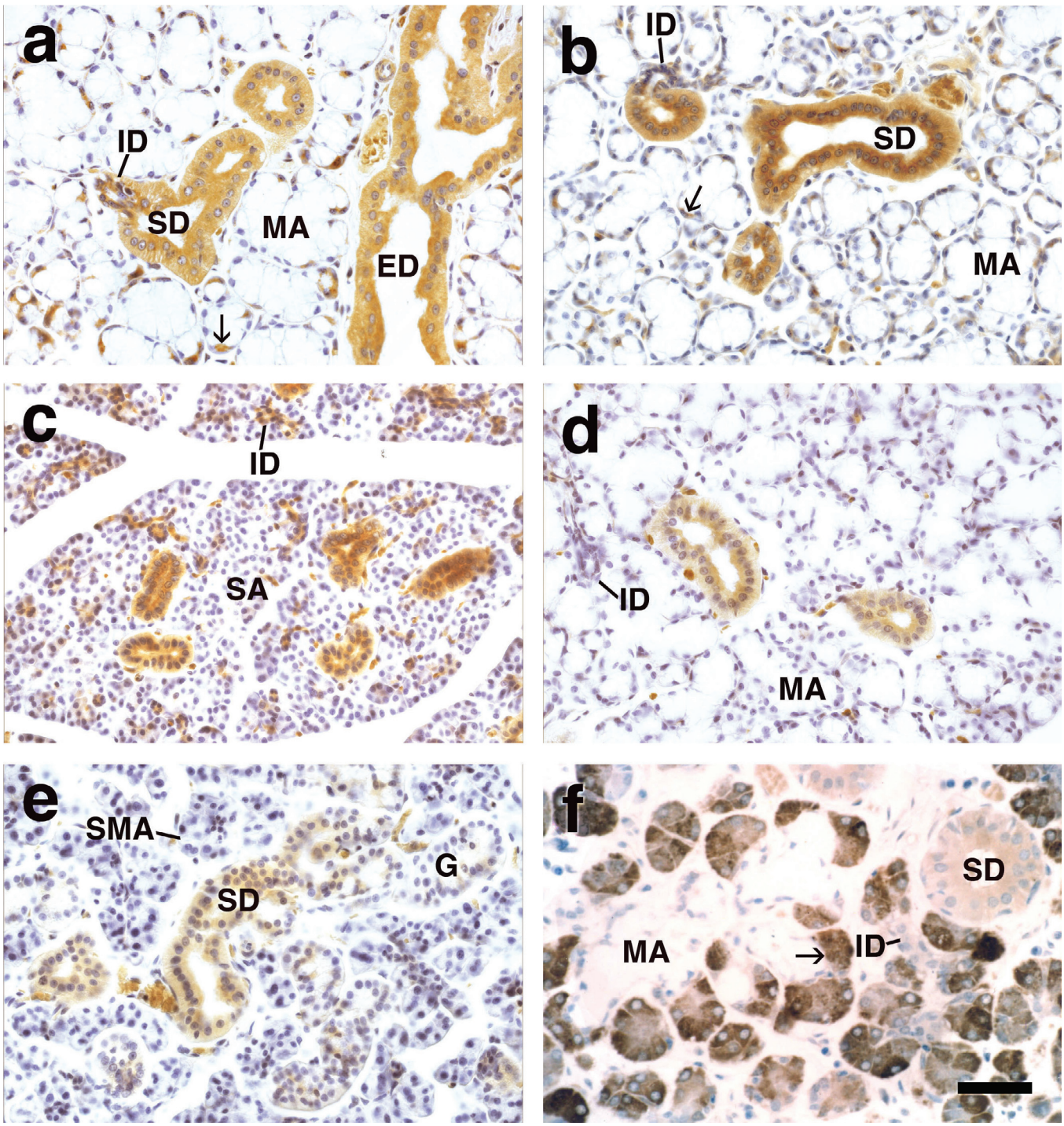

Fig. 1. Photomicrographs of sections from the study by Peagler et al. (1998) of immunohistochemically localized carbonic anhydrase isozymes (yellow to brown deposits of reaction product) in the parotid (P), sublingual (SL) and submandibular (SM) glands of 42 days old rats **(a-e)** and a human SM gland **(f)**. **a.** CA I, SL. **b.** CA II, SL. **c.** CA II, P. **d.** CA VI, SL. **e.** CA VI, SM. **f.** CA VI, human SM. MA: mucous acini; SA: Serous acini; SMA: Seromucous acini; G: Granular Convoluted Tubules; ID, SD, ED: Intercalated, Striated and Excretory Ducts; Arrows: serous demilunes. Hematoxylin counterstain. Scale bar: 50 μm.

oldest rats (Redman, 2025). In addition, anti-human saliva CA VI antibodies reacted as expected with the serous acini and demilunes of the human submandibular gland (Fig. 1f; positive control) and acini of VEG, but only with the striated and excretory ducts in the major rat salivary glands. Western blots demonstrated that the antibodies reacted strongly with extracts of the tissues of origin (human rbcs and saliva) and also, though more modestly, with extracts of the rat glands, in bands of appropriate molecular mass (~30 kDa for CA I and II, 40-42 kDa for CA VI; Feldstein and Silverman, 1984; Ogawa et al., 1992). However, CAs I and II did not react with the rat rbc extract nor rbcs in the sections, and rat rbcs have been shown to harbor these CA isozymes (Ogawa et al., 1992). Peagler et al. (1998) offered a "plausible explanation for the diversity and overlaps in the specificity of antibodies that can be produced against CA antibodies: the protein sequences (Hewett-Emmett and Tashian, 1996) and glycosylation (Hooper et al., 1995; Di Fiore et al., 2020) in the α-family of CA isozymes show both diversity and homology. For example, antibodies to human CA VI reportedly do (Murakami and Sly, 1987) and do not (Feldstein and Silverman, 1984; Fernley et al., 1995) cross-react with highly purified human CA II."

Secretion

Sand (1949) found that the concentration of bicarbonate in stimulated human submaxillary-sublingual saliva was many times higher than in unstimulated saliva, and that CA was present in these glands. He then demonstrated that the bicarbonate concentration of the saliva was independent of the bicarbonate concentration in the blood (Sand, 1951). In experiments with dog submaxillary [submandibular] salivary glands, it had been shown that glucose is metabolized (Anrep and Cannan, 1922), and the production of CO_2 is increased three to four times when secretion is stimulated (Barcroft, 1901). These results led Sand (1951) to postulate, then demonstrate, that most of the bicarbonate secreted from rabbit salivary glands originated from the CO_2 produced by their cellular metabolism, not the blood. His experiments and those of Yoshimura et al. (1959) indicated that the accurate comparison of the concentration of bicarbonate in saliva among glands requires samples that are either unstimulated or in a range of moderately to maximally stimulated regardless of the source of stimulation. Thaysen et al. (1954) confirmed the rise in bicarbonate in stimulated human parotid saliva with increased flow rate to a plateau after 1.5 ml/min. Na^+ and Cl^- concentrations continued to rise and K^+ remained low at flow rates up to 4.5 mL/min. These data were portrayed in comparison to blood values in their iconic graph. In general, the pattern of increases in HCO_3^-, Na^+ and Cl^- and low (or of decreasing to low) K^+ concentration in stimulated saliva was found in subsequent studies with the parotid and submandibular glands of other species such as mouse (Mangos et al., 1973c), rabbit (except submandibular) (Mangos et al., 1973a), and rat (Holsgreve et al., 1966; Young and Martin, 1971; Mangos et al., 1973b; Sommers et al., 1975; Young et al., 1979).

The use of a micropuncture technique enabled the establishment of electrolyte concentrations in the acini/intercalated duct region (i.e., in the primary saliva), the intralobar (striated) ducts, and the main excretory duct of mature rat salivary glands (Mangos et al., 1966) and to describe the differentiation of the ducts during their postnatal development (Mangos, 1978). The unstimulated primary saliva in the submandibular gland of adult rats was isotonic in terms of osmolality, NA^+ and Cl^- (Martinez et al., 1966), and in the transient, immature acini (terminal tubules) of the preweaned rat it was hypertonic, compared with serum from the same rats (Holgreve et al., 1966). In both of these studies, and the mouse parotid and submandibular saliva study of Mangos et al. (1973c), the osmolality, NA^+ and Cl^- levels were dramatically less between the juxta-

Table 8. Immunolocalzation of salivary gland sodium-bicarbonate cotransporters (NBCs).

NBC	Species	Gland	Acinus		ID		SD		ED		Reference
			L	BL	L	BL	L	BL	L	BL	
NBC1	Guinea Pig	PG	-	+			+	-	+	-	Li et al. (2006)
NBC1	Guinea Pig	SMG	-	-			-	+	-	+	
NBC3	Guinea Pig	PG	-	-			-	+	-	+	
NBC3	Guinea Pig	SMG	+	-			+	-	+	-	
NBC3, -5	Rat	PG	-	+			+	+	+/-	+	Rousa et al. (1999)
NBC3	Rat	SMG	-	-			+	+	+	+/-	
NBCe1-A	Rat	SMG					+/-	+	+	+/-	Brandes et al. 2007
NBCe1-B	Rat							+	+/-		
pNBC1	Mouse	SMG	-	+	-	+	-	+	-	+	Luo et al. (2001)
NBC3	Mouse	SMG	+	-	+	-	+	-	+	-	
NBC1	Human	PG	-	+							Park et al. (2002)

ID, SD, ED, intercalated, striated and excretory ducts; L, BL, luminal and basolateral membranes; PG, SMG, parotid and submandibular glands. +/-, some cells +, others -.

glandular portion and oral terminus of the main duct, indicating that the ions were re-absorbed by the intralobar and excretory ducts. These values did not change after ductal administration of inulin, indicating that ductal transfer of water was not a factor (Mangos et al., 1973c). These findings led to and supported the explanation of changes in electrolyte levels with increasing rate of secretion of stimulated saliva as being due to acinar and ductal excretion of HCO_3^- and the inability of reabsorbtion to keep up with the increases in amounts of secreted Na^+ and Cl^- and (Young et al., 1970; Sommers et al., 1975).

The secretion of HCO_3^- into the acinar and ductal lumina of salivary glands involves the interactions of Na^+/HCO_3^- cotransporters (*NBCs*) (Roussa et al., 1999; Luo et al., 2001; Park et al., 2002; Alvarez et al., 2003; Li et al., 2006; Brandes et al., 2007) in the luminal and basolateral membranes (Table 8) and Na^+/H^+ exchangers (*NHE*) (Melvin et al., 1988; Nguyen et al., 2000; Li et al., 2006; Lee et al., 2012; Namkoong et al., 2015; Lee and Hong, 2020). The location of NBCs in the acini and ducts is indicative of their intra- (basolateral) or extracellular (luminal) direction of activity. The immunolocalization of NBCs is compiled in Table 8.

The overall process of salivary secretion involves the coordinated interaction of many players that are initiated by transient increases in intracellular Ca^{2+} and cytosolic cyclic adenosine monophosphate (cAMP) (Nauntofte, 1992; Melvin et al., 2005; Jung and Lee, 2014). Diagrams illustrating some of the interactions involved in HCO_3^- secretion have been presented by Melvin et al. (2005) for serous acini (not illustrated) and by Jung and Lee (2014) for large ducts (Fig. 2). Further discussion of these processes is beyond the scope of this review.

The Glu143Lys (E143K) mutation in CA XII causes salt wasting, hyponatremia and hyperkalemia, resulting in marked dehydration, and dry mouth (Feldshtein et al., 2010) and others. The importance of CA XII in the secretion of fluid and HCO_3^- was shown in experiments with a mouse model with the E143K mutation (Hong et al., 2015). The mutation resulted in altered glycosylation such that most of the CA XII did not transit to its normal basolateral location in the acini and ducts, greatly decreasing its regulation of HCO_3^- transporters in the cell membranes). With inter- and intralobular ducts isolated from wild type mouse submandibular glands, CA XII provided most of the HCO_3^-, more than from CA I, II and IV combined, that transferred into the lumen. In the ducts from the CA XII mutant mice, much less HCO_3^- and fluid transited into the lumen. Salivary secretion was markedly reduced in the mutant mice. In a subsequent report the CA XII mutation was shown to also disrupt cell membrane stability and volume regulation of the water channel AQP5 (Hwang et al., 2019). AQP5 occupies the luminal and basolateral membranes only of acini of human, mouse and rat salivary glands, and intercalated ducts of mouse submandibular gland (D'Agostino et al., 2020), and micropuncture studies have shown that the larger ducts secrete very little fluid (Mangos et al., 1973c). Therefore, the major effects of CA XII on salivary secretion should be in the acini, with effects on the striated and excretory ducts being regulation of salivary HCO_3^-.

Salivary pH

Shafer et al. (1958) showed that the pH of

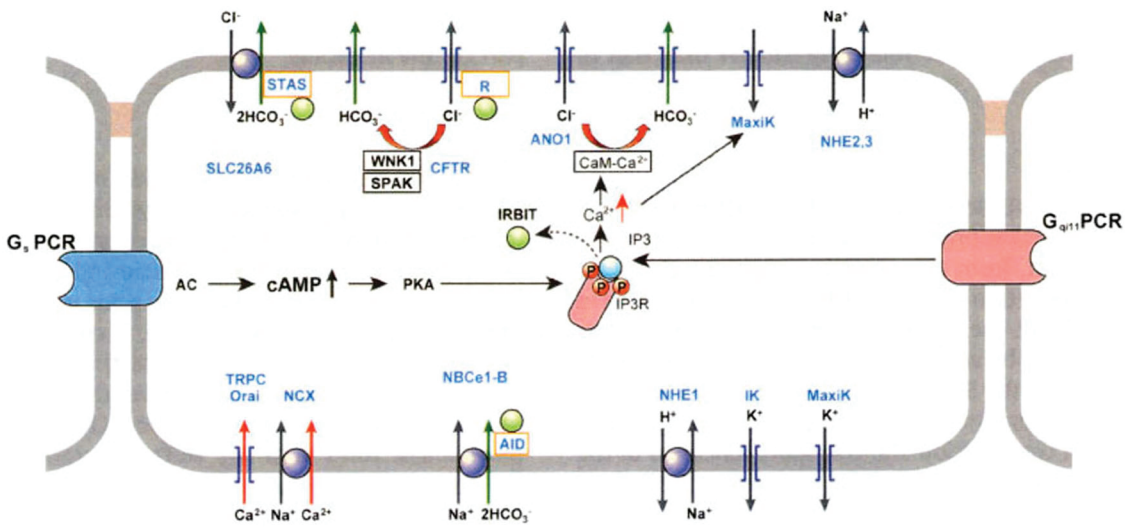

Fig. 2. Cell signals that augment epithelial HCO_3^- secretion. The HCO_3^- permeability of anion channels in the apical membrane is dynamically regulated by cell signals, which play important roles in transepithelial HCO_3^- secretion. The CFTR anion channel can be highly permeable to HCO_3^- by the low $[Cl^-]$-induced activation of with-no-lysine kinase 1 (WNK1) and STE20/SPS1-related proline/alanine rich kinase (SPAK). The ANO1 CaCC becomes highly permeable to HCO_3^- by a calmodulin (CaM)-dependent mechanism when $[Ca^{2+}]$ is above ~1μM. The key HCO_3^- -transporting proteins. Reproduced from Figure 2 in Jung and Lee (2014).

pilocarpine-stimulated whole saliva declined slightly from 8.6 to 8.3 to 8.0 in weanling, young adult and old adult rats, respectively, and that the pH was not appreciably affected when various combinations of the main excretory ducts of the parotid, sublingual and submandibular glands were ligated. On the other hand, the pH of unstimulated rat *parotid* saliva was 7.8 and a steady 7.5 regardless of flow rate when stimulated (Sommer et al., 1975).

Chauncey and Weis (1958) found no appreciable change in maximally stimulated human parotid salivary flow rate, HCO_3^-, NA Cl or pH (7.4) after administration of Diamox, a CA inhibitor. In a study by Dawes and Jenkins (1964) the pH of resting (unstimulated) human parotid saliva was 5.8, rising in an arc to 7.8 with increasing flow rate regardless of source of stimulation. Experiments by Bardow et al. (2000) showed that the pH of human *whole* saliva was 6.8 when unstimulated and 7.2 when stimulated by chewing paraffin, with no significant female/male differences in flow rate or pH. In the same study, these authors confirmed the conclusion by Lilienthal (1955) and many others that the buffering capacity of both stimulated and unstimulated human whole saliva is related mostly to HCO_3^-, and that this capacity increases in proportion to the rise in HCO_3^- in stimulated saliva. The concentration of phosphate, which is second in importance of salivary buffering capacity, did not increase with increasing salivary flow.

As noted in the Introduction, the pH and electrolytes of saliva are important to the function of salivary digestive enzymes. Some of the enzymes in human and/or rat saliva are α-amylase (Schramm and Danon, 1961), a type I deoxyribonuclease (Sreebny et al., 1965), an alkaline ribonuclease (Robinovitch et al., 1968), lysozyme (Jenzano et al., 1986), peroxidase (Mansson-Rahemtulla et al. 1986), an alkaline protease (Shafer et al., 1959), and a trypsinlike protease (Barrett and Ball, 1974). The optimal pH of α-amylase is 6.8 (Bernfeld, 1955), deoxyribonuclease I, 6.3 (Kurnick, 1962), lysozyme, 7.0 (Jenzano et al., 1986) salivary peroxidase, 7.27 (Mansson-Rahemtulla et al., 1986), alkaline ribonuclease, 7.8 (Kalnitsky et al., 1959), alkaline protease, 8.2 (Riekkinen and Niemi, 1968), and trypsinlike protease, 9.2 (Barrett and Ball, 1974). It is noteworthy that all of these enzymes are active in the pH range of stimulated and unstimulated rat and human whole saliva.

Salivary CA and oral diseases

Much of the early work on salivary and salivary gland CA (e.g., Sand, 1949, 1951; Lilienthal, 1955; Chauncey and Weiss, 1958) was in pursuit of what seemed a likely association between its regulation of salivary pH and buffering capacity, dental caries (favored by low pH), and periodontitis (high pH favoring deposition of dental calculus). The microenvironment of the salivary plaque also was recognized early on (Lilienthal, 1955).

Dental caries

Interestingly, a low concentration of CA VI in saliva has been associated with both a higher (Kivelä et al., 1999b) and a lower (Vasudevan et al., 2022) prevalence of caries. The mutant CA VI isozyme in C3H/HeN caries-resistant mice has greater affinity for hydroxyapatite and higher activity than the CA VI of WT mice (Oshima et al., 2022). However, caries development was reduced in CA VI deficient mice (Culp et al., 2011). These opposing effects of low salivary CA VI activity on dental caries may be partly explained by the work of de Sousa et al. (2021), who found that the higher salivary and biofilm CA VI activity in their early childhood caries group were transiently reduced to those of the caries free group by sucrose and sucrose plus starch rinses. Salivary pH and buffering capacity were reduced in both groups after the rinses.

A number of studies have shown a higher rate of dental caries in rats of dams fed a zinc deficient diet compared to those from dams fed a zinc sufficient diet, when challenged with a cariogenic diet (Brown et al., 1979; Cerklewski, 1980). Similarly, in a case-control study, children with systemic zinc deficiency had more dental caries, dental plaque and gingivitis than those with sufficient zinc (Atasoy and Ulusoy, 2012). The higher caries was attributed in part to less zinc in the tooth enamel and dentin of the zinc deficient groups. Oddly, the authors of these studies did not mention the studies cited above that had shown the effects of zinc deficiency on salivary CA activity. Effects on CA activity may not have been important in the studies by Brown et al. (1979) and Cerklewski (1980) as the cariogenic challenge diet fed after weaning was zinc sufficient and thus may have restored normal salivary CA activity. However, dental caries was higher in the zinc deficient variant of a cariogenic diet compared to the zinc sufficient variant pair-fed fed to weanling rats (Fang et al., 1980). It seems likely that the rats fed the zinc deficient diet would have had lower salivary CA activity.

In this regard, it is notable that CA VI is significantly reduced in whole saliva of patients with primary Sjögren's syndrome (Chan et al., 2012; Hall et al., 2017), an autoimmune disorder that destroys the ducts and acini of salivary and lacrimal glands. The resulting hyposalivation predisposes to dental caries. Anti-CA VI antibodies were detected in the serum of 60% of patients tested in early stages and 38% in late stages of Sjögren's syndrome, suggesting that these antibodies might be a useful biomarker for this condition (Beckman et al., 2017). Interestingly, type 2 diabetes patients with the TT genotype of CA VI polymorphism rs2274327 (C/T) gene have significantly lower unstimulated salivary flow rate, pH and buffer capacity and higher risk of developing dental caries, periodontitis,

xerostomia and taste impairment compared to patients with the CC and CT genotypes (Mrag et al., 2020). In healthy volunteers, the concentration but not the catalytic activity of CA VI in unstimulated whole saliva was lower in the TT compared to the CT and CC genotypes (Aidar et al., 2013).

Periodontitis

Intuitively it would seem that a higher pH and buffer capacity in resting whole saliva would favor the deposition of calcium phosphate in dental plaque as an important process in the formation of dental calculus, a critical factor in gingivitis and periodontitis. An association between higher calculus score and higher pH of saliva measured at the SLG/SMG orifices was found in a cross-sectional study of patients with good, fair and poor oral hygiene (D'souza et al. 2023). On the other hand, in the previously cited study by Mrag et al. (2020), the periodontal indices were higher, despite the lower salivary pH and buffer capacity, perhaps because of the lower salivary flow, in the CT and TT groups. Further, there was less CA VI in the unstimulated whole saliva in patients with periodontitis in patients with type 2 diabetes (Chan et al., 2012) and in patients with generalized aggressive periodontitis (Wu et al., 2009) compared with healthy controls.

Dawes (2006) suggested that the tendency for more calculus to form on the lingual surfaces of the lower anterior teeth is not because of a rise in the pH of saliva due to loss of CO_2 as it exits the SLG/SMG ductal orifices, as was widely believed at the time. Rather, the salivary film there has a high velocity, clearing acid and adding urea from the saliva, which favor the metabolism of bacteria that cause alkalinization of the plaque. In other words, what is going on in dental plaque is the deciding factor in whether the underlying tooth surface will demineralize (caries) or induce calculus deposition. This idea is supported by the observation that the pH of fluid in periodontal pockets bears no relation to the salivary pH. (Watanabe et al., 1996).

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