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SALIVARY COMPOSITION: EFFECTS OF AGE AND SEX

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SUMMARY

Saliva from healthy individuals and from patients with various diseases, between the ages of 10 to 90, were collected. The amylase and protein composition were correlated with age, sex and state of health. The results indicate that the specific activity of amylase decreased with increase in age. Men synthesize and secrete greater amounts of protein and amylase than women. Total protein and albumin increase with a simultaneous fall in IgA and IgG in octogenarians when compared with 10 year old healthy subjects. Amylase activity decreases also in patients with chronic pancreatitis and liver transplantation.

RESUMO

Composição da saliva: Influência do sexo e da idade

Procedeu-se à recolha de saliva de indivíduos, saudáveis ou sofrendo de diversas patologias, cujas idades oscilavam entre os 10 e os 90 anos. O teor de amilase e preteínas foi correlacionado com a idade, sexo e estado de saúde. Os resultados indicam que a actividade específica da amilase diminui à medida que aumenta a idade. Os indivíduos do sexo masculino sintetizam e secretam maiores quantidades de proteínas e amilase que os indivíduos do sexo feminino. A albumina e preteínas totais aumentam nos octogenários quando comparados com indivíduos saudáveis de 10 anos de idade, enquanto que a IgA e IgG diminuem. A actividade da amilase diminui em doentes com pancreatite crónica ou sujeitos a transplante hepático.

INTRODUCTION

Salivary concentrations of many substances provide an accurate index of blood free concentrations of these same substances. Saliva has many advantages as a body fluid subject to examination because it can be easily and painlessly collected and can be sampled repeatedly. However, despite such advantages the use of saliva in the diagnosis of disease states has received very little attention primarily because of the lack of understanding of the factors that control salivary flow rate and composition in health and disease. Diet, exercise and one's state of one's health are known to affect the rate of secretion and composition of saliva.[1] Thus, for example, the composition of the dietaru fat is known to influence the lipid composition of saliva,[2] while exercise is known to increase the protein and enzyme content and in at least one disease, cystic fibrosis, in which the parotid gland is known to be disturbed.[3] We have analyzed the composition of unstimulated whole saliva in healthy and diseased individuals in various age groups to define sex, age, and disease related relationships.

MATERIALS AND METHODS

Subjects:

Saliva samples were obtained from healthy male and female volunteers at local public schools, church groups and from the faculty and staff members of the University of Pittsburgh School of Medicine. Additional saliva samples were obtained from patients admitted to Presbyterian University and the Oakland Veterans Administration Hospitals, Pittsburgh, PA. The height, weight, sex and diagnosis of each subject was recorded at the time of saliva collection.

Collection of Saliva:

The subject was asked to spit out 4 to 5 ml of saliva into a sterile vacutainer tube. The tube was closed with a rubber stopper and was transported on ice immediately to the laboratory for analysis. An aliquot of the collected saliva was used immediately for estimation of amylase content. The remaining saliva was frozen at —70° C until it could be analyzed for protein content at a later convenient date.

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TABLE 1 **Protein and amylase content in saliva of males and females**

Age	Group	Male N=	Female N=	Protein +		Amylase + +	
				Male	Female	Male	Female
10	a	11	8	144 ± 27	79 ± 16	533 ± 163	236 ± 32
15	b	10	8	208 ± 40	114 ± 24	609 ± 182	389 ± 153
30-49	c	16	11	388 ± 42 a*b*	309 ± 74 a,b	807 ± 137	827 ± 179 a
50-79	d	8	8	293 ± 43 a	415 ± 82 a,b	669 ± 154	505 ± 113 a

\+ mg per 100 ml.
+ + μg per ml.
* mean ± S.E.M.
** $P < 0.05$ when compared with the group indicated.

Analytical Techniques

Amylase was estimated by the Phadebas amylase test with minor modifications.[4] Human salivary amylase (Sigma, type IX A) was used as the standard. The assay consists of preincubating an aliquot of the enzyme made to 4 ml with water at 37° C for 5 min. Phadebas amylase tablets were added to each tube and the incubation was carried out for an additional 30 min. The reaction was stopped by the addition of 1.0 ml of 0.5N NaOH. The blanks contained water alone and were treated identically with the test samples. For each test run, 126, 252 and 504 ng of a standard human salivary amylase were assayed in duplicate as controls. Following incubation and termination of the reaction, the reaction tubes were centrifuged for 10 min. and the blue color in the supernatant was measured at 620 nm using a spectrophotometer. In our hands the liberation of blue color into the supernatant was found to be proportional to enzyme concentration and the reaction was found to be linear up to 60 min. (maximum time tested). Units of activity were expressed as micrograms of amylase/ml/30 min. The protein content of the samples was estimated in suitably diluted aliquots of saliva by the method of Lowry et al.[5]

Quantitation of Salivary Proteins:

Saliva was clarified by low speed centrifugation (i.e., 800xg for 10 minutes) and the albumin, IgA and IgG content of the resultant solution quantitated by rate nephelometry (ICS, Beckman Instrument Co.);[6]

Isoelectric Focusing:

Agarose gels 1% Isogel, Marine Colloids containing 1.5% ampholytes Isogel pH 3.5-9.5, Marine Colloids, were cast and aged for 24 hr. at 4° C prior to use. Ten μl volumes of saliva were applied and electrofocusing was carried out at 1000 V. The initial current was 20-25 mA and the run was usually completed within 30 minutes (1.5-2.5 mA). Gels were dried and stained in the conventional manner,[6] prior to comparing the patterns obtained for the different age groups.

RESULTS

Normal healthy subjects were grouped according to age, into the following 5 groups: *a*) young (< 10 years); *b*) teenagers (< 15 years); *c*) middle age (30 to 49 years); *d*) old age (50 to 69 years); and *e*) senior citizens (70 to 90 years). In addition to examining differences in the composition of saliva with age, differences noted between the two sexes were observed as well.

The average protein, total amylase and specific activity of the amylase in saliva of both males and females are presented in Figure 1. These results indicate that the protein and amylase content of saliva increase progressively up to middle age and then remain constant in the older adult human populations. In contrast, the specific activity of amylase in saliva shows a progressive decline with advancing age.

The salivary content of albumin, IgA and IgG were assayed in young (< 10 years) and senior citizens (70 to 90 years) and the results are presented in Table 2. They indicate a significant increase in total protein and albumin content and a significant decrease in IgA and IgG in the saliva of senior citizens.

Protein and amylase contents of saliva of patients with various digestive diseases are presented in Table 3. Chronic pancreatitis and infective hepatitis show no alteration in protein content (400 and 570 mg/100 ml) but a significant drop in specific activity of amylase (142.3 units/mg protein). The protein and amylase in the normals of the same age group were found to be 388 ± 42 and 247.11 ± 42.6, respectively. Liver transplant patients in the same age group show identical protein but significantly lower amylase specific activities.

DISCUSSION

The composition of saliva is known to vary with the nature and type of stimulation used.[7] In order to establish base line values in various age groups, saliva was collected in unstimulated state and analyzed in subjects ranging from 10 to 90 years.

The protein and amylase in various age groups (Figure 1) increase progressively up to the middle age and then remain

TABLE 2 **Protein composition of saliva +**

Age Yr.	Specimens	Total Protein	Albumin	IgA	IgG
10	15	121.33 ± 22.0 + +	3.7 ± 0.91	11.3 ± 1.30	2.6 ± 1.0
80-90	5	296 ± 33 *	16.8 ± 5.8 *	6.98 ± 9.11 *	0.16 ± 0.11 *

\+ mg per 100 ml.
+ + Mean ± S.E.M.
* $P < 0.05$ when compared with 10 year olds.

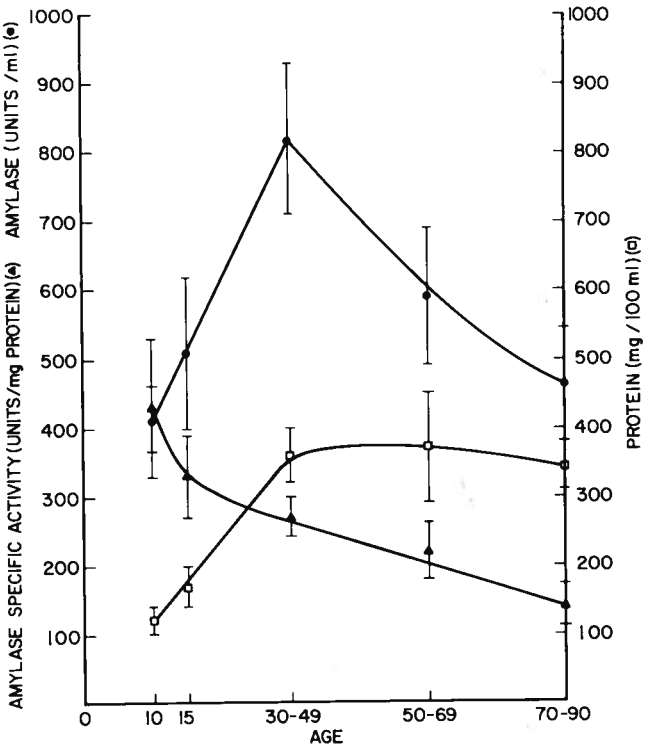

Figure 1: Amylase activity and protein content in saliva of normal healthy subjects. Each point represents Mean ± SEM. Units of enzyme activity are indicated in Materials and Methods.

constant through the rest of adult life. However, the specific activity shows a rapid and progressive decline with advancing age. This is in accordance with the observation that the flow rate of saliva and the protein and enzyme contents in saliva decrease with age.[8]

The salivary glands form their secretions by first elaborating an isotonic plasma like primary fluid in the end pieces and then modifying the composition of this secretion during its progressive passage through the duct system.[9] The results of the present investigation clearly establish that even in the unstimulated state the synthesis and secretion of enzymes decrease with advancing age, as is the case for many other organs.[10] If the flow rate of saliva is taken into consideration together with the specific activity of amylase, it appears that the adult saliva is inefficient in hydrolyzing the starchy deposits in the mouth, possibly contributing to bacterial growth and tooth decay with advancing years. Our results show similar increases in salivary protein and amylase content in males and females up through middle age and then the levels of these substances remain unchanged through the rest of adult life. It has been shown previously that men secrete more saliva per day than do women [11] and if this rate of secretion is taken into account, men obviously synthesize and secrete significantly greater amounts of protein and enzyme into saliva than do women (Table 1). It has been shown previously that the secretion of saliva is influenced by physical activity and in the present work varies with the sex of the subject.[8] For these reasons, we believe that the secretion of salivary constituents is influenced by sex hormones, a situation similar to what occurs in the pancreas [12] an organ not dissimilar from salivary glands.

Analysis of the protein composition of the younger and the older age groups (Table 2) shows clearly that in addition to the proteins and enzymes (amylase) being synthesized and secreted by the salivary gland, that the serum proteins which

TABLE 3 **Protein and amylase in saliva of patients with digestive diseases**

Disease	Age	Sex	Total protein +	Amylase specific activity + +
1. Renal failure	27	F	293	330.5
2. Ulcerative colitis	37	M	190	232.1
3. Chronic pancreatitis	38	M	400	142.3
4. Liver transplant - 1	42	M	293	17.8
Liver transplant - 2	44	M	620	30.5
5. Infective hepatitis	49	M	570	142.3
6. Hepatoma - 1	50	M	293	320.0
Hepatoma - 2	63	M	160	59.5

\+ mg per 100 ml.
\+ + μg of amylase mg protein

also diffuse into saliva from plasma show a significant decrease with advancing age. It is known that immunoglobulins, specifically IgA and IgG decrease in old people.[13] Thus, the composition of saliva reflects both: *a*) the activity of exocrine organs; and, *b*) the composition of plasma. This, observation ought to be exploited in the diagnosis of systemic diseases particularly as alterations of salivary constituents that are known to occur in some systemic diseases.[8]

With this possibility in mind and having established base line values in healthy individuals, we examined the composition of saliva of individuals with various digestive diseases (Table 3). Our results indicate that patients with pancreatic and liver diseases demonstrate a significant decrease in the synthetic activity of the salivary glands. The precise correlation between the composition of saliva, the concentration of its various constituents and the onset of digestive deseases remains a fertile area of clinical research.

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