A Study on the Rheological Property of Saliva

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Ⅰ. INTRODUCTION

The importance of saliva in oral health has become increasingly apparent in expanding aged population. Nowadays, advance in medical procedures and utilization of medication have been combined in an effort to maintain the quality of life. However, one of the common sequela to this effort is an increased prevalence of salivary dysfunction and the most common clinical manifestation of salivary dysfunction is a decreased output of secretion, termed "dry mouth"(hypo-salivation, xerostomia, or asialorrhea), which clinically may vary from a slight reduction in salivary flow with transitory inconvenience to a total xerostomia.[1]

Among diverse functions of saliva, lubrication is not only important in facilitating the movements of the tongue and lips during chewing and swallowing but also essential for clearly articulated speech.[2,3] Therefore, individuals with diminished salivary gland function are subject to serious alteration of the oral mucosa and may have difficulty with speech, mastication, and deglutition.[4-6] In addition, high rate of caries susceptibility and attrition of the dentition may be observed.[4-6]

While the many causes of xerostomia and its proper diagnosis have been addressed in the recent literature[4,5,7], two approaches have been utilized for the treatment of dry mouth : intrinsic and extrinsic.[1] Intrinsic reagents have been used to maintain or augment hypofunctional glands and can include pilocarpine[8-10], bromhexine[8], and anethole-trithione.[8] An obvious disadvantage of this approach is the potential side-effects, and the actual formulation and regimen of these compounds are still under experimental study.[8] As a extrinsic reagent, artificial saliva has served as a replacement modality for individuals exhibiting hyposalivation.[1,11,12] But, most commercially available artificial salivas generally lack the lubricatory protein, macromolecules, and antimicrobial factors found in normal saliva.[6,12]

The efficacy of saliva as a lubricant will depend on its viscosity and how this changes with shear rate, i.e. the rheological properties.[3,13] And, to reproduce the lubricating and protective properties of saliva substitutes, a thorough understanding of the rheological properties of human saliva is essential.[14] However, the information on the bio-rheology of saliva in literature is sparse. Until the 1970s, most rheological measurements of saliva were limited almost exclusively to Ostwald type U-tube viscometers or their modifications.[3] Therefore, none had overcome the problems of ensuring true quantitative measurements in this nonhomogeneous, non-Newtonian excretion. But, to solve this problem, the viscometer with small-angle, cone-and-plate configuration has been used to measure the rheological properties of saliva[13,15-17] because it has an advantage that the material in the gap is subject to a uniform shear. And, several studies have been attempted to apply the results from this type of viscometer to the diagnosis of disease[15] and assessment of various saliva substitutes.[13,16,17]

Yet, relatively little information exists concerning age-and sex-related changes of the viscosity values of saliva in normal healthy population.

The purpose of this study is : (1) to investigate the age-and sex-related changes in the viscosity values of stimulated whole saliva, (2) to examine the differences among the viscosity values of stimulated and unstimulated whole saliva, stimulated parotid saliva, and stimulated submandibular-sublingual mixed saliva, and (3) to present the equation representing the viscoelastic characteristics of saliva by regressional analysis.

II. MATERIALS AND METHODS

Subjects

The 240 healthy unmedicated subjects without history of suffering form dry mouth, were included in this study. The subjects were divided into six groups according to age and each group included 20 males and 20 females(Table 1).

Table 1. Demographic characteristics of the subjects.

Group	Age	Sex	Number	Mean age
1	0 - 9	Male	20	8.6
		Female	20	8.6
2	10-19	Male	20	17.4
		Female	20	16.8
3	20-29	Male	20	23.7
		Female	20	23.8
4	30-39	Male	20	34.9
		Female	20	35.1
5	40-49	Male	20	43.2
		Female	20	44.7
6	50-	Male	20	56.6
		Female	20	55.4

Saliva collection

Saliva collections were performed between 7 : 00 A.M. and 10 : 00 A.M. The subjects refrained from eating, drinking and brushing prior to sample collection(minimum one hour). The collections of stimulated whole saliva(S.W.S.) were done in all subjects except the twenties by having the subjects chew paraffin wax(1-2 g, melting point 42℃) with a constant rate(about 60-70 chews/minutes) and expectorate into test tubes. For the measurements of the viscosity values of unstimulated whole saliva(U.W.S.), stimulated whole saliva(S.W.S), stimulated parotid saliva(S.P.S.), and stimulated submandibular-sublingual mixed saliva(S.S.S.), four salivary specimens were collected from each subject of the twenties(group 3), always in the same sequence. The subject was instructed to rinse his or her mouth thoroughly with sip of water, to clear the oral cavity of existing secretions, and then to swallow the water. The unstimulated whole saliva was collected by the spitting method. The subject was seated comfortably for a period of two minutes, with eyes open and instructed to spit out every 30 seconds. The stimulated whole saliva was collected by the same method, chewing the paraffin wax. Secretion of the parotid saliva was stimulated by chewing wax, and the saliva was collected unilaterally by use of Lashley cup. The submandibular-sublingual mixed saliva was collected from the floor of the mouth with slight suction by an alternative and simple technic which was to block off secretion from the parotid glands with absorbent swabs, and a cotton roll was placed in the vestibule adjacent to the lower lip, as described by Fox et al.[4] Gustatory stimulus(lemon candy) was employed to stimulate the secretion of the submandibular-sublingual mixed saliva.

Viscosity measurement

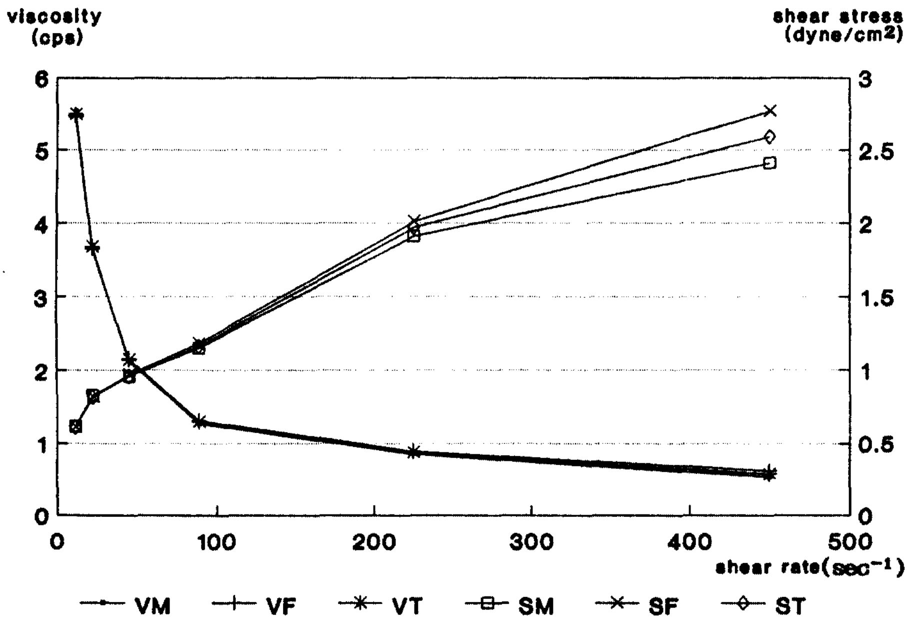
Viscosity measurements were performed at 37.0° ±0.2℃ by means of a model LVT Wells-Brookfield cone-and-plate digital viscometer (Brookfield Engineering Laboratories, Stoughton, MA, U.S.A.). A-0.8- degree cone(model No. CP-40) was used in all experiments. Shear rates were varied incrementally from 11.3 to 450.0(sec^{-1}) at six different speeds. The steady-shearing data were measured at the lowest rate of strain and then at progressively higher rates of strain in order to minimize shear-degradation of the high-molecular-weight mucins in the saliva samples. Viscosity values in centipoise(cps) were obtained from the displayed value multiplied by a factor supplied by manufacturer. Then, the values of shear stress and torque at different speeds were recorded. A 0.5-1.0ml volume of fluid was used in each test.

Statistical analysis

Data were inputed on a 16 bit IBM PC and all the statistical analyses were performed by SPSS PC^{+}(Microsoft Corp., U.S.A.). T-test, ANOVA, and Duncan's multiple range test were used to compare the mean values and to examine the effects of variables. Regressional analysis by variable transformation was also utilized.

Ⅲ. RESULTS

The viscosity values for saliva were dependent on the shear rate as seen in Fig. 1, 2, 3, and 5. In general, the viscosity values were inversely proportional to the shear rate with a large initial



(V:viscosity, S:shear stress, M:male, F:female, T:total)

Fig. 1. Graph of the viscosity and shear stress vs. the shear rate for stimulated whole saliva in all subjects.

decrease in value. From 45.0 to 450.0(sec^{-1}), the viscosity values decreased to a lesser extent. The relationships between the shear stress and shear rate were seen in Fig. 1 and 5. This pattern was typical of shear thinning, i.e. pseudoplasticity which reflected the non-Newtonian characteristics of saliva.

The mean values and standard deviations of the viscosity values of stimulated whole saliva in different shear rates were shown in Table 2, and these values at a shear rate of 450.0(sec^{-1}) were in Fig. 4. The mean values of the viscosity of stimulated whole saliva for male and female subjects at a shear rate of 450.0(sec^{-1}) were 0.45, and 0.41 in group 1, 0.66 and 0.54 in group 2, 0.45 and 0.53 in group 3, 0.46 and 0.77 in group 4, 0.43 and 0.70 in group 5, and 0.75 and 0.71 in group 6, respectively.

In male subjects, the stimulated whole saliva of group 2 and 6 displayed high values of viscosity (Table 2, Fig.2, and 4). And, significant differences ($p \leqq 0.05$) in the viscosity values were found between the other groups and group 2 and 6 at a shear rate of 450.0(sec^{-1}). In female subjects, there was a different pattern in the change of viscosity values with aging. The saliva samples of old subjects had viscosity values greater than those of young subjects. The stimulated whole saliva of group 4 displayed the highest values of viscosity, and there were significant differences ($p \leqq 0.05$) in the viscosity values between group 4 and group 1, 2 and 3 at a shear rate of 450.0 (sec^{-1}). Group 1 had the lowest values of viscosity and the significant differences($p \leqq 0.05$) were found between group 1 and group 4, 5, and 6 at a shear rate of 450.0(sec^{-1})(Table 2, Fig.3, and 4).

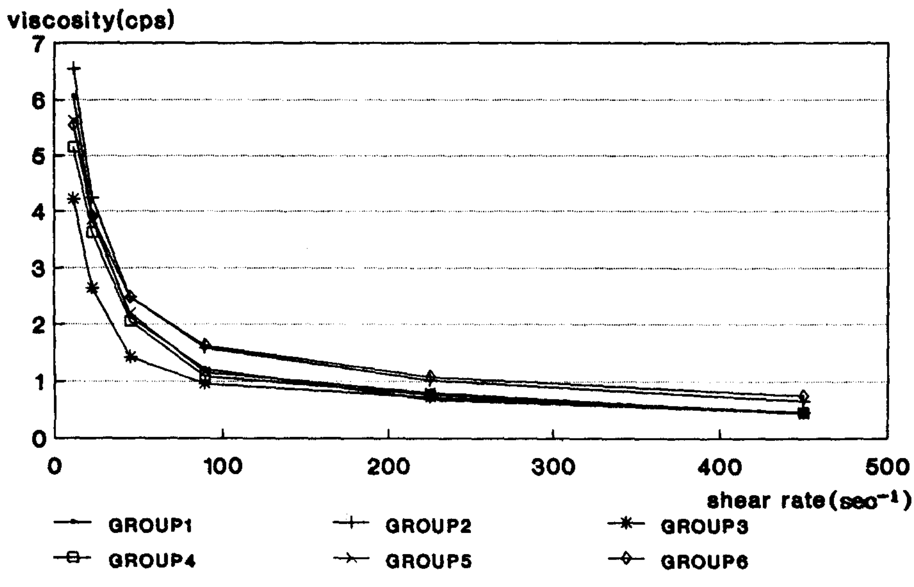


Fig. 2. Graph of the viscosity vs. the shear rate for stimulated whole saliva in male subjects according to age.

Table 2. Mean values and standard deviations of the viscosity values (cps) of stimulated whole saliva according to sex and age in different shear rates.

GROUP		GR1	GR2	GR3	GR4	GR5	GR6	TOTAL	Significance
Shear rate (sec^{-1})	AGE	0-9	10-19	20-29	30-39	40-49	50-		
	SEX	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	
11.3	M	6.07	6.55	4.21	5.15	5.63	5.56	5.53	* (2, 3)
		(1.51)	(2.50)	(1.88)	(3.90)	(2.51)	(2.27)	(2.60)	
	F	6.43	4.52	5.24	6.28	5.57	4.76	5.46	
		(2.82)	(3.43)	(3.17)	(2.83)	(2.41)	(2.77)	(2.94)	
	T	6.26	5.51	4.72	5.69	5.60	5.17	5.49	* (1, 3)
		(2.27)	(3.14)	(2.62)	(3.43)	(2.42)	(2.53)	(2.77)	
22.5	M	3.95	4.24	2.64	3.63	3.85	3.90	3.70	* (1, 3) (2, 3) (3, 4) (3, 5)
		(1.20)	(1.37)	(1.28)	(1.88)	(1.51)	(1.31)	(1.50)	* (3, 6)
	F	3.97	3.05	3.39	4.26	3.95	3.38	3.66	# (2)
		(1.42)	(1.76)	(1.73)	(1.64)	(1.91)	(1.51)	(1.68)	
	T	3.96	3.63	3.01	3.93	3.90	3.64	3.68	* (1, 3) (3, 4) (3, 5)
		(1.30)	(1.67)	(1.55)	(1.77)	(1.71)	(1.42)	(1.59)	
45.0	M	2.12	2.47	1.42	2.05	2.19	2.47	2.12	* (1, 3) (2, 3) (3, 4) (3, 5)
		(0.72)	(0.79)	(0.78)	(0.81)	(0.82)	(1.03)	(0.89)	* (3, 6)
	F	2.00	1.74	2.15	2.72	2.31	2.12	2.16	* (2, 4) # (2) # (3)
		(1.03)	(0.92)	(1.15)	(1.29)	(1.13)	(1.20)	(1.14)	
	T	2.06	2.10	1.79	2.37	2.25	2.30	2.14	* (3, 4)
		(0.89)	(0.92)	(1.03)	(1.10)	(0.98)	(1.12)	(1.02)	
90.0	M	1.22	1.59	0.96	1.09	1.16	1.63	1.28	* (1, 2) (1, 6) (2, 3) (2, 4)
		(0.52)	(0.58)	(0.53)	(0.41)	(0.31)	(0.75)	(0.58)	* (2, 5) (3, 6) (4, 6) (5, 6)
	F	1.03	1.15	1.25	1.69	1.43	1.39	1.32	* (1, 4) (2, 4)
		(0.46)	(0.52)	(0.65)	(0.77)	(0.70)	(0.78)	(0.67)	# (2) ## (4)
	T	1.12	1.36	1.10	1.38	1.30	1.51	1.30	* (1, 6) (3, 6)
		(0.49)	(0.59)	(0.60)	(0.67)	(0.56)	(0.77)	(0.63)	
225.0	M	0.68	1.02	0.72	0.77	0.80	1.08	0.85	* (1, 2) (1, 6) (2, 3) (2, 4)
		(0.27)	(0.38)	(0.43)	(0.26)	(0.22)	(0.49)	(0.38)	* (3, 6) (4, 6) (5, 6)
	F	0.60	0.80	0.87	1.09	1.02	1.02	0.89	* (1, 4) (1, 5) (1, 6)
		(0.21)	(0.40)	(0.43)	(0.36)	(0.46)	(0.58)	(0.45)	### (4)
	T	0.64	0.91	0.79	0.93	0.91	1.05	0.87	* (1, 2) (1, 4) (1, 5) (1, 6)
		(0.24)	(0.40)	(0.43)	(0.35)	(0.38)	(0.53)	(0.42)	* (3, 6)
450.0	M	0.45	0.66	0.45	0.46	0.43	0.75	0.53	* (1, 2) (1, 6) (2, 3) (2, 4)
		(0.17)	(0.24)	(0.20)	(0.13)	(0.12)	(0.45)	(0.27)	* (2, 5) (3, 6) (4, 6) (5, 6)
	F	0.41	0.54	0.53	0.77	0.70	0.71	0.61	* (1, 4) (1, 5) (1, 6) (2, 4)
		(0.11)	(0.25)	(0.20)	(0.23)	(0.27)	(0.43)	(0.29)	* (3, 4) #### (4) #### (5)
	T	0.43	0.60	0.49	0.61	0.57	0.73	0.57	* (1, 2) (1, 4) (1, 5) (1, 6)
		(0.14)	(0.25)	(0.20)	(0.24)	(0.25)	(0.44)	(0.28)	* (2, 6) (3, 6) (4, 6) (5, 6)

* denotes a significant difference between groups and # denotes a significant difference between sexes within same group (*, # : $p \leq 0.05$, ## : $p \leq 0.01$, ### : $p \leq 0.005$, #### : $p \leq 0.001$).
M : male, F : female, T : total, SD : standard deviation.

Table 3. Mean values and standard deviations of the the shear stress (dyne/cm²) of stimulated whole saliva according to sex and age in different shear rates.

GROUP		GR1	GR2	GR3	GR4	GR5	GR6	TOTAL	Significance
Shear	AGE	0-9	10-19	20-29	30-39	40-49	50-		
rate	SEX	Mean	Mean	Mean	Mean	Mean	Mean	Mean	
(sec^{-1})		(SD)	(SD)	(SD)	(SD)	(SD)	(SD)	(SD)	
11.3	M	0.68	0.73	0.47	0.57	0.63	0.62	0.62	* (2, 3)
		(0.17)	(0.28)	(0.21)	(0.44)	(0.28)	(0.26)	(0.29)	
	F	0.72	0.50	0.59	0.70	0.62	0.54	0.61	
		(0.32)	(0.38)	(0.36)	(0.32)	(0.27)	(0.31)	(0.33)	
	T	0.70	0.61	0.53	0.63	0.63	0.58	0.61	* (1, 3)
		(0.26)	(0.35)	(0.30)	(0.39)	(0.27)	(0.28)	(0.31)	
22.5	M	0.89	0.95	0.59	0.81	0.86	0.87	0.83	* (1, 3) (2, 3) (3, 4) (3, 5)
		(0.27)	(0.31)	(0.29)	(0.42)	(0.34)	(0.30)	(0.34)	* (3, 6)
	F	0.89	0.68	0.76	0.95	0.88	0.76	0.82	ǂ (2)
		(0.32)	(0.40)	(0.39)	(0.37)	(0.43)	(0.34)	(0.38)	
	T	0.89	0.81	0.67	0.88	0.87	0.81	0.82	* (1, 3) (3, 4) (3, 5)
		(0.29)	(0.38)	(0.35)	(0.40)	(0.39)	(0.32)	(0.36)	
45.0	M	0.95	1.11	0.63	0.94	0.98	1.11	0.96	* (1, 3) (2, 3) (3, 4) (3, 5)
		(0.33)	(0.36)	(0.35)	(0.35)	(0.37)	(0.47)	(0.40)	* (3, 6)
	F	0.90	0.78	0.96	1.22	1.05	0.95	0.97	* (2, 4) ǂ(2) ǂ(3)
		(0.47)	(0.42)	(0.52)	(0.58)	(0.53)	(0.54)	(0.52)	
	T	0.92	0.94	0.80	1.07	1.01	1.03	0.96	* (3, 4)
		(0.40)	(0.42)	(0.47)	(0.49)	(0.46)	(0.51)	(0.46)	
90.0	M	1.10	1.43	0.86	0.98	1.05	1.46	1.15	* (1, 2) (1, 6) (2, 3) (2, 4)
		(0.46)	(0.52)	(0.47)	(0.37)	(0.28)	(0.67)	(0.52)	* (2, 5) (3, 6) (4, 6) (5, 6)
	F	0.93	1.03	1.12	1.52	1.28	1.25	1.18	* (1, 4) (2, 4)
		(0.41)	(0.47)	(0.59)	(0.70)	(0.63)	(0.70)	(0.61)	ǂ(2) ǂǂ(4)
	T	1.01	1.22	0.99	1.24	1.17	1.35	1.16	* (1, 6) (3, 6)
		(0.44)	(0.53)	(0.54)	(0.61)	(0.50)	(0.69)	(0.57)	
225.0	M	1.54	2.31	1.62	1.75	1.81	2.44	1.91	* (1, 2) (1, 6) (2, 3) (2, 4)
		(0.60)	(0.86)	(0.96)	(0.58)	(0.49)	(1.11)	(0.85)	* (3, 6) (4, 6) (5, 6)
	F	1.37	1.78	1.95	2.46	2.30	2.30	2.01	* (1, 4) (1, 5) (1, 6)
		(0.48)	(0.90)	(0.97)	(0.81)	(1.04)	(1.31)	(1.00)	ǂǂǂ(4)
	T	1.45	2.04	1.79	2.08	2.06	2.37	1.97	* (1, 2) (1, 4) (1, 5) (1, 6)
		(0.54)	(0.91)	(0.97)	(0.78)	(0.84)	(1.20)	(0.93)	* (3, 6)
450.0	M	2.04	2.96	2.03	2.11	1.93	3.38	2.41	* (1, 2) (1, 6) (2, 3) (2, 4)
		(0.78)	(1.10)	(0.89)	(0.61)	(0.50)	(2.03)	(1.22)	* (2, 5) (3, 6) (4, 6) (5, 6)
	F	1.87	2.45	2.48	3.46	3.25	3.23	2.77	* (1, 4) (1, 5) (1, 6) (2, 4)
		(0.50)	(1.13)	(0.97)	(1.03)	(1.26)	(1.94)	(1.32)	* (3, 4) ǂǂǂǂ(4) ǂǂǂǂ(5)
	T	1.95	2.70	2.25	2.74	2.61	3.30	2.59	* (1, 2) (1, 4) (1, 5) (1, 6)
		(0.64)	(1.13)	(0.95)	(1.07)	(1.17)	(1.96)	(1.28)	* (2, 6) (3, 6) (4, 6) (5, 6)

* denotes a significant difference between groups and ǂ denotes a significant difference between sexes within same group (*, ǂ : $p \leq 0.05$, ǂǂ : $p \leq 0.01$, ǂǂǂ : $p \leq 0.005$, ǂǂǂǂ : $p \leq 0.001$).
M : male, F : female, T : total, SD : standard deviation.

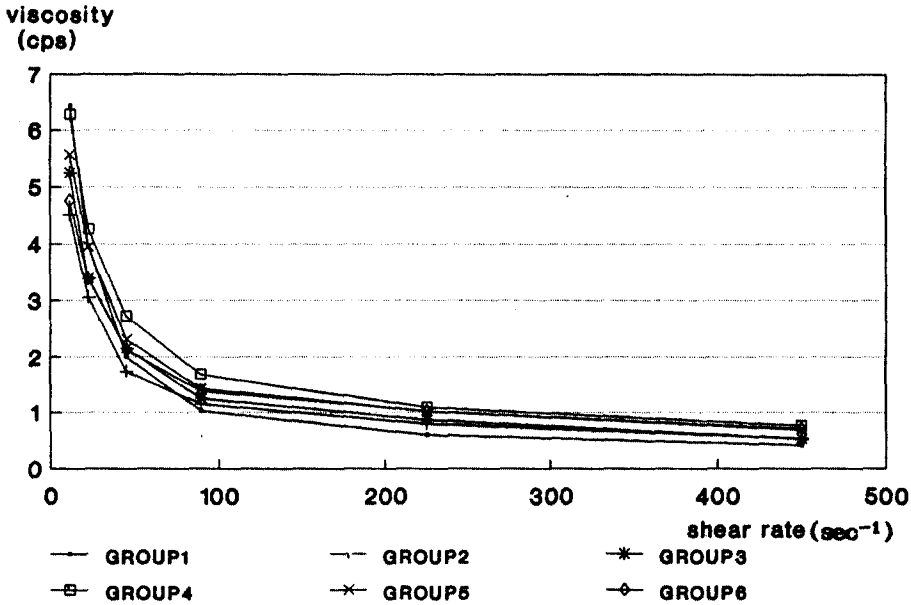


Fig. 3. Graph of the viscosity vs. the shear rate for stimulated whole saliva in female subjects according to age.

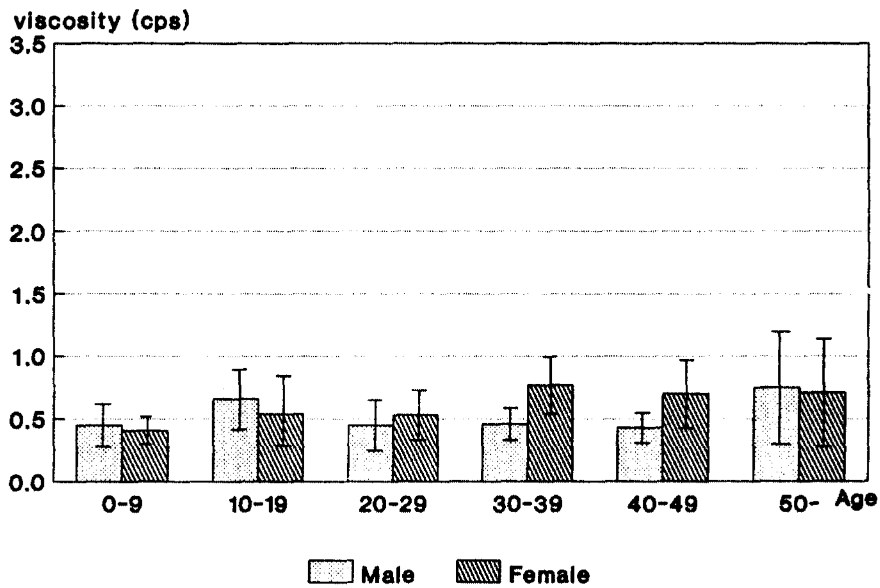


Fig. 4. The means and standard deviations of the viscosity values of stimulated whole saliva at a shear rate of 450.0 (sec^{-1}).

The mean values of viscosity of stimulated whole saliva in female subjects were greater than those of male subjects in group 3, 4, and 5. And, there were significant differences between sexes in group 4 at shear rates of 90.0($p \leqq 0.01$), 225.0 ($p \leqq 0.005$), and 450.0(sec^{-1})($p \leqq 0.001$)(Table 2).

And, the mean values and standard deviations of the shear stress of stimulated whole saliva in different shear rates were seen in Table 3, and the relationship with the shear rate was plotted in Fig. 1.

The mean values and standard deviations of the viscosity values of stimulated and unstimulated whole saliva, stimulated parotid saliva, and stimulated submandibular-sublingual mixed saliva in different shear rates were shown in Table 4, and these values at a shear rate of 450(sec^{-1}) were in Fig. 6.

Table 4. Mean values and standard deviations of the viscosity values(cps) of stimulated and unstimulated whole saliva(S. W. S., U. W.S.), stimulated parotid saliva(S.P.S.), and stimulated submandibular-sublingual mixed saliva(S. S. S.) according to sex in different shear rates.

Shear rate (sec^{-1})	SEX	N	S. W. S.	U. W. S.	S. P. S.	S. S. S.	Significance
			Mean(SD)	Mean(SD)	Mean(SD)	Mean(SD)	
11.3	M	20	4.21(1.88)	4.93(3.05)	4.78(2.45)	9.02(3.23)	≢
	F	20	5.24(3.17)	6.88(4.91)	4.33(2.47)	9.71(4.51)	≢
	T	40	4.72(2.62)	5.91(4.15)	4.56(2.44)	9.36(3.88)	≢
22.5	M	20	2.64(1.28)	3.31(1.75)	3.04(1.34)	5.90(1.77)	≢
	F	20	3.39(1.73)	4.66(3.03)	3.05(1.48)	5.86(2.61)	≢
	T	40	3.01(1.55)	3.99(2.53)	3.04(1.39)	5.88(2.20)	≢
45.0	M	20	1.42(0.78)	1.94(1.15)	1.71(0.72)	3.72(1.40)	≢
	M	20	2.15(1.15)≠	3.04(2.56)	1.82(0.91)	3.67(1.84)	≢
	T	40	1.79(1.03)	2.49(2.03)	1.76(0.81)	3.70(1.62)	≢
90.0	M	20	0.96(0.53)	1.29(0.64)	0.90(0.43)	2.26(1.10)	≢
	F	20	1.25(0.65)	1.68(1.25)	1.03(0.57)	2.29(1.08)	≢
	T	40	1.10(0.60)	1.48(1.00)	0.97(0.50)	2.27(1.07)	≢##++
225.0	M	20	0.72(0.43)	0.96(0.46)	0.63(0.35)	1.54(0.64)	≢#+*
	F	20	0.87(0.43)	1.07(0.58)	0.58(0.29)	1.45(0.51)	≢#++++*
	T	40	0.79(0.43)	1.01(0.52)	0.60(0.32)	1.50(0.57)	≢####++++*
450.0	w	20	0.45(0.20)	0.62(0.30)	0.38(0.18)	0.87(0.29)	≢#+++*
	F	20	0.53(0.20)	0.68(0.34)	0.37(0.15)	0.90(0.30)	≢#++++*
	T	40	0.49(0.20)	0.65(0.31)	0.37(0.17)	0.89(0.29)	≢####++++*

denotes a significant difference between stimulated whole saliva and unstimulated whole saliva. (: $p \leqq 0.05$)
# denotes a significant difference between stimulated whole saliva and stimulated parotid saliva.
(# : $p \leqq 0.05$, ## : $p \leqq 0.01$, #### : $p \leqq 0.001$)
+ denotes a significant difference between unstimulated whole saliva and stimulated parotid saliva.
(+ : $p \leqq 0.05$, ++ : $p \leqq 0.01$, +++ : $p \leqq 0.005$, ++++ : $p \leqq 0.001$)
≢ denotes a significant difference($p \leqq 0.001$) between stimulated submandibular-sublingual mixed saliva and the others.
≠ denotes a significant difference between sexes in the same shear rate. (≠ : $p \leqq 0.05$)
M : male, F : female, T : total, SD : standard deviation, N : number of subjects.

The mean values of viscosity at a shear rate of 450.0(sec^{-1}) were increased in order of stimulated parotid saliva, stimulated whole saliva, unstimulated whole saliva, and stimulated submandibular-sublingual mixed saliva. Significant differences in viscosity values at a shear rate of

450.0(sec^{-1}) were found between stimulated submandibular-sublingual mixed saliva and the others (p≦0.001), stimulated whole saliva and unstimulated whole saliva(p≦0.05), and stimulated whole saliva and stimulated parotid saliva(p≦0.05) in male and female subjects. And, significant differences were found between the viscosity values of unstimulated whole saliva and those of stimulated parotid saliva in male(p≦0.005) and female (p≦0.001) subjects(Table 4, Fig. 5, and 6).

Table 5. Mean values and standard deviations of the shear stress (dyne/㎠) of stimulated and unstimulated whole saliva (S. W. S., U. W. S.), stimulated parotid saliva (S.P.S.), and stimulated submandibular-sublingual mixed saliva (S. S. S.) according to sex in different shear rates.

Shear rate (sec^{-1})	SEX	N	S. W. S.	U. W. S.	S. P. S.	S. S. S.	Significance
			Mean(SD)	Mean(SD)	Mean(SD)	Mean(SD)	
11.3	M	20	0.47(0.21)	0.55(0.34)	0.53(0.28)	1.01(0.36)	≢
	F	20	0.59(0.36)	0.77(0.55)	0.48(0.28)	1.09(0.51)	≢
	T	40	0.53(0.30)	0.66(0.47)	0.51(0.27)	1.05(0.44)	≢
22.5	M	20	0.59(0.29)	0.74(0.40)	0.68(0.30)	1.32(0.40)	≢
	F	20	0.76(0.39)	1.04(0.68)	0.68(0.34)	1.31(0.59)	≢
	T	40	0.67(0.35)	1.89(0.57)	0.68(0.31)	1.32(0.49)	≢
45.0	M	20	0.63(0.35)	0.87(0.52)	0.76(0.33)	1.67(0.63)	≢
	F	20	0.96(0.52)≠	1.42(1.15)	0.81(0.41)	1.65(0.82)	≢
	T	40	0.80(0.47)	1.14(0.92)	0.79(0.37)	1.66(0.72)	≢
90.0	M	20	0.86(0.47)	1.16(0.58)	0.81(0.39)	2.03(0.99)	≢
	F	20	1.12(0.59)	1.57(1.11)	0.93(0.51)	2.05(0.97)	≢
	T	40	0.99(0.54)	1.36(0.90)	0.87(0.45)	2.04(0.96)	≢ # # + +
225.0	M	20	1.62(0.96)	2.15(1.04)	1.42(0.79)	3.45(1.44)	≢ # + *
	F	20	1.95(0.97)	2.41(1.30)	1.31(0.65)	3.30(1.16)	≢ # + + + + *
	T	40	1.79(0.97)	2.28(1.17)	1.36(0.72)	3.38(1.29)	≢ # # # # + + + + *
450.0	M	20	2.03(0.89)	2.79(1.33)	1.71(0.83)	3.92(1.28)	≢ # + + + *
	F	20	2.48(0.97)	3.05(1.51)	1.68(0.66)	4.06(1.33)	≢ # + + + + *
	T	40	2.25(0.95)	2.92(1.41)	1.69(0.74)	3.99(1.29)	≢ # # # # + + + + *

* denotes a significant difference between stimulated whole saliva and unstimulated whole saliva. (* : p≦0.05)
# denotes a significant difference between stimulated whole saliva and stimulated parotid saliva.
(# : p≦0.05, # # : p≦0.01, # # # # : p≦0.001)
+ denotes a significant difference between unstimulated whole saliva and stimulated parotid saliva.
(+ : p≦0.05, + + : p≦0.01, + + + : p≦0.005, + + + + : p≦0.001)
≢ denotes a significant difference (p ≦0.001) between stimulated submandibular-sublingual mixed saliva and the others.
≠ denotes a significant difference between sexes in the same shear rate. (≠ : p≦0.05)
M : male, F : female, T : total, SD : standard deviation, N : number of subjects.

No significant difference was found in the viscosity values between sexes at shear rates of 90.0, 225.0, and 450.0(sec^{-1}).

The values of the shear stress were shown in Table 5 and the relationship with the shear rate was plotted in Fig. 5.

The results of regressional analysis by variable transformation were shown in Table 6 and 7. The equations represented the curves of asymptotic form.

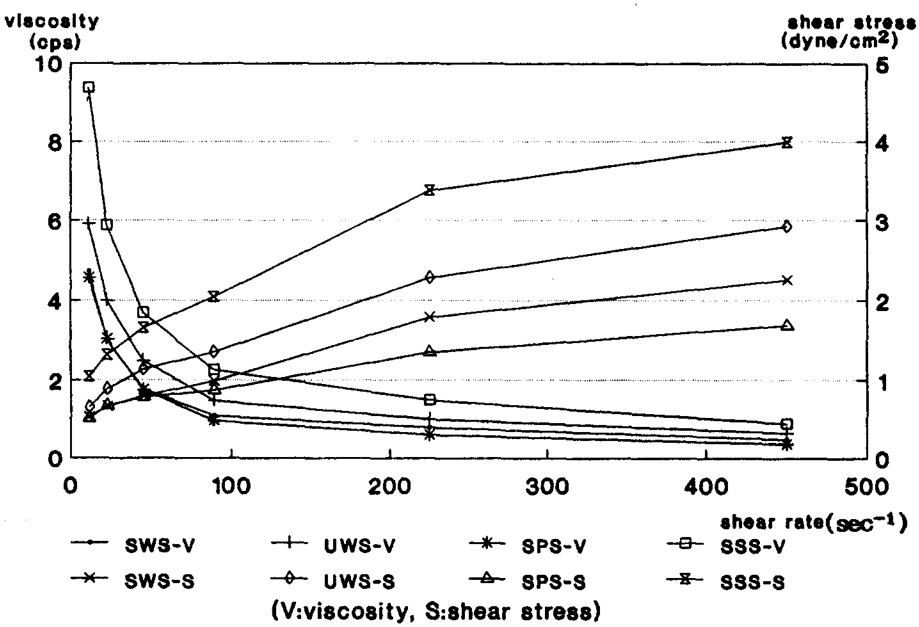


Fig. 5. Graph of the viscosity and shear stress vs. the shear rate for stimulated and unstimulated whole saliva (S.W.S., U.W.S.), stimulated parotid saliva (S.P.S.), and stimulated submandibular – sublingual mixed saliva (S. S. S.) in all subjects of group 3.

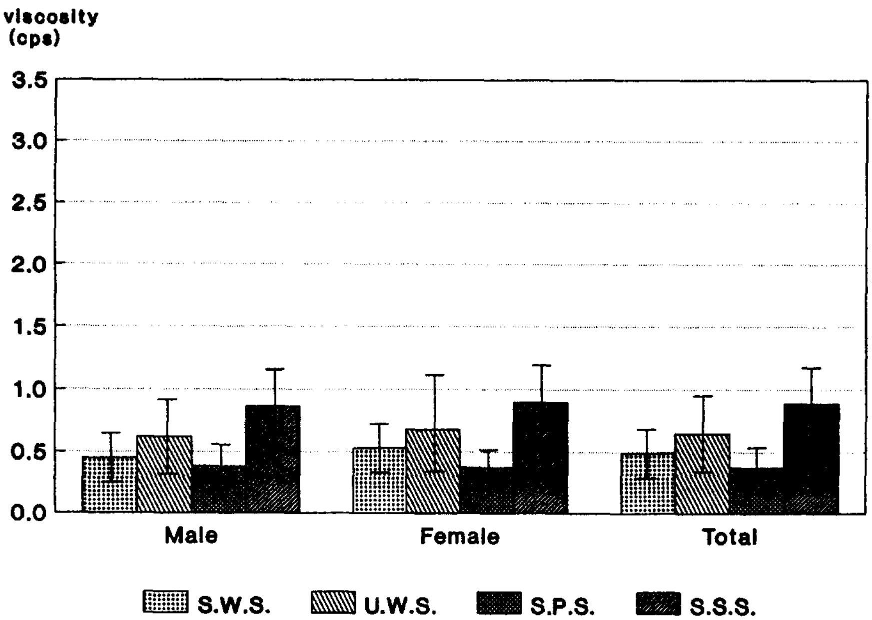


Fig. 6. The means and standard deviations of the viscosity values of stimulated and unstimulated whole saliva (S.W.S., U. W. S.), stimulated parotid saliva (S.P.S.), and stimulated submandibular – sublingual mixed saliva (S.S.S.) at a shear rate of 450.0 (sec^{-1}).

Table 6. The results of regressional analysis for stimulated whole saliva according to sex and age ($Y = aX^b$, Y : viscosity, X : shear rate).

Age	Sex	a	b
0 - 9	Male	34. 1	-0. 721
	Female	39. 3	-0. 768
	Total	36. 6	-0. 744
10 - 19	Male	27. 9	-0. 619
	Famale	17. 1	-0. 574
	Total	22. 2	-0. 599
20 - 29	Male	15. 6	-0. 589
	Female	22. 4	-0. 614
	Total	19. 1	-0. 604
30 - 39	Male	25. 8	-0. 663
	Female	24. 8	-0. 576
	Total	24. 8	-0. 613
40 - 49	Male	30. 9	-0. 696
	Female	21. 3	-0. 569
	Total	25. 0	-0. 624
50 -	Male	20. 5	-0. 546
	Famale	16. 0	-0. 516
	Total	18. 2	-0. 533
Total	Male	25. 0	-0. 635
	Female	22. 2	-0. 599
	Total	23. 6	-0. 617

Table 7. The results of regressional analysis for stimulated and unstimulated whole saliva (S.W.S., U.W.S.), stimulated parotid saliva (S.P.S.), and stimulated submandibular-sublingual mixed saliva (S.S.S.) according to sex ($Y = aX^b$, Y : viscosity, X : shear rate).

Type	Sex	a	b
S. W. S.	Male	15. 6	-0. 589
	Female	22. 4	-0. 614
	Total	19. 1	-0. 604
U. W. S.	Male	17. 6	-0. 552
	Female	32. 5	-0. 634
	Total	24. 5	-0. 599
S. P. S.	Male	24. 0	-0. 688
	Female	23. 8	-0. 684
	Total	24. 3	-0. 689
S. S. S.	Male	40. 4	-0. 620
	Female	42. 5	-0. 633
	Total	40. 9	-0. 625

Ⅳ. DISCUSSION

Biorheology which embraces elasticity, viscosity, and plasticity is the name given to the science of the deformation and flow of matter in biologic systems or in materials derived from such systems.[18]

Viscosity of a fluid is defined as the ratio of shear stress to shear rate. Shearing occurs as the result of the internal friction of the fluid layers moving over one another during flow. For simple fluids, this relationship is linear. But, the presence of colloidal suspension or macromolecular substances usually cause marked alinearity of this shear stress-shear rate relationship. In order to determine the viscosity of a fluid with anomalous flow properties, it is necessary to use an instrument with which all of the fluid under test is exposed to a uniform shear stress and shear rate. The viscometers with cone-and-plate or similar geometry which have an advantage that the material in the gap is subject to a uniform shear, were developed to overcome this limitation.[19]

Capillary tube viscometry which has been used to measure the viscosity traditionally, yields only a one point measurement on the shear stress versus shear rate curve that describes the rheologic behavior of a fluid. This is satisfactory for Newtonian fluids in which the shear stress-shear rate relationship is linear but for non-Newtonian fluids a single point on a curve of a nonlinear function will not allow a meaningful derivation since biologic fluids must experience a variety of different shear rates in vivo. Therefore, an accurate record of viscosity at physiologically significant rates of shear is essential.[19] For example, the shear rate can obtain high values, 60 and 160(sec^{-1}), during swallowing and speaking, respectively,[20] and may reach even higher values during chewing the hard food.

Some biologic fluids such as blood, sputum, gastric juice, synovial fluid, and saliva have been measured by various types of viscometers, and there were many attempts to apply the results clinically.

As the role of the viscosity of the blood in blood flow and peripheral resistance, a change in blood viscosity has been implicated in many clinical conditions. Studies on viscosity of plasma, various dextran solutions, and blood with different hematocrit levels have been performed using various types of viscometers.[19,21,22]

The rheological properties of tracheobronchial secretions have been regarded of great clinical importance, especially in patients with chronic bronchitis and bronchial asthma.[23,24] And, the viscosity of sputum could be an objective yard-stick of evaluating the effects of expectorants.[18]

Kurita et al.[25] found a significant positive correlation between the viscosity and macromolecular glycoprotein concentration of gastric juice, and suggested that the viscosity of gastric juice might be an index of the defensive factors of the stomach in gastric ulcer patients, and could be applicated in the staging of peptic ulcers.

Swann et al.[26] suggested that articular lubrication might be an inherent property of the articular cartilage and its macromolecular structure per se, and that a component of the articular lubricating fraction isolated from synovial fluid might occur there as a consequence of wear processes. Reimann[27] suggested that the viscosity and boundary lubricating properties were correlated to the different joint diseases or to the degree of alteration in the joint from which the synovial fluids were obtained, and described the correlation between the viscosity of synovial fluids and the amount of hyaluronic acid and its degree of polymerization.

While the viscosity values of saliva were observed to be non-Newtonian and shear-thinning in this present study, the overall values were lower than those of previously reported.[1,16] Such differences could be due to the manner of sample collection and handling, the method and time of testing, and individual variation in the chemical composition of the secretion.

Saliva is a complex system of glycoproteins, electrolytes, proteins, sugars, bacteria, enzymes, etc. Constituents such as proteins and polysaccharides in aqueous media form viscoelastic fluids, perhaps having internal structure. Also, glycoproteins are polyelectrolytes. For certain environmental conditions, intermolecular association result in a cohesive gel. Also natural saliva is labile and inhomogeneous. It is possible to have, simultaneously, a liquid phase, a gaseous phase(bubbles), and a gel phase.[13] And, studies by Shannon[28] and Nordbö[29] provided that flow rate, calcium level, and pH value could influence the salivary viscosity. Therefore, the rheological properties of whole saliva are highly variable and in general, there has been a lack of consistency among the values reported for salivary viscosity.[16]

In the present study, the author found that the viscosity values of stimulated whole saliva in male increased with aging after the twenties, though this pattern was not consistent in young subjects. These age-dependent changes of viscosity values could be explained by the decreased flow rate in the old. These changes were compatible with the premise that, at the very low levels of gland function, decrease in flow rate bring into dominance the process of passive diffusion of water from the saliva down an osmotic gradient produced by sodium reabsorption.[28] And, Shannon[28] suggested that an increase in total protein brought about by decreased flow rate was probably instrumental in producing the measured increase in viscosity.

Recently, though, several carefully controlled studies have reported unchanged stimulated secretion rates of whole,[30-32] of parotid[33], and of submandibular saliva.[34] Therefore, except the change of flow rate, other factors such as the changes of salivary composition and the degree of contributions of glandular secretions to whole saliva with age, etc., could be also involved.

In female, the viscosity values of stimulated whole saliva increased with aging until the thirties and decreased slightly thereafter. This phenomenon could be also explained by the change of flow rate with age and the effects of female sex hormones.

It was described that during the progesterone phase of the menstrual cycle, the viscosity values of the cervical secretions were much greater than during the estrogen phase. While, the hormonal balance could not be reflected in the saliva in the same way as in the cervical mucus, the fact that progesterone produced a marked increase in the viscosity of the salivary secretion and estrogen alone also produced a slight increase, was described by Dworkin and Mendelsohn[35] in their experiment of canine saliva.

Hormonal effects and difference in flow rate due to smaller gland size in famale[36] might explain the sex difference in the viscosity values. Although it has not been well known, Kullander and Sonesson[37] suggested that salivary changes in postmenopausal women might be due to an abscence of hormonal stimuli and / or age atrophy of the gland. These changes might be causal factor in sialosis and other oral diseases which increase in frequency at this period. In mice it was well known that the histological picture of the salivary gland changed after oophorectomy.

The viscosity values were increased in order of stimulated parotid saliva, stimulated whole saliva, unstimulated whole saliva, and stimulated submandibular-sublingual mixed saliva. Explanations for this order were the characteristics of each glandular secretions and proportional difference of glandular secretions in stimulated and unstimulated conditions. And, minor salivary secretions might be considered.[38] In the present study, the viscosity values of glandular salivary secretions were measured in the twenties only. The changes of the viscosity values of glandular secretions with age remained to be investigated.

The viscosity of saliva has been described as important in the lubrication of foodstuffs, protection of of oral mucosa, diffusion, and the retention of complete upper dentures.

One of the main functions of saliva is to cover the foodstuff with a lubricant layer to assist swallowing and to protect the lining cells of the mucosa against injury.[2] Glantz et al.[39] suggested that saliva had a high viscosity to fulfil this requirement. Yet, too high a viscosity might retard distribution of the saliva in the oral cavity and enclosure of boli.

Because the diffusion coefficient is inversely proportional to the viscosity of the medium, high viscosity influences not only the liquid flow but also the diffusion. Therfore, this might be important in demineralization and remineralization processes in the oral cavity.[40]

Vissink et al.[17] suggested that fixation of dentures would depend on the rheological properties of saliva at low shear rates. As movement of an ill-fitting denture will decrease the viscosity of the saliva, it will probably both impair the retention of the denture and increase the wear and tear of the mucosa thereby setting up a vicious circle.

The rheological properties of saliva have been also utilized to the development of artificial saliva which could reproduce natural saliva in physical properties. Vissink et al.[17] compared the rheological properties of saliva substitutes containing mucin, carboxymethylcellulose(CMC), and polyethylenoxide (PEO), and described that CMC and PEO solution had no viscoelastic properties and would not adjust to the viscosity needed for proper biological function in the oral cavity. They concluded that mucin-containing saliva substitutes appeared to be the best substitutes for natural saliva, as far as rheological properties were concerned.

There have been several attempts to relate the physical properties of saliva to the diseases. Slomiany et al.[15] studied the viscosity of parotid saliva in patients with Sjögren's syndrome. And, Braddock et al.[41] investigated surface tension and turbidity of submaxillary saliva in cystic fibrosis.

While reports in the literature have assumed that a correlation exists between viscosity and oral lubrication[29], there have been several studies to suggest that the properties of lubrication and viscosity might differ and be manifestated by different salivary molecules.[1,16,42] And, Swann et al.[43] suggested that the lubrication of mineralized tissues by synovial fluids was not dependent on the viscosity of the fluid per se, but rather on the presence of a glycoprotein capable of forming a boundary interface at the opposing surfaces moving relative to each other. Further studies will be needed to investigate the relationship between biophysical properties and certain biochemical constituents in saliva.

In the present study, the author focused on the viscosity values at high shear rate because the functional discomforts such as difficulties of swallowing, speaking, and chewing, were very common in patients with xerostomia. But, an important question was the reversibility of the breakdown under shear. Well et al.[19] suggested that to a certain extent the high torque imposed on the salivary samples might lead to physical dissolution of macromolecular structure and therefore rheologic curves might reflect in part the influence of these changes(rheodestruction) as well as pseudoplastic flow behavior.

The impaired salivary gland function and resulting decrease in the protection of the mucous membranes may partly explain why completely different external factors such as infections, mechanical, thermal, chemical and electrochemical injury seem to be able to influence the course of various intraoral soft and hard tissue diseases.

It is necessary to define the biochemical and biophysical basis of salivary lubrication and viscosity between oral hard and soft tissue interfaces by further studies. This information may provide parameters for diagnosing functional discomfort and a rationale for the development of substitute fluids that may truly provide comfort and function for xerostomic patients.

V. CONCLUSIONS

The rheological properties of biological fluids have been studied by a number of investigators. Several types of viscometers were utilized and their results were discussed for clinical purposes. And, it has been stressed the importance of the shear dependent viscosity values of natural saliva on the development of proper artificial saliva.

The author has investigated the age—and sex—related changes in the viscosity values of stimulted whole saliva, and the differences among the viscosity values of stimulated and unstimulated whole saliva, stimulated parotid saliva, and stimulated submandibular-sublingual mixed saliva by the viscometer with cone—and—plate geometry. The 240 healthy unmedicated subjects were included in this study. The author came to the following conclusions :

1. Saliva was the non-Newtonian fluid which displayed viscoelastic property. And, the relationship between viscosity and shear rate could be represented by the curves of asymptotic form.

2. There were age-dependent changes of viscosity values of stimulated whole saliva, and the viscosity values showed the increasing pattern with aging. The viscosity values of stimulated whole saliva displayed the highest values after the age of fifties in males and in the thirties in females.

3. The viscosity values of stimulated whole saliva of females were greater than those of males in the age of thirties, fourties, and fifties.

4. The viscosity values were increased in order of stimulated parotid saliva, stimulated whole saliva, unstimulated whole saliva, and stimulated submandibular-sublingual mixed saliva.

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타액의 유동학적 성질에 관한 연구

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국문초록

타액의 유동학적 성질은 타액의 윤활작용 및 구강내 경조직, 연조직 보호작용에 영향을 미친다. 그러므로 타액의 유동성을 잘 이해하면, 구강내 기능적 불편감의 평가와 인공타액의 개발에 필요한 중요한 정보를 얻을 수 있다.

저자는 전단율 변화에 따른 타액점도 변화의 연령 및 성별에 따른 차이를 알고자, 각 연령층별로 구강건조증으로 고통받은 병력이 없으며 타액채취시 약물을 복용하고 있지 않은 남녀 각 20명씩 총 240명을 대상으로 자극시 분비된 전타액의 점도를 cone-and-plate 형태의 점도계를 이용하여 전단율 11.3에서부터 450.0(sec^{-1})사이에서 측정하였다. 또, 20대 남녀 각각 20명의 경우, 자극시 분비된 전타액의 점도와 함께 비자극시 분비된 전타액, 자극시 분비된 이하선 타액, 자극시 분비된 악하선 설하선 혼합 타액의 점도를 같은 방법으로 측정한 결과 다음과 같은 결론을 얻었다.

1. 타액은 점탄성의 성질을 가지는 non-Newtonian 유체로서 점도와 전단율 사이의 관계는 점근선으로 표시될 수 있었다.
2. 자극시 분비된 전타액의 점도는 연령이 높을수록 증가된 양상을 보였으나, 남자의 경우 50대 이상에서 가장 높고 여자의 경우 30대에서 가장 높았다.
3. 자극시 분비된 전타액의 점도는 20대, 30대, 40대에서 여자가 남자보다 높았다.
4. 타액 점도는 자극시 분비된 악하선 설하선 혼합타액에서 가장 높았으며, 비자극시 분비된 전타액, 자극시 분비된 전타액, 자극시 분비된 이하선 타액 순이었다.

주요어 : 타액, 유동학적 성질, 점도