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Chloride Concentration in Parotid Fluid at Low Rates of Flow

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The chloride ion is held to be an active participant in renal ion-exchange reactions that lead to alterations in the chemical composition of intracellular and extracellular fluids. Chloride is the major anion of the extracellular fluid and is practically absent from intracellular fluid except in the case of erythrocytes and cells of the kidney tubules, stomach, and intestines. Cells of the latter organs contain sodium and chloride, since they are engaged in reabsorption or secretion of these elements.[1] The precise mechanism of renal tubular exchanges of chloride is not clear, but chloride is generally considered to be subject to at least some of the influences that act upon the sodium ion. It is known that the chloride ion is one of the principal anions in saliva, whole or parotid, and that its concentration may be varied by altering the rate of flow of the saliva. The mechanisms controlling the chloride level in saliva are not understood.

It was the purpose of the present study to determine (*a*) the physiologic level of parotid fluid chloride at an extremely low rate of flow, (*b*) the correlation between chloride concentration and flow rate as the flow rate varied between subjects in a narrow range, and (*c*) thus to gain insight into the processes controlling chloride concentration in parotid fluid.

MATERIALS AND METHODS

Experimental subjects were 527 males between the ages of seventeen and twenty-two years. Food and drink offered, times of arising and retiring, physical exercise, and environmental exposure were virtually identical in all instances. Each of the randomly selected participants had been found physically fit by recent complete medical examination. Although the state of oral health was not a consideration in this instance, candidates with fewer than 20 teeth or with periodontal involvement of more consequence than a mild gingivitis were disqualified, and a DMFS score was calculated for each subject. Studies relating the caries status of this population to body-fluid chemistry are reported elsewhere.[2]

Collection of parotid fluid was begun between 8:00 and 8:30 A.M., approximately 3 hours after breakfast. Participants were seated comfortably in a quiet, semi-isolated section of an air-conditioned laboratory, and a metal parotid cap was placed over the right Stensen's duct with an absolute minimum of manipulation.[3] The fluid was col-

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lected over a 2-hour period in graduated tubes, and the final volume read to the nearest 0.025 ml. Care was taken to drain the collection apparatus thoroughly to insure an accurate determination of volume. Blood was collected by venipuncture at approximately 10:30 A.M., and the serum was separated and retained. The chloride content of the two fluids was determined by automatic titration on the Cotlove chloridometer.[4, 5]

RESULTS

The flow rate of parotid fluid for all 527 subjects under these conditions of virtually no stimulation was 0.042 ml/minute (S.D. $= \pm 0.030$), and the chloride mean for these samples was 24.78 mEq/liter (S.D. $= \pm 7.64$). The serum chloride mean for all participants was found to be 101.90 mEq/liter (S.D. $= \pm 2.61$). The subjects were divided into six groups based upon parotid flow rate, and means and standard deviations were calculated for chloride concentrations in each of the fluids under study (Table 1). There were no significant differences between mean concentrations of serum

TABLE 1

CHLORIDE CONCENTRATION IN PAROTID FLUID AS RELATED TO FLOW RATE

PAROTID FLUID FLOW RATE (ML/MIN)	NO. OF SUBJECTS	PAROTID FLUID CHLORIDE (MEQ/LITER)		SERUM CHLORIDE (MEQ/LITER)	
		Mean	S.D.*	Mean	S.D.*
0–0.010	91	31.48	9.44	102.2	2.42
0.011–0.020	93	28.04	6.47	102.6	2.88
0.021–0.030	84	26.06	5.39	101.9	2.58
0.031–0.050	111	23.03	5.24	101.8	2.75
0.051–0.080	74	21.04	4.01	101.8	2.57
>0.080	74	17.32	4.57	101.6	2.28

* $\text{S.D.} = \left\{ \frac{Sx^2 - [(Sx)^2/n]}{n-1} \right\}^{1/2}$.

chloride in the six flow-rate groups. A significant ($p < 0.01$) negative correlation ($r = -0.25$) was found between chloride concentration in parotid fluid and rate of flow for the 527 subjects. In the extremely low-flow group—flow rate 0.010 ml/minute or less—a mean chloride concentration of 31.48 mEq/liter as found. As the flow rate increased to more than 0.080 ml/minute, the mean concentration of chloride fell progressively to 17.32 mEq/liter.

The correlation between parotid fluid flow rate and chloride concentration was then tested, with the subjects grouped according to dental caries status. The correlation coefficients within these groups, all negative, varied from —0.16 to —0.30, no significant differences being found between the group coefficients (Table 2).

DISCUSSION

Many studies have been carried out in which salivary chloride content has been found to vary with rate of flow. In 1933, Baxter,[6] working with dogs, found that a positive correlation existed between parotid fluid flow rate and chloride concentration.

He concluded, further, that some effect other than that of flow rate was present, since he found the chloride level much lower in parotid saliva activated by pilocarpine than in this fluid secreted at the same rate under meat extract or bread and meat-powder stimulation.

Bramkamp[7] used paraffin for slow flow-rate production and lemon for the collection of rapid parotid fluid samples. Doses of atropine or pilocarpine were also administered to these human subjects. The chloride content of parotid fluid showed a consistent and marked increase in concentration with more rapid secretion, the saliva produced at high rates having about three times the chloride concentration of that at lower rates. It was concluded that atropine and pilocarpine altered the chloride concentration only in proportion to the change in flow rate produced. The inference was also drawn that chlorides were actively handled by the gland cells in distinction from substances which entered the saliva by simple filtration processes.

TABLE 2

THE CORRELATION BETWEEN FLOW RATE AND CHLORIDE CONCENTRATION IN SUBJECTS CLASSIFIED AS TO DENTAL STATUS

DMFS* Grouping	No. of Subjects	Correlation Coefficient	DMFS* Grouping	No. of Subjects	Correlation Coefficient
0–10	77	−0.16	31–40	106	−0.26
11–20	93	−0.24	41–50	67	−0.30
21–30	110	−0.26	>50	74	−0.23
			Over-all	527	−0.25

* Sum of decayed, missing, and filled surfaces.

Thaysen, Thorn, and Schwartz[8] studied parotid fluid collected from 3 young women with uncomplicated essential hypertension. Subcutaneous beta-methylacetylcholine hydrochloride (4–6 mg. in 0.5–1.0 ml. isotonic saline) was employed to stimulate flow. Chloride was determined in 30 samples and was found to range from 10 mEq/liter at a flow rate of 0.31 ml/minute to 43 mEq/liter at 3.66 ml/minute.

Chauncey, Lisanti, and Winer[9] collected two samples of parotid fluid under flavored chicle stimulation from each of 50 subjects on 2 successive days. No significant correlation between chloride and rate of flow was found under these conditions.

Carter, Englander, and Weber[10] collected parotid fluid "50 times on each of 2 individuals using different methods of stimulation to produce different flow rates of parotid saliva during a 5-minute period." Neither data nor correlation coefficient was provided, but a positive correlation between chloride concentration and flow rate was reported.

Anders[11] measured chloride in whole saliva of 431 children and found a mean value of 16.25 mEq/liter (S.D. $= \pm 4.63$). No mechanical stimulation was employed. Nine children were also tested with paraffin stimulation, and no consistent relationship between flow rate and chloride was found.

Past reports from our laboratories have presented conflicting findings concerning the relationship of flow rate to chloride concentration. Over a 3-day period, 27 samples of whole saliva were collected under three-rubber-band stimulation from each of 29 sub-

jects. For 23 of the subjects a negative correlation between flow rate and chloride was found. Twelve of these coefficients were significant, while, in the entire study, only one significant positive correlation was found.[12] In another study,[13] whole saliva was collected without exogenous stimulation from 682 healthy young adults. For males and for females, significant negative correlations of —0.23 and —0.16, respectively, were calculated. The male flow-rate mean was 1.26 ml/minute, while that for females was 0.93 ml/minute. As another approach[14] the flow rate of whole saliva and the concentration of chloride were studied in 93 subjects, each sampled on 3 successive days with increasingly strong stimulation. As the mean rate of flow increased daily from 0.97 to 2.12 to 2.85 ml/minute, a significant increase in the mean concentration of chloride was found. In the same study, for 112 subjects sampled with no exogenous stimulation on 2 successive days, the flow rate increased from 1.16 to 1.36 ml/minute, and a significant negative correlation with chloride concentration was found. When 65 subjects were stimulated with three rubber bands on 2 successive days, the means for volume were 1.82 and 1.88 ml/minute, and the chloride means were not significantly different. With three-rubber-band stimulation, whole saliva was collected at a flow rate of 1.74 ml/minute for 323 males and at 1.25 ml/minute from 159 females. The correlation between flow rate and chloride concentration was significantly positive for males and significantly negative for females.[15] In further experimentation[16] samples of whole saliva were collected without exogenous stimulation from 537 young adult male subjects. One hundred and fifty-three of these subjects were caries-rampant, 213 were caries-resistant, and 171 presented at least 20 filled surfaces and no carious lesions. Within each of the three groups a negative correlation between flow rate and chloride concentration was found. In a parotid fluid study,[5] samples were collected from each of 25 subjects in an ascending-descending flow-rate pattern. As the mean rate of flow varied from 0.28 to 0.44 to 0.84 to 0.46 to 0.34 ml/minute, a highly significant positive correlation with chloride concentration was noted. In the present study, a consistent and significant negative correlation was found between flow rate and chloride concentration.

It was thus evident that the relationship between flow rate and chloride concentration varied markedly with the rate of flow. In studies carried out with only minimal stimulation, variations in flow rates between subjects were found to be negatively correlated with chloride concentration. As exogenous stimulation was increased slightly, the correlation between the two variables became unpredictable. Some subjects maintained the negative correlation as found in the very low flow-rate studies while, in a virtually equal number of subjects, definite positive correlations were found. In studies carried out at medium to high rates of flow, the correlation was consistently strongly positive.

It is not the purpose of this paper to discuss the mechanisms of electrolyte control in minute detail, but the above observations become more meaningful when they are discussed in the light of the following unreported studies. When parotid fluid samples were collected from 513 subjects under conditions identical with those of the present study, the mean flow rate was 0.027 ml/minute, and the mean concentrations of sodium and potassium were 2.61 and 36.68 mEq/liter, respectively. Parotid fluid bicarbonate concentration was determined in another as yet unpublished study on 502 subjects. At a mean flow rate of 0.042 ml/minute, the mean bicarbonate concentration was only 1.04 mEq/liter. In these studies some instances were noted in which sodium

and bicarbonate concentrations were so low as to be almost undetectable, while some of the samples contained potassium in excess of 80.00 mEq/liter.

The implication is that sodium is being reabsorbed in the ducts and that the concentration of this ion in the parotid fluid is a negative function of the length of time that the saliva remains in the duct system. That is, the lower the rate of flow, the longer the saliva remains in the ducts, and the lower is the sodium concentration. Virtually identical statements can be made concerning our findings for bicarbonate concentration. We have seen the concentration of this variable exhibit a positive correlationship with flow rate throughout our studies and have seen the concentration fall progressively from a mean of 24.77 mEq/liter at a flow rate of 0.858 ml/minute[5] to the extremely low mean concentration reported above at a very low mean rate of flow. Our experimental evidence indicates that the potassium ion may be actively secreted by the duct cells into the parotid fluid. Studies in renal physiology[17–19] not only have established that potassium is secreted into the urine within the nephron but have also localized secretion in the distal tubule. In the present study the very high potassium levels found at very low rates of flow are particularly suggestive of duct-cell secretion of this ion.

Although none of the foregoing experiments point out specifically the manner in which the chloride concentration of parotid fluid is controlled, it can be definitely stated that the chloride pattern is not identical with that seen for either sodium or potassium, the two ions with which chloride is most often studied. At the higher rates of flow, chloride is probably reabsorbed along with sodium. As flow is decreased, however, the sodium concentration continues to fall to almost the level of disappearance, while a chloride mean of at least 17.00 mEq/liter has been found in our lowest flow-rate groups. Rather than to postulate without evidence that chloride reabsorption is selectively halted at a given salivary concentration, our evidence points to both reabsorption and secretion of chloride by the duct cells. At higher rates of flow the secretion of chloride by the duct cells is probably rendered relatively ineffective by the high chloride concentration in the saliva resulting from the reduced reabsorption in the ducts. Conversely, at very low rates of flow, both processes—reabsorption and secretion—seem to become maximally effective in regulating chloride concentration. In this situation a great deal of chloride is being reabsorbed along with sodium ions, but the chloride level in the exiting fluid is much higher than that found for sodium, since chloride is also entering the saliva, along with potassium, from the duct cells. The very high potassium levels would seem to support this tentative working hypothesis. Further, the negative correlation between chloride concentration and flow rate found within the present study is compatible with our recent unpublished observation on 513 subjects that, at comparable flow rates, a significant negative correlation ($r = -0.47$) exists between flow rate and potassium concentration.

SUMMARY

Parotid fluid was collected from 527 healthy young adult male subjects under conditions of minimal stimulation. At a mean flow rate of 0.042 ml/minute (S.D. $= \pm 0.030$), the chloride mean was 24.78 mEq/liter (S.D. $= \pm 7.64$). The mean serum chloride concentration was 101.90 mEq/liter (S.D. $= \pm 2.61$). At this low rate of flow a significant ($p < 0.01$) negative correlation ($r = -0.25$) was found between flow rate and chloride concentration. A comparison of the results of this study with those

from other work on comparable subjects led to the hypothesis that the chloride concentration of the parotid fluid is controlled as follows: Chloride is reabsorbed along with sodium by the duct cells and is, at the same time, secreted into the saliva, along with potassium, by cells of the duct walls. At high rates of flow the reabsorption mechanism is overwhelmed, and the chloride concentration increases along with flow-rate increases. At the low rates of flow the secretion of chloride, along with potassium, into the saliva prevents the then very effective reabsorptive process from decreasing the chloride concentration to the very low levels found for sodium and bicarbonate. Thus parotid fluid chloride concentration appears to be dependent on the movement of sodium and potassium between the duct cells and the saliva.

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