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THE MECHANISM OF SECRETION OF FLUID AND ELECTROLYTES IN SALIVARY GLANDS

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INTRODUCTION

It has been realized since Ludwig's original studies that the mechanisms which underlay the secretion of saliva involve some type of active process in the gland (Ludwig *et al.,* 1851). It was demonstrated in those early experiments that the submaxillary ductal pressure in dogs will rise above arterial pressure when the duct is occluded during active secretion. Since then, experiments carried out in a wide range of epithelial tissues which are capable of transcellular transfer of large volumes of fluid and electrolytes (e.g., frog skin, toad bladder, gall bladder, small intestine, etc.) have provided ample evidence that water transport through cells is associated with active electrolyte transport (e.g. see Diamond's extensive treatment of this subject; Diamond, 1964). Thus, it seems reasonable to suspect that the fluid generating mechanism in salivary glands is associated with electrolyte secretion. However, there have been few efforts to examine relationships between electrolyte secretion rates and fluid generation at multiple physiological levels of function of the glands. Thaysen *et al.* (1954) reported on Na, K, Cl, and CO_2 in human parotid saliva in a study whereby stimulation was provided by acetylcholine administration, and Yoshimura, *et al.* (1963) have investigated the same electrolytes in human mixed saliva under resting conditions and during pilocarpine administration.

The study described below was done using human parotid secretions; it was designed to define the relationships between the rates of secretion of sodium, potassium and chloride fluid secretion rates, and to the osmolality of the secretory fluid under differing degrees of gland function. While the data have been reported elsewhere (Suddick and Shannon, 1970), this paper provides the opportunity to offer a theory of secretory cell transport processes which could explain the reported results, and which may provide some new insights into exocrine secretory processes.

METHODS AND MATERIALS

Two groups of healthy young adult males served as subjects – Group I, comprised of 513 subjects, was used to determine basal levels and population differences for parotid fluid sodium, potassium and chloride concentrations (hereafter concentration will be denoted by bracketed symbol) and for resting rates of flow in the absence of overt physiological stimulation (i.e. under "resting" conditions). Group II included a total of 271 subjects divided into two subgroups (IIA and IIB); this group was used to determine changes in parotid fluid $[Na^+]$, $[K^+]$ and $[Cl^-]$, osmolality, specific gravity, and per cent total solids at two different rates of physiologically stimulated rates of flow. Group IIA (125 subjects) served for the $[Na^+]$, $[K^+]$ and $[Cl^-]$ determinations, while IIB (146 subjects) was used for the other parameters. "Resting" secretion values for Group IIA parameters were also determined for comparison to the more comprehensive results obtained from Group I subjects. Parotid fluid sampling was performed by means of a metal collection

device (Shannon and Chauncey, 1967), the sampling undertaken at approximately 7:30 AM, with subjects in the fasting state.

For determination of "resting" values with respect to flow rate as well as $[Na^+]$, $[K^+]$ and $[Cl^-]$ in Group I subjects, a 120 minute sample was collected with the subjects isolated from extraneous interferences but awake and alert. The Group II "resting" secretion collection period was 45 minutes. Physiological stimulation of secretion for Groups IIA and IIB samplings was provided by having the subjects sequentially 1) chew on a rubber band, and 2) suck on a sour lemon drop. Such stimulated samplings were carried out in successive 10-minute collection periods, going from rubber band to lemon drop stimulation, then back to rubber band stimulation, then lemon drop, etc. This cycle was run three times (six 10-minute samples) in Group IIA and twice (four 10-minute samples) in Group IIB. Prior to each 10-minute test sample collection, the subject collected a 5-minute waste sample (discarded) to allow the gland to accommodate to the new stimulus and to clear the dead space in the collection apparatus.

Sodium, potassium, and chloride were measured on single samples collected during the described sampling periods. Sodium and potassium were measured by flame photometry (Shannon *et al.*, 1963), and chloride determinations were carried out on the chloridometer (Cotlove *et al.*, 1968). Determinations of osmolality were done by freezing point depression in an Advanced Instruments Osmometer, and specific gravity by a single drop method in the Banco Gradient Balance (Shannon, 1968). Total solids were measured by drying overnight to constant weight under low negative pressure.

RESULTS

For all 513 subjects of Group I, the mean for parotid flow rate was 0.027 (S.D. = 0.025) ml/min, for sodium 2.61 (S.D. = 2.00) mEq/l, and for potassium 36.7 (S.D. = 12.5) mEq/l. Comparable means for serum sodium and potassium were 141.1 (S.D. = 4.5) and 4.65 (S.D. = 0.32) mEq/l, respectively. The 513 subjects were divided into six flow rate subgroups extending from a rate of less than 0.011 ml/min to a flow greater than 0.080 ml/min. Sodium and potassium concentrations in both parotid fluid and serum for these groups are presented in Table 1. Neither sodium nor potassium in the blood serum was found to be related to parotid flow rate. Parotid fluid sodium means for the subgroups ranged from 2.45 to 2.96 (S.D. = 2.43) mEq/l and did not differ significantly between any of the subgroups. The between-subjects correlation coefficient for sodium and flow rate was 0.031, this figure not differing significantly from zero.

Mean parotid fluid potassium levels ranged from 46.3 mEq/l for the lowest flow rate subgroup to 25.5 in the fastest flowing subgroup (Table 1). The between-subjects correlation coefficient for these two variables was −0.468, and this figure was significantly different from zero at the 0.01 level.

Table 1: Effect of flow rate on Parotid fluid Na, K, and Cl

Flow Rate Grouping	Number of Subjects	Sodium (mEq/1)		Potassium (mEq/1)		Chloride (mEq/1)	
		Mean	S.D.	Mean	S.D.	Mean	S.D.
< .011	23	3.03	2.50	46.6	10.15	30.9	5.99
.011-.020	66	3.47	3.18	38.9	8.08	26.7	5.08
.021-.030	46	3.12	2.79	32.2	5.17	24.8	4.58
.031-.050	31	3.68	2.66	29.0	3.46	20.6	3.61
.051-.080	28	3.44	3.02	26.7	2.83	19.1	3.58
> .080	9	4.27	2.71	24.1	1.97	18.9	8.22

Thus, in this unstimulated state, subjects exhibiting higher levels of gland function as indicated by flow rate demonstrated lower levels of potassium concentration than did the slower-flowing subjects.

Effects at the different levels of stimulated secretory activity are presented in Table 2 reflecting changes in individual rates of secretion for each ion (flow rate X concentration) and in Figure 1 which simply reflects the changes in concentration at the different levels of secretion. The rubber band stimulus resulted essentially in a nine-fold increase in flow rate over "resting" levels, a seven-fold increase in $[Na^+]$ (excepting the first 10-minute sample), and a halving of $[K^+]$ and $[Cl^-]$ (Fig. 1). Because of the dramatic increase in $[Na^+]$ in contrast to actual decreases in $[K^+]$ and $[Cl^-]$, the minute secretion rate for sodium increased much more than that of potassium or chloride (sixty to seventy-fold vs. five to six-fold) (Table 2). Going from the rubber band stimulus to the lemon drop stimulus caused further increases in flow rate and $[Na^+]$ (Fig. 1), with minute secretion rates for sodium about seven-hundred times greater than at the resting level (Table 2).

While $[Na^+]$ underwent a three-fold increase in going to the lemon drop stimulus, $[K^+]$ remained at the rubber band stimulated level; potassium rate of secretion thus simply paralleled the flow rate increase (Table 2). Chloride, after undergoing the decrease in concentration from the resting level during the initial rubber band stimulation, underwent a three-fold concentration increase when going to sour lemon drop stimulation (Fig. 1). Its relative concentration increase and thus its relative minute secretion rate increase from the rubber band stimulated level, closely paralleled the secretion rate changes for sodium in going to the highest stimulated level of secretion.

Table 2: Parotid fluid sodium, potassium, and chloride concentrations and rates of secretion at different levels of gland activity.

FUNCTIONAL STATE OF GLANDS	SAMPLING ORDER	FLOW RATES	SODIUM		POTASSIUM		CHLORIDE	
			Concentration mEq/1	Rates of Secretion μEq/min	Concentration mEq/1	Rates of Secretion μEq/min	Concentration mEq/1	Rates of Secretion μEq/min
Unstimulated (0)	1	.03	3.50	.12	30.35	1.01	21.98	.73
Unstimulated (0)	1	.03	3.50	.12	30.35	1.01	21.98	.73
Rubber Band Stimulus (+)								
1st 10 min. sample	2	.20	7.13	1.41	20.62	4.07	13.86	2.74
2nd 10 min. sample	4	.30	23.16	6.89	18.40	5.47	11.89	3.54
3rd 10 min. sample	6	.32	24.07	7.64	18.94	6.01	12.87	4.08
Lemon Drop Stimulus (++)								
1st 10 min. sample	3	1.18	64.41	75.77	18.15	21.36	39.38	46.32
2nd 10 min. sample	5	1.19	66.26	78.87	18.18	21.64	39.02	46.45
3rd 10 min. sample	7	1.16	64.41	75.00	18.44	21.48	38.20	44.48

Each value is the mean from determinations done on 125 subjects. Multiple samples were collected on each subject in the order indicated. Each value at each level of gland activity is significantly different ($p < .01$) from any given value at any other level of gland activity (e.g. all Rubber Band flow rate values are significantly different from the unstimulated flow rate and from all Lemon Drop flow rates) with the exception of Rubber Band and Lemon Drop Potassium concentration values which are not significantly different. In addition, some significant differences appeared between the 1st and 3nd 10 min Rubber Band Stimulus collections for flow rates and Sodium values. Standard deviations of all values can be obtained from the authors on request.

Further examination of Table 2 and Figure 1 will reveal that the subjects were rotated through the two types of gland stimulations three different times, and these rotations were run consecutively. The closeness of the mean values obtained at specific stimulus levels during the described complex rotational procedure are demonstrated in Figure 1 and provide an indication of the reproduceability of these experiments; also, the striking correlation of $[Na^+]$ with the different levels of fluid secretion (flow rates) can be readily observed in the figure and compared to the $[K^+]$ and $[Cl^-]$ changes in relation to secretion rate.

The effects of the two levels of stimulation upon parotid fluid osmolality, specific gravity, and total solids in the fluid are shown in Table 3 (Group IIB). The higher level of gland stimulation (Lemon Drop Stimulus) caused essentially a doubling of fluid osmolality as determined by the freezing point depression. The corresponding changes in specific gravity and per cent solids were consistent with the changes in osmolality.

DISCUSSION

These findings should be compared to a previous study from these laboratories on the effects of atropine upon parotid flow rate, osmolality, total solids, and rates of secretion of Na, K, and Cl (Shannon, *et al.*, 1969). Depression of secretion from the resting level by atropine resulted in a parallel depression of the flow rate to the sodium rate of secretion; in other words $[Na^+]$ was not affected by the drug, but the levels of eleven other major constituents (including K^+) increased. The broad correlations of the atropine study and the present data then are that an inverse relationship has been demonstrated between $[K^+]$and rates of saliva flow, but $[Na^+]$ remains steady throughout all levels within these low flow rate ranges.

The different relationships of the electrolytes to fluid secretion rates at the minimal levels of secretion should be compared to relationships of the same electrolytes at the higher physiological levels of stimulation. It is evident that the increasing levels of stimulation (rubber band, and sour lemon drop induced) brought about progressively greater effects upon the minute secretion rates for sodium than for potassium or chloride; Na secretion increased 60-70 times over basal levels with rubber band stimulation and more than 700 times with sour lemon drop stimulation, effects much greater than those exhibited by K and Cl (Table 2). These results are quite similar to those reported in mixed human saliva by Yoshimura, *et al.* (1963), and also to results reported for human parotid secretions by Thaysen, *et al.* (1954) in which stimulation was provided by acetylcholine hydrochloride administration.

The increases in osmolality which occur during stimulated secretion and that which follows atropine appear to be caused by entirely different

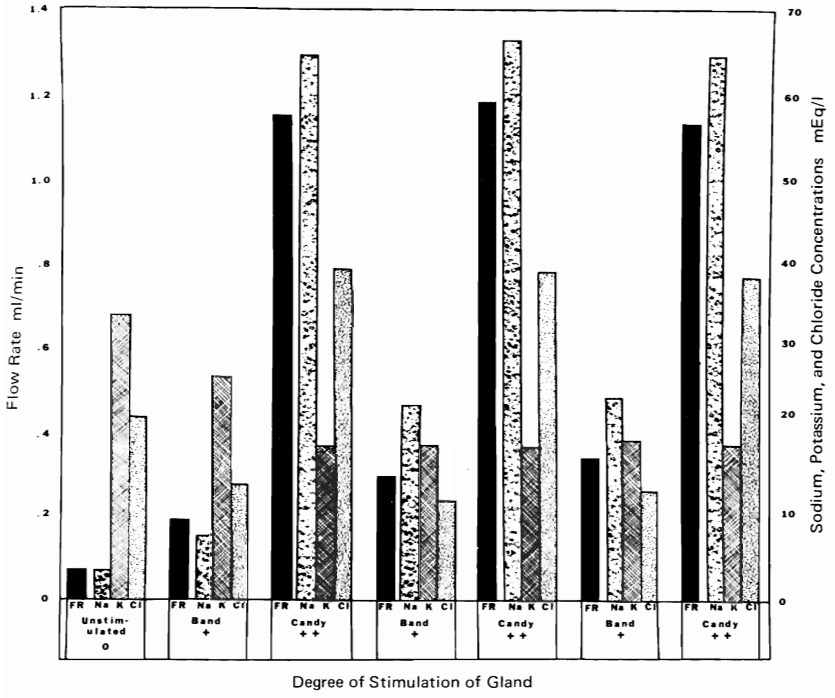

FIGURE 1

secretory phenomena. In atropine depressed secretion, the increases in osmolality appear to be due to a general concentration of electrolytes and non-electrolytes in the fluid with the exception of sodium. On the other hand, during increased levels of physiological stimulation, the greatly increasing sodium concentration in the secretory fluid would appear to be an important contributor to the increasing osmolality.

These different patterns of response of sodium secretion and osmolality during stimulated levels of secretion, during various stages of very low "resting" secretion, and during drug-induced depression of secretion have implications concerning the mechanism of fluid secretion. During very low levels of secretion, there is a one for one relationship between the amounts of sodium and water secreted over a broad range of the very low flow rate values. There are two different explanations which can be offered singly or in combination which could explain the steady [Na^+] at low levels of secretion. Under the assumption that the concentration of sodium in the final secretory fluid reflects the actual concentration in the lumen adjacent to the secretory cells, it would appear that there is a one for one relationship

TABLE 3 PAROTID FLUID OSMOLALITY, SPECIFIC GRAVITY, TOTAL SOLIDS, AND RATES OF SECRETION AT DIFFERENT LEVELS OF GLAND ACTIVITY

FUNCTIONAL STATE OF GLANDS	SAMPLING ORDER	FLOW RATES (ml/min)	OSMOLALITY (mosm/kg H_2O)	SPECIFIC GRAVITY	TOTAL SOLIDS (%)
Rubber Band Stimulus (3)					
1st 10 min. sample	1	.19	63.51	1.00195	.419
2nd 10 min. sample	3	.30	60.70	1.00249	.485
Lemon Drop Stimulus (33)					
1st 10 min. sample	2	1.05	136.90	1.00536	.984
2nd 10 min. sample	4	1.04	130.51	1.00516	.922

Each value is the mean from determinations done on 145 subjects. The collection sequence was identical to that shown in Fig. 1, with the exception that an unstimulated sample was not taken. All of the values given at the higher level of gland function (Lemon Drop Stimulus) are significantly different from any given value at the lower level (Rubber Band Stimulus) ($P < .01$).

between the actual secretion of sodium and the secretion of water over a broad range of low flow rates. However, if sodium is reabsorbed during passage through the secretory tubules, then the same relationship must hold for reabsorptive sites – that is, a one for one relationship for water and sodium at the different low flow rate values. A different picture is presented during physiologically stimulated secretion. Secretion rates of sodium (Na concentration X flow rate) increase more rapidly than do water secretion rates. Chloride secretion rates also follow this pattern, but only at the highest secretion rates (see Fig. 1), a decrease in Cl concentration being observed at an intermediate level of stimulation (the 1st rubber band stimulated sample). Thus, it would appear that [Cl^-] behavior mimics that of potassium at resting and at the lowest levels of stimulation – while at the higher levels of stimulation [Cl^-] behavior mimics that of sodium.

One possible explanation for these various findings can be offered. It is suggested that the water of secretion may be generated by local osmosis surrounding sites of active sodium transport into the lumen; these sodium pump-fluid generation sites are probably located on the luminal membranes of the secretory cells. Over the broad range of the low secretory rates, there appears to be a one for one relationship between the amount of sodium transported and the "secretory" water which enters the lumen. The sodium generated entrance of water at somewhat higher secretory rates (e.g. the first rubber band stimulation) results in dilution of potassium and chloride already present in the luminal fluid. At higher levels of stimulation, the increasing amounts of secretory water moving through the cell is generated by greatly increased activity of the luminal sodium pump. Thus, sodium concentration rises markedly and this, from electrochemical considerations, would tend to increase the rate of chloride transfer across the secretory cell.

Another factor which undoubtedly operates at higher secretory rates is solvent drag. With the increasing quantities of water (which is indeed enormous compared to the cellular water content of the glands) being transfered across the cell, some electrolytes must be carried with the solvent. At the higher rates of flow, the behavior of [K^+] definitely indicates a solvent drag effect (i.e. [K^+] levels off at the higher secretory rates and its rate of secretion changes thus parallels that of water). Thus, of the three principal osmolytes of saliva studied over the broad range of flow rates, sodium in its concentration and rates of secretion changes appears to be the only one for which a plausible case can be made as a primary mover of the secretory fluid by local osmotic effects across the luminal membrane of the secretory cell.

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