Excretion of Sodium, Potassium, Chloride and Carbon Dioxide in Human Parotid Saliva

JØRN HESS THAYSEN, NIELS A. THORN AND IRVING L. SCHWARTZ

From the Hospital of the Rockefeller Institute for Medical Research, New York City

Heidenhain (1) first demonstrated that the concentration of inorganic salts in the submaxillary saliva of dogs varies in proportion to the secretory rate. Gregersen and Ingalls (2) showed that the concentration of sodium increases with increasing rates of secretion, whereas the concentration of potassium is independent of salivary flow. This finding has been confirmed by later investigators working on submaxillary saliva of various animals (3-5) and on human mixed saliva (6).

In the present experiments the concentrations of sodium, potassium, bicarbonate, and chloride were measured simultaneously in human parotid saliva following stimulation of the secretion by the subcutaneous injection of beta-methyl-acetylcholine hydrochloride. The results obtained on the relative rates of excretion of potassium and sodium by the parotid gland are compatible with the hypothesis that both cations are brought into a precursor solution at a rate which varies in direct proportion to the rate of salivary flow, and that sodium is withdrawn from this precursor solution by a process of reabsorption which is limited by a maximal capacity.

EXPERIMENTAL PROCEDURE

Three young women with uncomplicated essential hypertension served as subjects for the experiments. Their daily intake of sodium chloride varied between 5 and 10 gm.

The subjects were sitting in a chair during the tests. The flow of saliva was stimulated by the subcutaneous injection of beta-methyl-acetylcholine hydrochloride, (Mecholyl), 4-6 mg in 0.5-1.0 ml of isotonic saline. Parotid saliva was collected from the opening of Stenson's duct in a lucite collection chamber 10 mm in diameter and 4 mm deep, similar in construction to those used by previous investigators; these collection methods have recently been reviewed by Curby (7) who modified and improved the technique. The saliva produced by the other salivary glands was drained away by suction.

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The parotid saliva was collected in syringes of 1-5-ml capacity during exactly timed periods of 3-2 minutes duration. A piece of thin-walled rubber tubing, 2 cm long, served as a connection between the draining tube of the collection chamber and the tip of the syringe. The saliva was drawn into the syringe by slight suction applied through the plunger. At the end of each collection period stronger suction was applied, thus drawing out the mucosa of the cheek in the papillary area to fill the collection chamber almost completely, and collapsing the thin-walled rubber tubing. This procedure minimized the 'dead space' of the collection unit. During change of syringes the rubber tubing was compressed between two fingers in order to avoid loss of saliva and carbon dioxide.

At the end of a collection period the syringe was removed and immediately closed with a rubber cap. The syringes with the rubber caps attached were weighed before and after each collection period for determination of the amount of saliva.

The 'dead space' of the collection system was measured by the amount of water which remained in the system after a known amount was injected and recovered as completely as possible. The cup was placed on the mucosa of the cheek close to, but not covering Stenson's duct, and the system was sucked free of air until the cheek mucosa filled the cup and the rubber tubing collapsed. With the rubber tubing compressed between two fingers a 1-ml syringe containing an exactly determined amount of water was connected and approximately 0.5 ml water was injected to fill the cup and rubber tubing; immediately afterwards strong suction was applied in order to recover the maximum amount of injected water. In five such experiments the 'dead space' was found to be 0.010 (%0.007) ml; this value is small as compared to the smallest volume of saliva collected in the present experiments, 0.20 ml, and negligible in relation to the usual amounts of 0.40-4.00 ml.

Syringes and weighing bottles were coated with silicone and carefully washed with distilled water to minimize contamination with sodium, potassium, and chloride. The procedure was the same as that previously described in the method for collection of small samples of sweat (8). A sample of venous blood was drawn at the end of each experiment and the plasma immediately separated for analysis by centrifugation under mineral oil.

ANALYTICAL METHODS

Saliva was kept in a syringe, protected from air with a rubber cap. A straight pipette, 0.2
ml in volume, was connected to the syringe with a piece of plastic tubing about 1 cm long. The saliva was injected into the pipette; the pipette was removed and the plastic tubing immediately closed with a hemostat to prevent evaporation and loss of carbon dioxide. The sample was delivered into the chamber of a Van Slyke machine and its content of carbon dioxide determined by a standard method (9). Determination of the content of carbon dioxide in the plasma was carried out by the same method.

Determination of sodium and potassium was carried out on a Perkin-Elmer flame photometer (model 52A), using lithium as an internal standard. The sample for analysis, 3-6 drops of saliva, was delivered from a syringe into a siliconed weighing bottle and the exact amount determined by weight increment. It was diluted with 25 ml of LiNO₃, 80 mEq/l. Sodium and potassium in plasma were determined after dilution by the same solution in the proportions 1:200 and 1:50, respectively.

Determination of chloride in the saliva was carried out by a modification of the differential potentiometric titration of MacInnes and Dole (10), acidifying the samples as suggested by Cunningham, Kirk, and Brooks (11) in the application of potentiometric titrations to biological fluids. A sample of saliva, approximately 0.1 ml in volume, was transferred into a preweighed weighing bottle containing 1.9 ml of 0.4 N H₂SO₄. The exact size of each sample was determined by reweighing the bottle to a precision of 0.05 mg; chloride was determined by titration with 0.01 N AgNO₃, carried out in the weighing bottle.

Chloride in the plasma was determined by the same method as above using 0.5 ml of plasma diluted with 3.5 ml of 0.4 N H₂SO₄. The accuracy and precision of the determination of carbon dioxide, sodium, potassium, and chloride were determined in recovery experiments.

A solution of sodium bicarbonate was delivered into nine syringes which were immediately stoppered. Thereafter, exactly the same procedure was followed as in an actual analysis of saliva. The solution contained 12.0 mM/l. of NaHCO₃, the amount of carbon dioxide found by analysis was 12.3 (±0.23) mM/l.

A solution containing 65.0 mEq NaCl and 30.0 mEq KCl/l. was delivered into 10 weighing bottles and treated thereafter like samples of saliva. The amounts of sodium and potassium found by analysis were 63.9 (±0.71) and 29.7 (±0.27) mEq/l., respectively.

The concentration of chloride in a sample of mixed saliva was found by analysis of five 0.1-ml aliquots to be 13.52 (±0.05) mEq/l. A second group of five aliquots received, in addition, 0.49 µEq of chloride. The concentration of chloride in the second group was found to be 18.34 (±0.09) mEq/l., closely approximating the expected value of 18.42 mEq/l.

RESULTS

Excretion of Potassium. The concentration of potassium was determined in 47 samples of

Fig. 1. Relation between the concentrations (mEq/l.) of sodium and potassium and the rate of salivary flow (ml/min.). Lines have been fitted by inspection to show the general trends.

Fig. 2. Relation between the concentrations (mEq/l.) of bicarbonate and chloride in the saliva and the rate of salivary flow (ml/min.). Dotted lines have been fitted by inspection to show the general trends.
parotid saliva in four experiments on three subjects. The secretory rates ranged from 0.20–4.26 ml/min. and the concentrations of potassium in the plasma varied between 3.2 and 5.7 mEq/l.

The concentration of potassium in the saliva was independent of the secretory rate and greater than the concentration of potassium in the plasma. The mean concentration of potassium in all 47 samples of saliva was 19.8 (±3.0) mEq/l. Inspection of the data revealed that the first samples obtained following stimulation of the secretion had a higher concentration of potassium than average in three out of four experiments. Similarly, the concentration of potassium was found to be elevated in nine samples which had been secreted at rates below 0.50 ml/min. The mean concentration of potassium in the three first periods was 23.2 (±0.58) mEq/l. These concentrations of potassium were found to be significantly ($P < 0.01$) different from the mean concentration of potassium in the remaining 35 samples, which was 18.8 (±2.3) mEq/l, or approximately 4 times the concentration in plasma. The concentration of potassium in these 35 samples of saliva was independent of the secretory rate; the coefficient of variation was 1.27%, not significantly greater than that for replicate analyses. Further analysis of variance showed that the difference between average values of different experiments was no greater than that expected from the fluctuation of values within experiments.

Excretion of Sodium. The concentration of sodium was measured in the same 47 samples of saliva. Unlike the concentration of potassium, the concentration of sodium in saliva varied in relation to the secretory rate and was smaller than the concentration in the plasma. These relations are shown in figure 1. In the first sample obtained following stimulation of salivary flow the concentration of sodium was found in three out of four experiments to be somewhat lower than expected from the secretory rate; in these samples the concentration of potassium was elevated. Presumably the initial discharge from the gland is not typical of the secretion produced during the brisk flow, and for this reason these initial values have been omitted from figure 1.

Excretion of Carbon Dioxide. The concentration of carbon dioxide was measured in 49 samples of saliva, including the 47 analyzed for sodium and potassium. It was found that the concentration was independent of the secretory rate at salivary flows above 1.5 ml/min. and approximately twice the concentration of carbon dioxide in the plasma, which ranged from 25.6–28.9 mM/l. At secretory rates below 1.5 ml/min. the concentration of salivary carbon dioxide dropped to levels equal to or lower than the concentration in plasma. These relations are shown in figure 2. In this figure carbon dioxide has been charted as bicarbonate, since saliva is alkaline at all rates of flow (12).

Excretion of Chloride. Chloride was determined in 30 of the 47 samples that had been analyzed for sodium, potassium, and carbon dioxide. The concentration of chloride in the saliva varied between 10 mEq/l. at a secretory rate of 0.31 ml/min. and 43 mEq/l. at a secretory rate of 3.66 ml/min. It was always lower than the concentration in the plasma which was measured once in each experiment and found in all cases to lie in the narrow range of 100.4 and 101.4 mEq/l.

The sum of the concentrations of carbon dioxide, computed as bicarbonate, and chloride amounted to 90 (±5) % of the two cations at all secretory rates. The mean con-
centrations of the four ions in the saliva at varying secretory rates are shown in figure 3, together with the mean concentrations of sodium, potassium, chloride, and bicarbonate in the plasma.

**DISCUSSION**

The demonstration by Gregersen and Ingalls (2), that the concentration of sodium in dog saliva increases with increasing secretory rate, whereas the concentration of potassium in saliva is independent of salivary flow, has been confirmed by the findings of the present experiments on human parotid saliva. Two exceptions from this generalization were noted. First, the concentration of potassium rose significantly above average when the secretory rate fell below 0.5 ml/min. A similar observation had been made by Langstroth and McRae (3) who examined the submaxillary secretion of cats following electrical stimulation of the chorda tympani. The reason for this rise in potassium concentration at low secretory rates remains obscure. Secondly, the initial samples of parotid saliva collected in the present experiments contained a higher concentration of potassium than average, and, usually, a lower concentration of sodium than might be expected from the rate of salivary secretion. A possible explanation for this phenomenon is that the brisk flow of saliva produced by the injection of Mecholyl is mixed in the ducts of the gland with saliva produced at the lower secretory rates prior to stimulation.

In figure 4 a comparison is made between the rate of excretion of sodium and potassium in \( \mu \text{Eq/min.} \) and the secretory rate in ml/min. At secretory rates above 1 ml/min, the rate of sodium excretion approximates a linear function of salivary flow, whereas at lower flows the rate of excretion somewhat exceeds the value predicted by the linear relation. A linear regression of the rate of sodium excretion on the rate of salivary flow, calculated for all data at or above a secretory rate of 1 ml/min., has a slope of 107 \( \mu \text{Eq/ml} \) and an intercept of \(-60 \mu \text{Eq/min.} \). In contrast, the rate of excretion of potassium is directly proportional to the rate of salivary flow within the whole range examined. This finding is compatible with the assumption that both sodium and potassium are transferred into a hypothetical precursor solution at rates varying in direct proportion to the secretory activity of the gland, but that in addition sodium is reabsorbed in part by a process of limited capacity. The values for slope and intercept of the straight line can be interpreted to mean that sodium is transferred into a precursor solution at a rate of 107 \( \mu \text{Eq/ml} \) of saliva discharged and that 60 \( \mu \text{Eq/min.} \) of sodium is reabsorbed/min., when the flow of saliva exceeds about 1 ml/min. These values probably underestimate actual rates since the straight line was fitted to points that had not quite reached their asymptote. Potassium appears to be transferred into the precursor solution at a rate of 17.5 \( \mu \text{Eq/ml} \) of saliva secreted and remains unaffected by any
rcabsorptive process. The concentrations of sodium and potassium in the hypothetical precursor solution cannot be stated from these results since the movement of water in the secreting gland remains undetermined.

The rate of excretion of bicarbonate showed a linear relationship to salivary flow at secretory rates above 1.5 ml/min. This finding is in agreement with the conclusion of Sand (10) who stated that the main source of salivary bicarbonate is the carbon dioxide formed in the gland by metabolic activity.

**SUMMARY**

The concentrations of sodium, potassium, chloride and carbon dioxide were measured in human parotid saliva at varying secretory rates and compared to the concentrations of these four substances in the plasma.

The concentration of sodium in the saliva increased with increasing secretory rates, but was always lower than the concentration of sodium in the plasma. Conversely, the concentration of potassium in the saliva was largely independent of the secretory rate and greater than the concentration of potassium in the plasma.

The concentration of chloride in the saliva, like that of sodium, increased with increasing secretory rates, but was always lower than the concentration of chloride in the plasma. The concentration of carbon dioxide in the saliva was independent of the secretory rate at salivary flows above 1.5 ml/min. and approximately twice the concentration of carbon dioxide in the plasma. At secretory rates below 1.5 ml/min. the concentration of carbon dioxide in saliva dropped to levels equal to or lower than the concentration in plasma. The sum of the concentrations of chloride and carbon dioxide, computed as bicarbonate, amounted to 90% of the sum of the concentrations of sodium and potassium at all secretory rates.

The differences in the rates of excretion of sodium and potassium by the parotid gland conform to the following hypothesis: both ions are delivered into a precursor solution in amounts which are simply proportional to the rate of salivary flow. Sodium, but not potassium, is in part reabsorbed from this precursor solution by a subsequent process of limited capacity.

**REFERENCES**