

## Excretion of $\text{Na}^+$ , $\text{K}^+$ , $\text{Cl}^-$ , and $\text{HCO}_3^-$ in the Saliva of the Rhesus Monkey<sup>1</sup> (34843)

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(Introduced by H. A. Waisman)

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The excretion of ions in the parotid and submaxillary saliva has been studied extensively in man, dog, rat, and cat, and to a lesser degree in other mammals (1). Since search of the literature revealed no information about the function of the same glands in subhuman primates, the present study of the salivary glands of the rhesus monkey was undertaken. The excretion of sodium, potassium, chloride, and bicarbonate was studied in the parotid and submaxillary saliva during stimulation of the glands with pilocarpine. The ability to measure secretory flow rates accurately, the similarities of the excretory patterns of ions in the monkey saliva to those observed in human saliva and the phylogenetic closeness of this animal to man make the rhesus monkey a suitable animal for the study of salivary gland function.

**Materials and Methods. Animals.** Five male rhesus monkeys (*Macaca mulatta*) from the inbred colony of the Wisconsin Regional Primate Research Center, weighing 3–8 kg were used in these experiments. The animals were fed Ralston Purina Monkey Chow and had unlimited access to water. Each animal was anesthetized by the intravenous injection of sodium thiamylal (19 mg/kg of body weight).

**Collections of saliva.** After anesthesia, the orifices of the right parotid and submaxillary glands were identified and the ducts were cannulated with tightly fitting polyethylene cannulae (PE 10, 20, 60, 100, Clay-Adams Inc., New York) with outside diameter from

0.61–1.52 mm and inside diameter from 0.28–0.86 mm. Since preliminary experiments showed that the ionic composition of saliva was a function of the flow rate, rather than the time from the onset of stimulation, the following method was used in this study. The glands were stimulated by the intravenous injections of pilocarpine nitrate (1.0–1.5 mg) and profuse salivation began in seconds; the saliva collected in the first 5 min was discarded to eliminate electrolyte transient gradients. Subsequently, saliva was collected in preweighed vessels under mineral oil; several samples were collected during each experiment until the flow rates returned to resting levels. The volume of saliva was determined gravimetrically. Three experiments were performed with each monkey during a 4-week period in the summer of 1968. At the end of the last experiment, the glands were removed surgically and weighed. The salivary flow rates were expressed as microliters of saliva per minute per gram of wet gland tissue ( $\mu\text{l}/\text{min} \cdot \text{g wt}$ ).

**Analytical methods.** Salivary sodium and potassium concentrations were measured as previously reported (2) by the use of the Instrumentation Laboratory Flame Photometer (Model 143). Salivary chloride concentrations were measured by electrometric titration of chloride ions in the Cotlove chloridometer. Bicarbonate concentrations were measured in a microgasometer by a modification of the Van Slyke technique (3).

**Results. Parotid gland** (Fig. 1). The sodium concentration in the parotid saliva varied with the flow rate. At low flow rates it was as low as 2–3 mEq/liter, but increased at flow rates from 5–30  $\mu\text{l}/\text{min} \cdot \text{g wt}$ , and reached a plateau at higher flow rates. The concentration of bicarbonate was similar to

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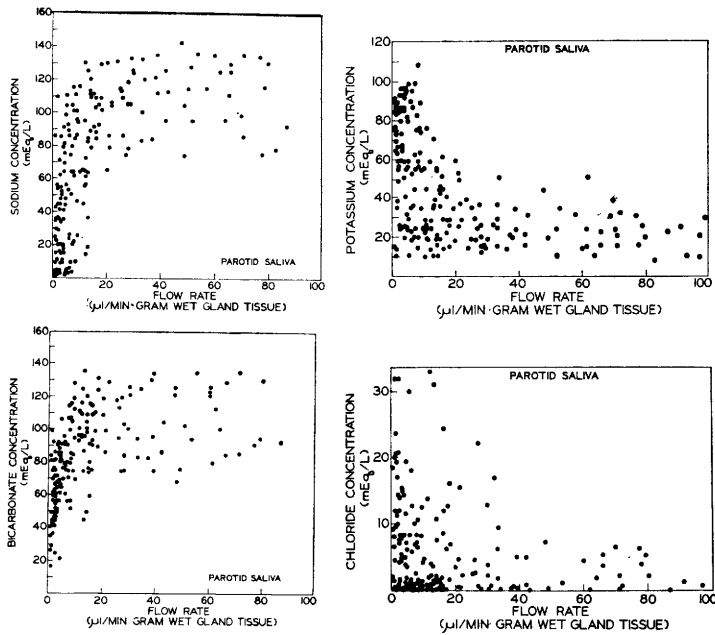


FIG. 1. The relation of flow rate to sodium, potassium, bicarbonate, and chloride concentrations in the parotid saliva of five rhesus monkeys.

that of sodium, with the lowest values (20 mEq/liter) observed at the lowest flow rates. The potassium concentration was high at low flow rates, and decreased gradually with

increasing flow rates to values as low as 10 mEq/liter at all flow rates. The highest values were observed at the lowest salivary flow rates. The concentrations of chloride were

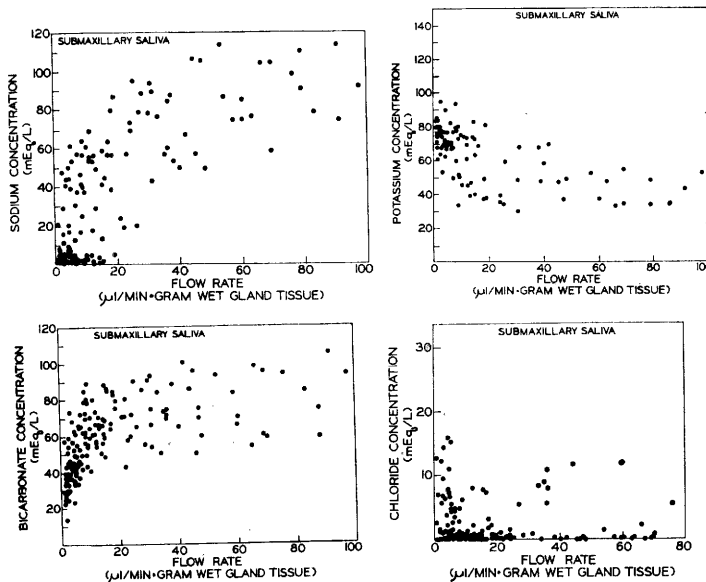


FIG. 2. The relation of flow rate to sodium, potassium, bicarbonate, and chloride concentrations in the submaxillary saliva of five rhesus monkeys.

below 34 mEq/liter at all flow rates with the highest concentrations observed at the low flow rates.

*Submaxillary gland* (Fig. 2). The sodium concentration in the submaxillary saliva varied with the flow rate as in the parotid saliva. A similar relationship was observed between bicarbonate concentration and flow rate. The potassium concentration was high at low flow rates and decreased with increasing flow rates. The chloride concentration was low (below 20 mEq/liter) at all flow rates. Table I shows the body weight and the weight of the parotid and submaxillary glands of the animals used in these experiments.

*Discussion.* This study presents data on the excretion of sodium, potassium, chloride and bicarbonate by the major salivary glands of the rhesus monkey. The significance of the present experiments lies in the similarities between the patterns of excretion of the above monovalent ions in the saliva of the monkey and the patterns observed in the same excretory product of man. The concentrations of these ions are related to the excretory flow rates: in general, sodium and bicarbonate concentrations are low at low flow rates and increase with progressively higher flow rates, while potassium and chloride concentrations are high at low flow rates and decrease with progressively higher flow rates. With the exception of chloride concentrations, the above patterns are identical to those observed in human saliva (4). In man, the most pronounced problem in the study of the salivary gland function arises from the inability to determine secretory gland mass.

Since there are no available substances that could be used for quantitative measurements of secretory activity and water fluxes across the ducts (as inulin is used for measurements of glomerular filtration rate in the kidney), the flow rates of saliva in all the reported studies in man were expressed as volumes of saliva per unit of time per gland. This introduces significant errors because the size of each gland may vary in one individual, and from individual to individual, as studies in other animals have shown. Furthermore, in disease states the salivary glands may become larger or smaller than in health (5, 6). In the presence of such changes in gland mass, and in view of the fact that electrolyte concentrations in the saliva are related to flow rate, which in turn is determined by the secretory gland mass, any comparison between the saliva of patients and the saliva of healthy men are meaningless unless the gland mass is known. The same is true in comparisons of experimental animals with salivary gland enlargement to control animals. Thus, the salivary glands of the rhesus monkey, with their functional similarities to the human salivary glands, could be used as valuable experimental models for the study of gland physiology and pathophysiology in an animal phylogenetically and functionally close to man.

The relation of salivary bicarbonate and chloride concentrations to flow rate may be of considerable interest. Saliva is assumed to be produced by the secretion of a primary fluid by the acini-intercalated ducts of the ducts of the parotid and submaxillary glands. Recently, by use of micropuncture and microanalytical techniques, it was demonstrated in the rat that this primary fluid is isotonic to plasma and contains sodium and potassium in concentrations identical to those of plasma (7-10). Salivary hypotonicity is produced by reabsorption of sodium in excess of water as the primary fluid flows along the duct system of the glands. The rate of sodium reabsorption is limited, and reaches its maximum at a certain flow rate. At higher flow rates, a gradual increase in the sodium concentration of the final saliva is observed. Since potassium is secreted by the ducts into

TABLE I. Age, Body Weight, and Parotid and Submaxillary Gland Weight in the Five Rhesus Monkeys Used in These Experiments.

Monkey no.	Age (years)	Body wt (kg)	Parotid wt (g)	Submaxillary wt (g)
1	6 $\frac{1}{2}$	7.70	7.90	2.40
2	3 $\frac{1}{2}$	4.25	4.50	1.82
3	3 $\frac{1}{2}$	3.15	3.37	1.73
4	6 $\frac{1}{2}$	7.32	8.10	2.66
5	4 $\frac{3}{4}$	4.45	4.53	1.97

the luminal fluid at low flow rates, which permit longer contact time of the fluid column with the transporting surface, high concentrations of potassium are observed. As the flow rate increases, the salivary potassium concentration decreases. These changes have been demonstrated by direct measurements of the composition of samples of duct fluid obtained through micropuncture (7-10). In the present experiments a relation of salivary bicarbonate and chloride concentrations to flow rate similar to that of sodium and potassium concentrations was observed. If the data of salivary sodium and potassium excretion by the rat are applicable to the monkey, the above similarity suggests that the predominant anion in the primary fluid may be bicarbonate which is subject to reabsorption as the fluid flows along the duct system. The electrophysiological studies of Lundberg (11) indicate that the basis of the secretion of primary fluid in salivary glands is an anionic pump mechanism located on the contraluminal side of the acinar cells. The present study suggests that the anion secreted in the salivary glands of the rhesus monkey could be primarily bicarbonate. This hypothesis could be confirmed only by direct ultramicroanalytical measurements of bicarbonate or chloride concentrations in fluid samples obtained from the intercalated ducts of salivary glands through micropuncture. The rhesus monkey appears to be an animal suitable for this type of physiological experiments.

*Summary.* The excretion of sodium, potassium, chloride and bicarbonate in the parotid and submaxillary saliva was studied in five young adult monkeys (*M. mulatta*). After stimulation of the glands with pilocarpine (1.0-1.5 mg) injected intravenously, saliva was collected at progressively decreasing flow rates until the flow of saliva stopped. The

glands were dissected and weighed, and flow rates were expressed as  $\mu\text{l}/\text{min}/\text{g}$  wet gland tissue. The concentrations of ions were related to flow rate. Sodium and bicarbonate concentrations were low at low flow rates and increased to a plateau with increasing flow rates. Potassium and chloride concentrations were high at low flow rates and gradually decreased with increasing flow rates. These patterns of excretion of ions were similar to those observed in man, suggesting that the rhesus monkey, an animal phylogenetically closely related to man, is suitable for experimental studies of salivary gland function.

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