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STIMULUS EFFECTS ON PROTEIN AND ELECTROLYTE CONCENTRATIONS IN PAROTID SALIVA

BY C. DAWES

From the Department of Oral Biology, Faculty of Dentistry, University of Manitoba, Winnipeg, Canada R3E 0W3

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SUMMARY

1. Twelve subjects collected ten 1 min samples and then a 2·5 ml sample of parotid saliva at a constant flow rate on five separate days with citric acid, salt, sugar, quinine sulphate, and sour lemon drops as gustatory stimuli.

2. The ten 1 min samples were analysed for protein and electrolyte content and the final 2·5 ml sample was used for electrophoretic separation of the different salivary proteins.

3. In most subjects, salt elicited the secretion of saliva with a much higher protein concentration than did the other stimuli, but none of the stimuli differentially influenced the relative proportions of the different proteins secreted.

4. There were several small but statistically significant effects of the nature of the stimulus on the concentrations of sodium, calcium and chloride, but not on potassium, magnesium or phosphate.

5. Since the nature of the gustatory stimulus can influence the composition of saliva, salivary composition could be influenced by the nature of the diet.

INTRODUCTION

If salivary composition were influenced by the nature of the diet, this would have important implications for studies on the relationship of diet to oral health.

There appear to have been no systematic studies of whether the composition of human saliva can be influenced by the nature of the gustatory stimulus when all other factors known to influence salivary composition are standardized.

Dawes & Jenkins (1964) reported that parotid saliva secreted in response to a predominantly salty gustatory stimulus contained a higher protein concentration than saliva secreted in response to a predominantly acid stimulus. Newbrun (1962), who studied parotid saliva, and Caldwell & Pigman (1966), who studied submandibular saliva secreted in response to various gustatory stimuli, also reported somewhat higher protein concentrations with salt as a stimulus. In an abstract, Ferguson (1981*a*) also reported that the secretion of protein in human parotid saliva was influenced by the nature of the stimulus. In none of these earlier studies was there adequate standardization of salivary flow rate, duration of stimulation, serial dependency or circadian rhythms, all of which can have marked effects on salivary composition (Dawes, 1981*a*), since their effects were only recently appreciated.

The objective of this study was to determine whether protein and electrolyte secretion in human saliva was specifically affected by any of the four common types of gustatory stimuli (sour, salty, sweet or bitter) when all other factors known to influence salivary composition were standardized as far as possible.

METHODS

Saliva collection

On five separate days, six male and six female young adult subjects collected ten successive 1 min samples of stimulated left parotid saliva at a constant flow rate (Dawes, 1967) followed immediately by collection of a 2·5 ml sample at the same flow rate. Briefly, after positioning a Lashley cannula over the parotid duct, saliva was collected into a graduated centrifuge tube positioned in front of a mirror. By observation the subject was able to calculate the flow rate with the aid of a stopwatch and regulate the degree of sucking on the stimulus to maintain a constant flow rate. The subjects were allowed to expectorate the stimulus at intervals during the parotid saliva collection if they so desired. The five gustatory stimuli, which were employed in random order for each subject for the five collections, were citric acid tablets (acid), sodium chloride tablets (salt), sugar cubes (sweet), quinine sulphate tablets (bitter) and sour lemon drops (SLD–the conventional stimulus). The acid-tasting tablets were compounded of 50 % citric acid in microcrystalline cellulose and the bitter-tasting tablets contained 20 mg of quinine sulphate in 800 mg microcrystalline cellulose. The flow rate chosen for each subject was constant and was the maximum (up to 1 ml/min) which could be maintained with each of the five stimuli. The flow rates for the twelve subjects were 0·40 ml/min, (3); 0·50, (2); 0·60, (2); 0·70, (1); 0·80, (1) and 1·00, (3) and practice sessions were held initially with each subject until they could easily maintain the constant flow rate with all the stimuli (number of subjects in parentheses). The collections were all carried out at about 13.30 h to minimize the effects of circadian variations in salivary composition (Dawes, 1981*a*) and 2 h after lunch to avoid serial dependency (Dawes & Chebib, 1972).

Analytical techniques

The ten 1 min samples of saliva were analysed for sodium, potassium, calcium and magnesium by atomic absorption spectroscopy (Dawes, 1967, 1969), chloride by a coulometric method (Cotlove, Trantham & Bowman, 1958), inorganic phosphate by the method of Chen, Toribara & Warner (1956) as modified for saliva (Dawes, 1969) and total protein by the method of Lowry, Rosebrough, Farr & Randall (1951). Anionic polyacrylamide slab gel electrophoresis of salivary proteins (about 350 μg) from the final 2·5 ml saliva sample was carried out as described previously (Dawes, 1981*b*) and the proteins were stained with a mixture of 0·25 % Coomassie Brilliant Blue 250 and 0·5 % Wool Fast Blue BL. For the last eight subjects, 5 or 10 μg of salivary protein samples from each 2·5 ml saliva sample were subjected to anionic slab gel electrophoresis on SDS–polyacrylamide as described by Merril, Dunau & Goldman (1981). The proteins were stained with a slight modification of the much more sensitive silver staining method of Merril *et al.* (1981) in which, after fixation, the gels were exposed for 30 min to dithiothreitol (5 μg/ml) as recommended by Morrissey (1981).

The analytical results for protein and electrolytes in the ten successive 1 min saliva samples were subjected to two factor factorial analysis of variance in randomized blocks. When a significant effect of stimulus type was revealed by analysis of variance, the means for the five stimuli (over all subjects and all time points) were compared using the Duncan new multiple range test (Steel & Torrie, 1960) which requires only the five means and the error mean square, derived from the analysis of variance.

RESULTS

The mean results for the twelve subjects for the effects of the five different gustatory stimuli on the salivary concentrations of protein, sodium, potassium, calcium, magnesium, chloride and phosphate are shown in Figs. 1–7, respectively. Variations in composition between subjects are always large in salivary studies and

the pooled between-subject standard deviations for the above seven components were 125 mg/100 ml, 13·7 mM, 3·6 mM, 0·19 mM, 7·2 μM, 7·2 mM and 0·88 mM, respectively. However, since each stimulus was used by all subjects, the analysis of variance distinguishes this source of variation from that due to the stimulus type and to the effect of time.

TABLE 1. Duncan new multiple range test comparisons of the five stimuli

Comparison	Protein	Sodium	Calcium	Chloride
Acid:salt	**	**	**	—
Acid:sweet	—	**	**	**
Acid:bitter	—	**	**	**
Acid:SLD	—	**	—	—
Salt:sweet	**	**	**	**
Salt:bitter	**	*	**	—
Salt:SLD	**	—	**	—
Sweet:bitter	—	—	—	—
Sweet:SLD	—	**	**	*
Bitter:SLD	—	*	*	—

*, $P < 0.05$; **, $P < 0.01$.

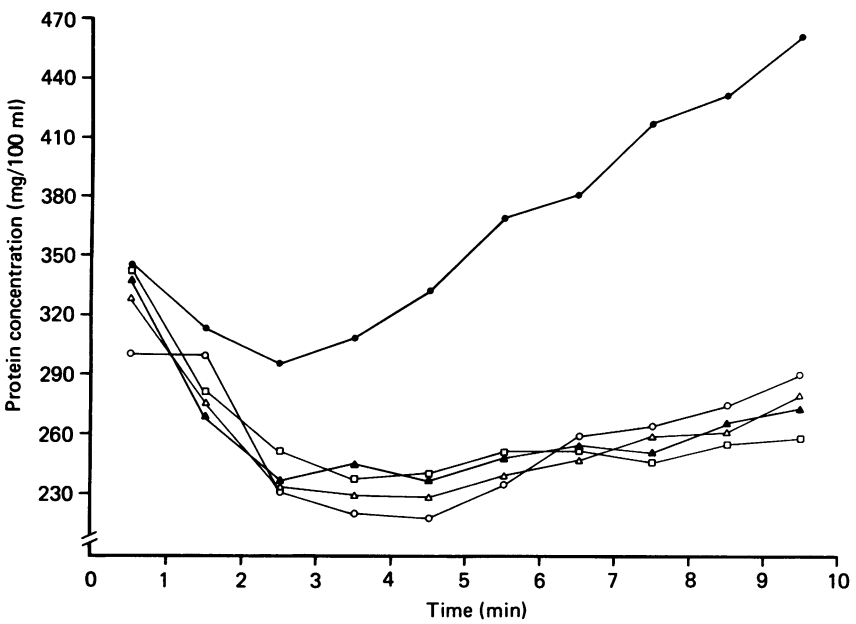

Fig. 1. Effect of the nature of the stimulus on the secretion of protein in parotid saliva. ○, citric acid; ●, salt; △, sugar; ▲, quinine; □, SLD.

Analysis of variance revealed significant effects of the nature of the stimulus only on the concentrations of protein, sodium, calcium and chloride and the results of the Duncan new multiple range test comparisons for the five different stimuli are given in Table 1. The analytical values for the samples collected during the first two minutes of stimulation were the most variable, because of inclusion of unstimulated saliva

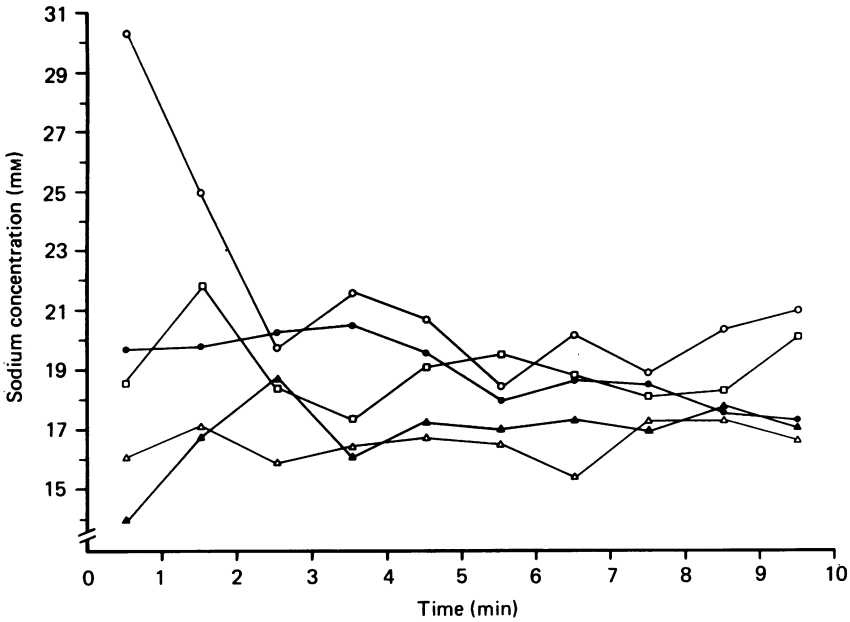

Fig. 2. Effect of the nature of the stimulus on the secretion of sodium in parotid saliva. Symbols as Fig. 1.

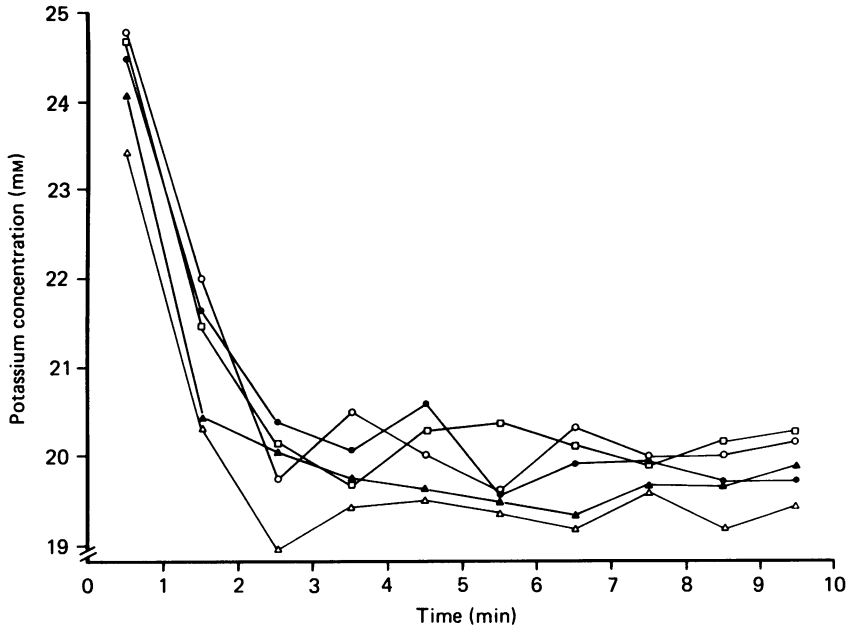

Fig. 3. Effect of the nature of the stimulus on the secretion of potassium in parotid saliva. Symbols as Fig. 1.

previously present in the duct system. However, even when these results were not included in the statistical analyses, the same levels of significance were obtained.

The most striking effect of the nature of the stimulus was on protein secretion (Fig. 1) where salt was a much more effective stimulus than any of the other four stimuli. In seven of the twelve subjects, salt was the most effective of the five stimuli,

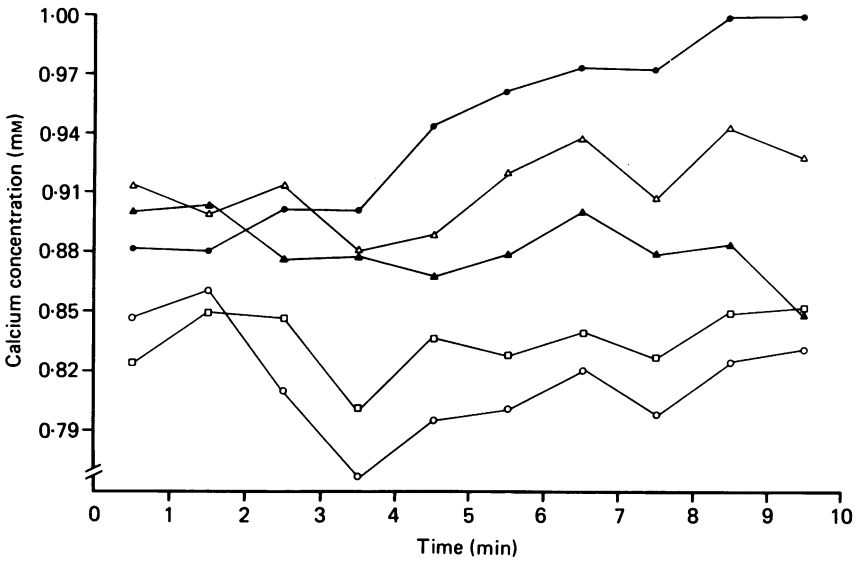

Fig. 4. Effect of the nature of the stimulus on the secretion of calcium in parotid saliva. Symbols as Fig. 1.

whereas in four of the subjects it was the second most effective. In these latter four subjects the most effective stimulus for protein secretion was citric acid (1), bitter (1) and sugar (2). When the protein results for the five subjects for whom salt was not the most effective stimulus for protein secretion were subjected to the same type of analysis of variance, the citric acid and SLD were significantly less effective than the other three stimuli.

Although Table 1 reveals many statistically significant differences between the effects of the different stimuli on sodium and chloride concentrations, Figs. 2 and 6 show that the actual differences were numerically very small.

The effects of the different stimuli on calcium secretion (Fig. 4 and Table 1) are proportionally larger than for sodium and chloride. As with protein, salt was the most effective stimulus for calcium secretion. However, the other four stimuli also had significantly different effects on calcium secretion (Table 1) even though they had no significantly different effects on protein secretion (Table 1). There were no significant effects of the nature of the stimulus on the concentrations of potassium (Fig. 3), magnesium (Fig. 5) or phosphate (Fig. 7).

Plate 1 illustrates the effects of the five different stimuli on the types of proteins present in the final 2·5 ml of parotid saliva from four of the subjects in which silver staining of the electrophoretically separated proteins was employed. Even though

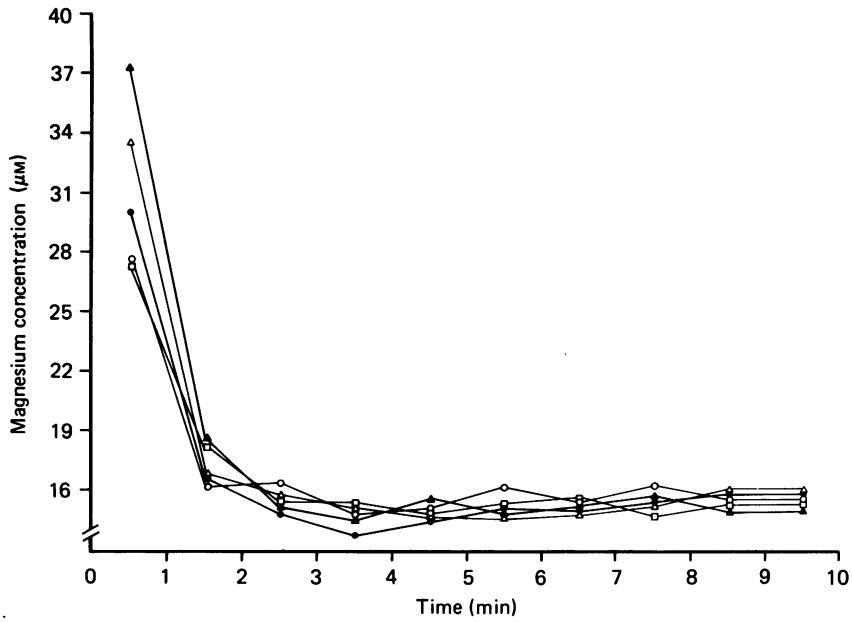

Fig. 5. Effect of the nature of the stimulus on the secretion of magnesium in parotid saliva. Symbols as Fig. 1.

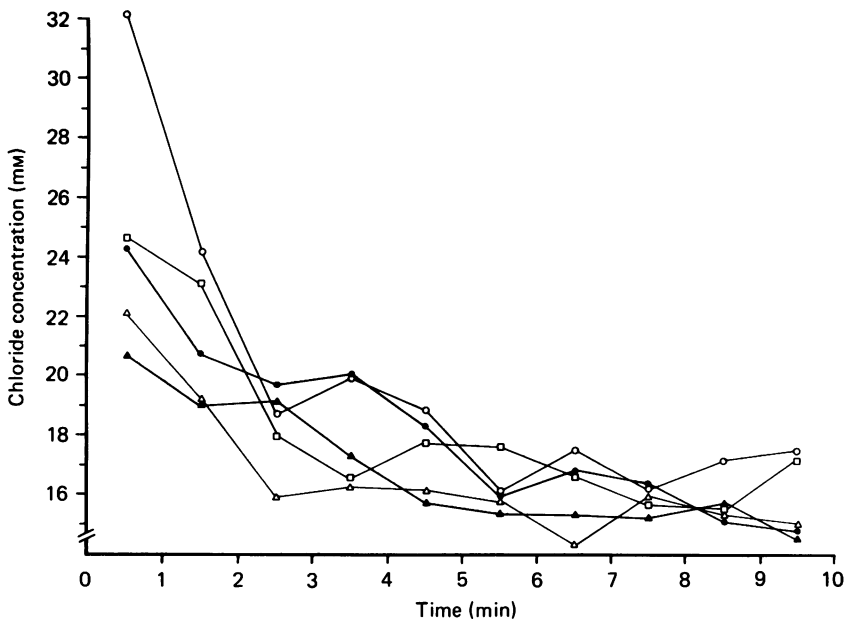

Fig. 6. Effect of the nature of the stimulus on the secretion of chloride in parotid saliva. Symbols as Fig. 1.

there are obvious differences between subjects in the relative proportions of the different proteins, in none of the twelve subjects could a differential effect of the nature of the stimulus be detected.

On two occasions with one of the subjects, two of the stimuli (bitter and SLD) elicited secretion of saliva with extremely low protein concentrations (about

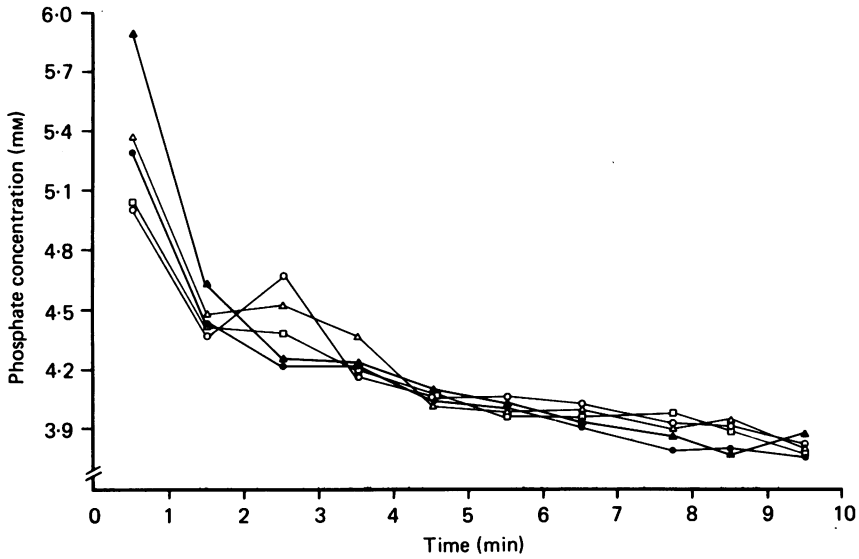

Fig. 7. Effect of the nature of the stimulus on the secretion of phosphate in parotid saliva. Symbols as Fig. 1.

35 mg/100 ml) but with no anomalous results for the electrolytes. However, electrophoretic analysis of these saliva samples revealed no changes in the relative proportions of the different salivary proteins in comparison with samples having normal protein concentrations from the same subject. On repetition in this subject with the bitter and SLD stimuli, entirely normal protein concentrations were obtained.

DISCUSSION

Previous studies on human subjects have shown that pharmacological stimuli such as pilocarpine elicit the secretion of saliva with a composition different from when gustatory stimuli are used (Dawes, 1966; Mandel, Katz, Zengo, Kutscher, Greenberg, Katz, Scharf & Pintoff, 1968). In addition sympathomimetic and parasympathomimetic agents have been shown to have characteristically different effects on human salivary composition (Mandel *et al.* 1968; Mandel, Zengo, Katz & Wotman, 1975). In general, β-adrenergic agonists are the most potent stimuli for protein secretion.

There appear to have been no previous studies in which different gustatory stimuli were compared under conditions when other known variables, and particularly flow rate, were maintained constant. The results from the present study show that the

composition of human parotid saliva can be influenced by the nature of the gustatory stimulus.

The effects on protein and electrolyte secretion (Figs. 1–7) of duration of stimulation seen over the 10 min of stimulation (Figs. 1–7) are quite consistent with those reported previously for parotid saliva (Dawes, 1967, 1969).

The finding that salt is the most potent for protein secretion of the gustatory stimuli tested, agrees with previous studies in which other variables were not as well controlled (Newbrun, 1962; Dawes & Jenkins, 1964; Caldwell & Pigman, 1966). The physiological advantage of the higher protein concentration in response to salt stimulation is not immediately apparent. Presumably a salt stimulus in some way increases the ratio of sympathetic to parasympathetic stimulation to the gland, thereby causing the higher rate of protein secretion (Speirs, Herring, Cooper, Hardy & Hind, 1974).

The finding that calcium concentrations were also significantly higher with salt as a stimulus agrees with results from previous studies where a correlation was shown between protein and calcium secretion (Windeler & Shannon, 1966). It was surprising that significant differences in calcium concentrations occurred in response to the other four gustatory stimuli in the absence of differences in protein secretion and there is no obvious explanation of the mechanism for these differences. In an abstract, Ferguson (1981*b*) also reported differences in the calcium concentration in submandibular saliva, depending on the nature of the stimulus.

The differences in sodium and chloride secretion due to the nature of the stimulus, although statistically significant, are quite small. A possible explanation for them is that they are due to differential sympathetic effects on the duct system, as Denniss, Schneyer, Sucanthapree & Young (1978) have shown that sympathomimetic agents can influence electrolyte transport by isolated salivary ducts. The reason for the higher sodium concentration at the beginning of stimulation with citric acid is uncertain but may have been due to an initial more rapid response to the acid by the glands.

There is no obvious explanation for the very low protein concentration in two of the saliva collections from one of the subjects. Although stop-flow is known to decrease the protein concentration in parotid saliva (Dawes, Dowse & Knull, 1980) there was no obvious blockage of the cannula during these collections and no evidence that electrolyte secretion was affected, as occurs during stop-flow.

In conclusion, the composition of human saliva does appear to be dependent on the nature of the gustatory stimulus employed and thus salivary composition may be immediately dependent on the type of diet consumed. Further studies are needed to determine the mechanisms involved for these effects of the nature of the stimulus and whether they are of physiological importance.

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EXPLANATION OF PLATE

Anionic SDS–polyacrylamide slab gel electrophoresis of parotid saliva proteins. There are five slots for each of four subjects and the five successive samples for each subject were obtained after stimulation with acid, salt, sweet, bitter and sour lemon drop, respectively. The anode is at the bottom.