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THE EFFECTS OF DIFFERENT STIMULI ON THE COMPOSITION OF SALIVA IN MAN

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Pavlov (1910), in a much quoted article, stated that the composition of saliva in dogs is adapted to the nature of the stimulus. Sand stimulated a flow of watery saliva, whereas raw meat resulted in the secretion of a highly viscous saliva. Pickerill (1912) also interpreted teleologically his finding in the human subject that the alkalinity of saliva is high with acid stimuli. However, there appears to have been little systematic study in man of the responses of the different salivary glands to a variety of stimuli.

Since parotid and submandibular saliva differ markedly in their composition, changes in the composition of mixed saliva could be due to variations in the proportions of saliva secreted by the different glands. Pickerill (1912) and Schneyer & Levin (1955*b*) found no evidence of this although Gore (1938) described one experiment in which chewing wax increased the flow of parotid saliva more than that of the submandibular gland. We therefore first report in this paper our results in one subject where simultaneous collection of parotid and submandibular saliva was successfully achieved.

Most of our observations were on the changes in composition of saliva from either the parotid or submandibular glands when the flow was increased by different stimuli in the mouth. Increase in salivary flow from a single gland is always accompanied by a change in composition of the saliva (Heidenhain, 1883; Langstroth, McRae & Stavraky, 1938; Hildes, 1955; Ferguson, Krahn & Hildes, 1958; Chauncey & Degler, 1958; Shannon, 1958).

The object of most of the experiments reported here was to ascertain whether different stimuli (acid, salt, sand, chewing wax, etc.) caused the saliva to have a composition different from that which would be explained solely by the changed rate of flow.

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METHODS

Collection of duct saliva

Parotid saliva was collected by means of a modified Lashley cannula (Lashley, 1916) constructed of acrylic resin of external diameter 1·9 cm and width 0·4 cm (Fig. 1*A*). The diameter of the inner chamber was 0·8 cm and this was placed over the parotid papilla and held in place by suction applied to the outer ring. Suction was not applied directly to the duct orifice. Submandibular saliva was collected from both ducts at once by means of a similar device but with the two polythene tubes inserted vertically instead of horizontally (Fig. 1*B*). If, during the course of an experiment, leakage of saliva down the suction tube was detected, that experiment was abandoned. In practice the submandibular cannula worked on only three of the six subjects tested, probably because of the great anatomical variations between subjects and also because they could not all devote the time necessary to acquire the technique. The saliva was collected in graduated tubes for periods of up to 7 min depending on the rate of flow, and in all experiments the first 0·5–1·0 ml. produced

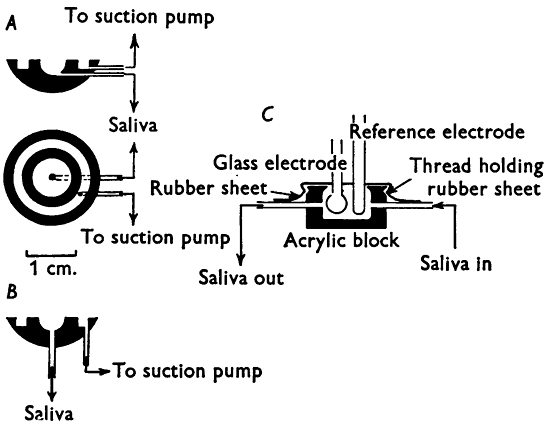

Fig. 1. Diagram of the cannula used for collecting saliva: *A* from the parotid gland; *B* from the submandibular gland. *C*, apparatus for measuring the pH of saliva collected directly from the gland without exposure to air.

was discarded, as this fraction has probably been stagnant for some time in the ducts. A wide variety of substances was used to stimulate salivary flow and they included paraffin wax (M.P. 45° C), starch, wholemeal bread and sand as mainly mechanical stimuli and fruit drops, portions of oxo cubes, salt, sodium bicarbonate crystals, citric acid crystals and 1 % acetic acid as sapid stimuli. Mechanical stimuli were always chewed on both sides of the mouth at once to avoid unequal stimulation of the two sides (Kerr, 1961) and sapid stimuli were distributed throughout the mouth as far as possible. When comparing different stimuli it is very difficult to produce successively exactly equal rates of flow. A range of rates of flow was therefore produced with each stimulus by applying it at different intensities so that the relation between composition and rates of flow could be plotted and compared for different stimuli. The order of application of different stimuli was varied randomly and an interval of a few minutes was allowed between the application of each.

Estimations

Sodium and potassium. Saliva was diluted 1:100 and tested against 0·1 M mixed sodium and potassium standard in an EEL flame photometer.

Inorganic phosphate. Analysis was carried out on 0·1 ml. samples by the method of Kuttner & Cohen (1927).

Calcium was estimated on 0·1 ml. samples by a micro modification (Dawes & Jenkins, 1962) of the method of Kramer & Tisdall (1921).

pH. As soon as saliva comes into contact with air carbon dioxide is lost and the pH rises. To avoid contact with air a device was constructed as shown in Fig. 1*C*. It consists of a block of acrylic resin hollowed out to accommodate the bulb of a glass electrode and the end of a reference electrode. Saliva passes directly from the cannulated duct of the gland being studied through a tube to the electrode chamber and then flows into a collecting vessel, where its volume and hence its rate of flow can be measured. The acrylic block is made watertight by covering the open end with a sheet of dental rubber dam tied in position in the groove round the block. Two holes in the sheet allow insertion of the electrodes and a tight seal around them. The dead space was 0·15 ml. and residual air was removed by elevating the outflow end. With buffer solutions passing through the apparatus at different rates of flow, the pH recorded by a 'Record' automatic graphical recording device, was independent of the rate of flow.

Amylase. This was determined by a modification of Nørby's method described by Largerlöf (1942) and the results expressed as Nørby units. It was found necessary to dilute parotid saliva 1:4000 and submandibular saliva 1:400 before estimation.

Protein nitrogen. The protein was digested by the method of Shaw & Beadle (1949), the resulting ammonia being estimated by the standard Conway technique. The S.D. of ten estimations of 70 μg of ammonia N was = 1·4 μg. Total N was estimated on 0·2 ml. samples of saliva and non-protein N was estimated in the supernatant after the protein of 0·2 ml. of saliva had been precipitated with 1 ml. of acetone containing 3 % glacial acetic acid. This mixture gave more complete protein precipitation than did trichloroacetic acid. Protein N was estimated as the difference between total N and non-protein N.

Sialic acid was estimated by the method of Warren (1959).

RESULTS

The proportional contributions of parotid and submandibular saliva at different rates of flow and with different stimuli

Only one subject (C.D.) was found who could, by virtue of the fact that his lower incisor teeth occluded anterior to his upper incisors, retain in position two parotid cannulae and the one submandibular cannula at the same time in the presence of mechanical stimuli. The results obtained in three experimental sessions are shown in Figs. 2 and 3 and have been analysed by linear regression analysis as detailed by Quenouille (1950). As the curve obtained by plotting the results when oxo was used as a stimulus appeared to deviate from linearity when the parotid flow was greater than about 2·9 ml./min, results above this value were not used to calculate the regression line.

If $y =$ the volume of submandibular saliva (ml./min) and $x =$ the

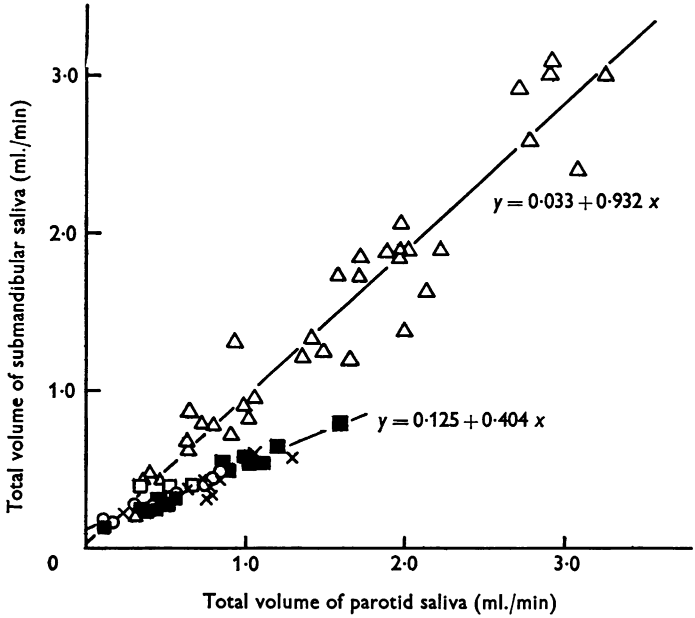

Fig. 2. Subject C. D. The volumes of saliva secreted by the submandibular and parotid glands in response to the mechanical stimuli of paraffin wax ○, sand ■, wholemeal bread × (lower curve), and the sapid stimuli of fruit drops △ (upper curve).

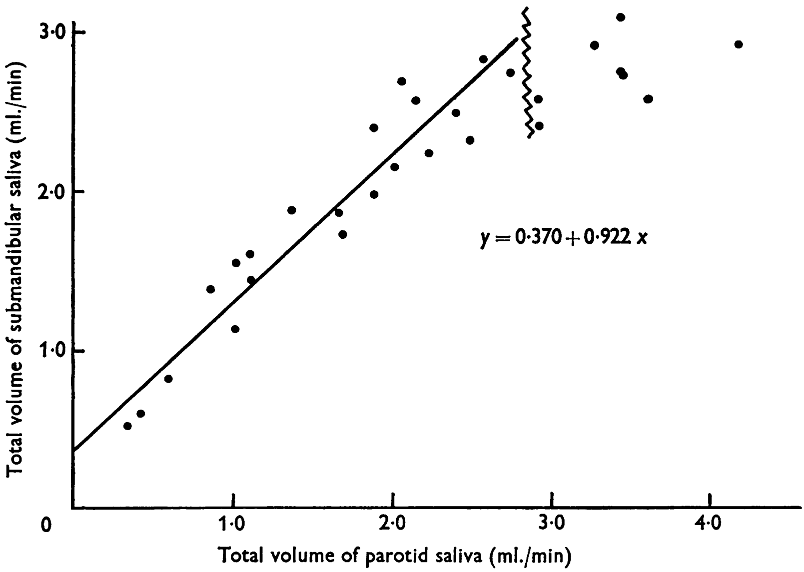

Fig. 3. Subject C.D. The volume of saliva secreted by the submandibular and parotid glands in response to stimulation by oxo.

volume of parotid saliva (ml./min), the equations of the three regression lines become:

Mechanical stimuli	$y = 0{\cdot}125 \pm 0{\cdot}404x$;
Fruit-drop stimuli	$y = 0{\cdot}033 + 0{\cdot}932x$;
Oxo stimuli	$y = 0{\cdot}370 + 0{\cdot}922x$.

The slope of the regression line obtained with mechanical stimuli was significantly different ($P < 0{\cdot}001$) from those of the other two lines. The slopes of the lines obtained for fruit-drop and oxo stimuli were not significantly different from each other, but the distance between the lines was significant at the $P < 0{\cdot}001$ level.

At higher rates of flow mechanical stimuli caused the production of about twice as much parotid saliva as submandibular, whereas sapid substances stimulated the production of about equal amounts of the two types of saliva. Unfortunately no other subject was found who could retain the three cannulae in the presence of mechanical stimuli. One subject, however, was occasionally able to retain all three cannulae during stimulation with fruit drops and the small number of results obtained followed a similar curve to that produced with fruit drop stimulation in C.D.

The effect of the rate of flow and the nature of the stimulus on some inorganic constituents of saliva

pH. A result typical of those obtained in three subjects is shown in Fig. 4; it is of interest that the pH may vary over a range of more than two units. It is seen that the pH is dependent solely on the rate of flow and not on the nature of the stimulus. The lowest pH recorded was 5·55 for parotid saliva and 5·90 for submandibular saliva with rates of flow of 0·064 ml./min and 0·10 ml./min respectively, which confirms the results of Schmidt-Nielsen (1946). The highest pH recorded was 7·90 for parotid saliva and 7·70 for submandibular saliva, with rates of flow of 1·88 ml./min and 2·5 ml./min respectively.

Inorganic phosphate. A result typical of those obtained in three subjects for submandibular saliva and six for parotid saliva is shown in Fig. 5, in which the line separates figures for the two types of saliva. As the parotid saliva was collected from one gland, and the submandibular from both, the rate of flow for parotid saliva was multiplied by two to make it correspond with the submandibular. In Fig. 5 the results from one subject on three different days were combined. Unlike the result with most other ions, the phosphate concentration fell quite markedly as the rate of flow was increased to slightly above resting levels, but at high rates of flow the concentration tended to a limiting value. In all experiments the phosphate concentration depended upon the rate of flow and not on the nature of the stimulus, and at a given rate of flow the concentration was higher in parotid than in submandibular saliva.

Calcium. A result typical of those found in two subjects for submandibular and in three subjects for parotid saliva is shown in Fig. 6 and, contrary to the results for inorganic phosphate, submandibular saliva

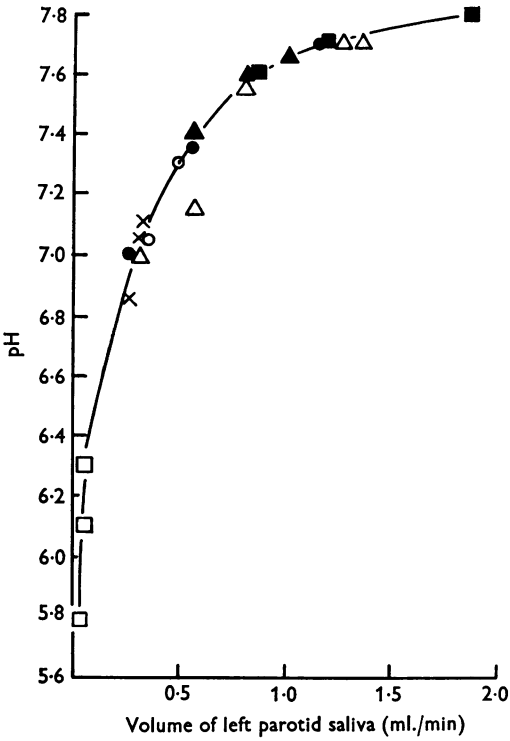

Fig. 4. The effect of variations in rate of flow and the nature of the stimulus on the pH of parotid saliva. Observations with C.D. in one session. Resting flow □: stimuli; wax ×, fruit drop △, oxo ●, sand ○, sodium bicarbonate ▲, citric acid ■.

contained a higher concentration of calcium than did parotid saliva, confirming the results of Schmidt-Nielsen (1946). A rise in the concentration of calcium with rate of flow was observed in submandibular saliva but it did not seem to depend on whether the stimulus was oxo or a fruit drop.

Sodium and potassium. A result typical of those in two subjects for submandibular saliva and in six subjects for parotid saliva is shown in Fig. 7. The potassium concentration was found to be independent of the rate of flow or the nature of the stimulus, whereas the sodium concentration was

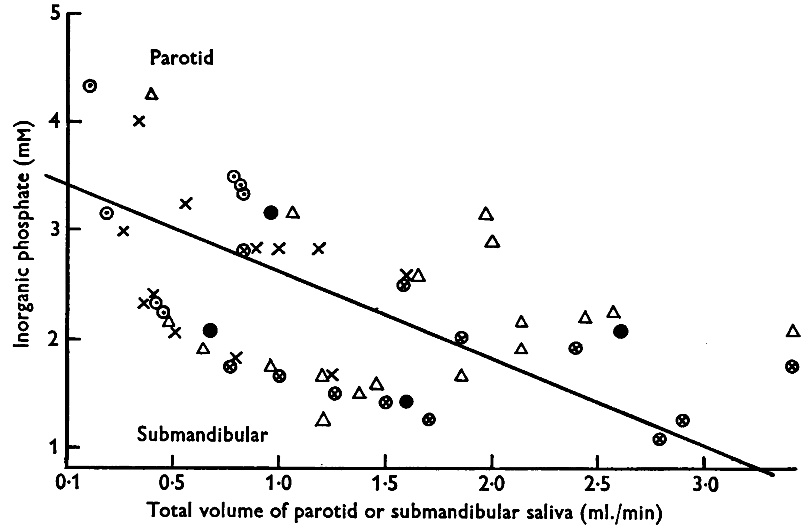

Fig. 5. The inorganic phosphate concentration in parotid and submandibular saliva. Subject C.D., 3 sessions. The oblique line separates the points referring to the parotid secretion from those referring to the submandibular secretion. Stimuli; wax ⊙, oxo ●, citric acid ⊗, sand ×, fruit drop △.

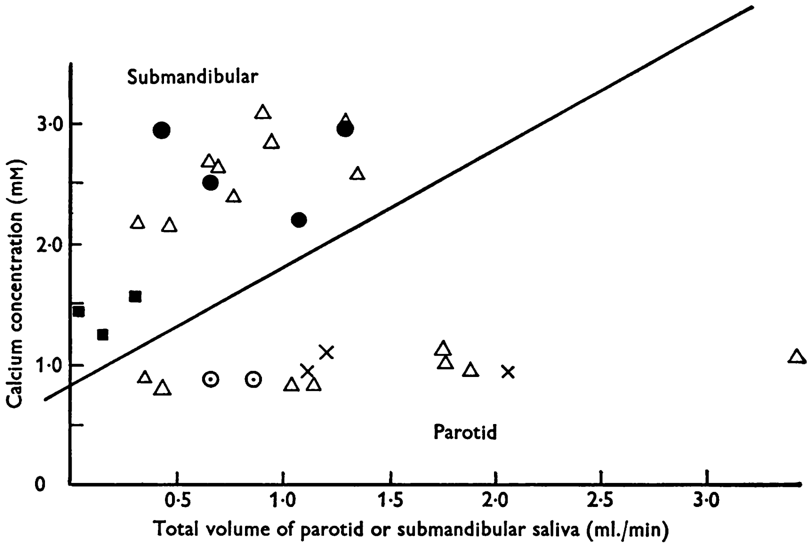

Fig. 6. Subject G.N.J. The calcium concentration in parotid and submandibular saliva. Points referring to the secretion from each gland are separated by the line. Stimuli; fruit drop △, wax ⊙, citric acid ×, oxo ●, submandibular resting flow ■.

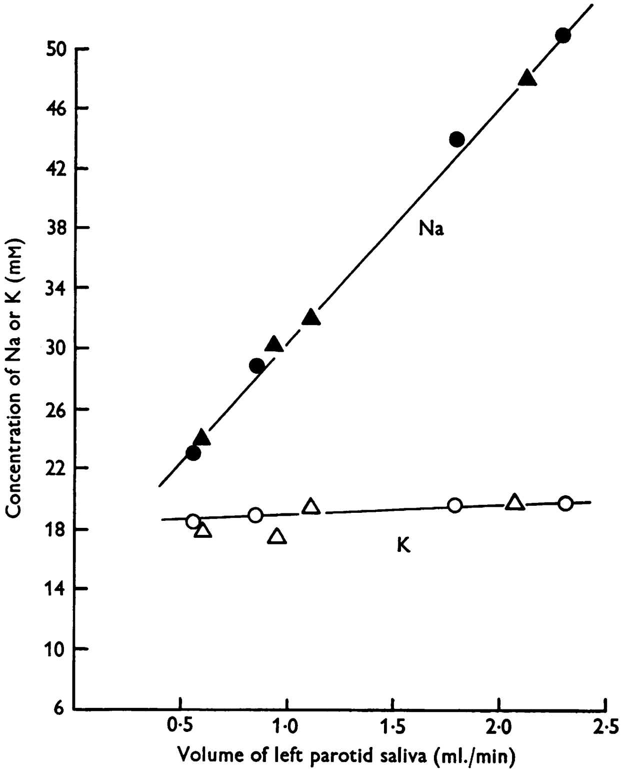

Fig. 7. The sodium (upper curve) and potassium (lower curve) concentrations in parotid saliva. Subject M.D. one sitting. Stimuli; fruit drop △ or ▲, oxo ○ or ●; ▲, ● Na; △, ○ K.

directly proportional to the rate of flow but quite independent of the nature of the stimulus.

The effect of the rate of flow and the nature of the stimulus on some organic constituents of saliva

Amylase. The amylase concentration in submandibular saliva secreted at different rates of flow and with a variety of stimuli was studied in three individuals, and results typical of all the experiments are shown in Fig. 8. It appears that the amylase concentration in submandibular saliva is independent of both the rate of flow and the nature of the stimulus.

The amylase concentration in parotid saliva was studied in six subjects; for two of them it was not found possible to produce rates of flow greater than 5 ml./min. In these two subjects, and also in the other four subjects when the rate of flow was less than about 1 ml./min, no consistent relation could be observed between the rate of flow and the amylase concentration and the different stimuli had no characteristic effect. However, with rates of flow greater than 1 ml./min the nature of the stimulus did affect the amylase concentration and three typical results are shown in Fig. 9. At higher rates of flow oxo tended to stimulate the production of saliva with a higher amylase concentration than that produced with fruit drops as the stimulus. In 11 experiments on one subject (C.D.) with oxo or salt (which is presumably the active constituent of oxo) as stimuli, there was

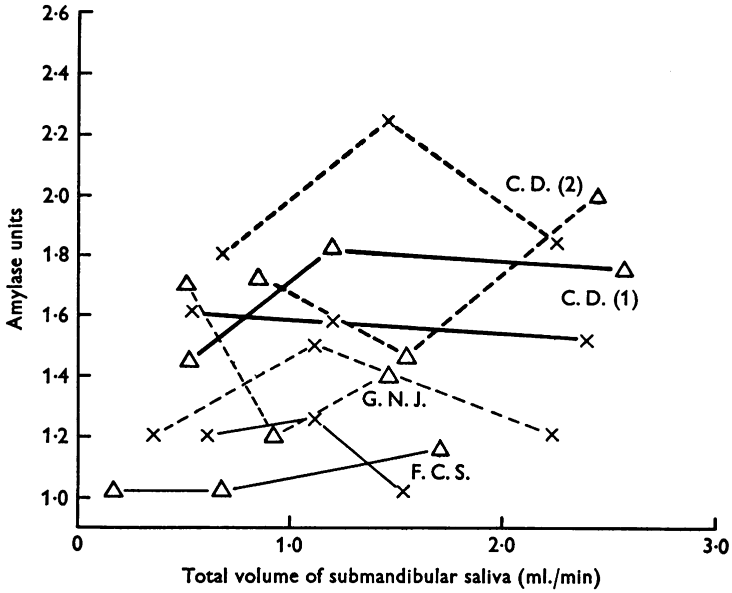

Fig. 8. The amylase concentration in submandibular saliva in three subjects. Stimuli; oxo ×, fruit drop △.

always a rise in amylase concentration at higher rates of flow (usually above 1 ml./min), in contrast to fruit drops, citric and acetic acids, starch and various mechanical stimuli, which usually caused no change, or occasionally a slight rise, in amylase concentration with rate of flow. Figure 10 shows the results of one of these experiments in which the protein N as well as the amylase concentration was estimated when oxo and 1 % acetic acid were used as stimuli. The rise in amylase concentration at high rates of flow when oxo was the stimulus was closely paralleled by a rise in protein N.

As the effect of salt-containing stimuli at high rates of flow might be due to some extra action on the parotid gland by perhaps the sympathetic or parasympathetic nerves, it was thought of interest to repeat the experiments after the subjects had been given 0·65 mg of atropine by mouth 2 hr before saliva collection, since this might be expected to affect any parasympathetic components of the mechanism. The results of three such experiments are shown in Fig. 11 and the maximum rate of flow obtained under these conditions was 1·37 ml./min. It is now seen that the

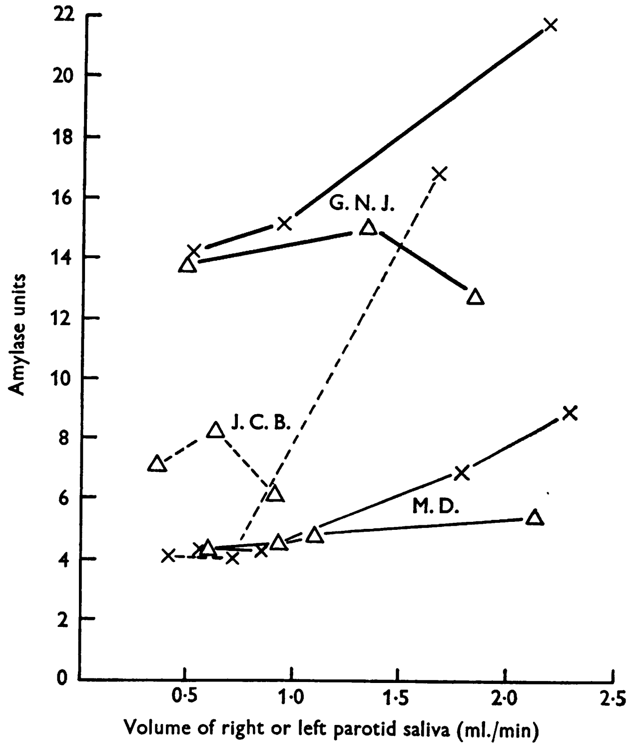

Fig. 9. The amylase concentrations in parotid saliva in three subjects. Stimuli; oxo ×, fruit drop △.

characteristic difference between the effects of oxo and fruit-drop stimuli occurs at rates of flow much lower than the limiting value of 1 ml./min previously found.

Protein N of submandibular saliva. As amylase accounts for only a small proportion of submandibular salivary protein, estimations of protein N were carried out on three subjects at different rates of flow and with a variety of stimuli. The results averaged 23·2 mg/100 ml., ranging from 8·0 to 45 mg/100 ml. However, no consistent relation between the protein N concentration and either the rate of flow or the nature of the stimulus could be detected.

Sialic acid of submandibular saliva. Sialic acid is one of the most specific components of sialomucins, of which salivary mucoid is an example, and it was considered that sialic acid estimations might reveal changes in the

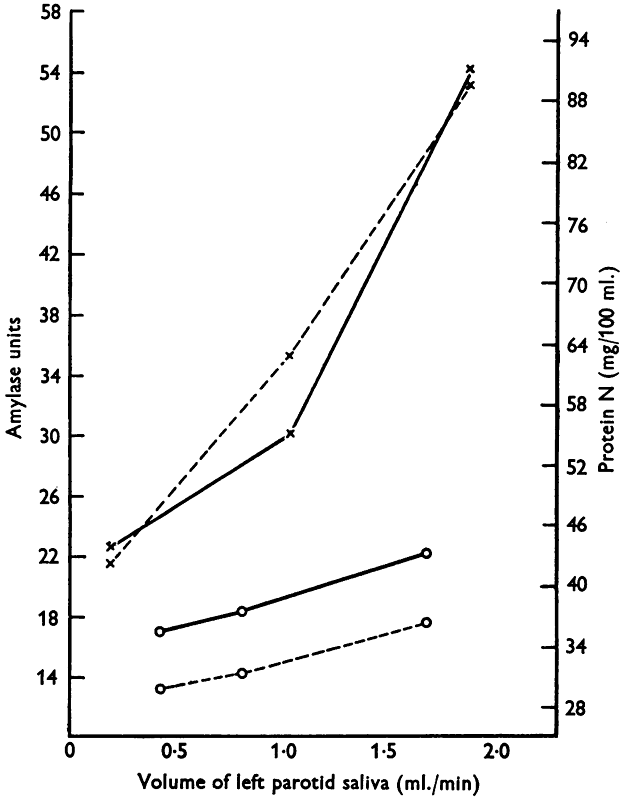

Fig. 10. The amylase (——) and protein N (---) concentrations in parotid saliva, in one experiment on C.D. Stimuli; oxo ×, 1 % acetic acid ○.

mucoid concentration in saliva under different conditions. The results on six subjects averaged 3·1 mg/100 ml., ranging from 1·6 to 5·4 mg/100 ml. However, no consistent relation could be detected between the sialic acid concentration in submandibular saliva and either the rate of flow or the nature of the stimulus.

DISCUSSION

The results on the proportional contributions of parotid and submandibular saliva at different rates of flow and with a variety of stimuli confirm the work of Schneyer & Levin (1955*a*), who showed that at low rates of flow the submandibular saliva makes a greater contribution to the total

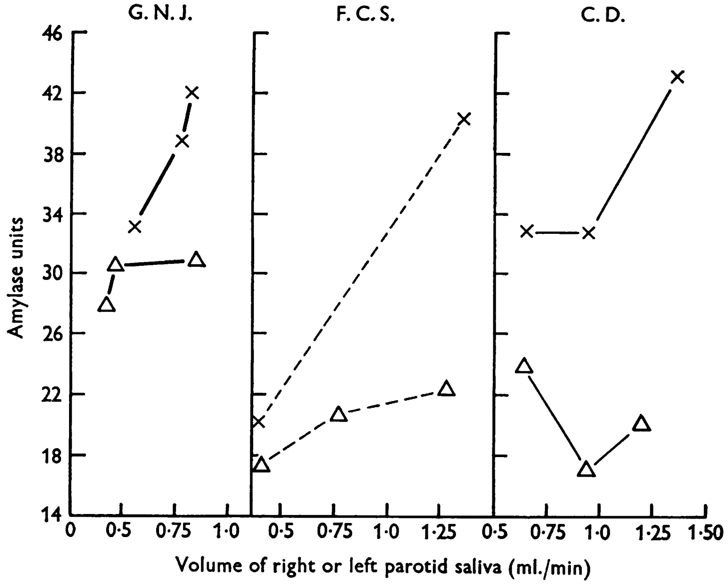

Fig. 11. Effect of stimulation by oxo (×) and fruit drop (△) on the amylase concentration of parotid saliva in three subjects after the administration of atropine 0·65 mg by mouth.

flow than does parotid saliva. Although the linear regression line for oxo cuts the y axis at 0·37 ml./min (Fig. 3), this is probably not very accurate, as at low rates of flow the points all fall below the line but still show a higher proportion of submandibular saliva. The results do show that different stimuli are able to vary the proportional contributions of saliva from the two main types of salivary glands. It is of interest that the three types of mechanical stimuli all gave results fitting on the same straight line, which suggests that they all stimulated the same type of afferent nerves, possibly those in muscle, but, as Kerr (1961) suggests, more probably periodontal membrane proprioceptors. The fact that salt and fruit drops produced responses significantly different from each other as well as from mechanical stimuli suggests that the afferent nerves from the different types of taste receptors are not distributed equally to the different salivary centres in the brain.

In no experiments on the inorganic constituents of saliva was there evidence that the nature of the stimulus affected the composition other than by the effect on rate of flow. Pickerill (1912) was mistaken in his belief that acid stimulates specifically an alkaline saliva, and it is seen from Fig. 4 that the high alkalinity of acid-stimulated saliva arises simply because acid stimulates a very high rate of flow. This non-specific effect of acid is particularly well illustrated in Fig. 4 by the points which show that if bicarbonate and acid are used at such concentrations as to stimulate the same rate of flow the resulting salivas have the same pH. The advantages of a high rate of salivary flow are that food debris around the teeth will be washed away more readily and that the alkaline saliva will more effectively buffer the acid, formed by the dental plaque, which is believed to be responsible for the initiation of dental caries.

The higher level of calcium in submandibular saliva than in parotid saliva may help to explain the fact that dental plaque around the lower incisor teeth has been found to contain a higher concentration of calcium than does plaque from other regions (Dawes & Jenkins, 1962). Calculus also forms most readily around the lower incisor teeth and is the main causal factor in periodontal disease, to which these teeth are particularly prone. The high level of calcium in submandibular saliva may also be responsible for the fact that the lower incisor teeth are the least susceptible to caries.

The results for amylase activity in parotid saliva differ from the results for all other salivary constituents tested in that above a rate of flow of about 1 ml./min the amylase concentration becomes dependent upon the nature of the stimulus as well as the rate of flow. There is no obvious physiological advantage to be gained by this effect of the salt-containing stimuli and the mechanism remains obscure. The experiments carried out after atropine administration, the effectiveness of which in blocking the parasympathetic was shown by the much lower maximum rates of flow, still produced the effect but at rates of flow much less than the previous critical figure of 1 ml./min. This suggests that parasympathetic nerves are probably not involved, as in the normal subjects without atropine the differential effect of salt-containing and other types of stimuli only occurred at high rates of flow which from animal experiments, might be expected to involve a high level of parasympathetic activity. As sympathetic stimulation in certain species such as the dog and cat causes the secretion of saliva with a high protein content (Burgen & Emmelin, 1961) it is possible that salt-containing stimuli at high levels of intensity may, in some way, increase the sympathetic activity to the parotid glands.

Previous investigators (Schneyer, 1956; Bates, 1958; Newbrun, 1962) have reported contradictory results on the effect of rate of flow and type of stimulus on the amylase concentration of parotid saliva, but the rates of flow in their experiments were below 1 ml./min. In the present work it was only when salt-containing stimuli were used and at rates of flow greater than 1 ml./min that a rise in amylase activity could be demonstrated in normal subjects without atropine. There appear to be no previous results obtained under these conditions with which these may be compared. The results confirm the work of Schneyer (1956), who found the amylase level in parotid saliva to be at least four times higher than that in submandibular saliva.

SUMMARY

1. An analysis has been made of the various ways in which different types of stimuli may influence the composition of saliva. Saliva from the parotid and submandibular ducts has been collected at different rates of flow after applying a variety of stimuli, and analysed for calcium, inorganic phosphate, sodium, potassium, pH, amylase, protein N and sialic acid.

2. The concentration of these constituents was found to be dependent only on the rate of flow and not on the nature of the stimulus, except that for parotid saliva at rates of flow greater than 1 ml./min the amylase concentration was higher when salt-containing stimuli were used.

3. This distinction between salt-containing and other stimuli was not abolished by the administration of small doses of atropine.

4. The proportions of parotid to submandibular saliva secreted in the one subject from whom it was possible to collect the separate secretions simultaneously were higher with mechanical than with sapid stimuli.

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