The origin and fate of salivary urea and ammonia in man

J. KOPSTEIN* AND O. M. WRONG
Royal Postgraduate Medical School, and Medical Unit, University College Hospital Medical School, London

(Received 27 April 1976; accepted 12 August 1976)

Summary

1. Saliva obtained from the parotid duct of normal and uraemic subjects had an average urea concentration of 86% of the plasma concentration whereas in mixed saliva obtained from the mouth the urea concentration was only 31% of the plasma concentration. Ammonia concentrations were low or unmeasurable in parotid saliva but varied between 0.6 and 26 mmol/kg in oral saliva, showing a positive correlation with the plasma urea concentration.

2. The urea in samples of mixed oral saliva incubated at 37°C disappeared by 290 min. Ammonia steadily increased during incubation; within the first 100 min, the increase could be largely accounted for by bacterial hydrolysis of urea, but later non-urea sources became relatively more important.

3. These findings suggest that the ammonia in mixed oral saliva is derived by bacterial hydrolysis of urea within the mouth. However, the concentration of ammonia plus urea nitrogen in oral saliva was only 76% of the urea nitrogen concentration of parotid saliva, which suggests that some ammonia is lost from the mouth by buccal absorption or by volatilization.

4. To assess the role of non-ionic diffusion of ammonia through the buccal mucosa, we studied the effect of pH on the disappearance of ammonia from buffered solutions retained in the mouth. Ammonia concentrations fell more rapidly at pH 9 than at pH 7, as also did those of hydrazine, a non-volatile analogue of ammonia which is known to be absorbed through other mucosae by non-ionic diffusion. These findings suggest that salivary ammonia is reabsorbed passively through the oral mucosa in the un-ionized phase.

Key words: ammonia, mouth, saliva, urea.

Introduction

Over 100 years ago Wright (1841–42) first demonstrated the presence of urea in saliva. Later Schmitz (1922) and Updegraff & Lewis (1924) showed that the salivary urea concentration is increased when the blood urea is raised and it has since been suggested that analysis of saliva provided a useful assessment of renal function (Hench & Aldrich, 1922, 1923; Forland, Shannon & Katz, 1964). Forland et al. (1964) found that saliva collected directly from the parotid duct, before it entered the mouth, has 87–95% of the urea concentration of plasma. Mixed saliva, collected from the mouth, has lower concentrations of urea, equivalent to 0–47% (average 30%) of the plasma concentration (Schmitz, 1922; Updegraff & Lewis, 1924). This difference might theoretically arise from admixture of parotid saliva with saliva from other glands if their secretion contained lower concentrations of urea, but there is no evidence that this is so and a more likely explanation is that the low urea concentration in mixed saliva is the result of hydrolysis of urea to ammonia by urease, which is produced by many of the commensal bacteria.

* Present address: Faculty of Medicine, University of Rio Grande do Sul, Porto Alegre R.G.S., Brazil.
Correspondence: Professor O. M. Wrong, University College Hospital Medical School, London WC1E 6JJ.
normally present in the mouth. If this process were entirely responsible for the difference between the urea concentrations of mixed and parotid saliva, the total concentrations of ammonia plus urea nitrogen in the two fluids should be identical. Schmitz (1922) and Updegraff & Lewis (1924) found that the urea plus ammonia nitrogen of mixed saliva was only 84% of the simultaneous plasma urea nitrogen, but apparently a direct comparison between mixed and parotid saliva has not been made.

We have compared the composition of mixed and parotid saliva in both normal and uraemic subjects. To study the role of non-ionic diffusion in buccal absorption of ammonia, we have compared its disappearance from the mouth with that of hydrazine, a non-volatile analogue which is known to diffuse across other membranes in un-ionized form.

**Methods**

**Collection of saliva**

Saliva was obtained from four healthy subjects and eight uraemic patients (plasma urea 11.7-30.9 mmol/kg), who chewed gum (previously shown to be free of ammonia and urea) throughout the period of collection. After an initial mouth wash and a preliminary period of 10 min to allow oral contents to equilibrate with newly secreted saliva, a modified Carlson-Crittenden suction device (Shannon, Prigmore & Chauncey, 1962) was applied to the aperture of one perotid duct and parotid saliva was collected until at least 0.7 ml had been obtained. Next, each subject then collected at least 5 ml of mixed saliva in a graduated cylinder and, finally, a specimen of mixed venous blood was obtained. The total time spent collecting the three specimens was about 30 min. Samples of saliva were kept under paraffin oil, and all estimations were performed as soon as possible, usually immediately and always within 1 h.

**Salivary incubation**

Five healthy subjects provided samples of unstimulated mixed oral saliva for two incubation studies. In each study three subjects were used. After an initial mouth wash with distilled water, saliva (totalling 36 and 47 ml respectively) was collected in a 50 ml sterile measuring cylinder, and thoroughly mixed. At zero time, immediately after collection and mixing, urea was added (0.1 and 0.3 mmol respectively) to bring the concentration into the normal plasma range, and each specimen was then tightly stoppered and incubated in a water bath at 37°C, with mixing by inversion at 10-15 min intervals. Periodically samples (1 ml) were removed for analysis of urea and ammonia up to 290 and 360 min, by which time the urea concentration had fallen to unmeasurable amounts and an odour of putrefaction was detectable.

**Studies of buffered solutions retained in the mouth**

Disappearance of ammonia and hydrazine from buffered solutions in the mouth was measured in four normal subjects. To avoid errors introduced by the presence of ammonia-containing saliva, it was necessary first to dry the mouth. This was achieved by oral propantheline bromide (0.7-1.0 mg/kg body weight, a dose which was found to be the maximally effective one in a pilot study). Half an hour later, the mouth was washed out with distilled water and dried with paper tissues.

For the ammonia experiments, a solution containing ammonium chloride (100 mmol/l) and Tris buffer (170 mmol/l) was prepared. This concentration of ammonium chloride was chosen to simulate conditions in the mouth of a severely uraemic patient; it corresponds to a urea concentration of 50 mmol/l if it is assumed that the urea is completely hydrolysed to ammonium salts. Aliquots were titrated to pH 7.0, 8.0 and 9.0 with HCl (0.1 mol/l). A portion (15 ml) of each of these solutions was retained in the mouths of four subjects without swallowing for 90 min, during which the retained solution was frequently moved through the mouth. At the end of this period the oral contents were collected in a graduated cylinder and covered with a thin layer of mineral oil; pH and ammonia content were determined after measurement of the volume.

For the hydrazine experiments, a solution of hydrazine hydrate (0.1 mmol/l) was made up in Tris buffer as above, and titrated to pH 7.0 and pH 9.0. Portions (15 ml) of this solution were retained in the mouths of three similarly prepared subjects for 15, 30, 45, 60 and 90 min,
each subject performing ten separate studies with five different time-intervals at the two different pH values. The samples were again measured in a graduated cylinder and pH and hydrazine concentration were estimated.

**Analytical**

Urea and ammonia determinations were made in duplicate, by the microdiffusion method of Conway (1957). This method measures urea by difference; it is not satisfactory for concentrations below 0.5 mmol/l and for ease of subsequent logarithmic transformation all such values have been expressed here as 0.5 mmol/l. Hydrazine concentrations were measured by the method of Dambrauskas & Cornish (1962). The pH of mixed oral saliva was determined by glass electrode; specimens of parotid saliva were too small for pH measurement.

The water content of mixed saliva was obtained by drying at 100°C to constant weight, and was found to vary between 98.7 and 99.4% (w/w) with a mean value of 99.0%. When making comparisons between saliva and plasma we have therefore multiplied salivary concentrations per litre of saliva by a factor of 1.010 and plasma concentrations by 1.067 (Sunderman & Boerner, 1949) to yield concentrations per kg of water.

**Results**

The pK of ammonia is approximately 9.1, so ionized ammonium is the predominant species at the pH of saliva; however, for convenience we have here used the term 'ammonia' (or NH₃) to include both ionized ammonium and free ammonia.

**Salivary composition**

Figs. 1–7 show the relationship between the urea and ammonia concentrations of saliva and plasma over a range of plasma urea from 4.0 to 30.8 mmol/kg of water. Initial inspection of the data revealed that the absolute differences between the urea concentration of mixed saliva and plasma (or parotid saliva) were proportional to the amount of urea present, but became relatively constant when urea concentrations were expressed logarithmically. Logarithmic transformations have therefore been performed on the data plotted on both the abscissa and the ordinate, and the significance of differences has been calculated from these logarithmic values by means of the paired t-test.

Fig. 1 shows the relationship of the urea concentration in parotid saliva to that of plasma, expressed as molal concentrations. Parotid salivary urea concentrations were closely correlated with plasma urea \( (r = +0.99, P<0.001) \), ranging from 75 to 105% (mean 86 ± 9) of the plasma urea concentration,
figures similar to those found by Forland et al. (1964). Although the difference in urea concentrations of the two fluids was small, it was significant at the 0.1% level ($P<0.001$). The concentration of ammonia in parotid saliva was very low (as reported by Schmitz, 1922), averaging 0.5 mmol/kg. The highest figure observed was 1.6 mmol/kg in a normal subject, and in many specimens ammonia could not be detected by the Conway method.

Fig. 2 shows the relationship of the urea concentration in mixed saliva to that of plasma. The correlation was poor ($r = +0.35$, $P = 0.13$), salivary urea concentrations being always lower ($P<0.01$), averaging only $31 \pm 20\%$ of the plasma values. Mixed salivary ammonia varied between 0.6 and 26 mmol/kg, with a mean of 3.8 in normal subjects and 13.6 mmol/kg in uraemic subjects; Fig. 3 shows that there was a positive correlation between mixed salivary ammonia and urea nitrogen ($r = +0.54$, $P<0.05$), but the ammonia was on average only $33 \pm 19\%$ ($P<0.001$) of the urea nitrogen concentration. Even the sum of mixed salivary ammonia and urea nitrogen, shown in Fig. 4, although strongly correlated with plasma urea ($r = +0.96$, $P<0.001$) was always less than the plasma urea concentration ($P<0.001$), being $64 \pm 16\%$ of the latter figure.

The above comparisons have been made between saliva and plasma, because this is the comparison which has been made by previous workers. However, in order to determine the fate of urea and ammonia inside the mouth, it is more appropriate to compare mixed saliva with parotid saliva, which is representative of saliva before it enters the mouth, and this comparison is made in Figs. 5–7. In Fig. 5 is shown the comparison between the urea concentration in the two fluids: there was a poor correlation ($r = +0.35$, $P<0.13$), the mixed salivary urea being consistently less ($P<0.05$) averaging $37 \pm 21\%$ of the parotid salivary urea.
Salivary urea and ammonia in man

The correlation between mixed salivary ammonia and parotid salivary urea (Fig. 6) is close \((r = +0.51, P<0.05)\), mixed salivary ammonia nitrogen averaging \(40 \pm 23\% \) \((P<0.001)\) of the parotid urea nitrogen. On theoretical grounds one might expect a particularly close relationship between mixed salivary urea plus ammonia nitrogen and parotid salivary urea nitrogen, but Fig. 7 shows that despite a strong positive correlation \((r = +0.96, P<0.001)\) the average urea plus ammonia nitrogen was still appreciably less \((P<0.001)\) than parotid salivary nitrogen, averaging \(76 \pm 19\%\) of the latter figure.

The pH of mixed saliva from uraemic subjects was more alkaline \((pH 8.02 \pm 0.75)\).

![Figure 6](image1.png)

**Fig. 6.** Comparison of mixed oral salivary ammonia and parotid salivary urea.

![Figure 7](image2.png)

**Fig. 7.** Comparison of mixed oral salivary urea and ammonia with parotid salivary urea.

![Figure 8](image3.png)

**Fig. 8.** Urea and ammonia concentrations in incubated mixed oral saliva. ■, Total NH₃; ▲, non-urea NH₃; △, urea NH₃; ●, urea. The contributions of urea and non-urea sources to the total increment in ammonia have been calculated, and are shown by broken lines.
than that of healthy subjects (pH 7.14 ± 0.75), a highly significant difference ($P < 0.01$).

**Salivary incubation**

The results of the first experiment are shown in Fig. 8. The urea concentration fell progressively from 5.0 mmol/l at the start of incubation to undetectable values at 290 min, and simultaneously ammonia rose from 8.0 to 28.7 mmol/l. This increase in ammonia was greater than could be attributed to hydrolysis of urea: at most 10 mmol/l (48\%) of the increment in ammonia might have been derived from this source, and the remaining 10.7 mmol/l (52\%) must have originated from sources other than urea.

A second experiment (not charted) gave similar results. Urea concentration fell from 7 mmol/l to undetectable amounts at 200 min, and pH fell from 7.73 to 7.53. During 360 min ammonia rose from 6.8 to 29.7 mmol/l. Of the total increment in ammonia concentration at most 61\% could be attributed to urea hydrolysis, and the remaining 39\% (predominantly in the second half of the incubation) must have been from non-urea sources.

**Buffered solutions retained in the mouth**

**Ammonia solutions.** The pH of each solution fell during the experiment, by mean values of

![Fig. 9. Disappearance of ammonia from solutions of different pH retained in the mouth for 60 min. Each symbol represents a different subject.](image)

0.40 for the pH 7 solution, 0.75 for the pH 8 solution and 1.20 for the pH 9 solution. The three solutions showed mean increases in volume of 2.8, 1.1 and 2.8 ml respectively.

The disappearance of ammonia at each pH value for all subjects, corrected for change in volume of the solution, increased with increasing pH of the solution, as is shown in Fig. 9. The mean loss of ammonia was 27\% at pH 7, 53\% at pH 8 and 63\% at pH 9 (significance of differences: pH 7–8 $P = 0.05$; pH 8–9 $P = 0.1$; pH 7–9 $P = 0.01$).

**Hydrazine solutions**

Changes in pH and volume were similar to those of the ammonia solutions; for example, at 60 min the mean reduction in pH was 0.81 for the pH 7 solution and 1.22 for the pH 9 solution, and the mean increase in volume of the two solutions was 1.0 and 2.5 ml respectively. Absorption of hydrazine showed considerable variation between different subjects (Fig. 10) and even within the same subject studied at different times, but in each subject the mean absorption at all times was greater at pH 9 than at pH 7. There was a significant difference
incubated saliva is rather slow, for it takes several hours for urea to disappear when it is added at a concentration close to that of plasma, whereas the urea concentration of mixed oral saliva is often well below that of plasma even if the saliva is collected as rapidly as possible and presumably within a few minutes of its formation. This discrepancy can probably be explained by the continuous contact of saliva within the mouth with dental plaque, in which the concentration of urea-splitting organisms is known to be particularly high.

If all the urea which is lost from saliva in the mouth were converted into ammonia one would expect the sum of urea and ammonia nitrogen in the two fluids to be identical, or the concentration to be slightly higher in oral saliva if it were supplemented by ammonia generated from sources other than urea. The fact that there was not enough ammonia in the mixed oral saliva to account fully for the absent moiety of urea suggests that some ammonia is lost from oral saliva, either because of further bacterial action or by diffusion. Ammonia is known to be a substrate of many widely distributed bacteria (Guirard & Snell, 1962), such as Escherichia coli, Staphylococcus aureus, Proteus vulgaris and strains of Pseudomonas and Clostridia (Mortenson, 1962), some of which can be demonstrated in the mouths of healthy subjects (Richardson & Jones, 1958). Diffusion of ammonia seems an even more probable explanation; ammonia may diffuse through the oral mucosa into the bloodstream, or be lost by diffusion into the atmosphere.

In our experiments with ammonia solutions retained in the mouth more ammonia was lost from solutions of higher pH, suggesting one of three possible mechanisms: (1) the oral mucosa is more permeable to un-ionized ammonia; (2) ammonia is transported by some other pH-dependent mechanism, either active or passive; (3) ammonia volatilizes more readily from an alkaline medium.

Our experiments with hydrazine were devised to distinguish between these possibilities. Hydrazine is a non-physiological compound with a close structural similarity to ammonia and similar physical properties except that it is not volatile at normal temperatures (Table 1). It exhibits pH-dependent movement through many biological membranes (Orloff & Berliner, 1956; Coe & Korty, 1967; Swales, Tange &
TABLE 1. Physical characteristics of ammonia and hydrazine

<table>
<thead>
<tr>
<th></th>
<th>Ammonia</th>
<th>Hydrazine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight</td>
<td>17</td>
<td>32</td>
</tr>
<tr>
<td>pK</td>
<td>9.2</td>
<td>8.1</td>
</tr>
<tr>
<td>Boiling point (°C)</td>
<td>−33</td>
<td>113</td>
</tr>
<tr>
<td>Partition coefficient</td>
<td>0.05</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Wrong, 1970; Swales, Papadimitriou & Wrong, 1973), a finding which suggests diffusion in the un-ionized form. In our present studies absorption across the oral mucosa was pH-dependent (Fig. 9) and very similar to the disappearance of ammonia.

Further evidence of diffusion of un-ionized bases through the oral mucosa is provided by the pH-dependent absorption of drugs such as amphetamine and methylenedamphetamine (Beckett & Triggs, 1967), substances with little chemical similarity to ammonia and hydrazine except for their weakly basic character. It is inherently improbable that these substances all share a common active pathway of absorption, and the most likely explanation is of passive diffusion in the un-ionized form.

Diffusion of ammonia through most biological membranes appears to be pH-dependent, by non-ionic diffusion. In mammalian organs this process has been found to account for diffusion equilibrium of ammonia across the renal tubular epithelium (Orloff & Berliner, 1956; Coe & Korty, 1967) and ammonia absorption through the mucosae of stomach (Fleshler & Gabuzda, 1965), ileum (Swales et al., 1973), colon (Swales et al., 1970; Price, Sawada & Voorhees, 1970; Castell & Moore, 1971; Down, Agostini, Murison & Wrong, 1972) and urinary bladder (Rosenfeld, Aboulafia & Schwartz, 1963).

Despite these strong arguments suggesting that ammonia was lost from the mouth in our experiments by non-ionic mucosal diffusion, we cannot absolutely discount the possibility of some loss by volatilization, which would also be pH-dependent, as only the un-ionized fraction of ammonia can assume the gaseous form. Volatilization of salivary ammonia has long been regarded as playing a role in uraemic foetor (Bennett, 1928), though we know of no studies in which it has been measured. Uraemic subjects commonly breathe through the mouth, whereas our normal subjects kept their mouths closed, but even in our studies some volatilization of ammonia could have occurred through the nasopharynx.

Urea is usually considered to be an end product of nitrogen metabolism, but Walser & Bodenlos (1959) showed that about 20% of the daily production is hydrolysed by bacteria in the body. This degradation is usually assumed to occur in the colon, which is heavily colonized by urea-splitting organisms, but recent work (Wolpert, Phillips & Summerskill, 1971; Gibson, Park, Sladen & Dawson, 1976) has shown that the colonic mucosa is not sufficiently permeable to permit the passage of more than 20% of the 120 mmol of urea hydrolysed daily. The main site of urea degradation is thus unknown, but a simple calculation suggests that the contribution by the mouth is very small. Unless urea destruction occurs within the oral mucosa itself, as suggested by Wolpert et al. for the colon, or the oral mucosa is much more permeable to urea than its stratified squamous structure and a comparison with the colonic mucosa suggests, one can estimate that the maximum possible salivary urea destruction, with a daily salivary flow of 700 ml, would be about 4 mmol/day, or 3% of the overall degradation rate.

Acknowledgments

This work was carried out with the aid of MRC grants G.968/42, G.971/25 and G.975/605. We are grateful to Professor J. D. Swales for help with the hydrazine studies.

References


absorption from the human colon. *Gastroenterology*, 60, 33-42.


WRIGHT, S. (1841-42) A case of ascites in which during a spontaneous ptyalism that occurred after tapping, urea was detected in the saliva. *Lancet*, ii, 753-758.