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CONCENTRATION OF SALIVARY IODIDE: A COMPARATIVE STUDY

By B. COHEN* and N. B. MYANT

From the Department of Dental Science, Royal College of Surgeons of England, London, W.C. 2

and The Medical Research Council Experimental Radiopathology Research Unit, Hammersmith Hospital, London, W. 12

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Iodide has been shown to be selectively concentrated in the saliva of humans (Schiff, Stevens, Molle, Steinberg, Kumpe & Stewart, 1947), hamsters (Cohen, Logothetopoulos & Myant, 1955), mice (Logothetopoulos & Myant, 1956*a*, *b*) and dogs (D. D. Adams, G. A. Robinson & G. W. Stavraky, personal communication). In each of these species, within a few minutes of an intravenous injection of radio-iodide, the concentration in the mixed saliva may reach a value of more than twenty times the concentration in the serum. There have been several attempts to identify the cells concerned in this mechanism. Honour, Myant & Rowlands (1952) showed that the saliva:serum radio-iodide concentration ratio is at least as high for saliva collected from the opening of the human parotid duct as that for mixed saliva. They concluded that the parotid gland concentrates iodide, but they could not exclude the possibility that the submandibular and sublingual glands also contribute to the high concentration of iodide in the mixed saliva. In hamsters the intralobular (or proximal) ducts of the submandibular glands have been shown to concentrate radio-iodide (Cohen *et al.* 1955). Selective concentration of radio-iodide does not occur in the mixed saliva or the salivary glands of rats (Logothetopoulos & Myant, 1956*b*). We have continued these investigations and have extended them to several other species.

METHODS

In experiments on animals, carrier-free radio-iodide (about 1 μc/10 g body weight) was given by injection. Mixed saliva was sucked out of the mouth with a pipette. In cats, dogs, rabbits and guinea-pigs, saliva was also obtained by cannulation of separate salivary ducts of the anaesthetized animal with nylon tubing. In some cases the ducts were cannulated intra-orally through

* Leverhulme Research Fellow.

their openings in the mouth. In others the ducts were cannulated extra-orally after dissection. In humans the parotid and submandibular ducts were cannulated with metal or polythene cannulae after the openings of the ducts had been stretched with a polythene probe; no anaesthetic was used. In cats and dogs the sublingual and submandibular ducts were identified at the end of the experiment by injection of Indian ink.

The term 'residual saliva' will be used for the secretion obtained from the mouth after ligating both parotid ducts and removing both submandibular and sublingual glands. This juice was obtained from rabbits, guinea-pigs, cotton-rats, rats, mice and hamsters following injection of ^{131}I and pilocarpine.

Tissue samples were blotted, weighed, and digested in 4 N-NaOH on a steam bath. The soft palate was wiped free from mucus before it was removed from the animal. All samples were made up to 10 ml. with water and their radioactivity measured in a scintillation counter. For the analysis of the ^{131}I in human salivary glands the tissues were ground in a mortar with powdered glass and extracted with 5 vol. of absolute alcohol. The residue left behind after the extraction was hydrolysed by boiling in 2 N-NaOH for 16 hr. The ^{131}I in the alcoholic extracts and in the hydrolysates was analysed by paper chromatography with a solvent mixture of *n*-butanol (4 vol.) and dioxane (1 vol.) equilibrated with 2 N ammonia (5 vol.). The markers were iodide and thyroxine. Radioactivity along the chromatograms was measured with a continuously recording scanner. In the experiments on animals other than mice, hamsters and *Mastomys*, blood samples were taken at intervals of 10–20 min and the concentration of ^{131}I in the serum at any time estimated by interpolation between successive values. The saliva:serum ^{131}I concentration ratio was estimated from the concentration of ^{131}I in the sample of saliva and the serum ^{131}I concentration at the mid point of the sampling interval. In the case of mice, hamsters and *Mastomys* a single blood sample was taken immediately after the sample of saliva. In experiments on humans the right parotid and right submandibular ducts were cannulated 10–20 min after the injection of ^{131}I and the saliva collected over 5 min intervals for half an hour. A blood sample was taken about half way through the period of sampling.

RESULTS

Cats

The saliva:serum and salivary gland:serum ^{131}I concentration ratios (hereafter called $S:P$ ratio for saliva and $S:P$ ratio for salivary gland) were measured in cats after intramuscular injections of radio-iodide. Since cats do not normally secrete saliva unless the salivary glands are stimulated, an intraperitoneal injection of pilocarpine (5 mg/kg) was given 15 min after the injection of radio-iodide. The serum ^{131}I concentration showed little change between the 20th minute and the end of the experiment. In most cases there was a slight rise to a maximum, followed by a fall during the second hour (Fig. 1).

When all the salivary glands were allowed to secrete into the mouth, a flow of saliva began about 20 min after the injection of pilocarpine and continued until the cat was killed 2–3 hr later. Under these conditions, the $S:P$ ratio for saliva sucked out from the floor of the mouth was always above unity. In different cats the ratio varied between 5 and 10. An attempt has been made to identify the glands responsible for concentrating iodide in the mixed saliva of cats.

Parotid saliva. Both parotid ducts were cannulated. The cat was then given an injection of radio-iodide, followed by an injection of pilocarpine. For all

the samples of saliva obtained from the parotid ducts, the $S:P$ ratio averaged $0{\cdot}93 \pm 0{\cdot}13$. Although there was slight concentration in four out of fourteen samples obtained from five cats, the highest ratio observed was 1·9. However, in every experiment the $S:P$ ratio for the saliva obtained from the floor of the mouth was several times higher than that for any sample of parotid saliva from the same cat.

Submandibular saliva. The low concentration ratios for parotid saliva showed that the parotid glands were not responsible for the high concentration of radio-iodide in the mixed saliva. In the next experiment, therefore, saliva

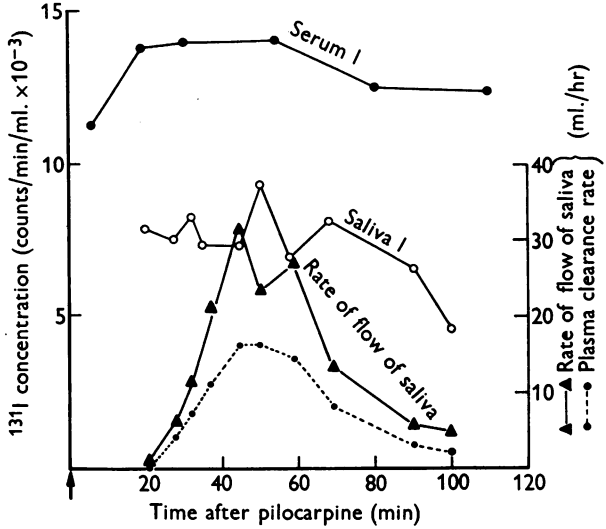

Fig. 1. $S:P$ ^{131}I ratio for cat's submandibular saliva, showing (left-hand ordinate) ^{131}I concentration in serum (●) and in saliva (○) obtained by cannulation of both submandibular ducts, and (right-hand ordinate) rate of flow of saliva (▲) and plasma radio-iodide clearance rate (●---●) by the salivary gland. In all figures, arrow ↑ shows injection of pilocarpine.

was obtained from the submandibular ducts by extra-oral or intra-oral cannulation. After insertion of the cannulae into the submandibular ducts on both sides, both parotid ducts were ligated and severed. Radio-iodide and pilocarpine were then injected as before.

In most cats, the $S:P$ ratio for the submandibular saliva obtained under these conditions was less than unity. The highest ratio observed for any sample was 2·1 and the average for all samples obtained from all cats was $0{\cdot}92 \pm 0{\cdot}09$. Fig. 1 shows the results of an experiment in which both submandibular ducts were cannulated extra-orally. Within 20 min of the injection of pilocarpine, saliva began to flow from the cannulae. The rate of flow rose during the first hour to a maximum of 20–30 ml./hr from the two ducts, and then gradually fell off. The $S:P$ ratio for all the samples averaged 0·6 and in all cases was less than unity.

During the course of every experiment a thick viscid material collected at the back of the mouth. When the concentration of ^{131}I in this material was measured, it was found to be several times higher than the concentration in the saliva collected through the cannulae in the same cat. In one experiment the $S:P$ ratio for the viscid saliva obtained from the back of the mouth was 13·4.

There was no correlation, either positive or negative, between the $S:P$ ratio for the submandibular saliva and its rate of flow, the $S:P$ ratio usually remaining more or less constant while the rate of flow of saliva rose and fell. Owing to the constancy of the $S:P$ ratio, the plasma clearance rate of radio-iodide by the submandibular gland ($S:P$ ratio × rate of flow) rose and fell in

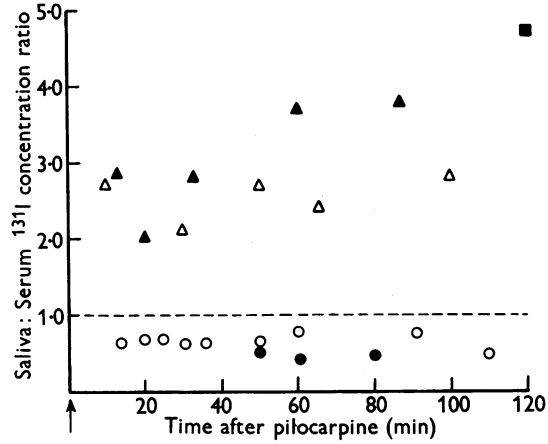

Fig. 2. $S:P$ ^{131}I concentration ratios for cat's saliva—submandibular, right, ○, left, ●: sublingual, right, △, left ▲; and residual saliva ■.

parallel with the rate of flow of saliva. In the experiment shown in Fig. 1, the clearance rate reached a maximum of 16·7 ml. of plasma cleared per hour through the two cannulae.

When the cannula had been passed intra-orally into the duct opening in the plica sublingualis, an injection of Indian ink at the end of the experiment showed that the cannula was in the submandibular duct in ten out of twelve cannulations. In the other two it was in the sublingual duct. It appears, therefore, that intra-oral cannulation is not an absolutely sure way of obtaining submandibular saliva, although the relationship between the openings of the submandibular and sublingual ducts is such that a cannula passed intra-orally nearly always enters the submandibular duct.

Sublingual saliva. Fig. 2 shows the results of an experiment in which sublingual saliva was obtained by extra-oral cannulation (Liddell & Sherrington, 1929). In this experiment both sublingual and both submandibular ducts

were cannulated extra-orally after ligation of the parotid ducts. For the 2 hr during which samples were taken, the $S:P$ ratio for the saliva from both sublingual ducts varied between 2 and 4. The $S:P$ ratio for the saliva from the submandibular ducts, on the other hand, never rose above 0·78. While the samples were being taken the same thick viscid material that had been observed before collected in the mouth. A sample of this was taken at 2 hr after the injection of pilocarpine. The $S:P$ ratio (5·2) was higher than that for any of the samples of sublingual or submandibular saliva. The rate of flow of sublingual saliva was only about 1/10 of the rate of flow of submandibular saliva. The plasma clearance rate of radio-iodide by the sublingual gland was also much lower than that by the submandibular gland. The average $S:P$ ratio for all samples obtained from the sublingual ducts of four cats was $1{\cdot}6 \pm 0{\cdot}3$.

Residual saliva. Up to this point the results showed that neither parotid nor submandibular nor sublingual saliva could account for the high $S:P$ ratio in the mixed saliva, although sublingual saliva appeared to be a contributory factor. They also showed that a thick viscid saliva is secreted into the mouth in conditions in which parotid, submandibular and sublingual saliva are excluded. This viscid material we have called 'residual saliva'. The $S:P$ ratio for the residual saliva was always higher than that for saliva secreted by any of the three salivary glands and was high enough to account for the $S:P$ ratio for the mixed saliva. In order to identify the source of the viscid secretion appearing in the mouth in the absence of the main salivary glands, a cat was anaesthetized; both parotid ducts were then cut between ligatures, and the submandibular and sublingual glands removed by dissection. The wounds were closed and the inside of the cat's mouth dried with swabs of cotton wool. The jaws were held open so that the back of the mouth could be seen clearly in a good light, and injections of radio-iodide and pilocarpine were then given intravenously. Within a few minutes of the injection of pilocarpine, numerous small drops of a clear liquid began to appear over the soft palate and the posterior part of the cheek. When they first appeared there were about 25 drops/cm^2 and they covered an area which extended to the posterior border of the soft palate, forward to the margin of the hard palate and laterally on to the cheek as far forward as the level of the molar teeth. No drops appeared over any part of the tongue, the hard palate or the mucosa covering the floor of the mouth. Gradually the drops increased in size and eventually formed a mass of viscid material filling the oral part of the pharynx and the floor of the mouth. The $S:P$ ratio for a sample removed with forceps at the end of the experiment was 5·7. Owing to the difficulty of removing this material from the mouth its rate of secretion could not be measured. As judged by eye, however, at least 5 ml. had accumulated in the mouth within 30 min of the first appearance of drops on the soft palate. The average $S:P$ ratio for the

samples of residual saliva obtained from all the cats so investigated was $7{\cdot}5 \pm 1{\cdot}4$.

Samples of salivary gland and soft palate. Whenever possible, samples of the salivary glands and soft palate were removed after the last sample of saliva had been taken (Table 1). In every case the concentration of ^{131}I in the gland was much less than that in the corresponding secretion. However, the highest $S:P$ ratios were found for the soft palate and the sublingual gland, the tissues responsible for the two secretions with the highest $S:P$ ratios. Table 1 shows the $S:P$ ratios for the salivary glands and soft palate.

Dogs

Observations were made on five dogs. The ducts were cannulated and ^{131}I given by intraperitoneal injection, followed by one or more intravenous injections of pilocarpine (0·5 mg/kg). Samples of saliva were collected during intervals of about 10 min. At the end of the experiment the position of the

TABLE 1. Salivary gland:serum ^{131}I concentration ratios

Species	Parotid	Submandibular	Sublingual	Soft palate
Cat	0·80±0·14	0·67±0·06	1·65±0·28	1·71±0·14
Dog	1·21±0·14	0·77±0·20	0·83±0·24	0·63±0·34
Rabbit	0·62±0·04	0·65±0·14	0·54	2·20±0·45
Guinea-pig	0·79±0·17	0·64±0·10	0·43±0·12	0·83±0·21
Cotton-rat	1·39±0·14	0·45±0·02	0·49±0·07	0·86±0·10
Rat	0·34±0·02	0·43±0·06	0·22±0·07	0·41±0·01
Mouse	0·59±0·02	5·1 ±0·27	0·53±0·02	0·54±0·04
Hamster	0·80±0·03	5·1 ±0·63	0·41±0·02	0·54
Mastomys	0·84	3·41±0·29	0·44	—
Man	4·6	6·9	11·2	—

The concentration of ^{131}I in a gland is estimated in the liquid obtained by digesting a biopsy sample of the gland with NaOH.

cannula in the submandibular or sublingual duct was confirmed by an injection of Indian ink. The $S:P$ ratios for each type of saliva varied considerably in different experiments and during the course of a single experiment (Table 2). For parotid saliva the average ratios varied from 5·5 to 14. For submandibular and sublingual saliva the average ratios varied from 0·9 to 2·2.

In three dogs (3, 4 and 5) observations were also made on residual saliva. All the ducts which could not be cannulated were exposed and tied with strong silk ligatures. Before the injection of pilocarpine the dog was placed on its back with the head pointing upwards and the jaws held widely open so as to expose the back of the mouth to view. Within less than a minute of the injection drops of clear viscid fluid began to appear over the soft palate. The parts of the soft palate and cheek over which the drops first appeared were the same as in cats, but the rate of flow was much greater. In two dogs the $S:P$ ratios for residual saliva were only a little above unity; in the other the ratios were as high as those observed for the parotid saliva (Table 2). Owing to the high viscosity of the residual saliva its rate of secretion could not be accurately

measured. However, in one dog 10 ml. was removed from the back of the mouth 6 min after it had been mopped dry with cotton wool. The rate of secretion was, therefore, at least 100 ml./hr. The maximum rate of flow from the cannulated submandibular duct of this dog, measured over the same 6 min interval, was 33·6 ml./hr and from the parotid duct, 19·2 ml./hr.

TABLE 2. $S:P$ ^{131}I concentration ratio for parotid, submandibular, sublingual and residual saliva of five dogs at 10 min intervals after injection of pilocarpine

Time (min)	Parotid (dog no.)					Submandibular (dog no.)				Sub-lingual (dog no.)	Residual (dog no.)		
	1	2	3	4	5	2	3	4	5	5	3	4	5
10	6·7	17·6	3·4	14·3	16·6	1·7	2·0	1·0	1·3	—	—	1·1	—
20	4·2	8·8	3·7	13·4	8·6	2·0	2·3	1·0	1·2	—	5·2	1·1	1·6
30	—	—	—	6·7	17·8	—	—	0·9	1·0	1·4	13·1	1·3	—
40	—	—	—	7·1	7·7	—	4·0	0·6	—	1·3	—	—	—
50	—	—	8·8	11·2	19·6	—	1·5	0·8	1·1	1·2	6·4	—	1·5
60	—	—	4·9	—	—	—	1·2	—	0·7	1·2	3·7	1·1	—
Average	5·5	13·2	5·2	10·5	14·1	1·9	2·2	0·9	1·1	1·3	7·1	1·2	1·6

In addition to the initial injection of pilocarpine, further injections were given to dog 4 at the 23rd min and to dog 5 at the 17th and 39th min.

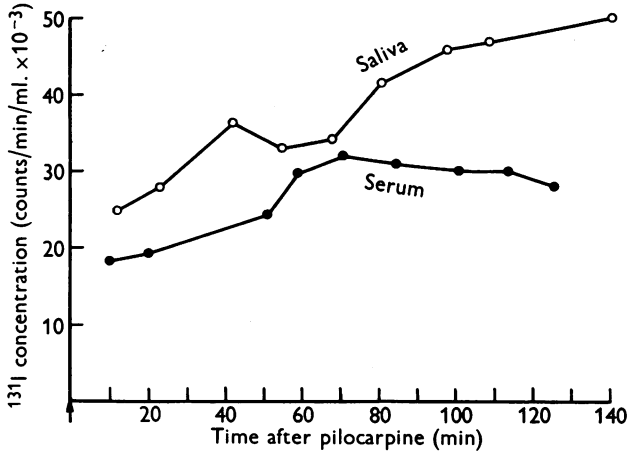

Fig. 3. $S:P$ ^{131}I ratio for rabbit's parotid saliva, showing ^{131}I concentration in serum (●) and in saliva (○) obtained by cannulation of one parotid duct

Rabbits

In rabbits given intraperitoneal injections of ^{131}I, the serum ^{131}I concentration rose to a maximum during the first hour and then fell slowly or remained constant during the next hour or two (Fig. 3). After injections of pilocarpine (3 mg/kg intravenously), the $S:P$ ratio for the mixed saliva averaged $3·1 \pm 0·75$. Saliva was also obtained from the parotid duct (Fig. 3). The average of the $S:P$ ratio for parotid saliva in nine rabbits was $1·8 \pm 0·15$. Residual saliva was obtained from eight rabbits. After stimulation with pilocarpine the

residual saliva was seen to appear first over the soft palate. Within 10 min of the injection the mouth was filled with thick viscid saliva, similar to the residual saliva of cats and dogs. The average $S:P$ ratio for this material was $4{\cdot}5 \pm 0{\cdot}70$.

The average $S:P$ ratio for biopsy samples of the soft palate was $2{\cdot}4 \pm 0{\cdot}45$. For all the salivary glands the $S:P$ ratio was less than unity (Table 1).

Guinea-pigs

Mixed saliva was obtained from guinea-pigs given intraperitoneal injections of pilocarpine (5 mg/kg). The average $S:P$ ratio was $15{\cdot}9 \pm 2{\cdot}1$. Measurements were also made on parotid saliva. Both parotid ducts were cannulated and

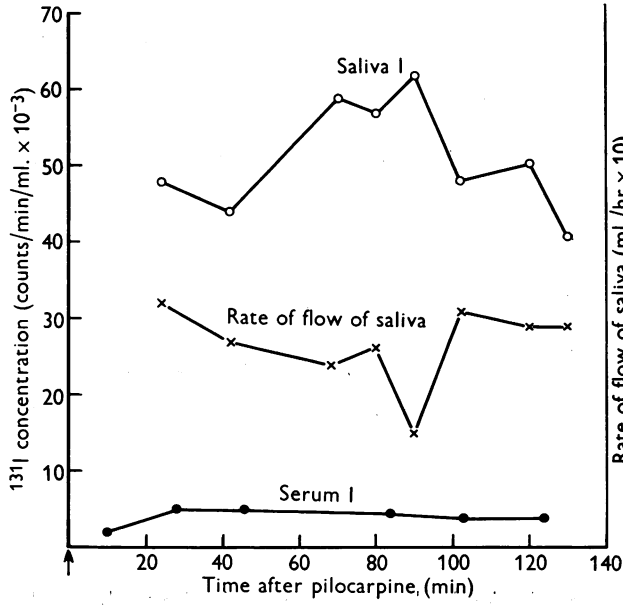

Fig. 4. $S:P$ ^{131}I ratio for guinea-pig's parotid saliva, showing (left-hand ordinate) ^{131}I concentration in serum (●) and in saliva (○) obtained by cannulation of both parotid ducts; and (right-hand ordinate) rate of flow of saliva (×).

an injection of ^{131}I given intraperitoneally; pilocarpine was given as before. Samples of saliva were then collected from the parotid ducts and from the floor of the mouth. Fig. 4 shows the results of a typical experiment in which the $S:P$ ratio varied from 9·7 to 14·3 and the rate of flow varied from 1·5 to 3·2 ml./hr during a sampling period of 2 hr. The average ratio for all the samples of parotid saliva obtained from eight guinea-pigs was $12{\cdot}7 \pm 1{\cdot}9$, and the average for all the samples of saliva obtained from the floor of the mouth was $3{\cdot}4 \pm 0{\cdot}7$.

Residual saliva was obtained from three guinea-pigs. With the amount of

pilocarpine used for obtaining parotid saliva no residual saliva was secreted. However, after larger injections of pilocarpine (up to 15 mg/kg) about 50 mg of thick mucus was obtained from the back of the mouth. The $S:P$ ratio for samples of this material averaged $1{\cdot}2 \pm 0{\cdot}4$. The $S:P$ ratios for biopsy samples of the salivary glands and soft palate were all less than unity (Table 1).

These results show that the parotid glands are mainly responsible for the high $S:P$ ratio observed for the mixed saliva of guinea-pigs. They also show that residual saliva in this species is secreted only after large doses of pilocarpine and does not contain ^{131}I at a high concentration. It is unlikely, therefore, that this secretion has any effect on the $S:P$ ratio for the mixed saliva secreted after smaller doses of pilocarpine. The saliva appearing in the floor of the mouth when both parotid ducts were cannulated must have come almost entirely from the submandibular and sublingual glands. It may be concluded, therefore, that either or both of these glands concentrate ^{131}I in the saliva, though to a much smaller extent than the parotid glands.

Cotton-rats (Sigmodon)

In cotton-rats anaesthetized with ether and injected with pilocarpine (1·5 mg/kg), the $S:P$ ratio for the mixed saliva averaged $13{\cdot}6 \pm 1{\cdot}8$. For residual saliva the ratio averaged $1{\cdot}8 \pm 0{\cdot}21$.

The parotid ducts were not cannulated but parotid saliva was obtained in two ways. In some cases both submandibular and both major sublingual glands were removed and a flow of saliva induced by an injection of pilocarpine. In other cases the parotid ducts were exposed by dissection and cut. An injection of pilocarpine was then given and the drops of saliva appearing at the proximal end of the parotid duct were removed with a fine pipette. For all the samples obtained by both methods, the $S:P$ ratio averaged $9{\cdot}2 \pm 0{\cdot}7$.

Saliva, presumed to come mainly from the submandibular and sublingual glands, was obtained by stimulation with pilocarpine after the parotid ducts had been exposed and cut. The $S:P$ ratio for this saliva averaged $6{\cdot}8 \pm 0{\cdot}85$. The $S:P$ ratios for biopsy samples of the salivary glands and soft palate are shown in Table 1.

Rats

Logothetopoulos & Myant (1956*b*) showed that iodide is not concentrated in the mixed saliva of rats. However, the high $S:P$ ratios for the residual saliva of cats and dogs raise the possibility that the rat's soft palate might also, under some conditions, secrete iodide at a concentration higher than that in the plasma. Accordingly, the $S:P$ ratio was measured for residual saliva obtained from albino rats of the Wistar strain.

The rats were anaesthetized with ether, followed by an intraperitoneal

injection of pentobarbitone. Both parotid ducts were severed, the submandibular and sublingual glands removed, and an injection of ^{131}I given intraperitoneally. Ten minutes later, pilocarpine (0·2 mg in 0·2 ml. of saline) was injected into the femoral vein. The jaws were held widely open and a light shone into the back of the mouth. Within a few minutes a thick viscid fluid appeared over the soft palate. This was removed with forceps and a sample of blood taken at the same time by cardiac puncture. In five rats the $S:P$ ratio for this material ranged from 1·1 to 2·3 and averaged $1·4 \pm 0·07$. This shows that the soft palate of the rat is capable of secreting radio-iodide at a higher concentration than that in the plasma, though its concentrating power is much less than that of the soft palate in cats and dogs.

In the experiments of Logothetopoulos & Myant (1956*b*), the flow of saliva was probably due to stimulation by the ether used as the anaesthetic. It is possible that the low $S:P$ ratio for the mixed saliva obtained under these conditions is due to dilution of the palatal secretion by a relatively large volume of parotid and submandibular saliva, in which iodide is not concentrated. On the other hand, it is possible that ether does not stimulate the soft palate to secrete. The latter explanation is supported by our finding that the $S:P$ ratio for the mixed saliva of rats injected with pilocarpine was often, though not always, greater than unity ($1·29 \pm 0·10$).

Since there are sex differences in the histological appearance of the submandibular glands of rats and mice (Lacassagne, 1940), the $S:P$ ratios were measured for the mixed saliva of male and female albino rats anaesthetized with ether. The average values in males and females did not differ significantly.

In order to see whether absence of the salivary iodide-concentrating mechanism extended to rats other than albinos of the Wistar strain, observations were also made on two Hooded rats of the Lister strain and on two wild rats (*Rattus rattus*). The average $S:P$ ratio for four samples of mixed saliva from the Hooded rats was $0·89 \pm 0·09$; the $S:P$ ratio observed for the wild rats averaged $1·04 \pm 0·11$.

Mice

An attempt was made to find out which salivary glands are responsible for the high $S:P$ ratio observed for the mixed saliva of mice. Small quantities of residual saliva were obtained after stimulation with pilocarpine (1·5 mg/kg, intraperitoneally). In six mice the average $S:P$ ratio was $0·84 \pm 0·12$. At the end of each experiment the soft palate was removed and the $S:P$ ratio measured (Table 1).

In other mice both submandibular and both major sublingual glands were removed, but the parotid glands and parotid ducts were left intact. Radio-iodide and pilocarpine were injected as before. The pilocarpine produced a copious flow of watery fluid, presumed to be parotid saliva. The $S:P$ ratio for this fluid averaged $1·8 \pm 0·2$.

A few observations were made on the $S:P$ ratios for the mixed saliva and for the salivary glands of male and female mice anaesthetized with ether, but not given pilocarpine (see section above). The values obtained for the mixed saliva and for the salivary glands did not differ significantly as between males and females.

Table 1 shows the concentration ratios obtained for the salivary glands and soft palate of mice. In this table the values for the salivary glands are derived from our own observations and those of Logothetopoulos & Myant (1956*b*).

Hamsters

In hamsters, the $S:P$ ratio for residual saliva averaged $2{\cdot}1 \pm 0{\cdot}32$. For parotid saliva, obtained by the methods used for cotton-rats and mice, the ratio averaged $2{\cdot}0 \pm 0{\cdot}65$. The $S:P$ ratios for the salivary glands (values from Logothetopoulos & Myant, 1956*a*) and soft palate are shown in Table 1.

Mastomys

In *Mastomys*, the average $S:P$ ratio for the mixed saliva from three males and three females was $6{\cdot}3 \pm 0{\cdot}34$. The $S:P$ ratios for the salivary glands are shown in Table 1.

Humans

Observations were made on four thyrotoxic patients who had been given test doses of radio-iodide (50–100 μc ^{131}I) by intravenous injection. In three patients the flow of saliva was stimulated by an 'acid drop' placed on the back of the tongue as soon as the cannulae were inserted.

TABLE 3. Human parotid and submandibular saliva: $S:P$ ^{131}I concentration ratio and rate of flow in four patients (average of all values in each patient)

	Parotid		Submandibular	
Patient	$S:P$ ratio	Flow (ml./hr)	$S:P$ ratio	Flow (ml./hr)
1	13·7	24·7	31·9	14·5
2	5·1	27·0	7·2	17·1
3	29·7	50·0	—	—
4	59·3	17·5	26·4	10·3

High $S:P$ ratios were observed for all samples of parotid and submandibular saliva (Table 3). There was no consistent difference between the $S:P$ ratios for the two kinds of saliva, nor was there a significant correlation between the $S:P$ ratio and the rate of flow of saliva. However, the low rates of flow usually occurring in humans under resting conditions were not observed here (see Discussion). Biopsy samples were not obtained, but the parotid, submandibular and sublingual gland, together with a piece of skeletal muscle and a blood sample, were taken from a patient who had been given a dose of 100 mc of ^{131}I 59 hr before death. When the samples were taken the body had been kept at 0° C for the 3 days since death. The concentration of inorganic ^{131}I

in the serum was measured after the organic ^{131}I had been precipitated with trichloroacetic acid. The concentration of total ^{131}I was also measured in the salivary glands and muscle. The muscle:serum ^{131}I concentration ratio was 0·36; the $S:P$ ratios for the salivary glands are shown in Table 1. The ^{131}I in portions of the parotid and submandibular glands was analysed by paper chromatography. The alcoholic extracts contained 95% of the ^{131}I in the parotid, and 78% in the submandibular. In the parotid, 99·5% of the total ^{131}I behaved chromatographically as iodide; in the submandibular 95·5% behaved as iodide.

All observations on the $S:P$ ratio for mixed saliva and for saliva from separate salivary glands are summarized in Table 4.

TABLE 4. $S:P$ ^{131}I concentration ratios for mixed saliva and for saliva from separate salivary glands of ten species

Species	Mixed	Parotid	Submandibular	Sublingual	Residual
Cat	+ +	0	0	+	+ +
Dog	+ + +	+ + +	0 to +	0 to +	+ to + +
Rabbit	+	+	—	—	+
Guinea-pig	+ + +	+ + +	+	+	+
Cotton-rat	+ + +	+ +	+ +	+ +	+
Rat	0	0	0	0	+
Mouse	+ + +	+	+ + +	0	0
Hamster	+ + +	+	+ + +	0	+
Mastomys	+ +	—	+ +	—	—
Man	+ + +	+ + +	+ + +	—	—

0 = <1, + = 1–5, + + = 5–10, + + + = >10.

We did not obtain mixed saliva from dogs, but since the $S:P$ ratio in parotid saliva was high we have assumed that it is also high in the mixed saliva. In guinea-pigs and cotton-rats, either the submandibular, or the sublingual, or both glands concentrate iodide in the saliva. The values for submandibular and sublingual saliva of mice and hamsters are based on evidence from autoradiography (Logothetopoulos & Myant, 1956*b*). In *Mastomys* the submandibular gland is assumed to be responsible for the high $S:P$ ratio for the mixed saliva, since this is the only gland for which the $S:P$ ratio is above unity (Table 1).

DISCUSSION

Unless the serum ^{131}I concentration remains constant during the course of an experiment, the ratio of the concentrations of ^{131}I in the saliva and in the serum is not exactly the same as the ratio of the concentrations of non-radioactive ^{127}I. If, for example, the serum ^{131}I concentration were to fall, the observed $S:P$ ^{131}I ratio (calculated from the serum ^{131}I concentration at the time when the sample of saliva was collected) would be higher than the true $S:P$ ^{127}I ratio. This is because the serum ^{131}I concentration would have been higher when the saliva was formed in the salivary gland than when it had reached the open end of the cannula. The difference between the $S:P$ ^{131}I ratio and the $S:P$ ^{127}I ratio is least when the serum ^{131}I concentration changes slowly, when the rate of flow of saliva is high, and when the internal volume of the salivary ducts and the cannula is small. (For discussion of this point, see

Honour *et al.* 1952). In most experiments on cats, dogs, rabbits and guinea-pigs, these conditions were satisfied. In the experiments shown in Figs. 1, 3 and 4, for example, the serum concentration seldom changed by more than 20% during any sampling period. In mice, hamsters and *Mastomys* only one blood sample was taken from each animal, so there is no evidence as to how quickly the serum ^{131}I concentration changed. However, observations on rats given intraperitoneal injections of ^{131}I showed that the serum concentration followed the same time course as in guinea-pigs. It seems likely, therefore, that the $S:P$ ratio gives a true indication of the iodide-concentrating power of the salivary glands in all the species investigated. The $S:P$ ratios in dogs, especially those for parotid saliva, show a greater degree of variability than that observed in the other species. Apart from the possibility that the repeated injections of pilocarpine, which were required to produce residual saliva in dogs 4 and 5, may have influenced the $S:P$ ratio, we have no explanation for this variability. However, it seems clear from Table 2 that the dog's parotid gland is capable of concentrating iodide to a degree comparable with that shown by the guinea-pig's parotid.

The absence of any relationship between rate of flow of saliva and $S:P$ ^{131}I concentration ratio is a little unexpected, especially since others have found that the concentration of several electrolytes in the saliva is related to the rate of flow (Thaysen, Thorn & Schwartz, 1954; Coats & Wright, 1957). Ferguson, Naimark & Hildes (1956) have shown that the $S:P$ ^{131}I ratio for human parotid saliva tends to be higher at low rates of flow than at moderate or fast rates. However, in none of our experiments on humans did we obtain the low rates of flow at which the relationship shown by Ferguson *et al.* (1956) is most obvious (their Fig. 2). Whatever the cause of this discrepancy, there is no evidence of an increase in the $S:P$ ratio with increasing rates of flow, such as would be expected if iodide were concentrated in the saliva by selective reabsorption of water by the ducts.

The results summarized in Table 4 show that the power of the salivary glands to concentrate iodide varies widely in different species. Concentrating power is high in humans, dogs, mice, hamsters, guinea-pigs and cotton-rats, moderate in *Mastomys* and cats, weak in rabbits, and almost absent from rats. Further, when concentration occurs it is not always brought about by the same salivary glands. In humans it is effected in the parotid and submandibular glands; in dogs in the parotid; in mice and hamsters it is brought about by the submandibular gland. In *Mastomys* also, indirect evidence points to the submandibular gland. In guinea-pigs and cotton-rats concentration is due mainly to the parotid gland, but the submandibular and sublingual may also contribute. In rabbits, and even more so in cats, concentration seems to be due almost entirely to the glands in the soft palate.

The $S:P$ ratio for each salivary gland is in all cases only a fraction of the

$S:P$ ratio for the corresponding saliva. This is not surprising, since in most glands the iodide-concentrating mechanism is confined to a small proportion of the tissue digested. In hamsters (Cohen *et al.* 1955) and mice (Logothetopoulos & Myant, 1956*b*), it is confined to the cells lining the intralobular ducts. There must also have been a certain amount of fat and connective tissue in all the tissue samples. For the most part there is a fair degree of correlation between the $S:P$ ratios for the salivary glands and for the saliva they secrete, but there are exceptions to this. In guinea-pigs, for instance, the $S:P$ ratio for the parotid is among the lowest observed for this gland (Table 1), whereas the $S:P$ ratio for the parotid saliva of the guinea-pig is high. In this case the discrepancy may be due to the high fat content of the guinea-pig's parotid. With regard to the high $S:P$ ratios for the human salivary glands, it would be unwise to draw firm conclusions from a single set of observations made under abnormal conditions. However, the high values are unlikely to be due to an error in measuring the concentration of ^{131}I in the serum, or to disappearance of ^{131}I from the serum after death, since the muscle:serum ^{131}I concentration ratio was close to that reported by other workers (Trunnell, Duffy, Godwin, Peacock, Kirschner & Hill, 1950).

It might be thought that a comparison between the histological appearance and the iodide-concentrating power of the different salivary glands would reveal something of the underlying mechanism. However, histological examination of the salivary glands from different species (Cohen & Myant, unpublished) has failed to reveal any particular type of cell or structure common to those glands in which the mechanism is most active. The finding that the iodide in the saliva of cats and rabbits is secreted chiefly in the mucus from the soft palate raises the possibility that in these species the iodide is carried into the saliva by a muco-polysaccharide. The slight concentration of iodide in cat's sublingual saliva is consistent with this, since in cats the sublingual saliva contains mucus. However, the ability to concentrate iodide is not a general property of mucus-secreting cells, since iodide is not concentrated by the sublingual glands of rodents or by the mucous cells in the stomach of the hamster (Logothetopoulos & Myant, 1956*a*). Nor is secretion of mucus a necessary condition for iodide secretion, since the iodide-concentrating mechanism has been shown to be present in the intralobular ducts of the submandibular glands of hamsters (Cohen *et al.* 1955) and mice (Logothetopoulos & Myant, 1956*b*); the cells lining the ducts do not contain mucus. Basal striations are conspicuous in the intralobular ducts of mice, hamsters and *Mastomys*. However, they do not appear to be related to the iodide-concentrating mechanism, since they are equally conspicuous in the intralobular ducts of rats, in which iodide is not concentrated, and are absent from the mucous glands of the soft palate in cats and rabbits.

There seems to be little relationship between the iodide-concentrating

power of the species we have examined and their biological affinity with one another. Hamsters and cotton-rats belong to the same family (*Cricetidae*) of the rodents, but iodide is concentrated by the submandibular in the one, and by the parotid in the other (Tables 1 and 4). Although iodide is concentrated in the mixed saliva of the cat and the dog, both carnivores, there is little similarity in the site of the concentrating mechanism. *Mastomys* belongs to the family *Muridae*, which also contains the rat and the mouse, but its exact position within this family has not yet been decided. In certain respects it is closer to a mouse, in others to a rat (D. H. S. Davis and A. G. Oettlé, personal communication). In the histology of its salivary glands and in the high $S:P$ ratio for the submandibular gland it is more like a mouse than a rat. The striking difference between rats and mice suggested the possibility that the failure of laboratory-bred rats to secrete iodide might be due to artificial selection having eliminated a gene controlling iodide secretion in the saliva. However, this appears to be ruled out by the fact that the iodide-concentrating mechanism is equally feeble in two different strains of hybrid rats, and also in wild rats. Any hypothesis as to the function served by iodide secretion in the saliva must take into account the fact that it does not occur in rats.

SUMMARY

1. After injections of radio-iodide the saliva:serum ^{131}I concentration ratio was measured for mixed saliva and for saliva obtained from separate salivary ducts in cats, dogs, rabbits, guinea-pigs, cotton-rats, mice, hamsters, *Mastomys* and humans. Observations on iodide concentration were also made on biopsy samples of the salivary glands.

2. Iodide-concentrating power varied widely in the different species and in different salivary glands of a given species.

3. Concentrating power was high in humans, dogs, mice, hamsters, guinea-pigs and cotton-rats, moderate in *Mastomys* and cats, weak in rabbits, and negligible in rats.

4. In humans concentration of iodide was brought about by the parotid and submandibular glands; in dogs by the parotid; in mice, hamsters and *Mastomys* by the submandibular; in guinea-pigs and cotton-rats by the parotid and possibly also by the submandibular and sublingual. In cats and rabbits concentration was due to secretion by the glands of the soft palate and the adjacent part of the cheek.

5. Histological examination of the salivary glands failed to reveal any special type of cell peculiar to the glands in which the iodide-concentrating mechanism was present.

6. The ^{131}I in the parotid and submandibular glands taken from a human who had died after a dose of radio-iodide was analysed by chromatography. In both glands more than 95% of the ^{131}I was identified as iodide.

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