Studies on the Concentration of Radioiodide and Thiocyanate by Slices of the Salivary Gland

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(*Received* 10 *October* 1955)

Human salivary glands concentrate iodide to an extent comparable with that of the iodide trap of the thyroid gland (Honour, Myant & Rowlands, 1952), and there is a very close analogy between the effects of antithyroid drugs on the concentration of iodide by the thyroid and salivary glands (Rowlands, Edwards & Honour, 1953). Thus it seemed likely that a study of the concentration of iodide by salivary glands might help to elucidate the mechanism of the iodide trap of the thyroid, which is much more difficult to study as it is only the first stage in the synthesis of thyroid hormone, whereas organic binding of iodide does not occur in salivary glands.

Edwards, Fletcher & Rowlands (1954) found evidence of antagonism between iodide, perchlorate, thiocyanate and nitrate for secretion in human saliva, the most likely mechanism being that they compete as substrates for receptors on a protein which transports them across the salivary cells. To study the mechanism in more detail it was necessary to obtain preparations of salivary gland which could be studied *in vitro*. Although the concentration of iodide in the saliva of both man and the guinea pig is about 30 times the plasma level, the concentration in their salivary glands is the same as, or only slightly greater than, the plasma iodide; the rat does not concentrate iodide in its saliva or salivary glands. We therefore used the mouse as an experimental animal, following Collins & Hellmann's (1955) observation that it concentrates iodide in its salivary glands, an observation which we have confirmed. This paper describes the effect of various factors on the concentration of [^{131}I]-iodide by slices of the salivary gland of the mouse.

METHODS

Paper chromatography and autoradiography. The procedures described below were adopted to exclude the possibility that synthesis of organic iodine was occurring in the salivary gland. A mouse was given 20 μc of ^{131}I as iodide by intraperitoneal injection. One hour later, it was killed and its salivary glands were removed. These were ground with sand and the resulting breis were completely extracted with *n*-butanol. After evaporation, samples of the butanol extract were applied to chromatography paper and developed either in *n*-butanol–dioxan–ammonia (Gross, Leblond, Franklin & Quastel, 1950), or in *n*-butanol–2N acetic acid. A similar technique was used to obtain chromatograms from slices of salivary glands which had been incubated for 40 min. in an oxygenated saline solution containing $^{131}I^-$ at 37°. Autoradiography of the dried chromatograms obtained by both methods showed all the activity to have migrated as a single spot corresponding to added carrier iodide; no other compounds were detected.

Preparation and distribution of tissue slices. In vivo, the concentration of iodide in the salivary glands of a mouse weighing approx. 35 g. is about eight times the plasma level, but this ratio increases up to 15 with increasing weight of the animal. Male albino mice (Tuck's no. 1 strain, The Mousery, Rayleigh, Essex.) weighing 40–55 g. were therefore used in these experiments. *In vitro*, it was found that slices of salivary glands from different mice varied in their power of concentrating $^{131}I^-$ from the surrounding medium, the concentration ratio varying from 5 to 10. As several mice were used in each experiment, the following technique was devised to eliminate the effects of this variation as far as possible. Three mice were killed and the submaxillary glands dissected out as rapidly as possible. Each gland was sliced into three pieces by the method of Deutsch (1936), and each of these pieces was cut in half and placed in dishes containing buffered-saline solution (adjusted to pH 7·4 by the addition of M-H_3PO_4) of the following composition: water 100 ml., NaCl 850 mg., KCl 42 mg., $CaCl_2$ 24 mg., glucose 100 mg., Na_2HPO_4 60 mg. The pieces were then gently blotted on filter paper, weighed on a torsion balance and then transferred to six Warburg flasks in such a way that each flask contained a portion of tissue from each gland, the average weight of tissue in each flask being approximately 120 mg.

Incubation of slices and estimation of $^{131}I^-$ concentration. Two of the six flasks in each experiment contained 3·5 ml. of saline and served as controls. The other four contained different concentrations of the substance under test, dissolved in saline solution to a final volume of 3·5 ml., except when dinitrophenol and triiodothyroacetic acid [3:5-diiodo-4-(4-hydroxy-3-iodophenoxy)phenylacetic acid] were used, these being placed in the side arm. Carrier-free ^{131}I, as iodide, dissolved in 0·5 ml. of the saline medium, was placed in the side arm of each flask. The flasks were gassed with O_2 (100%) for 5 min. and then placed in the bath at 37°. After equilibration at bath temp. (10 min.) the flasks were sealed and the $^{131}I^-$ was tipped into the main compartment. The slices were incubated for about 40 min. and O_2 consumption was measured in the conventional manner. This period of time was chosen because the concentration of $^{131}I^-$ in the tissue did not increase with longer periods of

incubation. After incubation, the tissues were removed from the flasks and blotted, and digested in 2N-NaOH. Samples of the saline medium were also taken from each flask, and after adjustment of volume the radioactivity of both tissues and fluid was determined in an M 6 liquid counter.

From the known weight of tissue in each flask the ratio of the concentrations of $^{131}I^-$ in tissue (T) and medium (M) was derived:

$$T/M \text{ for } ^{131}I = \frac{\text{counts/g. of tissue}}{\text{counts/ml. of medium}}.$$

Estimation of thiocyanate. The tissues were distributed and incubated as described above, except that ^{131}I was not added, and after incubation the slices were removed from the flasks and homogenized in a small, all-glass, Potter–Elvehjem homogenizer in a known volume of water. The homogenate (0·5 ml.) was treated with 0·3 ml. of 80% (w/v) trichloroacetic acid and centrifuged, and thiocyanate was determined on the supernatant by the method of Aldridge (1945). Thiocyanate estimations were also made on the saline medium in each flask.

RESULTS

The chromatographic studies showed that synthesis of organic iodine did not occur in the salivary glands during the period of these experiments. Concentrations of methylthiouracil varying from $4{\cdot}4 \times 10^{-5}$ to $1{\cdot}3 \times 10^{-3}$ M had no effect on the uptake of $^{131}I^-$ by the slices.

Effect of carrier-iodide $^{127}I^-$ and bromide on the concentration ratio (T/M) for $^{131}I^-$

When $^{127}I^-$ is added to the suspending medium in varying concentrations, the absolute amount of iodide in tissue and medium can be estimated by measuring the radioactivity in each, since the amount of iodide initially present in the gland is negligible. Fig. 1 shows the amount of iodide in the tissue at different concentrations in the medium; the concentration in the tissue rises with increasing concentrations in the medium but the concentration ratio (T/M) falls. However, even when large amounts of $^{127}I^-$ are added to the medium, the concentration ratio does not fall below about 1·0; indeed, it has not been possible to depress the ratio below 0·8, either by adding very high concentrations of powerful inhibitors to the suspending medium or even by boiling the gland after slicing it.

In Fig. 1 there is a considerable scatter of the experimental points about the curve because they represent the results of three separate experiments. In any one experiment, the slices are so distributed that differences in the concentrating power of the glands from different mice are reduced, but these differences may be considerable between one experiment and another. If, however, for each point on the graph the amount of iodide in the tissue is divided by the concentration ratio (T/M) of the corresponding control, the scatter is greatly diminished. In effect this expresses the amount of iodide in the gland per unit of concentrating power rather than per unit weight of tissue. In this way a straight-line relationship is obtained as shown in Fig. 2.

Potassium bromide in concentrations of 1×10^{-3} to 1×10^{-5} M had no effect on the concentration ratio (T/M) for $^{131}I^-$.

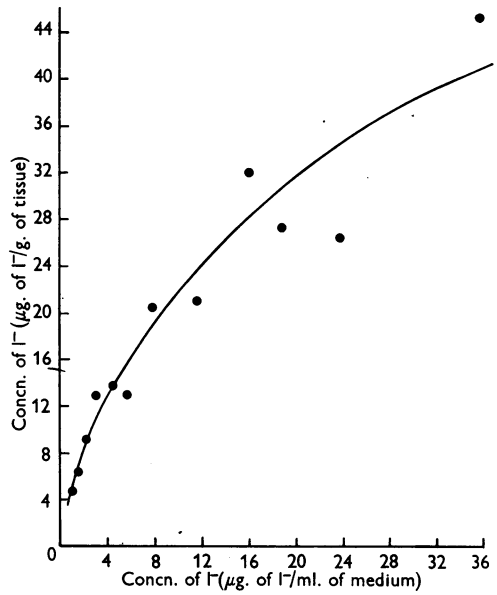

Fig. 1. Relationship between concentration of I^- in tissue and in medium.

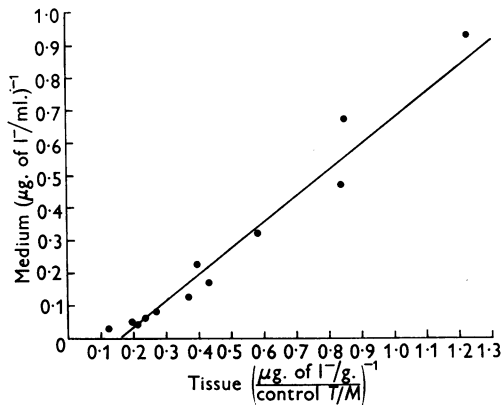

Fig. 2. Relationship between the reciprocal of the concentration of iodide/g. of tissue divided by the concentration ratio (T/M) of the control, and the reciprocal of the amount of iodide/ml. of medium.

Effect of ClO_4^-, SCN^-, *and* NO_3^- *on the concentration ratio* (T/M) *for* $^{131}I^-$

The potassium salts of each of these three anions depressed the uptake of $^{131}I^-$ when added in suitable concentration to the suspending medium. The range of concentrations used was 5×10^{-7} to $3{\cdot}8 \times 10^{-5}$ M of ClO_4^-, $2{\cdot}1 \times 10^{-5}$ to $4{\cdot}5 \times 10^{-4}$ M of SCN^-, and 2×10^{-4} to 4×10^{-3} M of NO_3^-. Perchlorate was 10 times as effective per equivalent as thiocyanate, and 80 times more effective per equivalent than nitrate, in lowering the concentration ratio (T/M). Fig. 3 shows the relative effects of ClO_4^- and SCN^- as inhibitors, and the effect of added $^{127}I^-$ is also plotted for comparison. The perchlorate ion is a very powerful inhibitor, since a concentration as low as 5×10^{-1} μM has a significant effect, and a concentration of 60–80 μM usually lowers the concentration ratio (T/M) to about 0·8. The ratio remains at about 0·8 even when enormous concentrations of the order of 4 mM are used.

Table 1. *Observed and calculated effect on the concentration ratio* (T/M) *for* $^{131}I^-$ *of adding* ClO_4^- *and* SCN^- *simultaneously to the medium*

T, $^{131}I^-$ counts/g. of tissue; M, $^{131}I^-$ counts/ml. of medium.

Concn. of ClO_4^- (μM)	Concn. of SCN^- (μM)	Observed T/M for $^{131}I^-$	Calculated T/M for $^{131}I^-$
5·06	345	14·5	17·4
15·1	129	14·7	16·7
7·54	43·1	19·0	24·2
5·02	108	20·4	23·7
2·51	134	22·1	25·2
1·76	32·4	36·2	39·5
0·754	32·3	41·9	46·0
1·76	10·8	43·2	48·0

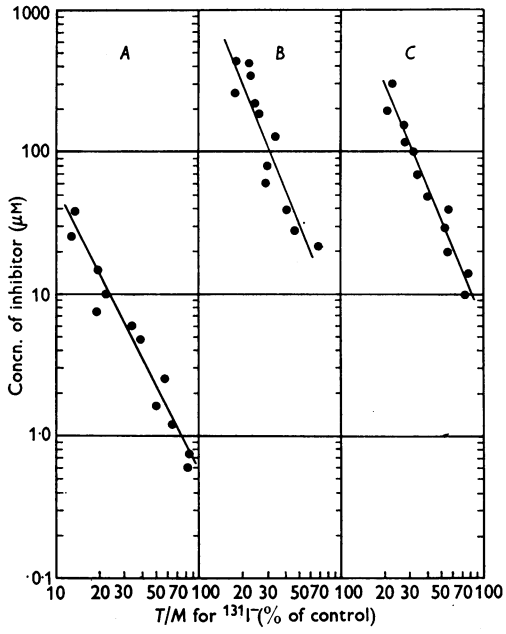

Fig. 3. Effect of ClO_4^- (*A*), SCN^- (*B*), and I^- (*C*) on the concentration ratio of $^{131}I^-$.

The similarity of the curves in Fig. 3 suggests that the mechanism of inhibition of iodide uptake may be similar for all three anions. Perchlorate and thiocyanate were therefore added simultaneously to the incubating medium to determine whether a summation of their individual effects occurred (Table 1). The third column in the table shows the observed concentration ratio (T/M) expressed as a percentage of the controls for each mixture of different concentrations of the two anions. Column 4 shows the calculated concentration ratios, assuming that the effect of the mixture represents a summation of the effect produced by either anion separately. The calculated concentration ratios were found in the following manner. The expected inhibitory effect of the ClO_4^- in each mixture was first read off from the curve for ClO_4^- in Fig. 3; then from the curve for SCN^- the amount of SCN^- necessary to produce this inhibitory effect was determined. The sum of the amount thus determined and the amount of SCN^- added to the mixture was found, and the inhibition by this total was read off from the SCN^- curve. The similarity of the observed and calculated results suggests a summation of the effects of these two anions.

Effect of endogenous SCN^- *on the concentration ratio* (T/M) *for* $^{131}I^-$

Table 2 shows the concentration of SCN^- in the tissue when different amounts of SCN^- are added to the suspending medium. It is evident that very large amounts have to be added to the medium to raise the concentration in the tissue significantly. Thus a concentration of SCN^- of $8{\cdot}6 \times 10^{-2}$ mM in the medium did not increase the concentration in the gland, yet this amount of added SCN^- depresses the concentration ratio (T/M) for $^{131}I^-$ by about 70 %.

The concentration of SCN^- present in the plasma of the mouse is 2·83 μg./ml. (48·8 μM; mean of six determinations, range 2·18–3·68). The concentration *in vivo* in the submaxillary salivary gland is 28·1 μg./g. (mean of three determinations, range 26·9–28·8). Thus the concentration ratio *in vivo* for SCN^- is about 10. It will be seen that the amount of SCN^- present in the gland is large compared with that which has to be added in order to depress T/M for $^{131}I^-$. Furthermore, from the data of Fig. 3 the amount of SCN^- in the plasma, 2·83 μg./ml. (48·8 μM), should cause considerable depression of iodide concentration.

Table 2. *Effect of the addition of* SCN^- *to the medium on the amount of* SCN^- *in the tissue and on the concentration ratio (T/M) for* SCN^-

Concn. of SCN^- in medium (μg./ml.)	Concn. of SCN^- in tissue (μg./g.)	Concn. ratio for SCN^- (T/M)
0·8	27·1	34·8
0·9	25·6	27·7
0·9	34·4	36·6
1·1	26·1	24·6
1·1	27·3	25·1
1·3	27·9	21·4
1·8	28·9	16·3
3·3	27·4	8·3
3·5	31·0	8·9
5·2	36·1	6·9
6·9	37·4	5·4
9·7	27·2	2·8
12·9	36·2	2·8
28·0	43·3	1·5
49·0	56·7	1·2

Table 3. *Comparison of the concentration ratio (T/M) for* $^{131}I^-$ *before and after leaching out* SCN^- *from the tissue*

Conc. of SCN^- in tissue (μg./g.)	T/M for $^{131}I^-$	Conc. of SCN^- in tissue after leaching (μg./g.)	T/M for $^{131}I^-$ after leaching
28·50	5·06	19·9*	6·74
30·91	8·54	16·5†	7·26
22·90	7·75	8·0†	6·43

* Leached for 30 min. in saline.
† Leached for 60 min. in saline.

The following observations were therefore made on tissue slices *in vitro* to study the effect of the high concentration of endogenous SCN^- in the glands on the uptake of $^{131}I^-$. It seemed possible that the concentration ratio for $^{131}I^-$ would rise if the endogenous SCN^- could be leached out of the tissue. Slices of gland were incubated in 20 ml. of saline solution for varying periods of time, and the amount of SCN^- remaining in the tissue was estimated (Table 3). Even after an hour's incubation, however, approximately 40% of the SCN^- was still present in the tissue, whereas only 10% should still be present under these experimental conditions to maintain the usual concentration ratio of about 30 between tissue and medium. In contrast to this it was found that $^{131}I^-$ could be leached out of a gland very rapidly. A mouse was injected with a dose of $^{131}I^-$ and killed an hour later, and its salivary glands were dissected out, sliced and immersed in 20 ml. of saline. Within 30 min. the usual concentration gradient between tissue and medium had been reached. Table 3 also shows the effect of incubating slices for 30 min. in saline to leach out some of the SCN^-, and then transferring them to Warburg flasks and measuring their uptake of $^{131}I^-$; the concentration ratio (T/M) for $^{131}I^-$ did not differ significantly from that of the controls in which the slices had not been previously immersed in saline.

Experiments were then carried out to determine whether the concentration of SCN^- in the slices could be lowered by adding either I^- or ClO_4^- to the medium. Table 4 shows that these anions had no effect on the endogenous SCN^- even in doses far exceeding those required to depress the concentration ratio for $^{131}I^-$ to 0·8.

Effect of dinitrophenol, triiodothyroacetic acid and thyrotrophic hormone

ClO_4^-, SCN^-, NO_3^- and $^{127}I^-$ depress the uptake of $^{131}I^-$ by the tissue without affecting its O_2 consumption significantly. On the other hand, 2:4-dinitrophenol in concentrations of 10^{-3} to 10^{-5} M depressed both O_2 consumption and T/M for $^{131}I^-$ (Table 5).

In view of the action of dinitrophenol on the tissue slices we tried the effect of adding triiodothyroacetic acid to the suspending medium, as it seemed possible from the results of Thibault & Pitt-Rivers (1955) that an increase in both O_2 consumption and uptake by the tissues of $^{131}I^-$

Table 4. *Effect on concentration of* SCN^- *in the tissue of adding* (1) 300 μM $^{127}I^-$, *and* (2) 100 μM ClO_4^- *to the medium*

Concn. of SCN^- in tissue (μg./g.)	Concn. of $^{127}I^-$ in medium (μM)	Concn. of ClO_4^- in medium (μM)
Expt. 1		
22·7	—	—
24·5	—	—
23·9	300	—
26·8	300	—
Expt. 2		
31·3	—	—
36·3	—	—
33·4	—	100
28·3	—	100

Table 5. *Effect of the addition of 2:4-dinitrophenol to the medium on the concentration ratio (T/M) for* $^{131}I^-$ *and on* q_{O_2}

Concn. of 2:4-dinitrophenol in medium (M)	T/M for $^{131}I^-$	q_{O_2} (μl. of O_2/mg. wet wt./hr.)
0	7·84	2·25
0	7·09	1·69
10^{-5}	5·56	1·88
5×10^{-5}	2·69	1·26
10^{-4}	2·18	1·12
10^{-3}	2·31	0·79

Table 6. *Action of various concentrations of triiodothyroacetic acid on the concentration ratio (T/M) for $^{131}I^-$ and oxygen consumption in salivary glands*

	Control		Triiodothyroacetic acid		
Concn. (μM)	T/M for $^{131}I^-$	q_{O_2} (μl. of O_2/mg. wet wt./hr.)	T/M for $^{131}I^-$	q_{O_2} (μl. of O_2/mg. wet wt./hr.)	Incubation times (min.)
1·0	3·94	2·03	5·46	2·26	20
			6·74	1·89	20
1·0	2·67	1·56	2·70	1·83	15
	2·81	1·82	2·60	1·85	15
			2·81	1·86	15
			2·37	2·13	15
1·0	3·64	1·99	2·32	0·79	20
	3·23	1·87	3·62	2·08	20
			3·93	1·90	20
			4·01	1·84	20
1·0	8·73	1·92	9·34	2·07	40
			9·94	1·90	40
1·61	2·18	1·82	3·49	1·91	15
			3·13	1·74	15
1·61	3·85	1·92	4·96	1·92	25
			3·74	1·78	25
1·61	2·51	1·82	3·06	1·64	10
			2·86	1·82	10
1·61	3·50	1·83	4·06	1·74	20
			4·26	1·60	20
2·0	3·39	1·96	3·92	2·20	20
	4·36	2·05	3·06	1·94	20
3·84	3·39	1·96	3·83	1·95	20
	4·36	2·05	3·72	1·98	20
5·0	6·11	2·27	8·00	1·95	20
			9·25	2·08	20
8·05			3·50	1·75	15
16·10	3·25	2·33	3·60	2·04	15
40·25	3·71	2·24	3·19	1·90	20
80·50			3·11	1·32	20

might be found. Using a range of concentrations of triiodothyroacetic acid from 0·96 to 80·5 μM and incubation times of 10, 15, 20 and 40 min. we have been unable to demonstrate consistent increases either in O_2 consumption or iodide uptake (Table 6).

Thyrotrophic hormone was added to the suspending medium in four concentrations ranging from 0·025 to 4·25 units (U.S.P.) without any significant effect on O_2 consumption or on uptake of $^{131}I^-$ by the slices. In this experiment the tissue was incubated for 105 min. instead of the usual 40 min., as it seemed likely from *in vivo* experiments that a longer period would be necessary to exclude an effect by thyrotrophic hormone.

Water content of tissue

The failure to depress T/M for $^{131}I^-$ below 0·8 suggested that the iodide of the medium was in diffusion equilibrium with the tissue water. The water content of the tissues was therefore determined. After incubation for 50 min. slices of gland were removed from the flasks, and blotted and weighed. They were then dried in an oven at 100° for 12 hr., and, after cooling, were reweighed. In duplicate experiments the ratio, weight of tissue water/weight of wet tissue, was 0·77 and 0·76.

DISCUSSION

These results show that the salivary gland of the mouse provides a relatively simple iodide-trapping system which can be studied *in vitro*. It resembles the iodide trap of the thyroid so closely in many respects that it is reasonable to suppose that the mechanism may be similar, but it can be studied more easily in the salivary gland as the iodide trap of the thyroid is intimately linked with the subsequent processes involved in the synthesis of organic iodine. There was no evidence that this occurred in the salivary gland during the period of these experiments, and the uptake of iodide by the gland was not affected by drugs such as methylthiouracil which act by inhibiting the synthesis of hormone. The relative efficacy of the anions ClO_4^-,

SCN^-, $^{127}I^-$ and NO_3^- in depressing the uptake of $^{131}I^-$ by the tissue slices was closely analogous to their effect on the iodide trap of the thyroid *in vivo* (Wyngaarden, Wright & Ways, 1952; Wyngaarden, Stanbury & Rapp, 1953) and *in vitro* (Freinkel & Ingbar, 1955), and on the secretion of radioiodine in human saliva (Edwards *et al.* 1954). The uptake of $^{131}I^-$ by the salivary gland is so sensitive to ClO_4^- that the tissue-slice technique could probably be used for biological estimation of this anion, which is notoriously difficult to estimate chemically; it has become important to study the pharmacology of this anion as it is now being used in the treatment of thyrotoxicosis.

Scatchard & Black (1949), using a pure solution of albumin, found that the combining powers of ClO_4^-, SCN^-, I^- and NO_3^- for protein were in the order $ClO_4^- > SCN^- = I^- > NO_3^-$. Fig. 2 shows that the concentration of iodide by the salivary slices satisfies the Langmuir adsorption equation and hence it is possible that adsorption of I^- is a stage in the trapping mechanism. Moreover, the similarity between the curves for ClO_4^-, SCN^- and I^- in Fig. 3, and the summation of the effects of ClO_4^- and SCN^- (Table 1) suggest that these ions act in a similar way and at the same point in the iodide trap. Competitive adsorption would thus appear to be a possible explanation of their inhibitory effects.

Our observations on the concentration of endogenous SCN^- in the tissue, however, seem to be in serious disagreement with this theory. Both ClO_4^- and $^{127}I^-$ failed to discharge the endogenous SCN^- from tissue slices, and only a proportion of it could be eluted even after an hour's immersion in a large volume of saline. On the other hand, although the tissue was almost saturated with endogenous SCN^-, the uptake of $^{131}I^-$ was inhibited by adding SCN^- to the suspending medium in a concentration which was small compared with that already present in the tissue. Moreover, the uptake of $^{131}I^-$ was not enhanced in tissue slices from which 60% of the endogenous SCN^- had been previously eluted by prolonged immersion in a large volume of saline. The most likely explanation of these findings is that the endogenous SCN^- in the tissue slices is not freely exchangeable with the medium and plays no part in inhibiting I^- uptake; experiments using radioactive SCN^- would show whether the salivary glands of the mouse contain a non-exchangeable pool of SCN^-. This high concentration of endogenous SCN^- may be peculiar to the mouse since the concentration in human and guinea-pig salivary glands is about the same as, or only a little above, that in the plasma. Wood & Kingsland (1950) were unable to demonstrate concentration of SCN^- by the thyroid tissue of the rat. Hence our experimental results with SCN^- are not necessarily inconsistent with the hypothesis of competitive adsorption of a variety of anions in the salivary gland. The observation that concentration of I^- by the salivary gland *in vivo* is not depressed by the relatively high amount of SCN^- present in the plasma could possibly be explained by inactivation of this SCN^- by binding to one of the plasma constituents.

Whatever the precise mechanism for concentrating I^- may be, the action of dinitrophenol in depressing both oxygen consumption and uptake of I^- indicates that high-energy phosphate bonds may provide most of the energy.

SUMMARY

1. It has been found that slices of salivary glands of the mouse concentrate [^{131}I]iodide 5–10 times from the surrounding medium.

2. All the ^{131}I in the gland is present as iodide; no evidence of organic synthesis has been obtained.

3. Concentration of [^{131}I]iodide is inhibited by the addition of perchlorate, thiocyanate, iodide and nitrate to the medium, the order of activity being perchlorate > thiocyanate = iodide > nitrate, as *in vivo*.

4. The amount of endogenous thiocyanate in the gland and plasma is high compared with the amount which has to be added to the medium to inhibit the concentration of [^{131}I]iodide.

5. The addition of 2:4-dinitrophenol to the medium depresses both the respiration of the slices and their concentration of [^{131}I]iodide, indicating that energy is probably necessary for the process of concentration.

6. Evidence is adduced for a possible competitive-adsorption process to explain the mechanism of concentration.

REFERENCES

Aldridge, W. N. (1945). *Analyst*, **70**, 474.
Collins, K. J. & Hellmann, K. (1955). *J. Physiol.* **128**, 49*P*.
Deutsch, W. (1936). *J. Physiol.* **87**, 56*P*.
Edwards, D. A. W., Fletcher, K. & Rowlands, E. N. (1954). *Lancet*, **266**, 498.
Freinkel, N. & Ingbar, S. H. (1955). *J. clin. Endocrin. Metabolism*, **15**, 598.
Gross, J., Leblond, C. P., Franklin, A. E. & Quastel, J. H. (1950). *Science*, **111**, 605.
Honour, A. J., Myant, N. B. & Rowlands, E. N. (1952). *Clin. Sci.* **11**, 447.
Rowlands, E. N., Edwards, D. A. W. & Honour, A. J. (1953). *Clin. Sci.* **12**, 399.
Scatchard, G. & Black, E. S. (1949). *J. phys. Chem.* **53**, 88.
Thibault, O. & Pitt-Rivers, R. (1955). *Lancet*, **268**, 285.
Wood, J. L. & Kingsland, N. (1950). *J. biol. Chem.* **185**, 833.
Wyngaarden, J. B., Stanbury, J. B. & Rapp, B. (1953). *Endocrinology*, **52**, 568.
Wyngaarden, J. B., Wright, B. M. & Ways, P. (1952). *Endocrinology*, **50**, 537.