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The Accumulation of Iodide by *Fucus ceranoides*

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(Received 6 May 1957)

The high concentrations of iodide found in certain marine algae suggest that these plants have mechanisms for accumulating iodide from sea water (e.g. Jacques & Osterhout, 1938). The uptake of radioactive iodide has been demonstrated in *Ascophyllum nodosum* (Kelly & Baily, 1951; Kelly, 1953), *Laminaria flexicaulis* (Roche & Yagi, 1952) and *Nereocystis luetkeana* (Tong & Chaikoff, 1955). In work described in the present paper the uptake of radioactive iodide by *Fucus ceranoides* from sea water appears to involve both a carrier mechanism and binding of the ion within the tissue.

EXPERIMENTAL

Special chemicals. Iodide, perchlorate, thiocyanate and nitrate were all used as solutions of their potassium salts. Carrier-free radioactive iodide ($^{131}\text{I}^-$) in 0.01 N-sodium thiosulphate from the Radiochemical Centre, Amersham, was diluted with water. Samples of 3-iodo-L-tyrosine and 3,5-di-iodo-L-tyrosine were dissolved in *n*-butanol for use as marker substances on paper chromatograms. Solutions of 2,4-dinitrophenol (DNP) in 0.025 N-NaOH and *p*-chloromercuribenzoate in 0.1 N-NaOH were neutralized with HCl before use. Methylthiouracil was dissolved in 0.1 N-NaOH and added to media containing equivalent amounts of HCl. Solutions of 2-mercapto-1-methylimidazole were made in

water. Sea water was filtered through Whatman no. 1 paper before use. It was assumed to have the usual iodide content of about 5 $\mu\text{g.}/100\text{ ml.}$ Artificial sea water was prepared according to Lyman & Fleming (1940).

Tissue preparation. *Fucus ceranoides* was collected at low tide from the shore of Southampton Water. Portions of the fronds from the region between 3 and 25 mm. behind the growing tip were cut into fragments of 10–20 mg. fresh wt. This tissue was washed eight times with filtered sea water, spread on filter paper for 30 sec. to remove excess of moisture and portions were weighed out for experiments. The dry weight of the tissue was 10–15% of the fresh weight.

Incubation procedures. All experiments (except measurements of oxygen uptake) were performed at room temperature (15–18°), which remained constant during individual experiments. The medium in all cases consisted of at least 90% (v/v) sea water. The concentration of thiosulphate due to $^{131}\text{I}^-$ added to the medium did not exceed 10 $\mu\text{M.}$ Even 1000 $\mu\text{M.}$ thiosulphate had no effect on uptake of $^{131}\text{I}^-$.

In experiments in which the radioactivity in the medium was measured at successive times (Tables 1 and 2) 2 g. of tissue was added to 50 ml. of medium in a 250 ml. flask. Except where inhibitors were allowed to act for longer periods, 2–5 $\mu\text{C.}$ of $^{131}\text{I}^-$ was added within 10 min. of mixing tissue and medium, and the flask placed on a shaker. Portions of the medium were decanted at intervals for measurement of radioactivity and then returned to the flask. In other experiments (except in Fig. 1, Expt. C) incubation was started by mixing the complete medium with the tissue. For comparison with uptake of thiocyanate, the uptake of $^{131}\text{I}^-$ by 3 g. of tissue was measured from 7.5 ml. of

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medium. The uptake of $^{131}\text{I}^-$ in duplicate flasks did not differ by more than 5% in the experiments reported in Tables 1 and 2 nor by more than 2% in other experiments.

The loss of $^{131}\text{I}^-$ from the tissue under conditions of uptake of iodide was measured as the radioactivity appearing in media shaken with tissue which had previously been incubated in the presence of $^{131}\text{I}^-$.

When tissue was washed free of perchlorate and thiocyanate it was shaken three times for 5 min. with 50 ml. of sea water.

Uptakes of thiocyanate and $^{131}\text{I}^-$ were compared by shaking 3 g. (fresh wt.) of tissue with 7.5 ml. of medium consisting of sea water to which was added either thiocyanate or iodide and $^{131}\text{I}^-$. Thiocyanate was added only after the tissue had been incubated with sea water for 10 min. in order to diminish its natural iodide content. At the end of incubation the medium was decanted for thiocyanate estimation or measurement of radioactivity. Data from duplicate vessels did not differ by more than 5%.

The release of $^{131}\text{I}^-$ from the tissue by heating was measured by the increase of radioactivity in the medium after heating the flask for 2.5 min. in a boiling-water bath, during which time the temperature of the flask contents rose to about 95°. When $^{131}\text{I}^-$ was released from the tissue by trichloroacetic acid (TCA), 10 ml. of 30% (w/v) TCA was added to 50 ml. of medium at the end of incubation and the radioactivity in the supernatant measured 2–3 hr. later.

Oxygen uptake was measured in the dark in Warburg manometers at 22° after equilibration for 10 min. Conical Warburg flasks contained 3 ml. of medium and 0.4 g. of tissue (fresh wt.) in the main compartment and 0.2 ml. of 2*N*-NaOH and filter paper in the centre well. The rate of oxygen uptake in duplicate flasks did not differ by more than 7%.

Measurement of radioactivity. A beta-counter tube (liquid, type M6, 20th Century Electronics Ltd.) was used. In experiments in which the radioactivity in individual flasks was measured at successive times (Tables 1 and 2) the radioactivity in 10 ml. of medium was measured for 1 min. In other experiments the radioactivity was measured for a time sufficient to give a standard counting error of less than $\pm 1\%$.

Chromatography. Chromatograms of material containing $^{131}\text{I}^-$ were run in both the collidine and butanol-acetic acid solvents of Taurog, Tong & Chaikoff (1950), with Whatman no. 1 paper. In all cases $^{131}\text{I}^-$ added to similar material derived from tissue incubated in the absence of $^{131}\text{I}^-$ served as a control. Tissue treated with TCA was used for chromatography either as a finely ground suspension or was first hydrolysed with 10% (w/v) $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$ for 8 hr. at 100°, and the pH adjusted to 2 with *N*-HCl, and extracted with *n*-butanol. Chromatograms were prepared from the butanol extract with and without the addition of mono- or di-iodotyrosine. Radioactive areas on the chromatograms were detected by exposing the paper to Kodak Code 6 X-ray film for 1–5 days. The film was then developed in Ilford ID-19 developer and fixed with Ilford IF-9 fixing salt. To identify the position of the added amino acids the chromatograms were sprayed with 0.25% (w/v) ninhydrin in water-saturated *n*-butanol and heated in an oven at 150°.

Iodide estimation. The medium was filtered and stored at 0°. After centrifuging for 30 min. at 2000 *g*, iodide in the medium was estimated by the method of Rogina & Dubravcic (1953). Standards were prepared by adding known concen-

trations of iodide to sea water which had previously been incubated with tissue for the same time as the test medium. Duplicate estimations did not differ by more than 2%.

Iodide was also estimated by making use of the fact that the rate of uptake of $^{131}\text{I}^-$ by the tissue depends on the iodide concentration in the medium. Test media and standards prepared as for chemical estimation were incubated with fresh tissue and $^{131}\text{I}^-$. The rate of uptake of $^{131}\text{I}^-$ in duplicate vessels did not differ by more than 5%. The results by this method agreed with those of chemical estimation.

Thiocyanate estimation. A 5 ml. sample of the medium was centrifuged after the addition of 1 ml. of 30% (w/v) TCA. Thiocyanate in the medium was estimated by the method of Aldridge (1945) when the concentration was less than 10 μM . Higher concentrations were either diluted or estimated by a modification of the method of Bowler (1944), in which 5 ml. of 0.2*M*- FeCl_3 in 2% HNO_3 was added to 5 ml. of TCA supernatant. Sea water which had previously been incubated with tissue under the same conditions as the medium containing thiocyanate was used as a blank and for preparing standards.

Expression of results. The radioactivity taken up by the tissue or recovered in the medium is expressed as a percentage of the total radioactivity added to the flask. The percentage of the total radioactivity taken up by the tissue is calculated as 100 minus the percentage of the total radioactivity remaining in the medium.

RESULTS

Uptake of $^{131}\text{I}^-$ from sea water. *F. ceranoides* tissue incubated aerobically at room temperature in sea water without added substrate rapidly took up $^{131}\text{I}^-$ (Table 1). After 15 min. 2 g. of tissue had taken up about 50% of the radioactivity in 50 ml. of sea water, so achieving a concentration of radioactivity in the tissue about 25 times higher than in the medium. Tissue which had been heated for 2.5 min. in a boiling-water bath was permeable to $^{131}\text{I}^-$, but the concentration of radioactivity in the tissue did not rise above that in the medium. The rate of uptake of $^{131}\text{I}^-$ by fresh tissue was diminished by about half by cooling to 0° and by the presence of 100 μM -cyanide, 10 μM -2,4-dinitrophenol (DNP), or 50 μM -*p*-chloromercuribenzoate. It was also found that the rate of uptake of $^{131}\text{I}^-$ was independent of illumination but was much slower in artificial sea water, with or without traces of added iodide, than in natural sea water.

In other experiments the oxygen uptake of fresh tissue remained constant for at least 1–1.5 hr. with a Q_{O_2} ($\mu\text{l. of O}_2/\text{mg. dry wt. of tissue/hr.}$) of approx. 1.5–2, and was unaffected by *p*-chloromercuribenzoate at concentrations up to 200 μM , but was inhibited (80%) by 1000 μM -cyanide and increased (50%) by 50 μM -DNP.

Effect of added iodide on uptake of $^{131}\text{I}^-$. The rate of uptake of $^{131}\text{I}^-$ from sea water was reduced by adding iodide (Table 2A) and above a concentration of 200 μM there was no measurable uptake of radio-

Table 1. Uptake of $^{131}\text{I}^-$ from sea water by *Fucus ceranoides* tissue

Fucus tissue (2 g.) was incubated with 50 ml. of sea water containing added $^{131}\text{I}^-$, and the radioactivity in the supernatant measured at 15 min. intervals. Temp., 18°, except in flasks incubated on ice at 0°. Tissue was inactivated by heating for 2-5 min. in a boiling-water bath. Inhibitors were added to the tissue 20 min. before beginning incubation with $^{131}\text{I}^-$.

Conditions	Concn. of inhibitor (μM)	Radioactivity in tissue at times shown (% of total added)			
		15 min.	30 min.	45 min.	60 min.
Control	—	50	71	81	84
Incubated at 0°	—	21	38	48	54
Heat-inactivated	—	3	3	4	3
Cyanide added	{ 100	21	38	48	56
	{ 1000	11	24	33	38
DNP added	{ 10	36	50	63	68
	{ 100	19	23	30	36
p-Chloromercuribenzoate added	50	26	41	51	55

Table 2. Effect of added iodide on uptake of $^{131}\text{I}^-$

Details are as for the control in Table 1 except that iodide was added to the medium either at the start of incubation (A) or after incubation for 30 min. (B). Temp., 17°.

Expt.	Concn. of added iodide (μM)	Radioactivity in tissue at times shown (% of total added)				
		15 min.	30 min.	45 min.	60 min.	75 min.
A	0	60	78	89	90	—
	5	25	43	56	67	—
	10	15	29	40	48	—
	50	6	8	11	16	—
	200	2	3	4	3	—
B	0	44	63	72	78	81
	5	44	63	58	62	66
	200	44	63	48	41	36

Table 3. Net uptake of iodide by *Fucus ceranoides* tissue

Combined data from three experiments showing radioactivity and iodide concentration remaining in the supernatant after 2 g. of tissue had been incubated for various times with 50 ml. of sea water containing $^{131}\text{I}^-$ and a known initial concentration of added iodide. Iodide was in all cases estimated chemically. The calculation of the iodide concentration remaining was based on the assumption that the specific activity of iodide in the medium remained constant and that the natural iodide content of sea water was negligible.

Initial concn. of added iodide (μM)	Radioactivity in supernatant after incubation (% of total added)	Concn. of iodide in supernatant after incubation (μM)	
		Calc.	Found
2.5	66	1.7	1.7
5	47	2.4	2.3*
5	{ 55	2.8	3
	{ 31	1.6	1.6
10	57	5.7	5.8

* Iodide was also measured by the rate of uptake of $^{131}\text{I}^-$ by fresh tissue. The latter rate was intermediate between the rates of uptake from comparable media containing 2.25 and 2.5 μM concentration of added iodide.

activity by the tissue from the medium. With concentrations up to 1000 μM of added iodide there was no effect on the respiration.

When iodide was added after the beginning of incubation (Table 2B) radioactivity was released from the tissue in a form chromatographically similar to inorganic iodide. This release of $^{131}\text{I}^-$, when brought about by adding 5 μM -iodide, was followed by a further net uptake of $^{131}\text{I}^-$ by the tissue. Iodide concentrations above 200 μM caused a progressive release of $^{131}\text{I}^-$ from the tissue, the greater part of which occurred within 15 min. of adding iodide. Only small additional amounts of radioactivity were lost after 45 min. In other experiments all concentrations of iodide above 200 μM released $^{131}\text{I}^-$ at about the same rate, 40-50 % of the tissue radioactivity being lost in 45 min.

Net uptake of iodide. Estimation of iodide in the medium showed that the tissue took up iodide during incubation. The concentration of iodide remaining after incubation, as found by chemical estimation, was the same as calculated from the percentage of the total radioactivity remaining in the medium, assuming that the specific activity of the iodide had not changed (Table 3). This was

confirmed in other experiments in which the concentration of iodide in the medium was measured by the rate of uptake of $^{131}\text{I}^-$ by fresh tissue. In the following experiments it is therefore assumed that the uptake of a certain percentage of the radioactivity represents the uptake of an equal percentage of the iodide in the medium.

Variation of initial rate of uptake of iodide with iodide concentration. The initial rate of uptake of iodide by the tissue (v) varied with the initial concentration of iodide in the medium (s) in a manner such that the plot of s/v against s was a straight line, as if the rate of uptake depended on a reversible combination of iodide with a site in the tissue (Lineweaver & Burk, 1934). Fig. 1 shows the results of experiments which together extend over the concentration range 1–20 μM of added iodide. At higher concentrations the rate of uptake of $^{131}\text{I}^-$ was too slow for accurate measurement. In several different experiments the calculated maximum velocity of uptake of iodide varied between 0.25 and 0.45 μmole of iodide/g. of fresh tissue/hr., but approximately constant values were found for the quantity K_m (iodide concentration at half-maximum velocity of uptake). The values of K_m in Expts. A and B (4.8 and 4.7 μM) become approx. 4.3 μM if sea water is assumed to contain 0.5 μM -iodide (K_m calculated from the value of s/v when concentration of added iodide = $-0.5 \mu\text{M}$).

These experiments had to be of sufficient duration to permit the uptake of a significant amount of radioactivity at high concentrations of iodide, but this meant that the radioactivity in the media with low concentrations of iodide diminished by as much as 50%. In Expt. C therefore the volumes of media with low concentrations of iodide were increased so that in all cases only about 20% of the radioactivity was taken up, and the overall rate of uptake at low iodide concentrations approximated more closely to the initial velocity. This experiment was performed in such a way that much of the iodide occurring naturally in sea water had been removed by the tissue before the beginning of incubation with $^{131}\text{I}^-$. The K_m value of 4.3 μM should therefore be compared with the corrected value (approx. 4.3 μM) for Expts. A and B.

Loss of $^{131}\text{I}^-$ from the tissue under conditions of uptake of iodide. When tissue which had been incubated in a medium containing $^{131}\text{I}^-$ was placed in a similar medium without $^{131}\text{I}^-$, radioactivity from the tissue appeared in the latter medium. This radioactivity was not extractable into carbon tetrachloride and behaved in the same way as inorganic iodide on paper chromatograms. The amount of $^{131}\text{I}^-$ so transferred reached a maximum in about 15 or 30 min. depending on the duration of the previous incubation with $^{131}\text{I}^-$, and then diminished as it was again taken up by the tissue (Table 4).

Fig. 1. Variation of initial rate of uptake of iodide with iodide concentration. Media containing $^{131}\text{I}^-$ and added iodide were incubated at 17° with 2 g. of tissue. Averages of duplicate data are plotted according to Lineweaver & Burk (1934). The initial rate of iodide uptake (v) is given by the amount of iodide taken up during a constant time. Since v is proportional to the initial iodide concn. in the medium (s) multiplied by % of total radioactivity taken up, $1/(\% \text{ of total radioactivity taken up})$ is plotted as the ordinate instead of s/v . In all cases '% of total radioactivity taken up' is expressed as the % taken up from 50 ml. of medium. The concn. of added iodide is plotted as the abscissa instead of the actual initial iodide concn. s . In this way data can be plotted directly and the concn. of iodide normally present in sea water neglected. Straight lines were fitted by the method of least mean squares. In Expt. A ($K_m = 4.8 \mu\text{M}$), tissue was incubated with 50 ml. of medium for 10 min. Expt. B ($K_m = 4.7 \mu\text{M}$) was similar except that incubation was for 30 min. and that the volume of medium in the flask with the lowest iodide concn. (2.5 μM) was 100 ml. In this flask 40% of total radioactivity was taken up, which was assumed to be equivalent to 80% taken up from 50 ml. In Expt. C ($K_m = 4.3 \mu\text{M}$), iodide and $^{131}\text{I}^-$ were added together to tissue already suspended in sea water and incubation was continued for 10 min.; 17–22% of total radioactivity was taken up in all flasks since the volumes of medium were 100, 75, 50 and 50 ml. in flasks containing respectively 1, 3, 6 and 9 μM concn. of added iodide. For flasks containing 100 or 75 ml. of medium the value for '% of total radioactivity taken up from 50 ml.' was derived as in Expt. B.

Table 4. *Loss of $^{131}\text{I}^-$ under conditions of net uptake of iodide*

Tissue (2 g.) and 50 ml. of medium containing added iodide as indicated were incubated at 17° either with or without $^{131}\text{I}^-$. After 5 or 30 min. the supernatant was decanted from those flasks which contained $^{131}\text{I}^-$. The tissue in these flasks was washed by shaking for 10 sec. with the supernatant from a similar flask without added $^{131}\text{I}^-$, and then incubated in a medium identical with that used for the washing procedure. The radioactivity which appeared in this medium is expressed as percentage of the total radioactivity originally added.

Concn. of added iodide (μM)	Time incubated with $^{131}\text{I}^-$ (min.)	Radioactivity in tissue (% of total added)	Radioactivity in initially non-radioactive medium at times shown (% of total added to radioactive medium)			
			5 min.	15 min.	30 min.	45 min.
0	5	23	1.5	2.0	1.4	1.2
	30	69	1.6	3.2	4.3	3.2
10	5	5	1.2	1.6	1.5	1.3
	30	21	1.5	2.4	3.4	3.2

Table 5. *Inhibition of uptake of iodide by perchlorate and thiocyanate*

Inhibitor, iodide and $^{131}\text{I}^-$ in 50 ml. of medium were added to 2 g. of tissue in flasks on a shaker. The supernatant was decanted for estimation of radioactivity after incubating at 18° for 10 or 20 min. (added iodide concn. 2.5 or 10 μM respectively).

Inhibitor	Concn. of added iodide (μM)	Concn. of inhibitor (μM)	Radioactivity in tissue (% of total added)	Uptake of radioactivity in the presence of inhibitor (% of uptake in control)
KClO_4	2.5	0	22	100
		0.5	13	59
		1	10	45
	10	0	17	100
		2	10	59
		4	8	47
KSCN	2.5	0	34	100
		100	19	56
		200	15	44
	10	0	28	100
		400	15	54
		800	11	39

The initial rate of release of $^{131}\text{I}^-$ from the tissue, as given by the amount lost in the first 5 min., did not decrease as the percentage of the radioactivity remaining in the medium decreased. It is therefore unlikely that the loss of $^{131}\text{I}^-$ took place from the intercellular spaces of the tissue. The amount of $^{131}\text{I}^-$ lost in the first 5 min. was small compared with the total amount of $^{131}\text{I}^-$ added initially, but was a fairly large proportion of the radioactivity taken up by the tissue and the initial rate of loss of $^{131}\text{I}^-$ was scarcely affected by the amount of $^{131}\text{I}^-$ which had been taken up by the tissue.

Inhibition of iodide uptake by perchlorate, thiocyanate and nitrate. Perchlorate (0.1 μM), thiocyanate (10 μM) and nitrate (1000 μM) reduced the rate of uptake of $^{131}\text{I}^-$ from sea water by about 50 %. Higher concentrations of all three ions were required to inhibit uptake of $^{131}\text{I}^-$ when iodide was added. However, these ions, when tested in other experiments at concentrations up to 500, 2000 and

8000 μM respectively, had no effect on the respiration of the tissue.

Table 5 shows that when the concentrations of added iodide and of perchlorate or thiocyanate were increased by the same proportionate amount the percentage inhibition of uptake of $^{131}\text{I}^-$ remained about the same. Other experiments showed that the effect of the inhibitors was the same whether they were added to the tissue together with iodide or beforehand and that the inhibition was reversible, because after washing with sea water the tissue took up $^{131}\text{I}^-$ at the same rate as an untreated control. These results suggest that perchlorate, thiocyanate and perhaps nitrate act as competitive inhibitors of uptake of iodide.

The effects of the three inhibitors were compared in the presence of 20 μM -iodide (Table 6). If n is the rate of iodide uptake in the presence of a competitive inhibitor (expressed as a percentage of the control rate) the expression $(n/100 - n) \times (\text{inhibitor}$

concn./iodide concn.) is equal to the ratio (inhibitor concn.:iodide concn.) at 50% inhibition. In Table 6 this expression gives only approximately constant values, perhaps because it assumes that the combining site is saturated, whereas 20 μM -iodide corresponds to about 80% saturation only. However, the results indicate that the affinity for the combining site decreased in the order perchlorate, thiocyanate, nitrate.

Release of $^{131}\text{I}^-$ from tissue by perchlorate and thiocyanate. The addition of perchlorate or thiocyanate to tissue which had been incubated with $^{131}\text{I}^-$ released radioactivity from the tissue in a form chromatographically similar to inorganic iodide. Thiocyanate released more $^{131}\text{I}^-$ in this way than did perchlorate but the effect of both ions was reduced by raising the iodide concentration (Table 7). Thus 400 μM -perchlorate or -thiocyanate apparently released only small amounts of radioactivity from tissue which had taken up $^{131}\text{I}^-$ from sea water containing 2.5 μM -iodide.

Uptake of thiocyanate. *Fucus* tissue rapidly took up thiocyanate from sea water. The greater part of this uptake occurred in the first 5–10 min., and

after 30 min. the rate of uptake was very slow. Table 8 shows that at all concentrations tested the tissue took up about two-thirds as much thiocyanate as iodide in 30 min.; at the lower concentrations the ratio (tissue concn.:medium concn.) was considerably less for thiocyanate.

Form of radioactivity in the tissue. When tissue which had taken up $^{131}\text{I}^-$ was heated for 2.5 min. in a boiling-water bath or treated with 5% TCA, the radioactivity within the tissue was released in a form which behaved on paper chromatograms in the same way as inorganic iodide. Although heating appeared to release all the radioactivity, a proportion, increasing in amount with the duration of previous incubation, was retained after adding TCA (Table 9), and was released as iodide only by heating in a boiling-water bath.

Chromatograms of finely ground TCA-treated tissue showed radioactivity both in the form of iodide and in material which remained at the origin. After hydrolysis of similar material with barium hydroxide almost all the radioactivity behaved like inorganic iodide, but chromatography of butanol extracts showed traces of radioactivity

Table 6. *Inhibition of uptake of iodide by perchlorate, thiocyanate and nitrate*

Details are as in Table 5 except that all flasks contained added iodide (20 μM) and were incubated for 40 min. at 16°. The expression $n/(100 - n) \times (\text{inhibitor concn.}/\text{iodide concn.})$ gives the relative affinity of iodide or inhibitor for a combining site involved in uptake of iodide, where n is the rate of iodide uptake in the presence of the inhibitor expressed as a percentage of the control rate (e.g. Krebs, Gurin & Eggleston, 1952).

Inhibitor	Concn. of inhibitor (μM)	Radioactivity in tissue (v) (% of total added)	$\left(\frac{v}{25} \times 100\right)$ (n)	$\left(\frac{n}{100 - n} \times \frac{i}{20}\right)$
—	—	25	100	—
KClO_4	2	15	60	0.15
	4	11	44	0.16
	8	8	32	0.19
KSCN	400	13	52	21.6
	800	9	36	22.5
	1600	7	28	31.2
KNO_3	4000	19	76	635
	8000	16	64	710

Table 7. *Release of $^{131}\text{I}^-$ from tissue by perchlorate and thiocyanate*

Tissue (2 g.) was incubated at 15° with 50 ml. of sea water containing $^{131}\text{I}^-$ and in some cases added iodide as shown. After 30 min. 400 μM -perchlorate or -thiocyanate was added. Release of $^{131}\text{I}^-$ is shown by the fall of radioactivity in the tissue as compared with the amount at 30 min.

Concn. of added iodide (μM)	Inhibitor added after 30 min.	Radioactivity in tissue at times shown (% of total added)				
		15 min.	30 min.	45 min.	60 min.	75 min.
0	—	48	72	83	86	86
	KClO_4	48	72	69	67	63
	KSCN	48	72	64	57	55
2.5	—	26	46	60	70	75
	KClO_4	26	46	46	45	45
	KSCN	26	46	46	45	44

Table 8. *Comparison of uptake of thiocyanate and iodide*

Thiocyanate or iodide plus $^{131}\text{I}^-$ in 7.5 ml. of medium was incubated with 3 g. of tissue for 30 min. at 15° . Thiocyanate was added only after the tissue had been previously incubated with sea water for 10 min. Tissue concn./medium concn. is taken as $(7.5 \times \% \text{ in tissue}) / (3 \times \% \text{ in medium})$.

Concn. of added iodide or thiocyanate (μM)	Iodide		Thiocyanate	
	In tissue (% of total radioactivity added)	Tissue concn. Medium concn.	In tissue (% of total added)	Tissue concn. Medium concn.
2.5	98	122	65	5
10	96	60	50	3
50	55	3	34	1
500	30	1	21	1

Table 9. *Release of $^{131}\text{I}^-$ from tissue by heating or with trichloroacetic acid*

Flasks containing 2 g. of tissue and 50 ml. of medium composed of sea water and $^{131}\text{I}^-$ were incubated at 18° for the times shown. Radioactivity in the supernatant was measured before and after either heating the flasks for 2.5 min. in a boiling-water bath or adding 10 ml. of 30% (w/v) TCA. Recovery of 96% of the total radioactivity in the supernatant after heating and 97% after adding TCA indicates an approximately similar concentration of radioactivity in tissue and medium.

Treatment	Time incubated (min.)	Radioactivity (% of total added)		
		In tissue after incubation	After treatment	
			In supernatant	Retained by tissue
Heated	0	0	96	0
	15	56	96	0
	30	70	96	0
	45	76	96	0
TCA	0	0	97	0
	15	53	90	7
	30	73	87	10
	45	78	85	12

Table 10. *Effect of methylthiouracil and 2-mercapto-1-methylimidazole on the accumulation of trichloroacetic acid-stable form of radioactivity*

Details are as in Table 9 except that $4000 \mu\text{M}$ -methylthiouracil or -2-mercapto-1-methylimidazole was added to flasks other than the control and that TCA was added to all flasks. After incubation for 45 min. the tissue had in all cases taken up 95% of the total radioactivity added.

Inhibitor	Time incubated (min.)	Radioactivity found after adding TCA (% of total added)	
		In supernatant	Retained by tissue
—	0	97	0
—	45	90	7
Methylthiouracil	45	96	1
2-Mercapto-1-methylimidazole	45	97	0

of similar R_f to those of added mono- and di-iodotyrosine.

Effect of methylthiouracil and 2-mercapto-1-methylimidazole. Both the rate of uptake of $^{131}\text{I}^-$ and of respiration were unaffected by methylthiouracil and 2-mercapto-1-methylimidazole even when these substances had been in contact with the tissue for 30 min. before incubation. However, both substances inhibited the accumulation within the tissue

of the TCA-stable form of radioactivity (Table 10). In order to demonstrate these effects high concentrations ($4000 \mu\text{M}$) had to be used.

DISCUSSION

The results presented in this paper show that, as with *Ascophyllum nodosum* (Kelly, 1953) and *Nereocystis luetkeana* (Tong & Chaikoff, 1955),

uptake of $^{131}\text{I}^-$ by *F. ceranoides* was reduced when the respiration was inhibited, suggesting that energy derived from respiration was required to establish the apparent concentration gradient between tissue and medium. Dinitrophenol may have inhibited uptake of $^{131}\text{I}^-$ by uncoupling oxidative phosphorylation, since it was shown to stimulate respiration.

Compared with the energy available from respiration the energy required for transport of iodide in *F. ceranoides* appears to be small. The change of free energy required to bring 1 g.mol. of iodide from concentration c_1 to c_2 is given by $RT \ln c_1/c_2$, where R is the gas constant (1.987 cal./degree) and T the absolute temperature. A maximum iodine content of about 0.1% in dried *Fucus* (e.g. Black, 1949) would correspond to approx. 1000 μM -iodide in the fresh tissue if all iodine occurred as iodide ions and assuming that the fresh weight:dry weight ratio of the tissue was 10. The energy required at 20° for iodide transport from 10 μM external concentration at a maximum velocity of 0.5 $\mu\text{mole/g.}$ of fresh tissue/hr. would then be about 1.3×10^{-3} cal./g. of fresh tissue/hr. In contrast the energy available from respiration was about 0.5 cal./g. of fresh tissue/hr., as calculated from a Q_{O_2} of 1.5 $\mu\text{l.}$ and assuming a phosphorylation quotient of 3. Therefore uptake of iodide at the maximum rate apparently required less than 1% of the respiratory energy. The actual energy requirement for uptake of iodide may have been even less since much of the iodine in the tissue probably does not behave as iodide ions.

The manner in which the rate of iodide uptake varied with the external iodide concentration suggested that the mechanism of uptake of iodide involved a reversible combination of the ion with a site in the tissue. Mitchell (1954) has presented similar evidence for a site which combines with phosphate in *Micrococcus pyogenes*. The fact that uptake of iodide by *F. ceranoides* was competitively inhibited by certain other ions also indicates the existence of a specific combining site for iodide. Similar conclusions regarding the absorption of Rb^+ and Br^- ions by barley roots have been drawn from the observation that rubidium absorption is inhibited competitively by K^+ and Cs^+ ions (Epstein & Hagen, 1952), and bromide absorption is inhibited in the same way by chloride (Epstein, 1953).

The suggested combining site for iodide may be a carrier group controlling the transport of the ion across some boundary in the tissue (e.g. Osterhout, 1952). The evidence that the combining sites for phosphate in *M. pyogenes* function only in a carrier capacity is supported by the fact that the number of sites is small compared with the amount of phosphate taken up (Mitchell, 1953, 1954). In this paper no direct evidence is presented that the number of

sites for iodide was small compared with the amount of iodide taken up. However, the fact that even high concentrations of competitive inhibitors of uptake of iodide released only a fraction of the $^{131}\text{I}^-$ which had been taken up by the tissue suggests that iodide stored within the tissue was not combined at the site concerned in its uptake from the medium. On the other hand, this does not necessarily imply that iodide accumulated within the tissue existed as the free ion.

Although $^{131}\text{I}^-$ was lost from the tissue under the conditions of uptake of iodide, it is unlikely that this loss occurred to any great extent from the region where the bulk of the iodide was accumulated. If the relationship between the initial rate of uptake of iodide and the external concentration is correctly interpreted to indicate the operation of a carrier mechanism, then a rapid and appreciable loss of $^{131}\text{I}^-$ during the initial period of incubation must have been due to a loss of iodide from a region other than that to which iodide was transferred by the operation of the carrier. It is possible that this loss of $^{131}\text{I}^-$ represented in part the dissociation of the carrier-iodide complex, since the combination of the carrier site with iodide in the medium appeared to be reversible. In keeping with this is the observation that the initial rate at which $^{131}\text{I}^-$ was lost from the cells was scarcely affected by the total amount of $^{131}\text{I}^-$ which had been taken up. On the other hand, the release of $^{131}\text{I}^-$ from the tissue by high concentrations of thiocyanate was presumably not due solely to the dissociation of a carrier-iodide complex since perchlorate, although a more effective competitive inhibitor of uptake of iodide, released less $^{131}\text{I}^-$ at high concentrations than did thiocyanate. Thiocyanate readily entered the tissue. It is therefore possible that, at very high concentrations relative to iodide, thiocyanate in some way displaced part of the iodide accumulated within the tissue.

Just as the $^{131}\text{I}^-$ remote from the carrier did not readily return to the medium under conditions of uptake of iodide, so the bulk of the iodide already in the tissue at the beginning of incubation did not participate in any transfer of I^- ions to the medium, since the specific activity of iodide in the medium remained constant during uptake of iodide. This failure of accumulated iodide within the tissue to return to the medium under physiological conditions may be due to the existence of permeability barriers or to binding of the ion in an indiffusible form. The breakdown of either of these would account for the release on heating of all $^{131}\text{I}^-$ which had been taken up. Cowie, Roberts & Roberts (1949) have suggested on similar grounds that potassium is concentrated within *Escherichia coli* in a bound form. As with concentration of iodide by *F. ceranoides*, the ability of *E. coli* to concentrate

potassium diminishes as the external concentration of the ion is raised, presumably because the binding sites for the ion approach saturation. Evidence for the binding of inorganic ions in plant cells has also been provided by studies of uptake of potassium by barley roots (Broyer & Overstreet, 1940) and of uptake of potassium and bromide by beetroot (Sutcliffe, 1954).

The only direct evidence for the formation of a labile complex involving iodine was that trichloroacetic acid failed to release about 10 % of the tissue radioactivity, and that this was in a form which remained at the origin on chromatograms but was released on heating. This trichloroacetic acid-stable complex was not formed in the presence of methylthiouracil or 2-mercapto-1-methylimidazole. In the thyroid gland these substances inhibit the formation of organic iodine compounds, probably by preventing the formation of iodine from iodide (Pitt-Rivers, 1950). The formation of the trichloroacetic acid-stable complex may therefore involve an oxidation of iodide. *F. ceranoides* did not, however, appear to form free iodine, unlike *Laminaria flexicaulis* (e.g. Roche, van Thoai & Lafon, 1949), although it resembled both the latter alga (Roche & Yagi, 1952) and *Nereocystis luetkeana* (Tong & Chaikoff, 1955) in forming small amounts of iodotyrosines.

The inhibition of uptake of iodide by perchlorate, thiocyanate and nitrate recalls the effect of these ions on uptake of iodide by the thyroid gland and also by certain other animal tissues, in particular salivary gland (Fletcher, Honour & Rowlands, 1956), mammary gland (Brown-Grant, 1957; Freinkel & Ingbar, 1956) and stomach mucosa (Halmi, Stuelke & Schnell, 1956; Logothetopoulos & Myant, 1956). Under natural conditions *F. ceranoides* and the thyroid both accumulate iodide from about the same external concentration. Metabolic inhibitors and sulphydryl reagents inhibit concentration of $^{131}\text{I}^-$ in the thyroid (Freinkel & Ingbar, 1955) as in *F. ceranoides*. Furthermore, perchlorate, thiocyanate and nitrate are competitive inhibitors of uptake of iodide by the thyroid gland and their relative activities are approximately the same as was found in the alga (Wyngaarden, Stanbury & Rapp, 1953). These ions, and also iodide, release $^{131}\text{I}^-$ from the thyroid (Vanderlaan & Vanderlaan, 1947; Stanley & Astwood, 1948; Wyngaarden, Wright & Ways, 1952) as from *F. ceranoides*.

On the other hand, iodide which is not in stable organic combination seems to be much more readily released by anions from the thyroid gland than from *F. ceranoides*. Iodotyrosines appear to be formed more rapidly and in larger amounts by the thyroid gland than by the seaweed. Furthermore, although both tissues take up thiocyanate, the thyroid, in

contrast to *F. ceranoides*, is unable to concentrate this ion from the medium (Wood & Kingsland, 1950). These differences may be due to the form in which iodide and perhaps thiocyanate accumulate in the two tissues, whereas the mechanism of uptake of iodide appears, in contrast, to be very similar in both.

SUMMARY

1. Pieces of *Fucus ceranoides* respiring without added substrate rapidly took up $^{131}\text{I}^-$ from sea water at room temperature. The rate of uptake of $^{131}\text{I}^-$ was diminished by about 50 % by incubation at 0° or by 100 μM -cyanide, 10 μM -dinitrophenol or 50 μM -*p*-chloromercuribenzoate. Respiration was inhibited by cyanide, increased by dinitrophenol and unaffected by *p*-chloromercuribenzoate.

2. The presence of added carrier iodide in the medium reduced the rate of uptake of $^{131}\text{I}^-$. Addition of carrier iodide after the beginning of incubation released $^{131}\text{I}^-$ from the tissue.

3. The uptake of a certain proportion of the total $^{131}\text{I}^-$ corresponded to the uptake of the same proportion of the iodide in the medium. The initial rate of uptake of iodide varied with the external concentration over the range 1–20 μM as if iodide uptake depended on a reversible combination with a site in the tissue. The half-maximum rate of uptake occurred at 4.3 μM -iodide.

4. During net iodide uptake there was a simultaneous movement of iodide out of the tissue cells, the rate of which was independent of the amount of iodide which had been taken up from any one external concentration.

5. Perchlorate, thiocyanate and nitrate acted as competitive inhibitors of uptake of iodide. At 50 % inhibition their respective concentrations were about 0.2, 30 and 700 times the concentration of iodide. At high concentrations perchlorate released small amounts of $^{131}\text{I}^-$ from the tissue. Thiocyanate released larger amounts, but in both cases this effect was diminished when added carrier iodide was present. Under similar conditions the tissue took up about two-thirds as much thiocyanate as iodide.

6. Heating the tissue apparently released all its $^{131}\text{I}^-$ in the form of inorganic iodide. Trichloroacetic acid released only 90 %. The remainder was in a form which remained at the origin on chromatograms but was released on heating. Hydrolysis with barium hydroxide gave rise to $^{131}\text{I}^-$ and traces of mono- and di-iodotyrosine. No radioactivity accumulated in the trichloroacetic acid-stable form during incubation in the presence of methylthiouracil or 2-mercapto-1-methylimidazole.

7. The data suggest that a specific site which combined reversibly with iodide transported the ion across a boundary in the cell and that iodide accumulated within the cells in a bound form. The

mechanism of uptake of iodide appeared to be very similar to that in the thyroid gland.

The author is grateful to Professor W. T. Williams for permission to use the Warburg respirometers in the Botany Department of Southampton University, and also wishes to thank Dr R. Pitt-Rivers, F.R.S., for a gift of 3-iodo-L-tyrosine, Imperial Chemicals (Pharmaceuticals) Ltd. for a gift of methylthiouracil, and British Schering Ltd. for a gift of 2-mercapto-1-methylimidazole.

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Studies in the Biochemistry of Micro-organisms

103. METABOLITES OF *ALTERNARIA TENUIS* AUCT.: CULTURE FILTRATE PRODUCTS*

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(Received 1 April 1957)

Raistrick, Stickings & Thomas (1953) have described the isolation of two metabolic products—alternariol (I) and one of its monomethyl ethers—from the mycelium of two strains of *Alternaria tenuis* auct. grown on Czapek–Dox solution. These

two compounds appear to constitute practically the whole of the ether-extractable, but petroleum-insoluble, fraction of the dried mycelium.

* Part 102: Birkinshaw, Chaplen & Lahoz-Oliver (1957).

It was noted during this work that the metabolism solution of these strains of *A. tenuis* gave a reddish brown colour reaction with ferric chloride. While small amounts of alternariol and its methyl ether could be isolated from the solution, it was soon clear that the material responsible for this ferric reaction was a complex mixture of these two compounds with a number of new substances.

It is the purpose of this paper to describe some of the main constituents of this mixture, and their separation and characterization. The study has been extended to two other strains of *A. tenuis*. Detailed consideration of the structures of the new compounds will be published later. It will be clear from the following account that the list of metabolic products is probably very incomplete, and indeed paper chromatography indicates the presence of