## IODINE EXCHANGE IN ASCOPHYLLUM

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In a preliminary study of iodine uptake in the brown algae (Kelly and Baily, 1951) the process was interpreted as involving an exchange between iodine in the cells and iodine in the sea water. The experiments described here were carried out to verify, elucidate and provide quantitative information on this process. In general, experimental data were obtained by measuring the radioactivity of segments of the brown alga, *Ascophyllum nodosum* (Linn.) LeJolis, and of solutions containing radioactive iodine to which the segments were exposed.

The experimental techniques followed those reported previously with the following exceptions:

1. The medium used as carrier for radioactive iodine was natural sea water rather than Van't Hoff artificial sea water plus iodide. The percentage of radioactive iodine removed from the natural sea water follows closely that from artificial sea water.

2. The liquid samples removed for counting were five ml. rather than two ml. in volume. The larger volume was used to reduce the variations in counting that result from pipetting errors.

3. The amount of radioactive iodine in solution was reduced from 14 to 0.1 microcuries per ml. since a more sensitive Geiger counter was used for sample counting.

4. By means of glycyl-glycine buffer, the pH of solutions during experimental periods was kept at eight to eliminate loss of iodine that occurs at lower pH.

To determine whether the exchange takes place only between living cells and sea water, the removal of radioactive iodine from solution by segments which had been killed by boiling and were no longer respiring was compared with that removed by living segments. After exposure to radioactive iodine for 1.6 hours, the living segments contained considerable radioactivity and the dead ones a negligible amount (Table I). The exchange, therefore, is characteristic of living cells only. This agrees with the previous finding (Kelly, 1953) that uptake of radioactive iodine is decreased by respiratory inhibitors.

To determine whether diffusion, a phenomenon occurring in non-living as well as in living systems, contributed to radioactive iodine removal from sea water

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	Counts per second in segments						
Exposure period (hours)	Living			Dead			
	1	2	3	1	2	3	
1.6 14	87.8	96.8	84.4	1.8 3.5	1.8 3.5	1.8 3.5	

Removal of radioactive iodine by living segments and by segments killed by boiling

during long exposure periods, the dead segments described above were replaced in their solutions and allowed to remain for fourteen hours. After this period, their radioactivity had increased only slightly (Table I). Diffusion into segments, then, does not materially contribute to the removal of radioactivity from solutions.

The form in which iodine is removed from sea water is of importance in elucidating the exchange process: iodides and iodates both occur in natural sea water. Removal of radioactive iodine was determined, therefore, in the presence of iodates only and in the presence of iodides only. A solution containing iodates only was obtained through conversion of iodides present in sea water (including added radioactive iodide) into iodates by oxidation with bromine water, followed by boiling to remove excess bromine. As seen in Table II, radioactive iodine removal by segments from solutions so treated is negligible. Exposure of segments for longer periods does not materially increase removal of iodate (Fig. 1). While an exponential uptake is indicated by this curve, the actual amount taken up by the algae is negligible when compared to our earlier results or to those which will be discussed in later sections of this paper. Iodine, then, is not removed from sea water in the form of iodates by this particular system.

A solution containing iodides only was obtained by reducing natural sea water (containing added radioactive iodide) with sodium bisulfite. The pH was adjusted to eight in order to prevent oxidation and iodine loss. The removal of radioactive iodine from such solutions was similar to that from untreated solutions

TABLE II

Removal of radioactive iodine by Ascophyllum segments exposed to solutions containing either iodides only or iodates only. Figures are those from a typical experiment

			Co	ounts per sec	ond		
Exposure period (hours)		Solu	ition		Segments		
	0	1	4	24	1	4	24
Iodide Iodate	16.3 14.9 52.2	7.9 14.9 50.2	49.6	49.3	100 0.8 0.7	0.8	2.1
Natural sea water	135	74.8	66.7 -	37.7	140	144	178



FIGURE 1. Exchange of potassium iodate ions between Ascophyllum and natural sea water containing I<sup>131</sup>. All iodine in this solution had been oxidized before the Ascophyllum segments were placed in the solution. These points represent the average of three determinations.

(Table II) and far exceeded that from solutions containing iodates only. The iodine removed from sea water by the alga is then in the form of iodides.

The removal of radioactive iodine from sea water over a fifteen- to twenty-hour period follows a definite pattern, interpreted previously as involving an exchange between iodine within the alga and iodine in the surrounding medium. It has further been suggested (Kelly and Baily, 1951) that this exchange process proceeds at two rates: a rapid rate between iodine in the intercellular spaces and the sea water, and a slower rate between iodine in the intercellular spaces and the cells themselves. Indeed, the physical makeup of the alga lends itself to this type of formulation, since the segments are made up largely of loosely packed cells.

The removal of radioactive iodine from sea water (Fig. 2) follows a curve with two distinct slopes. This curve can be obtained mathematically when the following mechanical model is assumed: We visualize a system made up of three separate compartments. The first such compartment is the environment in which the ascophyllum is placed, namely the radioactive sea water. The ascophyllum itself makes up the second and third compartments. These are the intercellular and cellular spaces.

The initial rapid increase of radioactivity in the segments comes about as iodide ions penetrate the outermost layer into the intercellular spaces of the "cortical"



FIGURE 2. Iodine exchange between Ascophyllum segments and a solution of natural sea water containing I<sup>131</sup>. This curve represents the results of a typical experiment.

region. Here, the entering ions exchange with those present in the intercellular spaces.

Since the ions in the intercellular spaces are readily accessible to the entering ions, the exchange rate between them is rapid. The ions in the intercellular spaces then penetrate the cell membranes and exchange with iodides within the cell. This exchange rate is less rapid, since penetration through membranes must also be achieved.

The initial slope of the curves indicates that the rate at which the iodide ions enter the intercellular spaces is much greater than that with which they enter the cellular space. However, as equilibrium between I<sup>128</sup> and I<sup>131</sup> ions in the intercellular space is approached, the slope of these curves approaches that which would be entirely due to the rate of ion transport from intercellular to cellular space. Finally, as the curve becomes truly exponential, its slope will be the rate at which iodide ions are moving from intercellular to cellular space. The model and the experimental evidence suggest, also, that the non-cellular material on the outer cells of the alga is more permeable to the iodide ion than are the cell membranes. The failure of the curve to reach zero slope suggests further that iodine may be used by the cells in metabolic processes. The equilibrium that was reported attained in previous work, was probably not an equilibrium at all but a levelling off of iodine removal because of iodine loss from the solution with time, since no attempt was made in the previous work to control pH. In preliminary experiments designed to determine the rate of iodine removal, the alga was exposed to sea water containing radioactive iodine for short periods of time, as indicated in Figure 3. While the data obtained could not be used to calculate the actual rate, the curve indicates a total uptake increasing exponentially and consequently an uptake rate which is a function of time.

Since it was found that the exchange process involved iodine which is in the form of iodides, the removal of radioactive iodine was studied in which all the iodine was reduced to iodides. The concentration of iodine in this experiment was 200 gamma per liter. This high concentration of iodide was used since it became evident that the amount of iodine exchanged was dependent upon concentration. Under natural conditions, the amount of iodine exchanged by the alga is limited obviously by the concentration rather than by the total amount of iodine available. This and subsequent experiments show that a concentration of 200 gamma per liter in the volume provided is not sufficient to bring the segments to a saturated condition (Figs. 4 and 5).







FIGURE 4. Exchange of iodide ions between Ascophyllum and natural sea water having a total iodide content of 200 gamma per liter. All iodine was converted to iodides. Solutions were buffered to pH 8 using glycyl-glycine.

If an exchange of iodine occurs, then segments pre-soaked in radioactive iodine solutions will release their radioactivity to similar but non-radioactive solutions. As indicated in Table IV, the release of radioactive iodine from presoaked segments followed the course which would be expected from an exchange process.

The removal of radioactive iodine from sea water by whole plants, using a volume of 825 ml. per plant, is similar to that removed by groups of segments.

In order to demonstrate that the iodide concentration influences the exchange rate, removal of iodine was studied when the external concentration of iodide was varied. If segment permeability is assumed to remain constant during the experimental period, the only other factor influencing the exchange rate is the relative concentration of iodine in the segments and in the surrounding medium. As the external concentration of iodide is varied, the per cent decrease in radioactivity of

TABLE III

Radioactive iodine removed from solution by Ascophyllum segments as a function of time and of initial iodide concentration. Each figure is the result of three determinations

Concentration of	Per cent of initial activity remaining in solution Exposure time (hours)					
	1	. 4	24			
0.5	89	69	29			
0.7	86		38			
0.75	89	67	22			
2.0	_	77	21			
5.0	_	80	27			
100		93	91			





the surrounding medium during a certain time interval should remain constant. As indicated in Table III, the amount of iodine exchanged is dependent upon external concentration for iodide concentrations of less than 5 milligrams per liter of sea water under the experimental conditions employed.

Table III also suggests that even at extremely high iodide concentrations (100 mg./liter) natural conditions are not duplicated. Since the alga has pre-

Time (hours) after radio-	Counts per second in solution					
placed in the solution	1	4	24	50		
	1.2	4.2	11.3	11.6		

TABLE IV

viously been in an environment capable of supplying all the iodine it was capable of absorbing, it is assumed to be in an iodine-saturated condition. If so, when it is exposed to a radioactive sea water solution, the total amount of radioactivity removed from this solution would not be dependent on iodide concentration provided the concentration is great enough to carry out the exchange process at the same level as would take place in its natural habitat.

Under conditions of saturation, the radioactivity removed from solution should reach a stable value. That is, a given amount dependent on the ratio of  $I^{128}$  to  $I^{131}$  would be removed, and no further depletion of radioactivity in the solution would take place as time progressed. This amount removed would then tell us the ratio of total iodine content within the alga to that in sea water.

This condition was satisfied experimentally in the following manner: alga segments were transferred hourly to 10-ml. sea water solutions containing one mg./ liter iodide, and the decrease in radioactivity of each solution measured. This procedure was repeated until the solutions no longer showed a decrease in radioactivity. Twenty-one to twenty-four hours was the time required for this condition to be reached.

To calculate the ratio of the initial concentrations of iodine in the segments to iodine in the solution, the following procedure was used:

Let 
$$I_0$$
 = the amount of I<sup>128</sup> in the initial solution  
 $f$  = the fraction of I<sup>131</sup> in the initial solution  
 $f_h'$  = the fraction of I<sup>131</sup> in solution at the end of  $h$  hours  
 $I_0'$  = the amount of I<sup>128</sup> initially in the segments  
 $f_h$  = the fraction of I<sup>131</sup> in the segments at the end of  $h$  hours.

Now the amount of I<sup>131</sup> in solution at t = 0 must be exactly equal to the amount of I<sup>131</sup> in solution at t = h plus the amount of I<sup>131</sup> in the segments at t = h.

$$\therefore fI_0 = f_h' I_0 + f_h I_0'.$$

At the end of n hours, when equilibrium is reached,

$$\left[ nf - \sum_{i=1}^n f_i' \right] I_0 = f_n I_0'.$$

At equilibrium, fn = f,

:. 
$$n - \sum_{i=1}^{n} \frac{f_i'}{f} = \frac{I_0'}{I_0}$$

or,

$$I_0' = \sum_{i=1}^n \left(1 - \frac{f_i'}{f}\right).$$

The average ratio (3 determinations) of  $I_0'$  to  $I_0$  by this procedure is  $4.20 \pm 0.16$ .

The volumes of solutions and segments were 10 ml. and 0.189 ml., respectively. These give a value of 220 for the ratio of the concentrations. On the basis of the fresh weight of the alga, the amount of iodine contained by the segments is 0.19 milligram per gram.

### SUMMARY

1. Through the use of radioactive iodine the following information concerning the removal of iodine from sea water by Ascophyllum has been established: the removal occurs as an exchange process between iodine in the sea water and iodine already present in the cells; the iodine is exchanged in the form of iodides, not iodates; the exchange takes place in living cells only and in entire plants as well as the isolated segments studied in detail.

2. The exchange rate is dependent upon concentration of iodine available. Two different exchange rates may exist, suggesting that a two-step process is involved.

3. The iodine concentration within the alga is about 220 times that of sea water and the absolute iodine content of the alga examined was 0.19 mg./g. fresh weight.

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