RESPIRATION AND IODINE UPTAKE IN ASCOPHYLLUM

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The brown algae are noted for their high iodine content. They have the property of removing iodine from sea water and accumulating it in their cells in concentrations many times that of sea water. This phenomenon of accumulation against a concentration gradient has been observed for several elements in a variety of tissues. There is considerable evidence that aerobic metabolism is necessary for accumulation (Hoagland, 1940), involving the cytochrome system (Robertson and Turner, 1945; Hoagland and Broyer, 1942), and, perhaps, the Krebs cycle (Machlis, 1944).

Since the mechanism of iodine uptake by the algae might well parallel the uptake of salts previously investigated, it is of interest to know whether iodine uptake is related to respiration. To determine this, three types of experiments were carried out:

1. General nature of respiration by the use of oxidizable substrates and inhibitors.
2. Effect of these substrates and inhibitors on iodine uptake.
3. Iodine uptake in nitrogen.

Materials and Methods

The brown alga, *Ascophyllum nodosum* (Linn.) LeJolis was selected as a suitable species for respiration and iodine uptake measurements because (1) it accumulates iodine; (2) reproducible samples could be obtained from day to day by cutting segments; (3) it remains vegetative in the locality used throughout the summer season; (4) it is readily available.

Respiratory measurements. The alga was gathered in the late afternoon, sliced transversely into segments 0.25 mm. thick with a razor blade, and washed overnight in darkness in a running sea water aquarium at a temperature of 18 to 22° C. Segments of this thickness had a respiratory rate of 0.72 μl. O_2 per hour per mg. dry weight (103 μl. O_2 per hour per mg. nitrogen), a rate considerably higher than segments one and ten mm. in thickness (Table I). This suggests that penetration of oxygen into the tissue is an important factor in determining the absolute respiratory rate of this alga. The segments were cut from the distal region between the first and second air bladders; the respiratory rate of segments from this region was lower than that of segments from younger tissue and higher than that of segments from older tissue (Table II). This is evidence that a respiratory gradient exists in this species. Fifty to 400 segments were introduced into each flask, the number being constant in any one experiment. When the latter number was employed,

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TABLE I

Respiratory rate of segments of various thicknesses. Each figure is the average of 2 to 4 determinations. Tissue starved

Thickness in mm.	No. segments in flask	μl. O_2 per hour		
		Per no. segments	Per mg. D.W.	Per mg. N
0.25	400	84.4	0.72	103
1.00	100	48.3	0.57	82
10.0	10	49.4	0.38	72

the number was determined volumetrically. The respiratory rate was proportional to the number of segments used, indicating that oxygen in the flask was not limiting.

Measurements of oxygen uptake were made in darkness in standard Warburg flasks of approximately 15 ml. volume, maintained in a water bath at 25° C. and shaken at 80 r.p.m. Manometer readings were made every half hour after an equilibration period of 30 minutes. Oxygen uptake was measured as the amount of oxygen taken up by the segments during the entire experimental period of three hours. In the preliminary experiments comparing segments of various thickness and from tissue of different ages, the calculations were based also on dry weight and total nitrogen content of the segments. In general, the basal medium consisted of van't Hoff sea water minus calcium.

The effect of the following substances on respiration was measured: sucrose, mannose, succinic acid, malic acid, sodium pyruvate, malonic acid, sodium azide, iodoacetic acid, sodium fluoride, and potassium cyanide. The experiments with sucrose, mannose, azide, iodoacetic acid and cyanide were carried out at pH 6.8, with segments in van't Hoff sea water solution minus calcium as controls. The experiments with succinic, malic, pyruvic and malonic acids and fluoride were carried out in phosphate-citrate buffers of pH 4.5, 5.5 and 6.5, with segments in buffers of pH range 4.5 to 6.5 as controls. The buffers were made up, and the substances tested dissolved, in van't Hoff sea water solution minus calcium. The respiratory rate was 15 per cent less in van't Hoff sea water than in natural sea water; at pH 6.5 (phosphate-citrate buffer) the rate was decreased an additional 15 per cent.

TABLE II

Respiratory rate of segments of different ages. Each figure is the average of 2 to 3 determinations

Age of tissue	μl. O_2 per hour			
	Starved		Not Starved	
	Per mg. D.W.	Per mg. N	Per mg. D.W.	Per mg. N
Youngest	1.07	200	1.52	659
Intermediate	0.60	109	1.19	361
Old	0.23	69	0.71	380

TABLE III

Effect of sucrose and glucose on respiration. Each figure is the average of 2 to 4 determinations. Tissue starved

Concentration in *M*	Per cent change over control	
	Sucrose	Glucose
0.02	8	0
0.05	20	0
0.10	− 2	0
0.20	− 7	15

Iodine uptake measurements. Segments for iodine uptake measurements differed from those used in respiration studies in being one cm. in thickness. Despite the difference in respiratory rate (Table I), the departure was made to obtain a more uniform counting geometry of iodine taken up than was possible with the thinly sliced segments. They were, however, similarly gathered and washed. The segments were placed in 25 ml. rubber-stoppered Erlenmeyer flasks (10 to a flask) containing 3 ml. of van't Hoff sea water solution to which had been added potassium iodide to give a concentration of 0.05 p.p.m. iodide (the accepted concentration in natural sea water). Iodine^{131}, supplied in $NaHSO_3$, was added to the solution in amounts giving approximately 14 μc. per experimental flask. The flasks were rotated slowly on a turntable.

The iodine^{131} removed by the segments during the experimental period (3 to 6 hours) was determined by obtaining counts of the radioactivity present in the seg-

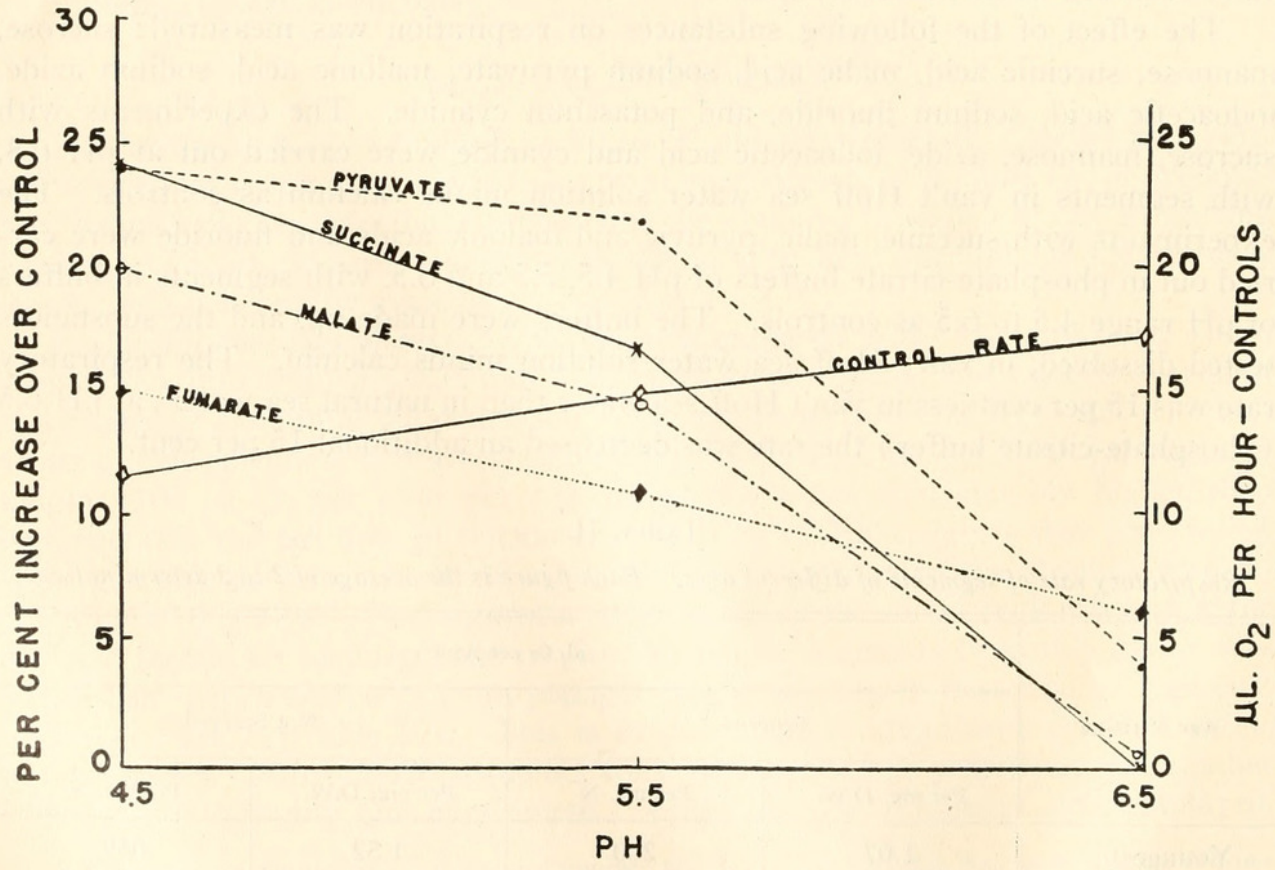

FIGURE 1. Oxygen uptake by Ascophyllum segments in the presence of respiratory intermediates, 0.025 *M* (except pyruvate, 0.0025 *M*.). Segments in phosphate-citrate buffered van't Hoff sea water minus calcium.

ments and in the solutions before and after the segments were exposed to them. Before the segment counts were made, the segments were removed from the solutions and washed by six successive rinses with a non-radioactive solution, otherwise similar to that in the flasks. Each rinse contained 15 to 20 ml. of solution. By the sixth rinse no further removal of radioactivity from the segments took place. The excess rinse solution was blotted from the segments with a paper towel, and the segments transferred to a counting pan. They were evenly spaced so that a constant geometry

TABLE IV

Effect of various concentrations of respiratory inhibitors on oxygen uptake and on uptake of iodine^{131}

Inhibitor and concentration (M)	Per cent inhibition	
	O_2 uptake	I^{131} uptake
Potassium malonate		
pH 4.5		
0.005	16	—
0.010	47	—
0.025	87	79
0.050	81	—
Sodium azide		
pH 6.5		
0.0001	8	77
0.001	84	94
0.01	87	96
pH 6.5		
0.0001	—	71
0.001	13	95
0.01	75	96
Potassium cyanide		
pH 7.5		
0.00001	42	—
0.0001	66	—
0.001	71	—
0.005	58	42
0.05	61	84
Potassium fluoride		
pH 4.5		
0.0025	29	—
0.005	52	—
0.025	81	—

was established for each set of segments counted. After counting, they were returned to the radioactive solutions for the next time interval.

The radioactivity of the solutions was determined by counting two-ml. aliquots of the solutions before and after the segments were exposed to them.

All counting rates were determined by taking at least 2×10^3 total counts, so that the statistical error is below 2 per cent. The counting rates thus determined were then corrected for background and decay of the iodine^{131}.

When a nitrogen atmosphere was desired, the flasks containing the segments were kept in darkness in a vacuum desiccator filled with nitrogen. Before nitrogen was

admitted the desiccator was evacuated and the gas passed through alkaline pyrogallol.

The effect of the following substances on iodine uptake was measured: sucrose, glucose, iodoacetic acid, potassium cyanide, and sodium azide. These substances were dissolved in van't Hoff sea water solution, pH 6.8, minus calcium, to which the isotope was added.

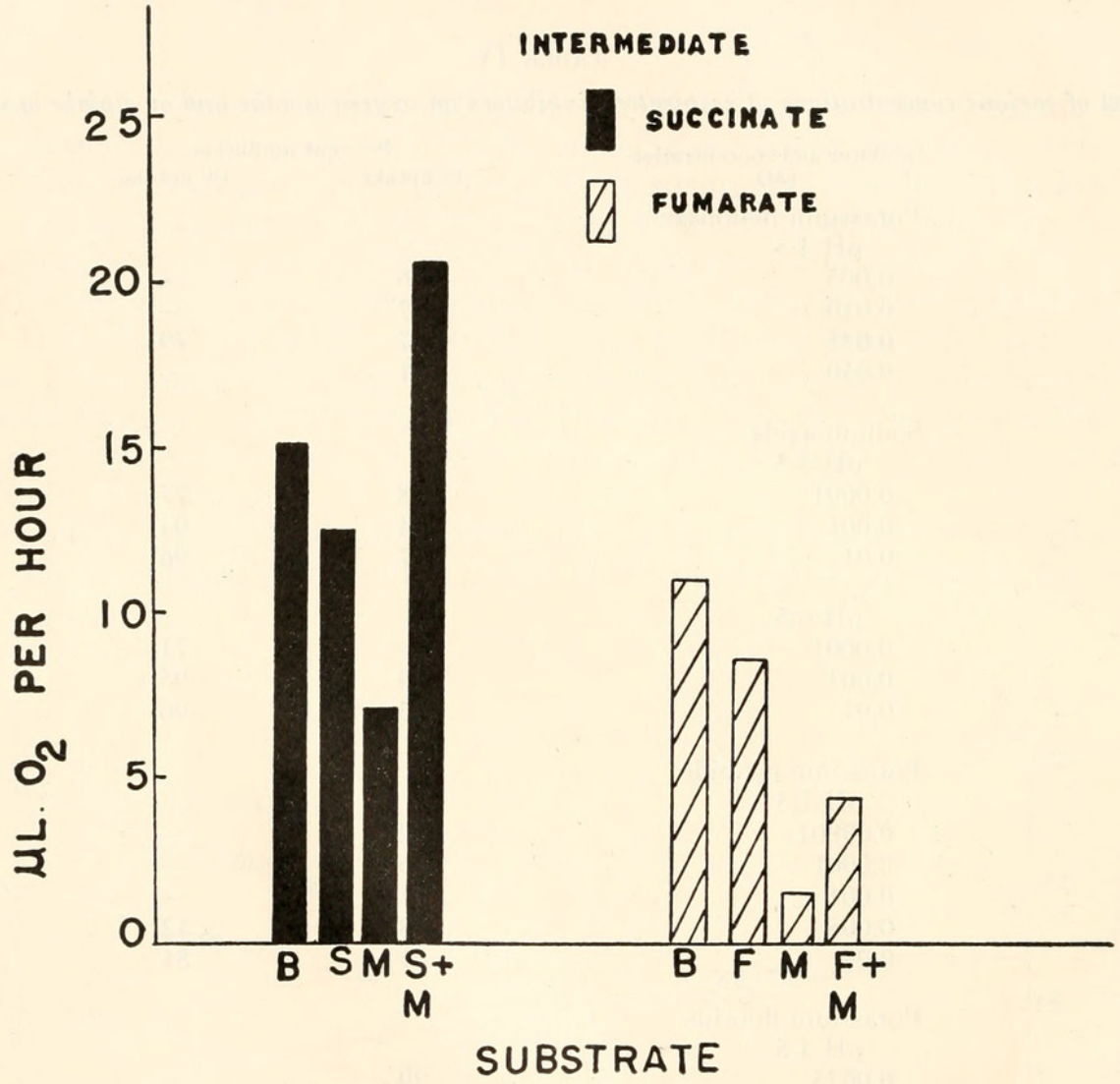

FIGURE 2. Reversal of malonate inhibition of oxygen uptake by Ascophyllum segments. B = basal medium (van't Hoff sea water minus calcium, buffered with phosphate-citrate at pH 4.5); S = succinate; M = malonate; F = fumarate. Concentration of intermediates, 0.025 *M*. Malonate concentration in succinate reversal, 0.01 *M*; in fumarate reversal, 0.05 *M*.

RESULTS AND DISCUSSION

The respiratory quotient of the Ascophyllum segments was 0.9. Since the enzyme systems involved in Ascophyllum respiration are not known, it was thought feasible to make a survey of the effect of substances that are known to serve as respiratory substrates and inhibitors in other types of cells. Of the sugars tested, sucrose and glucose stimulated oxygen uptake of the segments up to 20 per cent, as indicated in Table III. Mannitol, a storage product of Ascophyllum, had no effect

in concentrations of 0.01 and 0.001 *M* and was inhibitory at 0.1 *M*. When the acids, succinic and malic, were tested for their effect on oxygen uptake in van't Hoff sea water at neutral or alkaline pH they stimulated only in high concentrations or after prolonged exposure. When tested at pH 4.5, however, these acids were stimulatory in the concentrations and to the extent given in Figure 1. The respiratory rate of the control at the low pH values was less than at pH 6.5 (Fig. 1). The fact that these acids were effective only at the low pH is in accord with the theory that they enter the cell chiefly in the undissociated state (Baron, 1950; Simon and Beevers, 1951).

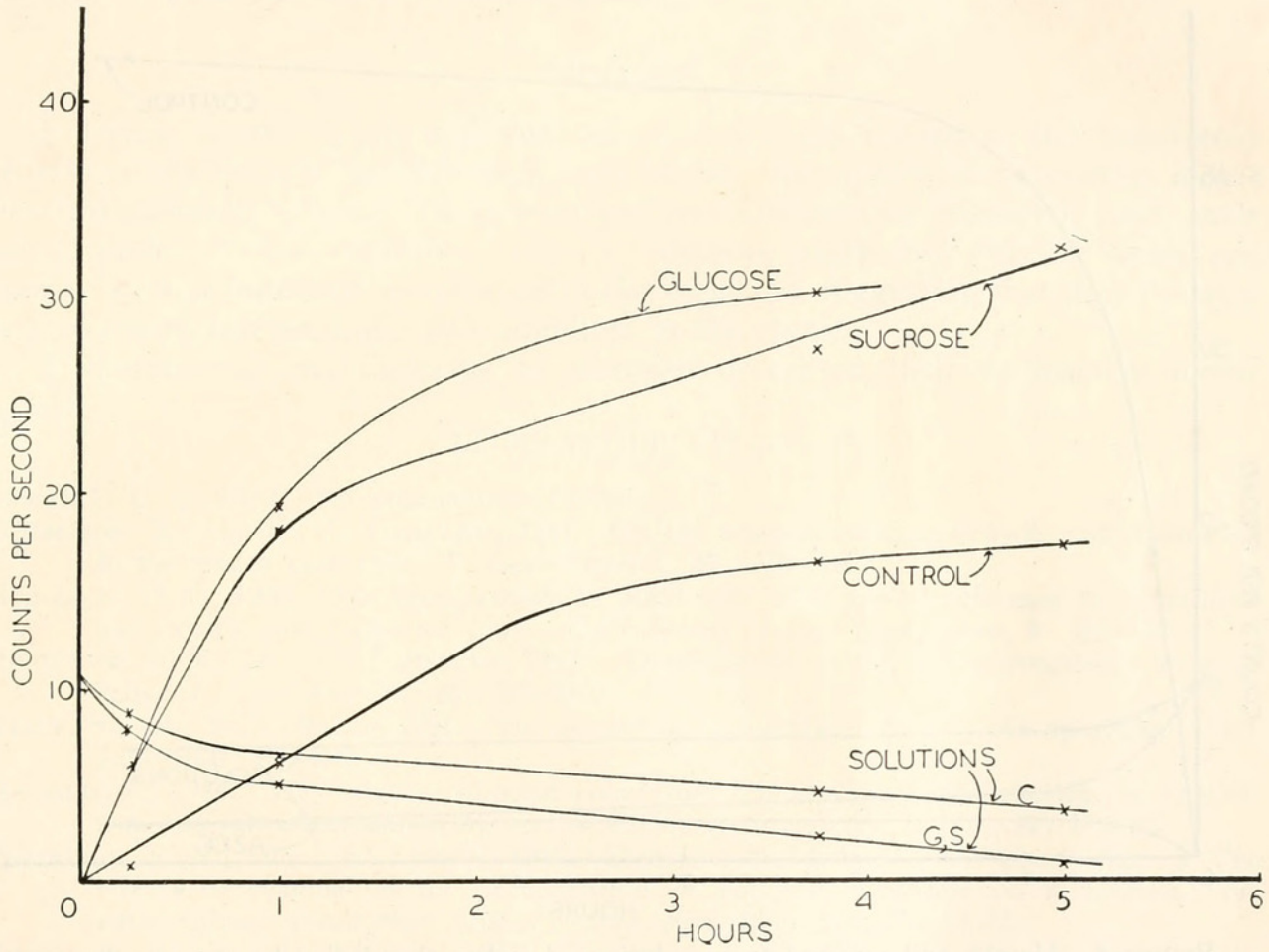

FIGURE 3. Uptake and removal from solution of radioactive iodine by Ascophyllum segments in the presence of added sugars, 0.025 *M*. Segments in van't Hoff sea water minus calcium, containing 0.05 p.p.m. iodide.

The data showing that these acids, known to be intermediates in metabolism of various animal and plant cells, can be oxidized by Ascophyllum segments are suggestive of the presence of a 4-C acid system in this alga.

Additional evidence that this system is present was found in the action of substances inhibitory to respiration of other types of cells: potassium cyanide, sodium azide and iodoacetic acid inhibited oxygen uptake (Table IV). Malonic acid and sodium fluoride inhibited when the pH was lowered to 4.5 but did not inhibit at pH 6.5 (Table IV), again compatible with the theory that these acids enter in the undissociated state. Attempts to reverse the inhibition caused by malonic acid by the

addition of succinic and fumaric acids were successful only when the intermediates were present from the start along with the inhibitor (Fig. 2).

These results suggest that heavy metal enzymes, dehydrogenases, and glycolases are functioning in Ascophyllum segments. Anaerobically the segments utilized malic acid, glucose and pyruvic acid, in the order given, while respiring in nitrogen when carbon dioxide production was followed from a bicarbonate buffer.

The amount of iodine^{131} taken up from van't Hoff sea water by the segments increases up to the first hour of exposure to the element; beyond that time no further increase in radioactivity takes place. Since the segments already had iodine in

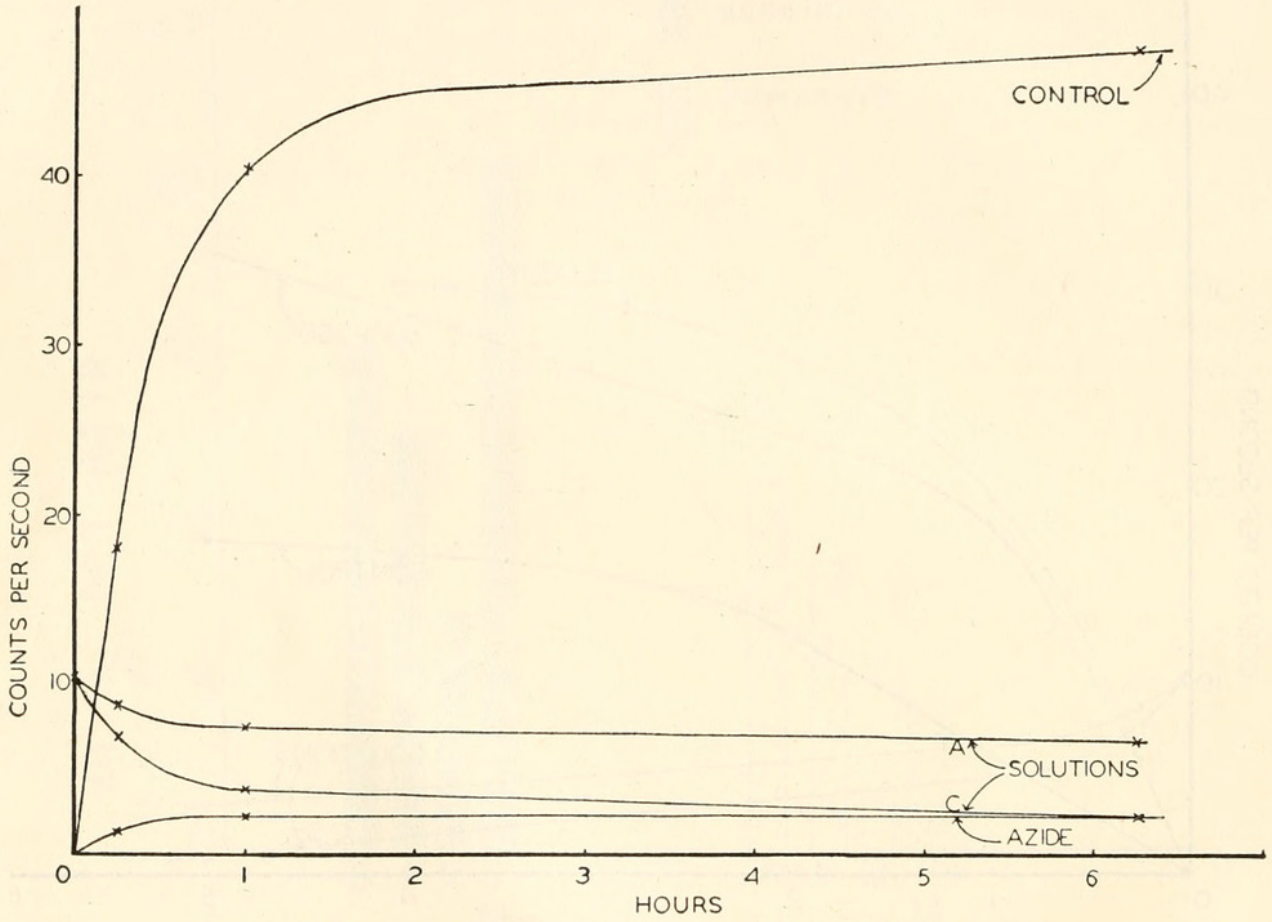

FIGURE 4. Uptake and removal from solution of radioactive iodine by Ascophyllum segments in the presence of sodium azide, 0.001 *M*. Segments in van't Hoff sea water minus calcium, containing 0.05 p.p.m. iodide.

them, having lived in natural sea water until the experimental period, the removal of radioactive iodine has been interpreted as meaning that iodine in the solution is being exchanged for iodine in the segments (Kelly and Baily, 1951). When the ratio of iodine^{131} to iodine^{128} inside the segments becomes equal to the same ratio outside, then net changes in radioactivity cease, indicating an equilibrium has been reached.

When glucose and sucrose were added to the solution in a concentration of 0.025 *M* the uptake of iodine was stimulated, as measured by the increase in radioactive iodine in the segments and its decrease in the solutions. This stimulation was apparent more or less immediately (Fig. 3). Potassium cyanide, iodoacetic acid and sodium azide inhibited iodine uptake (Fig. 4). A comparison of the in-

hibitory concentrations and amount of inhibition of both iodine uptake and respiration (Table IV) shows that iodine uptake is more completely inhibited than respiration by comparable concentrations. This suggests that only a fraction of aerobic metabolism is involved in iodine uptake, an interpretation analogous to that given by Commoner and Thimann (1941) to their experiments in which iodoacetic acid completely inhibited growth at concentrations only partially inhibitory to respiration.

The inhibition by cyanide and azide suggests that iodine uptake is an aerobic process. This interpretation is further strengthened by the fact that segments maintained in a nitrogen atmosphere during the period of iodine uptake show a 50 to 75 per cent decrease in amount of iodine uptake.

Summary

1. Iodine uptake by Ascophyllum was found to be related to the respiratory process on the basis of the following experiments: the uptake of radioactive iodine was stimulated by glucose and sucrose and was inhibited by iodoacetic acid, azide and cyanide. These substances and, in addition, malic and succinic acids and fluoride in turn influenced respiration of the segments, suggesting that they function as respiratory intermediates and inhibitors in the species.

2. Maintaining the segments in nitrogen decreased their radioactive iodine uptake.

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