PHYSIOLOGY OF IODINE UPTAKE AND DISTRIBUTION IN *LAMINARIA SACCHARINA* (PHAEOPHYTA)

by

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Maitrise, Univ. P. et M. Curie, Paris, 1978

D.E.A., Univ. P. et M. Curie, Paris, 1979

THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in the Department

of

Biological Sciences

SIMON FRASER UNIVERSITY

December 1985

APPROVAL

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DEGREE: Doctor of Philosophy

TITLE OF THESIS: Physiology of iodine uptake and distribution in *Laminaria saccharina* (Phaeophyta)

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Physiology of iodine uptake and distribution in Laminaria saccharina (Phaeophyta)

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ABSTRACT

Little is known about the role of iodine in the physiology and growth of brown algae. Blade discs and intact plants of the kelp Laminaria saccharina (L.) Lamour. (Phaeophyta) were used to investigate the uptake, translocation and distribution of iodine. These studies were related to the field growth of Laminaria.

Using discs from mature blade, $^{125}I^-$ uptake rates of 0.39 to 3.9 nmol $I^- cm^{-2} h^{-1}$ were recorded and shown to be dependent on experimental conditions. No difference was seen in uptake in light and dark unless discs were preincubated in the dark. The uptake was both temperature and ATP dependent which strongly suggests that it is an active process. The apparent internal iodide pool ranged from 12.0 to 106.0 μmol g^{-1}d.wt..

Most of the extracted tissue iodine was found as the inorganic form iodide. After tissue hydrolysis, only trace amounts of mono-iodotyrosine (MIT) and di-iodotyrosine (DIT) were detected.

^{125}I was found to be translocated basipetally following a 'source to sink' pattern at velocities of 2 to 3.5 cm h^{-1} from the blade towards the meristem where it accumulated.

The growth of tagged L. saccharina plants was followed in a kelp bed from July 1983 to July 1985 at Brockton Point, Vancouver, British Columbia, Canada. The growing season extended

from late January to the beginning of fall. Highest blade elongation rates of 2.4 cm day^{-1} were recorded in late August. In relation to growth, iodine levels were determined by neutron activation analysis (N.A.A.) in different parts of the thallus. Much higher levels were found in the holdfast, stipe and meristem than in the blade. Iodine concentration increased in all parts throughout the year, especially in the fall.

The growth of young L. saccharina sporophytes with added potassium iodide (KI) was followed in the laboratory. In the 10 and 100μM KI media, sporophytes grew fast, were highly pigmented and had a high dry weight. The sporophytes in the control (no added KI) and the 1μM KI media showed signs of necrosis, decay and decreased growth.

In summary, the facts that iodine is translocated towards the meristem where it accumulates and that exogenous KI promotes the growth of young sporophytes suggest that iodine may have a direct or indirect growth promoting role. It is also suggested that iodine may have some antibacterial properties.

RESUME

Le rôle de l'iode dans la physiologie et la croissance des algues brunes est peu connu. Des échantillons de lame ainsi que des thalles entiers de Laminaria saccharina (L.) Lamour. (Phaeophyte) ont été utilisés pour étudier l'absorption, la translocation et la distribution le long du thalle de l'iode. Ces études ont été comparées à la croissance sur le terrain de Laminaria.

Des taux d'absorption d'iode de 0.39 à 3.9 nmol $I^{-}cm^{-2}h^{-1}$ ont été trouvés pour des échantillons de lame; ces taux dépendant des conditions expérimentales. L'absorption d'iode n'est pas différente en présence ou en absence de lumière, à moins que le tissu ait été préalablement incubé dans le noir. L'absorption d'iode dépend de la température et de la présence d'ATP, ce qui suggère que l'absorption est un transport actif. Le "pool apparent interne" varie de 12 à 106.0 umol g^{-1} de poids sec.

La plupart de l'iode extrait du tissue est sous la forme inorganique I^{-}. Après l'hydrolyse du tissue des traces de mono-iodotyrosine (MIT) et de di-iodotyrosine (DIT) ont été trouvées.

La translocation de l'iode ^{125}I se fait à des vitesses de 2 à 3.5 cm/h et de façon basipète de la lame au méristème, où l'iode s'accumule.

La croissance de plantes de L. saccharina a été suivie dans un peuplement de laminaires à Brockton Point, Vancouver, Colombie-Britannique, Canada. La saison de croissance s'étend de la fin janvier au début de l'automne. Un taux d'élongation maximal de lame de 2.4 cm/j a été trouvé à la fin août.

En parallèle à la croissance sur le terrain, les niveaux d'iode le long du thalle ont été déterminés par l'analyse par activation de neutrons. Des niveaux d'iode bien supérieurs à ceux dans le thalle ont été trouvés dans le crampon, le stipe et le méristème. La concentration d'iode le long du thalle augmentait tout au long de l'année, et encore plus à l'automne.

La croissance de jeunes sporphytes de L. saccharina exposés à des niveaux croissants de KI dans leur milieu, a été suivie in vitro. Les sporophytes ont dénoté des taux de croissance, de concentration de pigments et de poids secs plus élévés dans les milieux de 10 et 100 uM de KI. Les sporophytes témoins (sans KI ajouté) et dans 1 uM de KI, ont montrés des signes de nécroses, pourrissement et croissance décrue.

Le fait que l'iode est transporté vers le méristème où il s'accumule, et que l'addition de KI promouvoit la croissance de jeunes sporophytes suggère que l'iode est impliqué soit directement ou indirectement comme agent de croissance. On peut aussi suggérer que l'iode a des propriétés anti-bactériennes.

ACKNOWLEGMENTS

I sincerely wish to thank my senior supervisor, Dr. L.M. Srivastava for his guidance, advice and editorial efforts in the making of this thesis. I am indebted to other members of my committee, Dr. J. d'Auria and Dr. W.L. Vidaver, the latter with whom I had many a discussion after vigorous games of squash.

I am very grateful to the World University Service of Canada for having financed this project for four years.

Special thanks go to Dr. V. Bourne for always providing time and advice in all the steps of this thesis; to Dr. L. Druehl for valuable 'kelp discussions'; to my lab companions, S. Cheong, C. Dykstra, Z.-H. Liu, J. Meng, N. Yalpani and of course K. Rosell for helping with the field work and for always supplying me with encouragment; to A. Cifarelli and A. Erdman for assistance in the hot lab and friendy discussions; to Mr. J. Humphries from Novatrack, TRIUMF, for running the NAA analyses.

I wish to thank my family who have encouraged me all along to pursue a career in this field.

F. Dittman, R. Smith, T. Smith, R. So, K. & G. Van Frankenhausen, A. Vezina, S. Villeneuve, I. Walker, not forgetting Babouche and Igor, have all contributed through their humor and moral help to the realisation of this work. Last, my deepest gratitude go to my companion, Dave Trotter, for his unlimited support and help to preserve my sanity throughout this endeavour.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PREFACE

Iodine is a rare element and only a few organisms, such as some marine invertebrates and algae, concentrate it. It has been known since the early 1800's that marine algae, especially those of the order Laminariales, can concentrate iodine up to 1% of their dry weight. Iodine is a necessary nutrient for the good functioning of the mammalian thyroid gland. Although we obtain our iodine from mineral deposits, these seaweeds constitute a practically untapped source for iodine. So far, only the Chinese cultivate these algae for this purpose.

There is a lack of information regarding the significance of iodine accumulation in algae. Thirty years ago, this topic attracted some interest but no conclusive results or interpretations emerged and many questions remained unanswered. Past work did show that iodine is an essential nutrient for some brown and red algae, without which the thalli died or failed to reproduce. This fact suggests a possible involvement of iodine in the metabolism of the algae and further that it might be necessary either directly or indirectly for the growth of the algae. My study explores this possibility by studying an iodine rich kelp, Laminaria saccharina. A literature survey follows this Preface to give the reader more background information on the biogeochemical cycle of iodine and the importance of algae in that cycle. This survey includes a review of the current advances in iodine research in algae and only then will I

present the points on which I have focused in my thesis research.

PART A

Literature review

N.B. All through this thesis the term iodine is used in a general sense to include the molecular as well as all ionic forms of iodine. When it is certain that we are dealing with molecular iodine, it is specified as such.

I. Iodine in the environment

Little is known about the biogeochemical cycle of iodine. Unlike most nutrients, the weathering of rock material provides less iodine than the atmosphere. Low levels of iodine are found in soils, rivers, lakes and terrestrial plants.

Iodides (I^-), iodates(IO_3^-) and organoiodines (I-R) are the different forms of iodine found in fresh and seawater.
In seawater the total iodine concentration ranges from 0.3 to 0.5 μM. The distribution of total iodine in the oceans is fairly well known, but the relative concentrations of IO_3^- and I^- are still a matter of debate due mainly to imprecise or inaccurate methods of measurement (Truesdale and Chapman, 1976). Iodate is the stable form of iodine in seawater but high levels of iodide are found especially in the euphotic zone. This suggests that either iodate is reduced to I^- by reducing substances as found in runoffs (Truesdale, 1978) or by biological activity of organisms such as bacteria, phytoplankton and algae. The reduction of IO_3^- by biological activity is substantiated by the facts that tropical surface waters are rich in I^- where little runoffs exist and, further, that in areas of high productivity iodide levels may be up to 50% of the total iodine (Butler *et al*, 1981). Hirano *et al*. (1983), with the use of radioisotopes, examined the effect of living material on the conversion of IO_3^- to I^-. Without algae, there was a slow oxidation of $^{125}I^-$ to $^{125}IO_3^-$ which became noticeable after 25 days. With the addition

of a macroalga, IO_3^- was converted to I^- and this was noticeable after 2 days. These observations lend weight to the idea that biological activity influences the concentration of I^- in seawater, although no seasonality to this phenomenon has been found (Truesdale, 1978).

The vertical distribution of I^- shows levels decreasing quasi exponentially with depth to non-detectable levels below the euphotic zone. Iodate concentrations, on the other hand, are low in surface waters and increase with depth. On the whole the vertical distribution of IO_3^- resembles that of NO_3^- and seems to be correlated with apparent oxygen utilization[1], thus displaying a nutrient-like behavior in seawater (Wong and Brewer, 1974).

The organically bound I_2 could result from the combination of I_2, produced by the photochemical oxidation of I^-, with unsaturated organic compounds. The total iodine in inshore waters is about 0.08 μM lower than in offshore waters which can be explained by the formation of organoiodine or its disappearance by bio-assimilation (Truesdale, 1975).

Molecular iodine (I_2) and methyliodide (CH_3I) are important components in the cycle. High levels of methyliodide were found

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[1] The mineralization of organic matter requires oxygen, the consumption of which is proportional to the quantity of organic matter mineralized. There is thus a constant ratio between various dissolved nutrients (PO_4, NH_4, NO_2, NO_3 and CO_2) and the oxygen deficit. The apparent oxygen utilization is the difference between a saturating O_2 concentration at the surface and what is measured in situ and gives an approximation of the nutrients present.

by Lovelock et al (1973) while surveying for halogenated compounds in and over the Atlantic ocean. The source of CH_3I is oceanic, and most likely of biological origin as high levels of CH_3I are found in close proximity to kelp beds. Other authors consider molecular iodine to be the transient species at the air-sea interface (Wong and Brewer, 1974), where photochemical oxidation of iodide releases iodine into the atmosphere (Tsunogai and Sase, 1969). Marine algae are thought to liberate molecular iodine into the air, a process called iodovolatilization (see later).

As to the speciation of iodine by living organisms, Tsunogai and Sase (1969) reported that bacteria with a nitrate reductase capacity could reduce iodate to iodide. The reduction of iodate, although more difficult than that of chlorate, thermodynamically, is nonetheless easier than the reduction of nitrate (standard redox potential of +0.67 V, +0.74 V and +0.42 V at pH 7, respectively). This suggests that organisms with a nitrate reductase activity can reduce iodate when nitrate, a preferred substrate, is in limiting quantities. However, these data on bacteria are not substantiated by work using phytoplankton (Butler et al, 1981).

Seaweeds can influence iodine speciation by converting IO_3^- to I^- as was described earlier (Hirano et al., 1983). Also, higher levels of I^- are reported in seawater close to kelp beds (Lovelock et al., 1973).

II. Iodine and Marine Algae

1. Iodine levels in Marine Algae

Iodine was first discovered in kelp by Courtois in 1811. Since then many algae, particularly Rhodophyta and Phaeophyta, have been found to concentrate iodine. In the latter division, Laminariales in particular can concentrate iodine up to 30,000 times the seawater concentration and this element can constitute up to 1% of their dry weight (80 μmol g^{-1} d.wt.) (Black, 1948).

Table A1 shows concentrations of iodine in terms of μmol g^{-1} I^- dry weight in different groups of algae.

Table A1. Concentrations of iodine in μmol $I^{-}g^{-1}$ dry weight.

Alga	μmol g^{-1}d.wt.	Reference
A. Chlorophyta		
Ulva fenestrata	1.04	Saenko *et al.*. 1978
Enteromorpha clathrata	0.17	"
B. Rhodophyta		
Myriogramme sp.	60.0	"
Ptilota filicina	33.6	"
C. Phaeophyta		
Alaria esculenta	2.4-7.2	Haug and Jensen, 1957
Laminaria saccharina	16.0-40.0	"
L. hyperborea	20.0-56.0	"
L. digitata	32.0-88.0	"
Desmarestia sp.	9.6	Saenko *et al.*, 1978
Alaria marginata	12.0	"
L. japonica	44.8	"
L. longicruris	13.6	Young and Langille, 1958
L. digitata	20.0	"

It should be noted that different species of *Alaria* and *Laminaria* and the same species of *L. digitata* are reported as

having widely different concentrations of iodine. These differences may be due to different methodologies used and also due to seasonal and/or local variations in the environment.

2. Distribution of iodine in a plant

a. In cells and tissues

Structures named 'ioduques' or ioducts were described for species of red algae such as Polysiphonia, Falkenbergia, Asparagopsis (Sauvageau, 1925; Codomier et al., 1983). Sauvageau (1925) reported that Polysiphonia, when crushed in a starch solution, would turn the solution blue thus indicating the presence of iodine. Sauvageau (1925) and Mangenot (1928) also used brilliant cresyl blue to locate those cells that contained iodine: free iodine as found in the ioducts turned the blue colouration slowly green. Red crystals were formed by the binding of the dye with I^-. In this manner, Mangenot (1928) studied sections of 3 species of Laminaria: L. Cloustonii, L. flexicaulis and L. saccharina. Red crystals formed in the vacuoles, indicating the presence of I^-. Iodide-containing vacuoles were found in the cells of the medulla of stipes, blade and holdfast in the 3 species of Laminaria and also in the meristoderm cells in L. flexicaulis and L. saccharina. The cortex cells showed no presence of I^-. Interestingly, I^- was found in the mucilage ducts.

Much later, with more modern techniques at hand such as X-ray microanalysis in conjunction with electron microscopy, several workers have looked at the presence of iodine in algal cells. Codomier et al. (1983) used cryosections of Asparagopsis armata to analyze the ioducts. This method has the advantage of preserving very diffusible ions such as Na^+, K^+ and halogens. These ioducts contained high levels of iodine and bromine. Westlund et al (1981) localized and quantified iodine and bromine in tissues of Phyllophora truncata, a red alga. The two elements were found in the outer cell wall, the epidermal cells and their middle lamellae, the epidermal cells showing concentrations of 1.2 and 0.9 % for iodine and bromine, respectively. None was detected in the cells of the inner cortex or medulla. There was a good correlation between the location and the concentration of the two compounds which suggested that they could occur together perhaps as part of a larger molecule. Pedersen et al. (1983) studied the localization of iodine in cells of 3 Laminaria species. They fixed the material before analysis which might have led to a leaching of I^- out of the cells (Simon and Peyron, 1968); only bound or organic iodine would thus be measured. In L. saccharina stipes, iodine was found in physodes (a vacuole-type organelle with phenolic compounds) and the outer cell wall. In older stipes, where the typical zonation of dark and light rings appeared, the dark physode-rich rings showed iodine content whereas the light rings showed few physodes and no iodine. The physodes of young L. saccharina stipes also did not show any presence of iodine. No

iodine was found in the chloroplasts of L. saccharina stipes. The authors mention, however, that the levels of iodine might be too low to be detected by their methods.

To summarize specifically for Laminaria, labile I^- has been shown to be present in the mucilage ducts and in the vacuoles of the medulla and meristoderm cells. On an ultrastructural level, bound iodine is found in the physodes of outer cells, outer cortex and middle lamellae of the stipe. Young stipes do not show iodine in their physodes nor do the chloroplasts of L. saccharina show any presence of iodine.

b. In different parts of the plant

The distribution of iodine within a plant shows variations. Young and Langille (1958) noticed differences of iodine content between the blade and stipe tissues in two species of Laminaria. In L. digitata the blade and the stipe showed 37.6 and 13.1 μmol g^{-1} d.wt. iodine, respectively. In L. longicruris, by contrast, iodine content in the stipe was more than in the blade; 9.2 vs 5.6 μmol g^{-1} d.wt., respectively. Rinck and Brouardel (1949) investigated the distribution of iodine in Laminaria flexicaulis. Their data showed high levels of iodine in the holdfast, decreased levels in the stipe, a peak at the stipe-blade junction, then increasing levels in the blade again (Fig A1). Larsen and Haug (1961) analysed iodine levels in different tissues of a Laminaria stipe and found decreasing levels of iodine towards the centre of the stipe (Table A2).

Figure A1 Distribution of iodine along a L. flexicaulis blade
Values are given in % d.wt.
(Data copied from Rinck and Brouardel, 1949).

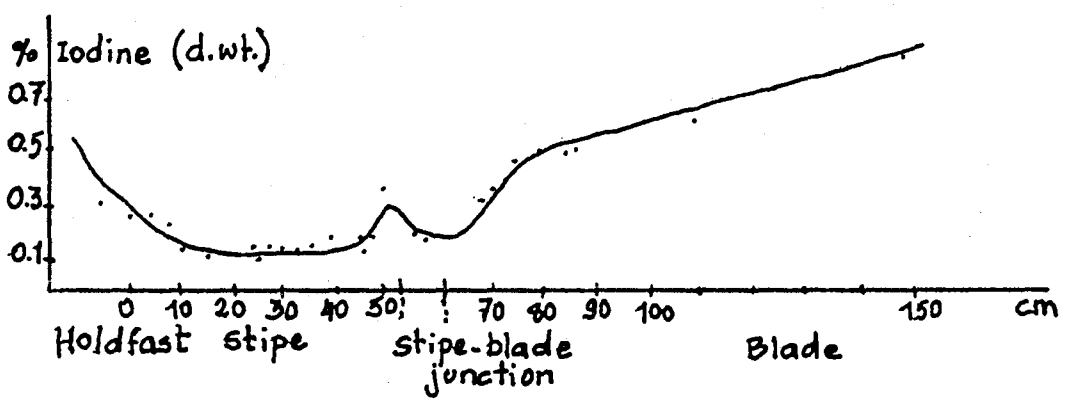

%
Iodine (d.wt.)
0.7
0.5
0.3
0.1
0
10
20
30
40
50
70
80
90
100
150
cm
Holdfast
Stipe
Stipe-blade junction
Blade

Table A2 Composition of stipe tissues of Laminaria hyperborea (Larsen and Haug, 1961).

Tissue	Iodine μmol g^{-1} d.wt.
Peripheral tissue	264.8
Outer cortex	76.0
Inner cortex	5.2
Medulla	5.7

Sargassum pallidum showed a different accumulation of iodine in its various parts with the maximum concentration found in the leafy segment (Saenko et al , 1978). Adachi et al (1978) surveyed the concentrations of iodine in the upper part of the blade, the blade, and stipe of Laminaria and Undaria. Their data are shown in Table A3.

Table A3 Distribution of iodine in Laminaria and Undaria in μmol g^{-1} d.wt. (Adachi et al., 1978).

Tissue	L.japonica	L.j.var.ochotensis	Undaria sp.
top	18.8	5.2	0.008
blade	4.5	6.0	0.96
stipe	10.3	11.6	0.48

3. Variations in iodine levels with location and season

Saenko et al. (1978) reported increased levels of iodine in Laminaria inclinatorhiza with depth (Table A4), a result in conformity with that of Black (1949, as cited in

Table A4 Increase in iodine with depth in Laminaria inclinatorhiza (Saenko et al., 1978).

Depth(m)	μmol g^{-1}d.wt. iodine
13	46.4
16	64.8
18	91.2

Whyte and Englar, 1978) who had shown that a higher I^- level was associated with plants growing at the lower level of their distribution. Saenko et al (1978), also reported that the concentration of iodine increases with latitude in different species of algae.

Haug and Jensen (1957) studied the seasonal variation in iodine content of 4 species of brown algae: Alaria esculenta, Laminaria digitata, L. saccharina and L. hyperborea. Alaria showed the lowest content (2.4-7.2 μmol g^{-1} d.wt.), but levels of iodine, in all cases showed a maximum in winter and a minimum in early summer. In the Laminaria species slightly higher levels were found in the stipes (24-40 μmol g^{-1} d.wt.) than in the

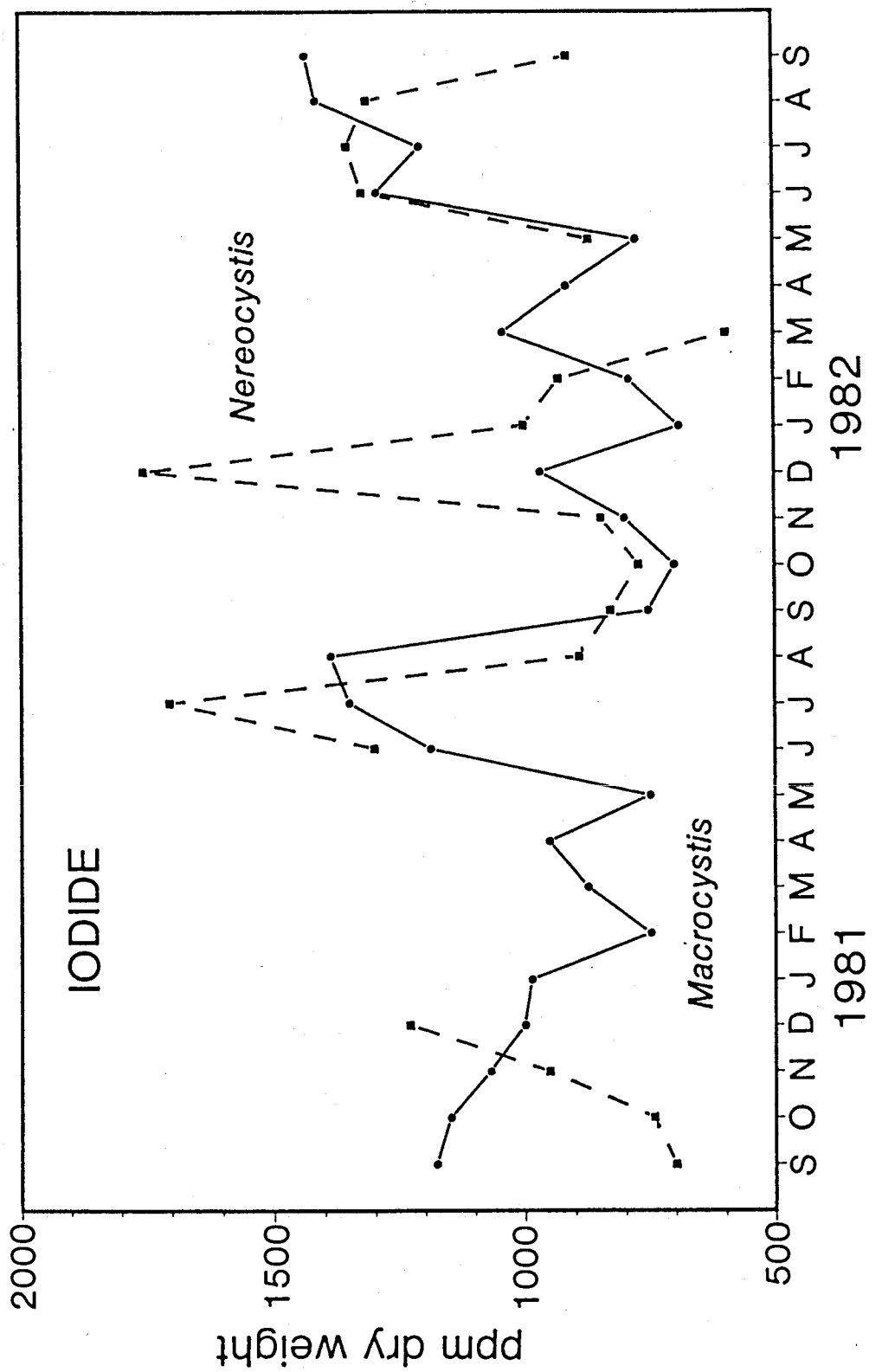
blades (16-40 μmol g^{-1} d.wt.), but they did not show as clear a seasonal trend as did the blades. No differences were found in levels of iodine in plants growing in open coast or sheltered localities.

Whyte and Englar (1978) determined the I^- content in samples of Macrocystis integrifolia collected during the growth season (April-October). Their data were as follows:

Month	Iodide (μmol g^{-1} d.wt.)
April	8.7
May	13.8
June	15.5
July	11.0
August	12.4
September	11.7
October	13.3

Rosell and Srivastava (1984) determined levels of iodide in monthly samples of M. integrifolia and N. luetkeana over a period of 2 years. Higher values were recorded in summer for both Nereocystis and Macrocystis and in Nereocystis, iodine levels showed another peak in winter. The values ranged from 5.6 to 14.0 μmol g^{-1} for Nereocystis and from 4.2 to 11.0 μmol g^{-1} for Macrocystis (Fig A2).

Figure A2 Seasonal variation of iodide in Macrocystis and Nereocystis
Levels are given in ppm.
(Data from Rosell and Srivastava, 1984).


IODIDE
Nereocystis
Macrocystis
ppm dry weight
2000
1500
1000
500
S O N D J F M A M J J A S O N D J F M A M J J A S
1981
1982

Although iodine levels vary in different parts of the thallus of a species and in the same species with location and season, there is no question that ultimately the iodine levels in a plant are genetically influenced.
By selective breeding, Chinese scientists (Section of Seaweed Genetics and Breeding, Academia Sinica, 1976) obtained two strains of Laminaria sp. with considerably higher iodine content than the original strains (20 and 58% more iodine than the control). The same authors also indicated that plants of the same variety planted in different coastal areas showed differences in iodine levels, a finding which reinforces the importance of environmental factors.

Vinogradov (as cited in Saenko et al, 1978) showed that there exists a very good correlation between the iodine content of an alga and its taxonomic position, which also supports the idea that iodine content is genetically controlled.

4. Leaching of iodine

Very little work has been done on the leaching of iodine with different solvents. Young and Langille (1958) showed that a thorough washing of Ulva lactuca in tap water reduced the content of I^- from 0.56 to 0.48 μmol g^{-1}. Whyte et al. (1981), in contrast, showed that sequential water leachings of Macrocystis and Nereocystis left only 47% and 15% of the

original iodide content in the tissues, respectively:

	Unleached	Leachings		
		1	2	3
Nereocystis	100%	36%	15.2%	15%
Macrocystis	100%	66%	51%	47%

Rosell and Srivastava (1984) indicated that an acidic extraction of Macrocystis tissue was more effective in extracting I^-:

	Unleached	% of original weight left in residue after extraction with:		
	umol g-1	Water	0.1 N HCl	0.1 N HCl+ 2% Na2CO3
Iodide	8.3	67.0	36.4	14.4

These observations suggest that a good proportion of iodine in a plant occurs in a water-soluble form and a still larger proportion is extractable with dilute acid.

5. Iodine requirement of algae

Several authors have tried to determine whether iodine is an essential element for algal growth. The main problem in these studies is to get a seawater medium which is totally free of I^-. This ion is often a contaminant in different salts (used in

preparing artificial seawater) in concentrations greater than in seawater. However, experiments carried out with I^- free medium using specially purified chemicals and parallel cultures containing different concentrations of KI or organoiodine have shown in some cases the absolute need for iodine. Fries (1966) working on red algae in axenic culture showed that Polysiphonia urceolata had an absolute requirement for either organically-bound iodine or inorganic I^-; without these sources of iodine the thalli died. The growth was linear between 1 and 8 μM I^-; above 8 μM I^- inhibition of growth occurred. Nemalion sp. was indifferent and Goniotrichum (both Rhodophytes) was inhibited by an I^- concentration close to that of natural seawater. Codomier et al. (1979) studied the effect of KI or KIO_3 on the growth of Asparagopsis armata. Maximum growth occurred at 5 μM. At 30 μM the thalli died after 25 days and at 300 μM the thalli died the next day. These authors indicated a direct correlation between the presence of secretion cells (ioducts, see section on Distribution of iodine in a plant) as in some red algae and the non-tolerance to high levels of iodine in the medium. Brown algae do not have such cells and tolerate much higher levels. Hsiao (1969) studied the development of Petalonia fascia (Phaeophyta) and showed that iodine at a concentration of 40 μM KI or more was required to obtain the Ralfsia stage[1] and that a concentration of at least 4 μM was necessary to keep the blades of the sporophytes alive. A slight

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[1] This is the crustose gametophyte of the leafy sporophyte, Petalonia.

inhibition of blade growth was found with 20 mM, a concentration 50,000 times that of seawater. Protonemata and plethysmothalli stages were formed in I^- free media. Continuing that work Hsiao and Druehl (1973) showed that I^- or IO_3^- were not essential for antheridial or oogonial production in L. saccharina but did have a stimulatory effect. Inhibition also occurred at I^- concentrations 60,000 times that in seawater. At low concentration iodide was more effective than iodate for meiospore germination, but at high concentration it was also more toxic than iodate. The authors suggested that Cl^- ions reduced the inhibition caused by high I^- concentrations. Hsiao (1969) observed no difference in growth of Petalonia fascia whether NaI or KI was used. Pedersen (1969) showed an absolute need for iodine in Ectocarpus fasciculatus (Phaeophyta) with an inhibition of growth occurring at 64 μM I^-. Woolery and Lewin (1973) confirmed these results for E. siliculosus and also showed that any appreciable vegetative growth required at least 1.5×10^{-3} μM I^-, whereas the normal formation and maturation of plurilocular sporangia required higher concentrations of 2.5×10^{-2} μM I^-. A concentration of iodine half that in seawater is necessary for normal development. No iodine in the medium led to a lack of growth and even death of the thalli but growth could be restored by the addition of KI. The authors found no difference between the use of KI or KIO_3.

The studies reviewed above indicate that in several benthic algae the requirement of iodine for growth and/or reproduction is absolute, a finding which suggests that this element plays an

important role in the metabolism of some marine algae.

6. Pathway of iodine in marine algae

Radioisotopes of iodine have been widely used to label the iodine present in algae, either by incubating the plants in radioactive solutions (Scott, 1954; Tong and Chaikoff, 1957; Klemperer, 1957; Knappe and Werner, 1975) or by adding the radioisotopes to minced algae or an extract of the algae (Scott, 1954; Tong and Chaikoff, 1955). Various solvents such as HCl or t-butanol have been used to extract the iodinated compounds (Knappe and Werner, 1975). More commonly, a water or ethanol extraction is followed by a barium hydroxide hydrolysis (Scott, 1954; Klemperer, 1957; André, 1971) or by proteolysis of the residue (Tong and Chaikoff, 1955; Knappe and Werner, 1975) after which chromatography with collidine-water is used to separate I^-, mono- and diiodotyrosines as well as some other minor iodinated compounds (Klemperer, 1957). Tong and Chaikoff (1955) showed that cell-free extracts of Nereocystis luetkeana converted $^{131}I^-$ to organic iodine enzymatically in presence of tyrosine. Such conversion did not occur if boiled cell-free extract was used:

Cell-free extract $+^{131}I$ + Tyrosine = iodinated amino acids

Boiled cell-free extract $+^{131}I$ + Tyrosine = no organic iodine

Cell-free extract $+^{131}I$ - Tyrosine = no organic iodine

When tyrosine was replaced by monoiodotyrosine (MIT),

diiodotyrosine (DIT) was produced. When DIT was added, very little triiodotyrosine was detected.

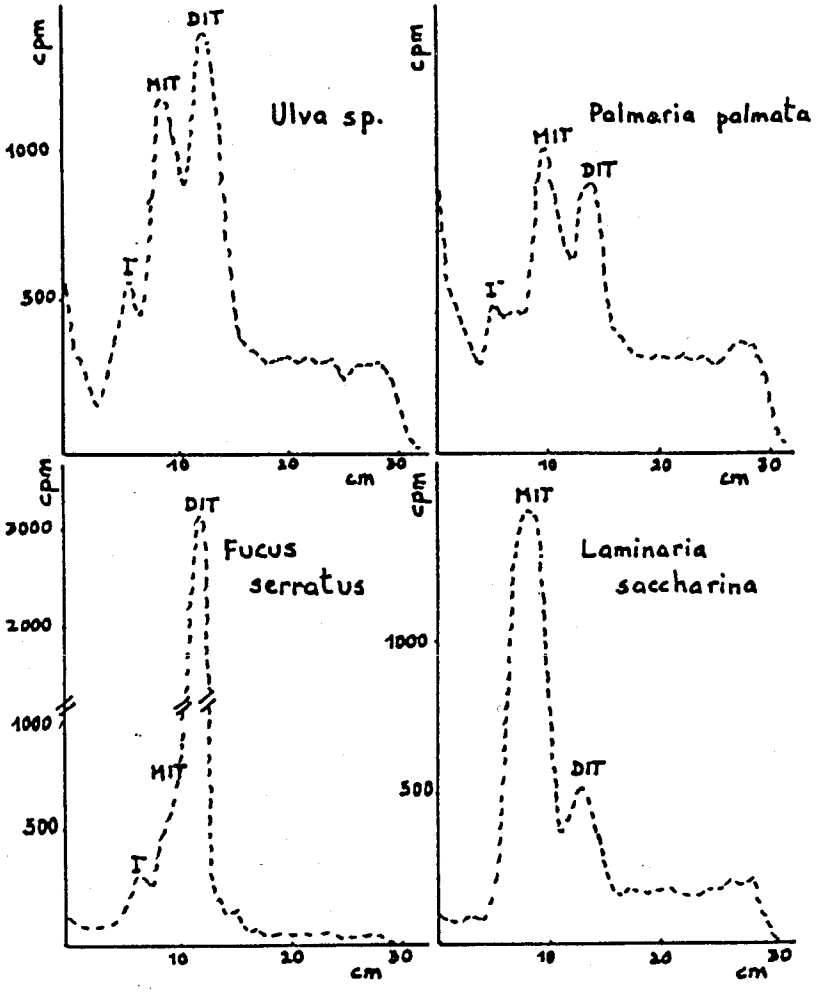
The same authors showed that 2/3 of the ^{131}I taken up by Nereocystis ended up as organic iodine. Other authors, by contrast found very little organic iodine in Fucus (Klemperer, 1957), which led André (1971) to look at the ratios of inorganic/organic iodine in different species of algae:

	Labelled iodide under I- form
Fucus serratus	80-90%
Laminaria saccharina	"
L. flexicaulis	"
Ulva sp.	10-15%
Enteromorpha compressa	"
Palmaria palmata	35-40%

André, also, found that the ratio MIT/DIT varied greatly in these species (see Fig A3) where the algae were first extracted with EtOH to remove the soluble iodide.

It seems that MIT and DIT are part of an iodoprotein, the proportions of which vary according to the species. Also, the

Figure A3 Relative proportion of MIT and DIT in some algae
The radioactivity curves were obtained chromatographically from butanolic extracts of hydrolysates in 4 species of algae. These show the different proportions of MIT, DIT (and I^-). All tissue was first extracted with EtOH to remove I^- and hydrolysed in NaOH for 5-8 h.(Data copied from André, 1971).
The X axis represents the distance migrated by the various compounds on the chromatogramm.


cpm
1000
500
DIT
MIT
I
Ulva sp.
10
20
30
cm
cpm
MIT
DIT
I
Palmaria palmata
10
20
30
cm
cpm
3000
2000
1000
500
DIT
MIT
I
Fucus
serratus
10
20
30
cm
cpm
MIT
1000
500
DIT
Laminaria
saccharina
10
20
30
cm

difference between organic and inorganic I^- (according to André, 1971, I^- is found mainly in the cell free space and is "pulled in" to the cells for organification) reflects a different use of I^- in the various species. Scott (1954), examined the levels of iodoamino acids in different algae and found that in Laminaria digitata inorganic iodine was present only in the stipe but both organic and inorganic forms were present in the blade.

7. Uptake of iodine

The mammalian thyroid gland concentrates iodine and has been used as a model in attempts to explain iodine uptake in algae. Mono- and diiodotyrosine are found in the thyroid and in algae. For this reason, many workers studying algae have based their methods and use of inhibitors on the work which has been done on the thyroid. Most authors seem to agree that the uptake of iodine in algae is an active process and involves enzymes such as oxidases and/or peroxidases which oxidize I^- before its passage through the plasma membrane. Subsequently, it may be stored in the cell as I^- or iodotyrosine. This very basic pattern has variants as reviewed below and many of the steps or details are still not understood.

As iodate is the main form of iodine in seawater, it would seem likely that it is taken up by algae. Baily and Kelly (1955) compared the uptake of I^- and IO_3^- in Ascophyllum and found that only iodide was taken up by the alga. More recently, Hirano et

al.. (1983) showed that Hizikia sp could take up both forms of iodine , $^{125}I^-$ and $^{125}IO_3^-$, although the rate of uptake was one order of magnitude less for iodate than for iodide.

Klemperer (1957) reported an uptake rate of 0.25-0.45 μmol I^-g^{-1} f.w. h^{-1} for Fucus ceranoides and further that the uptake was linear with time.

When uptake of iodide in the light was compared to that in the dark, darkness seemed overall to have little effect on the uptake. Even over a seven day dark period, there was apparently no effect on ^{131}I uptake by red algae (Knappe and Werner, 1975). Shaw (1959) recorded no differences in uptake in darkness unless nitrogen was bubbled through the medium. By contrast, Roche and André (1966) noted a higher uptake in the dark which they thought was due to the existence of a diurnal rhythm in the uptake of iodine. The uptake seems also to be independent of illumination intensity (Klemperer, 1957).

A reduction of temperature from 15°C to 0°C decreased the uptake of ^{131}I by half (Klemperer, 1957). Anaerobic conditions blocked the uptake (Tong and Chaikoff, 1955) and respiration seemed necessary for the process to take place (Baily and Kelly, 1955). Also, the presence of oxidative phosphorylation was shown to be needed as dinitrophenol (DNP) blocked the I^- transport into the cell reversibly (Roche and André, 1966; Klemperer, 1957). Inhibitors of photosynthesis such as dichloromethylurea (DCMU) and CCCP (Carbonylcyanid-m-chlorphenylhydrazon) were

reported to be ineffective in the uptake of I^- (Knappe and Werner, 1975; Shaw, 1959).

As to the actual uptake process and possible organification of iodide, many authors (e.g., André, 1965; Baily and Kelly, 1955; Klemperer, 1957; Shaw, 1959,1961; Tong and Chaikoff, 1955) have shown that inhibitors, including some thyroid specific inhibitors, have an effect on these two processes. Perchlorate, a characteristic inhibitor of the thyroid, was found to compete for iodide uptake (Klemperer, 1957) or had no effect at all (Tong and Chaikoff, 1955; André, 1965). André suggested that ClO_4^- may not permeate the tissues of certain algae. Ouabain, which was then found to inhibit iodide transport in the thyroid and is now considered a specific inhibitor of Na^+K^+-ATPase (Lehninger, 1975), showed no effect on iodine uptake in algae (André, 1965). Many metabolic inhibitors, such as cyanide, inhibited iodide uptake reversibly. Klemperer found that 2-mercapto-1-methylimidazole inhibited the organification process of I^-, but not its uptake, and that thiocyanate and nitrate (see bacterial activity section) were two competitive inhibitors (with ClO_4^-) of I^- uptake in concentrations 30 and 700 times that of I^- which reduced the uptake by half. It was also noted that the addition of iodide ions reduced the radioactivity in the tissue (with 0.1 mM in Shaw, 1959) or abolished it altogether (over 200 μM in Klemperer, 1957). Shaw (1959) demonstrated a stimulation of I^- uptake by the addition of I_2.

As to the mechanism of iodine uptake, Shaw (1959) postulated a system consisting of an oxidase on the outer wall of the algae which oxidizes I^- into I_2 or HIO; the latter two compounds then enter cells and in turn are reduced to I^- or organified into MIT or DIT. But no molecular iodine has been found in algal tissues (Tong and Chaikoff,1955) and more recent work has cast doubt on the existence of HIO in seawater. Baily and Kelly (1955) suggested an ion-exchange mechanism involving exchange of iodide ions between the medium and that of the cells. This mechanism was considered unlikely by Woolery and Lewin (1973) as they showed a necessity of iodine in the growth of Ectocarpus without which the thalli exhibited nutrient deprivation symptoms. Also, a simple exchange would not explain internal concentrations of iodine up to 30,000 times those in the seawater. Klemperer (1957) proposed that a specific site combines reversibly with iodide, transports the ion across a boundary in the cell and iodide is then accumulated within the cells in a bound form. Tong and Chaikoff (1957) suggested the involvement of a peroxidase system that could iodinate tyrosine. More recently Murphy and OhEocha (1973) showed that cell free extracts of Laminaria digitata could catalyse the oxidation of iodide as well as the formation of iodoamino acids. This required H_2O_2 and the activity was strongly inhibited by azide and cyanide. Vilter et al. (1983) determined the peroxidase activity and iodine content in 33 species of Phaeophyceae. Although no clear trend was shown, the authors suggested a possible link between the peroxidase activity in the algae and its iodine content. The

peroxidase activity seemed to be I^- dependent and showed more activity in spring than in autumn. Peroxidases of two different types were postulated to be present: one in the cell walls between cortex and medulla and the other on the thallus surface (Vilter, 1983). Peroxidase activity was stimulated by illness and infection and, although these algae have high levels of tannic substances that might act as antimicrobial agents, the authors suggested that the peroxidase-dependent formation of iodine may also have an important antimicrobial function.

8. Iodovolatilization

A special mention should be made of the phenomenon called iodovolatilization that triggered much interest at the beginning of this century. Dangeard (1957) wrote a review on this topic, the important points of which can be summarized as follows.

Laminariales, known to be rich in iodine, can release molecular iodine, a phenomenon that is accentuated at low tides especially at the two equinox spring tides. This process requires oxygen; bubbling nitrogen through seawater inhibits the release of iodine. Also, when the surface of the lamina is scraped, the resulting scrapings can oxidize I^- to I_2 which indicates the involvement of an oxidase in this phenomenon. Iodovolatilization can be enhanced by stress conditions such as heat, desiccation and certain poisons (e.g., chloroform) and also by seawater containing high levels of I^- (800μM). According

to Dangeard, iodovolatilization could be the result of stressing agents or, and this he favours, it is a mechanism enabling the plant to free itself of excess iodide, since it may have no mechanism to prevent its entry. This interpretation would suggest that iodide uptake is not an active process. It must be noted that Fucales and certain red algae can also iodovolatilize although their iodine contents are much lower than those of the Laminariales.

9. Aims

The above literature review has pointed out the extent of variability of current knowledge concerning iodine and its uptake, distribution and role in algae. In part, this is due to different methods used by various authors and in part due to different behaviour of algae with respect to iodine. For example, the concentration and distribution of iodine in algae varies greatly from species to species or according to the authors' analyses. Similarily, the chemical state of iodine found in algae is still very controversial; the proportion of organic to inorganic iodine appears to be species dependent and it is only after hydrolysis with an alkali that chromatograms indicate the presence of MIT, DIT and sometimes tri-iodotyrosine. Authors agree that the environment could have an important influence on the physiology of algae with respect to iodine. It seems indubitable that iodine is a necessary nutrient in the growth and reproduction of some red and brown

algae.

In this thesis, I wish to concentrate on some of the above topics by studying the following points:

1- Does exogenous iodine affect the growth of young Laminaria saccharina sporophytes?. Previous work in this area has always been done using the gametophytic stages of algae.

2- What are the parameters involved in the uptake of iodine in L. saccharina.

3- What is the iodine distibution in L. saccharina?. Under what chemical state is iodine found in this alga?.

4- Is iodine translocated in L. saccharina?.

5- In addition to the above points, the field growth of L. saccharina will be monitored. This study should indicate whether there is any relationship between the growth of L. saccharina and its content in iodine or its uptake of iodine. It will also enable me to see whether iodine uptake rates or iodine levels in various parts of the plant vary with season. This study will precede all the others.

6- A short chapter will conclude on the findings of the various points of study.

PART B

Growth of *Laminaria saccharina* in the field

Although the field growth of L. saccharina is well documented in other parts of the world (Parke, 1948; Lüning, 1979) and in the present study site (Druehl 1965, Druehl and Hsiao 1977), it was necessary to follow the growth of the plants concurrently with other aspects of this study; the uptake of iodine could fluctuate with season. Similarly, the distribution of iodine within a plant might be related to growth, and if this were the case, it would be necessary to know exactly when and at what rates these plants were growing. The growth of these plants was followed over 2 years (1983-1985) at Brockton Point, Stanley Park, Vancouver, B.C., Canada, the identical site from where plants were collected for physiological experiments and for monitoring seasonal levels of iodine along the thallus.

I. Material and Methods

A 'kelp farm' with about 40 tagged plants of Laminaria saccharina was established in situ at Brockton Point (49°18' N, 123°7' W). The farm comprised of a 10 m rope running parallel to the shore, held down by two chains and locatable by a float (Fig B1). The float provided access to the plants even at a 1.2 m tide. The plants were tagged and monitored monthly from August 1983 to July 1985 following the method by Parke (1948). A hole was punched at 10 cm from the stipe-blade junction every month and measurements were made of the following parameters:

-total length of blade

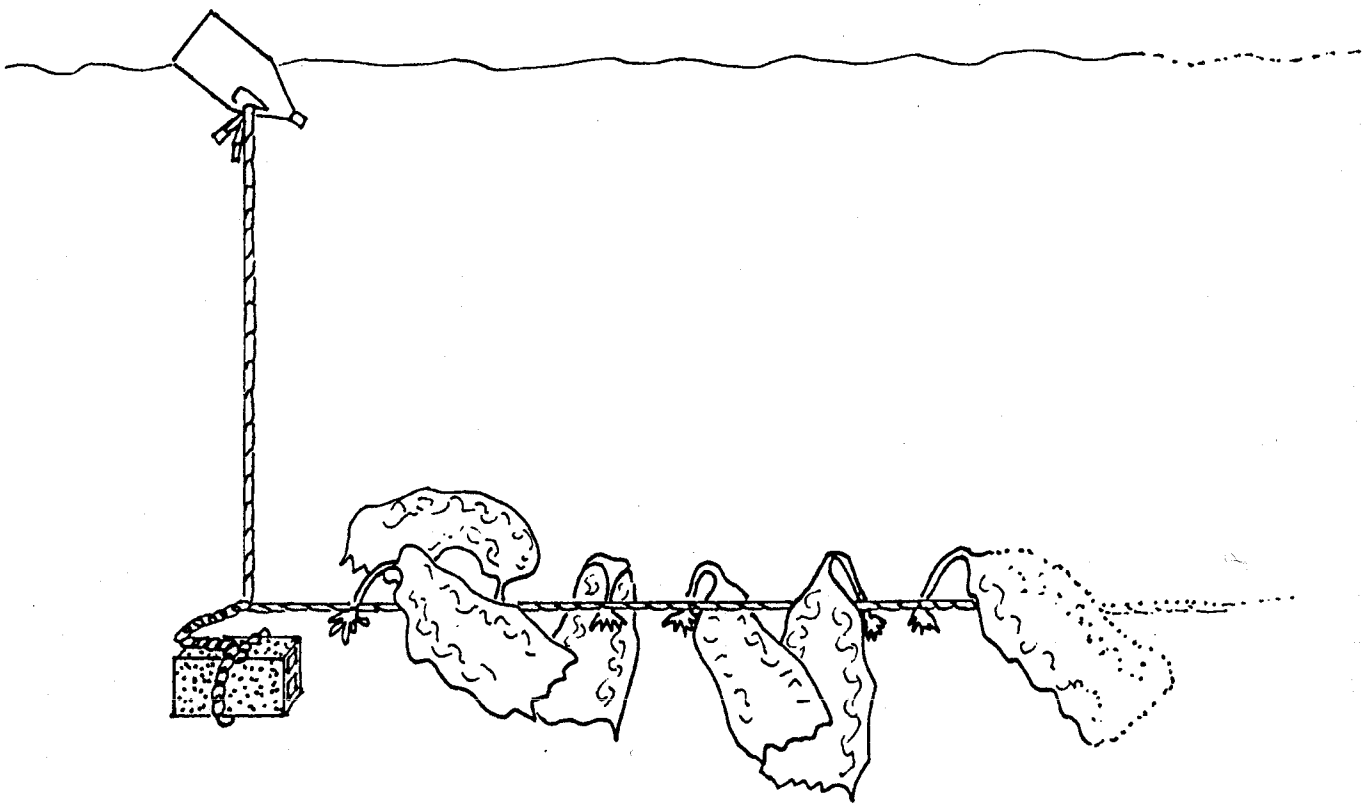
-width of the blade at hole newly punched (new hole)

-length of the blade between new hole and previously punched hole (old hole)

-width of blade at old hole

Unfortunately, a continuous set of plants could not be used as many plants died at the summer spring tides of 1984, which coincided with very hot weather, and winter spring tides in 1983-84 and 1984-85 when the temperature was well below freezing. When this happened, new plants were tagged and monitored (indicated in figure legends).

Figure B1 Kelp farm
About 40 plants were attached to a polypropylene rope, both ends were weighted down by chains and a cement brick. A float locates the set up.



II. Results

The raw data for the growth, width at new hole and old hole and the total length of the tagged plants appear in Appendix I. Mean growth rates, mean lengths and mean widths at both holes were calculated together with the standard error and appear beside each set of raw data. The mean blade length has been plotted (Fig B2) and a visual representation of a typical plant's growth from 1983 to 1985 using widths at both holes and the mean blade length appears in Fig B3. The plants have been drawn to scale using the mean values of each variable at every measuring time.

The following observations were made. In February 1984, only small sporophytes 2-3 cm in length were found at the study site. All the large sporophytes had died following heavy frost in December 1983 but the gametophytes obviously survived and gave rise to the new 1984 population. Plants in April showed a mean length of 65 cm, indicating a growth rate between February and April of 1.06 cm d^{-1}. This elongation rate may appear small, but it must be noted that the increase in surface area is much greater. In May the growth rate increased to nearly 2 cm d^{-1} and then decreased to 1.5 cm d^{-1} in June and 1.2 cm d^{-1} in July. The total length of the blades also dropped dramatically from 100.0 cm in May to 71.3 and 58.2 cm in June and July. This decrease in growth which was accompanied by an increase in erosion was due to the summer solstice spring tides concurrent with very hot weather which resulted in massive tissue damage. This phenomenon

Figure B2 Mean blade length for 1983-1985.
N=30-40 ±S.E.
Plants were retagged where indicated in Fig B3.

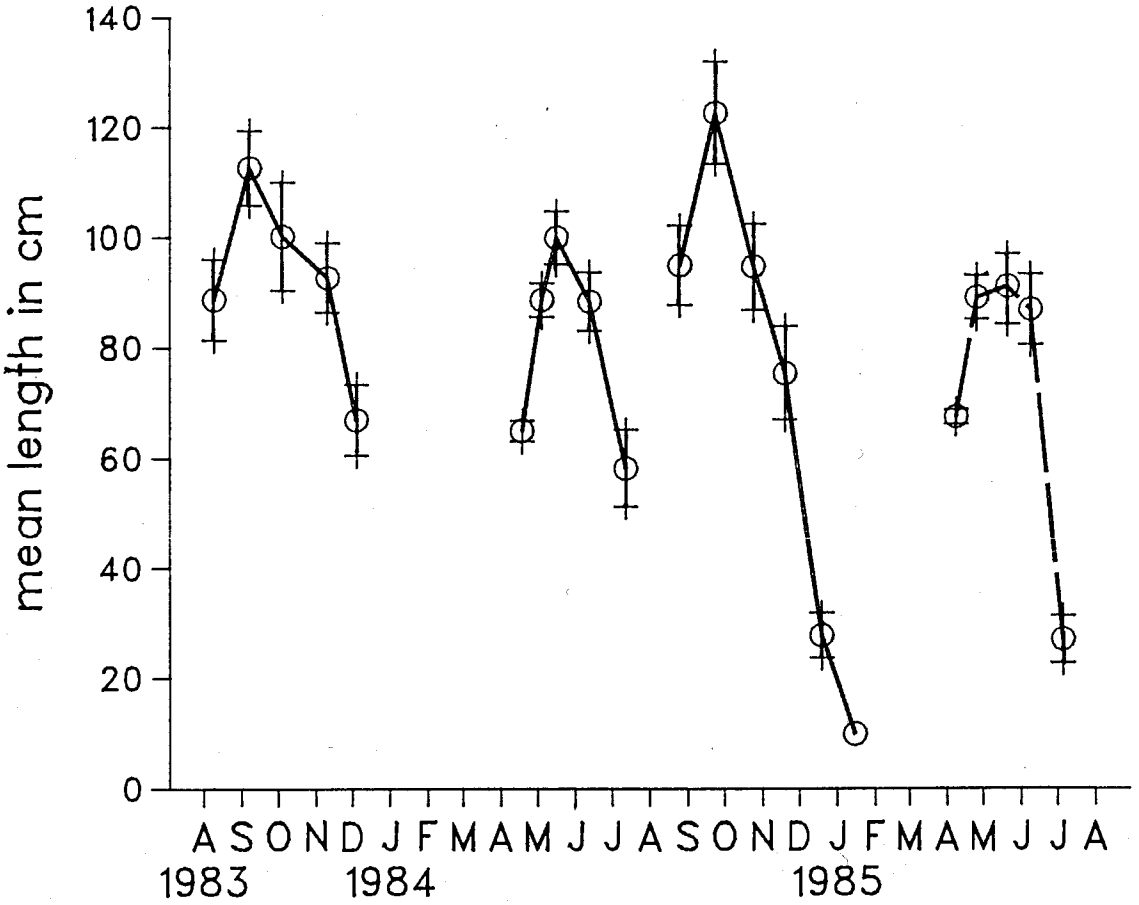
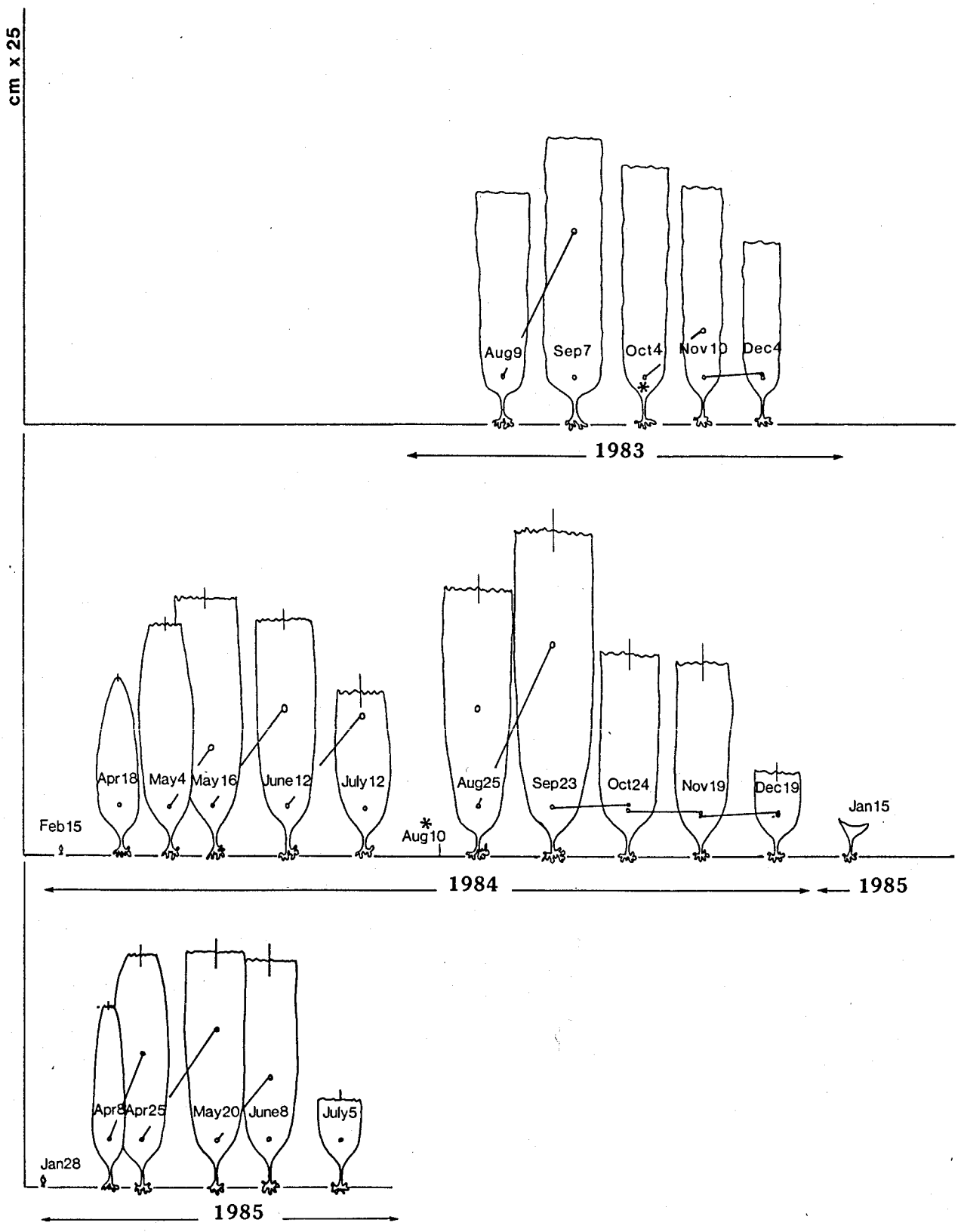

mean length in cm
140
120
100
80
60
40
20
0
A S O N D J F M A M J J A S O N D J F M A M J J A
1983
1984
1985

Figure B3 Visual representation of the growth of Laminaria plants
The plants have been drawn to 1/25 scale. The shape of the plants is that of a typical plant at one given time. An asterisk indicates that a new series of plants was tagged within the growing season. The S.E. of the blade length is drawn at the tip of each plant.
On August 10, 1984, the data were lost so no plant outline appears at that date.


cm x 25
Aug9
Sep7
Oct4
Nov10
Dec4
1983
Apr18
May4
May16
June12
July12
Feb15
Aug10
Aug25
Sep23
Oct24
Nov19
Dec19
Jan15
1984
1985
Apr8
Apr25
May20
June8
July5
Jan28
1985

reoccured at the beginning of July 1985. By August the growth again reached 2.4 cm d^{-1} and blades were at their maximum length in September with an average length of 122.6 cm. Similar results were found in 1983 when growth was monitored from August to December. The growth rate in September was still high, 2.14 cm d^{-1}, but this soon dropped in October, when plants barely grew. It was estimated that Laminaria produced on an average 258 cm of blade tissue from May 4 to December 19, 1984, when erosion was not taken into account. All tagged plants carried sori in October of both years, which in some cases nearly covered the entire blade. This was the case until December. In January 1985, hardly any blade tissue remained on the tagged plants, a situation prevalent in the whole kelp bed. Only very few plants with blades, and these were in poor condition, could be found in the kelp bed. In February 1985, and later, in April, a total of 6 tagged plants had started to grow, indicating, as was later seen, that these plants can live a second year. These second year plants displayed a lower growth rate than the first year plants: a maximum of 1.5 cm day^{-1} in April 1985 versus 2.2 cm day^{-1} for first year plants during the same period.

III. Discussion

The elongation rate was used to estimate the growth of Laminaria saccharina plants. It is one approach to measuring growth which, although not as precise as the added measurement of thickness and dry weight, should give a reasonable understanding of periods of maximum and minimum growth. One must keep in mind that blade width and thickness are important parameters in influencing blade elongation rates.

" A plant with a wider blade can elongate at a lower rate and still produce more biomass than a narrower plant" (Gagne et al., 1982).

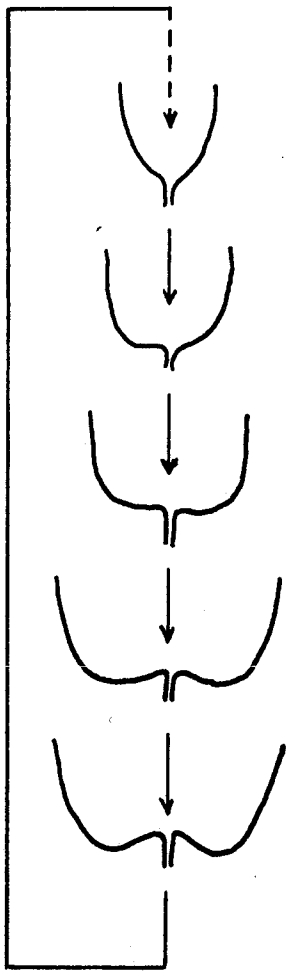
The data presented above displayed a maximum elongation rate of 2.4 cm day^{-1} for Laminaria saccharina in late August 1984. This rate was similarly high in August-September 1983 with a value of 2.17 cm day^{-1}. This elongation rate falls well within the range found for other Laminaria species : 1.9 cm day^{-1} in L. digitata in Nova Scotia (Mann, 1972), a maximum of 3.5 cm day^{-1} in L. longicruris (Anderson et al., 1981). Druehl (1965), however, found a maximum rate of 1.4 cm day^{-1} for L. saccharina in the same study site in September 1962. The reason for this discrepancy is not clear. As was shown in the results, second year plants displayed smaller growth rates than first year plants. It is possible that Druehl (1965) monitored the growth of second year plants. Alternatively, the nutrient conditions in seawater near Brockton Point may have changed since the time of Druehl's study.

The duration of growth as recorded here corresponds very well with the growth data of Druehl and Hsiao (1977). As in Druehl and Hsiao's study, the elongation rate of plants decreased rapidly in the fall to cease in November-December, when light and maybe temperature become too limiting to sustain growth which rapidly declines. Gagné et al. (1982) have indicated that plants growing in a nutrient-rich environment follow the seasonal light availability. Vancouver Harbour represents such a nutrient-rich environment. These plants grow in summer and fail to store carbohydrate reserves which, as in the case of nutrient-limited plants, would enable them to grow in winter. The growth cycle of these nutrient limited plants is regulated by the nitrogen availability; an extreme case of this was shown by Chapman et al (1980) who monitored the growth of L. solidungula in the Canadian arctic. The growth of L. solidungula was found to be closely correlated only with the seasonal variation of nitrogen and was inversely related to seasonal light availability. Plants grew mostly in winter (2 mm d^{-1}) when there was a total ice cover and arctic winter light regimen. However, other studies have shown that light availability plays an important role in the growth cycle (Dieckmann, 1980) and some plants reduce their growth rate in early summer when light and nitrogen are in good supply (Lüning, 1979). This suggests that there might be some genetic control over at least some parts of the growth cycle. It is interesting to note that similar first year plants of the species L. groenlendica had nearly halted their growth in August-September on the West coast of Vancouver

Island (Villeneuve, 1985); this is when L. saccharina had maximum blade elongation rates in Vancouver Harbour. The raw data in Appendix I also indicate the monthly width increase. A good representation of the change in width and shape of these plants is given in the diagram by Perez (1969) which I have included in Fig B4.

One could summarize the growth pattern of these plants in the following way. The new sporophytes appearing in February represent the majority of the population. Although these plants display a maximum elongation rate in the period between August and September, their maximum biomass production probably occurs in the spring when these plants are much smaller. A clear bimodal pattern of blade length is caused by two different phenomena. The first factor is external to the plant: the summer spring tides cause considerable blade damage which is followed by a massive blade erosion (also see Druehl, 1965, Druehl and Shaw 1977). However, there is only a slight growth decline during that time. The second factor is due to the biology of the kelp itself and occurs in the fall-winter. At this time, growth is halted and cannot compensate blade loss through erosion. Besides, most plants produce sori after September which often cover the whole blade. Lüning, (1979) has also observed that Laminaria plants in a reproductive phase show minimal growth. In December, another setback may occur, as in 1983 when plants were frozen during winter spring tides and many were killed. However, and although this occured in only a small proportion of the

Figure B4 Change in shape of the basal part of Laminaria
This evolution of the shape of the basal part of Laminaria was observed in the present study.
Diagram copied from Perez (1969).



tagged plants, it was remarkable to observe that a debladed stipe could regenerate to a full sized plant in the second year though these plants never grew as well as the first year plants on the farm.

As Druehl and Hsiao (1977) remarked, the majority of L. saccharina plants at Brockton Point act as annuals although they have the potential of being perennials.

PART C

Effect of added KI on the growth of sporophytes and blade discs

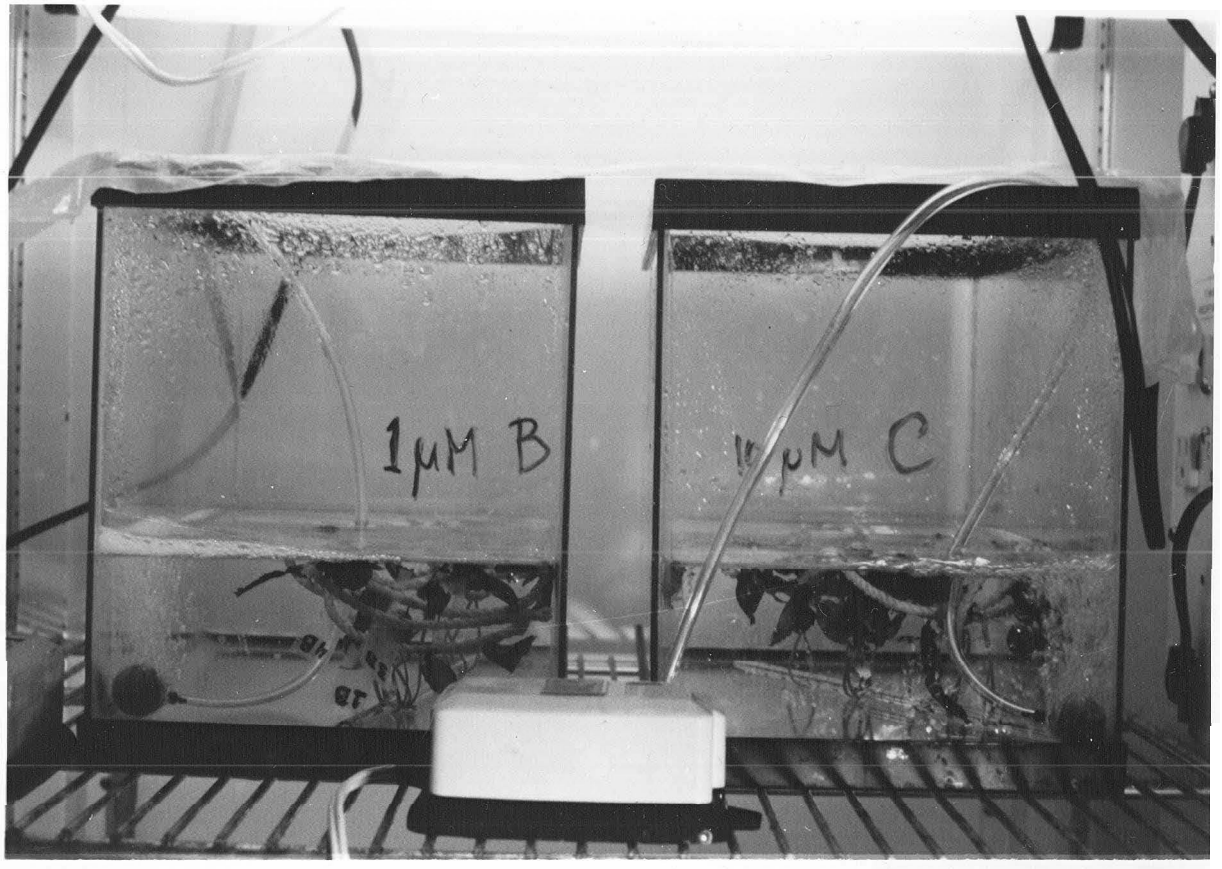
To test whether exogenous iodide could influence the growth of *Laminaria saccharina* sporophytes, young sporophytes were collected for *in vitro* feeding experiments. The use of large plants, as found in the field, was impractical due to their size. Instead, blade discs punched out of the growing area of blades of these large plants were used and their growth in media containing various amounts of KI was measured. In addition to overall growth, effects of added KI on cell number per given area, pigment content and chlorophyll fluorescence were measured.

I. Materials and Methods

A supply of small sporophytes (2-3 cm in length) was collected from a wooden wharf in the marina at Coal Harbour, near the entrance to Stanley Park, Vancouver, B.C. in April 1984. The plants were in very good condition as in this location they do not get damaged by tides, waves, rocks, etc. Plants collected were categorized in size classes in order to have a group of plants as homogeneous as possible for the experiment. The plants were cleaned with a fine paintbrush and fixed on a 10 cm long polypropylene rope (6.5 mm thick) by untwining it and inserting the holdfast of a plant into the rope. Three to 4 plants per rope were inserted in this way and each rope was tagged for recognition. Ropes were held in the aquaria by elastic bands fixed onto the ropes and looped around glass pipettes lying on the bottom of the tanks (Fig C1 a,b). In this fashion, plants were kept totally submerged instead of being left floating as trapped air bubbles in contact with blade tissue could have deleterious effects on the plants (Steve Fain, pers. comm.). This experiment was repeated in March 1985 where large Erlenmeyer flasks were used instead of the aquaria. The use of flasks allowed more efficient air bubbling.

Treatments consisted of 5 L of filtered seawater (Millipore 0.22 μm) plus 1 L of distilled water to bring the salinity to 20 g l^{-1} as measured in Stanley Park. The salinity was not corrected the following year. Varying quantities of KI were

Figure C1 Experimental set-up for young sporophytes in culture
a-The young sporophytes were inserted on a rope that was looped around a glass pipette.
Air was vigorously bubbled through the medium which consisted of 0, 1, 10 or 100μM KI.
b-Incubator with 2 treatments.
Two day light and 2 cool-white fluorescent tubes on each level constituted the light source.


1μM B
1μM C

added yielding concentrations of 1, 10 and 100μM. The control contained no added KI and the iodide concentration was that found naturally in seawater (about 0.5 μM). In addition, in 1985, the experiment was carried out using 100 μM NaI to insure that any observed effect was due to iodide and not to the potassium content of KI. The media were vigorously bubbled with air and changed every day.

To estimate growth, the ropes were flattened on photographic paper and each plant was held down with a glass slide. Light was shone from above and the paper was developed, thus revealing the outline of each plant. Subsequent measurements with a planimeter were made on the photographic print of each plant to give its integrated surface area. Plants were measured in this way every 3 days.

Pigments were extracted according to the method of Seely et al (1972) but the results were inconclusive and this method was abandoned. Instead, the total pigment density (chlorophyll a, c, and fucoxanthin) was measured by extracting 0.3 g of freeze dried tissue twice in acetone and once in DMSO (adapted from Woolery and Lewin, 1973). The extracts were combined and an aliquot was measured spectrophotometrically (Pye Unicam SP8-100 UV Spectrophotometer) at 435 nm. The results are expressed as optical density.

In addition to the above, an estimate of cell size and of photosynthetic efficiency by measurement of chlorophyll

fluorescence (Papageorgiou, 1975) were also attempted. These data were not conclusive and the results for the estimate of cell size appear in Appendix II.

The above experiments conducted on small sporophytes were also carried out in August 1984 on blade discs, 1.95 cm in diameter. A preliminary test indicated that the discs punched closer to the meristem (5-10 cm from the stipe junction) grew better than those punched from the mature blade tissue. Thus, younger tissue was used. Thirty discs per treatment were put into 2 L of filtered seawater with 0, 1, 10, 100μM KI and bubbled with air. Temperature of the incubator was set at 12°C. The diameter (twice at a=180° as discs tend to grow as ovals) was measured every 2 days and the medium was replaced at the same time. The experiment was carried out for 14 days and the data were expressed as mean diameter for each measuring time. Pigment and chlorophyll fluorescence analyses were also performed on these discs and the fluorescence data appears in Appendix II.

II. Results

1. Young sporophytes

Four days after the beginning of the experiment, visual differences could be observed between treatments. Controls and plants kept in 1μM added iodide displayed necrotic areas at the tip of the blades, which may have been due to a fungal or bacterial infection (Dr. J. Craigie, pers. comm.). No such symptoms were observed in the 10 or 100μM treatments which displayed healthy, darker and bigger plants. These differences were more apparent by the end of the experiment 12 days later, although the colour differences had diminished. A repetition of this experiment in 1985 gave similar results. In the control and 1 μM treatment 1/3 of the plants had virtually disintegrated by day 12 of the experiment as opposed to the 10 and 100μM treatments where plants remained healthy and very few were lost during the experiment.

The photographic data analyzed by integrating the surface area with a planimeter appear in Appendix III. These data were analyzed by using a statistical package (SPSS: Statistical Package for the Social Sciences, 1979) and the results of the analysis of variance appear in Figs C2(1984) and C3(1985) as the increment of growth at each measuring time from the plant size at time 0. Although true replicates of each treatment were not run due to logistics, it was felt that inferential statistics could be applied to the data as the experiment was carried out

Figure C2.Effect of added KI (0, 1, 10, 100μM) on the growth in surface area of young sporophytes
Data obtained in 1984.
S.E. appears at each data point.
No difference between treatments at a 95% confidence interval was found where an arrow is pointing.

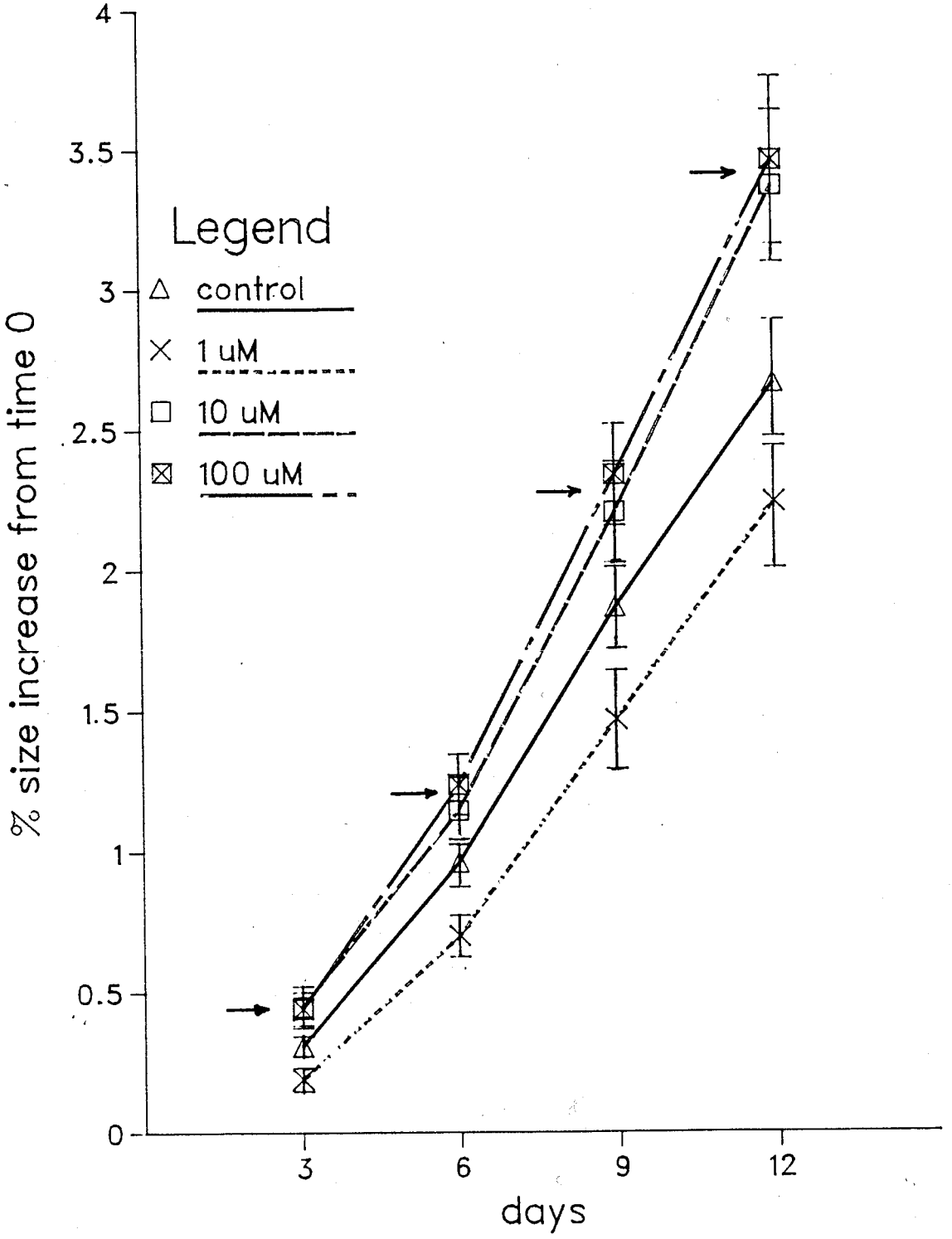
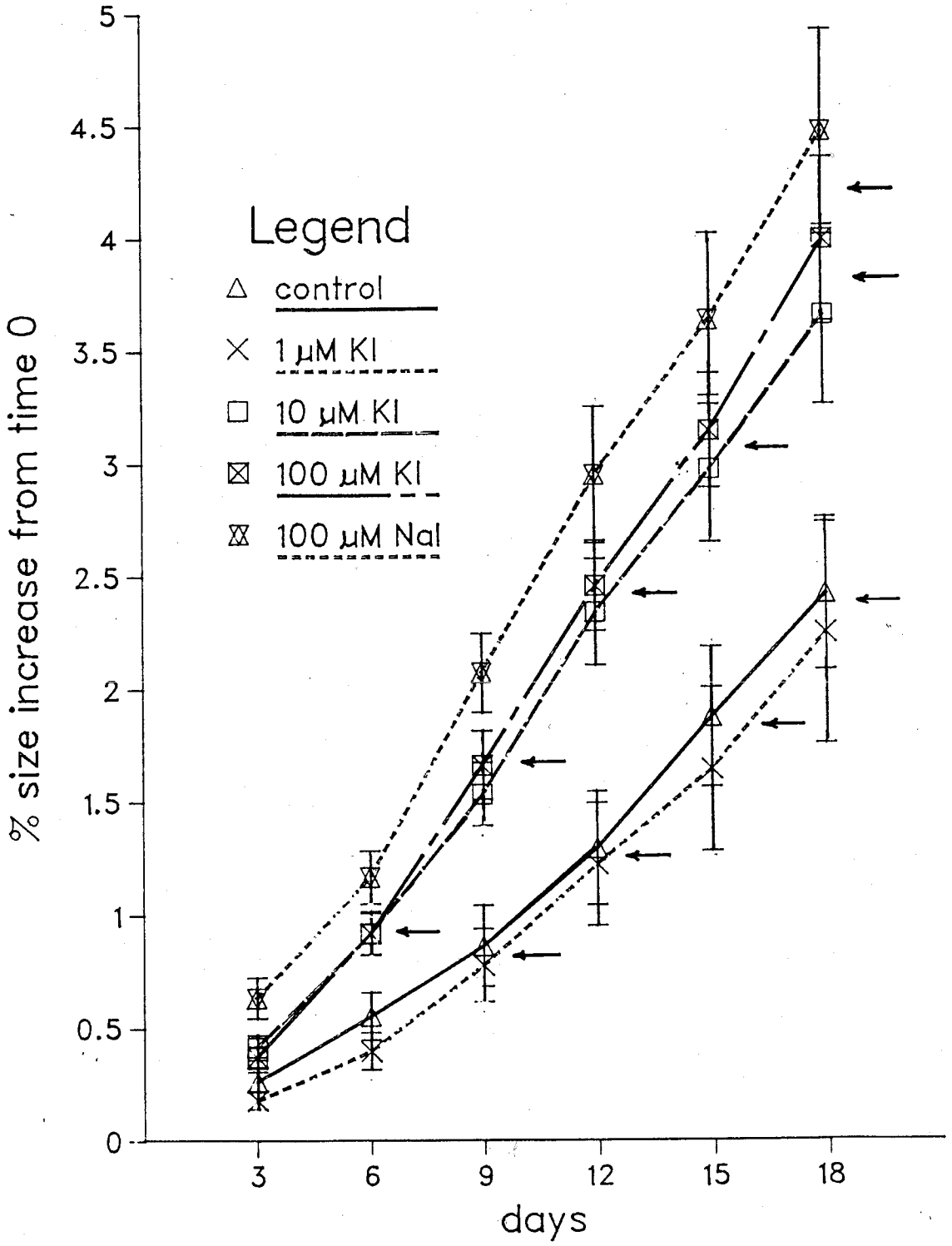

Legend
control
1 uM
10 uM
100 uM
% size increase from time 0
days
0
0.5
1
1.5
2
2.5
3
3.5
4
3
6
9
12

Figure C3.Effect of added KI (0, 1, 10, 100μM) on the growth in surface area of young sporophytes
Data obtained in 1985.
See Fig C2 for additional comments.


Legend
control
1 µM KI
10 µM KI
100 µM KI
100 µM NaI
% size increase from time 0
days
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
3
6
9
12
15
18

twice and results were comparable in both cases. The following conclusions were made. Differences between treatments at the 4 growth measuring times are not significant for the 10 and 100μM treatments (Fig C2). But they are significant between the 10-100μM treatments on the one hand and the 1μM treatment and the control on the other and indicate a higher growth rate for the treatments with 10 and 100μM added KI. Control plants show more growth than the 1μM treatment, but both developed necrotic areas over the course of the experiment. These trends were identical in 1985.

Results obtained with 100 μM KI and 100 μM NaI were statistically not different indicating that we are really looking at the effect of iodide and not that of potassium.

The effect of added KI on dry weight/fresh weight X 100 showed the following values of 9.49%, 9.98%, 10.44% and 10.51% for the control, 1 μM, 10 μM and 100 μM, respectively. NaI showed a value of 10.55% dry weight.

The data on cell counts per given area (in Appendix II) showed that no increase in cell size could be attributed to the addition of KI. This indicates that the resulting increased growth observed in the 10 and 100 μ M treatments can only be due to cell division (see Appendix II for the data).

The colorimetric determination of pigment content showed the following optical densities at 435 nm: 0.319, 0.429, 0.416, 0.698 and 0.818 for the control, 1, 10, 100 μM KI and 100 μM

NaI, respectively. These results reflect an increase in pigment density with iodine content in the media confirming the observed visual colour differences seen between the treatments.

2. Blade discs

In addition to the young sporophytes, blade discs from young parts of mature thallus were used to determine the effects of added KI in the medium.

Disc measurements, a mean of 2 orthogonal diameters, showed growth in nearly all discs at each measuring time (every 2 days). At the end of the experiment, after 12 days, the mean size of discs of each treatment was calculated and is given in Table C1. Size differences between treatments are small and show no marked trend. When calculated as a percent diameter increase the following values were found after 16 days: 30%, 31%, 25% and 24.8% whereas on a % surface area increase the values of 66%, 71%, 56% and 54% were obtained for the control, 1, 10 and 100μM KI, respectively. It is difficult to evaluate whether these differences are meaningful as there is no general trend in these values, the 1μM concentration giving the highest % size increase. No trend was found either in the weights of discs in the different treatments.

Pigment analyses also showed no effect of the treatments in levels of chlorophyll a and c or fucoxanthin. Although there seems to be an increase in the pigment levels with an increase

Table C1 Growth of Laminaria discs in 0, 1, 10, 100μM KI. Values in cm diameter.

Day	Treatment			
	control	1 uM KI	10 uM KI	100 uM KI
2	2.016	2.033	2.011	1.995
4	2.193	2.197	2.114	2.063
6	2.321	2.346	2.266	2.175
8	2.397	2.404	2.346	2.285
10	2.462	2.428	2.398	2.368
12	2.490	2.428	2.416	2.410
14	2.523	2.455	2.436	2.427
16	2.536	2.560	2.451	2.435

of KI in the medium as seen with the young sporophytes but not the blade discs, the fluorescence data suggest that there is no direct effect on the mechanism of photosynthesis itself (see Appendix II).

III. Discussion

The experiments dealing with the effect of added KI on the growth of blade discs failed to show any clear results. This is not altogether surprising as the growth of the discs was not very marked: they increased only 20-23 % of their initial diameter in 12 days, so differences between treatments would be hard to distinguish. The young sporophytes, however, after 12 days in culture had increased their size by 200-344 % depending on the treatment. Drew (1983) assessed the growth of blade discs for 3 species of Laminaria: L. hyperborea, L. digitata and L. saccharina. L. digitata discs, from similar tissue as that used in this experiment, doubled in surface area after 15 days in bubble culture. L. hyperborea discs expanded at a rate close to that of L. digitata discs. L. saccharina discs, on the other hand, failed to grow after 4 days incubation. In my case I found an increase in size of about 10% after 4 days which is small compared to that of L. digitata. Intrinsic factors particular to the species may determine whether isolated discs can grow in culture. Alternatively, growth of L. saccharina blade discs may require nutrients or environmental conditions which are still unknown. The rest of this discussion will focus on the results obtained with the young sporophytes.

A clear effect of added iodine on the growth of the young sporophytes was seen. With no added iodide or only 1 μM additional KI, many plants showed depigmentation at their distal

tips, necrosis and a general unhealthy appearance. This was the case in both years this experiment was carried out. In 1985, only 17 plants out of 30 control plants survived till the end of the experiment, the remaining plants seemed to grow well albeit being very light in colour. The 10 and 100 μM treatments were comparable in their results. In both treatments plants were healthy, showed no signs of tissue damage and both sets were darker in colour than the control or the 1 μM KI treatments. No significant difference was found between the use of KI of NaI indicating that the observed effect cannot be attributed to potassium. This confirms the results by Hsiao and Druehl (1973) where no difference was found between KI or NaI when used on Laminaria saccharina gametophytes.

The measurement of cortical and meristodermic cell sizes for all treatments showed too great a variation to estimate whether the addition of KI could influence cell enlargement. The lack of a clear trend in cell size between treatments coupled with the fact the sporophyte size increased much more in 10 and 100μM KI treatments than in 0 and 1μM KI treatments clearly indicates that there must have been a greater increase in cell number in the 10 and 100μM KI than the 0 or 1μM KI treatments. The data on dry weight values support this conclusion and show that the dry weight increased as the external concentration of KI was raised. Both these facts indicate that the increase in growth is due to cell division, not cell enlargement per se, and that there is synthesis of cellular material which is promoted by an increase

of I^- in the medium.

Culture work involving iodine as an added nutrient has previously been done mainly on the gametophytic stage of kelps. These are microscopic plants which makes their handling easy for laboratory work. All artificial seawater media contain iodide as an added nutrient as iodide has been found to stimulate or promote the growth or the production of gametes of these gametophytes (see Literature Survey). Of the Phaeophyceae more relevant to this study, Macrocystis gametophytes have been shown to need I^- for their survival (Kuwabara and North, 1980). The gametophyte growth of Desmarestia ligulata was promoted between 8 and 80 μM KI (Nakahara, 1984). Iodide enhanced meiospore germination and gametogenesis in Laminaria saccharina (Hsiao and Druehl, 1973). The only paper found to date concerning sporophytes showed a stimulation of growth of Laminaria by iodine (Harries, 1932 as cited in McLachlan, 1982). Unfortunately no role of iodine has been suggested by these various authors, only the fact that iodine might have an important role in the metabolism of these algae.
Several propositions can be made as to why iodide improves the growth of young Laminaria saccharina sporophytes in vitro:

1. Iodide might be affecting some organism directly in the medium by preventing its development without a direct influence on the plant itself. This is doubtful as the iodide levels measured in these plants at the end of the experiment by Neutron Activation Analysis (NAA) showed an increase in iodide content

with an increase of iodide in their medium: 90, 1258 and 2059 μg I g^{-1} for the control, 10 and 100 μM KI plants, respectively. There is clearly an assimilation of iodide by these plants.

2. Iodide could have a direct effect on plant growth, and it may be essential for some biochemical step in growth. This is a possibility because the surviving control plants showed a growth rate that might be close to that of plants in higher KI concentrations had they not been affected by decay.
The pigment analysis showed an increase in total pigment density with increased KI concentrations. The Kautsky curve results (Appendix II) suggest that the mechanism of photosynthesis is not affected by increasing external iodide levels. The pigment increase could be a reflection of the better growth and health of those plants in higher KI concentrations. The above considerations suggest that iodine may play a growth promoting role although it is uncertain how this is carried out. As will be seen in Chapter E, trace amounts of organic iodine as MIT and DIT were detected in L. saccharina tissue. Could I go so far as to speculate whether these substances are precursors to a growth hormone as is the case in the mammelian thyroid gland?. Or maybe, as seen in the next paragraph, Laminaria can use iodine in other ways that would still indirectly promote its growth.

3. Woolery and Lewin (1973) mention that tissue bleaching and depression of growth are characteristic of nutrient starvation as in iodide deprived Ectocarpus. This can barely be the case here as the medium was changed every day and the ratio of plant

tissue to water was 1 to 500 at the end of the experiment. Also, the natural level of iodine in seawater, 0.5μM, supports growth of plants in the field. Why should seawater with no KI added or 1μM KI not support the growth of plants in culture?. It is possible that the observed decay at blade tips was due to a pathogen. These cultures are not axenic and a Scanning Electron Microscope observation of the sporophyte tissue in all treatments revealed that all the sporophytes were covered with bacteria. However, the data are not extensive enough to decide whether there was any correlation between the concentration of KI in the medium and the extent of bacterial coverage. It still remains possible that adding iodide to the medium enables the algae to use it against potential pathogens. Kelps have been shown to release molecular iodine through the process of iodovolatilization (Dangeard, 1957) which suggests an antibacterial role of iodine (Vilter, 1983). This phenomenon is increased under stress conditions which is a likely situation for small sporophytes grown in vitro. One would need to repeat the above experiments in axenic and bacteria free conditions in order to prove this point.

PART D

Uptake of ^{125}I by blade discs

It was suggested in the preceding section that the exogenous addition of iodide influences the growth of young Laminaria sporophytes, and further that as the concentration of external iodide is raised, there is an increase in the iodide levels in the tissue. Thus there is an assimilation of iodide by these plants. In the present section, experiments were carried out to determine the uptake kinetics of iodide by Laminaria blade discs and the energy dependence of the uptake. The effects of various environmental parameters, including season, and the effects of several possible competitors on uptake were also assayed. Blade discs, rather than young sporophytes, were used in these experiments because they were available in larger quantities as well as most of the year. ^{125}I was used to monitor the uptake.

I. Materials and Methods

1. Plant material

Laminaria saccharina plants were collected at low tide at Brockton Point, Vancouver, B.C., Canada in an area adjacent to where the field growth study was carried out. Plants were brought to the laboratory in a cooler and were stored for a maximum of 6 days at 8-12°C in a cooled seawater tank with a photoperiod of 16 h light/8 h dark (fluorescent lights yielding 6 W m^{-2}). Plants were wiped with tissue paper and cleaned of visible epiphytes before any experiments. Seawater used for incubations was filtered through a 0.22 μm Millipore filter and stored at 10°C. The pH and salinity were in the order of 8.1 and 26 g L^{-1}, respectively.

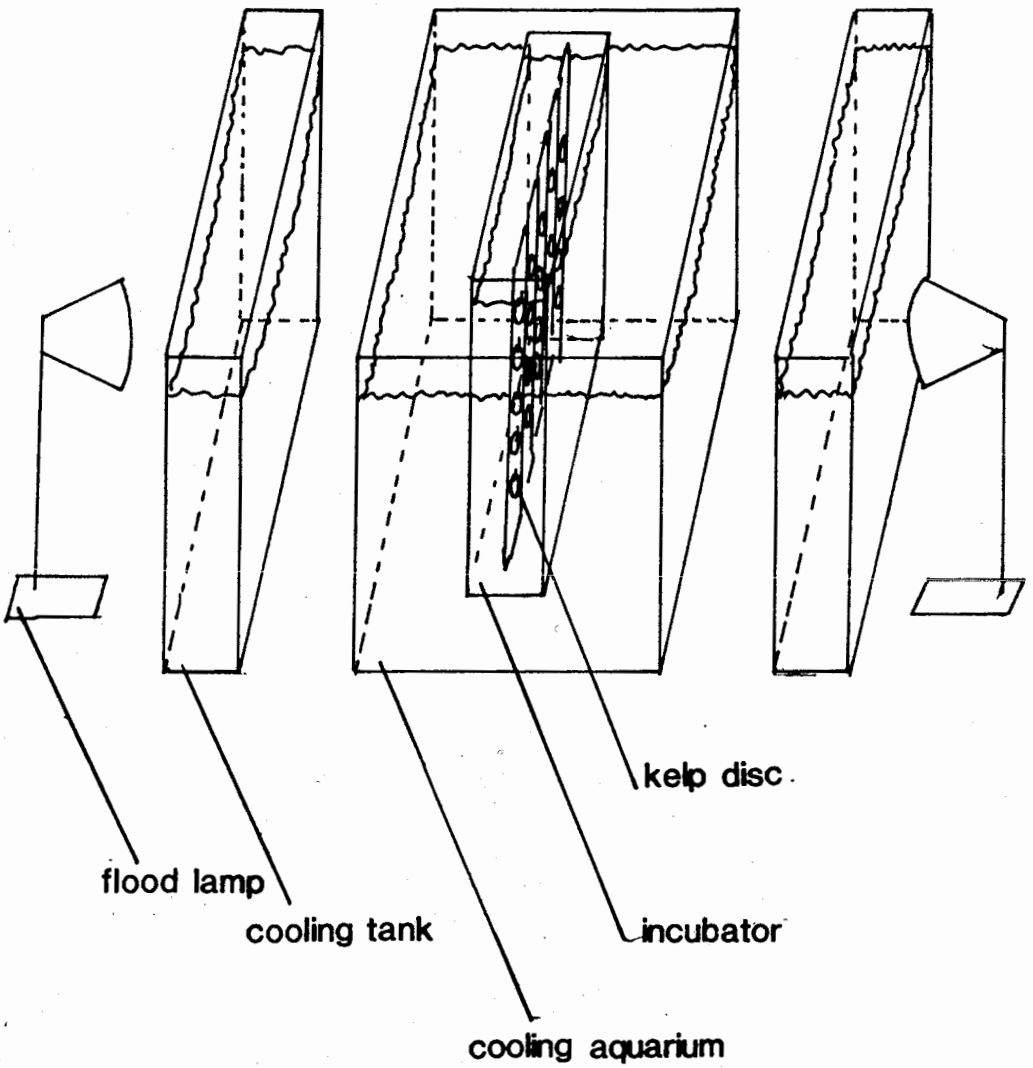
Discs 1.8 cm in diameter, punched out of *L. saccharina* blades, were used for uptake experiments. A Plexiglas incubator with a total volume of 1 L was placed in an aquarium which in turn was cooled by an annex cooler (Haake F3; Karlsruhe, West Germany) that kept the temperature within 0.2°C of the desired temperature for most experiments, 10 or 11°C. In the incubation chamber, vertical Plexiglas bars with metal hooks held the discs perpendicular to the two light sources. Initially, lighting consisted of 2 fluorescent light banks that produced a light intensity of 6.3 W m^{-2}. These were later replaced by 2 flood lights which gave a light intensity of 46.0 W m^{-2} measured by a

Figure D1 Apparatus for uptake experiments

Tap water was passed through the two cooling tanks on either side of the cooling aquarium and served to dissipate heat produced by the flood lamps.

The cooling aquarium, containing the incubator, was connected to a Haake F3 cooler which maintained the temperature within 0.2°C of the experiments' temperature.

Kelps discs were held by hooks on vertical Plexiglass bars in the incubator. Bubbling air served to mix and aerate the medium.


kelp disc
flood lamp
cooling tank
incubator
cooling aquarium

Li-Cor inc. LI-185B Photometer. Two separate tanks with running tap water were used to dissipate the heat produced by the flood lights (see Figure D1).

The incubation medium consisted of filtered seawater, 1 μM NaI and about 37 KBq of radioactive iodine ^{125}I ($Na^{125}I$, New England Nuclear, Boston MA; spec. act. 643.8 GBeq ml^{-1}). In several experiments radioactive iodate $^{125}IO_3^-$ was used. In order to obtain $^{125}IO_3^-$, $^{125}I^-$ was oxidized by adding Br_2, which converts I^- to IO_3^-, and then boiling off the excess Br_2 (Hirano et al., 1983). The half life of the isotope is 60 days. A Nuclear Chicago γ counter was used to count all radioactive material. The background was in the order of 60 cpm.

For an experiment, discs punched out of the same area of a L. saccharina blade were used. Discs were preincubated for about 15 min at the conditions of the experiment before addition of labelled iodine by which time the mucopolysaccharides had stopped exuding from the discs. During incubations, the medium was mixed and oxygenated using an air bubbling device linked to a timer that would operate every 7-8 min for 17 s. Dyes were used to see whether the mixing was adequate and these showed an even distribution of colour after one bubbling. The position of discs in the incubator was also taken into account when comparing the cpm of replicates. There was no relation between the site in the incubator and the cpm. In several experiments, as detailed in the text, bubbling was continuous.
Tissue incubated in radioactive iodine was rinsed for 10 min in

seawater containing 1μM KI in order to exchange any radioactive iodine adsorbed on the surface of the tissue and was immediately counted in the γ-counter for 1 min. In most experiments, 3 discs were used as replicates for each data point. It was found that as the incubation time increased so did the variation between discs, thus up to 5 replicates were used for the longer incubation times.

Another setup was devised where discs at each incubation time were γ-counted and subsequently replaced in the incubation chamber for further ^{125}I uptake; these discs are called "reusable" discs. In this manner, fewer discs were used and uptake curves were representative of one disc, thus giving much smoother curves (there was no variation associated with different replicates). Also the incubation period could be prolonged as desired as curves were determined simultaneouly with the progression of the uptake experiment. In all of these experiments, five replicates were used.

II. Results

1. General considerations

a. Calculations

It is assumed that the kelp cannot differentiate between the 2 isotopes of iodine and takes them up in the same ratio that is present in the medium. Thus, using the formula

$$\frac{\text{cpm in the medium}}{\text{cold I in the medium}} = \frac{\text{cpm in the kelp}}{\text{cold I in the kelp}}$$

the quantity of cold iodide taken up by discs equals:

$$\text{cold I in the kelp} = \frac{\text{cpm in the kelp}}{\text{cpm in the medium}} \times \text{cold I in the medium}$$

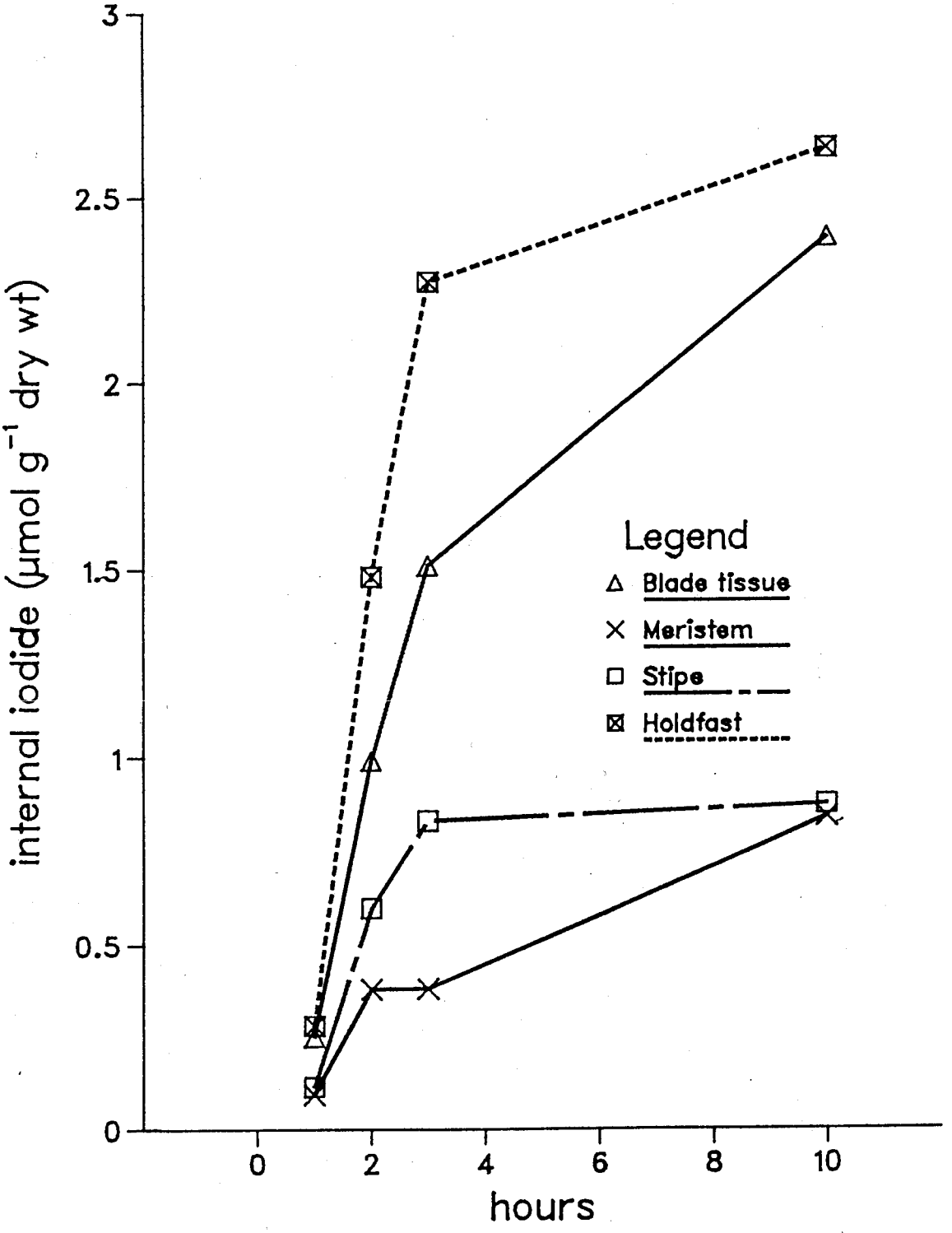
The values are expressed in nmoles $disc^{-1}$. The advantage of this calculation is that all experiments can be compared to one another. Most curves given in the figures carry the date at which the experiment was done. In addition, standard error bars are given for each data point.

b. Uptake of ^{125}I by different parts of the thallus

In order to assess the uptake of I^- by different parts of Laminaria thallus, plants were cut up into: holdfast, stipe, meristem (blade-stipe junction) and 2 discs 15 cm distal from the blade/stipe junction (young tissue). These parts from several plants were pooled, weighed, and incubated in seawater with ^{125}I . The dry weight values were calculated according to previously determined dry weight average values for the different thallus parts. The highest iodide levels were recorded in the blade and holdfast but after 10 h the blade discs were still taking up iodide (Fig D2); at 10 h they displayed a mean uptake rate of 0.12 μmol I^- g^{-1}d.wt. h^{-1} , whereas the holdfast showed 0.051, the meristem 0.06 and the stipe 0.006 μmol I^- g^{-1}d.wt. h^{-1}. For convenience, as well as because they took up iodide readily, blade discs were used in subsequent experiments.

In order to choose the blade area from which discs should be taken, it was necessary to compare the uptake of iodide of young, mature and old tissues. These areas were determined using the growth in the field data (Part B). There, the growth data showed that 85% of the growth of the blade occured within 30 cm of the blade-stipe junction. Thus tissue up to about 30 cm from the blade-stipe junction is considered young. Above that for another 30-50 cm the tissue is considered mature and at the proximal end of the blade the tissue is considered old.
The uptake of iodide by these 3 types of tissues was compared and the curve slopes were: 1.11, 0.96, 0.75 after 90 min for

Figure D2 Uptake of iodide by different areas of the Laminaria thallus
The values of internal iodide were converted to μmol per g dry weight.
All parts taken out at each data point belonged to one plant.


3
2.5
2
1.5
1
0.5
0
internal iodide (µmol g⁻¹ dry wt)
0
2
4
6
8
10
hours
Legend
Blade tissue
Meristem
Stipe
Holdfast

young, mature and old tissue, respectively (data not shown). The young tissue displayed the highest uptake rate followed closely by the mature tissue. However, the mature tissue was chosen in all subsequent experiments because more tissue was available.

The iodide uptake for discs of different sizes and of different thicknesses punched from the same blade were compared. Thick discs originated from the central portion of the blade, whereas the thin ones originated from the margin or 'wing' of the blade; both were of the same age.

Size	dry weight (mg)	
	midrib	wings
1.8 cm diam.= 2.54 cm^2	15.3	9.6
1.0 cm diam.= 0.78 cm^2 (one side)	4.8	3.2

The uptake curves are shown in Fig D3. At one hour, discs of same size but different thickness displayed a comparable uptake. After this, the thicker discs took up more iodide than the thinner ones, the latter showing signs of levelling off of uptake after 6 h. The smaller discs of either thickness took up about one third the iodide of the larger discs, in direct proportion to their surface area which was about one third that of the bigger discs. From these experiments it was concluded that thin discs take up iodide at the same rate as thicker discs, for a short while, but their long term capacity is lower

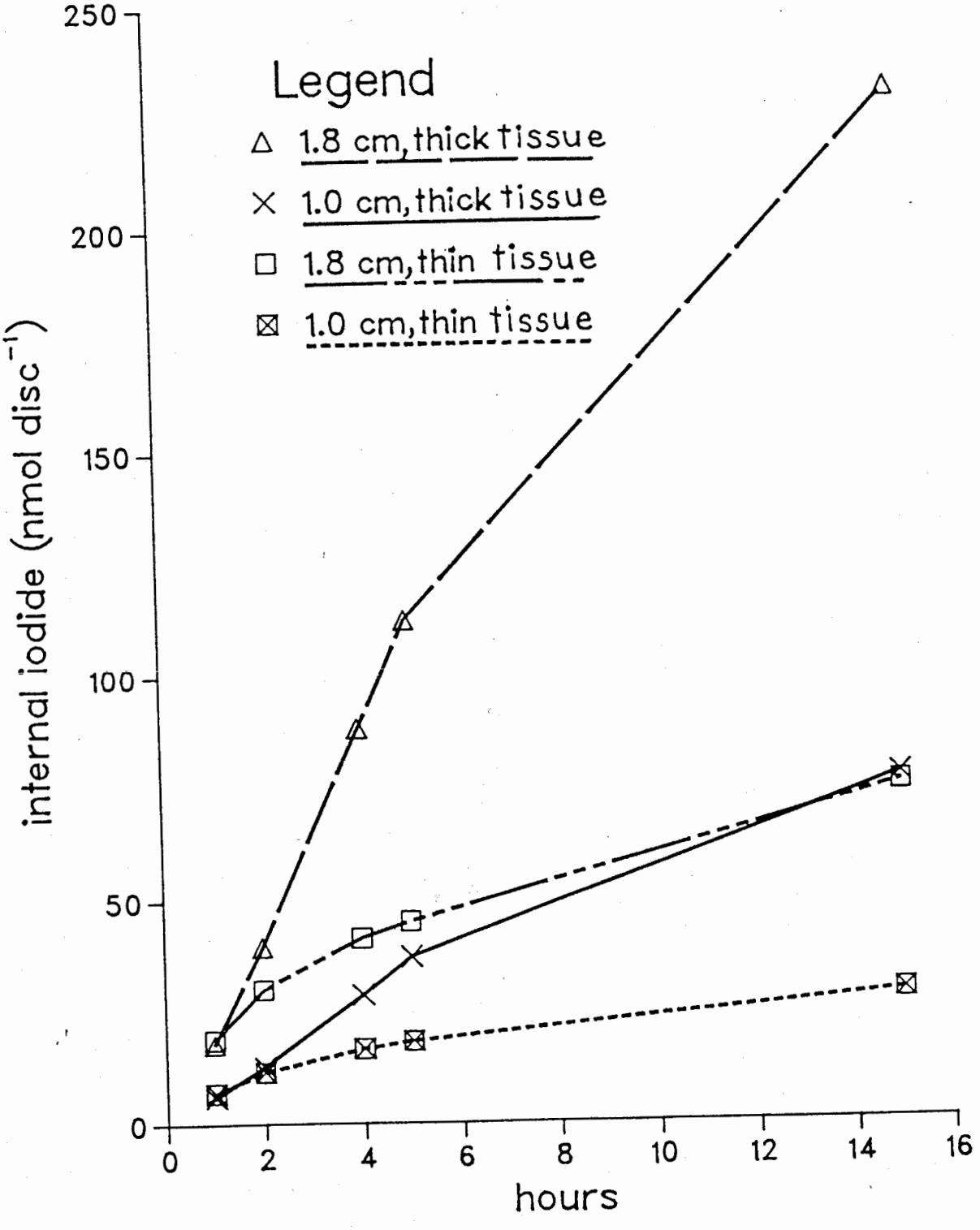
than that of thicker discs. In subsequent experiments uptake was calculated mainly on the basis of suface area (discs all of 1.8 cm diameter) and discs were generally punched out of the central portion of the mature tissue of the blade as tissue is homogeneous and plentiful.

In order to see whether sorus tissue (fertile tissue) could take up iodine, discs punched from blade and sorus tissue were incubated in ^{125}I. The blade tissue, albeit thinner and lighter than the sorus tissue, showed a higher uptake rate (data not shown) further substantiating the importance of surface area. This may also indicate that sorus tissue may have little or less need for iodide.

c. Comparative uptake of $^{125}I^-$ and $^{125}IO_3^-$

The uptake of $^{125}IO_3^-$ by blade discs was compared to that of $^{125}I^-$. The experimental protocol was similar to that of the other uptake experiments. Calculation adjustments were made at t=1 h between the counts in the seawater for IO_3^- and I^- in such a manner that the cpm in the discs could be compared (there were 20% more cpm in the IO_3^-medium, so the cpms in discs of the I^- were raised by 20%, this percentage was kept at each data point). Uptake rates were comparable although lower for IO_3^- with 2.0 nmol h^{-1} disc $^{-1}$ vs 4.2 nmol h^{-1} $disc^{-1}$ for I^-. Labelled iodide was then used in all experiments.

Figure D3 Uptake of iodide as a function of disc size and thickness
Continuous bubbling, n=3.


Legend
1.8 cm, thick tissue
1.0 cm, thick tissue
1.8 cm, thin tissue
1.0 cm, thin tissue
internal iodide (nmol disc⁻¹)
250
200
150
100
50
0
0
2
4
6
8
10
12
14
16
hours

d. Release of activity

Discs previously incubated in radioactive medium for 30 h were transferred into nonradioactive 1μ KI medium with air bubbling to assess whether any labelled iodide was released. The results of the experiment are shown in Fig D4. Most counts were released in the first 20 to 30 min and these are assumed to arise from the labelled iodide present in free space. After that period labelled iodide continued to be released, albeit at a much slower rate. This release may represent an exchange of hot for cold iodide or a release of labelled iodide in one form or another. Two and a half percent and 4 % of the total initial counts in the discs were released after 40 min and 2 h, respectively. After 48 h nearly 17 % of the activity had been released (data not shown).

Discs in all experiments were rinsed for 10 min before γ counting as most of the free space release occurs in that interval and this length of time was convenient for experimental logistics.

2. Kinetics of uptake

a. Rates of uptake

Typical uptake curves for short and long term uptake under intermittent and continuous bubbling conditions are shown in Fig D5. The results of many other comparable uptake experiments performed between 1982 and 1985 are regrouped in Table D1. From

Figure D4 Release of radioactive iodide
The different curves indicate different replicate discs. Total cpm were recorded in the seawater in which the individual discs were bathed. Each disc had in the order of 80,000 cpm before the start of rinsing.

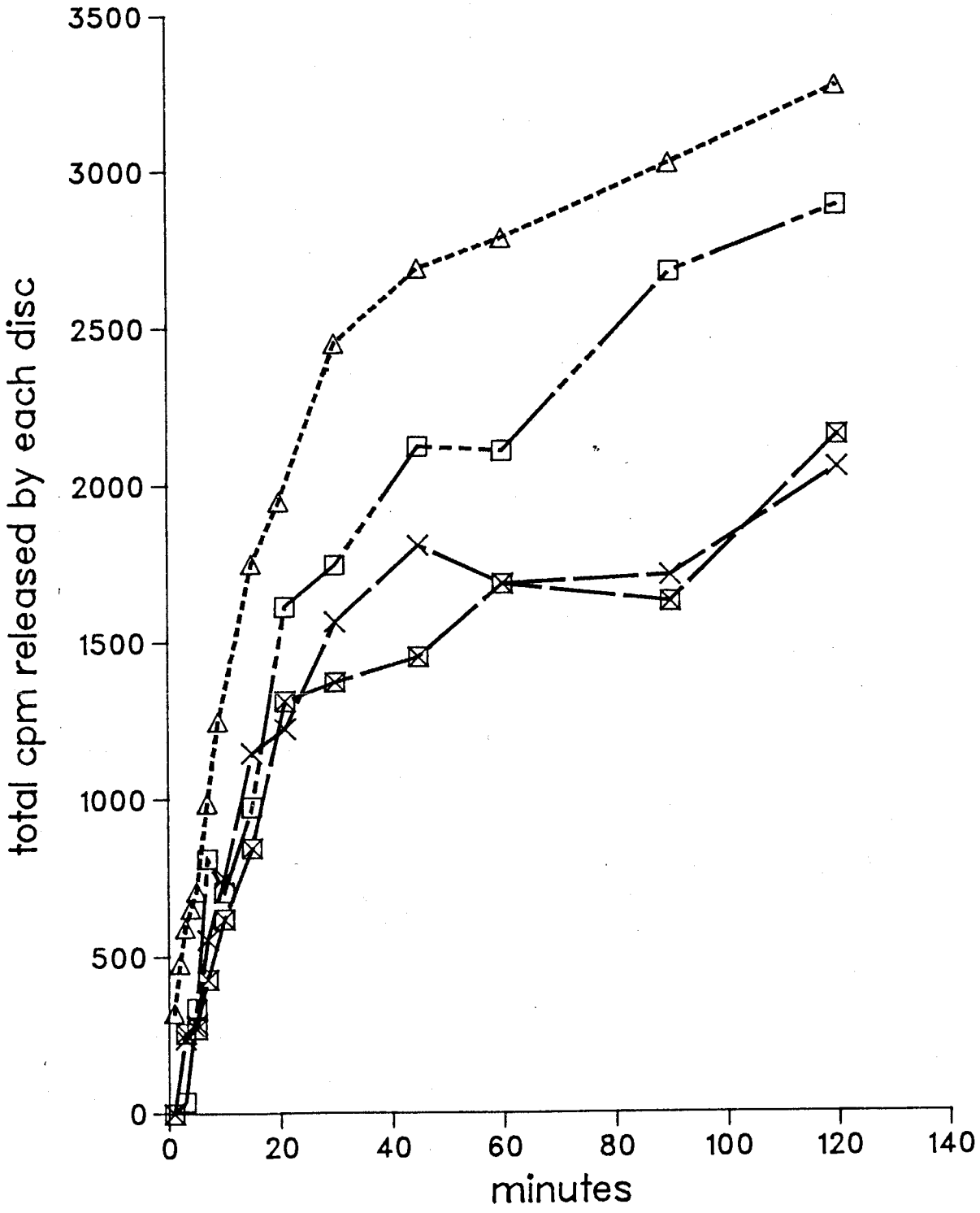

3500
3000
2500
2000
1500
1000
500
0
total cpm released by each disc
0
20
40
60
80
100
120
140
minutes

Figure D5 Uptake of ^{125}I by blade discs under intermittent and continuous bubbling
All discs in these and subsequent experiments, unless specified, are 1.8 cm in diameter.
The medium contained 1 μM KI. S.E. bars appear at each data point unless smaller than the symbol.
Fig D5 a: three replicates were taken out at each data point
Fig D5 b,c and d: five "reusable" discs were used in each of these experiments.
Note the differences in the abscissae values.

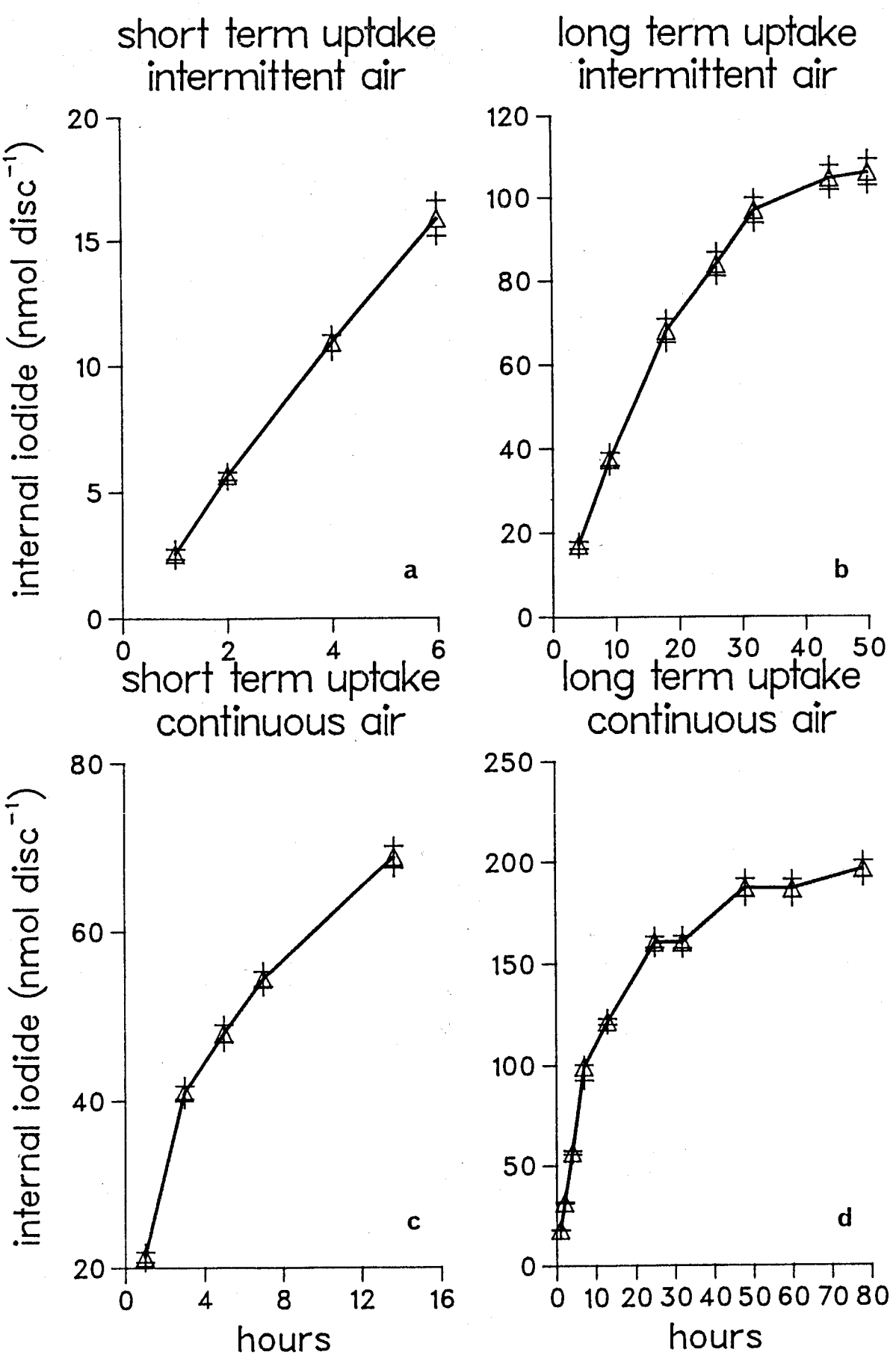

short term uptake
intermittent air
long term uptake
intermittent air
short term uptake
continuous air
long term uptake
continuous air
internal iodide (nmol disc^{-1})
hours
a
b
c
d

Table D1 Comparative uptake of ^{125}I by blade discs.
Data for experiments done between 1982-1985.
The internal iodide levels are given in nmol per disc for different incubation times.
All experiments were run at 10-11°C under similar light conditions. All discs were of the same size but not necessarily of the same thickness (see Discussion).

Date	Length of incubation (hours)						
	1	2	4	8	24	32	72
1982							
June 9	2.3	5.1	11.3	20.9			
July 3	2.5	5.7	10.9	20.0			
July 9	2.5	5.7	11.5	23.6			
1983							
December 6	2.5	5.3	10.8	22.7			
1984							
June 4	4.0	7.6	16.0	35.0			
August 25			13.1		95.8		115.8
October 7	3.1	6.3	15.6	29.2			
October 15			17.9	34.0	82.1	97.0	
November 2		6.8	9.4	15.3	36.8		
December 11		8.0	13.0	25.4	40.1	45.0	
1985							
March 13	5.1	12.2	16.2	30.0			
March 18	4.1	7.1	14.4				
March 25	3.7	7.2	14.0				
April 4	4.0	8.1	15.0				
April 10	3.5	7.4	14.6	27.0			
April 24	4.3	7.5	13.8	27.3			
April 26		5.4	11.1	22.3			
May 9		6.9	14.4	29.8			
May 11		7.5	14.8	30.4			
Continuous air:							
May 11		29.9	51.1	87.5			
May 14		26.4	35.0	51.0			
May 21	21.3	31.0	44.5	56.5			
May 24	14.4	28.7	51.8	96.4			
June 4	17.8	32.7	62.0				
July 11	20.2	34.7	48.2	60.2	105.2		

Fig D5 and Table D1, it can be seen that the initial uptake rate (first hour) varies between 2 to 5 nmol $h^{-1}disc^{-1}$ under intermittent bubbling conditions whereas it may be up to 20 nmol $h^{-1}disc^{-1}$ under continuous bubbling. As expected, the rate of uptake declines with time. This decline in rate is plotted for several experiments in continuous air bubbling in Figure D6. There is a steady and rapid decline in rate of iodide uptake in the first 5 to 10 h, after which the rate declines less rapidly which may correspond to the beginning of saturation of internal iodide pool.

The effect of increasing concentrations of cold iodide in the incubation medium on ^{125}I uptake was studied. Concentrations of carrier KI ranged from 0.2 to 250 μM. Before γ counting, discs were rinsed for 10 min in seawater at the same concentration of unlabeled NaI as used in the experiment. In two separate experiments concentrations of NaI of : 0.2, 0.5, 1.0, 10.0, 100 μM and 0.5, 1.0, 10.0, 100.0 , 250 μM were added to the incubation medium. These experiments, performed in late March 1982 and early July 1982 are shown in Figs D7 a, b. At both times the uptake was greatly enhanced with increasing concentrations of iodide; a 3 fold increase of accumulated iodide between 1 and 10 μM and 10 and 100 μM was observed. These uptake experiments were repeated in fall-winter 1984 with three concentrations: 1, 10, 100 μM KI , and longer incubation times, up to 46 h (Fig D8 a, b). Rates were comparable to those of July 1982 which was only a 6 h experiment and showed an increase of

Figure D6 Decline in iodide uptake rates with time
The rate of iodide uptake was calculated, at each data point, as the iodide taken up over the time it took to take it up. The rate is plotted vs the time at which each of these rates was recorded. N=5, with "reusable" discs.

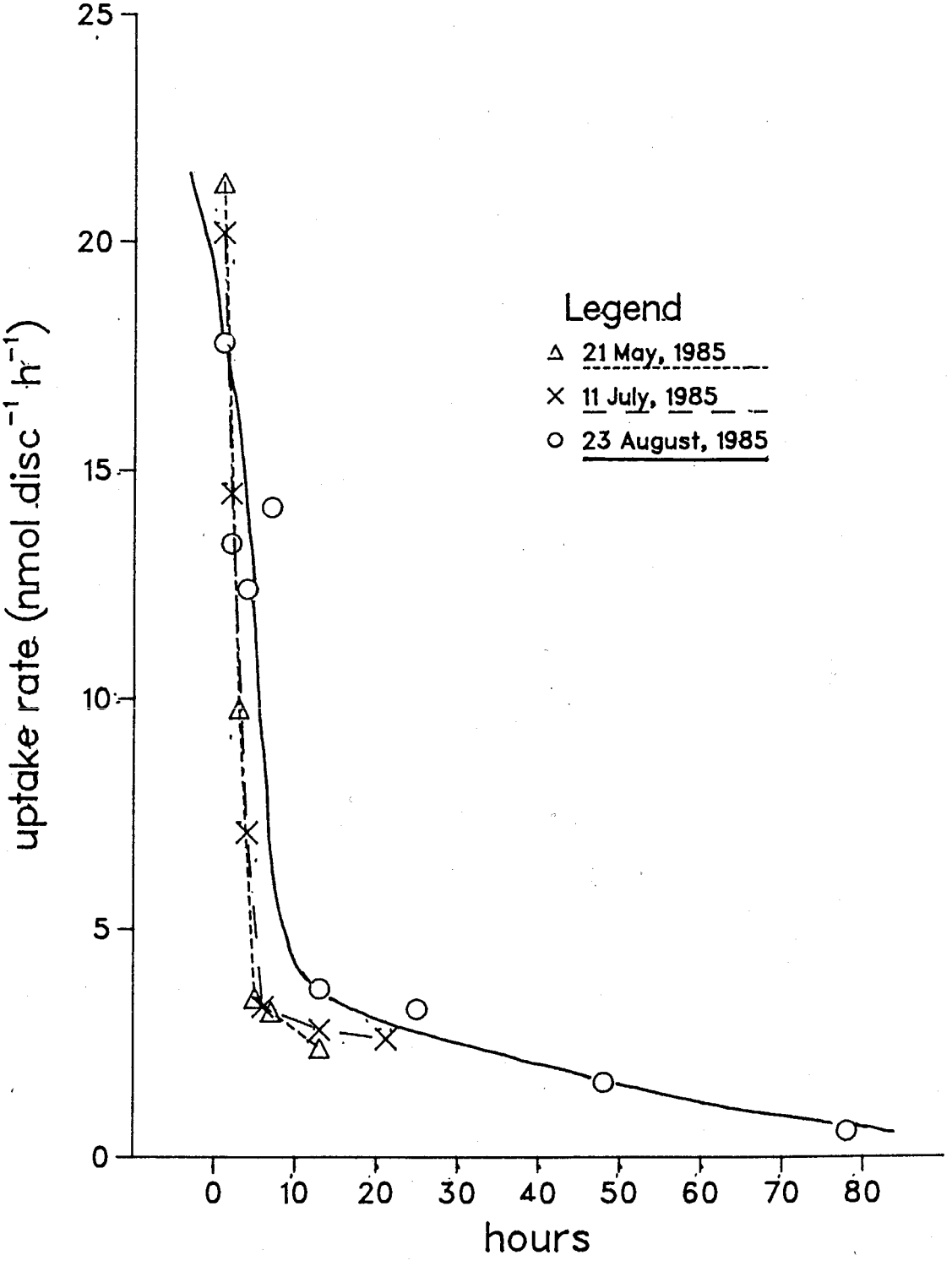
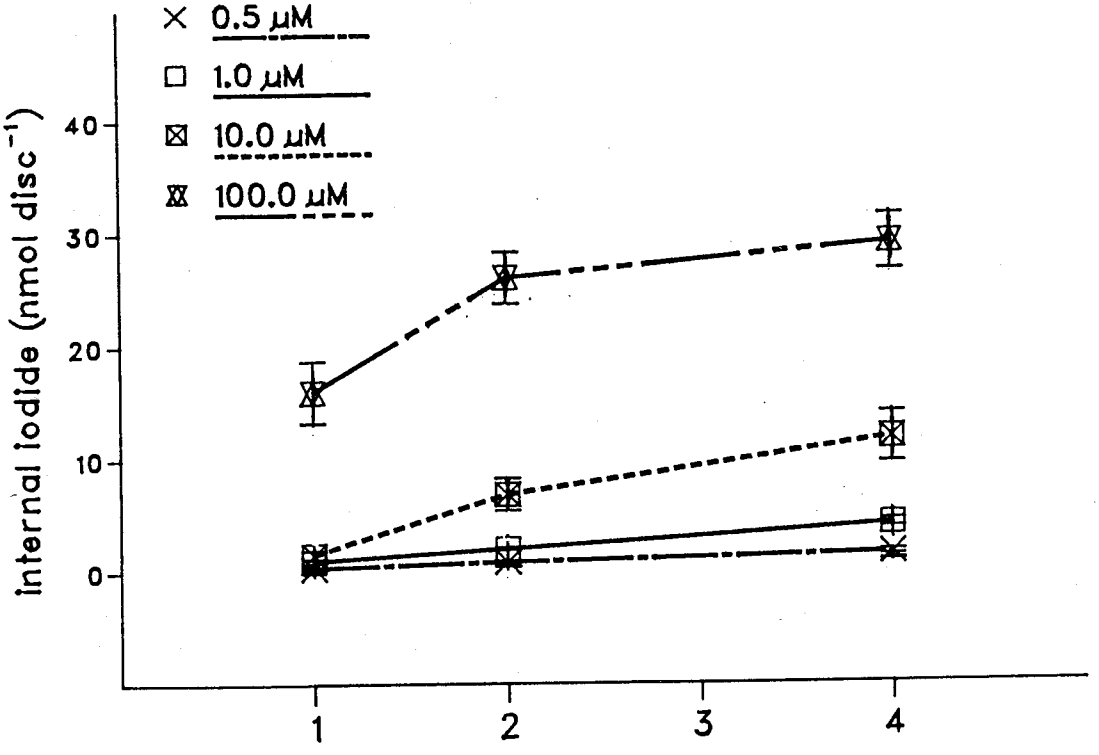

25
20
15
10
5
0
uptake rate (nmol disc^{-1} h^{-1})
0
10
20
30
40
50
60
70
80
hours
Legend
21 May, 1985
11 July, 1985
23 August, 1985

Figure D7 Effect of varying concentrations of added cold KI on ^{125}I uptake
The bubbling was intermittent and 3 replicates were taken out at each data point. Error bar represent S.E. and when smaller than the symbol are not represented.

March 30, 1982



July 1, 1982

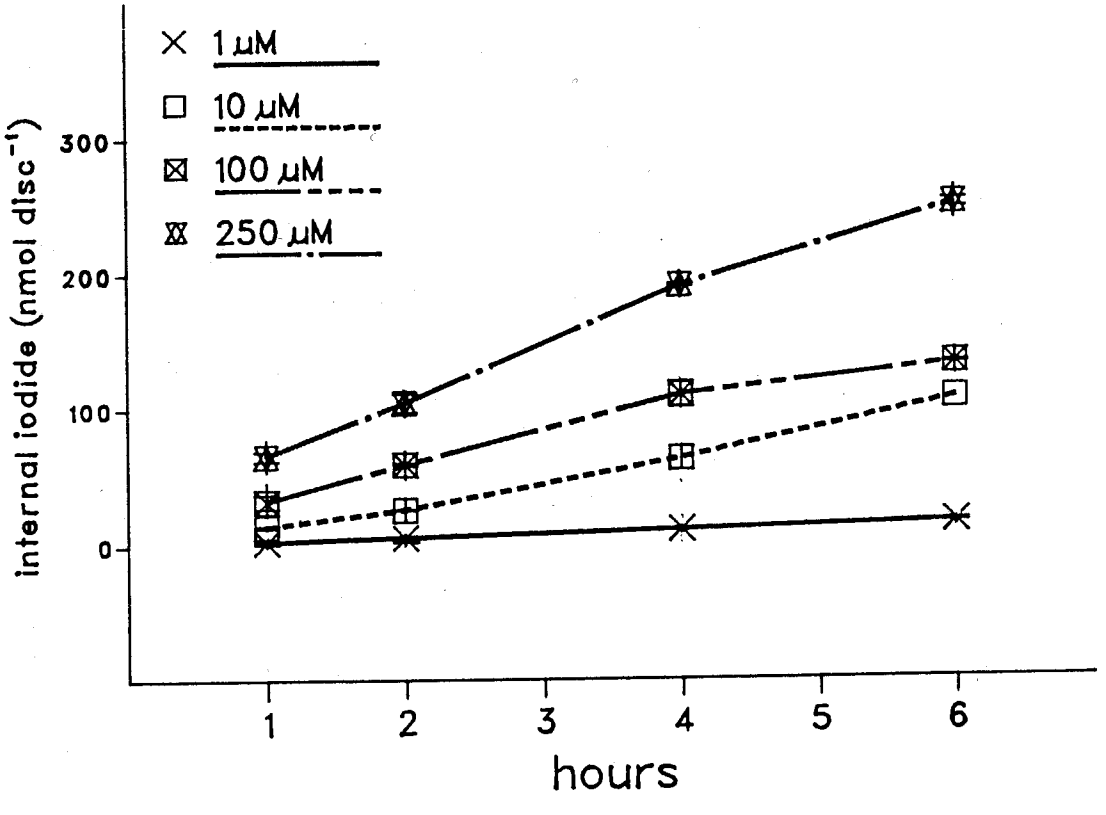
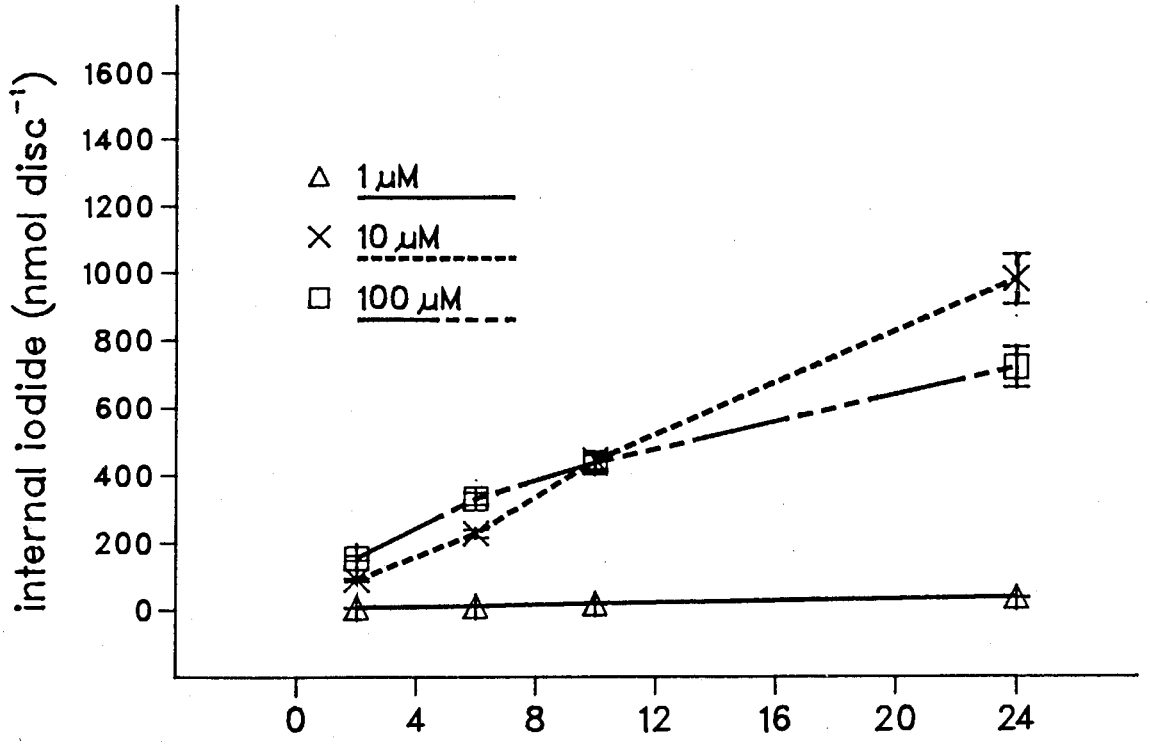
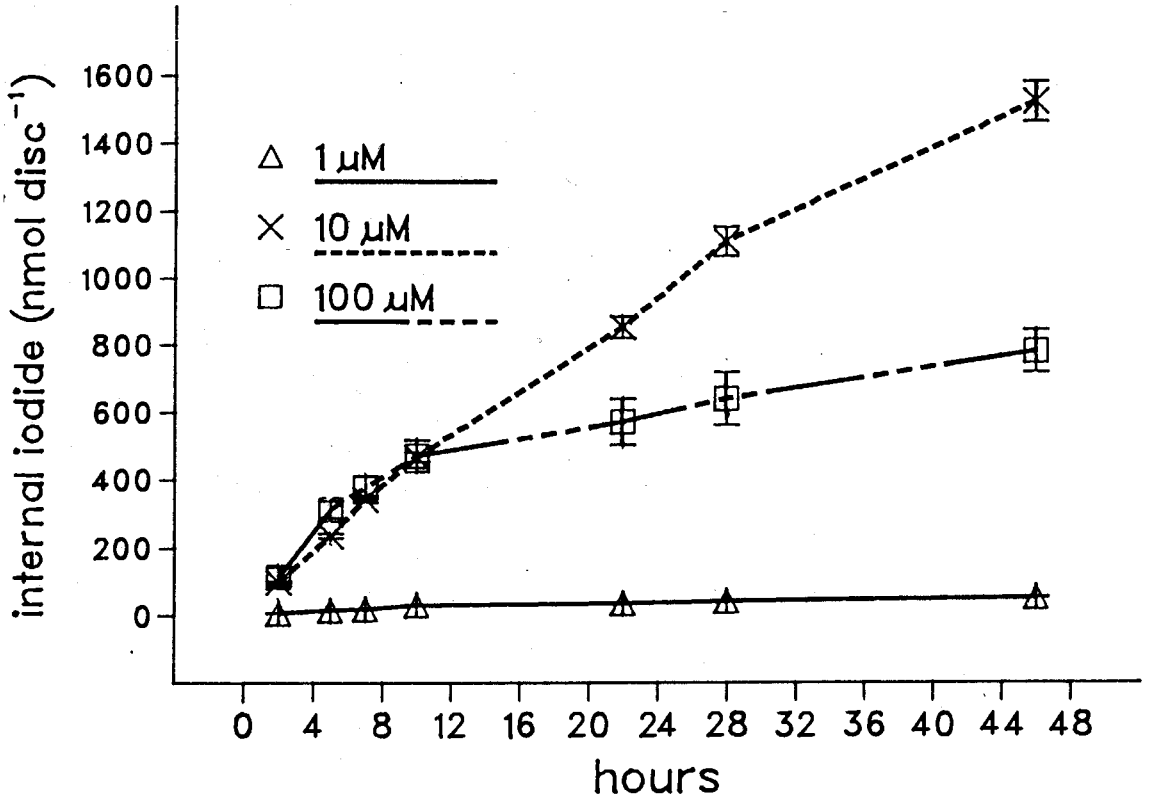


Figure D8 Effect of varying concentrations of added KI on ^{125}I uptake
For details see legend to Fig D7. Here, for longer incubation times, 5 replicates were taken out.

November 2, 1984



December 11, 1984



initial uptake rate of iodide with increased external concentration of iodide; initial rates of about 4.0, 46.0 and 67.0 nmol $disc^{-1}$ h^{-1} were recorded for the 1, 10 and 100μM, respectively.

It is clear that in continuous bubbling conditions the initial uptake rate is increased about 5 fold over intermittent bubbling conditions; stirring had the same effect than continuous bubbling conditions. When external iodide is added to the medium this rate is increased even further: initial rates of 21.3, 95.3 and 93.5 nmol $disc^{-1}$ h^{-1} are recorded for the 1, 10 and 100μM concentrations, respectively (Fig D9). Also, in Fig D9, under continuous bubbling, the uptake rate in 100μM after one hour is lower than the rate in the 10μM concentration; this occurs after about 10 h under intermittent bubbling conditions (see Fig D8). This feature is considered later in Discussion.

The effect of added KIO_3 on $^{125}IO_3$ uptake was also investigated and showed that the uptake increased with the external concentration of KIO_3 (Fig D10).

b. Estimates of pool sizes

As seen in Fig D5 b, with 1μM KI, a levelling off of the uptake occurs after about 50 h or when the internal iodide concentration reaches roughly 100 nmol $disc^{-1}$. With continuous bubbling a levelling occurred after 30 h but the internal iodide concentration was already in the order of 180 nmol $disc^{-1}$ (Fig D5d). With increasing concentrations of external iodide in the

Figure D9 Effect of varying concentrations of added KI on ^{125}I uptake
The bubbling was continuous and "reusable" discs were used with 5 replicates. Error bars represent S.E. and are not represented if smaller than the symbol.

21 May 1985

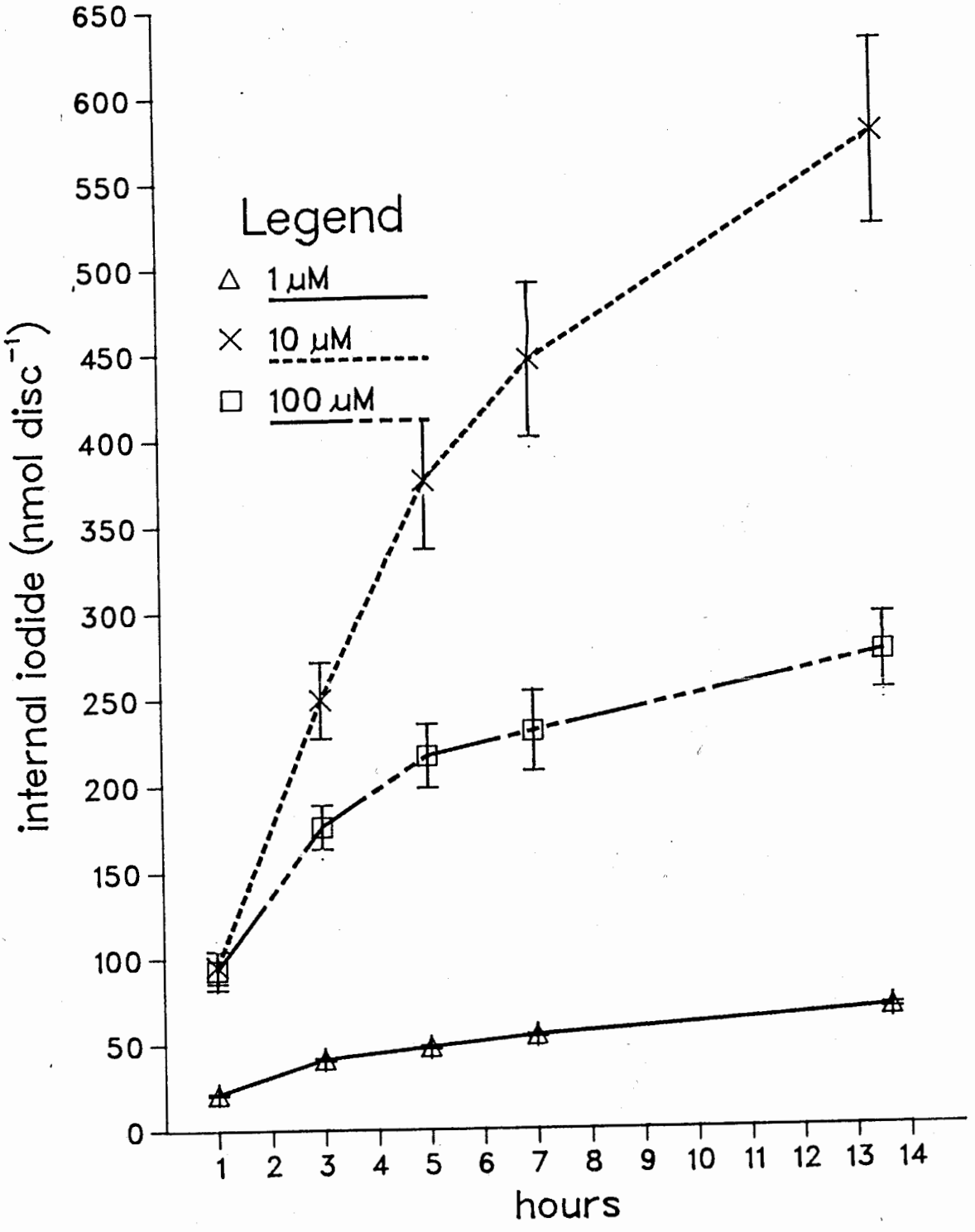
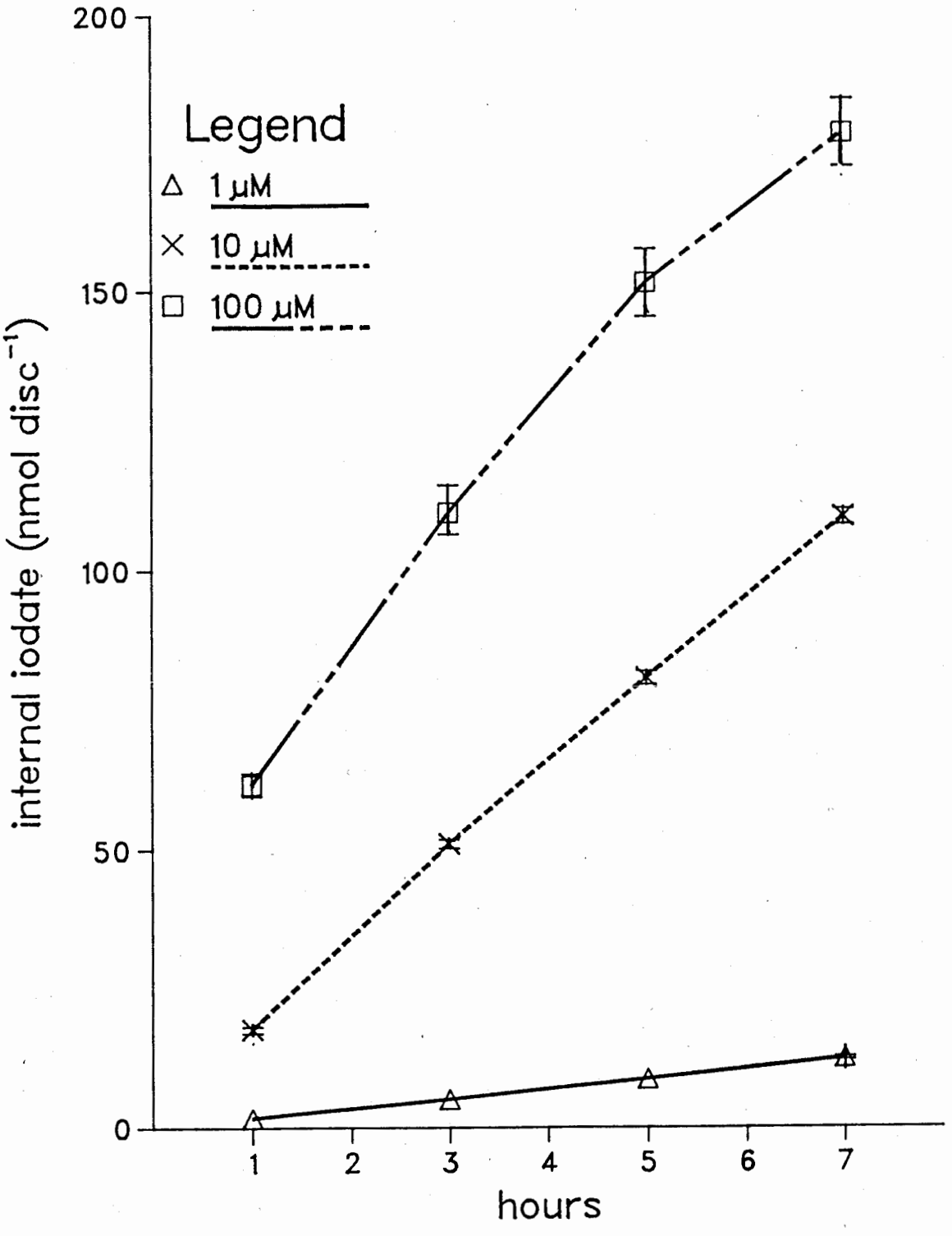

Legend
1 µM
10 µM
100 µM
internal iodide (nmol disc^{-1})
650
600
550
500
450
400
350
300
250
200
150
100
50
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14
hours

Figure D10 Effect of added KIO_3 on $^{125}IO_3$ uptake
The bubbling was intermittent. Three replicates were taken out at each data point and the internal iodate was calculated as for KI. Error bars represent S.E. and are not represented if smaller than the symbol.


Legend
1 µM
10 µM
100 µM
internal iodate (nmol disc⁻¹)
200
150
100
50
0
1
2
3
4
5
6
7
hours

medium the uptake in the 100μM concentration seems to saturate at around 700 nmol disc^{-1} (Fig D8 a,b), but in the 10μM medium there appears to be no saturation, and the internal iodide levels have reached 1.5μmol disc^{-1} or roughly 8.3 μmol g^{-1}f.wt..

In many of the experiments, a levelling off of ^{125}I uptake (saturation) seems to have occurred, whereas in others despite relatively high internal or tissue iodide concentrations no saturation seems to have occurred. Saturation data can be used to estimate internal pool sizes but in the present series of experiments, I use the term apparent pool size, because the pool is estimated only on the iodide taken up during the experiment; the iodide present in the tissue before the experiment is not taken into account. Also, the utilization of iodide during the course of the experiment is unknown. In an earlier experiment, in continuous bubbling and at 1μM KI, a levelling off of the uptake occurred around 0.18 μmol disc^{-1} (Fig D5 d), whereas in a later experiment with 10μM external KI, saturation was not evident even at 1.5 μmol disc^{-1} after 46 h (Fig D8b). It is possible that in the experiment shown in Fig D5d the iodide in the medium was insufficient to sustain the uptake. A rough computation shows that a concentration of 1.5 μmol disc^{-1} represents 13.3 mg iodide g^{-1} d.wt. which is 1.3% iodide. From the above data and other experiments (see Figs D3, D5, D7, D8, D9, D17 and Table D1) an estimate of the pool size can be given as ranging between 0.18 and 1.5μmol disc^{-1}. Furthermore, the pool size is dependent on the thickness of tissue, whereas the

rate of uptake is dependent on intermittent vs. continuous bubbling, the external iodide concentration and probably the internal iodide concentration of the tissue at the start of an experiment.

The following experiments were undertaken to establish whether the uptake is energy dependent. In this manner, light and dark could have an effect if photosynthesis is involved as an energy (ATP) producing system. Similarily, oxygen may be required through its involvment in respiration as electron acceptor; this again would show the uptake is ATP dependent. An effect of temperature might indicate whether the uptake is carrier mediated.

3. Effect of Light and Dark on ^{125}I uptake

In order to determine the effect of light or dark on $^{125}I^-$ uptake, the incubator was replaced by two 500 ml beakers, one of which was left uncovered (light) while the other was covered by black tape and aluminum foil (dark). Both beakers had magnetic stirrers and were placed in the cooling aquarium on top of 2 stirrers. Discs were sampled at various time intervals, the "dark" beaker being only exposed to green light when opened. It should be noted that the use of beakers with stirring bars enables a continuous motion of the medium resembling the effect produced by continuous bubbling.

Several types of experiments were conducted in order to ascertain the role of light and dark on iodine uptake.

1- Discs were kept alternately in the light and in the dark to see if any quick change in the uptake could be observed.

2- Discs were preincubated up to 48 h in the dark with bubbling air before an experiment. Then parallel series of discs, some kept in the light and some in the dark, were used to establish whether any differences in uptake existed between the two conditions. Preincubation in the dark would result in depletion of reserves or "starving" the discs of photoassimilates. Thus, a more pronounced difference between the effects of light and dark should be seen.

3- In another variation on these experiments involving parallel series of discs in light and in dark, the discs in the dark were put in the light after a certain time of incubation to see whether uptake would reach the rate in the light.

Without preincubation in the dark, discs showed little response to alternating light-dark treatments (Fig D11 a,b). This indicated that the discs may possess sufficient metabolic reserves in order to continue functioning more or less "normally" or at least they could take up iodide efficiently for many hours.

A greater difference between light and dark uptake was noted when the discs were previously "starved" in the dark for 44 to 48 h (Fig D12a,b). This experiment was repeated with discs of 1.3 cm diameter, instead of 1.8 cm, to reduce the tissue area

Figure D11 Effect of light or dark on ^{125}I uptake by blade discs
All experiments involving light and dark were run in beakers, the medium being mixed by stirring bars.
N=3 and error bars represent S.E.
a and b: The discs were put alternatively in the light (fluorescent lights) and in the dark.

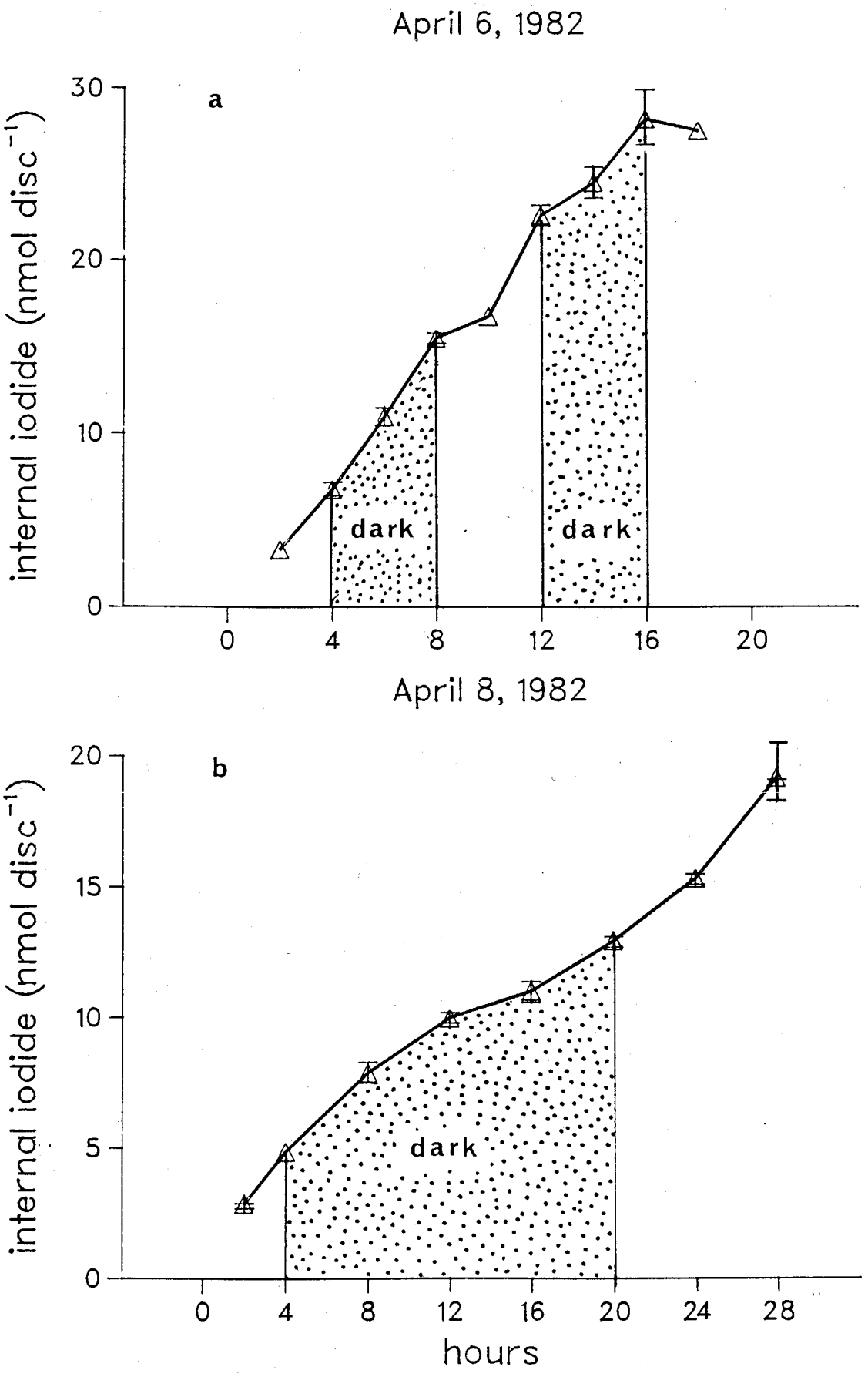
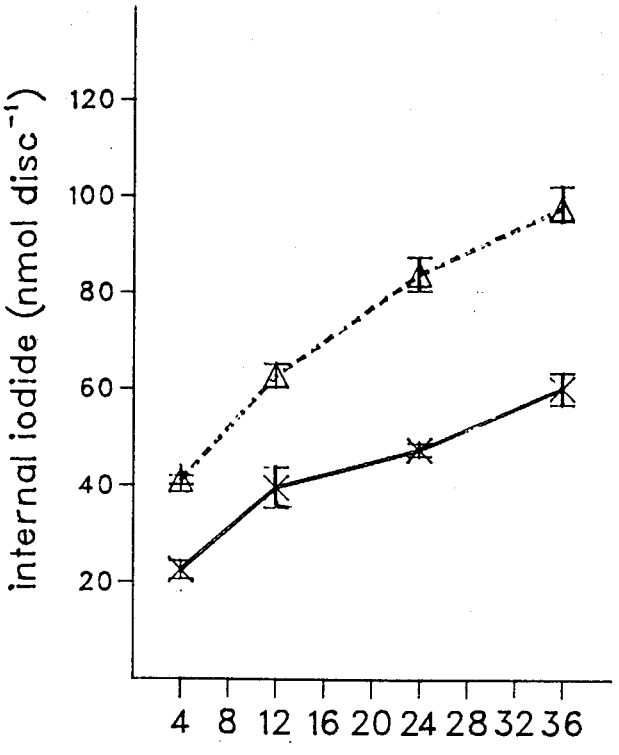
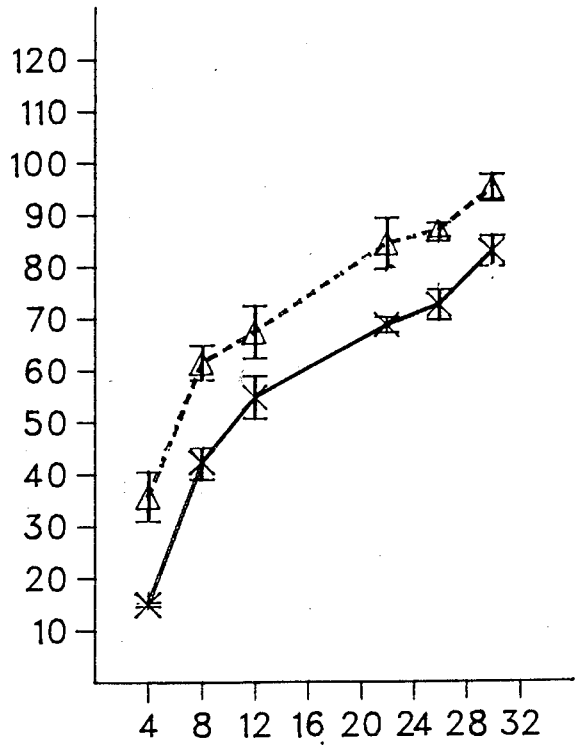

April 6, 1982
a
internal iodide (nmol disc^{-1})
30
20
10
0
dark
dark
0
4
8
12
16
20
April 8, 1982
b
internal iodide (nmol disc^{-1})
20
15
10
5
0
dark
0
4
8
12
16
20
24
28
hours

Figure D12 Effect of light or dark on ^{125}I uptake by blade discs
Discs were preincubated in the dark, one series was put in the light and one was kept in the dark.
N=3, error bars represent S.E.
a : 48 h preincubation in the dark, fluorescent lights.
b : 44 h preincubation in the dark, flood lights.
c: 18 h preincubation in the dark, flood lights, discs 1.3 cm diameter (instead of 1.8 cm).

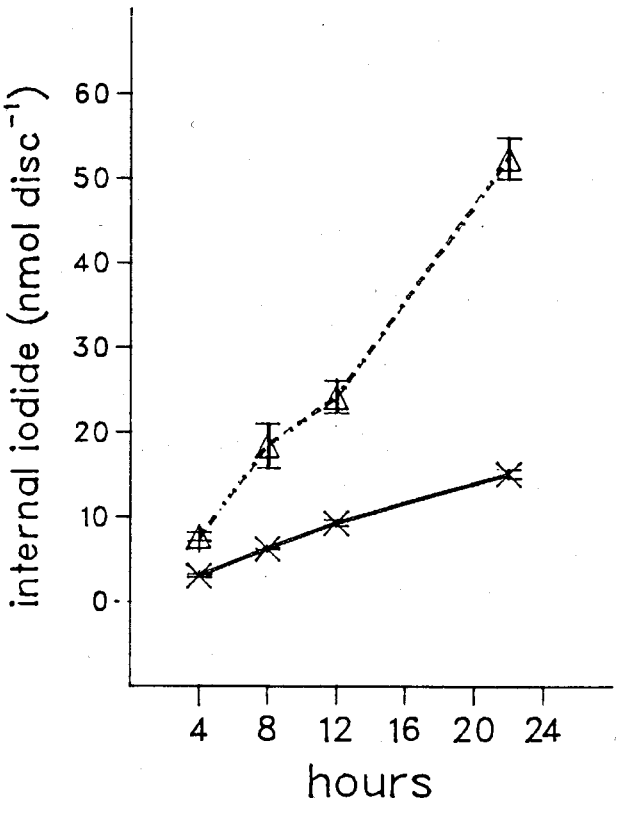
April 19, 1982



January 29–30, 1984



February 1, 1984



Legend

△ light

× dark

used and increase the number of replicates for the longer incubation times. The two resulting curves appear in Fig D12 c. Besides showing much less variation among discs, they show a clear enhancement of I^- uptake in light.

In a series of experiments, discs previously incubated in the dark were put in the light in parallel with a series of discs which were continuously in the dark. Figs D13 a, b and c show that discs previously incubated in the dark resume the uptake rate in the light when irradiated.

4. Effect of temperature on $^{125}I^-$ uptake

Blade discs from the same plant were used for each temperature in an experiment. In the first experiments, discs were preincubated for 12 h at the temperature of the experiment. Two experiments were run in July 1982 and June 1984 and both showed a decrease in uptake at temperatures below 10°C (data not shown). In July 1982, the maximum uptake was recorded at 10°C, a temperature of 20°C showing a lower uptake. However, in June 1984, the reverse was seen; the uptake at 20°C was greater than that at 10°C (see Discussion).

In June 1985, discs were only preincubated for 1.5 h at the temperature of the experiment. Results are shown in Fig D14. Iodide uptake is clearly highest at 10°C. At 5°C, the iodide content is 78 % and at 0°C 34 % that at 10°C after 6 h. At a temperature higher than 10°C uptake was reduced.

Figure D13 Effect of light or dark on ^{125}I uptake by blade discs
Discs were preincubated in the dark, one series was put in the light, one in the dark and the light was switched on where indicated.
a : 48 h preincubation in the dark, fluorescent lights, n=3.
b : 44 h preincubation in the dark, flood lamps, n=3.
c : 39 h preincubation in the dark, flood lamps, the stirring bars were replaced with continuous bubbling air, n=5, "reusable" discs.

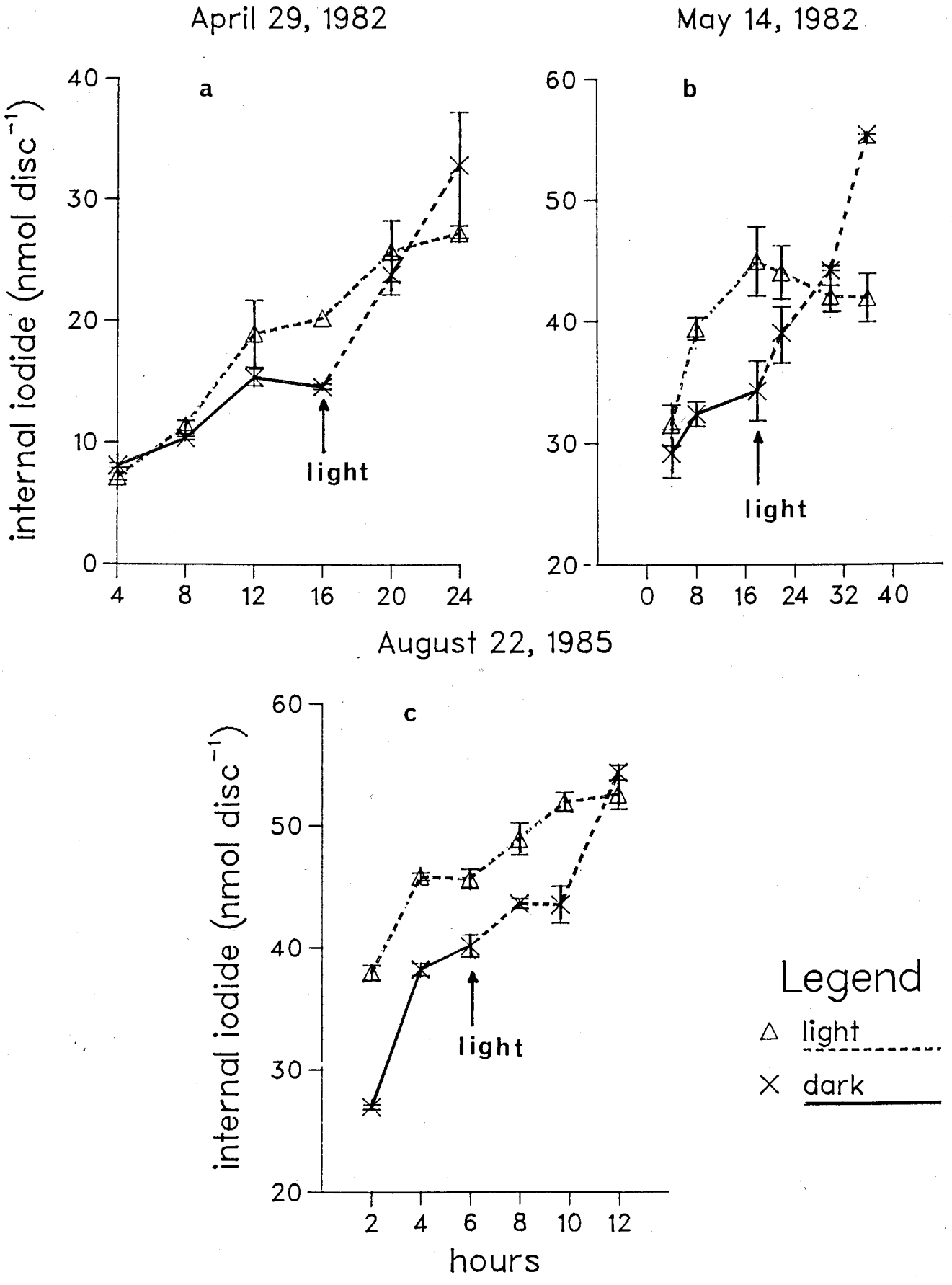
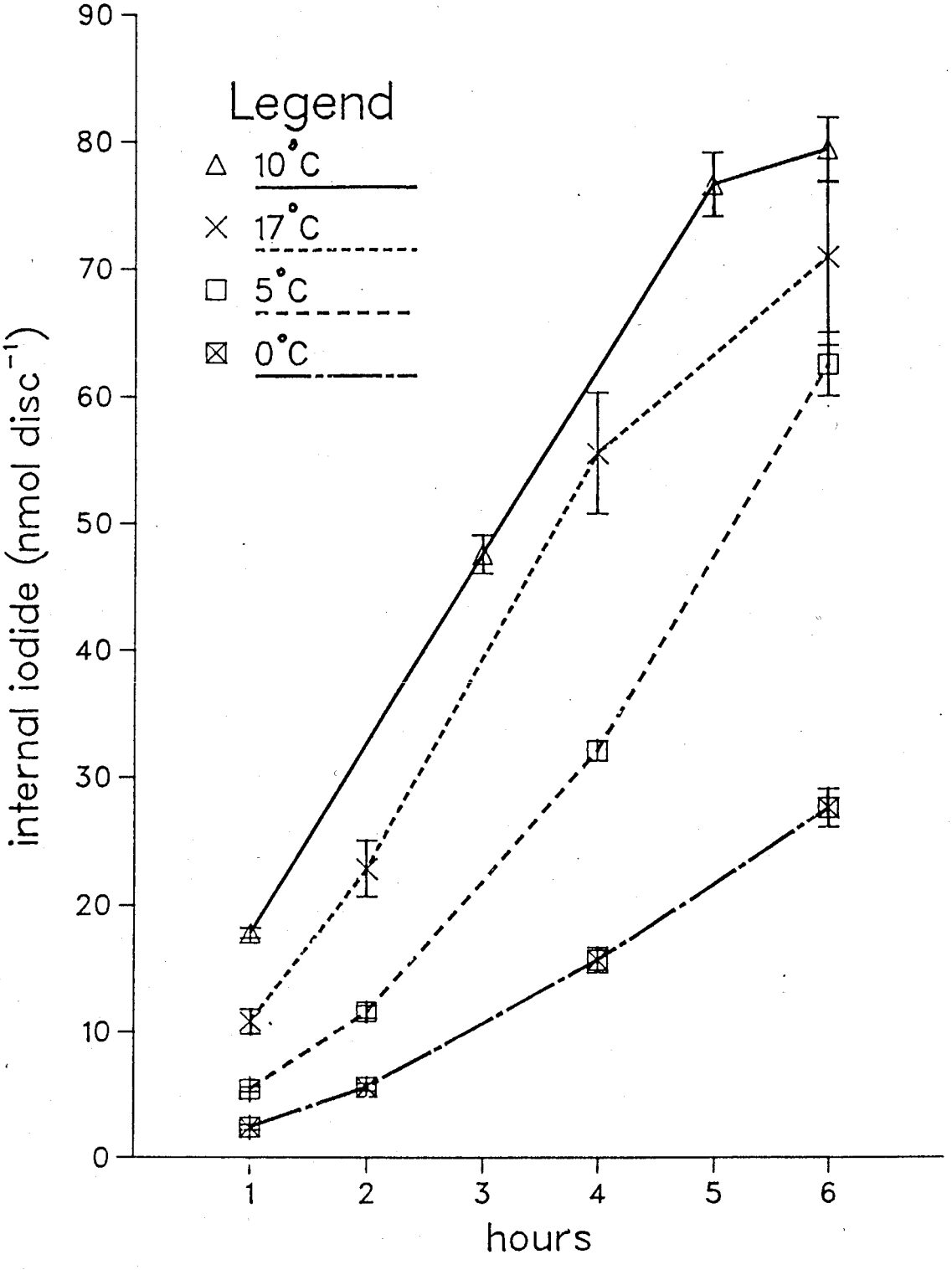

April 29, 1982
May 14, 1982
August 22, 1985
a
b
c
internal iodide (nmol disc^{-1})
light
hours
Legend
light
dark

Figure D14 Effect of temperature on iodide uptake
Discs were incubated 1.5 h at the given temperature of the experiment. Bubbling was continuous. N=5, "reusable" discs and error bars represent S.E.

June 4,1985


Legend
10°C
17°C
5°C
0°C
internal iodide (nmol disc⁻¹)
hours
0
10
20
30
40
50
60
70
80
90
1
2
3
4
5
6

5. Effect of anaerobic conditions on ^{125}I uptake

For these experiments, nitrogen or helium was bubbled continuously rather than intermittently to obtain as anaerobic conditions as possible. In the control, the air was bubbled continuously. No difference in the uptake could be detected in the light with bubbling nitrogen or helium compared to the control which was bubbled with air (Fig D15). Slightly higher uptake was recorded when O_2-enriched air (40-46% O_2) was used for bubbling but this was not statistically different. A similar experiment with the first 5 h in the dark showed that the uptake of ^{125}I in the presence of nitrogen was almost totally stopped in the dark whereas it continued at a high rate in air or air enriched with O_2 (Fig D16). When the discs were put in light at the end of 5 h, the ones in nitrogen resumed uptake of iodide at an increased rate similar to the initial uptake rate of those in air and oxygen enriched air (40-46% O_2). These experiments suggested that oxygen or light were required for uptake to take place. In light, O_2 is produced through photosynthesis whereas in the dark exogenous O_2 must be added for uptake to occur. Oxygen is necessary as the electron acceptor of respiration. This indicates that the uptake is energy dependent and requires ATP production through respiration or photosynthesis. To test this hypothesis, an inhibitor of electron transport from PS II to PS I in photosynthesis, DCMU (dichloromethyl urea) at a concentration of $10^{-5}M$, was added to the incubation medium after 2 h of uptake (Fig D17). DCMU had no effect on iodide

Figure D15 Effect of continuous bubbling of nitrogen, air and oxygen enriched air on ^{125}I uptake in light (oxygen concentration ranged from 40 to 46% in O_2 enriched air).
Reusable discs were used with 5 replicates. These curves are not statistically different.

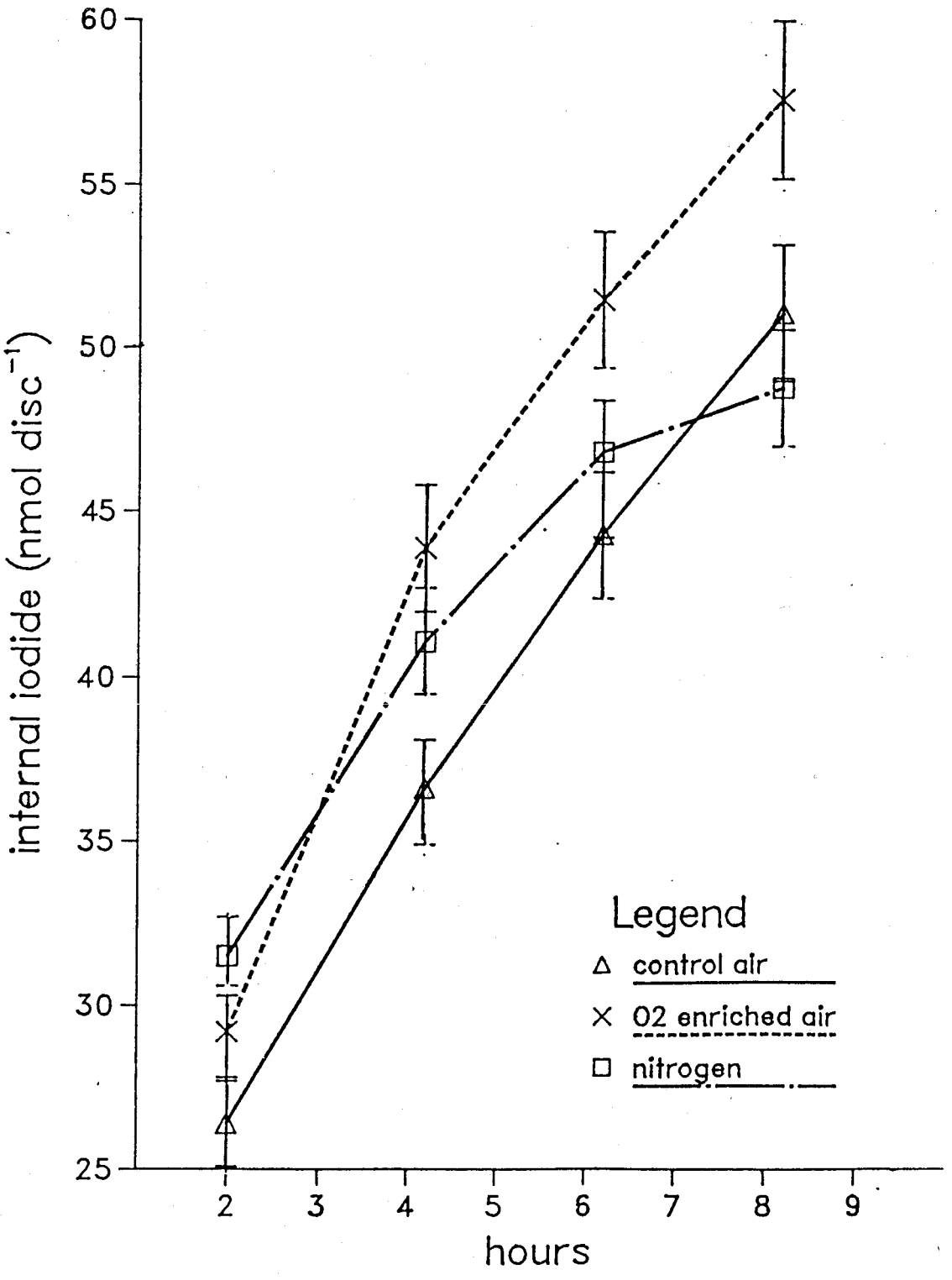

60
55
50
45
40
35
30
25
internal iodide (nmol disc^{-1})
2
3
4
5
6
7
8
9
hours
Legend
control air
02 enriched air
nitrogen

Figure D16 Effect of dark and bubbling nitrogen on ^{125}I uptake
The experiment was first run in the dark for 5 h and light was given where indicated.
Reusable discs were used with 5 replicates, error bars represent S.E.

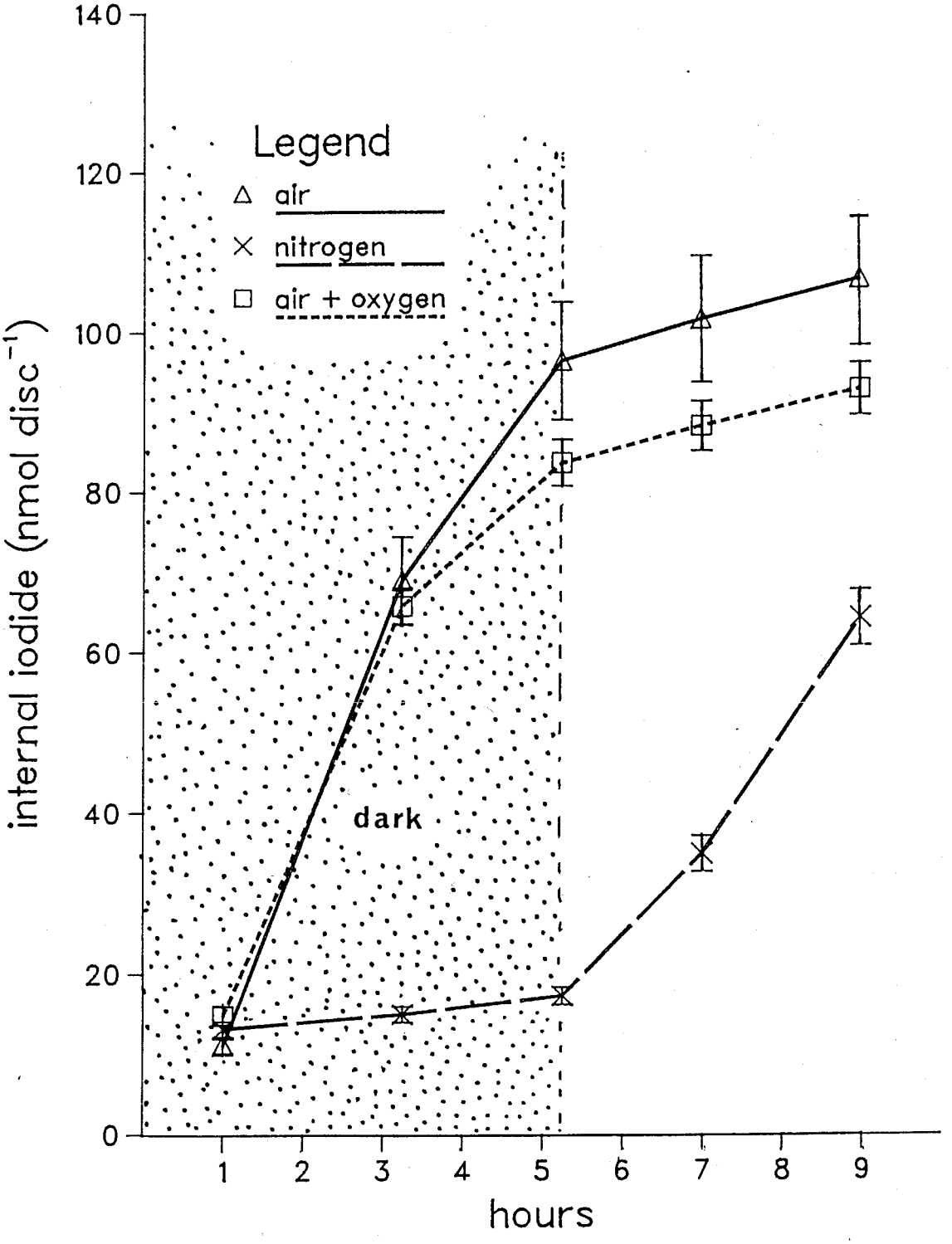
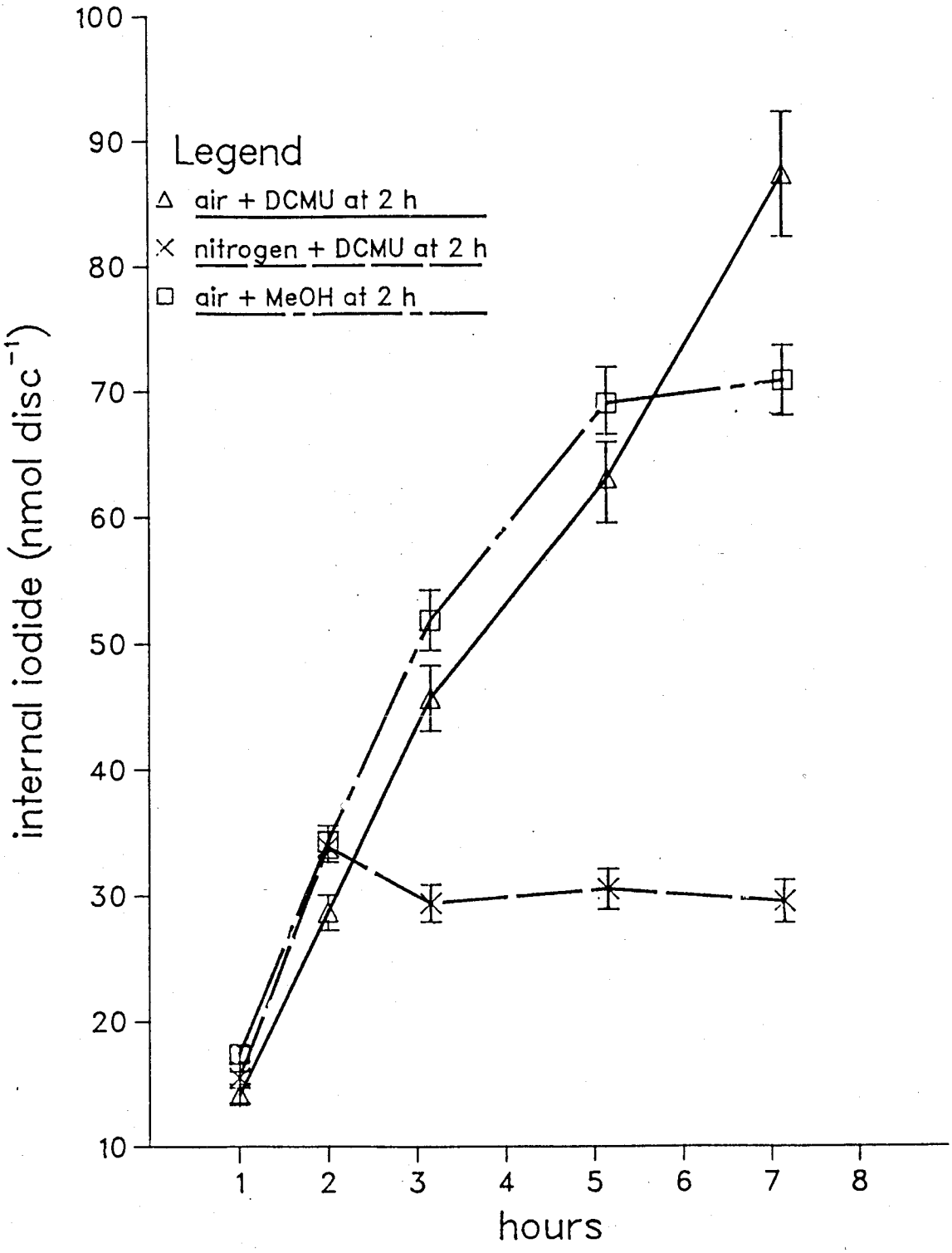

Legend
air
nitrogen
air + oxygen
dark
internal iodide (nmol disc^{-1})
hours
0
20
40
60
80
100
120
140
1
2
3
4
5
6
7
8
9

Figure D17 Effect of DCMU on iodide uptake in the light
DCMU was added where indicated. One control had methanol added instead of DCMU as this is the DCMU solvent. The bubbling was continuous and 5 replicates were used, error bars represent S.E.


Legend
air + DCMU at 2 h
nitrogen + DCMU at 2 h
air + MeOH at 2 h
internal iodide (nmol disc^{-1})
hours
100
90
80
70
60
50
40
30
20
10
1
2
3
4
5
6
7
8

uptake in the light and in air but stopped all uptake when air was replaced by nitrogen.

To see whether the uptake required ATP originating from respiration, carboxyatractyloside, an inhibitor used to block the availability of ATP from respiration, was used at a concentration of 10^{-5}M and 2 x 10^{-5}M. This compound specifically inhibits the enzyme ATP translocase and although ATP is still formed in the mitochondria it is not available to the cell. No effect of carboxyatractiloside was found on iodide uptake in light and in air (data not shown). Photosynthesis was consequently blocked by using DCMU and discs were incubated in conjunction with carboxyatractyloside. This also failed to affect the iodide uptake in the light and air (see Discussion).

6. Competition experiments

a. Competition between $^{125}I^-/IO_3^-$ and $^{125}IO_3^-/I^-$

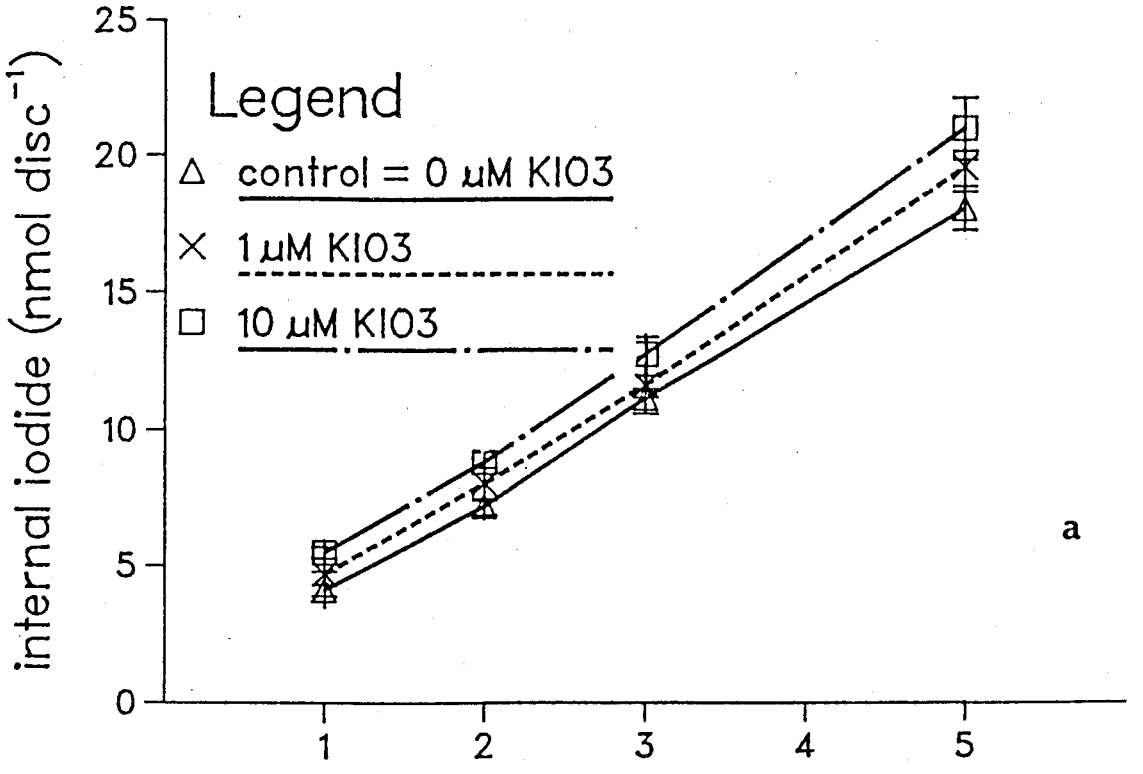
1. $^{125}I^-/IO_3^-$

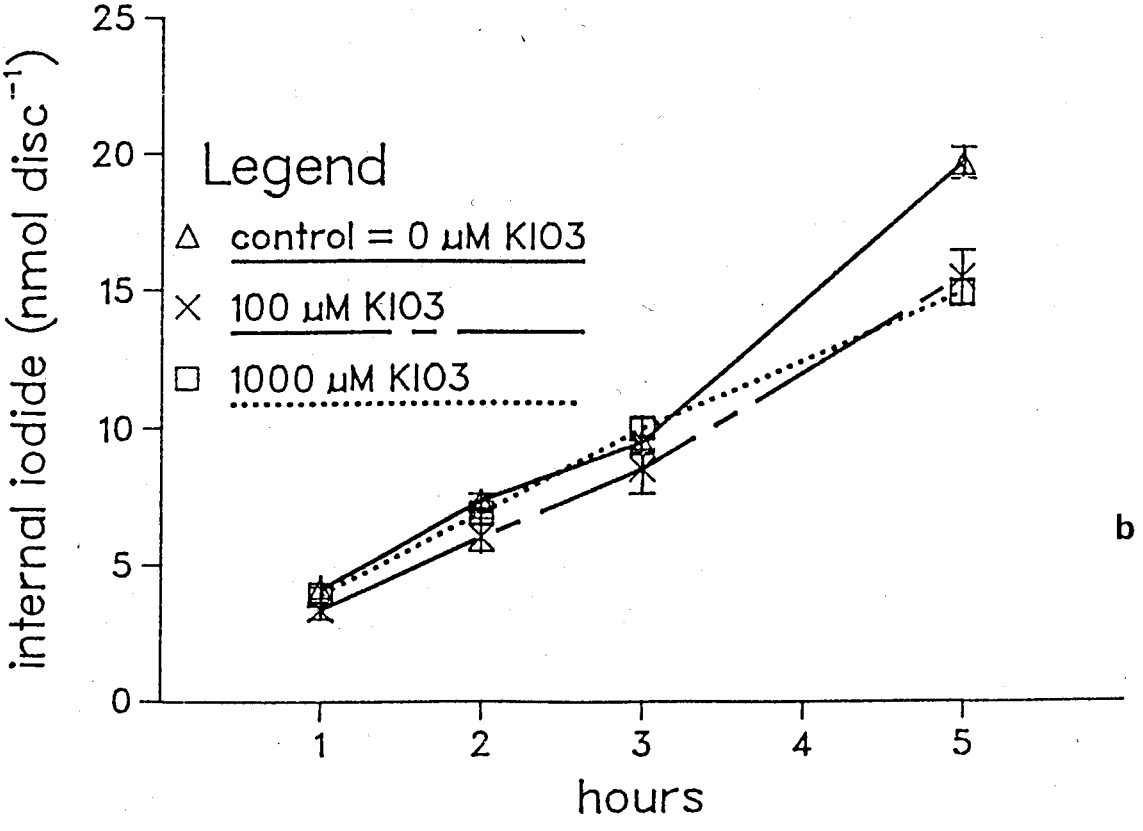
Labelled iodide and seawater with 1 μM KI was used. KIO_3 levels of 1, 10, 100, 1000 μM were added to the medium and the uptake of ^{125}I was followed for 5 h (Fig D18a,b). Only 100 and 1000 μM KIO_3 lowered the uptake of ^{125}I slightly after 5 h.

2. $^{125}IO_3^-/I^-$

In a reverse experiment, blade discs were incubated in a seawater medium with 1 μM KIO_3 and an appropriate amount of

Figure D18 a and b: Effect of added iodate on labelled iodide uptake
A new control was run in each experiment. The bubbling was intermittent and 5 "reusable" discs were used. Error bars represent S.E. and are shown if smaller than the symbol.


Legend
control = 0 µM KIO3
1 µM KIO3
10 µM KIO3
internal iodide (nmol disc^{-1})
25
20
15
10
5
0
1
2
3
4
5
a


Legend
control = 0 µM KIO3
100 µM KIO3
1000 µM KIO3
internal iodide (nmol disc^{-1})
25
20
15
10
5
0
1
2
3
4
5
hours
b

$^{125}IO_3^-$. To this medium KI concentrations ranging from 1, 10, 100, 500 μM were added and uptake of $^{125}IO_3^-$ monitored for 7 h (Fig D19 a,b). The 100 and 500 μM concentrations of KI very clearly decreased the uptake of IO_3^-.

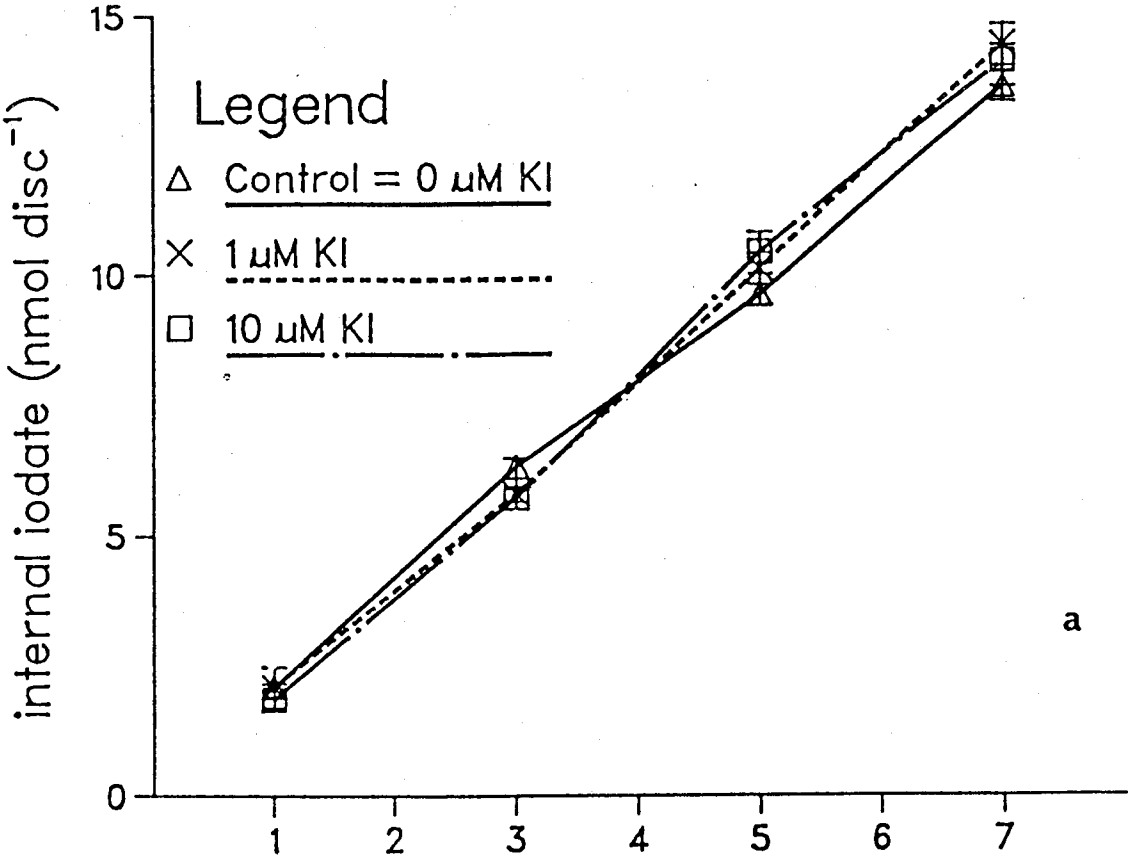
b. Competition with ClO_4^-

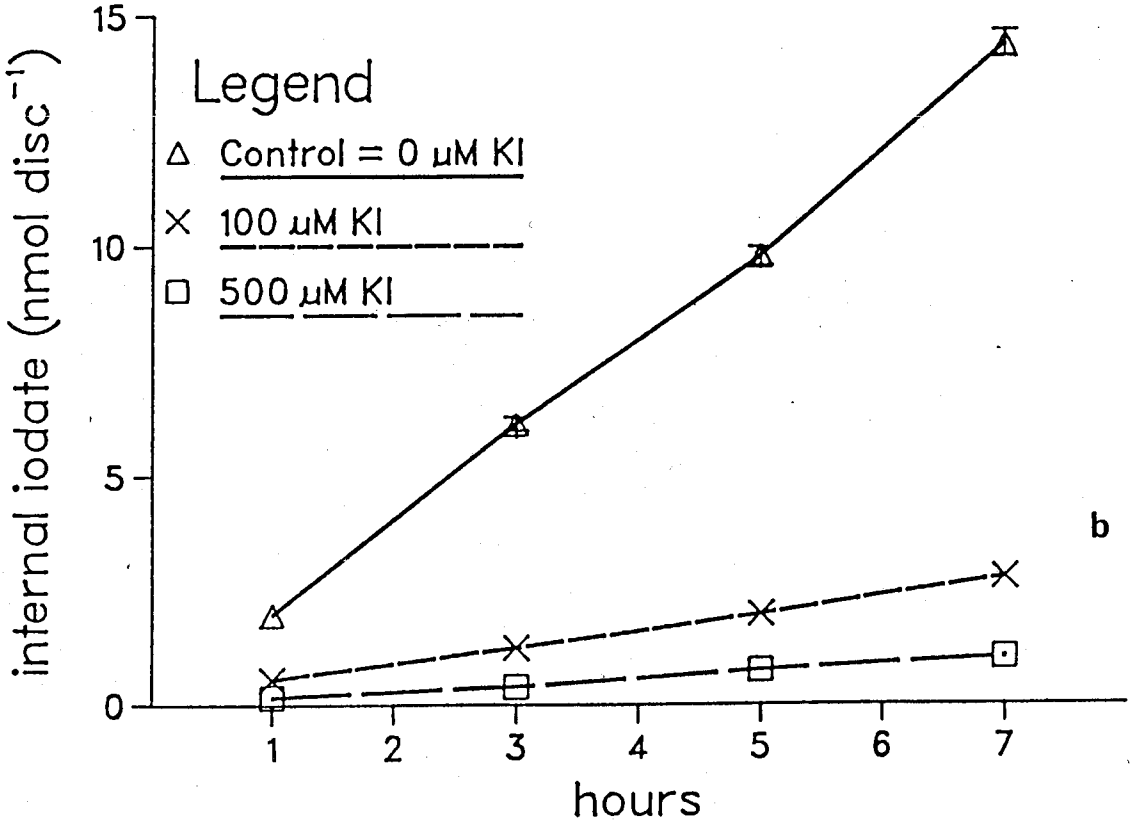
I looked at the effect of 0.2 and 1.0 μM $KClO_4$ on the uptake of iodide. Neither concentration showed an effect on the uptake of iodide (data not shown).

c. Competition with nitrate

In order to test the hypothesis in which bacteria with nitrate reductase can reduce iodate (see Literature review), thus regulating the uptake of iodate, radioactive IO_3^- was incubated with various concentrations of NO_3^-: 50, 100, 500, 1000μM $NaNO_3^-$. None of these concentrations induced a decrease in $^{125}IO_3$ uptake.

Figure D19 a and b: Effect of added iodide on labelled iodate uptake
A new control was run in each experiment. The bubbling was intermittent and 5 "reusable" discs were used. Standard error bars represent S.E. and are not shown if smaller than the symbol.


Legend
Control = 0 μM KI
1 μM KI
10 μM KI
internal iodate (nmol disc⁻¹)
15
10
5
0
1
2
3
4
5
6
7
a


Legend
Control = 0 μM KI
100 μM KI
500 μM KI
internal iodate (nmol disc⁻¹)
15
10
5
0
1
2
3
4
5
6
7
hours
b

III. Discussion

The use of tissue discs in these iodide uptake experiments presented many advantages; they are much more convenient to use than whole thalli, many replicates can be run, and, in addition, environmental conditions such as temperature and lighting can be more rigorously controlled. According to Drew (1983), physiological experiments using tissue discs actually reflect what occurs in a living plant. He cautions, however, that "Laminaria tissue is notoriously variable in its physiological response, between plants, within a given lamina and even within apparently homologous regions of a single lamina". It was found here, similarly, that young areas of the blade took up more iodide than distal blade tissue. Also, the holdfast paralleled the blade tissue in the level of iodide taken up. The meristem and stipe took up the least iodide on a per g d.wt. basis; this could be a consequence of their small surface area. But all these differences in iodide uptake varying with tissue origin are no doubt also a consequence of the different physiological roles of these tissues. In the next chapters the importance of this different iodide uptake capacity in the light of distribution of iodide in the plant and iodide translocation will be demonstrated.

When comparing iodide uptake by tissue of different thickness, I showed earlier that the area of the disc is an important factor in short term uptake experiments. For a longer

incubation time, and for an uptake on a per g d.wt. basis, the thicker tissue displayed a higher uptake rate. This could be due to the greater storage capacity of the thick tissue. Also, a thickening of tissue is often caused by an increase in thickness of the cortex and medullary tissues. This shows that iodide could be stored in these tissues or simply transferred towards the medulla for its potential translocation (see Chapter on Translocation of Iodide) although translocation would not be a possibility in cut tissue discs. In addition, Mangenot (1928) observed I^- in the vacuoles of the medullary and meristodermic cells but none in the vacuoles of the cortical cells a finding which adds credence to the aforementioned suggestion.
The difference in iodide uptake between tissues of different thickness might explain the variation recorded between some experiments. Discs were generally punched out of the central part of the mature tissue, but this could vary according to the plant as the healthiest part of the tissue was always chosen. Also, blade thickness varies from plant to plant. Arnold and Manley (1985) assessed the variability in photosynthesis and respiration rates according to disc origin and thickness and also showed a wide variation in the rates dependent on these two factors.

I have shown that the uptake could be optimized through continuous bubbling or stirring. This is probably a result of the turbulence created at the tissue surface which reduces the boundary layer (Wheeler, 1980) and which would enable more

iodide to be taken up.

As to the uptake of iodine, the form of iodine readily taken up by Laminaria is I^- although $^{125}IO_3$ can also be taken up. This differs from Baily and Kelly's (1955) data on Ascophyllum where iodate was not taken up. More recently, however, Hirano et al. (1983) have shown that Hijikia fusiforme takes up iodate although to a lesser extent than iodide. The present competition experiments demonstrate a preference by the plant for I^- over IO_3^- and support the data of Hirano et al. (1983).

The uptake of iodide shows that for ^{125}I previously taken up, 17% of the activity is lost after 48 h in non radioactive medium. Part of this release, 2.5% of the total label, is due to release of iodide from cellfree space that occurs in the first 40 min. This finding contrasts with the data of Manley and North (1980) for Macrocystis where 90 % of the cellfree space release of ^{125}I occured in 10 min. Therefore, 14.5% of the activity is lost in a further 46 h at a slower rate. Guimaraes et al. (1985) showed that Sargassum filipendula , after 3 days in non radioactive medium, loses 50% of its counts but then does not lose any further counts. Hirano et al. (1983) showed that Hizikia fusiforme, when transferred to a cold medium, lost very little activity even after 20 days whereas Sargassum sagmiamum lost 50% of its activity after 20 days. This release of activity seems to be species dependent and could be due to an exchange of hot for cold iodine. It could also be a release by the tissue of a certain form of iodine or iodinated compound for a

physiological purpose (see Discussions of Chapters C and E).

Iodide is taken up at an almost constant rate of 0.1-0.5 μmol I g^{-1} d. wt. h^{-1} for at least 30 h. However, in continuously bubbling air, the uptake reaches $\simeq$ 2 μmol g^{-1} d. wt. h^{-1} but levels off earlier, after about 10 h or when the concentration reaches $\simeq$ 10 μmol g^{-1} d. wt.. This is quite comparable to the values of 0.25-0.45 μmol I^- g^{-1}f.wt. h^{-1} recorded for Fucus ceranoides by Klemperer (1957).

When cold iodide was added to the medium this increased the uptake of I^-. A larger increase was often observed for 10 μM KI compared to 100 μM which might indicate that 10μM (or a concentration between 10 and 100 μM) is an optimum for the plant. The 100μM concentration might be toxic to the tissue which explains the lower uptake levels recorded and this is accentuated in the continuous air bubbling, where already after 1 h the uptake rate is lower than in the 10μM KI concentrations (Fig D10). It is interesting to note, as seen in the previous chapter, that young sporophytes do as well in the 100μM KI medium as in the 10μM one and appear very healthy. This indicates that cut discs may behave differently from whole plants or that mature tissue behaves differently than young, fast growing tissue. This latter suggestion appears likely in view of the possible role of iodine in growth (see previous chapter). Klemperer (1957) and Shaw (1959) reported that adding around 100-200 μM KI to Fucus and Laminaria respectively, nearly stopped all ^{125}I uptake. It is true that the overall counts of

the tissue decrease with added KI, but this is due to a decrease of the specific activity of the isotope by this dilution. The above authors do not mention that they corrected for this dilution and their data would be explained if indeed they had overlooked this correction factor.

Apparent pool sizes ranged between 0.18 and 1.5 μmol disc^{-1} (roughly 12.0-106.0 μmol g^{-1} d.wt.) the highest internal iodide level having been obtained with an incubation in 10μM KI. If we consider the blade to contain about 8μmol I^- g^{-1} d.wt. (1000 ppm) the tissue has acquired during the length of the experiment between 1.5 and 13 times its original iodide concentration. In these experiments we have fed the tissue 3 to 200 times (1-100 μM) the iodide concentration found in seawater. This is reflected in a substantial uptake of iodide which could be stored presumably in the vacuoles as was suggested ealier (also see next chapter) and by Mangenot (1928). It could also be utilized in some fashion for a metabolic purpose or a process like iodovolatilization.

The data recorded for iodide uptake levels or rates at different times of the year failed to show any seasonal influence on iodide uptake. This is a very interesting point because as will be shown in the next chapter, the iodide levels in various parts of Laminaria thalli increase as the growing season progresses, especially in the fall. Two points could be made as to why no seasonal influence on uptake of iodide by cut discs can be detected. 1) The variability linked to the tissue

itself (thickness and origin) could mask any seasonal trend. 2) As we cannot detect higher uptake rates by cut discs late in the year, the high tissue levels could be due to the accumulation of iodide or its non-use by the plant. We reported earlier that young sporophytes in 10 or 100μM KI grew better. Mature sporophytes barely grow after September; would their need for iodide, if such a need existed, consequently decline?. One could draw a comparison with nitrate uptake which is seasonally dependent; the uptake of NO_3^- in Macrocystis integrifolia (Wheeler and Srivastava, 1984) is inversely related to the blades' internal nitrate concentration. This in turn is dependent on the ambient seawater nitrate concentration which generally is low in summer and high in winter. Seawater iodide levels do not fluctuate in a similar way (Druehl and Hsiao, 1977) and levels recorded are much lower than those of nitrate. The nitrate/iodate competition experiment clearly showed that iodate is not a competitor of nitrate. As seen in the literature review, some organisms with a nitrate reductase enzyme could also reduce iodate, thus regulating the entry of iodate. Tsunogai and Sase (1969) demonstrated the capacity of bacteria-isolated nitrate reductase to reduce iodate but when large amounts of NO_3^- were added, this diminished the reduction IO_3^-. When large amounts of nitrate were added in the medium with IO_3^- and $^{125}IO_3^-$, no difference in uptake of ^{125}I could be detected, indicating that maybe nitrate reductase in kelp does not reduce iodate as seen in bacteria.

The effect of temperature on the uptake often showed an increased uptake around 10°C. However, in one case (June 1984) the uptake at 20°C was greater than at 10°C. This might reflect the physiological state of the plant when picked for the experiment; it may have had a "warm" preconditioning which would enable the plant to perform better at higher temperatures. Below 10°C, the uptake rate decreased. At 0°C it was about 30 % that at 10°C. Klemperer (1957) similarly found that at 0°C the uptake was reduced by 50 % in Fucus. This temperature dependence indicates the possibility of a carrier mediated system that would have an optimum around 10°C. Also, Laminaria is a temperate (cool) water species which cannot live at high temperatures. This might explain why the plant performs well in cool temperatures as opposed to higher temperatures.

The uptake seems light independent unless discs were previously dark incubated for up to 48 h. Only after this time did the illuminated discs show an increase in the uptake of iodide. This shows the importance of metabolism on iodide uptake. Reserves get depleted in the dark and discs in the light obtain their energy from photosynthesis which enables them to carry out metabolic processes. At the light intensity used of 46.0 W m^{-2} photosynthesis is at its maximum rate (Lüning, 1981).

No direct effect of anaerobiosis was found on iodide uptake unless discs were kept in the dark and this is in good agreement with Shaw (1959). This suggests that photosynthesis can supply the oxygen necessary for the uptake to take place. The

photosynthetic oxygen can be replaced by exogenous oxygen as seen when photosynthesis was blocked by DCMU. In the presence of air (oxygen) the uptake of iodide was unimpeded whereas in anaerobic conditions the uptake is stopped. Is oxygen itself necessary for the uptake, or is it oxygen for respiration or oxidative phosphorylation that is necessary for the uptake? Past authors (Klemperer, 1957; André, 1965) showed that DNP blocked the iodide uptake reversibly. However, although DNP inhibits oxidative phosphorylation by rendering the inner mitochondrial membrane permeable to protons, it also affects the chloroplast membrane and inhibits ATP production there too. Here, we used carboxyatractyloside to stop the input of energy from respiration energy, as this inhibitor is specific to mitochondrial ATP production. The inhibitor had no, or little effect on iodide uptake suggesting that ATP produced by photosynthesis was sufficient to support iodide uptake. When photosynthesis was blocked using DCMU and discs were incubated in conjunction with carboxyatractiloside, no effect was seen on iodide uptake. DCMU is known to block water-splitting by PS II but not cyclic phosphorylation (Moreland, 1980), meaning there is still an energy producing source left. But a likely suggestion could also be that carboxyatractiloside cannot penetrate the tissues in sufficient quantities to affect the mitochondria. When working with brown algae it is quite frequent that certain substances cannot penetrate the tissue of these plants (André, 1965). I am left with the possibility that oxygen *per se* is necessary for the uptake to take place, but this is

unlikely as this type of system has never been shown to exist (Gresser, pers. comm.).

The above conclusions suggest very strongly that iodide uptake is an active process. It is temperature dependent, no doubt carrier mediated and it is enhanced in photosynthetically active tissue, but moreover it is ATP dependent. Tissue incubated with DCMU showed no uptake of iodide in anaerobic conditions; oxygen is thus necessary in photosynthetically inactive tissue indicating that the ATP required for the uptake to take place is likely to be of mitochondrial origin.

PART E

Iodine in *Laminaria* tissue

I. Iodine levels in the plant and its seasonal variation

1. Methods

Prior to analysis, all tissue was frozen in liquid nitrogen, freeze-dried, and reduced to a fine powder with a Waring blender.

Samples were analysed for iodine using Instrumental Neutron Activation Analysis (NAA) (Law, 1975) by Novatrack, TRIUMF (Tri Universities Meson Facilities), UBC campus. This method measures the iodine content regardless of the physicochemical state of the sample, so one achieves a high level of accuracy. The method involves subjecting samples to thermal neutron bombardment and correlating the measured activity from the induced radionuclide ^{128}I (half-life, 25 min) of samples with that of standards. The making of standards was not easy as one needs a sample with a homogeneous distribution of iodine. This was finally achieved using mannitol as a matrix dissolved in a standard solution of NaI and then freeze dried. Standards were then consistent. A liquid standard was also made which impregnated graphite before being freeze-dried. All data in this section are expressed on the basis of per g dry weight.

2. Results

For the NAA method, Law (1975) describes an agreement within 2% between replicates and a lower detection limit of 7 μg per sample (0.15 to 0.4 g each). In our case the detection limit was 10 μg or 80 nmol g^{-1}d.wt. for samples weighing 0.5 g but kelp samples always had higher levels of iodine. Replicates were within 100 μg g^{-1} of each other.

In a preliminary analysis, plants were collected in September 1982 at Brockton Point, Vancouver, B.C., and cut up into different parts before analysis by NAA. The plant parts examined, with number of samples for each in parentheses, were: holdfast (28), stipe (27) separated in two parts: top of stipe (close to the meristem) and lower stipe, meristem (15) and blade (15). The results showed more iodine in the lower part of the plant such as the meristematic area with 36.0 μmol g^{-1}, the stipe with 31.2 μmol g^{-1}, and the holdfast 23.2 μmol g^{-1}. The blade showed lower levels with 6.4 μmol g^{-1}.

Plants were collected monthly from July 1983 to July 1985 for iodine content. The following parts were examined: blade, top of meristem, meristem, stipe and holdfast. The data for the seasonal variation of iodine in different parts of the plant appear in Fig E1 and E2. Due to limited possible usage of the TRIUMF facilities, only a few replicate samples could be run. The replicates were within 100 μg g^{-1} of each other; they are not represented in the figures. Data are lacking for several

Figure E1 Seasonal variation in iodide content as determined by NAA
Seasonal variation of iodine content in the blade, top of meristem and meristem.

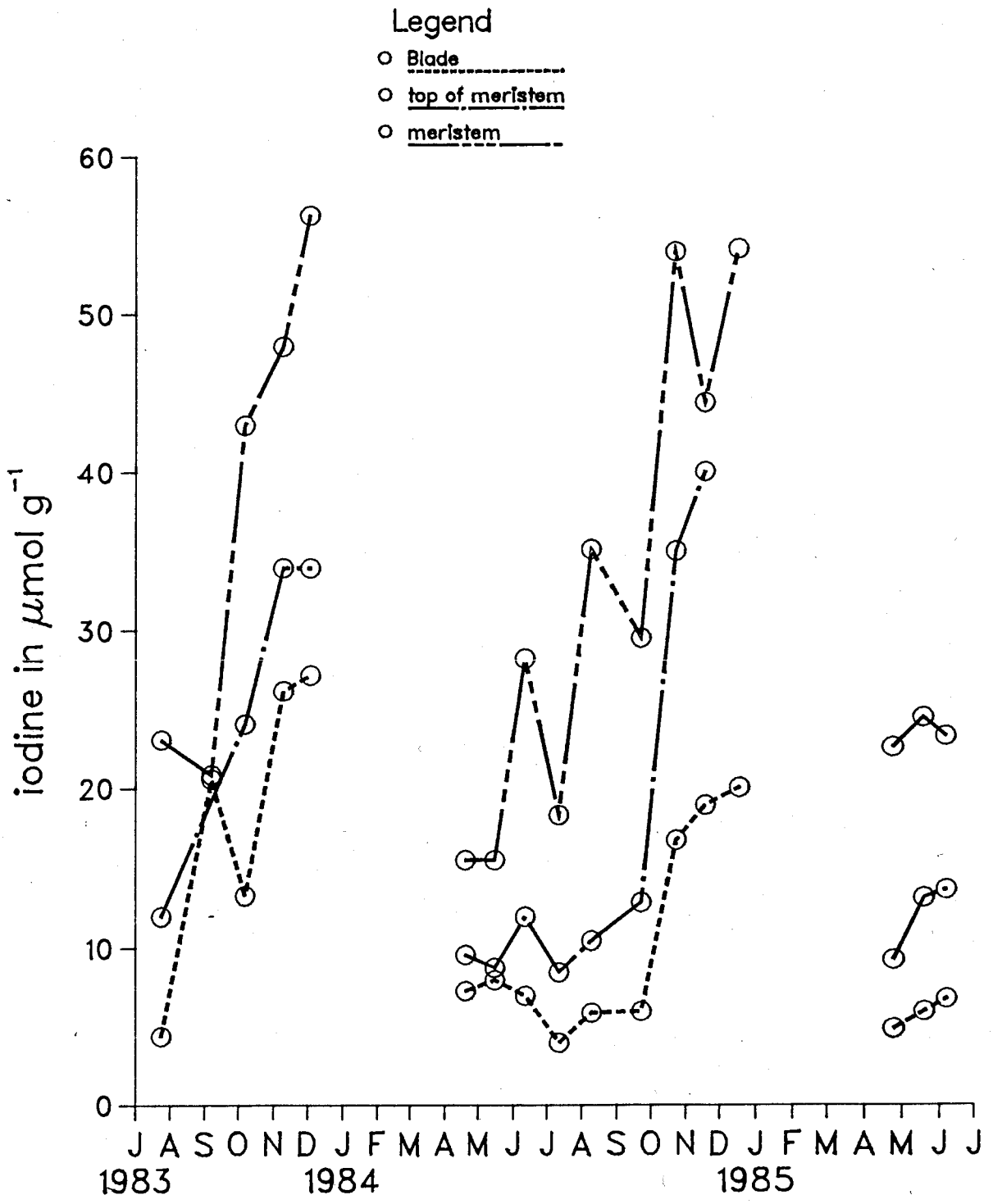
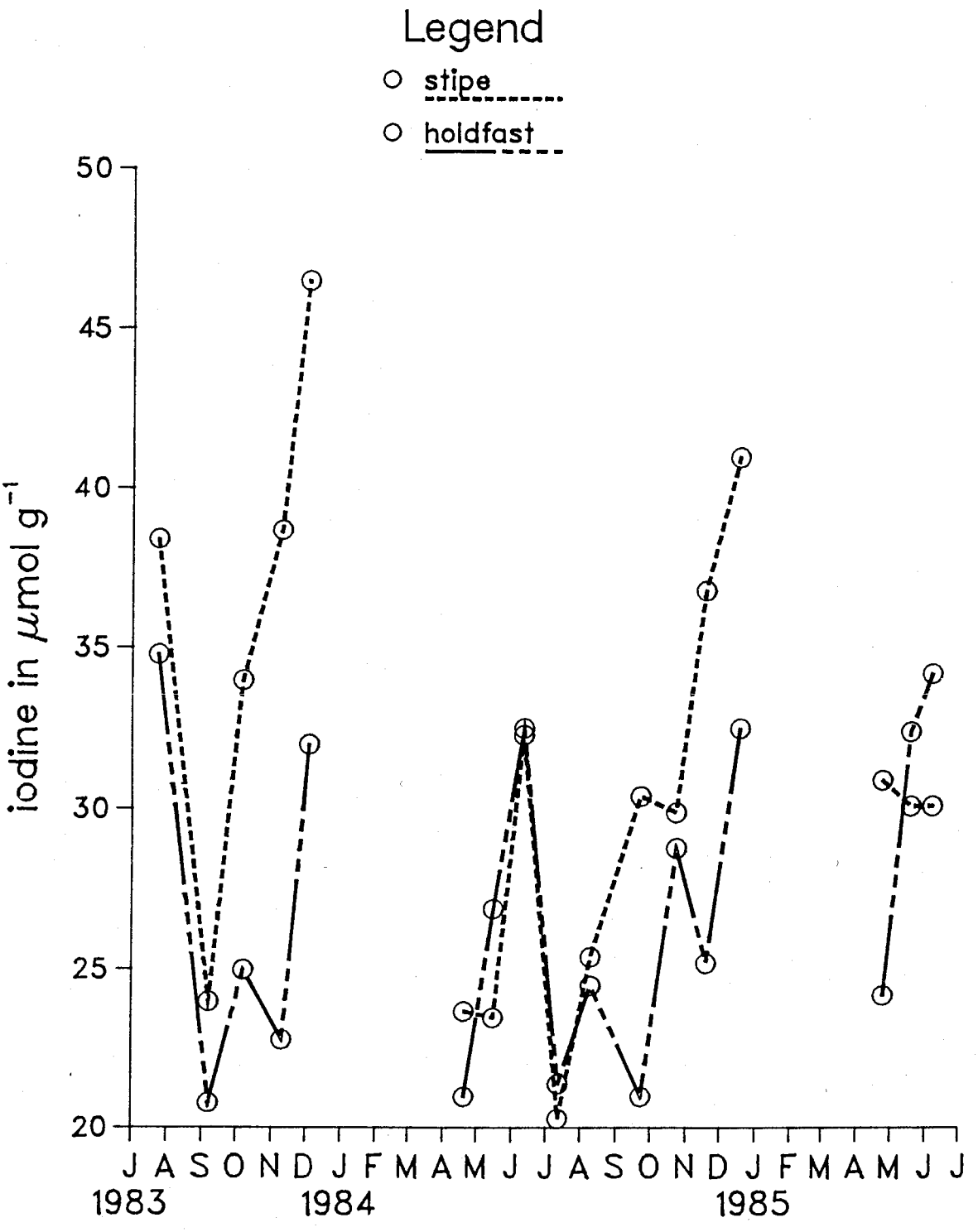

Legend
Blade
top of meristem
meristem
iodine in µmol g⁻¹
0
10
20
30
40
50
60
J A S O N D J F M A M J J A S O N D J F M A M J J
1983
1984
1985

Figure E2 Seasonal variation in iodide content as determined by NAA
Seasonal variation of iodine in the stipe and holdfast.
Please note how the y axis scale differs from Fig E1.


Legend
stipe
holdfast
iodine in µmol g⁻¹
50
45
40
35
30
25
20
J A S O N D J F M A M J J A S O N D J F M A M J J
1983
1984
1985

months at the beginning of 1984 and 1985 as heavy frost killed blade tissue of all plants in the kelp bed, leaving only stipes and holdfasts (see Chapter on Growth of Laminaria saccharina in the field). By April of each year, plants were big enough to be harvested, cut up, freeze-dried and analyzed.

From Fig E1 and E2, it is apparent that the lower part of the plant, namely the meristem, stipe and holdfast, showed the highest levels of iodine. This was the case at every sampling month. One can also observe a seasonal variation with low levels of iodine throughout the plant in the earlier part of the year; these levels slowly rise as the growing season progresses, reaching a maximum in late fall to early winter. Most of this increase in iodine content occurs in the fall or when plants have stopped growing. Levels reach up to 56 μmol g^{-1} d.wt. in the meristem by the end of the year. The stipe, similarly shows high levels reaching 46 μmol g^{-1} d.wt.. Unfortunately, it was not possible to know what the levels would have been in late winter-early spring.

The Novatrack data were double checked for the same samples with the standard method of iodine determination by titration with thiosulfate (see next section) for the same samples. The comparative results appear in Table E1. The titration method indicates levels of iodine systematically lower and even sometimes disproportionately lower than those from Novatrack. The possibility of losing some chemical form of iodine during the titration procedure must be considered, especially when the

Table E1 Comparison of iodide levels determined by NAA and titration

All values are in $\mu g\ g^{-1}$. Those determined by NAA are in brackets and replicates are indicated. Two sample weights were assayed for the titration to evaluate whether this would influence the results; those weights are given in brackets in g.

Part of plant	1983 July	Sept.	Oct.	Nov.	Dec. ..	1984 April	May
Top of Blade	390 (475)	465 (1260)	973 (1590)				
Blade	313(4g) 317(4g) (555)	751(2g) 742(4g) (2580)	2414 2340 (3280)	516 (3400)		608 (920)	810 (1150)

sample is heated in the furnace for 2 h at 500°C. Nevertheless this loss should remain constant in proportion, unless the amount of that chemical form is variable in the alga itself.

II. Partitioning of iodide in Laminaria saccharina tissue

1. Methods

N.B. All aqueous extractions or dilutions were done with distilled water.

1-thiosulfate titration method for quantitative determination of iodine.

The kelp powder was mixed with K_2CO_3 and heated for 2 h at 500-600°C in a muffle furnace. The mixture was then thoroughly extracted with boiling water and neutralized using 85% H_3PO_4. A few drops of bromine were added and boiled off. This step oxidized I^- to I_2. A few crystals of salicylic acid were added to the solution as it cooled. 85% H_3PO_4 and KI were added in excess before titrating the solution with thiosulfate (0.005 or 0.01 M) using starch as an indicator. This method proved to be lengthy but accurate and reproducible.

This method could be used for the titration of aqueous or ethanolic extracts of kelp tissues, fresh or freeze dried. Ethanol extracts precipitated the starch indicator so those samples were initially evaporated to dryness and taken up in water.

2-radiolabelled tissue

When radiolabelled tissue was used, the various extracted fractions were γ-counted in order to estimate the proportion of ^{125}I in each.

2. Results

In a first experiment, two Laminaria plants were fed 10 μM KI for 3 days to see whether the enrichment of iodine would occur in the soluble or insoluble fraction of the tissue. The control was kept in the same conditions as above without added KI. Plants were frozen, freeze-dried and reduced to powder. Tissues were extracted with water, the residues were dried and analyzed by NAA. Results were as follows:

Tissue	iodine level μmol g^{-1}d.wt.
control	6.1
control residue after 2 water extractions (2 h + overnight)	4.2
KI fed kelp	8.7
residue after 2 water extractions (2 h + overnight)	5.4

About 2.6 μmol iodine g^{-1} d.wt. tissue were acquired during the 3 days of incubation, and of that 1.4μmol were extractable with water in about 16 h; the rest remained in the insoluble fraction.

For labelled partitioning, several experiments were run in which discs were first incubated for up to 48 h in a seawater medium containing 1μM KI and 2-3 μCi of $Na^{125}I$. Discs were sampled at set times and extracted individually in hot EtOH for several hours. The EtOH fractions, the discs before and after extraction were all γ-counted. The residual ^{125}I in the disc after extraction was considered the non-soluble or bound iodine. The reverse experiment was also carried out where discs previously incubated in radioactive medium were transferred to plain seawater. Discs then were sampled at set times and treated as described above. Results of the 2 types of experiments appear in Table E2 A and B. The results show that about 90-91% of the total ^{125}I taken up is in a soluble state, leaving 4-7% of the iodine bound to the tissue; the recovery rate was 94-97%. After 48 h chase time in cold medium (Table E2 B) about the same proportion of soluble/insoluble iodine was found; the slight difference could be attributed to the different percent recovery in this experiment and also that a different plant was used.

Table E2 Partitioning of soluble/insoluble iodine

The results are a mean of 8 blade discs used.

A. Discs were incubated in ^{125}I and sampled at the given times. The counts are given for each step of the experiment and the percentages have been calculated.

B. Discs previously incubated with ^{125}I for 48 h were transferred to cold medium and sampled at the given times.

Both experiments were done on different plants in February 1984.

A.

Incub. time	cpm in: Disc	EtOH fract.	Disc after EtOH extr.	% cpm in: EtOH fract.	Disc	% recovery
1 h	7765	6997	548	90.1	7.0	97.1
12 h	42990	39349	1925	91.5	4.4	95.9
24 h	54753	49414	2254	90.2	4.1	94.3
48 h	81129	74403	3391	91.7	4.1	95.8

B.

Incub. time in cold I	cpm in: Disc	EtOH fract.	Disc after EtOH extr.	% cpm in: EtOH fract.	Disc	% recovery
0 h	55438	48138	2755	88.6	4.9	93.5
2 h	50714	44719	2055	88.1	4.0	92.1
24 h	54454	47944	3409	88.0	6.2	94.2
48 h	67142	58948	4358	87.8	6.5	94.3

III. Chemical form in which iodine occurs in Laminaria tissue

1. Material and Methods

a. Extraction procedures

1-Water or EtOH extractions

Water or ethanol extracts of freeze dried kelp powder or fresh tissue were prepared. If ethanol was used it was evaporated after extraction and the sample was taken up in water. These extracts were partially purified by passing them through ion exchange columns which have the property of "fixing" positive or negative ions depending on their characteristics. Thus a kelp extract introduced on a Dowex 50 WX8 (180x12 mm) column--H+ form--would bind negative ions. The mobile phase containing the neutral fraction or positive ions/molecules was then passed through a Dowex 1 WX10 (140x13 mm) --HCOO- form-that would retain the positive ions, letting the neutral fractions pass through. To collect the various fractions, the first column was eluted with a strong base which released the negative ions. A strong acid was used to elute the second column to release the positive ions. The eluates were reduced to near dryness, resuspended in water and used for TLC as described below.
The aqueous extract could also be partially purified for iodoamino acids by adapting Stouffer's method (1969) for isolating thyroid hormones from blood serum. In a separatory funnel, 10 ml of aqueous kelp extract were shaken with a mixture

of MeOH-chloroform (1:1) and 0.03 N H_2SO_4. The chloroform phase was rewashed in MeOH-H_2O and discarded. The MeOH phase after filtration through a Buchner funnel was adjusted to pH 7 before evaporation in vacuo to dryness. A small Sephadex LH-20 column was prepared in a 5 ml syringe, the sample introduced and washed through with water. A mixture of MeOH-NH3 eluted the iodoamino acids. The eluate was evaporated to dryness, taken up in MeOH, and used for TLC.

2-Hydrolysis of kelp tissue

Kelp powder or kelp residue after a water or ethanol extraction was introduced into a 5 ml Pyrex tube with 2 N NaOH. The tube was sealed and left in a 100°C oven overnight for hydrolysis to be completed. This was later improved by replacing the Pyrex tubes with a reflux system that enabled more tissue to be hydrolysed at the same time. The hydrolysate was acidified to pH 1.2 with 1 N HCl and centrifuged (André, 1971). The supernatant was then extracted with butanol, evaporated to dryness and taken up in MeOH for chromatography. A control of this method was made by spiking the kelp tissue before hydrolysis with mono-iodotyrosine (MIT) to see whether the hydrolysis would degrade the iodoamino acid and whether it was retained in the butanol extraction.

Another control was run, where tyrosine and I_2 were hydrolysed in NaOH 2N. The hydrolysate was adjusted to pH 8 and plated for chromatography.

b. Detection procedures

Thin Layer Chromatography TLC

Standards of KI, mono- and di-iodotyrosine (MIT and DIT), tyrosine and various kelp extracts were spotted on silica gel TLC plates (Whatman, Silica gel 60). The following solvent mixtures were tried:

n-Butanol-n-Propanol-n-propionic acid-H_2O (400:175:285:373) =solvent 1.

n-Butanol-acetic acid-H_2O (780:50:170) BAW =solvent 2.

95% acetic acid-benzene-xylene (3:1:1) =solvent 3.

To reveal the migrated compounds the following methods were used:

Patterson and Clement's specific test for iodoamino acids (1964).

This test is based on the catalytic action of traces of iodide upon the reduction of ceric sulfate by arsenic acid. Theoretically, 1.0 ng of I^- and 2 ng of DIT can be determined with this reaction but in practice quantities of between 0.01 and 0.1 µg per spot are determined. Compounds with phenol or thiol groups and ascorbate also give a positive reaction when present in quantities of 1 µg or more per spot. A mixture of 3 solutions is used to spray the plates which reveals prussian blue spots where the iodoamino acids (and other compounds!) are located.

Solution 1: 2.7% $FeCl_3x6H_2O$ in 2 N HCl

Solution 2: 3.5% aqueous solution of pure $K_3[Fe(CN)_6]$

Solution 3: 3.8 g As_2O_3 heated with 25 ml 2 N NaOH, cooled to 5°C; 50 ml 2 N H_2SO_4 also cooled to 5°C is added. The mixture was diluted to 100 ml.

The three solutions were mixed just before use in the ratio 5:5:1 and sprayed onto the plates, which were subsequently rinsed with water.

Ninhydrin test: 5% ninhydrin in butanol was sprayed on the plates that were then heated thus revealing spots containing amino acids.

If two dimensional TLC was performed using 20x20 cm TLC plates two solvent mixtures of different characteristics were used for each dimension, such as solvents 2 and 3 for instance. The ninhydrin test was used to locate the various spots.

Gas Liquid Chromatography GLC

A nonpolar capillary column, 18 m long and 1.5 mm I.D., packed with 3% SE-30 was used. Derivatizing the standard amino acids such as tyrosine, MIT and DIT was tried in several ways:

1-BSA (bis-trimethylsilyl acetamide) with acetonitrile as a solvent is a one step derivatization. The amino acid is heated with BSA at 150°C for 30 min. But the derivatized compounds are known to be unstable especially when in contact with water molecules.

2-TFA (trifluoroacetyl).

First the amino acid was esterified with a butanol-HCl mixture,

sonicated and heated at 100°C for 15 min. The mixture was then evaporated to dryness in vacuo. $MeCl_2$ and TFA were added to the sample and heated in a teflon lined cap vial for 5 min at 150°C. This mixture is highly volatile and can explode, thus a room temperature derivatization overnight was tried.
The TFA derivatives proved to be more stable but column retention times for the standards fluctuated greatly. No kelp extracts were assayed with this technique.

2. Results

TLC was first developed on standards. The Rf values of various standards for the different solvents are shown below.
Solvent 1 (see Methods)
KI: 0.60; DIT: 0.64; MIT: 0.57; Tyr: 0.50.
Solvent 2
KI: 0.50; DIT: 0.44; MIT: 0.35; Tyr: 0.23.
Solvent 3
KI: 0.18; DIT: 0.51; MIT: 0.35; Tyr: 0.25.
These 3 solvent mixtures were mainly used as they gave good separation of compounds.
The Patterson and Clements' test showed the characteristic prussian blue colouration with Tyr, MIT, DIT, Cyst and Thre. It did not react with Ala, Gly, Glu, Phe. The results of this test were not of great interest as the resolution of spots was not good and too many compounds seemed to react with the test thus reducing it's specificity. Also, the use of As_2O_3 proved to be

hazardous to my health.

The ninhydrin test was the most useful, even though it is not extremely specific--it reacts with most amino acids, even those containing iodine. With good standards and known Rf values, however, one is able to estimate the presence or absence of a substance. It should be noted that the standard MIT migrates into two spots one of which is tyrosine.

Laminaria tissue was extracted with water and this extract was passed through a Dowex 50 WX8 followed by a Dowex 1 WX10 column. The different fractions obtained were plated but no good resolution of spots was obtained. Only a massive presence of I^- could be detected which may have masked other compounds. The residue obtained after the water extraction and the whole kelp tissue were hydrolysed with 2 N NaOH and extracted with butanol. The residue, after aqueous extraction, and whole tissue gave similar spots with ninhydrin. However, standards of MIT and DIT, which were cochromatographed, failed to show the presence of these compounds in the extracts.

Thus a systematic approach to hydrolysis followed by TLC was undertaken on various kelp samples; fresh tissue, freeze-dried tissue, and/or EtOH extracted tissue, one of which was spiked prior to hydrolysis with MIT to see whether hydrolysis affected the stability of MIT and whether it can be traced by TLC using this methodology. The samples used prior to hydrolysis were:

-fresh blade tissue extracted with several washes of EtOH, half the sample was spiked with MIT

-fresh blade tissue

-freeze dried powdered blade tissue

All samples were hydrolyzed under reflux with 2 N NaOH and hydrolysates were adjusted to pH 4 before preparation for TLC. The presence of MIT was detected in the spiked sample, indicating that the hydrolysis does not affect MIT to the point of destroying its structure. Other samples did not show defined spots as they presented a great mixture of compounds. A butanolic extraction was performed on all these samples to purify them before preparative TLC. Areas of interest, that is, where the standards of MIT and DIT that were cochromatographed had migrated were scraped and redissolved in MeOH, evaporated to near dryness and replated. Both samples of fresh Laminaria displayed weak spots in the same Rf values as MIT and DIT. This gives an indication as to the existence of these compounds, although they would be in very low concentrations. The freeze-dried tissue did not show these spots.

The detection limit for MIT in TLC, using ninhydrin as a detector, was found to be 0.38 μg per spot. This means that the tissue would contain, after a rough calculation and assuming that nothing was lost during the extraction procedure or the TLC, 0.2 μg per g fresh tissue or 2 ppm dry tissue (16 nmol g^{-1}). If blade tissue contains 1000 ppm iodine, this would be 0.2% of the iodine content. It is obvious that, under the conditions of extraction described, only a very small proportion of the iodine is in the form of mono- or di-iodotyrosine. The main form remains I^-.

The results of the hydrolysis of tyrosine with I_2 showed the following products when cochromatographed with standards: MIT, DIT and I^-. It is thus possible that hydrolysis in an alkaline solution can provoke the formation of these products. Kelp is known to produce I_2 as in the process of iodovolatilization, and this would explain why traces of organic iodine were only found in fresh tissue.

IV. Discussion

Very little work has been done on the distribution of iodine in the thalli of algae. From the literature review it seems to be species-dependent and even author-dependent whether higher levels of iodine are found in the blade or in the stipe of Laminaria spp. Among the various authors, only Rinck and Brouardel (1949) examined the iodine levels in different parts of a L. flexicaulis thallus but their data were taken at only one time in the year. They found levels of about 24 μmol g^{-1} d.wt. in the holdfast, about 12 μmol g^{-1} d.wt. in the stipe, a peak of 24 μ mol g^{-1} in the meristem and then a steady increase in the blade up to 56 μmol g^{-1} d.wt. at 1 m from the meristem (see Fig A1 in the Literature Review). The present results in Laminaria saccharina differ in that the highest iodine levels occur in the holdfast-stipe-meristem tissues with the following respective maxima: 32, 45, 56 μmol g^{-1} d.wt. Levels in the blade and the distal end of the blade are much lower than any of the preceding values.

On a seasonal basis, the general trend observed is that of an increase of iodine in tissues with time, especially in fall winter. This was the case in both years 1984-1985. Similarly, Haug and Jensen (1957) followed the iodine content of blades and stipes of Laminaria saccharina and L. hyperborea for a year and found a maximum in the winter months (December-February) in the blade tissue. The stipes, although showing much variation, had

maximum iodine contents in February. A minimum value was found in June-July for both stipe and blade tissue of L. hyperborea. An explanation for this increased concentration of iodine with time is a build-up through accumulation. Young sporophytes show minimum iodine content, and as the growing season progresses, blade tissue gets thicker and sturdier, and there is an increase in iodine content. This also explains why the stipe and holdfast are rich in iodine as they grow more slowly than the blade or meristem. On the other hand the youngest tissue of the mature plant, the meristem, registers the highest iodine content. Roche and André (1962) also found that areas of growth of various seaweed species displayed high iodine contents. This could be due to the high number of cells per unit area as found in the meristematic tissues. It can also be due to iodine being translocated to the meristem from more mature parts (see next chapter). Both, the low iodine content in young sporophytes early in the season and translocation of iodine to the meristem in older plants suggest utilization of iodine in the growth of these plants. One can also speculate that an accumulation of iodine, especially in the fall, represents a lack of use of iodine when plants are barely growing. Young plants grow actively and present the least content of I^- g^{-1}d.wt. as compared to slower growing tissues. This draws attention to the results found in the 2nd chapter (Effect of added KI on the growth of young sporophytes) which also suggested an effect of iodine on growth as external iodide promoted the growth and health of young sporophytes in culture. Would this accumulation

of iodine, especially in the fall, prepare the plant for next year's growth? It should be noted, however, as we saw in the chapter "Growth of Laminaria saccharina in the field", that many plants are killed in winter, but they do have the capacity to grow a second year (or more?) as shown by survival of tagged plants.

Thus it seems that the iodine content of Laminaria thalli is not related to their seasonal growth pattern; high iodine levels are not recorded in spring when the growth rate is high, and in the fall, high iodine levels are found when growth is minimal.

The literature survey indicated the extent of controversy regarding the chemical state of iodine found in algae. The reported ratios of organic/inorganic iodide varied greatly and the available data seemed very genus and species-dependent and maybe even method-dependent. To recall only the data on Laminaria, Roche and Yagi (1952) were amongst the first to determine that 80 % or more of the ^{131}I fixed in L. flexicaulis was in the inorganic form of I^-. Later, André (1971) confirmed this and also showed that this ratio was genus-dependent. In the organic fractions as well, the ratio of MIT to DIT were genus related, Laminaria saccharina showing a predominance of MIT (see Literature Survey).

I found that about 90 % of the ^{125}I fixed was readily released from the tissue with ethanol. The remaining ^{125}I after many EtOH extractions was continuing to leach out. This points

to two important facts. Iodine is easily extractable by water or ethanol which indicates that it is most likely in solution as would be found in the vacuoles. This confirms histological studies at the beginning of the century when Mangenot (1928) detected I^- in the vacuoles with cresyl blue. That iodide continues to leach out with EtOH extractions indicates that the binding of iodide to the tissue, to macromolecules such as proteins for instance, is quite labile. Of this bound iodine, only trace amounts of MIT and DIT were detected and represented less than 1 % of the total iodine present in Laminaria. Also, I found that it was only in fresh tissue that MIT and DIT were detected. This may indicate that a higher proportion of MIT and DIT exist in the tissue before extraction or freeze-drying, but that the iodide is readily detached from tyrosine by EtOH extractions or the freeze-drying process. Also, it has been shown that the binding of iodide to tyrosine can be a spontaneous reaction that does not require an enzyme (Morrison and Schonbaum, 1976) and hence it is possible that these compounds are formed spontaneously in the algae. Acland (1952) and Stanley (1953) both showed that hydrolysing tissue in NaOH together with the migration of extracts in solvents for chromatography can lead to the formation of artifacts. As I showed, the simple hydrolysis of tyrosine and molecular iodine in NaOH caused the synthesis of organic iodine as MIT and DIT. It is then quite possible that past authors have been induced in error by this methodology; this would explain why so much variation exists in the ratios of organic/inorganic iodine found

by various authors in various species of algae. It would only be necessary to have present a strong oxidant such as I_2, together with tyrosine, to cause the spontaneous formation of MIT and DIT in alkaline conditions in any extract of algae.

Some halogenated compounds synthesized by algae are volatile and would not be detected by the used methods. This organification of elements detoxifies these compounds; this process is common in algae (Cooney et al., 1978) which then release these compounds to the environment. Gschwend et al. (1985) list a few iodinated compounds such as iodoalkanes and dihalomethanes (containing iodide) which are released by Fucus and Ascophyllum. These same authors had also shown that there was an avoidance response by Littorina littorea (periwinkle) to the release of CH_2I_2 by algae. This avoidance was confirmed by the use of this compound in agar based media and it was also shown that it could not be replaced by CH_2Br_2. I found highest iodine levels in the tissues critical for the existence of the kelp: the holdfast, stipe and meristem. These three tissues are fundamental to the survival of the plant and it must preserve them. Maybe these tissues can also release larger quantities of volatile halogenated organic compounds to fend off potential grazers, bacteria or epiphytes.

PART F

Translocation of ^{125}I

I. Introduction

We have seen that iodine is readily taken up by all tissues of Laminaria and especially well by blade tissue. In the preceding chapter we have also seen that the levels of iodine within a Laminaria plant show high values in the meristem, stipe and holdfast. Is this accumulation a result of the transport of iodine from the blade tissue to the basal part of the plant?. Translocation of photoassimilates and ions is well established in the Laminariales and Fucales (see review by Schmitz 1982, also see, Nicholson 1976, Moss 1983, Shih et al 1983). Although research has focused mainly on the transport of photoassimilates, the transport of inorganic elements such as rubidium (an analogue of K), sulfur, phosphorus and iron (Schmitz and Srivastava 1979, Manley 1981, Floc'h 1982, Diouris and Floc'h 1984) have also been studied. No reported work has described the translocation of iodine in algae.

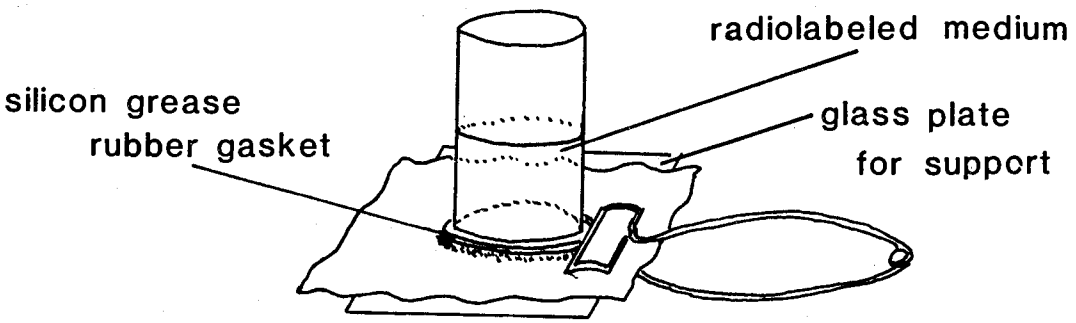
II. Material and Methods

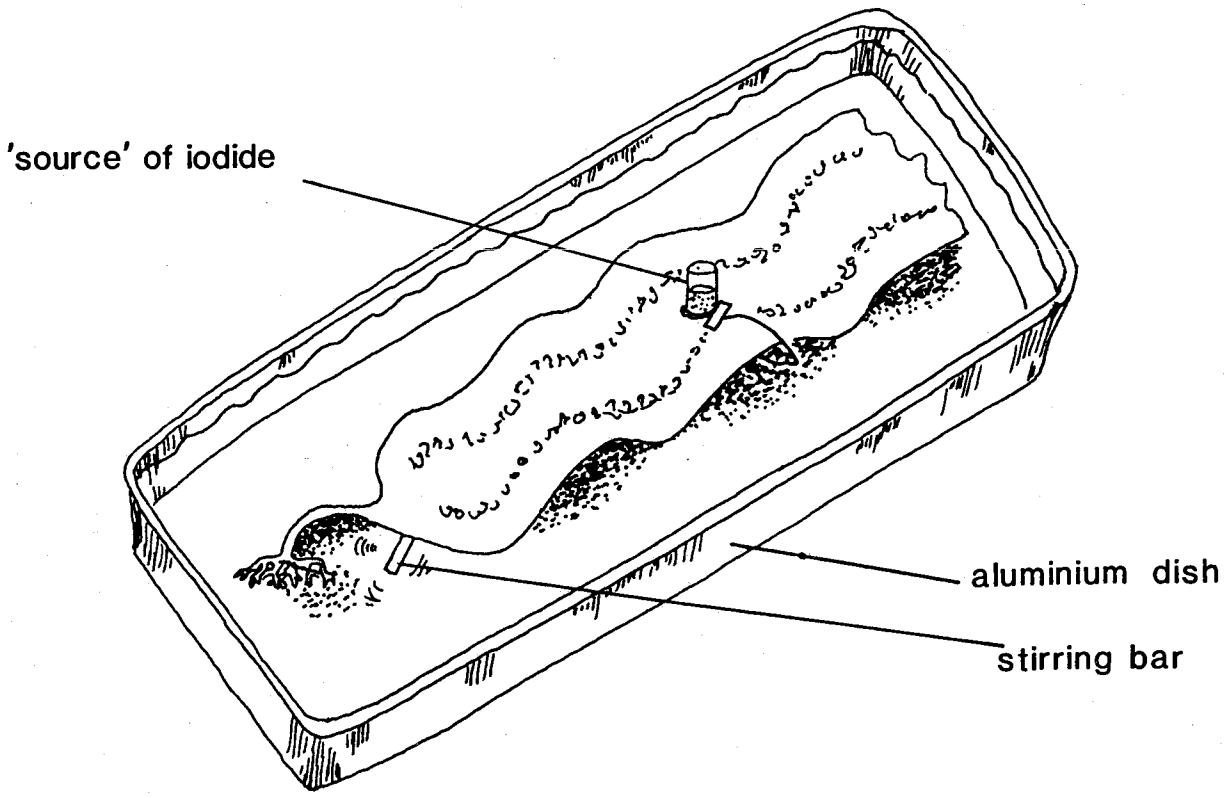
1. Feeding with ^{125}I

Plants were cleaned with tissue paper to remove any visible epiphytes. A glass cylinder (6 cm high, 3.3 cm i.d.) was fixed to the blade at about 30 cm above the stipe-blade junction with the help of a test tube clamp, a rubber gasket and silicon grease (Fig F1). The use of instant glues was discarded as they caused the formation of a ring of discoloured cells under the cylinder after about 1 h. Ten ml of seawater with 1 μM NaI and sufficient $Na^{125}I$ to give levels of activity of 35 to 110 KBq ml^{-1} were introduced in the glass cylinder. The seawater in the cylinder was bubbled intermittently to mix the medium. Attachment of the cylinder to the blade tissue was tested for leaks before any introduction of radioactive iodide. In addition, after the feeding, seawater in the bathing dish was tested for radioactivity.

Plants were immersed horizontally in a dish containing 6 L of filtered seawater and kept at 15°C. The seawater was kept in motion with two magnetic stirrers. The plant was illuminated from above by a flood lamp yielding 46.0 W m^{-2}.
In most cases an incubation of 6 h was followed by a chase period of 12 h, the radioactive medium having been replaced after thorough rinsing by 20 ml of 1 μM NaI seawater. In other experiments varying feeding times with or without chase periods were used. These are indicated at appropriate places.

Figure F1 Incubation chamber for translocation experiments
A glass cylinder was fixed onto the blade with a test tube clamp, a rubber gasket and silicone grease.


radiolabeled medium
silicon grease
rubber gasket
glass plate
for support


'source' of iodide
aluminium dish
stirring bar

2. Detection of transport

To determine the transport of ^{125}I, following feeding, 1 cm tissue discs were punched in different parts of the blade and γ-counted. In some experiments, the entire blade was rinsed off, dried, wrapped in an acetate sheet and left in contact with X-ray film (Kodak XAR-5) and fluor screen for approximately one week at 4°C for autoradiography.

To compare the transport of ^{14}C-labelled photoassimilate and ^{125}I, some experiments were run with a source of $NaH^{14}CO_3$ applied in parallel with one of $Na^{125}I$ on the same blade. Discs punched for ^{14}C were extracted in 80 % boiling ethanol and an aliquot of the extract was counted in a scintillation counter (Beckman LS 8000). Discs for ^{125}I were counted directly.

In order to investigate the form in which ^{125}I is translocated, radioactive discs were extracted in hot 80 % ethanol. The residue was then hydrolysed and used for TLC as described in the preceding chapter. The chromatographic plates were either scraped and γ-counted or used for autoradiography.

III. Results

1. Directionality of ^{125}I transport

Experiments were done to determine the directionality of ^{125}I transport. Figure F2 shows typical results of an experiment with the radioactive source placed on the mature part of a blade. Radioactivity was detected only in a basipetal direction from the source, that is, toward the meristem at the stipe-blade junction, and the meristem showed an accumulation of activity. No activity was detected distally or laterally to the source. The lower part of the stipe was not radioactive but the holdfast was slightly radioactive .

In other experiments the source was placed at different sites on the plant. When the source was placed just above the meristem, no radioactivity was detected above it or at any other site in the blade. Low levels of activity were recorded in the stipe and holdfast as shown in Fig F3. When the source was positioned at the holdfast, by immersing the holdfast in a vial containing the $Na^{125}I$ solution while the blade was held in seawater in a dish as before, no radioactivity was detected distally to the source (data not shown).

Figure F2 Translocation of labelled iodide in L. saccharina
The source was placed about 20 cm away from the meristem. Feeding time of 6 h was followed by 12 h chase. Circles represent discs punched out at the end of the experiment, and gamma counted. Numbers represent cpm, which are an average of 2 adjacent discs between the source and the beginning of the stipe. Other numbers represent cpm in areas defined on the diagram. A minus sign indicates that no cpms were recorded above background.

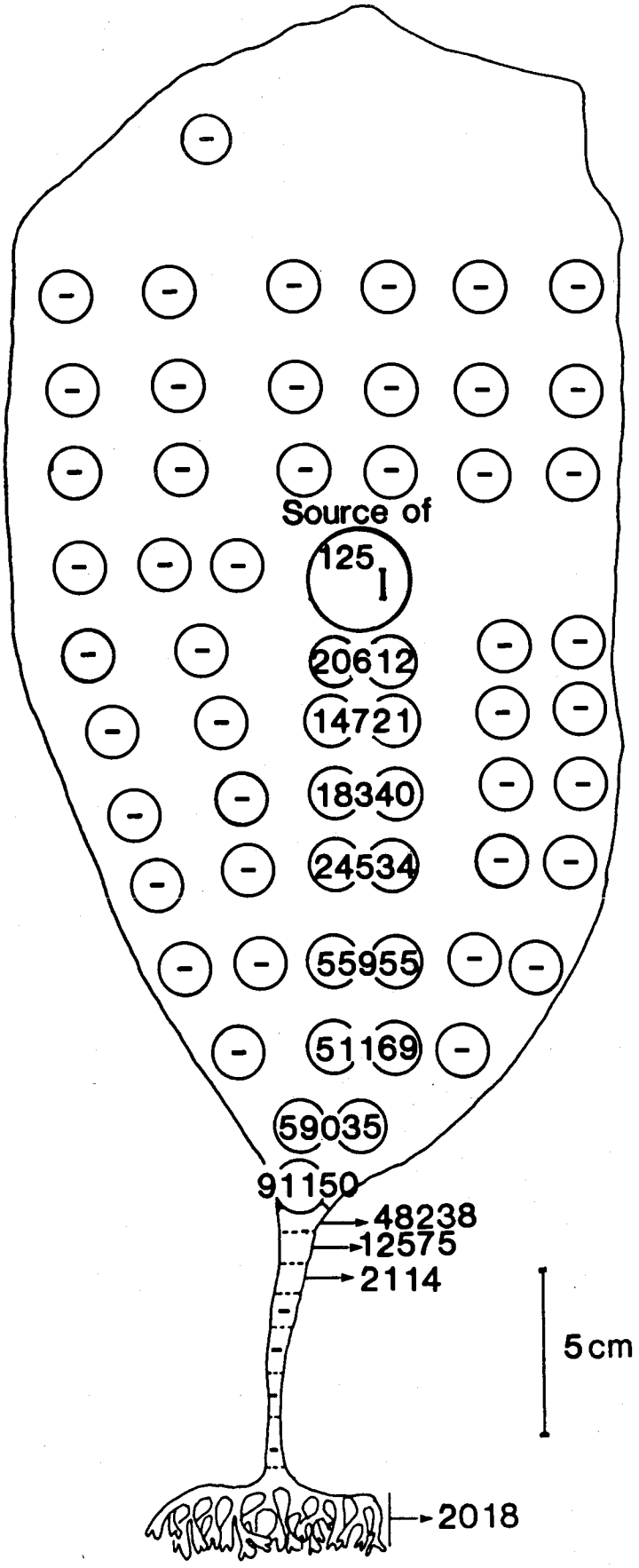
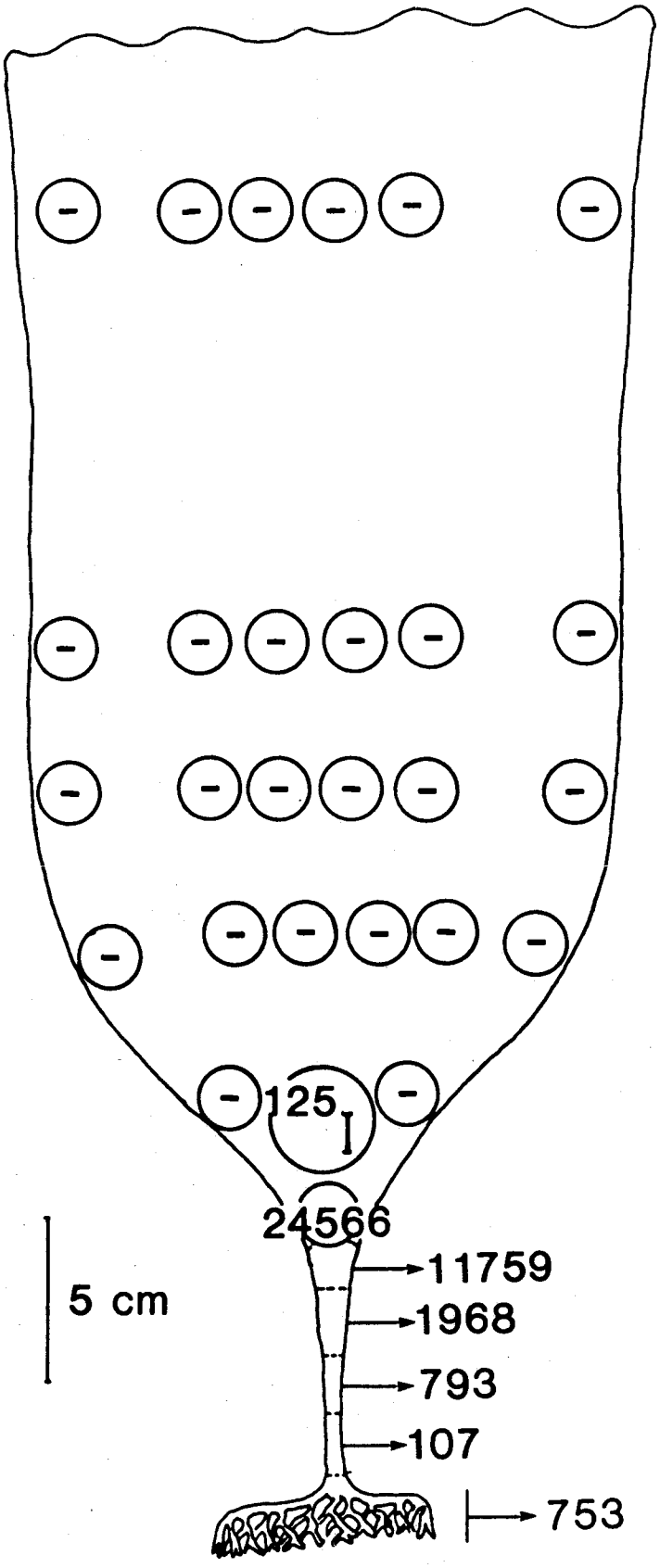

Source of
125 I
20612
14721
18340
24534
55955
51169
59035
91150
48238
12575
2114
5 cm
2018

Figure F3 Translocation of labelled iodide when source is placed at the meristem
Numbers represent cpmp for areas that were gamma counted.
For other details see legend to Fig F2.


125 I
24566
11759
1968
793
107
753
5 cm

2. Velocity of transport

In various experiments where the source of $Na^{125}I$ was placed 20 to 30 cm distally to the meristem a transport velocity of 2 to 3.5 cm h^{-1} was indicated. A summary diagram shown in Fig F4 summarizes several experiments undertaken on different plants in April 1984, where feeding times varied from 3 to 24 h. There was some variation in transport velocity but in all plants transport was toward the meristem and in the 24 h feeding time considerable radioactivity had accumulated in the meristematic area.

The transport velocity of $^{14}CO_3$ was compared to that of ^{125}I by placing two sources, one containing $NaH^{14}CO_3$ and the other $Na^{125}I$ at identical distances from the meristem on the same blade. The results of one such experiment are shown in Fig F5. During the 6 h feeding period, ^{125}I had migrated basipetally only 11 cm from the source, whereas the ^{14}C had migrated all the way to the meristem, 31 cm away from the source. In both cases no radioactivity was detected in discs punched distally to the source.

3. Pathway of transport

From the above experiments, it was clear that transport was from the source towards the meristem. As shown in Fig F6 when the source was applied near the margins of the blade, the pathway of ^{125}I was basipetal and mainly parallel to the edge of the blade

Figure F4 Profiles of labelled iodide along the thallus
Incubation times spanned from 3 to 24 h. One curve represents the activity measured along the thallus of one plant at one given time of incubation.

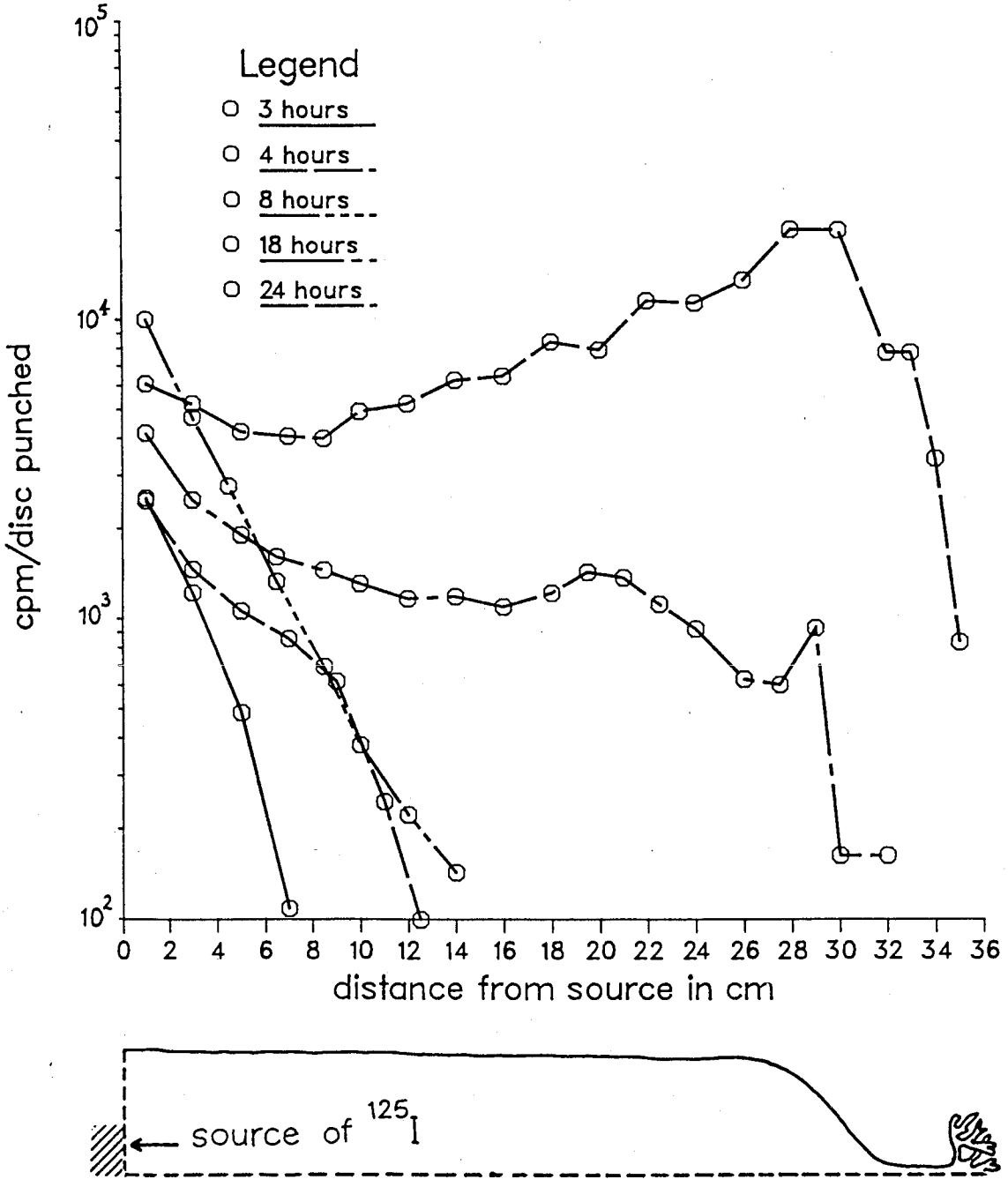

Legend
3 hours
4 hours
8 hours
18 hours
24 hours
cpm/disc punched
distance from source in cm
source of 125I

Figure F5 Translocation of labelled iodide and carbon
Both sources were placed at an equal distance from the meristem. No chase period was given following the 6 h incubation period. Numbers represent cpm of discs counted for ^{14}C and ^{125}I in a scintillation and gamma counter, respectively.

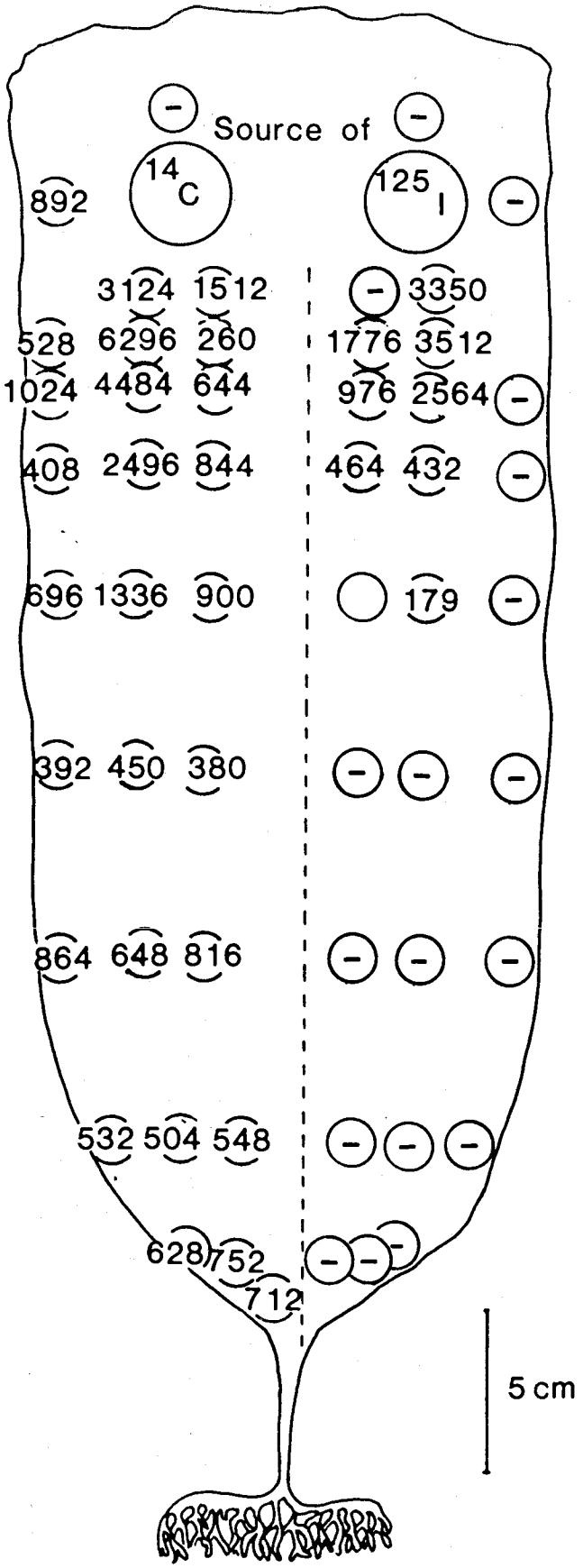
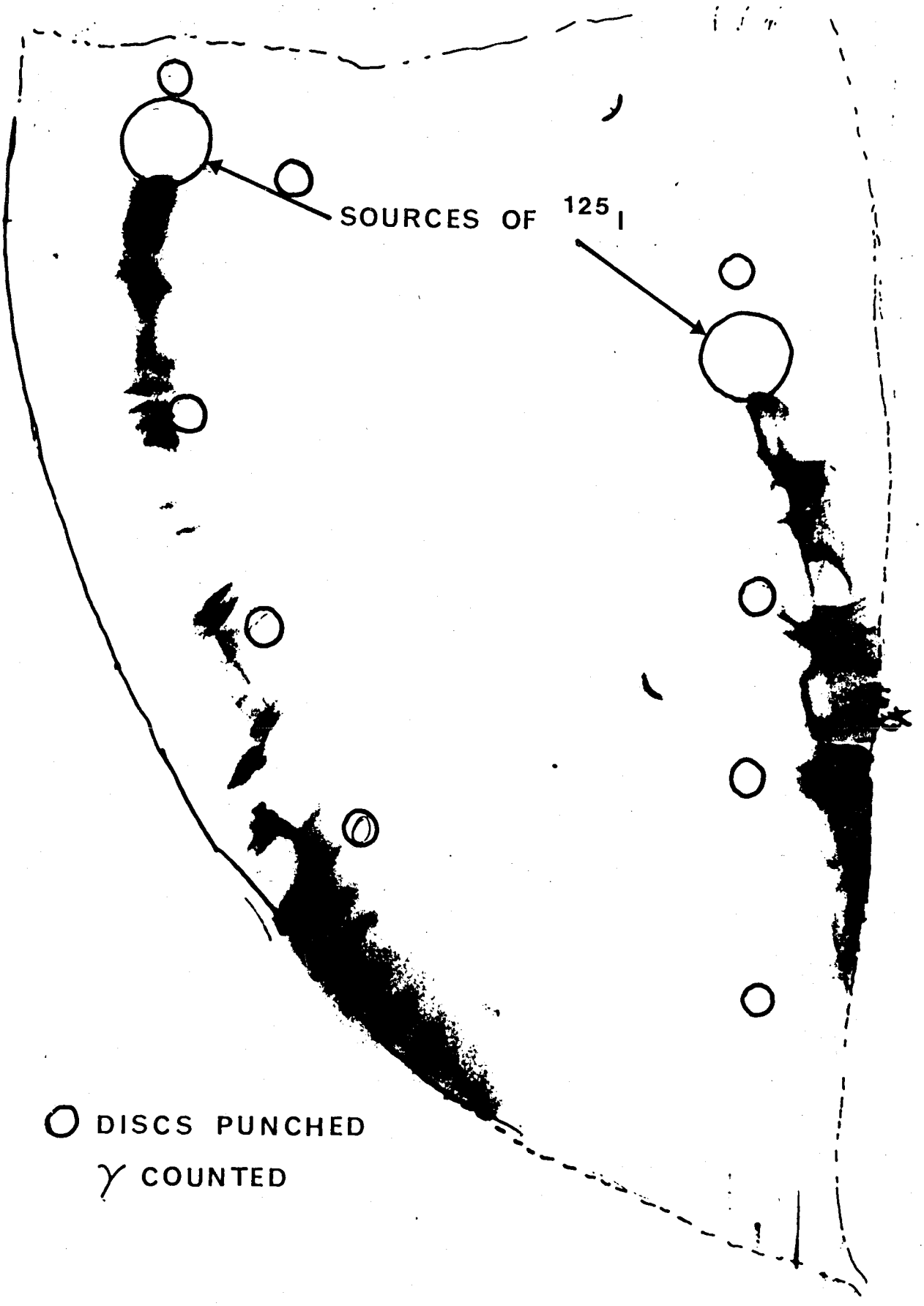

Source of
14C
125I
892
3124 1512
3350
528 6296 260
1776 3512
1024 4484 644
976 2564
408 2496 844
464 432
696 1336 900
179
392 450 380
864 648 816
532 504 548
628 752
712
5 cm

Figure F6 Autoradiograph of a L. saccharina blade with 2 labelled iodide sources
An 18 h feeding time was given. An outline of the blade as also of the 2 sources has been sketched in. Discs were punched where indicated and gamma counted.


SOURCES OF ^{125}I
O DISCS PUNCHED
γ COUNTED

with very little lateral diffusion.

4. Translocation in a plant at a reproductive stage

It was of interest to see whether a plant would transport iodine towards an area with a sorus in formation. This would indicate whether there was a need for iodine for the formation of the spores.

A plant was chosen for its healthy thallus with a sorus in formation at a good distance from the meristem. The chamber was placed 16 cm away from the meristem and about 5-10 cm from the sorus which was distal to the source. The radioactive pathway was well marked towards the meristem, with an accumulation of counts there. Towards the sorus, counts for 3 discs together recorded only 25-30 cpm above background, about the same as found laterally to the source at 2 cm. Nevertheless these counts are higher than those found at the margin of the blade (background) thus indicating perhaps a very slight influx of iodine towards the sorus. This shows the possibility for iodine to go against the normal distal to proximal direction.

5. Form in which ^{125}I is translocated

Tissue discs from the translocation pathway were pooled and extracted with hot 80 % ethanol. Ethanolic extracts and the NaOH hydrolysates of the residue were analyzed by TLC and only showed the presence of iodide as ^{125}I, an estimated 95% of which had

leached out into the ethanol extraction. No monoiodotyrosine or diiodotyrosine were detected in either of the two extracts.

IV. Discussion

Labelled iodide has been shown to be translocated in the blade of L. saccharina when applied at various sites on the plant. Diffusion, as a mechanism of transport, is unlikely as velocities of transport as high as 3.5 cm h^{-1} were recorded. Also, whatever the area of blade fed, the transport of iodide was well defined, unidirectional and basipetal to the young meristematic areas at the junction of blade and stipe, and to the holdfast with young growing haptera. These areas are the "sink" regions in the plants where the photoassimilate is also translocated (Schmitz and Lobban 1976, Floc'h 1982). No radioactivity was recorded above or laterally to the source whereas a diffusion would show activity in those sites. Finally, the distribution of cold iodine in the thallus, as determined by NAA (former Chapter, Fig E1,E2), shows much higher levels of iodine in the meristem, the stipe and holdfast than in the mature parts of the blade on a per g dry weight basis. This distribution could well be the result of translocation of iodide from mature to younger growing parts (for phosphorus, see Floc'h 1982) but such a distribution of iodine in the thallus argues against diffusion as the mechanism of translocation of ^{125}I.

The velocities of ^{125}I transport recorded here are similar to those previously reported by Floc'h (1982) for the transport of ^{32}P and ^{86}Rb, 2.6 and 2.8 cm h^{-1}, respectively, in Laminaria digitata. As for the transport of ^{14}C-labelled photoassimilate,

velocities in the range of 3 to 10 cm h^{-1} have been reported for Laminaria (Steinbiss and Schmitz 1972, Schmitz 1982) which compares favourably with our data showing that ^{14}C had reached the meristem 31 cm away after 6 h feeding. Notably, however ^{125}I fed at the same time migrated only 11 cm from its source (Fig F5). This difference in transport velocity of ^{125}I and ^{14}C (the same might apply to ^{32}P and ^{86}Rb as well) may be caused by a differential loading rate of photoassimilates on the one hand and ions and phosphorylated compounds on the other into the sieve elements.

The directionality of transport and the narrowness of the pathway between the pulse site and the base of the blade strongly suggests that iodide is transported via the sieve elements. The sieve elements run longitudinally along the blade in a parallel fashion, converging towards the meristem and stipe (for a schematic diagram in Nereocystis blades, see Nicholson 1976). The autoradiogram in Fig F6 where the iodide source was located marginally on the blade, closely approximates this arrangement of the sieve elements.

The only form of labelled iodide found along the transport pathway was that of the anion I^-. This, together with the fact that Parker (1966) and Manley (1983) both found levels of I^- in sieve tube sap of Macrocystis of 0.21 and 0.5 mg ml^{-1}, respectively, seems to indicate that this is the form under which iodine is transported in the Laminariales.

In summary, iodide is readily translocated to the meristematic areas of the plant where it accumulates. This is necessary to account for the high levels of iodine found in these areas because, as I showed earlier, the meristem and stipe take up iodine at a much lower rate than the blade and holdfast (Chapter on Uptake of ^{125}I). The holdfast can accumulate iodine by its own action as its uptake capacity is high but we have shown that translocation cannot be acropetal--iodine cannot migrate from the holdfast to the meristem--. It is thus necessary for the blade tissue to export iodine which then accumulates to the levels found in the meristem and stipe (see Chapter on Iodine in Laminaria tissue).

PART G

Conclusion

This study has contributed to our understanding of the effects of iodine on the biology of kelp. It was known that iodine was indispensable in culture media for gametophytic stages of brown algae without which the thalli failed to reproduce or grow. We have shown that without the addition of 10 or 100μM KI the young sporophytes of L. saccharina grow very poorly in vitro. They exhibit a bleaching of their thalli with a consequent sloughing off of tissue and this occured both times this experiment was carried out. An addition of KI to the medium resulted in an increased pigment level, increased dry weight and growth of the sporophytes. These plants showed an acquisition of iodine per g d.wt. which, when studied on the mature plants with ^{125}I is translated by a rapid uptake of I^-. All tissues of Laminaria can take up iodide, particularly the blade tissue at rates of 0.39 to 3.9 nmol $cm^{-2}h^{-1}$.

Discs, depleted of photoassimilates, take up less iodide and discs in anaerobic conditions and in the dark stop all uptake of I^-. This suggests that the uptake is an active process requiring ATP. Most of the iodide taken up by the tissue is easily extractable by water or better still by EtOH. This strongly suggests that iodide (or another chemical form of iodine) is stored in the vacuoles from where it can be extracted for use or release. The remainder of the iodide taken up could be bound, possibly to some protein as two iodinated amino acids were found after hydrolysis: MIT and DIT. This binding is quite labile as continued EtOH extractions continue to extract I^- and because in

freeze dried tissue no iodinated amino acids were found. The presence of these compounds, in very small quantities of less than 0.2% the iodine content, might suggest a destiny for the iodide taken up. Are these amino acids iodinated for a purpose?. Maybe these compounds are ultimately used as a growth promoting substance which explains why they exist in such small quantity; this is reminiscent of what occurs in the human thyroid gland where tri-iodothyronine is a precursor to the hormone thyroxine. It is also possible, as I have shown through the hydrolysis of a control containing no algae, that the presence of iodinated organic compounds are due to the iodination of tyrosine in the chemical procedure. If this is the case then most, if not all, the iodine in the algae is under I^- form. It has been shown that kelp can release molecular iodine when the tissue is under stress, such as when it is heated (Shaw, 1962). Dangeard (1957) explains this phenomenon as a means for the kelp to rid itself of excess iodine. I would rather suggest that this release is not incidental. Other studies have shown that algae can release some iodinated compounds, some of which have some antigrazing properties. Also, is molecular iodine not a recognized antiseptic?. I found iodide to be translocated from the blade to the meristem, and secondarily to the holdfast, where it is accumulated. The meristem is also the fastest growing area of the plant so it is tempting to combine the two suggestions that iodine has a growth promoting role and acts as an antifouling agent.

PART H

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PART I

Appendix

Appendix I.

Raw data for the growth of plants in the field. All numbers are in cm. The various columns represent: 1-Plant number. 2-Plant series (i.e. plant #2 of series 2 is the same throughout the series 2). 3-Length to hole- this represents the growth from the time when the hole was punched until the measurement was made. 4-Width at the old hole (O.H.). This does not occur if a new series of plants has been set up. 5-Width at new hole (N.H.), i.e., the width of the blade at the hole presently being punched. 6-Total length of blade. Missing data indicate loss of plants.

Set of plants 9 Aug. 1983

1	2		13	100
2	2		13	80
3	2		11	55
4	2		16	50
5	2		25	120
6	2		15	60
7	2		22	110
8	2		15	70
9	2		18	65
10	2		18	100
11	2		15	80
12	2		12	140
13	2		20	130
14	2		13	120
15	2		30	90
16	2		13	50

Mean length: 88.7
S.E.: 7.3
Mean width NH: 16.8
S.E.: 1.3

growth data from 9 August-7 Sept. 1983

1	2	40	18	17	110
2	2	92	18	15	130
3	2	75	19	15	130
4	2	86	20	20	130
5	2	40	28	32	83
6	2	40	18	18	70
7	2	47	35	20	90
8	2	45	25	25	80
9	2	30	28	20	120
10	2	86	30	20	140
11	2	71	21	22	140
12	2	52	16	16	160
13	2	62	26	31	140
14	2	59	17	25	110
15	2	80	27	42	80
16	2	90	20	20	90

Mean length: 112.6
S.E.: 27.4
Mean growth: 62.1 = 2.14/day
S.E.: 5.2
Mean width NH: 22.3
S.E.: 1.8

Set of plants 4 Oct. 1983

1	3		22	70
2	3		15	25
3	3		15	90
4	3		19	150
5	3		10	120
6	3		11	110
7	3		25	110
8	3		15	55
9	3		14	180
10	3		20	100
11	3		12	110
12	3		20	60
13	3		16	150
14	3		14	100
15	3		12	120
16	3		17	90
17	3		25	80
18	3		13	25
19	3		10	60
20	3		10	160
21	3		14	110
22	3		20	200
23	3		11	30

Mean length: 100.2
S.E.: 9.8
Mean width NH: 15.6
S.E.: 0.9

Set of plants 17 Oct. 1983

24	3		10	65
25	3		12	50
26	3		12	50
27	3		13	70
28	3		8	10
29	3		13	100
30	3		19	80

Mean length: 60.7
S.E.: 10.7
Mean width NH: 12.4
S.E.: 1.3

Growth from 4-17 Oct. to 10 Nov. 1983

1	3	20	20	9	70	
2	3	20	26	13	35	
3	3	12	13	12	85	
4	3	14	20	18	120	
5	3	12	10	10	125	
6	3	12	11	10	98	
7	3	22	16	11	130	
8	3	13	15	15	60	
9	3	25	15	12	170	
10	3	20	21	20	110	
11	3	0			68	
12	3	51	22	11	70	
13	3	15	18	16	190	
14	3	15	15	12	100	
15	3	13	13	12	110	
16	3	20	18	13	100	
17	3	32	19		80	
18	3	14	15	14	30	
19	3	61	11	11	100	
20	3	15	11	10	130	
21	3	27	16	12	120	
22	3	32	25	16	140	
23	3	30	12	10	45	
24	3	16	11	10	55	
25	3	11	11	11	45	
26	3	38	14	11	72	
27	3	12	11	11	70	
28	3	11	8	10	19	
29	3	21	15	13	95	
30	3	11	19	19	85	
31	3			13	130	=newly tagged
32	3			11	90	" plants
33	3			10	110	"
34	3			14	100	"
35	3			15	90	"

Mean length: 92.8
S.E.: 6.3
Mean growth: 20.5
S.E.: 2.3
Mean width NH: 12.5
S.E.: 0.5
Mean width OH: 16.4
S.E.: 0.2

Growth from 10 Nov. to 4 Dec. 1983

1	3					
2	3					
3	3					
4	3	0	19	19	90	
5	3	.5	10	10	90	
6	3	.5	11	10	50	
7	3	0	11	11	90	
8	3	0	14	14	21	
9	3	0	11	11	110	
10	3	1	16	16	60	
11	3	0	11	11	41	
12	3	0	11	11	70	
13	3	0	16	16	115	
14	3	0	12	12	55	
15	3					
16	3	0	12	12	110	
17	3	0	26	26	13	
18	3	1	13	13	30	
19	3	6	12	10	88	
20	3	0	11	11	60	
21	3	1	12	11	15	
22	3					
23	3					
24	3	2	10	10	55	
25	3					
26	3					
27	3					
28	3					
29	3					
30	3	0	19	19	70	
31	3	0	14	14	85	
32	3	.5	12	12	80	
33	3	0	10	10	22	
34	3	1	13	12	80	
35	3	0	15	15	110	

Mean length: 67.0
S.E.: 6.4
Mean growth: .56
S.E.: .25
Mean width NH: 13.1
S.E.: .8
Mean width OH: 13.3
S.E.: .7

Set of plants 18 April 1984

1	4	19	55	
2	4	12	55	
3	4	11	67	
4	4	14	80	Mean length:65.0
5	4	17	55	S.E.: 1.9
6	4	14	61	Mean width NH:13.6
7	4	13	83	S.E.: 0.5
8	4	15	65	
9	4	15	73	
10	4	16	61	
11	4	11	65	
12	4	11	52	
13	4	13	52	
14	4	13	51	
15	4	13	70	
16	4	15	78	
17	4	11	80	
18	4	12	78	
19	4	7	60	
20	4	15	66	
21	4	15	70	
22	4	16	53	
23	4	15	56	
24	4	10	63	
25	4	15	69	
26	4	17	72	

Growth from 18 April to 4 May, 1984

1	4	25	19	80	
2	4	20	16	80	
3	4	19	17	110	
4	4	18	15	35	Mean length:88.6
5	4	25	27	100	S.E.: 3.1
6	4	27	24	50	Mean width NH:16.0
7	4	25	18	110	S.E.: 0.5
8	4	28	20	55	Mean width OH: 0.7
9	4	27	19	95	
10	4	29	22	110	
11	4	23	15	90	
12	4	20	15	90	
13	4	19	14	90	
14	4	20	14	95	
15	4	19	14	70	
16	4	25	16	90	
17	4	21	12	115	
18	4	22	13	115	
19	4	18	10	90	
20	4	24	15	100	
21	4	27	17	105	
22	4	22	17	80	
23	4	21	14	70	
24	4	17	12	100	
25	4	26	14	110	
26	4	25	16	60	
27	4		17	90=newly tagged	
28	4		14	100" plants	
29	4		15	100"	
30	4		16	70"	
31	4		16	110"	
32	4		15	95"	
33	4		13	75"	
34	4		16	75"	
35	4		15	100"	
36	4		16	67"	
37	4		16	110"	
38	4		14	80"	

Growth from 4 May to 16 May, 1984

1	4	18	28	22	95
2	4	24	25	16	75
3	4	33	28	14	120
4	4	18	25	15	60
5	4	30	36	20	120
6	4	28	31	25	80
7	4	24	29	16	140
8	4	30	31	17	90
9	4	29	30	16	120
10	4	30	39	18	140
11	4			17	27
12	4	23	24	13	105
13	4	21	22	13	105
14	4	25	25	15	110
15	4	10	19	17	68
16	4	26	26	14	115
17	4			12	20
18	4	28	22	12	140
19	4	23	18	12	105
20	4	21	24	14	110
21	4			20	50
22	4	23	27	19	105
23	4	11	16	14	65
24	4	20	16	12	110
25	4	20	27	17	125
26	4	19	21	19	80
27	4	32	37	18	130
28	4	31	28	12	130
29	4	25	26	16	120
30	4	23	28	16	100
31	4	26	30	17	130
32	4	25	33	14	125
33	4	28	24	15	105
34	4	18	24	16	88
35	4	15	23	14	72
36	4	20	26	20	75
37	4	25	30	16	130
38	4	33	31	14	113

Mean length:99.9
S.E.: 4.8
Mean growth:23.8 = 1.98/day
S.E.: 0.9
Mean width NH:15.9
S.E.: 0.6
Mean width OH:26.5
S.E.: 0.9

Growth from 16 May to 12 June 1984

1	4				
2	4	53	28	18	146
3	4			16	62
4	4	59		15	115
5	4			19	25
6	4			18	90
7	4	50	29	19	95
8	4			20	50
9	4	50	26	18	90
10	4	55		20	95
11	4	38	20	16	70
12	4	26	18	14	45
13	4	34		13	104
14	4			12	35
15	4				
16	4	43		12	70
17	4			9	25
18	4	42		11	127
29	4	46		11	76
20	4				38
21	4				
22	4	38		19	85
23	4	25	17	13	58
24	4			10	30
25	4	18	25	21	116
26	4	53		19	90
27	4			21	35
28	4			16	25
29	4	55		20	98
30	4	45		18	130
31	4	41		15	70
32	4			14	50
33	4			13	45
34	4				
35	4	20		11	50
36	4	18			43
37	4			22	106
38	4	60		14	90
39	4			12	75
40	4			17	45
41	4			11	41

Mean length:71.3
S.E.: 5.4
Mean growth:41.4 = 1.53/day
S.E.: 2.9
Mean width NH:15.6
S.E.: 0.6
Mean width OH:23.3
S.E.: 1.8

Growth from 12 June to 12 July, 1984

1	4					
2	4	30		16	61	
3	4			13	22	
4	4	69		13	140	Mean length:58.2
5	4					S.E.: 7.0
6	4	45		23	79	Mean growth:38.0 = 1.26/day
7	4	55		14	152	S.E.: 4.5
8	4					Mean width NH:15.6
9	4					S.E.: 1.8
10	4			15	20	Mean width OH:22.0
11	4			15	46	(only 2 values)
12	4			15	41	
13	4					
14	4	29		14	52	
15	4					
16	4	39		11	84	
17	4					
18	4	55		13	97	
19	4			9	35	
20	4	9		9	29	
21	4					
22	4	44	22	16	94	
23	4	46		11	66	
24	4			12	29	
25	4					
26	4			18	23	
27	4			15	40	
28	4	11			27	
29	4					
30	4	44	22	16	94	
31	4					
32	4					
33	4			13	40	
34	4					
35	4					
36	4					
37	4	19		14	40	
38	4	21		16	59	
39	4	55		55	86	
40	4					
41	4			10	44	

Plants tagged 10 Aug. measured 25 Aug. 1984

1	5	17		19	32	
2	5	12		20	30	
3	5	22		20	45	
4	5	5		14	18	Mean length:94.9
5	5	38	19	21	75	S.E.: 7.2
6	5	26	15	21	52	Mean growth:36.1 = 2.40/day
7	5	42	20	14	120	S.E.: 2.2
8	5	39	16	19	74	Mean width NH:18.2
9	5	35	29	32	90	S.E.: 0.9
10	5	53	25	15	124	Mean width OH:27.6
11	5	48	26	15	117	S.E.: 1.1
12	5	5		9	41	
13	5	32	29	27	113	
14	5	52	33	13	168	
15	5	45	28	15	98	
16	5	38	27	20	109	
17	5	48	22	12	99	
18	5	38	27	22	111	
19	5	35	34	21	137	
20	5	44	22	13	118	
21	5	27	28	16	50	
22	5	50	34	16	185	
23	5	42	38	14	80	
24	5	45	31	22	128	
25	5			17	33	
26	5	43	31	15	130	
27	5	43	28	21	103	
28	5	45	30	18	122	
29	5			19	60	
30	5			16	66	
31	5	21	40	38	104	
32	5	34	28	9	194	
33	5	32	20	15	80	
34	5	49	26	17	115	
35	5	43	40	31	127	
36	5	36	22	17	102	
37	5	38	28	22	79	
38	5	7	8	15	22	
39	5	59	33	8	180	
40	5	49	28	22	96	

Growth from 25 Aug. to 23 Sept. 1984

1	5	68	25	37	85	
2	5	75	23	30	95	
3	5	54	30	52	90	
4	5	25		22	40	
5	5	74	30	19	160	
6	5	63	29	28	103	
7	5	48	28	16	116	
8	5	90	31	19	157	
9	5	57	41	59	140	
10	5	75	28	16	173	
11	5	90	31	20	195	
12	5	23		19	43	
13	5			21	43	
14	5	93	37	18	244	
15	5			13	82	
16	5			25	56	
17	5	100	32	15	190	
18	5	40	32	22	136	
19	5	44	31	20	170	
20	5	79	27	13	175	
21	5			19	53	
22	5	95	33	19	186	
23	5	79	36	16	165	
24	5	22	33	30	136	
25	5	23			60	
26	5					
27	5					
28	5	75	33	22	170	
29	5	78	37	20	139	
30	5	65	30	13	136	
31	5			12	21	
32	5			18	41	
33	5			17	95	
34	5	67	49	36	178	
35	5	56	32	17	148	
36	5	38	26	15	144	
37	5	68	43	28	129	
38	5					
39	5					
40	5					

Mean length: 122.6
S.E.: 9.3
Mean growth: 63.0 = 2.17/day
S.E.: 4.3
Mean width NH: 22.6
S.E.: 1.8
Mean width OH: 32.3
S.E.: 1.2

Growth from 23 Sept. to 24 Oct. 1984

1	5	0	14	14	25	
2	5	0	21	21	105	
3	5					
4	5	.5	26	26	50	
5	5	.5	16	16	130	
6	5	1	23	23	84	
7	5	0	16	16	94	
8	5	0	19	19	150	
9	5					
10	5	0	15	15	160	
11	5	.5	17	17	80	
12	5					
13	5	0	17	17	50	
14	5					
15	5	0	13	13	55	
16	5					
17	5	0	15	15	44	
18	5	0	21	21	97	
19	5	.25	19	19	115	
20	5					
21	5					
22	5					
23	5	.25	17	17	113	
24	5	0	20	20	28	
25	5					
26	5					
27	5					
28	5					
29	5	0	18	18	20	
30	5	.5	12	12	85	
31	5	0	20	20	14	
32	5					
33	5	0	17	17	75	
34	5	0	13	13	30	
35	5	0	14	14	68	
36	5	.5	13	13	38	
37	5	.5	38	38	100	
38	5					
39	5					
40	5					

Mean length: 75.4
S.E.: 8.4
Mean growth: 0.18 = 0.006/day
S.E.: 0.05
Mean width at both holes: 18.0
st. error: 1.1

Growth from 24 Oct. to 19 Nov. 1984

1	5	0	14	14	25	
2	5	0	21	21	105	
3	5					
4	5	.5	26	26	50	
5	5	.5	16	16	130	
6	5	1	23	23	84	
7	5	0	16	16	94	
8	5	0	19	19	150	
9	5					
10	5	0	15	15	160	
11	5	.5	17	17	80	
12	5					
13	5	0	17	17	50	
14	5					
15	5	0	13	13	55	
16	5					
17	5	0	15	15	44	
18	5	0	22	22	97	
19	5	.25	19	19	115	
20	5					
21	5					
22	5					
23	5	.25	17	17	113	
24	5	0	20	20	28	
25	5					
26	5					
27	5					
28	5					
29	5	0	18	18	20	
30	5	.5	12	12	85	
31	5	0	20	20	14	
32	5					
33	5	0	17	17	75	
34	5	0	13	13	30	
35	5	0	14	14	68	
36	5	.5	13	13	38	
37	5	.5	38	38	100	
38	5					
39	5					
40	5					

Mean length: 75.4
S.E.: 8.4
Mean growth: 0.18
S.E.: 0.05
Mean width at both holes: 18.3
S.E.: 1.1

Growth from 19 Nov. to 19 Dec. 1984

1	5	0			24	
2	5	0			43	
3	5					
4	5					
5	5	0	16	16	50	
6	5	rag				
7	5	0	16	16	60	
8	5	0	19	19	30	
9	5					
10	5	1.5	14	14	22	
11	5	0	16	16	60	
12	5					
13	5	rag	10	10	10	
14	5					
15	5	0			35	
16	5					
17	5	0	13	13	25	
18	5	rag				
19	5	0			18	
20	5					
21	5					
22	5					
23	5	.5			22	
24	5					
25	5					
26	5					
27	5					
28	5					
29	5					
30	5	0			15	
31	5	0			10	
32	5	rag			18	
33	5					
34	5	rag				
35	5	stub				
36	5	0			5	
37	5					
38	5					
39	5					
40	5					

Mean length: 27.7
S.E.: 4.1
Mean growth: 0.1
S.E.: 0.08

Set of plants 8 April, 1985

1	6	14	54
2	6	13	58
3	6	15	78
4	6	10	80
5	6	14	76
6	6	17	57
7	6	19	62
8	6	12	64
9	6	14	70
10	6	10	72
11	6	10	69
12	6	10	80
13	6	10	60
14	6	10	62
15	6	13	67
16	6	10	62
17	6	17	81
18	6	19	67
19	6	14	70
20	6	11	87
21	6	13	66
22	6	16	64
23	6	13	59
24	6	12	75
25	6	11	57
26	6	11	56
27	6	12	77
28	6	15	66
29	6	15	70
30	6	12	57
31	6	11	83
32	6	15	60
33	6	13	69
34	6	16	70
35	6	14	71
36	6	14	70
37	6	12	58
38	6	13	58
39	6	11	74
40	6	16	64

Mean length:67.5
S.E.: 1.3
Mean width NH:13.2
S.E.: 0.4

Growth from April 8 to April 25, 1985

1	6	33	24	20	84
2	6	41	24	18	95
3	6	51	30	34	130
4	6	43	16	10	102
5	6	50	27	19	127
6	6	37	27	23	95
7	6	43	31	24	100
8	6	46	22	13	108
9	6	45	24	11	113
10	6	38	19	15	108
11	6	38	17	12	80
12	6	31	22	14	114
13	6	18	15	12	51
14	6			16	52
15	6	33	20	17	90
16	6			13	56
17	6	30	27	22	77
18	6	19	27	21	53
19	6	39	25	17	115
20	6	43	23	23	125
21	6	35	22	17	70
22	6	43	31	21	92
23	6				
24	6	48	23	16	120
25	6	47	20	18	98
26	6	42	22	16	100
27	6	42	23	12	73
28	6	32	20	10	90
29	6	37	28	22	105
30	6	30	20	20	87
31	6			12	50
32	6			16	38
33	6				
34	6	27	22	17	81
35	6	32	24	15	93
36	6	37	24	15	97
37	6			14	54
38	6	44	23	14	103
39	6	22	17	11	68
40					

Mean length:89.0
S.E.: 3.9
Mean growth:37.3 = 2.2 cm/day
S.E.: 1.5
Mean width NH:16.7
S.E.: 0.8
Mean width OH:23.0
S.E.: 0.7

Growth from 25 April to 20 May, 1985

1	6					
2	6					
3	6	68	42	23	160	
4	6	48	17	12	105	Mean length:91.0
5	6	72	37	16	120	S.E.: 6.9
6	6	46	34	12	90	Mean growth:47.0 = 1.88 cm/day
7	6					S.E.: 3.7
8	6	60	27	15	130	Mean width NH:16.3
9	6					S.E.: 0.9
10	6	34	22	13	84	Mean width OH:27.4
11	6			11	65	S.E.: 1.8
12	6					
13	6					
14	6					
15	6					
16	6					
17	6					
18	6	26	23	23	60	
19	6					
20	6					
21	6					
22	6					
23	6					
24	6					
25	6	65	30	19	115	
26	6					
27	6	46	24	12	122	
28	6					
29	6					
30	6	37	25	19	83	
31	6					
32	6					
33	6					
34	6	33		23	57	
35	6	40	23	18	120	
36	6	39	25	14	70	
37	6			13	43	
38	6	44	28	15	90	
39	6					
40				19	73	
1	7			17	80	
2	7			17	62	

Growth form 20 May to 8 June, 1985

1	6					
2	6					
3	6					
4	6					Mean length:86.8
5	6	37		17	100	S.E.: 6.4
6	6	28	30	22	112	Mean growth:26.8 = 1.4 cm/day
7	6					S.E.: 2.0
8	6	35	25	14	140	Mean width NH:14.6
9	6					S.E.: 1.0
10	6	19	17	10	48	Mean width OH:23.8
11	6	17	14	12	70	S.E.: 1.8
12	6					
13	6					
14	6					
15	6					
16	6					
17	6					
18	6	20	31	24	76	
19	6	39	26	14	66	
20	6					
21	6					
22	6					
23	6					
24	6					
25	6	33		14	67	
26	6					
27	6	25	19	9	120	
28	6					
29	6					
30	6	22	25	15	95	
31	6					
32	6					
33	6					
34	6					
35	6	20	22	15	85	
36	6	16		14	67	
37	6					
38	6	22	20	13	95	
39				10	30	
40	6	34	33	17	100	
1	7					
2	7	36			62	

Appendix II 1-Cell size estimate for young sporophytes grown in 0, 1, 10 and 100 μM added KI.

The cell size was estimated by photomicrography (objective 25X, occular 10X) of a small part of the blade tissue, 4 mm from the stipe blade junction, at the end of the experiment.

Photomicrographs of identical areas of thallus were taken at X 250 and enlarged prints were made to count the numbers of meristodermic and outer cortical cells in a given area. For meristodermic cells, a print area 10 x 10 cm (=100 cm^2) was used. For the outer cortical cells, which are larger, a larger print 12 x 20 cm (240 cm^3) was used unless some parts of the picture were out of focus; this was standardized accordingly. Average cell size was determined by dividing the area by the cell number. The results of meristodermic and cortical cell counts for 4 replicates of each treatment are shown in Table 1. The meristodermic and cortical cell numbers both fluctuated greatly between replicates of a given treatment and therefore I could not conclude to any definite trends between treatments, although it seemed that the cell size of sporophytes did not increase with added KI in the medium.

Table 1 Cell size of young sporophytes grown in 0, 1, 10 and 100μM KI

A 2 mm disc was punched out at 4 mm from the blade-stipe junction of the young sporophytes at the end of 18 days experiment (run in 1985). Photomicrographs were taken at X 250 and enlarged prints made.

The cell size was estimated by dividing the number of cells for a given area: 100 cm^2 for the meristodermic cells and 240 cm^2 for the cortical cells.

Meristodermic cells

treatment	cell counts per unit area (4 replicates)	mean	S.E.	range
Time 0	61 88 90 100	84.7	8.3	76.4-93.0
Control	68 83 88 100	84.7	6.6	78.1-91.3
1 uM	96 81 72 63	78.0	7.0	71.0-81.0
10 uM	77 60 78 120	83.7	12.7	71.0-96.5
100 uM	73 78 88 70	77.2	3.9	73.3-81.1

Cortical cells

Treatment	cell counts per unit area (4 replicates)	mean	S.E.	range
Time 0	25 21 19 26	22.7	1.6	21.1-24.4
Control	39 55 42 52	47.0	3.8	43.2-50.9
1 uM	43 37 30 34	36.0	2.7	33.3-38.7
10 uM	51 32 38 76	49.5	9.7	39.8-59.3
100 uM	31 39 35 33	34.5	1.7	32.8-36.2

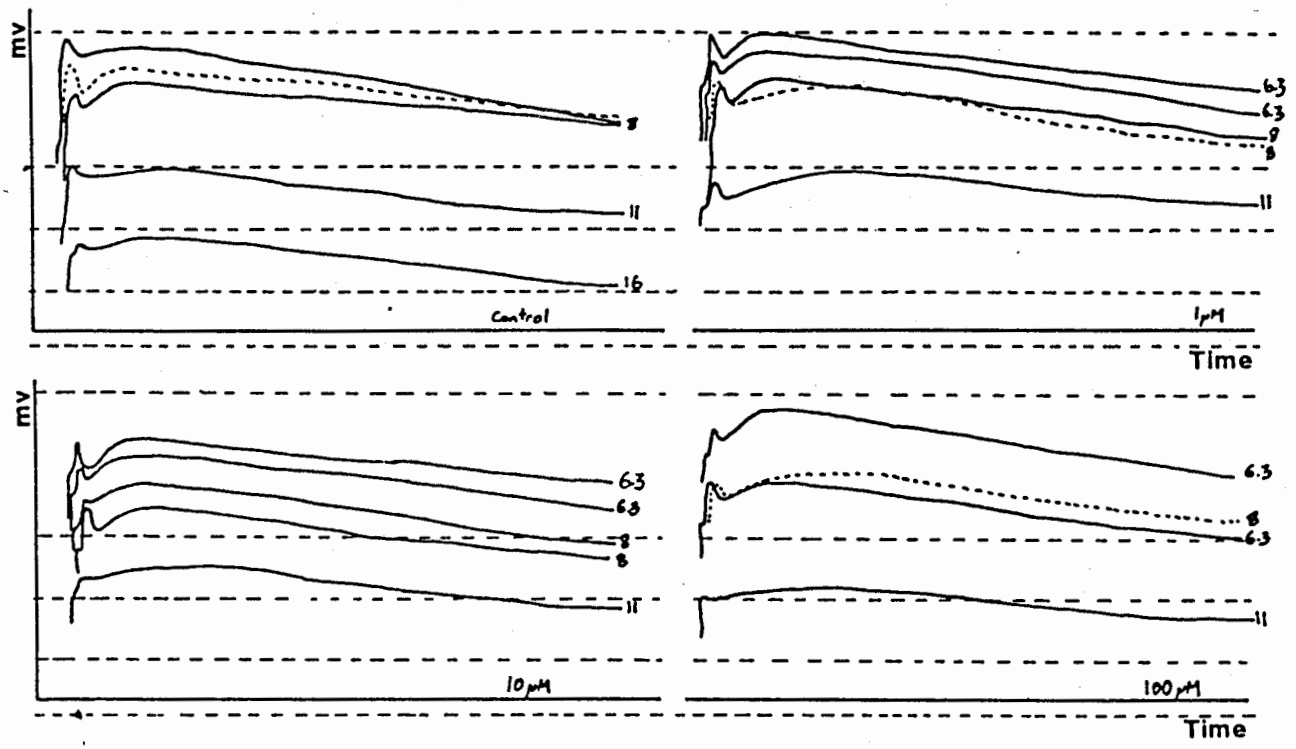
Appendix II continued. 2. Effect of added KI on chlorophyll fluorescence of young sporophytes grown *in vitro*

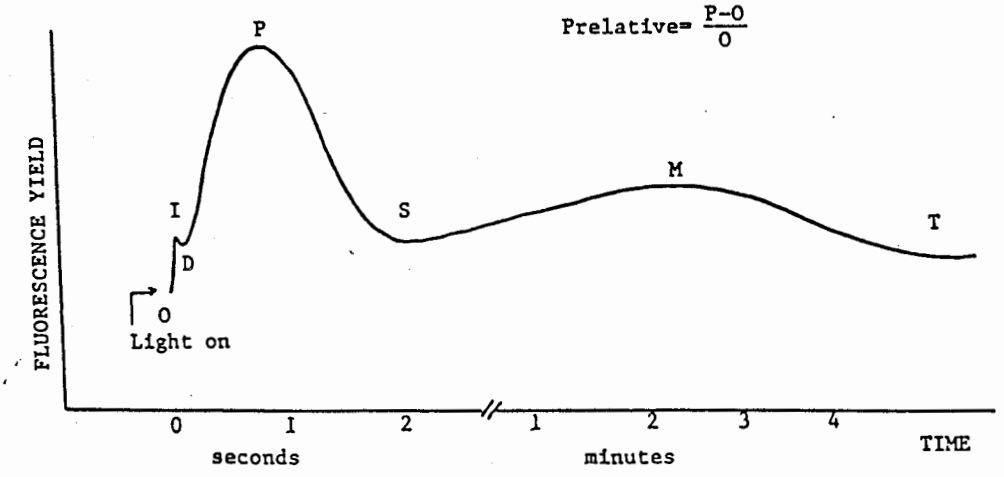
The effect of added KI on variable chlorophyll fluorescence was also examined. The plants or discs were dark adapted for a minimum of 1.5 h; then they were held in a vial of seawater while being exposed to varying intensities of blue light. The fluorescence emission (the Kautsky curve) was recorded on a plotter.

The following results apply only to the work done with the discs as many more replicates were used. The resulting Kautsky curves appear in Fig 1 and show differences in their shape especially at low light intensity. For instance, at the aperture 11 (low light) an increase in KI, notably at the 10 and 100μM concentrations, seems to reduce the height of M and also P (see model curve, Fig 1). However, on calculating the various curve coefficients (IP rise, P-relative, the height of M these observations were not confirmed and no trend was detected. Although there seems to be an increase in the pigment levels with an increase of KI in the medium as seen with the young sporophytes but not the blade discs, the fluorescence data suggest that there is no direct effect on the mechanism of photosynthesis itself.

Figure 1 Kautsky curves for discs grown in medium with different concentrations of KI

The apertures at which the fluorescence curves were obtained are indicated. 6.3 is the maximum aperture indicating the maximum light intensity.


mv
6.3
6.3
9
8
11
16
Control
1μM
Time
mv
6.3
6.3
8
8
11
6.3
8
6.3
11
10μM
100μM
Time


Prelative= P-O / O
P
I
D
S
M
T
O
Light on
FLUORESCENCE YIELD
0
1
2
1
2
3
4
seconds
minutes
TIME

Appendix III: Effect of added KI on size of young sporophytes.

The surface area of the plants was measured with a planimeter and the values appear in the tables.
The date of measurement is indicated, the number of plants being too important to be carried by one line, the data is presented in two blocks.
Values are in x 0.1 cm2. Data for the experiment carried out in 1984.

Control

Date of measurement	Plant surface area x 0.1 cm2 — Plant # 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Apr. 9	31	17	27	26	37	21	27	26	18	32	18	20	21	34	35
Apr.12	40	23	35	39	47	22	35	34	21	42	25	28	28	43	44
Apr.15	65	37	55	55	66	34	44	46	31	58	40	42	43	71	65
Apr.18	72	54	82	92	90	57	63	72	48	95	66	64	55	94	88
Apr.21	102	71	105	116	98	72	86	102	69	116	93	76	63	119	113

	Plant # 16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Apr. 9	24	28	29	26	29	21	20	18	23	38	23	23	24	40	22
Apr.12	32	32	37	32	38	31	27	26	28	48	32	32	34	53	27
Apr.15	52	47	54	46	58	45	40	44	41	70	35	50	56	77	47
Apr.18	73	70	80	71	83	67	57	57	57	109	50	75	83	111	71
Apr.21	104	88	96	86	98	85	78	73	75	135	53	99	101	143	90

Treatment of 1 uM KI

Date of measurement	Plant surface area x 0.1 cm2 — Plant # 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Apr. 9	24	28	31	23	25	21	22	22	22	32	19	36	24	20	33
Apr.12	30	37	42	23	28	21	25	26	26	36	28	47	28	24	35
Apr.15	45	55	54	32	37	36	35	37	37	54	39	69	34	33	52
Apr.18	72	84	75	38	53	59	36	68	46	82	55	110	35	47	58
Apr.21	102	108	92	45	66	71	48	92	56	110	70	140	41	63	93

	Plant # 16	17	18	19	20	21	22	23	24	25	26	27	28	29
Apr. 9	14	17	18	33	29	23	17	20	25	19	17	19	18	18
Apr.12	18	22	18	41	34	27	23	22	28	24	20	22	21	21
Apr.15	27	34	27	47	51	45	30	28	39	35	32	31	31	29
Apr.18	37	37	26	53	87	67	38	44	55	45	44	51	43	43
Apr.21	51	52	36	71	115	87	49	58	76	56	56	67	58	57

Continuation of Appendix 3. Same legend.

Treatment 10 uM KI

Date of measurement	Plant surface area x 0.1 cm2 — Plant #														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Apr 9	39	22	41	14	33	42	36	34	27	16	27	21	28	30	24
Apr12	61	32	46	22	45	48	55	47	38	31	34	36	44	33	34
Apr15	96	45	53	36	64	73	87	65	55	43	50	53	66	51	51
Apr18	142	62	95	52	97	110	124	109	101	68	70	77	92	74	81
Apr21	179	79	158	77	131	146	173	139	131	95	95	99	122	96	108

	Plant #														
	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Apr. 9	31	30	20	22	42	18	24	27	39	15	18	22	35	21	17
Apr.12	41	45	32	32	61	26	26	38	54	23	26	33	46	35	31
Apr.15	60	63	45	46	87	37	42	61	69	38	41	51	71	49	43
Apr.18	92	97	74	65	140	65	55	88	96	20	60	69	100	67	64
Apr.21	129	116	102	94	188	93	72	127	137	68	75	86	127	98	99

Treatment 100 uM

Date of measurement	Plant surface area x 0.1 cm2 — Plant #														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Apr 9	24	27	23	30	27	21	21	24	24	26	19	32	14	23	41
Apr12	42	41	35	43	36	27	37	35	35	33	27	40	21	30	56
Apr15	66	58	54	48	54	39	55	55	61	51	39	72	30	47	84
Apr18	97	90	86	74	85	53	84	78	82	81	67	105	43	73	124
Apr21	132	111	112	86	109	68	110	103	104	99	89	107	57	91	153

	Plant #												
	16	17	18	19	20	21	22	23	24	25	26	27	28
Apr. 9	17	36	23	22	32	20	43	28	16	15	12	49	21
Apr.12	25	52	36	29	43	38	55	39	23	25	21	63	32
Apr.15	45	78	56	44	62	68	92	69	37	37	32	102	52
Apr.18	73	103	82	65	98	112	92	83	57	61	43	143	74
Apr.21	93	139	111	132	132	156	171	121	79	87	55	178	99

Continuation of Appendix 3.
The following data applies to the experiment carried out in 1985. The first column indicates the plant number, the following columns indicate the size of the plants at each measuring date: March 7, 10, 13, 16, 19, 22 and 25. Values in X 0.1 cm2.

Control

1	14	16	-1	-1	-1	-1	-1
2	23	30	-1	-1	-1	-1	-1
3	14	16	21	29	34	37	44
4	36	47	61	76	87	97	122
5	27	33	46	58	66	73	95
6	52	61	65	65	69	-1	-1
7	16	19	24	27	35	38	49
8	25	34	36	-1	-1	-1	-1
9	82	113	142	189	234	294	367
10	47	59	74	96	128	154	194
11	17	22	30	37	44	51	65
12	20	24	30	31	-1	-1	-1
13	26	41	65	71	87	98	100
14	40	-1	-1	-1	-1	-1	-1
15	24	29	32	-1	-1	-1	-1
16	42	-1	-1	-1	-1	-1	-1
17	39	55	65	85	111	131	157
18	29	33	35	37	-1	-1	-1
19	18	25	30	45	58	70	77
20	26	34	36	41	45	-1	-1
21	52	53	64	83	101	101	130
22	13	18	23	-1	-1	-1	-1
23	16	18	20	21	25	-1	-1
24	27	33	34	37	46	51	54
25	36	48	60	71	79	95	108
26	21	27	29	33	40	50	58
27	18	21	26	37	47	50	61
28	39	45	64	73	82	97	123
29	11	14	20	15	17	-1	-1
30	25	33	39	54	64	88	94
31	21	28	31	-1	-1	-1	-1

1 uM KI

1	58	61	82	86	102	116	131
2	18	21	28	33	37	44	53
3	27	-1	-1	-1	-1	-1	-1
4	43	51	54	68	70	71	81
5	16	19	20	-1	-1	-1	-1
6	14	-1	-1	-1	-1	-1	-1
7	20	24	25	-1	-1	-1	-1
8	24	28	33	39	50	58	71
9	15	19	23	26	32	34	47
10	30	30	37	48	60	70	83
11	13	20	26	35	52	65	81
12	16	17	17	17	21	31	37
13	35	40	45	60	-1	-1	-1
14	14	16	19	21	35	47	58
15	39	48	-1	-1	-1	-1	-1
16	22	28	37	46	58	65	84
17	17	20	22	30	31	34	42
18	17	22	26	33	39	45	58
19	14	16	18	32	38	47	56
20	52	67	72	97	120	139	147
21	19	21	24	-1	-1	-1	-1
22	34	37	40	48	-1	-1	-1
23	24	28	40	49	56	63	81
24	35	39	54	63	78	110	133
25	24	28	40	53	61	69	87
26	53	60	66	67	77	88	98
27	32	36	-1	-1	-1	-1	-1
28	39	47	52	64	69	75	97
29	18	22	26	42	57	70	96
30	16	19	20	23	32	39	43

10 uM KI

1	25	32	48	65	79	94	108
2	38	50	66	91	109	123	143
3	14	23	34	41	62	73	90
4	30	40	60	83	119	144	159
5	22	26	37	53	67	75	85
6	41	55	76	104	136	165	181
7	17	20	-1	-1	-1	-1	-1
8	24	28	40	58	72	85	87
9	37	56	74	101	130	167	204
10	35	45	61	90	-1	-1	-1
11	25	37	53	68	95	128	152
12	36	54	72	97	122	133	144
13	24	35	48	70	96	106	122
14	18	29	35	47	53	78	91
15	43	59	79	94	124	152	163
16	23	40	47	55	70	86	103
17	16	24	28	38	57	71	87
18	21	32	47	56	72	81	107
19	29	44	67	102	142	171	197
20	20	28	37	54	71	81	98
21	21	28	37	44	63	72	89
22	39	55	63	82	104	126	147
23	30	44	48	62	80	91	116
24	11	17	26	32	46	58	67
25	45	58	64	70	85	89	97
26	19	32	42	58	73	93	105
27	23	31	41	61	74	82	89
28	23	29	41	56	75	82	105
29	25	35	50	58	80	96	124
30	29	40	57	65	85	89	105

100 uM KI

1	19	27	43	63	78	94	120
2	13	-1	-1	-1	-1	-1	-1
3	33	45	62	84	104	124	151
4	21	28	43	59	77	91	102
5	14	24	27	39	56	64	78
6	13	19	26	37	51	59	78
7	23	35	47	73	98	121	152
8	17	22	32	48	64	77	98
9	24	32	45	61	84	97	111
10	22	30	47	67	81	93	121
11	25	38	51	68	93	116	135
12	28	45	72	92	130	154	200
13	51	72	98	131	163	189	231
14	20	27	-1	-1	-1	-1	-1
15	35	47	70	90	113	139	157
16	20	23	34	50	65	77	95
17	21	27	35	48	61	69	82
18	28	39	59	75	100	116	146
19	18	24	35	43	63	77	86
20	35	49	61	93	110	126	138
21	31	38	52	70	88	95	112
22	23	29	46	66	85	110	129
23	24	30	41	53	68	90	104
24	14	18	21	31	42	50	62
25	16	21	26	34	46	66	67
26	14	20	29	44	45	55	67
27	47	65	91	108	151	182	214
28	12	18	26	40	49	60	73
29	24	27	34	47	60	67	83

100 uM NaI

1	13	23	36	43	81	95	112
2	16	27	40	54	71	88	97
3	27	36	53	75	93	109	129
4	30	50	59	73	82	87	95
5	38	56	76	97	-1	-1	-1
6	18	32	39	53	77	79	95
7	18	26	36	-1	-1	-1	-1
8	36	56	78	102	120	136	152
9	13	20	28	40	61	70	78
10	40	70	93	124	145	169	207
11	18	27	38	57	75	88	104
12	20	35	48	63	82	96	115
13	16	30	35	64	71	81	98
14	24	33	52	62	-1	-1	-1
15	17	23	27	38	53	64	75
16	26	40	51	73	91	116	124
17	14	27	33	47	59	66	83
18	21	38	53	78	102	115	135
19	12	24	32	42	55	64	79
20	33	42	53	87	93	102	118
21	23	44	49	76	88	102	118
22	9	20	27	39	47	60	73
23	15	28	33	45	60	70	81
24	14	17	26	40	47	60	72
25	27	42	58	76	98	118	148
26	23	37	55	77	84	104	117
27	30	41	54	87	110	120	146
28	13	22	27	42	53	67	78
29	15	27	30	50	55	65	82
30	23	31	43	58	71	87	106