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APPLICATION OF TOXIC AGENTS IN THE STUDY OF THE ECOLOGICAL RESISTANCE OF INTERTIDAL RED ALGAE

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(Text-figs. 1 and 2)

Biebl (1939*a*, *b*) has found that algae from various ecological groups show marked differences in their resistance to changes in the temperature of the surrounding sea water. He regards this temperature effect as an important factor influencing the distribution of different intertidal algae and points out that a change of only 2° C may prove critical with certain species. One of us (A.D.B.) has studied the resistance of sporelings of various common intertidal red algae to changes in environmental temperature and has obtained results similar to those of Biebl.

The present study is an attempt to assess the importance of the lipid moiety of the cell membrane in determining the degree of this resistance. Evidence of the amount of lipid material has been obtained by measuring the relative rates of penetration into the plants of substances of different lipid solubilities, it being assumed that if the cell membrane contains a large quantity of lipid material then substances of high lipid solubility will penetrate far more rapidly than those of low lipid solubility; whereas, if it has little then the relative rates of penetration of substances of high and of low lipid solubility will be much closer in value. Analysis of the contents of the plant cells for traces of the penetrating substance was considered too complex a task; therefore poisons were used, for their rates of penetration can readily be estimated by examining the toxic effects of the compounds on the growth and viability of the test organisms. The poisons chosen were *n*-propylmercuric chloride (n-C_3H_7HgCl) and mercuric chloride, compounds which have markedly different lipid solubilities (Corner & Sparrow, 1957) and are toxic to red algal sporelings (Boney, Corner & Sparrow, 1959).

In general, the results obtained support the view expressed by Blinks (1951) that lipids do, in fact, play an important role in determining the resistances of intertidal red algae to increased sea-water temperatures; moreover, the present findings indicate that these lipids are located in the cell membrane.

METHODS

Test species

The plants used were (1) *Polysiphonia lanosa* (L.) Tandy; (2) *Plumaria elegans* (Bonnem.) Schm.; (3) *Spermothamnion repens* (Dillw.) K. Rosenv.; (4) *Ceramium flabelligerum* J. Ag.; (5) *Ceramium pedicellatum* (Duby) J. Ag.; (6) *Antithamnion plumula* (Ellis) Thur. *var. plumula*. Most of these were collected from Church Reef, Wembury, where their shore habitats cover a fairly wide range. Thus, (1) grows on *Ascophyllum nodosum* (L.) Le Jol.; (2) and (3) are found in the subflora below *Ascophyllum*; (4) grows prolifically on *Laurencia pinnatifida* (Huds.) Lamour. in the landward crevices and overhangs from just above M.T.L. down to M.L.W.N.T.; (5) is found in tide pools and is particularly abundant at M.T.L.; (6) is generally sublittoral but, for the present study, spores were obtained from a persistent growth of the plant in the 'Drake's Island' tank at the Plymouth Laboratory.

Settlement of spores

Tufts of each plant, bearing tetrasporangia, were floated on to glass slides lying in an enamelled dish containing filtered sea water from outside Plymouth breakwater. The fruiting tufts were left overnight and the spores released were allowed to remain undisturbed for 2 days at 16° C before being used in the experiments. Usually, settlements of 150–200 sporelings were obtained on each slide.

Toxicity studies

Stock solutions of n-C_3H_7HgCl (5 mg Hg/l.) and $HgCl_2$ (50 mg Hg/l.) in sea water were prepared freshly for each experiment.

The methods used were those described by Boney *et al.* (1959) for experiments with sporelings of *Plumaria elegans*.

Estimations of lipid content

A known dry weight of the plant under test was extracted with absolute ethanol by maceration in a Waring blendor. The alcohol extract was then evaporated to dryness and the residue was extracted with warm petroleum ether (b.p. 40–60° C). The quantity of lipid material was then estimated by evaporating off the petroleum ether and weighing the dried residue.

RESULTS

Resistance to increased sea water temperatures

Estimates were made of the times for which 50% of a crop of sporelings of each plant survived in sea water at different temperatures. The results are shown in Table 1, from which it will be seen that sporelings of plants that grow in intertidal habitats where there is some exposure to air show a greater susceptibility to increased water temperatures, the one exception being *Ceramium flabelligerum*. Table 1 also includes the critical temperatures of the plants and these results correspond with those of Biebl (1939*a*) in that only three intertidal plants could tolerate 35° C and all were killed at 42° C.

TABLE 1. VIABILITIES OF SPORELINGS OF VARIOUS INTERTIDAL RED ALGAE IMMERSED IN SEA WATER AT DIFFERENT TEMPERATURES

Time (h) for which 50 % of sporelings survived

	Species readily withstanding exposure to air				Species with low resistance to exposure to air	
Temp (° C)	*Plumaria elegans*	*Polysiphonia lanosa*	*Spermothamnion repens*	*Ceramium flabelligerum*	*Ceramium pedicellatum*	*Antithamnion plumula*
25	32	—	—	—	—	—
27	17·5	27·5	37	—	—	—
30	0·33	10	13	36	32	32
32	0·25	0·49	—	8	—	15
34	0·08	0·16	1·0	—	2·5	2·0
36	—	—	0·50	1·0	1·5	1·0
38	—	—	—	0·16	—	—
40	—	—	—	0·08	0·08	0·08
42	0	0	0	0	0	0

Lipid contents of selected species

Experiments were carried out to test if the lipid contents of selected algae showed any marked differences, and if these differences correlated with corresponding ecological resistances. The results of this study are shown in Table 2 and demonstrate that although the total lipid contents of the three plants are very low, nevertheless the species which have a higher lipid content are those which are more susceptible to increased water temperature. However, these results did not give any indication of the distribution of lipid material within the plant cells, and to investigate this point experiments with poisons were made.

TABLE 2. TOTAL LIPID CONTENTS OF CERTAIN INTERTIDAL RED ALGAE

Species	Lipid content as % dry wt.
Plumaria elegans	0·91
Polysiphonia lanosa	0·55
Ceramium flabelligerum	0·34

Resistance to poisons

The toxicities of $HgCl_2$ and n-C_3H_7HgCl were compared with 6 species of algae as the test organisms. The results are shown in Table 3, from which it will be seen that a very high ratio of LD_{50} values was obtained with those species that are most susceptible to increased sea-water temperatures (1, 2 and 3). The findings are therefore consistent with the view that these species possess a considerable amount of lipid material in the cell membrane.

TABLE 3. RELATIVE TOXICITIES OF $N\text{-}C_3H_7HgCl$ AND $HgCl_2$ TO SPORELINGS OF VARIOUS INTERTIDAL RED ALGAE

	Low resistance to raised sea-water temperature			High resistance to raised sea-water temperature		
Species	(1) *Plumaria elegans*	(2) *Polysiphonia lanosa*	(3) *Spermothamnion repens*	(4) *Antithamnion plumula*	(5) *Ceramium flabelligerum*	(6) *Ceramium pedicellatum*
LD_{50} in $HgCl_2$ (mg Hg/l.)	6·7	8·0	3·0	5·0	3·2	4·2
LD_{50} in $n\text{-}C_3H_7HgCl$ (mg Hg/l.)	0·021	0·030	0·0125	0·045	0·080	0·15
Ratio of above	319	266	240	111	40	28

Effect of temperature

It seemed probable that the lipid moiety of the cell membrane would be so distorted by increased temperature as to become more readily penetrable. For this reason a study was made at different temperatures of the effects of a poison of low lipid solubility on *Antithamnion* and *Plumaria*, species with markedly different resistances to temperature changes. The results obtained (see Table 4) showed that the enhanced toxicity of $HgCl_2$ conferred by increased temperature was far more marked in experiments with *Plumaria* than in those with *Antithamnion*, and these findings provided further evidence in support of the view that the lipid moiety of the plasma membrane is closely involved in temperature sensitivity.

TABLE 4. LD_{50} VALUES FOR $HgCl_2$ AT DIFFERENT TEMPERATURES

Sporelings immersed in the toxic solutions for 0·5 h at each temperature.

	LD_{50} (mg Hg/l.)	
Temp °C	*Antithamnion plumula*	*Plumaria elegans*
5	—	9·0
16	5·0	6·5
25	—	0·75
29	1·5	—
34	<0·5	—

Speed of toxic effect

It was considered likely that if the lipid moiety of the cell membrane was more developed in a species that was particularly temperature sensitive, this species would be only slowly penetrated by a poison of low lipid solubility. Accordingly, sporelings of several species were treated with $HgCl_2$ and examined for signs of toxic effects after 1 and 7 days. The results (Fig. 1) demonstrated that sporelings of *Plumaria*, *Polysiphonia* and *Spermothamnion* showed considerable resistance to poisoning by $HgCl_2$ when examined after 24 h, but that after 7 days the cumulative toxic effects of the poison had become

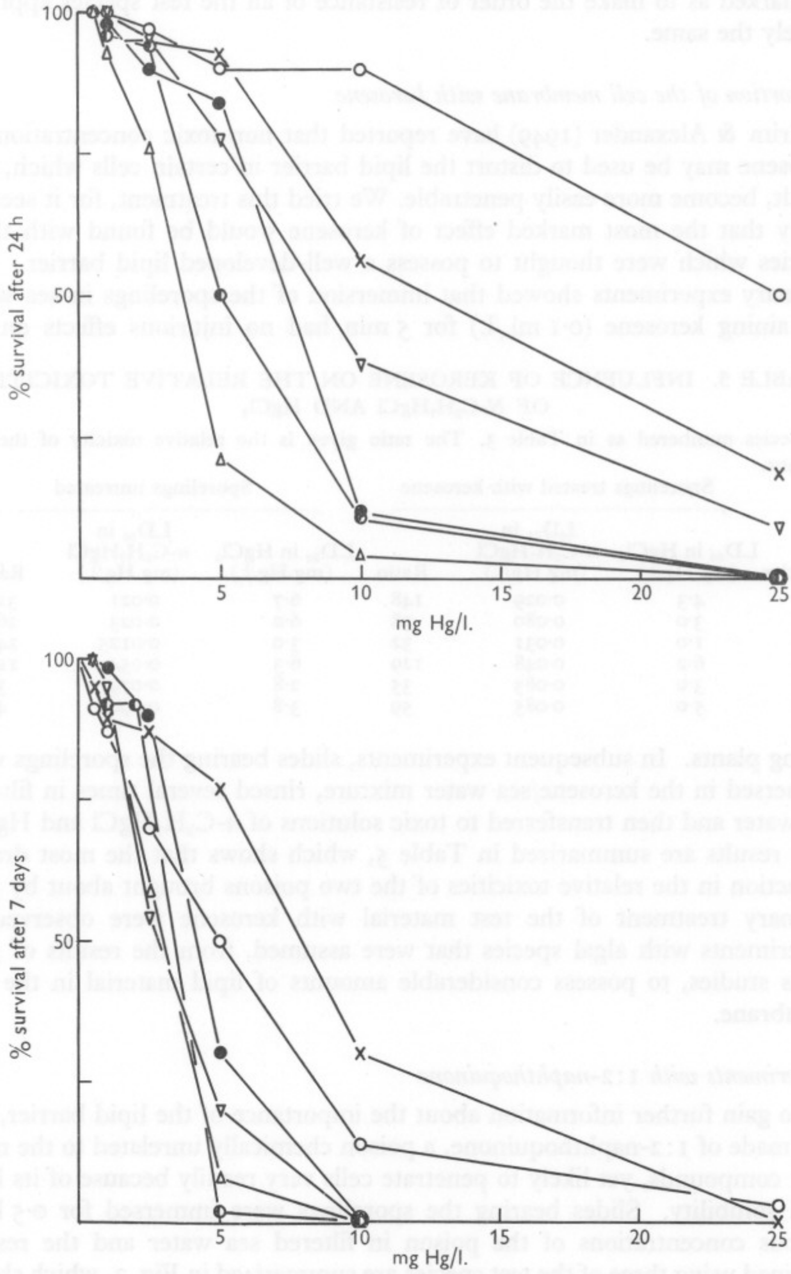

Fig. 1. Sporelings immersed 0·5 h in sea water containing various concentrations of mercury (as $HgCl_2$) then transferred to fresh sea water and examined 24 h (top figure) and 7 days (bottom figure) afterwards. ○—○, *Plumaria*; ×—×, *Polysiphonia*; ●—●, *Ceramium pedicellatum*; ◐—◐, *Antithamnion*; ▽—▽, *Spermothamnion*; △—△, *Ceramium flabelligerum*.

so marked as to make the order of resistance of all the test species approximately the same.

Distortion of the cell membrane with kerosene

Trim & Alexander (1949) have reported that non-toxic concentrations of kerosene may be used to distort the lipid barrier in certain cells which, as a result, become more easily penetrable. We tried this treatment, for it seemed likely that the most marked effect of kerosene would be found with those species which were thought to possess a well-developed lipid barrier. Preliminary experiments showed that immersion of the sporelings in sea water containing kerosene (0·1 ml./l.) for 5 min had no injurious effects on the

TABLE 5. INFLUENCE OF KEROSENE ON THE RELATIVE TOXICITIES OF N-C_3H_7HgCl AND $HgCl_2$

Species numbered as in Table 3. The ratio given is the relative toxicity of the two poisons.

	Sporelings treated with kerosene			Sporelings untreated		
Species	LD_{50} in $HgCl_2$ (mg Hg/l.)	LD_{50} in n-C_3H_7HgCl (mg Hg/l.)	Ratio	LD_{50} in $HgCl_2$ (mg Hg/l.)	LD_{50} in n-C_3H_7HgCl (mg Hg/l.)	Ratio
1	4·3	0·029	148	6·7	0·021	319
2	3·0	0·080	38	6·0	0·023	260
3	1·0	0·031	32	3·0	0·0125	240
4	6·2	0·048	129	6·3	0·054	115
5	3·0	0·085	35	2·8	0·079	35
6	5·0	0·085	59	3·8	0·090	42

young plants. In subsequent experiments, slides bearing the sporelings were immersed in the kerosene/sea water mixture, rinsed several times in filtered sea water and then transferred to toxic solutions of n-C_3H_7HgCl and $HgCl_2$. The results are summarized in Table 5, which shows that the most drastic reduction in the relative toxicities of the two poisons brought about by preliminary treatment of the test material with kerosene were observed in experiments with algal species that were assumed, from the results of previous studies, to possess considerable amounts of lipid material in the cell membrane.

Experiments with 1:2-naphthoquinone

To gain further information about the importance of the lipid barrier, use was made of 1:2-naphthoquinone, a poison chemically unrelated to the mercury compounds, yet likely to penetrate cells very readily because of its high lipid solubility. Slides bearing the sporelings were immersed for 0·5 h in various concentrations of the poison in filtered sea water and the results obtained using three of the test species are summarized in Fig. 2, which shows the percentage of the sporelings that survived 24 h after their immersion in the poison. It will be seen that these results accord well with those obtained using n-C_3H_7HgCl, in that 1:2-naphthoquinone is more toxic to sporelings of the species known to be highly sensitive to increased water temperature.

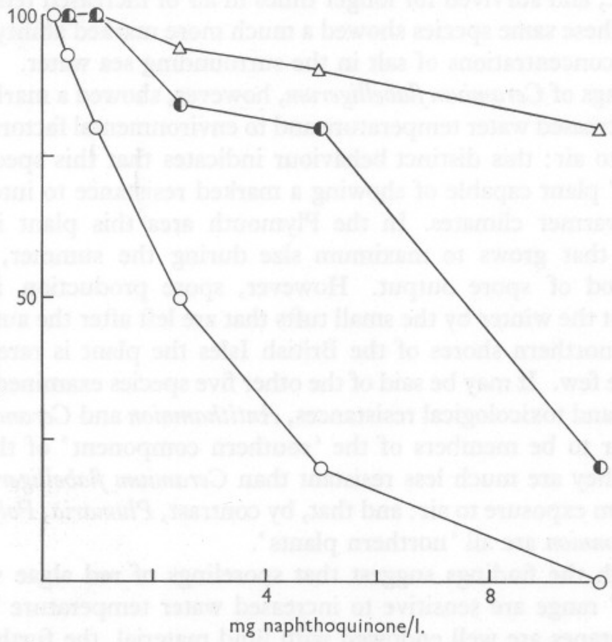

Fig. 2. Sporelings immersed 0·5 h in sea water containing various concentrations of 1:2-naphthoquinone then transferred to fresh sea water and examined 24 h later. △—△, *Ceramium flabelligerum*; ◐—◑, *Antithamnion*; ○—○, *Plumaria*.

DISCUSSION

Macpherson & Young (1949), examining the chemical composition of certain marine plants, found measurable quantities of lipid material in the red algae *Rhodymenia palmata* (3·78 % of dry weight) and *Halosaccion ramentaceum* (2·82 % of dry weight); but none in *Gigartina stellata*, *Chondrus crispus* and *Ahnfeltia plicata*. In the present study it was found that although three of the test species had only small lipid contents (0·34–0·9 % of dry weight), nevertheless the alga most sensitive to temperature (*Plumaria elegans*) had the highest content and the species least sensitive to temperature (*Ceramium flabelligerum*) had the lowest. Furthermore, the use of toxic agents provided a good deal of evidence in support of the view that the cell membranes of species that were temperature-sensitive contained more lipid material than those of species that were temperature-resistant.

A complementary investigation of the resistances of sporelings of the test species to a number of environmental factors showed that sporelings of *Plumaria*, *Polysiphonia* and *Spermothamnion* were able to withstand exposure to air in the intertidal zones more readily than sporelings of *Ceramium pedi-*

cellatum and *Antithamnion*. Thus, sporelings of the first three species, when enclosed in a water film, were more resistant to prolonged exposure to air of 100% R.H., and survived for longer times in air of increased temperature. In addition, these same species showed a much more marked ability to withstand increased concentrations of salt in the surrounding sea water.

Sporelings of *Ceramium flabelligerum*, however, showed a marked resistance to both increased water temperature and to environmental factors arising from exposure to air: this distinct behaviour indicates that this species may be a 'southern' plant capable of showing a marked resistance to intertidal conditions in warmer climates. In the Plymouth area this plant is a common perennial that grows to maximum size during the summer, which is its main period of spore output. However, spore production is carried on throughout the winter by the small tufts that are left after the autumn defoliation. On northern shores of the British Isles the plant is rare and fruiting records are few. It may be said of the other five species examined that by their ecological and toxicological resistances, *Antithamnion* and *Ceramium pedicellatum* appear to be members of the 'southern component' of the algal flora, although they are much less resistant than *Ceramium flabelligerum* to factors arising from exposure to air: and that, by contrast, *Plumaria*, *Polysiphonia* and *Spermothamnion* are all 'northern plants'.

Although the findings suggest that sporelings of red algae which have a 'northern' range are sensitive to increased water temperature because their cell membranes are well endowed with lipid material, the further possibility cannot be excluded that temperature sensitivity may also be due to denaturing of proteins (*vide* Blinks, 1951). In fact it is possible that both these changes may occur and together account for subsequent inhibition of metabolic processes.

There seems no reason why the methods used in the present work should not be applied to many problems of ecological interest and further studies along these lines are planned with sporelings of several 'southern' and 'northern' species of red algae. For example, it would be of considerable interest to examine *Ceramium acanthonotum* Carm. ex Harv. and *Callithamnion arbuscula* (Dillw.) Lyngb. for, although these two species are clearly 'northern' plants, whereas the former also occurs on 'southern' shores the latter does not. In addition, similar studies on ecological resistances might be made using material collected from different localities within the geographical range of a particular species to supplement information about the form range of that species. For, as Parke (1953) has indicated, this is one of the most pressing problems in marine algal ecology.

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SUMMARY

Sporelings of the intertidal red algae *Plumaria elegans*, *Polysiphonia lanosa* and *Spermothamnion repens* readily withstand exposure to air but have low resistance to increased sea-water temperature; those of *Antithamnion plumula* and *Ceramium pedicellatum* readily withstand increased sea-water temperature but have low resistance to exposure to air; those of *Ceramium flabelligerum* are resistant to both factors.

Poisons of high lipid solubility (n-C_3H_7HgCl and 1:2-naphthoquinone) are far more toxic than one of low lipid solubility ($HgCl_2$) to sporelings of *Plumaria*, *Polysiphonia* and *Spermothamnion*; but when *Antithamnion*, *Ceramium pedicellatum* and *Ceramium flabelligerum* are used as the test material the relative toxicities of the poisons are much closer in value.

The toxic effects of $HgCl_2$ to sporelings of *Plumaria* develop slowly at normal temperature and much more quickly when the temperature is raised. However, when sporelings of *Antithamnion* are used, toxic effects develop rapidly at both normal and raised temperatures.

The relative toxicities of n-C_3H_7HgCl and $HgCl_2$ to sporelings of *Plumaria*, *Polysiphonia* and *Spermothamnion* are markedly reduced when the test material is first treated with sub-toxic amounts of kerosene, a substance known to distort the lipid moiety of the cell membrane. However, when *Antithamnion*, *Ceramium pedicellatum* and *Ceramium flabelligerum* are used as the test material, the effect of the kerosene is negligible.

These results are consistent with the view that the proportion of lipid material present in the cell membrane is important in determining the susceptibility of an intertidal red alga to increased sea-water temperature and have been discussed with especial reference to the geographical distributions of the various species examined.

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