

REQUIREMENT FOR THIAMINE AMONG SOME MARINE AND SUPRA-LITTORAL PROTISTA

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(Text-fig. 1)

It has been known for some years that a need for an exogenous source of thiamine exists among algal flagellates (Lwoff & Lederer, 1935; Provasoli & Pintner, 1953) and indeed the requirement coupled with one for B₁₂ is quoted as characterizing auxotrophic algae (Provasoli, McLaughlin & Droop, 1957). Nevertheless, published data for marine species concerning thiamine are limited to a recent paper by J. J. A. McLaughlin (1958) which establishes an absolute requirement for thiamine on the part of *Prymnesium parvum*. The reason for this is partly due to interest in thiamine taking second place to interest in vitamin B₁₂ and partly to the fact that thiamine-free media are not easily prepared, so that the requirement for the vitamin appeared as stimulatory rather than absolute (Droop, 1953*a*, 1957).

The following is an attempt to remedy this state of affairs as regards a few species maintained in bacteria-free culture at Millport.

METHODS

The strains used

In the first instance B₁₂ requiring strains were chosen for the study, then two known to have no B₁₂ requirement were added and finally some dinoflagellates on which there were no data (Table 1).

Culture media

Basal media were as follows: for *Skeletonema*, S36 with thiamine omitted (Droop, 1955*b*); for *Hemiselmis*, S46 with thiamine omitted (Provasoli *et al.* 1957); and for the remainder, excepting *Oxyrrhis*, S50 with thiamine omitted. S50 is given in full in Table 2. It differs from my previously published media chiefly in the composition of the trace metal mixture and the replacement of the pH buffer tris(hydroxymethyl)aminomethane by glycylglycine (which is very much less toxic to some species than TRIS) and glycine (which increases the buffering of the medium in the region of pH 9-10).

The media for *Oxyrrhis* are to be discussed fully in a paper on the nutrition of this species shortly to be published. Thiamine data are included here for the sake of completeness.

Both stocks and experimental cultures were kept in Pyrex test-tubes 15 × 150 mm. Oxo aluminium test-tube caps were employed to begin with, but were replaced by cotton wool when it was realized that contamination from this source was certainly less than 10 mμg/l.

TABLE 1. STRAINS USED
(Millport reference numbers in parentheses)

| | | |
|--|------|--------------------------------|
| Chrysophyceae | | |
| <i>Monochrysis lutheri</i> | (60) | Droop, 1953 a, 1954 |
| <i>Prymnesium parvum</i> Carter | (65) | Droop, 1954 |
| <i>Syracosphaera elongata</i> | (62) | Droop, 1954, 1955 a |
| <i>Microglena arenicola</i> | (72) | Droop, 1955 a, 1957 |
| Cryptophyceae | | |
| <i>Hemiselmis virescens</i> | (64) | Droop, 1955 a, 1957 |
| Chlorophyceae | | |
| <i>Nannochloris oculata</i> | (66) | Droop, 1955 a |
| Bacillariophyceae | | |
| <i>Skeletonema costatum</i> (Grev.) Cleve | (73) | Plymouth strain, Droop, 1955 b |
| <i>Phaeodactylum tricornutum</i> Bohlin (syn. <i>Nitzschia closterium</i> f. <i>minutissima</i> (W. Smith) Allen & Nelson) | (14) | Finnish strain, Droop, 1953 a |
| Dinophyceae | | |
| <i>Glenodinium foliaceum</i> Stein | (47) | Finnish strain, Droop, 1953 a |
| <i>Peridinium trochoideum</i> (Stein) Lemm. | (88) | From the Clyde |
| <i>Oxyrrhis marina</i> Dujardin | (18) | Finnish strain, Droop, 1953 b |

TABLE 2. MEDIUM S50

| | | | | | | | |
|--------------------------------------|--------|----|--------|----------------------|---------|---------------------------------|---------|
| NaCl | 15 g | Br | 22 mg | Na ₂ EDTA | 50 mg | Glycylglycine | 500 mg |
| MgCl ₂ ·6H ₂ O | 2.5 g | Sr | 3.8 mg | Fe | 500 μg | Glycine | 250 mg |
| KCl | 400 mg | Al | 28 μg | Mn | 50 μg | KNO ₃ | 100 mg |
| CaSO ₄ ·2H ₂ O | 500 mg | Rb | 61 μg | Zn | 5.0 μg | K ₂ HPO ₄ | 10 mg |
| | | Li | 6.0 μg | Cu | 5.0 μg | B ₁₂ | 100 mμg |
| | | I | 20 μg | Co | 500 mμg | Thiamine | 1.0 mg |
| | | | | Mo | 500 mμg | H ₂ O to | 1.0 l. |
| | | | | | | pH adjusted to 8 | |

Where possible, growth was measured optically, in which case it is expressed as percentage transmission or as $OD \times 100$, $\left(= 100 \log_{10} \frac{100}{\% \text{ transmission}} \right)$. It was necessary to count in other cases; growth is then expressed as cells per mm³.

RESULTS

Serial transfer experiments

The concentrations over which growth is a function of thiamine concentration in other micro-organisms is 0–500 mμg/l. (Snell, 1951); and since my stock media contained up to 1 mg/l. of the vitamin, at least two transfers were necessary to reduce the concentration sufficiently in the blanks (cultures receiving no addition of the vitamin). This naturally depended on the size of the inoculum which was, therefore, made as small as possible. With *Monochryses*, *Prymnesium*, *Syracosphaera*, *Microglena*, *Nannochloris*, *Phaeodactylum*

and *Skeletonema* the inoculum could be reduced to 0.02 ml., thus allowing a 300-fold reduction at each transfer, so that the blanks of the second should have contained about 10 m.µg/l. With *Hemiselmis* and the dinoflagellates, however, an inoculum of 0.2-0.3 ml. was advisable which only allowed a 20-fold reduction, with the consequence that four transfers were necessary to deplete the blanks to the same extent.

Transfers were made from the blanks. To the other cultures the vitamin was added initially in the form of the complete molecule (0.1 mg/l.), but later

TABLE 3. SERIAL TRANSFER EXPERIMENTS WITH THIAMINE

(-, blank; +, with 0.1 mg/l. thiamine. OD × 100.)

| Transfer No. | Species | | | | | | | | | | | | | |
|--------------|---------------------|-----|----------------------|-----|---------------------|-----|-------------------|----|----------------------|----|-------------------|----|-------------------|-----|
| | <i>Nannochloris</i> | | <i>Phaeodactylum</i> | | <i>Mono-chrysis</i> | | <i>Prymnesium</i> | | <i>Syracosphaera</i> | | <i>Microglena</i> | | <i>Hemiselmis</i> | |
| | - | + | - | + | - | + | - | + | - | + | - | + | - | + |
| 1 | 66 | 68 | 57 | 70 | 122 | 110 | 80 | 92 | 44 | 41 | 35 | 52 | 32 | 70 |
| 2 | 92 | 92 | 89 | 100 | 30 | 140 | 0 | 80 | 16 | 39 | 11 | 48 | 7 | 110 |
| 3 | 110 | 100 | 92 | 89 | 4 | 150 | 2 | 80 | 27 | 59 | 22 | 43 | 2 | 105 |

TABLE 4. *SKELETONEMA COSTATUM*, SERIAL TRANSFER EXPERIMENTS

(OD × 100. standard errors in parentheses, n=5.)

| Transfer no. | Treatment | | |
|--------------|-----------|---|--------------------|
| | Blank | 1.0 mg/l. Na ₂ S ₉ H ₂ O | 0.1 mg/l. thiamine |
| 2 | 27 (5.4) | 21 (9.0) | 40 (3.7) |
| 3 | 39 (1.0) | 39 (0.8) | 39 (0.7) |
| 4 | 0.4 (0.4) | 29 (1.25) | 21 (3.4) |
| 5 | 21 (0.7) | 30 (1.05) | 32 (1.1) |

TABLE 5. DINOFLAGELLATE SERIAL TRANSFER EXPERIMENTS

(Numbers per mm³.)

| Transfer no. | Treatment | | | | |
|-------------------------------|-----------|----------|-----------------------|----------|------------|
| | Blank | Thiamine | Thiazole + pyrimidine | Thiazole | Pyrimidine |
| <i>Oxyrrhis marina</i> | | | | | |
| 1 | | 119 | 113 | 125 | 25 |
| 2 | 32 | 73 | 129 | 72 | 5 |
| <i>Peridinium trochoideum</i> | | | | | |
| 2 | 9 | 8 | 13 | 13 | 7 |
| 3 | 6 | 8 | 6 | 13 | 3 |
| 4 | 9 | 7 | 8 | 6 | 9 |
| <i>Glenodinium foliaceum</i> | | | | | |
| 2 | 34 | 26 | 27 | 30 | 24 |
| 3 | 20 | 28 | 27 | 33 | 28 |
| 4 | — | 33 | 39 | 28 | 31 |
| 5 | 28 | 21 | 26 | 26 | 25 |

the molecule was 'split' into its components, 4-methyl-5- β -hydroxyethyl-thiazole and 2-methyl-4-amino-5-aminomethyl-pyrimidine, and the following treatments given: (a) blank, (b) complete molecule, (c) thiazole and pyrimidine in equal amounts, (d) thiazole alone, and (e) pyrimidine alone.

The results of the transfer experiments are shown in Tables 3-5.

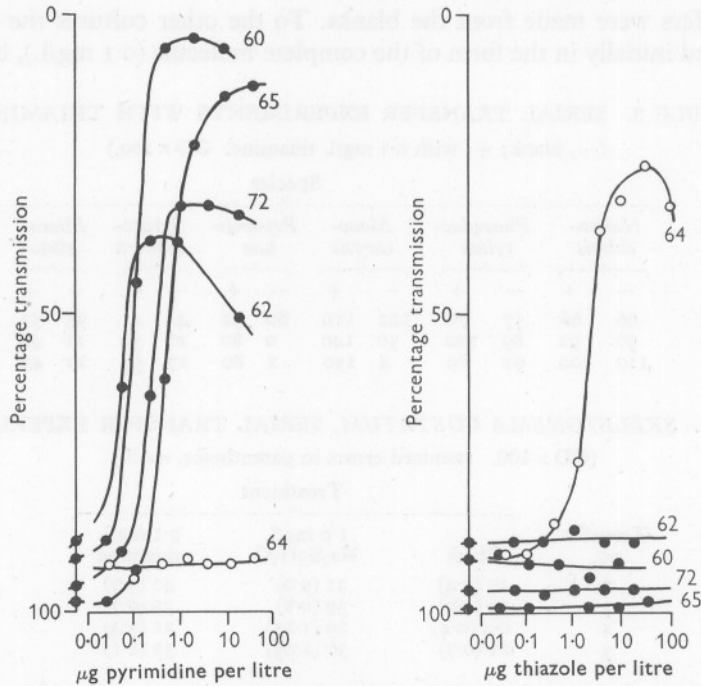


Fig. 1. Response to pyrimidine (left hand) and thiazole (right hand). 60, *Monochrysis lutheri*; 62, *Syracosphaera elongata*; 64, *Hemiselms virescens*; 65, *Prymnesium parvum*; 72, *Microglena arenicola*.

Dose/response experiments

Where time and the material allowed the transfer experiments were confirmed by dose/response experiments in which was measured yield in response to graded doses of thiamine and its component parts. Response curves for the chrysoomonads and *Hemiselms* to pyrimidine and to thiazole are set out in Fig. 1.

DISCUSSION

Table 6 summarizes the results and includes data of other requirements, notably that for B₁₂. Previous reports regarding *Phaeodactylum*, *Nannochloris* and *Skeletonema* are confirmed (Peach & Drummond, 1942; Hutner, 1948; Droop, 1955a, b). All but two of the species under discussion in this paper

are of littoral or supra-littoral origin, the pelagic strains being *Skeletonema* and *Peridinium trochoideum*: neither had an absolute requirement for thiamine.

Skeletonema has been rather difficult to work with in defined media, because the vigour of cultures seems to depend to a large extent on such factors as pH, oxygen tension and the state of the inoculum—whether, for instance, it is in the exponential or stationary phase of growth, and its relation to the auxospore cycle. Under favourable conditions growth is quite independent of thiamine, and at such times thiamine does happen to be beneficial (as in the fourth transfer, Table 4) it can always be replaced by inorganic sulphide;

TABLE 6. SUMMARY OF RESULTS, WITH DATA ON OTHER HETEROTROPHIC TENDENCIES

| | Requirement for thiamine | Portion of vitamin required | Requirement for vitamin B ₁₂ | Other heterotrophic tendencies |
|----------------------|--------------------------|-----------------------------|---|--|
| Chrysophyceae | } | Pyrimidine | + | { Can utilize some amino acids as N source (Droop, 1955 a) |
| <i>Monochrysis</i> | | | | |
| <i>Prymnesium</i> | | | | |
| <i>Syracosphaera</i> | | | | |
| <i>Microglena</i> | | | | |
| Chlorophyceae | } | - | - | { Can utilize some amino acids as N source (Droop, 1955 a) |
| <i>Nannochloris</i> | | | | |
| Bacillariophyceae | | | | |
| <i>Phaeodactylum</i> | | | | |
| <i>Skeletonema</i> | - | - | + | Organic compounds sometimes stimulatory in an unspecific way (Droop, 1957) |
| Cryptophyceae | } | Thiazole | { | { Amino N obligatory (Droop, 1957) Amino N obligatory; acetate as C source; phagotrophic in Nature; other B vitamins (unpublished data) |
| <i>Hemiselmis</i> | | | | |
| Dinophyceae | | | ? | |
| <i>Oxyrrhis</i> | | | | |
| <i>Glenodinium</i> | } | - | + | ? |
| <i>Peridinium</i> | | | | |

consequently, it is unlikely that the vitamin is functioning here in this same way as in other organisms. Incidentally, neither *Hemiselmis* nor *Oxyrrhis* could utilize inorganic sulphide in place of thiazole. The significance of sulphur-containing compounds in diatom growth has been discussed by Harvey (1939, 1955); Lewin (1954) and Droop (1957)

The results appertaining to two photosynthetic dinoflagellates, *Peridinium trochoideum* and *Glenodinium foliaceum* (Table 5) are not completely satisfactory owing to the lightness of growth normally obtained with these two species. It is noted, therefore, simply that no requirement was demonstrable even after four transfers. On the other hand, a requirement for vitamin B₁₂ became apparent on the first transfer and it was not possible to carry either species through more than two transfers in B₁₂-free media.

The four chrysomonads all responded to the pyrimidine half of the vitamin, whereas *Hemiselmis* and *Oxyrrhis* responded to the thiazole half (Fig. 1).

Possibly this represents a phyletic difference between the Chrysophyta and Pyrrophyta, though it is early to tell. With the exception of *Oxyrrhis*, all the species which proved to need thiamine had already been found to have a requirement for vitamin B₁₂ (Droop, 1957), but the reverse was not the case.

The Chrysomonads are quite indifferent to the presence or absence of thiazole in the medium, so their response to pyrimidine can be regarded as simple. *Hemiselmis* and *Oxyrrhis*, on the other hand, appear to obtain some benefit from pyrimidine: pyrimidine does not support growth and, in the presence of thiazole, affects yield only slightly, nevertheless, *Hemiselmis* bleaches more quickly in its absence and *Oxyrrhis* fails to produce the fine pink colour characteristic of heavy healthy cultures.

It is not possible to arrive at more than a rough estimate of the magnitude of the thiamine requirement from dose/response curves based on optical measurements (Fig. 1). Furthermore, the basal medium S50 was developed to suit *Monochrysis* and was not necessarily that most suitable for the other species. Half-maximal growth of the pyrimidine-requiring organisms appeared to be given by 100–300 mμg/l. pyrimidine or thiamine; half-maximal growth of both *Oxyrrhis* and *Hemiselmis* (requiring thiazole) by ten times as much, i.e. 2000 mμg/l., thiazole or thiamine. Such a difference in magnitude in thiamine requirement between pyrimidine and thiazole-requiring species has rather interesting implications should it prove to be real and of general occurrence. It suggests either that thiamine has a different function according to whether the requirement is met by one-half of the vitamin or by the other, or, alternatively, that the functions of pyrimidine and thiazole are divorced from each other and that the function of thiamine here is merely to supply either thiazole or pyrimidine as the case may be. Which alternative is biochemically less improbable it is difficult to say. On the other hand, it is possible that thiazole is merely more labile than pyrimidine.

SUMMARY

The requirement for thiamine was examined in eleven marine protists of littoral, supra-littoral or neritic origin. Six were found to have an absolute requirement for the vitamin.

The thiamine-requiring Chrysophyta responded to the pyrimidine half of the vitamin, whereas the two thiamine-requiring Pyrrophyta responded to the thiazole half.

All the species requiring thiamine were auxotrophic with respect to at least one other factor (usually vitamin B₁₂).

For half-maximal growth, species responding to pyrimidine required 100–300 mμg/l. pyrimidine or thiamine and species responding to thiazole 2000 mμg/l. thiazole or thiamine.

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