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A PELAGIC MARINE DIATOM REQUIRING COBALAMIN

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A bacteria-free culture of the important centric diatom *Skeletonema costatum* has recently been established at Millport, and some exploratory nutritional experiments have been carried out with it. *S. costatum* is a photo-autotroph whose autotrophy is, apparently, limited to cobalamin (vitamin B_{12}).

Details concerning isolation and maintenance of *S. costatum* differ from those already published (Droop, 1954*a*, 1955) only in the matter of pH control, a vital factor in the successful culture of this species whose range of tolerance is narrow (pH 7·5–8·5).

A synthetic solution (*S 36*) was developed for and used in the experiments to be described:

S 36

KNO_3	100 mg	Cobalamin	100 mμg
K_2HPO_4	10 mg	'S.W. 1'*	250 ml.
$Na_2SiO_3 . 9 H_2O$	100 mg	'S.W. 2'*	5 ml.
Tris-(hydroxymethyl)-aminomethane	500 mg	'T.M. 2'*	10 ml.
Thiamin	1 mg	Glass distilled water	735 ml.

S. costatum has been carried through more than ten transfers in this medium and growth has been as good as in any containing soil and liver extracts and natural sea water. The medium was adjusted to pH 8·1 before being decanted into test-tubes and autoclaved for a few moments at 15 lb., inoculations being carried out 18 hr later from exponentially growing cultures.

The sensitivity of *S. costatum* to pH was such as to require the use of replications in all vitamin experiments. This rendered conventional dose-response layouts very unwieldy. Consequently, the requirement for cobalamin was demonstrated qualitatively by the use of a serial transfer technique in which cultures receiving a full dose of the vitamin were compared with those receiving none. At each stage inoculations were taken from a vitamin-free culture of the previous stage to one batch of medium containing the vitamin and to one vitamin-free, the two batches having been prepared and adjusted as one, then divided for the addition of the vitamin, and autoclaved simultaneously.

The results of these experiments are shown in Table I. The magnitude of the errors occasioned by the difficulty of adequately controlling pH is not sufficient to obscure the fact that significantly poorer yields were obtained in the vitamin-free controls in each trial after the first. Inocula for the latter

* For composition of these metal solutions, see Droop (1955).

were from stock, hence carry-over of cobalamin in this instance would not be negligible. The results show that cyanocobalamin, the vitamin proper, can be spared by the variants, pseudo-vitamin B_{12}, factor A and factor B; but the experiments were not such as to be able to determine the relative activities of these variants, nor indeed to determine whether the requirement for cobalamin is absolute.

TABLE I. EFFECT OF COBALAMIN AT A CONCENTRATION OF 100 mμg/l. ON GROWTH OF *Skeletonema costatum* ON SERIAL TRANSFER IN MEDIUM *S 36* LESS COBALAMIN

Inocula were taken from controls of previous transfer (dilution factor: 100). Final yields measured optically (per cent absorption).

	Mean percentage absorption	Standard error	Replications
First transfer			
With cyanocobalamin	17	1·6	5
Control	16	0·81	5
Second transfer			
With cyanocobalamin	26	1·1	5
Control	7	0·84	5
Third transfer			
With cyanocobalamin	15	3·8	7
With factor A	13	2·2	7
With factor B	10	2·2	7
With pseudo-B_{12}	8	5·0	7
Control	2	0·62	7
Fourth transfer			
With cyanocobalamin	39	7·5	7
With factor A	44	4·6	7
With factor B	33	9·0	7
With pseudo-B_{12}	37	5·9	7
Control	7	1·9	7

S. costatum resembles *Bacterium coli* in being able to utilize factor B as the sole source of the vitamin, factor B being that part of the molecule, common to the various forms, which remains after removal of the respective nucleotides (Ford & Porter, 1952). The requirement for cobalamin in this instance, therefore, is likely to be occasioned by a synthetic disability concerning this non-nucleotide portion, as in the case of *B. coli* (Ford, Holdsworth & Kon, 1955). It cannot, however, yet be concluded that *S. costatum* is autotrophic with respect to the nucleotide, because the experiments were undertaken with a culture medium containing thiamin; but this may be the case.

A similar series of experiments with thiamin (in the presence of cobalamin) failed to demonstrate a significant requirement for this vitamin, but the use of cotton-wool plugs may account for this.

The culture of *S. costatum* in a state of bacteriological purity has allowed the first insight into the nutritional requirements of an important pelagic organism. It also provides a measure of hope that success will attend efforts to culture other pelagic species so necessary to an understanding of the biochemical ecology of the sea. Indeed, the concept of non-predatory relationships, though more than fifty years old (cf. Lucas, 1938), has, for want of proper experimental material, yet received no detailed support in the field of phytoplankton studies. The need for accessory substances for growth of marine diatoms, first postulated by Allen (1914) and later by Harvey (1939) and others, is paralleled by nutritional deficiencies in investigated euryhaline diatoms and flagellates (Hutner & Provasoli, 1953; Provasoli & Pintner, 1953; Lewin, 1954; Droop, 1954*b*; Sweeney, 1954). Latterly, each investigation has tended to make more probable the general importance of cobalamins in the economy of the sea, though the species investigated have mostly been supra-littoral or estuarine. The requirement in *S. costatum* is, therefore, of interest, and it is noteworthy that in this species, which possibly resembles other pelagic diatoms, there is a lack of specificity in the form of the vitamin preferred.

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