

# MONTHLY SURVEY OF VITAMIN B<sub>12</sub> CONCENTRATIONS IN SOME WATERS OF THE ENGLISH LAKE DISTRICT

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## ABSTRACT

The vitamin B<sub>12</sub> activity for *Euglena gracilis* of water from nine lakes of the English Lake District was assayed at 15 consecutive monthly intervals. Filtration through glass-fiber filters was used to differentiate between dissolved vitamin and that in gross particulate matter. The average vitamin B<sub>12</sub> concentrations correlated with what has previously been described as the evolutionary series of the lakes: the "primitive" rocky lakes of low productivity are low in vitamin B<sub>12</sub>, the "evolved" silted lakes, richer in sediments, nutrients, and organisms have higher levels of the vitamin.

## INTRODUCTION

Investigations of the nutritional requirements of algal flagellates led Provasoli and Pintner (1953) to consider the ecological implications of their findings. Axenic culture in chemically defined media had revealed the need for exogenous vitamin B<sub>12</sub>, and a source of this vitamin, in natural waters where the algal species investigated were seen to flourish, had therefore to be assumed.

Since then, about half the algal strains grown in pure culture have shown this requirement for vitamin B<sub>12</sub>; needs for biotin and thiamine are also common (Droop 1962). Obviously, in any particular aquatic environment where the ecology of the algae or of other organisms dependent on them is being studied, the possibility exists that populations are limited in growth or influenced in their species composition by the availability of vitamin B<sub>12</sub> or other trace-substance requirements. Ideally these trace substances should be measured together with the grosser nutrients and other variables.

In the sea, there are considerable variations of vitamin B<sub>12</sub> concentration with season (Cowey 1956; Vishniac and Riley 1961; Menzel and Spaeth 1962) and with depth (Kashiwada et al. 1957; Daisley and

Fisher 1958; Menzel and Spaeth 1962; Carlucci and Silbernagel 1966). However, few measurements of vitamin B<sub>12</sub> have been made in freshwater. Robbins, Hervey, and Stebbins (1950) carried out a ten-month survey of a pond in the New York Botanic Garden and found that vitamin B<sub>12</sub> concentration fluctuated between 100 and 2,000  $\mu\text{g}/\text{ml}$ . Benoit (1957) assayed a series of samples from different depths in a Connecticut lake and listed values from 60 to 150  $\mu\text{g}/\text{ml}$ —higher than any in this survey.

The lakes of the English Lake District form a graded series, from those with rocky shores and clear water, lying among steep slopes (e.g., Buttermere, Crummock Water) through intermediate (Derwent Water) to silted lakes with greater quantities of suspended and organic matter, surrounded by gentler soil-covered slopes (e.g., Bassenthwaite Lake, Windermere, and Esthwaite Water). This series was considered by Pearsall (1921, 1930) to represent an evolutionary sequence (primitive rocky  $\rightarrow$  evolved silted). Round (1957), classifying 17 lakes according to their sediments, achieved a grouping close to Pearsall's.

Esthwaite, Windermere, and another "evolved" lake, Blelham Tarn, have been much studied (*see* Lund 1961; Heron 1961). Lund (1949) discussed the differing characteristics of the north and south basins of Windermere (the south basin is more

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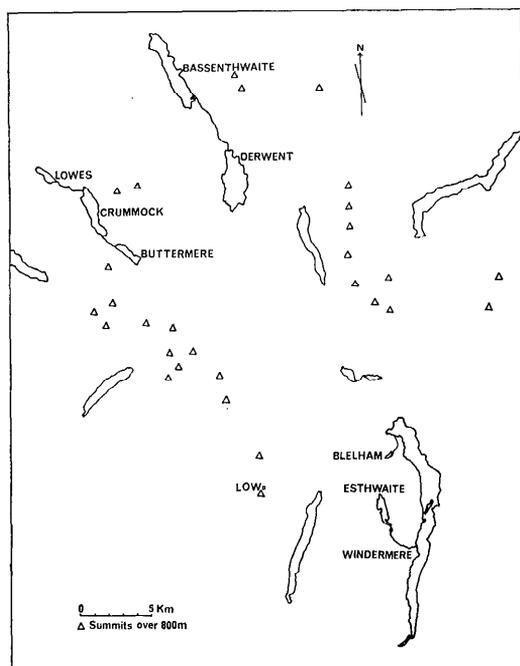


FIG. 1. Index map of lakes from which water samples for vitamin B<sub>12</sub> assay were taken (English Lake District).

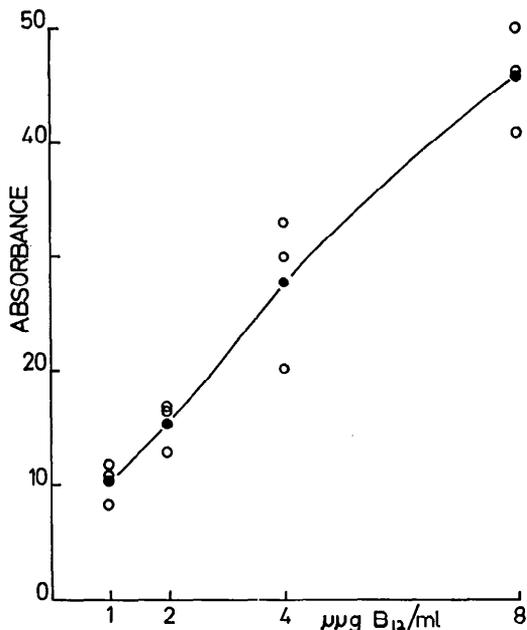


FIG. 2. Vitamin B<sub>12</sub> dose-response curve of *Euglena gracilis*. Assay flasks run in triplicate and individual readings (open circles) averaged (closed circles).

productive) and treated them separately, as I have done here also. One lake not described in the references cited is Low Water. This is an ultraoligotrophic rocky pool, in a corrie (cirque) on a mountainside (The Old Man of Coniston). To the descriptions of the lakes already published, this paper adds information on vitamin B<sub>12</sub> concentrations.

I am grateful to Dr. J. W. G. Lund, F.R.S., who invited me to undertake this study and collected and dispatched all the samples; and to Prof. S. K. Kon, C.B.E., for his encouragement.

#### METHODS

Nine lakes were sampled at monthly intervals (Fig. 1). The Windermere North and South, Blelham Tarn, and Esthwaite samples were taken at points where the water is 60, 32, 15.5, and 13 m deep respectively; other samples were from open rocky shore areas, over a water depth of about 1 m. All can be considered as represent-

ing the main lake water masses, with no nearby disturbing influence such as a stream or sewage entering.

Samples (0.5 liter) were poured without vacuum suction through GF/C glass-paper filters, which retain almost completely those organisms larger than bacteria. Portions of the filtered water were frozen in polythene bottles and these, with the filter pads and a packing of solid carbon dioxide, were transported to the laboratory. There the *Euglena gracilis* Klebs strain  $\alpha$  assay of Hutner, Bach, and Ross (1956) was used, except that culture densities were read by the method of Epstein and Weiss (1960). A typical standard curve is shown in Fig. 2.

The vitamin B<sub>12</sub> in the materials retained on the filters was extracted by heating in the presence of cyanide (Ford 1953): macerating the filters in 20 ml of 0.02 M phosphate-0.01 M citrate buffer pH 4.6 and 0.1 ml of 0.01% sodium cyanide solution and heating this slurry in flowing steam for

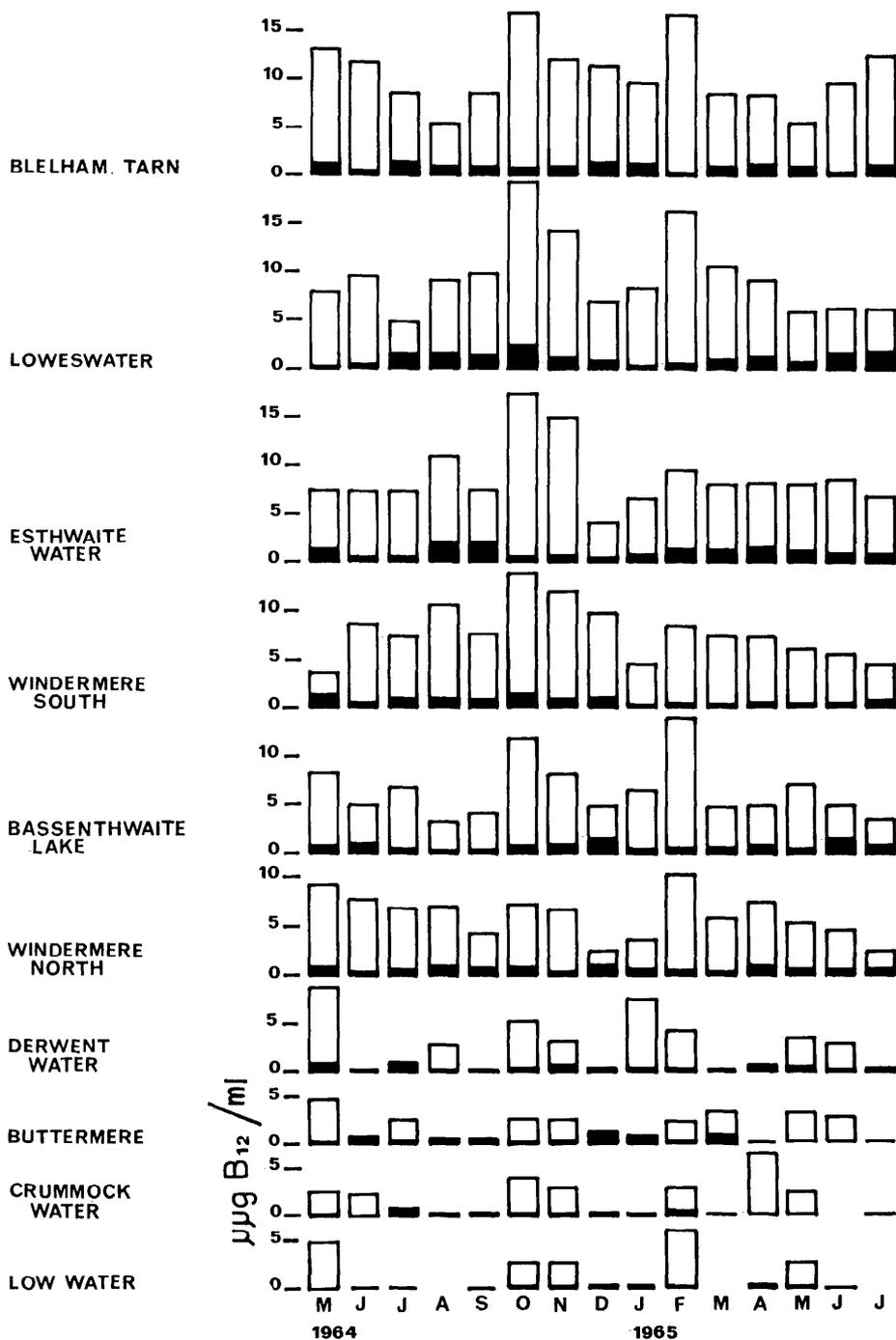


FIG. 3. Vitamin B<sub>12</sub> concentrations in  $\mu\text{g}/\text{ml}$  in the lake waters, May 1964–July 1965: open blocks, filtered water; filled blocks, retained by filter. Absence of a block, readings too low to be calculable in the assay (*see text*).

30 min before filtering. The volume was made to 50 ml before assay.

#### RESULTS

The results are shown in Fig. 3. In the filtered lake water assays, values of less than 1  $\mu\mu\text{g/ml}$  were not considered calculable from the assay standards and have been omitted; for the material off the filters, where in effect, 50 ml of extract represented 500 ml of original lake water poured through the filter, the corresponding lower limit is 0.1  $\mu\mu\text{g/ml}$ .

#### DISCUSSION

Two limitations of the method used must first be emphasized.

First, *E. gracilis* responds to a range of vitamin B<sub>12</sub> analogues (Ford 1953) wider than would be effective for some other species; for example, pseudovitamin B<sub>12</sub>, Factor A, and Factor F can substitute for vitamin B<sub>12</sub> in maintaining growth of *Euglena* but not of *Ochromonas malhamensis*, which has a specificity like that of higher animals. Information on the natural occurrence of such analogues in lake waters, and on the specificities of the component organisms of the lakes' populations towards them, is inadequate for any further comment on this aspect.

Second, autoclaving the assay flasks before inoculation with *Euglena* can be expected to liberate vitamin B<sub>12</sub> from bound forms (complexed with proteins or other substances) that may have existed in the original samples. Such bound vitamin B<sub>12</sub> would have an ecological importance different from that of free vitamin B<sub>12</sub> (see Daisley 1957; Ford 1958; Droop 1966).

The vitamin found in any one sampling must represent a momentary state of the balance between vitamin-producing and vitamin-removing factors. Vitamin B<sub>12</sub> is synthesized by microorganisms, including some of those in pond water, though it should be remembered that the production of vitamin B<sub>12</sub> in laboratory culture by a given microorganism does not necessarily mean that it will in nature produce the vitamin in excess of its own requirement.

Any vitamin producers in the water, in the bottom mud, and in the soil of the surrounding watershed will make contributions to the total vitamin present that will fluctuate not only with their populations but also with such factors as water turbulence and rainfall. Alteration in the local numbers of humans, farm animals, and wild animals will also affect the amount of vitamin-rich sewage entering the lakes.

Vitamin-removing factors are no less complex. Both dissolved and particulate vitamin may be lost by streams leaving the lakes, depending in part on rainfall. Organisms living in the lakes will take up the vitamin—the event which gives the vitamin its ecological importance—but here there will be differences between dissolved and particulate vitamin B<sub>12</sub>. Some species will use the vitamin in solution, some the particulate form, and others perhaps both. Some organisms taking up the vitamin may release it later, at death or before; others may degrade the molecule beyond further use. There may therefore be some recycling of vitamin B<sub>12</sub>, but within an overall pattern of fluctuating synthesis and removal.

The results (Fig. 3) indicate that some lakes (Blelham Tarn, Loweswater, and Esthwaite) tend to be richer in vitamin B<sub>12</sub> throughout the year than are others (Buttermere, Crummock, and Low Water). There is some indication of seasonal variation at least in some lakes (e.g., Loweswater and Esthwaite both show high values in October and November, followed by low levels), but the differences are much smaller than the seasonal algal changes (J. W. G. Lund, personal communication); nor is there any consistent ratio of filterable to filter-retained vitamin, nor any close resemblance between the May-June-July values of the two years.

The correlation that can be observed is not a seasonal one, but an overall one seen when the lakes are listed (as in Fig. 3) in their comparative vitamin B<sub>12</sub> richness, averaged for the period of the survey. When this listing is compared with those made previously on other bases, the

"evolved" silted lakes, comparatively rich in other nutrients, sediments, and organisms, are also richer in vitamin B<sub>12</sub> and the "primitive" rocky, clearwater lakes of low productivity are poorer.

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