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A PRELIMINARY INVESTIGATION OF THE VARIATION OF VITAMIN B_{12} IN OCEANIC AND COASTAL WATERS

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(Text-figs. 1–4)

The presence in the sea of vitamin B_{12}, or of its analogues, has been found necessary for the growth of several species of unicellular plants (e.g. Provasoli & Pintner, 1953; Droop, 1954, 1955).

Bioassays of the quantities in inshore waters during winter have been made by Lewin (1954) and by Droop (1954). The present investigation, carried out in 1955, was designed to extend this study and compare different seasons of the year.

Experimental

In the work reported here the vitamin B_{12} content of oceanic and coastal waters in areas investigated regularly by vessels of the Scottish Home Department laboratory at Aberdeen was examined over a period of several months. Surface sea water and plankton samples were collected from the Butt of Lewis (58° 40′ N., 6° 10′ W.), the Norwegian Deeps (60–61° N., 3–4° E.), the Northern North Sea (58–60° N., 0–2° W.), the Faroe Channel (60° N., 7° W.) and the North Atlantic (58° N., 17° W.) during 1955. These areas contrast markedly in their content of planktonic indicator organisms (see for example, Fraser, 1952), and it seemed feasible that their biological differences might be causally related to differences in their content of micronutrients, including vitamin B_{12}. Thus it was hoped that if spatial and temporal differences in the vitamin B_{12} content of sea water occur, both would become evident during the course of the work.

In general three 4 l. samples were collected at each station and treated as follows: (*a*) filtered and preserved, (*b*) unfiltered and unpreserved (stored in darkness at 15° C), and (*c*) unfiltered and preserved. In addition, when circumstances permitted, samples were Seitz-filtered, thus removing any bacteria present, and preserved. The preservative used was that recommended by Hutner & Bjerknes (1948), consisting of a mixture by volume of 1 part *o*-fluorotoluene, 2 parts *n*-butyl chloride, and 1 part 1:2-dichloroethane. Plankton hauls were made at each station with vertically towed Hensen and Standard non-closing nets.

The Hensen hauls were made with a 70 cm diameter net of 60 meshes to the inch, from 100 m (or near the bottom in shallower water) to the surface. The Standard hauls were made with a 50 cm diameter net of 180 meshes per inch, towed from 50 m to the surface.

Preliminary experiments indicated that none of the organisms that can conveniently be used for assaying vitamin B_{12} grew in normal sea water, probably because of the salinity and the low concentration of the vitamin. Hence it was necessary to reduce the concentration of salt in the sea water and to concentrate the vitamin.

After experimenting with several approaches the following standardized procedure was adopted:

The pH of a 4 l. sample of sea water was adjusted to 5 by the addition of 8 g of potassium dihydrogen phosphate, 0·2 ml. of a 1 % solution of sodium cyanide were added and the mixture was maintained at 80–90° C for 20 min. After cooling the solution was saturated with liquid phenol (about 230 ml.) and the whole extracted with 10 successive 30 ml. portions of liquid phenol.

The phenolic extracts were combined and 150 ml. portions were shaken with 300 ml. diethyl ether. The aqueous layer that separated at this stage was collected and the phenolic phase was further extracted with ten 10 ml. portions of distilled water. The combined aqueous extracts so obtained were buffered with sodium-β-glycerophosphate at the rate of 0·5 g/100 ml., and the pH was brought to 7·4 by the addition of a drop or two of concentrated hydrochloric acid. The extract was then concentrated under reduced pressure (at 45° C) to about 70 ml. Traces of phenol were removed from the solution by shaking it with three 20 ml. portions of diethyl ether. Ether was then removed from the aqueous solution by distillation under reduced pressure and the solution was made to the desired volume (40–60 ml.). This extract was then suitably diluted for assay.

Plankton, which was preserved in 60 % acetone, was extracted by steaming off the acetone, adding a few drops of a 1 % solution of sodium cyanide, reducing the pH to 5·0 with 0·1 N-HCl and maintaining at 80° C for 20 min. The extracts were made to a desired volume, filtered through a Whatman no. 42 filter-paper and then diluted appropriately for assay.

Extracts were assayed with *Ochromonas malhamensis* (Ford, 1953) and with *Lactobacillus leichmannii*, ATCC 4797, by the method of Skeggs, Nepple, Valentik, Huff & Wright (1950) modified as described by Ford (1953).

It has been known for some time that, besides vitamin B_{12} (cyanocobalamin) itself, there exist in natural materials several analogues of vitamin B_{12} (see, for example, Kon, 1955). These are active to different extents for various organisms (Coates, Ford, Harrison, Kon & Porter, 1953; Droop, 1955) and organisms requiring 'vitamin B_{12}' differ in the extent to which they can utilize different analogues. Thus higher animals are generally narrowly specific in their

utilization of vitamin B_{12} analogues but certain micro-organisms (e.g. *Escherichia coli*) are non-specific. Table I (compiled by Dr J. E. Ford) indicates the relative potencies of vitamin B_{12} analogues of natural occurrence for *Lactobacillus leichmannii* and *Ochromonas malhamensis*. Clearly, if the vitamin B_{12} activity of a given natural material is consistently higher for

TABLE I. RELATIVE ACTIVITY OF VITAMIN B_{12} ANALOGUES AND DEOXYRIBOSIDES FOR ASSAY ORGANISMS

Factor	*Ochromonas malhamensis*	*Lactobacillus leichmannii*
Vitamin B_{12}	100	100
Vitamin $B_{12\,III}$	40	50
Factor B	Inactive	Inactive
Factor A	Inactive	20
Pseudo-vitamin B_{12}	Inactive	50
Factor C	Inactive	Inactive
Deoxyribosides	Inactive	Active

TABLE II. RECOVERY FROM SEA WATER, BY 'PHENOL PASSAGE' TECHNIQUE, OF VITAMIN B_{12} ADDED TO IT

Sample	Vitamin B_{12} present. Portion extracted directly and assayed (μg/l.)	Vitamin B_{12} added to second portion (μg./l.)	Vitamin B_{12} found in second portion (μg/l.)	Percentage recovery
$3\frac{1}{2}$% saline	—	0·02	0·017	85
Unfiltered unpreserved sea water from 58° 40′ N., 6° 10′ W. (23 April)	0·003	0·004	0·006	75
Filtered preserved sea water from 60° N., 4° E. (1 June)	0·0003	0·004	0·0036	80
Unfiltered unpreserved sea water from 59° N., 1° E. (3 August)	0·0015	0·0006	0·002	83
Unfiltered unpreserved sea water from 61° N., 3° E. (10 August)	0·0035	0·001	0·0044	90
Unfiltered unpreserved sea water from 58° 40′ N., 6° 10′ W. (30 August)	0·0066	0·003	0·0094	90
Unfiltered unpreserved sea water from 59° N., 1° W. (3 October)	0·0033	0·004	0·0071	95

Lactobacillus leichmannii than for *Ochromonas malhamensis* and provided the higher *leichmannii* response is shown, by appropriate controls, not to be due to the presence of deoxyribosides in the material, it is reasonable to infer that vitamin B_{12} analogues active for *Lactobacillus leichmannii* but inactive for *Ochromonas malhamensis* are present in the material. Thus the presence of vitamin B_{12} analogues in natural materials can be detected by differential assays of this sort. Recovery tests (Table II) were performed by dividing a 4 l. sea-water sample into two 2 l. portions. One of these was extracted directly;

vitamin B_{12} in about the concentration expected in the sample was added to the other which was then extracted. The two extracts were then assayed and the percentage of recovered vitamin B_{12} determined.

Results

The results obtained are shown in Table III. Values for filtered preserved samples are taken as indicating the concentration of vitamin B_{12} in the sea water; those for unfiltered unpreserved samples as an indication of the concentration which vitamin B_{12} might reach as a result of bacterial multiplication and action on detritus and organic matter contained in the water; those for the unfiltered preserved samples indicate the total amount of vitamin B_{12} present in the water (i.e. that present in solution and in suspended matter), they form controls to the corresponding unfiltered, unpreserved samples.

The vitamin B_{12} content of filtered preserved samples throughout the period under investigation varied between 0·006 μg/l. in coastal water (Wee Bankie, 56° 11′ N., 2° 08′ W., in January) and 0·0001 μg/l. in North Atlantic water (57° 55′ N., 17° W., in July); since the Seitz-filtered, bacteria-free, samples so far examined contained vitamin B_{12} in the same concentration as paper-filtered preserved samples collected at the same time, it is not unreasonable to assume that all the 'filtered preserved' values represent vitamin B_{12} present in solution in the sea. Droop (1954) gives a value of 0·005–0·01 μg vitamin B_{12}/l. for West Scottish coastal waters in February and March 1954, and Lewin (1954) found 0·01 μg vitamin B_{12}/l. in sea water of the North-west Arm (Halifax, Nova Scotia) during the winter of 1952–53; their values agree adequately with that of 0·006 μg/l. for Wee Bankie in January and 0·016 μg/l. for Loch Fyne in March. Droop goes on to state that a cobalamin concentration of this order would not be a limiting factor in the growth of diatoms and other vitamin B_{12}-requiring protista. The cobalamin concentration of 0·0001 μg/l. recorded in North Atlantic water in July might, however, prove limiting, though vitamin B_{12} is probably only one of several factors (e.g. nitrate and phosphate concentrations) likely to be limiting at this time of the year.

Droop (1955, private communication) has also drawn attention to the fact that certain chrysomonads, and the only two diatoms known to require vitamin B_{12} (*Skeletonema costatum* and *Amphora perpusilla*) respond to analogues of vitamin B_{12} as well as to cyanocobalamin itself, and states 'it is likely that the analogues of vitamin B_{12} will have an importance equal to that of the vitamin in the ecology of the sea'. Differential assays of our sea-water extracts have shown the presence of vitamin B_{12} analogues in sea water. Water collected at Aberdeen Bay in January contained 0·006 μg vitamin B_{12}/l. when assayed with *Lactobacillus leichmannii*, and 0·004 μg/l. when assayed with *Ochromonas malhamensis*. Since vitamin B_{12} analogues are less

TABLE III. VITAMIN B_{12} CONTENT OF SEA WATER AND PLANKTON

NORTHERN NORTH SEA

Sample	Locality and date: 59° N., 0° 40′ E. 29 May	60° 43′ N., 0° 30′ E. 3 June	59° N., 1° E. 3 Aug.	60° N., 00° 00′ 9 Aug.	58° 45′ N., 00° 00′ 7 Sept.	57° 15′ N., 1° 30′ W. 12 Sept.	59° 00′ N., 1° 00′ W. 3 Oct.	57° 00′ N., 1° 00′ W. 10 Oct.
Sea water								
Filtered preserved (μg/l.)	0·0002	0·0002	0·00013	0·0002	0·0007	0·0006	0·0009	0·0012
Unfiltered preserved (μg/l.)	0·00035	0·0002	0·00015	0·00018	0·001	0·005	—	—
Unfiltered unpreserved (μg/l.)	0·0005	0·0006	0·0015	0·004	0·0075	0·008	0·0083	0·0042
Plankton: Hensen net haul								
Total (μg)	0·6	1·2	0·2	0·07	—	—	—	—
Wet wt. (μg/g)	0·06	0·07	0·03	0·01	—	—	—	—
Plankton: Standard net haul								
Total (μg)	0·07	0·4	0·01	0·02	—	—	—	—
Wet wt. (μg/g)	0·03	0·06	0·01	0·003	—	—	—	—

Sample	BUTT OF LEWIS Locality and date: 58° 40′ N., 6° 10′ W. Feb.	58° 46′ N., 6° 30′ W. 4 Apr.	58° 40′ N., 6° 10′ W. 23 Apr.	58° 40′ N., 6° 10′ W. 30 Aug.	NORWEGIAN DEEPS Locality and date: 61° 01′ N., 3° 30′ E. 4 Apr.	60° 00′ N., 4° 00′ E. 1 June	61° 01′ N., 3° 30′ E. 10 Aug.
Sea water							
Filtered preserved (μg/l.)	0·002	0·001	0·0004	0·0006 0·00055 (Seitz-filtered)	0·002	0·0003	0·0005
Unfiltered preserved (μg/l.)	—	—	0·0008	0·0006	0·003	0·0002	0·0005
Unfiltered unpreserved (μg/l.)	0·004	0·006	0·003	0·0066	0·006	0·0005	0·004
Plankton: Hensen net haul							
Total (μg)	0·02	0·06	0·1	0·27	—	0·1	0·13
Wet wt. (μg/g)	0·05	0·05	0·07	0·04	—	0·03	0·08
Plankton: Standard net haul							
Total (μg)	0·005	0·02	0·5	0·05	—	0·006	0·03
Wet wt. (μg/g)	0·02	0·02	0·06	0·06	—	0·06	0·003
Seitz filter: used in filtering 4 l. sea water (μg)	—	—	—	0·0004	—	—	—

WEE BANKIE, LOCH FYNE, FAROE CHANNEL AND NORTH ATLANTIC

Sample	Locality and date: 56° 11′ N., 2° 08′ W. Wee Bankie Jan.	Loch Fyne Mar.	60° 23′ N., 7° 49′ W. (F.C.) 23 Apr.	59° 56′ N., 7° 27′ W (F.C.) 31 Aug.	59° 54′ N., 14° 15′ W. (N. Atl.) 8 July	57° 55′ N., 17° 00′ W. (N. Atl.) 19 July
Sea water						
Filtered preserved (μg/l.)	0·006	0·16	0·0003	0·00073 0·0007 (Seitz-filtered)	0·00025	0·0001
Unfiltered preserved (μg/l.)	—	—	0·0002	0·0009	0·0003	0·0002
Unfiltered unpreserved (μg/l.)	—	—	0·008	0·005	0·005	0·003
Plankton: Hensen net haul						
Total (μg)	0·02	—	—	0·3	0·2	0·02
Wet wt. (μg/g)	—	—	—	0·04	0·03	0·005
Plankton: Standard net haul						
Total (μg)	0·01	—	—	0·08	0·01	0·002
Wet wt. (μg/g)	—	—	—	0·02	0·008	0·003
Filter-paper: used in filtering 4 l. sea water (μg)	—	—	—	0·0007	—	—
Seitz filter: used in filtering 4 l. sea water (μg)	—	—	—	0·0008	—	—

Notes. The vitamin B_{12}, found in the filter-paper (0·0007 μg) after filtering 4 l. of sea water through it, presumably represents the vitamin B_{12} present in suspended matter in the water. There would then be 0·0007/4, i.e. 0·00017, μg vitamin B_{12} present in suspended matter in each litre of sea water. If this value is added to that obtained for the filtered preserved water (0·00073 μg/l.) the sum tallies well with the value obtained for the unfiltered preserved sample, from which suspended matter had not been removed. A similar agreement was obtained with the Seitz-filtered and unfiltered preserved samples.

active for *Lactobacillus leichmannii* than vitamin B_{12} itself (e.g. factor A has only 20% of the activity of vitamin B_{12}) they are clearly present in this instance in a concentration greater than 0·002 μg/l. In the oceanic waters I have so far examined the concentration of analogues present when compared with that of vitamin B_{12} was very low, and a situation has not yet been encountered where the relative concentrations of vitamin B_{12} analogues and vitamin B_{12} were such as to limit the growth of organisms able to utilize vitamin B_{12} only, while permitting growth of organisms responding to vitamin B_{12} analogues and to vitamin B_{12}. In Table III only one value for vitamin B_{12} concentration is quoted for each sample from each station, since values obtained from *L. leichmannii* and *Ochromonas malhamensis* assays were not significantly different. When sea-water extracts used for routine assays were further concentrated and chromatographed cyanocobalamin alone could be detected. However, chromatograms of an extract of a 90 l. bulk of sea water showed that, besides vitamin B_{12}, factor B was also present.

TABLE IV. VITAMIN B_{12} ANALOGUES DETECTED IN MARINE ANIMALS

	Factor B	Factor A	Pseudo-vitamin B_{12}	Vitamin B_{12}
Haddock, gut contents	+	+	+	+
Cod, gut contents	+	o	+	+
Crangon allmani gut and contents	+	o	+	+

Ericson & Lewis (1953) have demonstrated that marine bacteria, isolated from sea water and from marine algae, are able to produce vitamin B_{12} factors. Similarly, the presence of such factors has now been shown chromatographically in the contents of the alimentary tract of various marine animals (Table IV), where they doubtless arise as a result of bacterial metabolism. Excretion by marine animals must contribute to some extent to the vitamin B_{12} analogues to be found in the sea.

The most obvious feature of the results to date is a seasonal variation in the vitamin B_{12} content of the waters examined; it is amply demonstrated in Fig. 1 for the three areas sampled. Although lack of time has made it impossible to obtain results for all three areas over a 12-month period, the similarity in seasonal trends in these areas is already apparent. Furthermore, the concentration of vitamin B_{12} in the few samples of North Atlantic and Faroe Channel water that have been examined was similar to the concentration in water from the other three areas at that time. From a winter maximum of about 0·002 μg vitamin B_{12}/l. the concentration fell to a level of about 0·0002 μg/l. by July and then gradually increased again to the winter level. Though there were slight temporal differences between the sequences in the three areas, the vitamin B_{12} content of the waters was broadly similar at any given time and none of these waters was characterized by a consistently

higher vitamin B_{12} content than others. Thus in the biologically distinct areas so far examined a significant difference in the concentration of vitamin B_{12} has not been established.

The vitamin B_{12} content of the water decreased in March–April, the period during which plankton organisms were multiplying most rapidly. Figs. 2 and 3 show for the Butt of Lewis and Northern North Sea areas the total vitamin B_{12} content of Standard net and Hensen net plankton hauls taken at the same time as water samples. Though the volume of plankton taken must depend to some extent on weather conditions at the time, it can be seen that

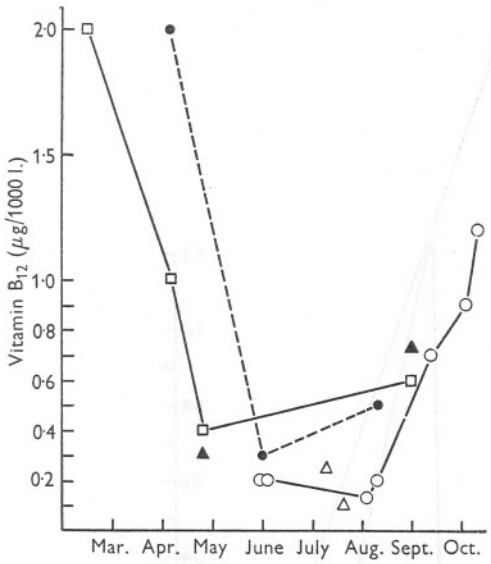

Fig. 1. Vitamin B_{12} content of filtered, preserved sea-water samples collected during 1955. □, Butt of Lewis; ●, Norwegian Deeps; ○, Northern North Sea; ▲, Faroe Channel; △, North Atlantic.

the quantity of vitamin B_{12} present in plankton was greatest when the vitamin B_{12} content of the water was lowest. Whether or not this was the result of a direct uptake of vitamin B_{12} from the sea water by diatoms is uncertain, since very little is known about the numbers of phytoplankton species that require vitamin B_{12} and the species, if any, that synthesize vitamin B_{12}, but the finding at least suggests the possibility. No estimate is as yet available of the relative contributions of the various factors affecting the regeneration of vitamin B_{12} in the sea during the late summer and autumn, though supposedly excretion by marine animals, bacterial action on detritus, and possibly mixing of surface waters with lower layers are all important in this respect.

In untreated sea water (unfiltered unpreserved samples) the number of bacterial species present falls during the first 3–6 h of storage, and there then follows an increase of many hundred per cent of the surviving species

(ZoBell & Anderson, 1936). It can be seen from Table III that, although the unfiltered unpreserved samples contained more vitamin B_{12} than the unfiltered preserved controls, the difference was never very great. Observations were not made of types and numbers of bacteria that multiplied in the unfiltered unpreserved samples and it is unlikely that similar types multiplied in each sample, but at least some of those that multiplied had a definite, if comparatively restricted, ability to produce vitamin B_{12} under conditions which

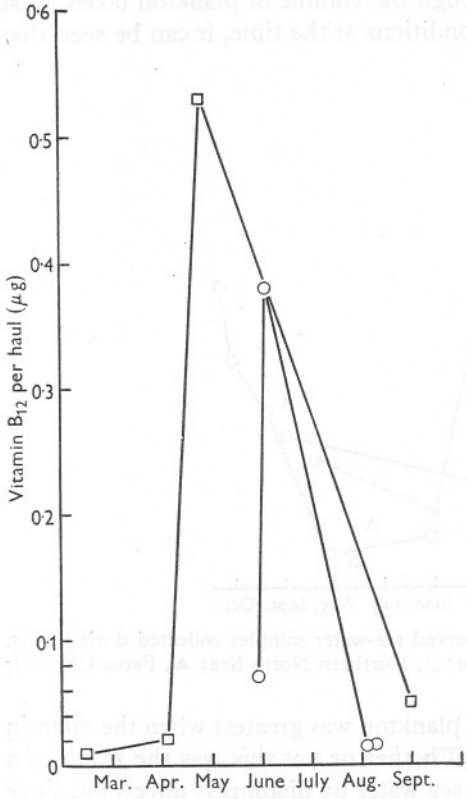

Fig. 2

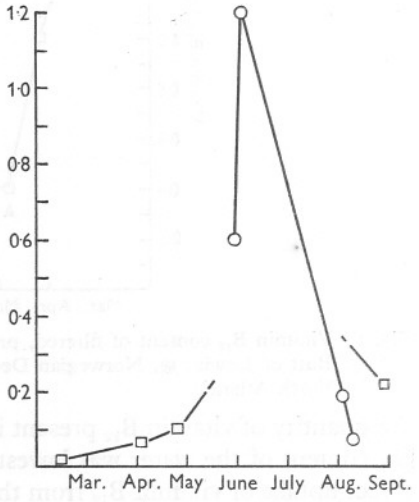

Fig. 3

Fig. 2. Vitamin B_{12} content of plankton taken in Standard net hauls during 1955. □, Butt of Lewis; ○, Northern North Sea.

Fig. 3. Vitamin B_{12} content of plankton taken in Hensen net hauls during 1955. □, Butt of Lewis; ○, Northern North Sea.

simulated the natural ones. The vitamin B_{12} content of the unfiltered unpreserved samples showed similar trends to those of the filtered preserved samples (Fig. 4), i.e. generally more vitamin B_{12} was produced in samples collected during autumn and winter than in samples collected during the summer.

The physical conditions (such as relationships between solid surface area and volume) likely to control bacterial multiplication in untreated sea water stored in bottles were maintained constant from sample to sample. If one can assume that the vitamin B_{12} content of these samples was in some measure a reflexion of bacterial activity, it seems that under these conditions sea water collected during autumn and winter was more conducive to production of vitamin B_{12} by bacteria than water collected in summer.

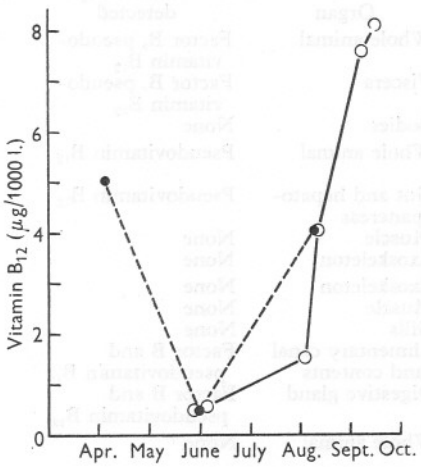

Fig. 4. Vitamin B_{12} content of unfiltered, unpreserved, sea-water samples collected during 1955. ○, Northern North Sea; ●, Norwegian Deeps.

The large zooplankton collections contained mainly *Calanus finmarchicus*, and their vitamin B_{12} content was 0·06–0·07 μg/g wet weight. Collections of *C. finmarchicus* alone contained 0·09 μg vitamin B_{12}/g wet weight (Table V). Another mixture of zooplankton, including the siphonophore *Lensia*, and the copepods *Rhincalanus*, *Pleuromamma*, and *Calanus finmarchicus*, gave 0·05 μg vitamin B_{12}/g wet weight. They could thus constitute an adequate source of vitamin B_{12} for higher marine forms. By comparison, the richest animal sources of vitamin B_{12} (e.g. cow liver) contain up to 1·3 μg vitamin B_{12}/g fresh tissue (Ford, Holdsworth & Porter, 1953).

There is not yet sufficient information to show whether species of the zooplankton from different sea-water masses differ in their vitamin B_{12} content. Indications are that they do not: thus *Meganyctiphanes norvegica* and *Euchaeta norvegica* both contained 0·05 μg vitamin B_{12}/g wet weight no matter where they were collected.

Phytoplankton contained much less vitamin B_{12} per unit wet weight than zooplankton. Thus samples of live phytoplankton in hauls were found to contain 0·02–0·03 μg vitamin B_{12}/g wet weight. The comparative richness of

zooplankton may indicate a rapid rate of grazing; on the other hand, zooplankton might derive its vitamin B_{12} largely from a bacterial flora in the gut.

TABLE V. VITAMIN B_{12} IN MARINE INVERTEBRATES

Species	Organ	Analogues of vitamin B_{12} detected	Activity expressed in terms of vitamin B_{12}. Wet weight (μg/g) *Escherichia coli*	*Ochromonas malhamensis*
Crangon allmani Kinahan	Whole animal	Factor B, pseudovitamin B_{12}	0·14	0·12
	Viscera	Factor B, pseudovitamin B_{12}	0·5	0·4
	Bodies	None	0·09	0·08
Pandalus bonnieri Caullery	Whole animal	Pseudovitamin B_{12}	0·14	0·11
	Gut and hepatopancreas	Pseudovitamin B_{12}	0·52	0·45
	Muscle	None	0·07	0·06
	Exoskeleton	None	0·13	0·09
Nephrops norvegicus L.	Exoskeleton	None	0·03	0·02
	Muscle	None	0·03	0·03
	Gills	None	0·03	0·02
	Alimentary canal and contents	Factor B and pseudovitamin B_{12}	0·08	0·05
	Digestive gland	Factor B and pseudovitamin B_{12}	0·12	0·11
Meganyctiphanes norvegica (M. Sars)	Whole animal	None	0·06	0·05
Euchaeta norvegica Boeck	Whole animal	None	0·05	0·06
Thysanoessa raschii (M. Sars)	Whole animal	None	0·08	0·07
Calanus finmarchicus (Gunnerus)	Whole animal	None	0·09	0·09
Aurelia spp. and *Ctenophores*	Whole animal	None	0·02	0·02

REMARKS

This work is exploratory and hence, of necessity, is only capable of giving pointers in new directions. No decisive conclusions are possible. Because of the time involved in the extraction procedure, results had to be based on single 4 l. samples from each station, i.e. there is no statistical basis for the values quoted. This drawback can best be overcome, and work of this nature generally facilitated, by acquiring marine micro-organisms that could be used for rapid direct assay of micronutrients in sea water. Furthermore, values obtained by direct assay of sea water are perhaps intrinsically more reliable than those obtained otherwise.

Besides the need for information on the concentration in sea water of vitamins, amino acids, and growth factors generally, the nutrition and synthetic powers of marine bacteria require examination, more especially of

any forms associated with diatoms *in vivo* or inhabiting the alimentary tracts of marine animals. Only then can we construct a reasonable picture of the interplay between different factors, upon which productivity in the sea is ultimately dependent.

I am grateful to the Director of the Scottish Home Department Laboratory at Aberdeen for providing facilities for the collection of sea water and plankton samples, and to Drs R. Johnston and J. H. Fraser of that Laboratory for constructive discussion in the initiation and indeed throughout the course of this work. I am especially indebted to Dr Johnston for supervising the collection of samples.

I have derived much benefit from the interest, advice and critical approach during discussions of Drs L. R. Fisher, J. E. Ford, E. S. Holdsworth and S. K. Kon.

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Summary

The vitamin B_{12} concentration in biologically distinct sea waters has been measured over a period of several months during 1955, a solvent-extraction technique being used to concentrate the vitamin and reduce the salt concentration in the water.

Similar seasonal changes in vitamin B_{12} content were found in water from the Butt of Lewis, Northern North Sea, and Norwegian Deeps, with a high winter level (about 0·002 μg/l.) and low summer level (about 0·0002 μg/l.). There was no evidence of a spatial variation in vitamin B_{12} concentration of the waters examined.

The vitamin B_{12} contents of plankton from hauls taken throughout the year (1955) at stations where water samples were collected, and of collections of individual zooplankton species were also determined and are reported on.

REFERENCES

Coates, M. E., Ford, J. E., Harrison, G. F., Kon, S. K. & Porter, J. W. G., 1953. Vitamin B_{12}-like compounds. 1. Vitamin B_{12} activity for chicks and for different micro-organisms of gut contents and faeces. *Brit. J. Nutr.*, Vol. 7, pp. 319–26.

Droop, M. R., 1954. Cobalamin requirement in Chrysophyceae. *Nature, Lond.*, Vol. 174, p. 520.

—— 1955. A pelagic marine diatom requiring cobalamin. *J. mar. biol. Ass. U.K.*, Vol. 34, pp. 229–31.

Ericson, L.-E. & Lewis, L., 1953. On the occurrence of vitamin B_{12}-like factors in marine algae. *Ark. Kemi*, Bd. 6, pp. 427–42.

Ford, J. E., 1953. The microbiological assay of vitamin B_{12}. The specificity of the requirement of *Ochromonas malhamensis* for cyanocobalamin. *Brit. J. Nutr.*, Vol. 7, pp. 299–306.

FORD, J. E., HOLDSWORTH, E. S. & PORTER, J. W. G., 1953. The occurrence of cyanocobalamin and related compounds in natural materials. *Proc. Nutr. Soc.*, Vol. 12, p. xi.

FRASER, J. H., 1952. The Chaetognatha and other zooplankton of the Scottish area and their value as biological indicators of hydrographical conditions. *Mar. Res. Scot.*, No. 2, 52 pp.

HUTNER, S. H. & BJERKNES, C. A., 1948. Volatile preservatives for culture media. *Proc. Soc. exp. Biol., N.Y.*, Vol. 67, pp. 393–7.

KON, S. K., 1955. Other factors related to vitamin B_{12}. *Symp. biochem. Soc.*, No. 13, pp. 17–35.

LEWIN, R. A., 1954. A marine *Stichococcus* sp. which requires vitamin B_{12} (cobalamin). *J. gen. Microbiol.*, Vol. 10, pp. 93–6.

PROVASOLI, L. & PINTNER, I. J., 1953. Ecological implications of *in vitro* nutritional requirements of algal flagellates. *Ann. N.Y. Acad. Sci.*, Vol. 56, pp. 839–51.

SKEGGS, H. R., NEPPLE, H. M., VALENTIK, K. A., HUFF, J. W. & WRIGHT, L. D., 1950. Observations on the use of *Lactobacillus leichmannii* 4797 in the microbiological assay of vitamin B_{12}. *J. biol. Chem.*, Vol. 184, pp. 211–21.

ZOBELL, C. E. & ANDERSON, D. Q., 1936. Observations on the multiplication of bacteria in different volumes of stored sea water. *Biol. Bull., Woods Hole*, Vol. 71, pp. 324–42.