津久井湖表層水におけるビタミンB12・チアミン・ビオチンの季節変化

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著者名	大和田,紘一 乙幡,恵 多賀,信夫
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Seasonal Cycles of Vitamin B_{12}, Thiamine and Biotin in the Surface Water of Lake Tsukui*

Kouichi OHWADA**, Megumi OTSUHATA*** and Nobuo TAGA**

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Vitamin B_{12}, thiamine and biotin concentrations in the surface water of Lake Tsukui have been determined over a period of two years, in 1970 and 1971, by the use of microbiological assay methods. The assay organism used for the determination of vitamin B_{12} was *Lactobacillus leichmannii* ATCC 7830, for thiamine was the marine yeast, *Cryptococcus albidus*, and for biotin the marine bacterium, *Achromobacter* sp. strain yH-51. Dissolved vitamin B_{12} concentration decreased remarkably and particulate portions increased considerably in the surface water at most times when diatoms, *Cyclotella* sp., *Fragilaria crotonensis* and *Synedra acus*, were dominant. Thiamine and biotin concetnrations increased very much in both dissolved and particulate forms in the surface water in such times when diatoms were dominant. From the results, the possible importance of vitamin B_{12} as one of the ecological factors in Lake Tsukui is suggested.

Vitamins such as vitamin B_{12}, thiamine and biotin have been suggested as one of the important factors which might regulate the growth and/or succession of phytoplankton in nature.[1–4] This assumption has been based on the fact that many species of phytoplankton require the three vitamins singly or in these combinations when they are cultured in the laboratory.[2,5–7] However, there remains a need for an evaluation of the importance of the vitamins ascertain whether they really affect the growth of phytoplankton in natural waters.

In 1970 and 1971, the present authors measured the concentrations of vitamin B_{12}, thiamine and biotin in Lake Sagami and Lake Tsukui to correlate the seasonal changes in their concentrations with the cycles of phytoplankton abundance. Some of the results have already been reported.[8,9] This paper describes the seasonal cycles of the vitamin concentrations in the surface water of Lake Tsukui and their relations with appearance of dominant phytoplankton during 1970 and 1971.

Materials and Methods

Lake Tsukui is an artificial lake located at the north-western part of Kanagawa Prefecture. The lake was impounded in 1965 as the reservoir for power generation and drinking water supply by damming River Sagami, which flows through Kanagawa Pre-

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** Ocean Research Institute, University of Tokyo, Nakano, Tokyo, Japan (大和田紘一・多賀信夫: 東京大学海洋研究所)

*** Nagasawa Filter Plant, Water Works Bureau of Tokyo, Ikuta, Tama-ku, Kawasaki-shi, Kanagawa-ken, Japan (乙幡 恵: 東京都水道局長沢浄水場)

fecture and empties into Sagami Bay. It has an area of 2.39 km^2 at the highest level which is 124 meters above sea level, with a storage capacity of 6.23×10^7 cubic meters. Lake Sagami has been formed as reservoir at the upstream along River Sagami. Map of the study area is shown in Fig. 1. Limnological features of the lake have been studied by

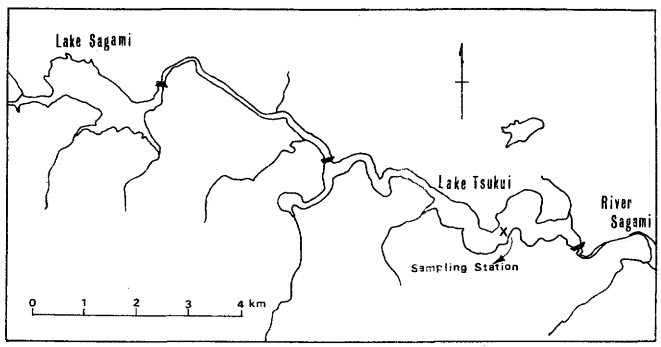

Fig. 1. Map showing the study area.

Hashimoto.[10] Sampling was carried out for two years at monthly intervals from December, 1969, to November, 1971, at the fixed station in the central part of Lake Tsukui. Only surface water samples were collected in the present study. Chemical analyses were made according to the procedures of the standard methods.[11] Chlorophyll *a* content of particulate matter was determined by the fluorescence method.[12]

The water samples were used directly or after being concentrated by centrifugation, according to the density of phytoplankton contained. Then, each 0.1 ml portion of the sample was pipetted on to a ruled slide glass, and numbers of phytoplankton in the sample were counted and identified under microscope.

Enumeration of heterotrophic bacteria was carried out by the surface colony counts method. The inoculated plates were incubated at 20°C for two weeks before bacterial colonies on agar plates were counted. The medium[13] used in the present investigation had following composition: polypeptone, 2.0 g; bacto-yeast extract, 1.0 g; glucose, 1.0 g; agar, 15.0 g; tap water, 1,000 ml; final pH, 7.0–7.2.

Water samples for vitamin assays were immediately filtered through HA Milliporer® filters (porosity, 0.45 μm), and the filtrates were then frozen at −20°C. Particulate matters were collected on HA Millipore filters and were kept frozen until the time of the vitamin assays.

Amounts of vitamin B_{12}, thiamine and biotin in the samples were determined by microbiological assay methods. The assay organism used for the determination of vitamin B_{12} was *Lactobacillus leichmannii* ATCC 7830.[14] Thiamine was assayed with the marine yeast, *Cryptococcus albidus* (Saito) Skinner.[15] Biotin was assayed with the marine bacterium, *Achromobacter* sp. strain yH–51[16]. Some modifications from the original

methods were made for the assay of thiamine and biotin in lake water samples. Turbidity of the culture of each assay organism in external and internal standards after a defined incubation period was used to calculate the amount of each vitamin in the sample. Calculated values for several different dilution levels of each sample, carried out in duplicate, fell within $\pm 10\%$ of the average.

Extraction of the vitamins from particulate matters were carried out according to the procedures of the Association of Vitamin Chemists[17] with some modifications as follows: Samples for vitamin B_{12} assay were extracted for 10 min at 120°C with phosphate-citrate buffer (pH 4.5) containing 0.01% of potassium cyanide. Samples for thiamine assay were extracted for 30min at 100°C with 0.1N hydrochloric acid solution with occasional shaking. The extracts were, then, cooled to room temperature and pH of each extract was adjusted to 4.5 with 2.5 M sodium acetate solution. Ten mg of Taka-diastase (Sankyo Co.) was added to each extract, and it was incubated at 37°C for 12 hr being covered with toluene to enzymatically hydrolyze bound thiamine. Samples for biotin assay were extracted for 30 min at 120°C with 1 N sulfuric acid solution. Each extract was adequately diluted and its pH was adjusted, then used for determination of the vitamins.

Results

The temperature of the surface water during 1970 and 1971 varied in the range between 6.5°C and 29.3°C, being highest in August and lowest in February. Presumably as a result of photosynthesis by phytoplankton, surface water became considerably alkaline from spring to summer. Maximum pH of the surface water was 9.8 from May to August, while pH of the water during the circulation period was 7.2.

Species and densities of dominant phytoplankton in the surface water of Lake Tsukui during 1970 and 1971 are shown in Table 1. In order to summarize the species composition of the dominant phytoplnakton in the lake as a whole, only phytoplankton which appeared more than 500 cells/ml were listed in the table. Diatom, *Cyclotella* sp., began to develop in February, and it dominated in the water of Lake Tsukui during spring both in 1970 and 1971. On 10 August, 1970, a bloom of blue-green alga, *Anacystis cyanea* (=*Microcystis aeruginosa*),[18] was observed. During the bloom cell density of the blue-green alga increased up to 19,850/ml and chlorophyll *a* content 12.19 mg/m^3.

Fig. 2 shows annual variation of the dissolved vitamin concentrations, chlorophyll *a* content and population of heterotrophic bacteria in the surface water during 1970. Concentration of dissolved vitamin B_{12} began to decrease with the development of diatom, *Cyclotella* sp., in February, and level of vitamin B_{12} concentration was always very low while the diatom has dominated in the lake except on 13 July. When a bloom of *Anacystis cyanea* occurred, vitamin B_{12} concentration became very high. In autumn season when

Table 1. Dominant phytoplankton (Number/ml) observed in the surface water of Lake Tsukui during 1970 and 1971.

1970				1971			
Date		Dominant phytoplankton*	No/ml	Date		Dominant phytoplankton*	No/ml
March	9	*Cyclotella* sp.	640	March	8	*Cyclotella* sp.	870
April	14	*Cyclotella* sp.	11,600	April	13	*Cyclotella* sp.	860
		Unknown species (*Monas*)	560			*Peridinium* sp.	670
May	18	*Cyclotella* sp.	570	May	12	*Cyclotella* sp.	11,300
		Unknown species (*Monas*)	26,000			Unknown species (*Monas*)	3,820
June	8	*Scenedesmus quadricauda*	670	June	14	*Fragilaria crotonensis*	1,220
		Cryptomonas sp.	500			*Synedra acus*	520
						Scenedesmus quadricauda	1,130
						Unknown species (*Monas*)	1,430
July	13	*Cyclotella* sp.	5,450	July	12	*Synedra acus*	4,670
		Nitzschia paleacea	2,140			*Scenedesmus quadricauda*	500
		Nitzschia acicularis	570			*Cryptomonas* sp.	650
Aug.	10	*Synedra acus*	770	Aug.	9	*Scenedesmus quadricauda*	720
		Anacystis cyanea	19,850				
				Nov.	12	*Stephanodiscus subsalsus*	2,160

* "Dominant phytoplankton" in the table indicates phytoplankton which appeared more than 500/ml in cell number.

chlorophyll *a* content was becoming very low, vitamin B_{12} concentration remained at high level compared with that found in spring. On the other hand, both dissolved thiamine and biotin concentrations showed a different seasonal variation from that of vitamin B_{12}. Namely, thiamine and biotin rapidly increased in concentration with the rapid development of diatom, *Cyclotella* sp., in spring. Although their concentrations fluctuated with months, their concentration levels continued to be considerably high in spring and summer when chlorophyll *a* content was high. In autumn season their concentrations became very low again. Thus, significant relationships were found between the fluctuations of the dissolved vitamins and abundance of dominant phytoplankton in the surface water of Lake Tsukui during 1970. No clear relationships were, however, observed between the seasonal variations of the vitamin concentrations and population of heterotrophic bacteria.

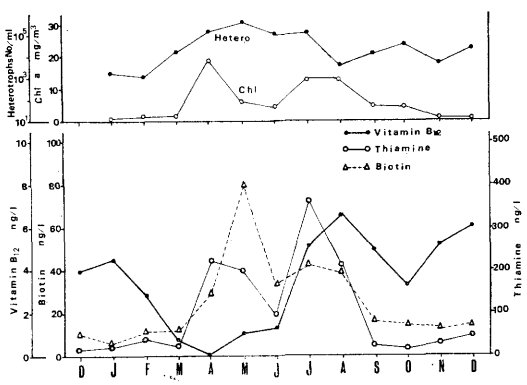

Fig. 2. Annual variation of vitamin concentrations, chlorophyll *a* and number of heterotrophic bacteria in the surface water of Lake Tsukui during 1970.

In order to investigate further on the ecological significance of the vitamins in Lake

Tsukui, the three vitamins of particulate form were also measured in the surface water in addition to the dissolved ones during 1971. The results obtained are given in Table 2. As observed in spring of preceding year, dissolved vitamin B_{12} concentration decreased markedly (0.5 ng/l) and those of thiamine and biotin increased (276 ng/l of thiamine and

Table 2. Seasonal variation of dissolved and particulate vitamins in the surface water of Lake Tsukui during 1971.

Date	Vitamin B_{12}			Thiamine			Biotin		
	Dissolved	Particulate	P/D	Dissolved	Particulate	P/D	Dissolved	Particulate	P/D
	(ng/l)		(%)	(ng/l)		(%)	(ng/l)		(%)
May 12	0.5	7.3	1,460	276	158.5	58	34.8	3.55	10
June 14	u*	20.3	—**	197	82.6	42	13.0	4.24	33
July 12	u	10.5	—	436	67.5	15	49.8	0.55	1
Aug. 9	1.9	9.9	521	274	106.1	39	58.0	1.83	3
Oct. 13	4.2	1.9	45	75	30.5	41	15.0	0.53	4

* U represents the amount less than the present assay limit.

** Percentage value of P/D cannot be calculated because of the lower amount of dissolved vitamin B_{12} than the present assay limit.

34.8 ng/l of biotin) on 12 May when *Cyclotella* sp. increased in number. It was observed further that vitamin B_{12} concentration in particulate matter was found to be very high (7.3 ng/l) in contrast to the low amount of dissolved one. As a result, amount of the particulate vitamin B_{12} (P) was found to be as much as 14.6 times higher than that of the dissolved one (D) in the surface water at that time. Such situation was observed also on 14 June and 12 July, 1971, when diatoms have dominated in the surface water. As number of phytoplankton decreased markedly in autumn circulation period (13 October), dissolved vitamin B_{12} concentration became higher and particulate one became lower. Such remarkable variations of P/D values with months were not recognized in the case of both thiamine and biotin, and P/D values fluctuated between 15 and 58% in thiamine and between 1 and 33% in biotin in the surface water during 1971.

Discussion

Above results demonstrate some ecological relationships between the seasonal variations of the vitamin concentrations and the dominant phytoplankton in Lake Tsukui, even though only surface water samples have been investigated in the present study. It was observed that dissolved vitamin B_{12} concentration decreased remarkably and particulate one increased considerably in the surface water of Lake Tsukui in most times when diatoms, such as *Cyclotella* sp., *Fragilaria crotonensis* and *Synedra acus*, were dominant. While, thiamine and biotin concentrations increased very much both dissolved and particulate forms during the time. It can be considered from the results that dissolved vitamin B_{12}

in the surface water would be taken up by the dominant diatoms and some other phytoplankton during their growing seasons. On the contrary, thiamine and biotin would be produced and released to lake water by them. Such phenomena have already been demonstrated experimentally with the axenic cultures of some unicellular marine algae.[19] OHWADA and TAGA[8,9] found such marked decrease of dissolved vitamin B_{12} concentration and increase of particulate one in the surface water of Lake Sagami during summer stagnation period when diatoms were abundant. From the results they suggested the possible role of vitamin B_{12} as one of the ecological factors which might regulate the growth and/or succession of phytoplankton in Lake Sagami. Though species composition of the dominant diatoms and the phase of their abundant season were somewhat different from each other, fairly corresponding relationships between the fluctuations of vitamin B_{12} and dominant diatoms were recognized in Lake Tsukui as well as in Lake Sagami. This observation is considered to support the previous suggestion by the present authors.

Recently, Hagedorn[20–22] studied thiamine in some lakes of Eastern Holstein, Germany. He found by adding thiamine into natural lake water that certain kinds of phytoplankton in the lake, such as *Chlorococcales*, started an increase of the exponential rate of growth after 24 hr, which continued 4 days. From the results he has pointed out the possible importance of thiamine for the regulation of the succession of phytoplankton in the lake.

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