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6 June 1960

Molybdenum as a Factor Limiting Primary Productivity in Castle Lake, California

Abstract. A trace-element deficiency is evident from carbon-14 bioassays of Castle Lake's natural phytoplankton populations. Increase in photosynthetic rates with the addition of molybdic acid or sodium molybdate has been demonstrated throughout the year. It is likely that other trace elements may also be found to be limiting factors in lakes having a limited watershed.

A study of primary productivity and limiting factors in a number of northern California lakes was started in March 1959. Castle Lake was among the lakes selected for this study. It is in a cirque basin at an elevation of 5200 feet in the Klamath Mountains, 11 miles southwest of Mt. Shasta, California. Its surface area is 19.4 hectares, and the lake has a maximum depth of 36.5 m. Studies on trout production in the lake have been in progress since 1938 (*1*).

A rapid carbon-14 bioassay technique developed for limiting-factor investigations in Alaskan lakes in 1957 (*2*) revealed changes in photosynthetic rate under various nutrient conditions. The lake's natural, unconcentrated phytoplankton population was studied under both field and laboratory conditions. Cultures in the field were contained in sterile, screw-cap, 500-ml Pyrex erlenmeyer flasks fastened to a floating crib anchored in the lake near shore. This arrangement provided lake-surface conditions of temperature and light and maintained the plankton population in suspension through wave motion of the crib. In the laboratory, cultures were held at a constant (10°C) temperature under a 40-watt fluorescent light.

In June 1959 cultures in both the lake and laboratory showed very significant increase in carbon fixation with the addition of potassium, sulfate, or a trace-element mixture. Subsequent cultures in which trace elements were added separately demonstrated that molybdenum was the stimulating micronutrient. Addition of 0.100 part of molybdenum (as Na_2MoO_4) per million increased the rate of photosynthesis of the lake phytoplankton over a 4-day period in June (Fig. 1). This was accompanied by a high rate of primary productivity in the lake. The addition to cultures of 0.100 part of molybdenum (as molybdic acid) per million had the same effect as the addition of Na_2MoO_4.

Other experiments started throughout the summer, fall, and winter of 1959 gave nearly identical results. Responses early in the season were greater; responses diminished somewhat with the seasonal decline in primary productivity and perhaps with rainfall in August and September. By October, 0.050 part of molybdenum per million was more effective than higher concentrations. In cultures maintained in the lake under 1 m of ice on 10 January 1960, the addition of 0.025 part of molybdenum per million gave a significant response during a 4-hour period. Although an increase in the rate of photosynthesis was consistently evident with the addition of from 0.001 to 0.050 part of molybdenum per million, the optimum was about 0.025. In the early summer of 1960, the addition of 0.005 part per million gave a greater response than higher concentrations.

The essential role of molybdenum in the growth of higher plants has been recognized since 1939 (*3*). It was demonstrated as a requirement for *Chlorella* in 1953 (*4*) and for *Scenedesmus* in 1955 (*5*). The low levels of molybdenum evident in a number of inland waters and the involvement of molybdenum in nitrogen reduction and fixation have suggested its possible importance in lakes (*6*). Although this is the first time molybdenum has been reported to be a limiting factor in lakes, it is probable that it and other micronutrients will be found in limiting concentrations in lakes with restricted watersheds.

The importance of molybdenum in the formation of nitrate reductase and in nitrogen fixation is well established (*7*). In attempting to identify more specifically the factors involved in the molybdenum deficiency in winter, Mo. NH_4^+ and ND_3^- were added singly and in combination. An almost totally nannoplankton population is characteristic of Castle Lake under ice conditions. Lack of response in *in situ* winter cultures with NH_4^+ or NO_3^-, and response to the addition of Mo and NH_4^+ would seem not to favor a reductase requirement. Nitrogen fixation by these microplankton remains a possibility as does a deficiency in more than a single enzyme system.

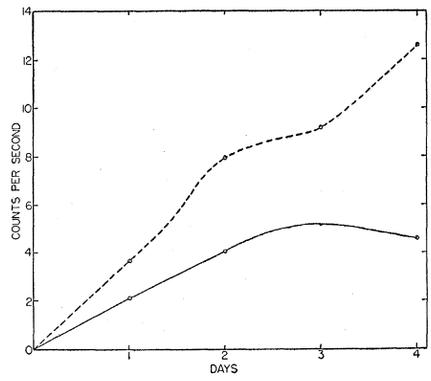

Fig. 1. Stimulation in the rate of carbon fixation (as measured by C^{14} uptake) with the addition of molybdenum to a culture of Castle Lake's natural phytoplankton population maintained at lake-surface temperature and light. Solid line, the control; broken line, the culture with 0.10 part of molybdenum per million added. This experiment was started on 29 June 1959.

The alder trees (*Alnus tenuifolia* Nutt) that are abundant along the east shore of Castle Lake make an appreciable nitrogen contribution to the lake, principally in the form of NO_3^- (*8*). Reduction of this nitrate by phytoplankton presumably would require molybdenum, which is in the lake in suboptimal concentrations. Analysis of the alder leaves showed molybdenum to be present in trace quantities (< 0.1 part per million). The nitrogen-fixing alder trees and other plants may be competing with the lake for the available Mo, K^+, and SO_4^{--} which would otherwise be added by the springs draining the alder-covered shore line. Culture experiments carried out during June and July of 1960 showed a lower response to the addition of molybdenum than those of the previous year. Precipitation between March and August of 1959 was only 35 percent of the mean annual precipitation for those months, and thus it seems likely that replenishment of molybdenum by inflow is a critical factor in reducing the level of deficiency (*9*).

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8 August 1960

Simple Technique for Study of Cortical Arousal Response

Abstract. A new method is described for eliciting a stable spindle burst response after the production of unilateral lesions in the reticular formation of the cat. This permits quantifying the effects of stimulation of the reticular activating system, either electrically or by injection of epinephrine, on the cortical arousal response.

The cortical arousal response, produced by either direct or indirect stimulation of the reticular activating system, has been used extensively as a test for drug action since its first description in 1949 (*1*). Unfortunately, unless facilities for elaborate electronic analyses are available, the evaluation of the magnitude and duration of this response is quite subjective. We herein describe a method for quantification of this response in cats by the use of unilateral brain stem lesions which produce a stable ipsilateral cortical spindle burst response. This is abolished during electrical or pharmacological stimulation of the reticular activating system.

Bremer in 1935 (*2*) discovered that transection of the anterior portion of the midbrain (*cerveau isolé*) led to spindle bursts which recurred during several hours. Lindsley *et al.* (*3*) reported on lesions in different areas of the brain stem and demonstrated stable spindle burst activity after mesencephalic and basal diencephalic bilateral lesions.

As an extension of their work we placed complete unilateral electrolytic lesions stereotaxically, using the coordinates described in the atlas of Jasper and Ajmone-Marsan (frontal, 3; horizontal, −2; vertical, 0 to 4) (*4*). The exact frontal placements were not critical for the production of the spindle bursts. There was an indication, however, that the response decayed somewhat more rapidly as the lesion was moved caudad or cephalad.

Surgery was performed under ether anesthesia; the animal was subsequently immobilized with gallamine triethiodide and maintained on artificial respiration and local anesthesia with 1 percent procaine hydrochloride. The animals were in a sound proof room and were kept as comfortable as possible during the recording sessions.

Some experiments were carried out using the *encephale isolé* preparation and, although the burst characteristics and background activity were slower, the response remained as stable as that seen with the immobilized animal. After a 1 hour recovery period, spontaneous electrical activity recorded from the cortex ipsilateral to the lesion showed the spindle burst phenomenon. The rate of bursting varied from 8 to 15 bursts per minute from animal to animal, but was relatively constant for a given animal. A stable response could be maintained for 3 to 4 hours if the animal remained in good condition. The spindle bursts could always be recorded from the suprasylvian and posterior sigmoid gyri and sometimes from the anterior sigmoid gyrus.

Since the contralateral side of the brain stem was intact, electrical or drug stimulation of the reticular activating system could still produce a cortical arousal response. This response was manifested by a disappearance of the spindle burst activity and, in some cases, by an increase in the frequency of the background activity of both sides of the cortex. The duration of the abolishment of the spindle bursts was directly correlated with the strength of the electrical stimulus or the dose of epinephrine, within normal physiological ranges. Figure 1 illustrates the effects of sciatic nerve stimulation and of intravenous epinephrine on the cortical spindle bursts in a cat with a unilateral brain stem lesion.

The disappearance of the spindle bursts lasted throughout the duration of the sciatic stimulation and about 10 to 15 seconds after the end of the stimulation. The rate and frequency of the electrocorticogram on the contralateral cortex seemed to return to prestimulus activity about 4 or 5 seconds after the return of the bursts on the ipsilateral side. Intravenous epinephrine caused the burst response to vanish about 10 seconds after the beginning of the injection (see *5*). The bursts began to reappear about 30 seconds later and sometimes showed a secondary effect of an increased rate for about 10 to 20 seconds before returning to the preinjection rate (*6*).

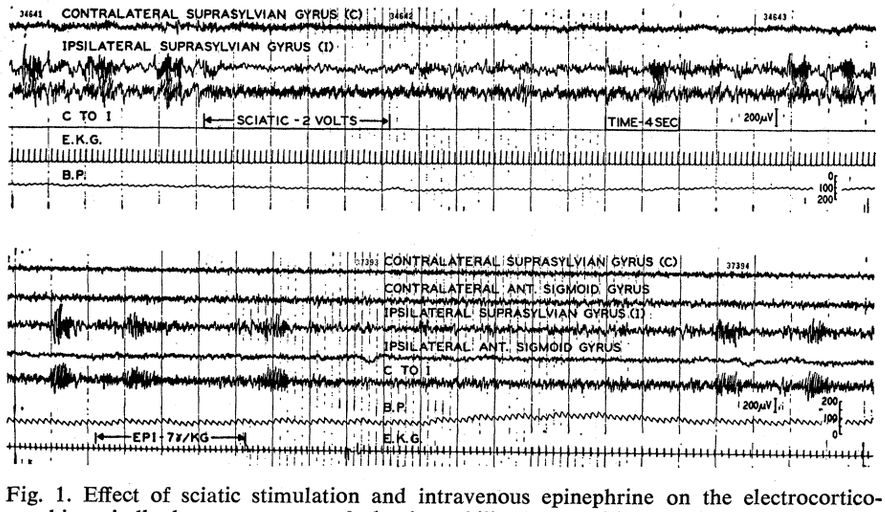

Fig. 1. Effect of sciatic stimulation and intravenous epinephrine on the electrocorticographic spindle burst response of the immobilized cat with a unilateral brain stem lesion. Upper section: Sciatic nerve stimulation (10 pulse/sec) of a 3.0 kg male cat. Lower section: Epinephrine injection into femoral vein of a 2.8 kg male cat.

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