contain many HBsAg-positive cells. The population enriched for HBsAg with the cell sorter was markedly reduced for the proportion of EBNA positive cells. Attempts to clone the HBV-infected cells are underway. However, it is possible that the RAC/BM cells requires the presence of the population infected with Epstein-Barr virus or factors produced by the latter cells for continuous growth in culture.

The proportion of cells infected in the mixed RAC/BM culture fluctuates from 10 to 20 percent, and the amount of HBsAg produced appears to be low. Nevertheless, positive cells were still present after more than 10 months in culture, and the proportion remained higher than the proportion of cells that were positive in the fresh bone marrow population.

The presence of HBcAg in some of the cells and the detection of virus-like particles at the density expected for Dane particles suggests that the RAC/BM culture might produce infectious HBV. Again, however, based on the amount of HBsAg detected, the numbers of virus-like particles produced is probably quite low.

The only pathological lesions regularly associated with HBV infections are those found in the liver. Perhaps because of this many virologists have assumed that hepatocytes and liver macrophages (Kupffer cells) are the only major cellular target for infection in vivo. Several cultures have been established from patients with primary liver cancer. At least two, PLC/PRF/5 and Hep 3B, appear to contain HBV genome and to make HBsAg in large quantities (*3*, *4*). Both are composed of adherent epithelial cells which presumably represent malignant hepatocytes, but neither culture appears to make whole virus. Our studies suggest that bone marrow should also be considered as a possible site for HBV infection in vivo and that various cell populations of hematopoietic origin should be evaluated for susceptibility to HBV replication both in vitro and in vivo.

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Low Nitrogen to Phosphorus Ratios Favor Dominance by Blue-Green Algae in Lake Phytoplankton

Abstract. *An analysis of growing season data from 17 lakes throughout the world suggests that the relative proportion of blue-green algae (Cyanophyta) in the epilimnetic phytoplankton is dependent on the epilimnetic ratio of total nitrogen to total phosphorus. Blue-green algae tended to be rare when this ratio exceeded 29 to 1 by weight, suggesting that modification of this ratio by control of nutrient additions may provide a means by which lake water quality can be managed.*

The ability to predict and manage algal biomass and transparency in lakes has been greatly improved by the development of empirical models of eutrophication due to phosphorus loading (*1*). However, whether specific lake restoration measures currently in use will significantly reduce the proportion of nuisance blue-green algae (Cyanophyta) in the epilimnetic phytoplankton cannot yet be predicted with confidence.

Numerous hypotheses have been proposed to explain the success of blue-green algae in eutrophic lakes (*2*). Although the importance of nitrogen to phosphorus (N:P) ratios in determining algal blooms has been discussed since the work of Pearsall (*3*) and Hutchinson (*4*), there have been comparatively few direct studies of the relation between N:P ratios and the presence of blue-green algae (*5*, *6*). I report a dramatic tendency for blue-green algal blooms to occur when epilimnetic N:P ratios fall below about 29:1 by weight, and for blue-green algae to be rare when the N:P ratio exceeds this value.

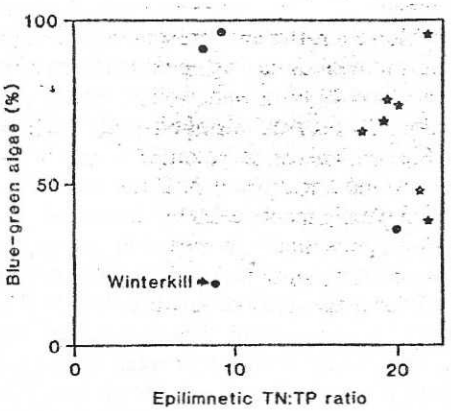

Fig. 1. Relation between the growing season mean proportion of blue-green algae and epilimnetic total nitrogen (*TN*) to total phosphorus (*TP*) ratios in Lake Trummen, Sweden. All blue-greens (unicellular, colonial, heterocystous, and nonheterocystous filamentous species) were included in the calculation of their proportion in the phytoplankton (*11*). Symbols represent data from one growing season for 11 years of measurements (*13*); circles indicate years before dredging, and stars, years after the lake was dredged.

The nutrient physiology of the Cyanophyta differs from that of other algae in that many blue-green species are capable of nitrogen fixation. This ability allows nitrogen-fixing species to maintain high growth rates in habitats deficient in inorganic nitrogen, and they should thus be superior nutrient competitors under conditions of nitrogen limitation. In addition, blue-green species that apparently do not fix nitrogen, such as *Microcystis aeruginosa*, may be as abundant as nitrogen-fixing forms during times of nitrate deficiency (*7*). In contrast, Tilman *et al.* (*8*) suggested that blue-green algae (both those that fix nitrogen and those that do not) are generally inferior to diatoms as phosphorus competitors, indicating that blue-green algae should typically be dominant in lakes with low N:P ratios (in which most phytoplankton species would be nitrogen-limited) and rare in lakes with high N:P ratios. Flett *et al.* (*9*) found that nitrogen-fixing blue-green algae were typically associated with lakes

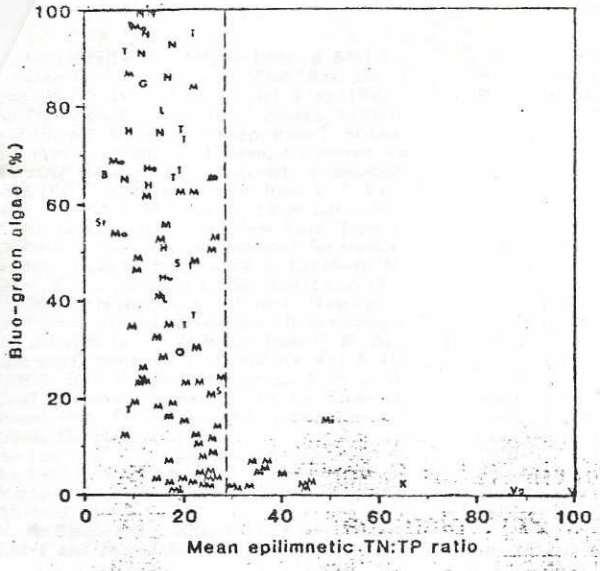

Fig. 2. Relation between the growing season mean proportion of blue-green algae by volume and epilimnetic total nitrogen (*TN*) to total phosphorus (*TP*) ratios in 17 lakes worldwide. The lakes are: *S*, Sammammish; *L*, Loch Leven; *Mo*, Moses; *N*, Norrviken; *O*, Ontario; *T*, Trummen; *H*, Hjälmaren; *V*, Vättern; *Vn*, Vänern; *M*, Mälaren (11 bays); *Hu*, Huron; *St*, Stone; *B*, Bysjön; *He*, Heart; *Mi*, Michigan; *G*, George; and *K*, Kinneret. See (*14*) for data sources. Each symbol represents data from one growing season. Boundary for *TN:TP* = 29 is shown by the dashed line.

having N:P supply ratios less than 10:1 by weight and were mostly absent from those with a greater N:P supply ratio. Their conclusions, however, were based on data of presence or absence rather than on quantitative measures of dominance (*10*).

In order to test the hypothesis that low N:P ratios promote dominance by blue-green algae, I compiled data from 17 lakes worldwide. For each lake, I calculated the average proportion of all blue-green algae using biomass in the epilimnion during the growing season (*11*). I also obtained data for the growing season mean epilimnetic total nitrogen (*TN*) and total phosphorus (*TP*) concentrations (*12*).

Lake Trummen, Sweden, showed a clear reduction in the proportion of blue-green algae as a result of changes in the epilimnetic *TN:TP* ratio. Although sewage and waste water were diverted from this lake in 1958, it did not recover until the nutrient-rich upper sediments were removed by suction dredging in 1970–1971 (*13*). This procedure greatly reduced internal nutrient loading, and a sharp decline in the proportion of blue-green algae accompanied the subsequent increase in the epilimnetic *TN:TP* ratio (Fig. 1). When the data from Lake Trummen and 16 additional lakes (*14*) were plotted together (Fig. 2), an apparent boundary was evident between a region where blue-green algae tended to dominate (*TN:TP* < 29 by weight) and a region where blue-green algae tended to be rare (*TN:TP* > 29 by weight). These data are consistent with those from other studies (*5*, *6*, *9*), and they provide strong support for the hypothesis that nutrient ratios are one important determinant of the species composition of natural phytoplankton communities. They also suggest that blue-green algae are generally better nitrogen competitors, but poorer phosphorus competitors, than other groups of algae.

More information is needed before an empirical model that accurately predicts dominance by blue-green algae in lakes can be designed. The N:P hypothesis alone is not sufficient to explain the presence or absence of blue-green algae in all lakes, since it is evident from Fig. 2 that many lakes having *TN:TP* ratios < 29 were dominated by non–blue-green algae (*15*). For example, despite a low *TN:TP* ratio, Cyanophyta were not dominant in Lake Trummen after a winter fishkill (Fig. 1), suggesting that the structure of other trophic levels can alter the response of phytoplankton to nutrients.

However, the data presented here suggest that lakes having epilimnetic *TN:TP* ratios > 29 by weight will typically exhibit low proportions of blue-green algae (Fig. 2). This is of practical significance since modification of N:P ratios can be achieved in many lakes by sewage diversion, phosphorus removal from waste water, or nutrient precipitation within the lakes themselves. Epilimnetic *TN:TP* ratios typically increase as a result of these lake restoration techniques (*16*), and the data in Fig. 2 suggest a preliminary N:P ratio toward which agencies concerned with water quality might aim (*17*). Nitrogen removal is often practiced by advanced waste water treatment plants, and in some cases this may be counterproductive if it results in low N:P ratios in downstream lakes. Leonardson and Ripl (*6*) suggested that waste water nitrogen treatment can be optimized to maintain proper water quality in lakes receiving such effluent. Alternatively, in other lakes, nitrogen fertilization may be of practical value (*6*, *9*).

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17. Note that an epilimnetic *TN:TP* ratio of 29:1 should not be equated with such a value in loading. The retention and recycling of nitrogen within lakes differs from that of phosphorus, and an epilimnetic *TN:TP* ratio > 29:1 by weight may result from much lower loading ratios (*9*). Nitrogen-loading models such as that of R. W. Bachmann [in *Restoration of Lakes and Inland Waters* (EPA-440/5-81-010, Office of Water Regulations and Standards, Washington, D.C., 1981), pp. 320–324] can be coupled with phosphorus loading models (*1*) to predict epilimnetic *TN:TP* ratios.
18. I thank V. J. Bierman, Jr., W. T. Edmondson, G. G. Ganf, N. P. Holm, J. Kalff, D. W. Schindler, J. Shapiro, E. B. Swain, D. Tilman, and an anonymous reviewer for help and comments, and I thank J. DePinto for the use of unpublished data. Supported by NSF grant DEB-7921755 to J. Shapiro and a NATO postdoctoral fellowship to V.H.S. Contribution 190 from the Limnological Research Center, University of Minnesota.

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Naltrexone Modulates Tumor Response in Mice with Neuroblastoma

Abstract. *Naltrexone, an opiate antagonist, had both stimulatory and inhibitory effects, depending on the dosage, on the growth of S20Y neuroblastoma in A/Jax mice. Daily injections of 0.1 milligram of naltrexone per kilogram of body weight, which blocked morphine-induced analgesia for 4 to 6 hours per day, resulted in a 33 percent tumor incidence, a 98 percent delay in the time before tumor appearance, and a 36 percent increase in survival time. Neuroblastoma-inoculated mice receiving 1 milligrams of naltrexone per kilogram, which blocked morphine-induced analgesia for 24 hours per day, had a 100 percent tumor incidence, a 27 percent reduction in the time before tumor appearance, and a 19 percent decrease in survival time. Inoculation of neuroblastoma cells in control subjects resulted in 100 percent tumor incidence within 29 days. These results show that naltrexone can modulate tumor response and suggest a role for the endorphin-opiate receptor system in neuro-oncogenic events.*

In addition to their analgesic and behavioral effects, opioid compounds are known to alter cell function and growth, particularly in developing neural systems (*1*, *2*). Zagon and McLaughlin have reported that long-term administration of heroin to mice with transplanted neuroblastoma inhibits tumor growth and prolongs survival time (*3*). These antitumor effects were blocked by concomitant administration of naloxone, an opiate antagonist. Paradoxically, in subsequent studies in which only naloxone was used and at concentrations that also interact at the opiate receptor level (*4*), this agent was found to be extremely effective in preventing or retarding tumor appearance and improving the survival of neuroblastoma-inoculated mice. The mechanisms underlying heroin's and naloxone's actions in regard to neural neoplasia are unknown but may involve the endorphin-opiate receptor system (*3*, *4*).

In view of the antitumor properties of naloxone alone, we were prompted to examine the chemotherapeutic potential of naltrexone, a narcotic antagonist that is eight times as active and three times as long-acting as naloxone (*5*). We chose the C1300 murine neuroblastoma, a well-characterized tumor that resembles human neuroblastoma in many respects (*6*), to assess naltrexone's actions. The results show that naltrexone can promote tumorigenesis at a dosage that continuously prevents morphine-induced analgesia but exerts an antineoplastic effect at a dosage that only temporarily blocks antinociception by morphine.

Male syngeneic A/Jax mice were inoculated with S20Y neuroblastoma cells and, beginning 2 days later, received daily subcutaneous injections of either 0.1, 1, or 10 mg of naltrexone per kilogram of body weight or sterile water (control). On day 29 after inoculation with tumor cells, all mice in the 10 mg/kg and control groups had measurable tumors, whereas only 75 percent of the 1 mg/kg group and no animal in the 0.1 mg/kg group had developed measurable tumors. By day 75, when every other tumor-bearing mouse had died (Fig. 1), 10 of 12 mice (83 percent) and 4 of 12 mice (33 percent) receiving 1 and 0.1 mg of naltrexone per kilogram, respectively, had developed tumors. The percentage of mice developing tumors in the latter group differed significantly from that of the controls ($P < 0.01$, chi-square test). Observations on the remaining mice for the next 25 days (that is, until 100 days after tumor cell inoculation) revealed no tumor development.

The survival time (Fig. 1) of mice receiving naltrexone (1 mg/kg) was comparable to that of control animals (mean and median life-spans = 50 days). Mice receiving naltrexone (10 mg/kg) survived for a significantly ($P < 0.02$) shorter time than control mice (mean and median life-spans 19 and 22 percent shorter, respectively, than controls). Moreover, the latency prior to tumor appearance for this group was reduced 28 percent from control values (21.25 ± 1.23 days). For those mice injected with naltrexone (0.1 mg/kg) that developed tumors, an increase in mean and median survival times of 42 and 36 percent, respectively, were recorded relative to controls, as well as a 98 percent increase in latency time prior to tumor onset.

In general, the patterns of tumor growth for mice in the control and 0.1 mg/kg groups were similar throughout

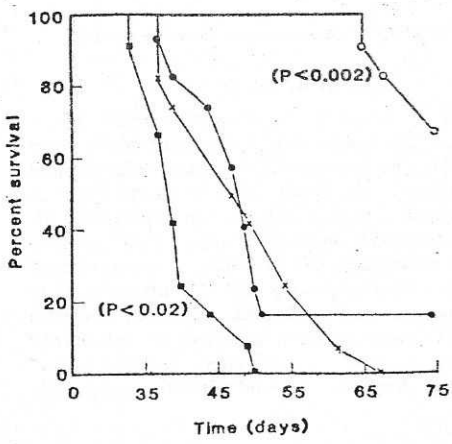

Fig. 1. Effect of daily subcutaneous injections of naltrexone (Endo Laboratories, Garden City, New York) on survival time (days after tumor cell inoculation) of mice inoculated with 10^6 S20Y neuroblastoma cells. The S20Y cells were cloned from the A/Jax mouse C1300 neuroblastoma and obtained from M. Nirenberg (National Institutes of Health, Bethesda, Maryland). Tumor cells were injected in the dorsal surface of the right shoulder. Survival curves for mice receiving naltrexone at dosages of 0.1 mg/kg (○), 1 mg/kg (●), or 10 mg/kg (■) or sterile water (×) were analyzed by the Mann-Whitney U test.