receptor-hormone complex. Excess (1000-fold) unlabeled hormone was added to prevent detectable recombinations of [^{3}H]DHT with receptor. Using the charcoal-dextran method of Korenman (*10*) we measured the half-life of the complexes under pseudo first-order conditions. The half-life was 74.5 hours at 0°C, with a rate constant of dissociation of 1.55×10^{-4} min^{-1}; at 0°C, all of the known extracellular androgen binding proteins, including the above-mentioned ABP and testosterone-estradiol binding globulin, have half-lives of dissociation in minutes (*6*). Using sucrose gradient centrifugation to separate bound and free steroid we measured the equilibrium constant (K_d) to be $2.99 \times 10^{-10}M$ (*2*).

In order to examine further the subunit structure of the DDT_1 cytoplasmic receptor, we utilized sucrose gradient analysis (*2*). Gradients (containing 0.4*M* KCl) analyzing the partially purified receptor peaks for DEAE and phosphocellulose or from cytosol treated with 0.4*M* KCl yielded receptor peaks sedimenting at 4.6 to 5*S* (Fig. 3). Similar gradients (without KCl) in similar cytosol preparations not treated with KCl revealed a peak sedimenting at 7*S* (Fig. 3). These data indicate that the 7*S* cytosol receptor in these cells has salt-dissociable 4.8*S* subunits that each bind androgen and are separable by DEAE chromatography. These results, in addition to our previous demonstration that this DDT_1 receptor enters the nucleus (*2*), exclude the possibility that the molecules are ABP's or other protein resembling the testosterone-estradiol binding globulin.

Our data indicate that the DDT_1 cytoplasmic receptor has several physical-chemical homologies with the highly purified chick oviduct progesterone receptor described by Schrader and O'Malley (*4*). The chick oviduct progesterone receptor has been suggested to have a subunit structure in which two 4*S* monomers combine to form a 6*S* dimer and also higher aggregates. The monomers appear to have diffferent functions in the nucleus, the A subunit binding preferentially to DNA and the B monomer binding to chromatin. This has led to the proposal (*11*) that the actual cytoplasmic form of the receptor is a 6*S* dimer, consisting of an A and B monomer, which moves into the nucleus and is bound to the chromatin acceptor site by the B subunit. The A subunit then dissociates from the B monomer and searches along adjacent chromatin regions until it locates and binds to a specific regulatory site on naked DNA. This model would explain the presence of subunits with different functions, but both binding steroid hormone.

We are not aware of a previous demonstration that the A and B monomers exist in only one cell type. Using DDT_1 cells, we have now shown that putative receptor subunits are present in a cloned cell line. This finding excludes the possibility that the multiple receptor forms from steroid target tissues are the result of mixing cytoplasm from different cell types during homogenization. These findings substantiate the hypothesis that the A and B monomers are subunits of a larger dimer existing within a single cell.

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Blue-Green Algae: Their Excretion of Iron-Selective Chelators Enables Them to Dominate Other Algae

Abstract. *During blue-green algal blooms, other algae can be completely suppressed. This ability of blue-green algae to suppress other algae may be determined by the availability of iron. Iron deprivation induces the production of hydroxamate chelators, which appear to be the agent suppressing other algae.*

The availability of iron for microbial uptake appears to be an important variable in determining the stability and composition of aquatic ecosystems. Twice in a eutrophic lake the sudden dominance of blue-green algae coincided with the production of strong iron chelators and the rapid uptake of iron. The increased demand for iron appears to result from high rates of nitrogen fixation. We believe that the *Anabaena* species, the dominant alga during these periods, excreted chelators that enhanced the growth of blue-green algae or directly restricted the growth of competing species (or both).

This type of competition between aerobic microbes has been intensively studied by terrestrial ecologists and pharmacologists. In microbes, the demand for iron often induces the excretion of siderochromes, which are trihydroxamates (or catechols) of low molecular weight that can selectively chelate ferric iron. Ferric iron is chelated by acetohydroxamic acid several orders of magnitude greater than are other cations (*1*). Neilands (*2*) believes that many of these chelators act as carrier molecules transporting iron across membranes. Species with stronger iron uptake systems would thus be able to preferentially utilize the available iron. This has been best documented in mammalian systems where the competition for iron between the microbial siderochromes and mammalian proteins, such as transferrins and lactoferrins, determines the virulence of the microbe (*3*). Some microbes produce antibiotics that are structural homologs of a competitor's siderochromes. These antibiotics bind iron and are inducible by low concentrations of iron (*3*). Because these antibiotics are actively taken up by the siderochrome uptake system and this toxicity results from intracellular reactions, the toxicity can be reduced or eliminated by the addition of another siderochrome. Thus, the ability of a species to survive a period of low iron availability may depend both on its ability to take up iron and its capacity to antagonize competing species.

Although the processes controlling the uptake of iron in aquatic ecosystems are not as well understood, there are many reports that are consistent with the results obtained in terrestrial systems. Iron often stimulates photosynthesis in many diverse aquatic ecosystems (*4, 5*). Also,

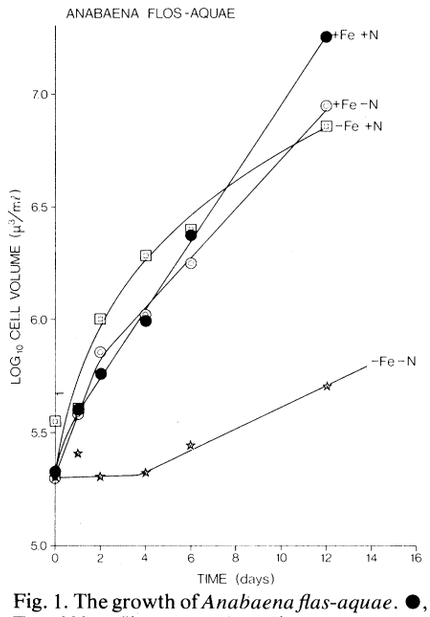

Fig. 1. The growth of *Anabaena flas-aquae*. ●, Fe, 1000 μg/liter; N, 250 mg/liter. ○, Fe, 1000 μg/liter; no combined N. □, N, 250 mg/liter; no added Fe. ★, no combined N, no added Fe.

ethylenediaminetetraacetic acid (EDTA) alone can often stimulate photosynthesis (*4*); hence the availability of iron rather than the concentration is critical. In upwelling areas of the ocean, the "older" waters possess chelation properties that enhance the growth of phytoplankton in freshly upwelled water (*6*). Fogg and co-workers have shown that blue-green algae can excrete polypeptides with chelation properties (*7*) and that in iron-deficient growth nitrogen excretion is enhanced (*8*). Another frequent observation is that filtered water from blue-green algal blooms inhibits the growth of other algae (*9*). The complexity of iron-organic interactions in lake water has retarded the formation of any hypotheses based on these observations.

The importance of hydroxamate chelators in lakes was first detected during a field investigation in the Bay of Quinte, a eutrophic bay on the northern shore of Lake Ontario. We used plastic enclosures (limnocorrals) to study the importance of phosphate and nitrate loading on algal production and biomass (*10*). The limnocorral supplied with nitrate and phosphate neither fixed nitrogen (*11*) nor showed a large demand for iron (*12*). In limnocorrals not supplied with nitrate, two periods of nitrogen fixation (August 1973, period A; July 1974, period B) coincided with the rapid uptake of ^{55}Fe, supplied as $FeCl_3$. Since the limnocorrals were open to the sediments, a flux of iron occurred from the reducing sediments. Since this was a closed system and the average residence time of iron in the water was less than a day, the settling rate of particulate iron was a good estimate of the flux of iron from the reduced sediment. During these periods of high ^{55}Fe uptake, the flux of iron from the sediments was unchanged. Thus, it appeared either that nitrogen fixation increased the demand for iron or that chemical assays of the iron supply did not measure the biologically active forms (or both).

To further test the hypothesis that nitrogen fixation increased the demand for iron, we used axenic cultures of *Anabaena flos-aquae*, a known nitrogen fixer. *Anabaena* will grow equally well with or without an inorganic nitrogen source as long as the supply of iron is adequate. Also, it grows at very low iron concentrations if supplied with nitrate; however, if *Anabaena* has to fix nitrogen and iron is not readily available, there is a long lag phase, the growth rate is reduced, and the cells quickly become chlorotic (Fig. 1).

Although nitrogen fixation may initiate the need for more iron, nitrogen fixation does not always coincide with rapid uptake of ^{55}Fe. In the field study, there was a third period of nitrogen fixation that did not result in rapid uptake of ^{55}Fe (period C). This discrepancy may be due to higher concentrations of biologically available iron that chemical techniques cannot distinguish or to variations in algal species requirements and uptake rates of iron.

The important difference between period C and periods A and B is the composition of the algal species. When iron was not in high demand, the blue-green algae comprised about 30 percent of the phytoplankton biomass. During periods A and B the blue-green algae rapidly increased to more than 90 percent of the phytoplankton biomass. In 2 weeks these blue-green blooms were followed by 65 percent (period A) and 50 percent (period B) reductions in the phytoplankton biomass. Although the high iron demand was transitory, the effects persisted for 2 months (period A) and 1 month (period B).

The blue-green algal suppression of other algae may have been mediated by hydroxamate siderochromes. An ultrafiltration experiment showed that during period B the microbes produced an organic compound having a molecular weight of less than 1000, which solubilized iron (*12*). A colorimetric assay (*13*) showed that bound hydroxamates, organics that need to be digested with acid to enable the hydroxylamine groups to react, were present only during the periods of rapid ^{55}Fe uptake. It was not possible

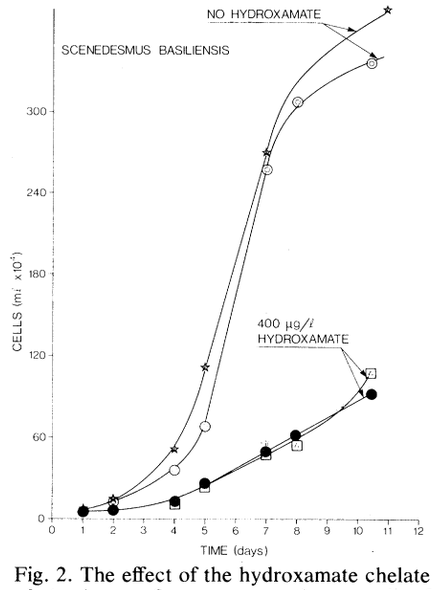

Fig. 2. The effect of the hydroxamate chelate of *Anabaena flos-aquae* on the growth of *Scenedesmus basiliensis*.

to determine which species were producing the hydroxamates in the field. In a survey of seven blue-green algae and ten green algae, only *Microcystis aeruginosa*, *Phormidium autumnale*, and *Anabaena flos-aquae* produced hydroxamate chelators in iron-deficient media. Bacteria (three species of *Pseudomonas* and an *Aerobacter*) isolated from Lake Ontario also produced hydroxamate chelators in media deficient in iron. The simplest hypothesis to explain our field observations is that hydroxamate chelators were produced by blue-green algae or associated bacteria and that growth of other algae was inhibited by these chelators.

To test this hypothesis we studied the effect of the hydroxamate chelate of axenic *Anabaena flos-aquae* (Lyngbye) on *Scenedesmus basiliensis* (Vischer) (Fig. 2). These algae were chosen because they are representative of the algae present when hydroxamates were detected in the Bay of Quinte. *Anabaena flos-aquae* was present in period A. In period B, *Anabaena affinis* was the dominant alga and *Scenedesmus bijuga* and *Scenedesmus abundans*, which earlier had represented 3 percent of the biomass, became undetectable.

Sephadex chromatography was used to isolate the chelate. Since uncomplexed iron binds to the column (*14*), any eluted ^{55}Fe is being carried through the column by an organic compound. The Csaky test (*13*) and a bioassay (*15*) confirmed that the hydroxamate chelator was in the same fraction as the ^{55}Fe. The bioassay was also used to verify that the chelate was still active after autoclaving, which is a vital precaution in these stud-

ies. The addition of the hydroxamate chelator to *Scenedesmus* suppressed growth. The colonies broke into single cells, and the cells quickly became chlorotic. There are two reasons for believing that the activity of this fraction is due to the hydroxamate and not a toxin that may have been in the same fraction. The uptake of iron per cell in *Scenedesmus* was only 20 percent of the control, and the addition of excess iron overcame the toxicity of this fraction. The addition of excess iron does not stimulate growth if the hydroxamates are not added to the culture. *Scenedesmus* does produce small peptides that can solubilize iron, but the *Scenedesmus* iron uptake system cannot compete with the hydroxamate system that is found in at least some blue-green algae and probably many planktonic bacteria.

It thus appears that the availability of iron may be an important factor in determining the stability and composition of aquatic ecosystems. The availability of iron is both a function of the biological demand for iron and the chelators excreted by microbes into lake water.

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Neural Properties of Cultured Human Endocrine Tumor Cells of Proposed Neural Crest Origin

Abstract. *Cells from human endocrine tumors of proposed neural crest origin—five pheochromocytomas, two medullary carcinomas of the thyroid, and two bronchial carcinoids—were grown in monolayer culture. Cells from all nine tumors, including epithelial forms of medullary carcinoma of the thyroid and bronchial carcinoid cells, and epithelial and neuron-like pheochromocytoma cells demonstrated all-or-nothing, short-duration action potentials.*

Embryological studies employing ablation, grafting, and tissue culture techniques have demonstrated that the neural crest is the origin of sensory and sympathetic ganglia, melanocytes, and adrenal chromaffin cells (*1*). Migrating neural crest cells are able to decarboxylate and store precursors of aromatic amines which fluoresce after exposure to formaldehyde vapor, and studies using this fluorescence as an endogenous marker suggest that the neural crest also gives rise to widely dispersed endocrine cells including enterochromaffin and related cells of the gut, argyrophil cells of the bronchi, islets of Langerhans, and parafollicular cells of the thyroid. These endocrine cells have been collectively termed the amine precursor uptake and decarboxylase (APUD) system (*2*).

This report demonstrates that cells from three types of human endocrine tumors of the proposed APUD system are capable of generating all-or-nothing, short-duration action potentials, consistent with their neural crest origins. In the case of pheochromocytomas, the cultured cells can also morphologically resemble neurons.

Cells from five adrenal pheochromocytomas, two medullary carcinomas of the thyroid (MCT's), and two bronchial carcinoids (BC's) were grown in monolayer culture (*3–5*) sometimes in medium supplemented with nerve growth factor (NGF) (*6*). These tumors are derived, respectively, from the APUD cells of the adrenal medulla, thyroid, and bronchi (*2*). Each tumor was diagnosed from multiple histologic sections by at least two of the pathologists in two teaching hospitals, using accepted morphologic criteria. Tumor cells from each of the pheochromocytomas grew as polygonal epithelial cells, 15 to 20 μm in diameter, which usually formed clusters, and which to varying degrees produced thin, branching, argyrophilic processes (> 100 μm)

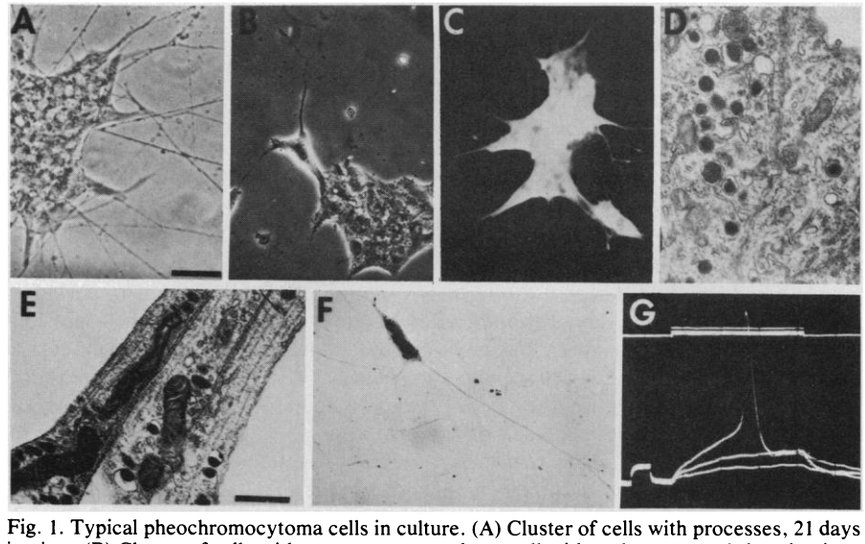

Fig. 1. Typical pheochromocytoma cells in culture. (A) Cluster of cells with processes, 21 days in vitro. (B) Cluster of cells without processes, and one cell with early process, 9 days in vitro. (A) and (B) are phase contrast pictures of live cells; scale bar, 50 μm. (C) Formaldehyde-induced fluorescence of cell bodies and processes. (D and E) Electron micrographs of flat-embedded cell bodies and proximal processes, showing large secretory granules typical of pheochromocytes 7 days in vitro; scale bar, 1 μm. Cells were fixed in 3 percent glutaraldehyde, *p*H 7.3, and postfixed in OsO_4. Processes often coursed side by side, forming fascicles. (F) Cluster of cells with branching fascicle of processes stained by the Holmes silver stain (*20*) with fibroblasts in background. (G) Typical intracellular recording from pheochromocytoma cells, showing three superimposed sweeps. (Upper trace) Current injected into cells. (Lower trace) Electrical potential responses: two subthreshold depolarizations and one suprathreshold depolarization triggering an action potential. Calibration pulse, 10 mv and 5 msec. Such action potentials could be elicited from all of the cell types illustrated.