age to the interpeduncular nucleus ($p = .05$).

The foregoing results strongly support the notion that two different neurological systems function in the retention of avoidance conditioning. The neural system involved in visceral conditioning probably contains the posterior thalamic region as one component, but does not include the interpeduncular nucleus or the visual cortex. In contrast, all three anatomical structures studied in this report are implicated with the neural system involved in skeletal conditioning, particularly the interpeduncular nucleus and the posterior thalamic area.

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3. This study was supported in part by research grant M-2529 awarded to the Department of Psychology, George Peabody College, from the National Institute of Mental Health of the National Institutes of Health, U.S. Public Health Service.
4. I acknowledge the assistance of Rita Thompson for preparation of the histological materials.
5. A control group of six untrained rats with moderate damage to the interpeduncular nucleus (mean damage of 48 percent) did not reveal any squeaking responses to the onset of light during ten presentations in the conditioning apparatus.
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Possible Explanation of Fluoride-Induced Respiration in Chlorella pyrenoidosa

Abstract. Low concentrations of sodium fluoride significantly increase oxygen consumption and total phosphorylated nucleotides in respiring *Chlorella pyrenoidosa*. Measurements of gas exchange at several *p*H values indicate that the stimulation is probably related to the undissociated hydrogen fluoride concentration in the suspending media.

Although fluoride has long been regarded as an inhibitor of respiration, recent investigations have demonstrated that it will also stimulate respiration. The stimulatory effect is usually produced at low concentrations. This phenomenon has been reported for algae (*1*), yeast (*2*), seedlings (*3*), and leaf tissue (*4*), but little interpretation has been offered as to the processes involved. The investigation reported here was an attempt to relate the fluoride-induced increase in oxygen uptake with some phase of the metabolism of *Chlorella*.

Chlorella pyrenoidosa Chick, Emerson strain type D, was grown at 24 to 26°C in a modified Knop's solution with added micronutrients. Iron was supplied as the salt of ethylenediaminetetraacetic acid. Air enriched to 3 percent CO_2 was continually supplied to the cultures. Unilateral illumination was provided by daylight-type fluorescent tubes, which supplied 600 ft-ca (6480 lux). After 72 hours' growth in constant light the cultures were subjected to 12-hour cycles of light and darkness for 48 hours and were harvested 6 hours after the last dark period.

The cells were harvested by centrifugation at approximately 500*g* for 15 minutes. The packed cells were resuspended in a volume of distilled, sterile water equal to the volume of packed cells. Cell counts were made on the resuspended algae, and all measurements were referred to these cell counts. Manometric measurements of the gas exchange in respiring *Chlorella* suspensions were made in 15-ml Warburg vessels in total darkness at 25°C. After a 110-minute treatment with NaF in 0.022*M* phosphate buffer at *p*H 4.0 it was apparent that $1.05 \times 10^{-4}M$ NaF produced an 8-percent increase in oxygen consumption and $1.05 \times 10^{-3}M$ NaF produced a 60-percent increase, while at $1.05 \times 10^{-2}M$ the rate was reduced to less than 70 percent of that of the nontreated system.

Experiments at other *p*H values, from 4.0 to 7.0, did not yield comparable results when the rate of oxygen consumption was compared to the total fluoride concentration of the solution. However, if the oxygen consumption was plotted relative to the calculated concentration of the undissociated HF, the results at different *p*H values were comparable (Fig. 1). The respiratory quotient did not vary greatly in the treated system as compared to the control.

To further define the mechanisms of fluoride-induced stimulation of oxygen uptake, the phosphate metabolism-respiration relationship was investigated. Respiratory-rate measurements were made on algae suspended in a nonbuffered water system in which the *p*H was initially adjusted to 4.0 with HCl or KOH. Similar suspensions of algae were placed in 40-ml test tubes that were attached to the manometer supports, and from these, aliquots were removed for phosphate determinations. Fluoride was added as sodium fluoride at zero time.

Measurements of phosphorylated nucleotides were made with a modification of the Norit A method (*5*). This procedure excludes sugar phosphates and other phosphorylated metabolic intermediates. The determinations of inorganic phosphate, after hydrolysis, were made according to the Fiske-Subbarow method (*6*).

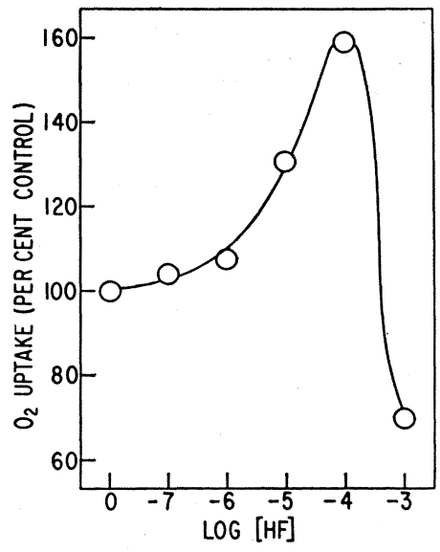

Fig. 1. Oxygen uptake in relation to the calculated HF concentration. The total fluoride concentration varied from 1.05×10^{-3} to $1.05 \times 10^{-1}M$ at *p*H 7.0, and from 1.05×10^{-4} to $1.05 \times 10^{-2}M$ at *p*H 4.0 in 0.022*M* phosphate buffer.

Concentrations of fluoride of 1.05×10^{-4}, 1.05×10^{-3}, and $1.05 \times 10^{-2}M$ increased oxygen consumption and at the same time increased the total phosphorylated nucleotides (Fig. 2). At $1.05 \times 10^{-4}M$ the oxygen consumption increased to 107 percent of that of the

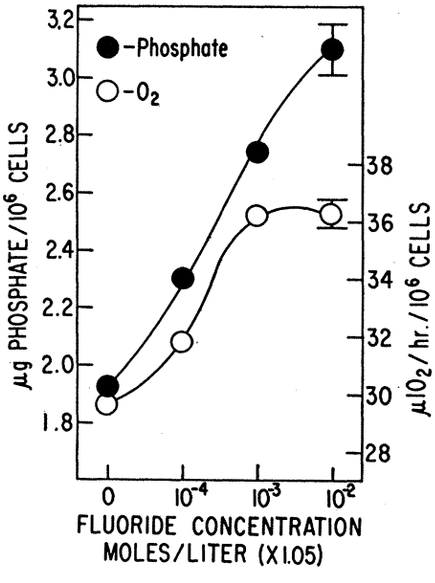

Fig. 2. Total nucleotide phosphate and rate of oxygen uptake in different concentrations of fluoride in the suspending solution. Undissociated HF concentrations were not calculated since this was a nonbuffered system. Bars at terminal points indicate 95-percent confidence limits.

control while the esterified phosphate increased to 119 percent. At a concentration of $1.05 \times 10^{-3}M$, oxygen consumption increased and esterified phosphate increased to 153 percent of that of the control. At the highest concentration reported here, $1.05 \times 10^{-2}M$, there was no further increase in the rate of oxygen consumption, but the esterified phosphate increased to 160 percent of that of the control.

The increase in esterified phosphate at fluoride concentrations above the concentration at which oxygen consumption fails to increase may indicate that fluoride was affecting multiple enzyme systems; this would be in accord with Reiner's (*7*) theoretical mechanism of multiple enzyme inhibition resulting in a stimulation of the oxygen consumption. The application of Reiner's theoretical treatment depends essentially upon the increased esterified phosphate's being adenosinetriphosphate (ATP).

Hackett (*8*) has indicated that the general over-all control of the rate of respiration in plants is dependent upon the concentration of the acceptor, adenosinediphosphate (ADP), rather than the donor, ATP. In addition, Krebs (*9*) has proposed that the control of respiration is dependent upon an interrelationship between inorganic phosphate, ADP, and ATP, ADP or phosphate acceptor being the most significant factor in the control mechanism.

Since there was a significant increase in phosphorylated nucleotides in these experiments, the following interpretation may apply. Fluoride probably disrupts the basic energetics of the cell in some manner and increases the oxygen consumption by increasing the phosphate acceptor or donor or at least by disturbing the interrelationships between inorganic phosphate, ADP, and ATP (*10*).

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Packaged Organic Materials as Monitoring Tools for Radionuclides

Abstract. Pint-size perforated polyethylene bags were used as containers to test preserved tea, spinach, ion-exchange resin, live filamentous green algae, and dead filamentous green algae for the sorption and concentration of radionuclides from natural aquatic habitats and from a variety of laboratory controlled nutrient media. These packaged materials have been used to detect trace levels of radionuclides not found by the usual methods of analysis of the raw water itself for dissolved radionuclides.

In measuring nuclide radioactivity levels in the water environment at points downstream from nuclear-energy facilities, problems are encountered that require large-volume samples for analysis because of the dilution that has taken place. Even if the levels of specific radionuclides released are known, analysis of downstream media is necessary to determine the fate of the radioactive materials in the particular environment and to define parameters of dispersion and diffusion necessary for evaluating the movement of specific nuclides and the time required for their passage through the system in question.

In concentrating trace amounts of nuclides from large volumes of sample, particularly where evaporation, precipitation, or ion-exchange techniques are used, the stable salt concentrations in the diluting medium interfere with the subsequent separation of the specific radionuclides. To avoid these difficulties a technique utilizing dead organic and living biological concentration under natural stream conditions was investigated. Radioanalysis of algae from natural aquatic habitats has shown a greater variety and higher concentration of radionuclides than an analysis of the water in which the algae live.

Considerable data exist in the literature regarding concentration of fission products by organisms (*1*). Phytoplankton are noted for accumulating substances in inorganic form without known metabolic function. Average concentration factors up to 7000 have been reported for fission products (*2*), whereas some induced radionuclides have been concentrated several hundred thousand times or more. This characteristic is particularly noted in lower forms of organisms in media low in metabolically required trace elements, as for example, the high uptake of phosphorous-32 by plankton in the Columbia River, which is low in stable phosphorous. Thus, under certain circumstances, sampling by biological concentration may avoid the need of collecting large volumes of water for radioanalysis. Furthermore, knowledge of the movement of radionuclides into organic materials may reflect useful information necessary to evaluate the disposal of radionuclides into the hydrosphere.

High concentrations of potassium and calcium were found not to interfere, respectively, with the uptake of cesium-137 and strontium-85 by many nonliving organic materials in laboratory trials. Several kinds of biological material were selected for study under field conditions and in the laboratory, including preserved materials—tea, spinach, and filamentous algae—and living filamentous algae. Because the filamentous green alga, *Pithophora oedogonia,* grows easily in the laboratory and has a high concentration factor for many radionuclides, it was selected as the living test organism. It was grown free of silt and interfering radionuclides and in low concentration of stable nuclides. Thirty grams of blotted wet algae, about 2 g by dry weight, were placed in a pint-sized polyethylene bag having 400 evenly spaced pores about 0.8 mm in diameter. About 200 of these packaged sampling materials have been tested by

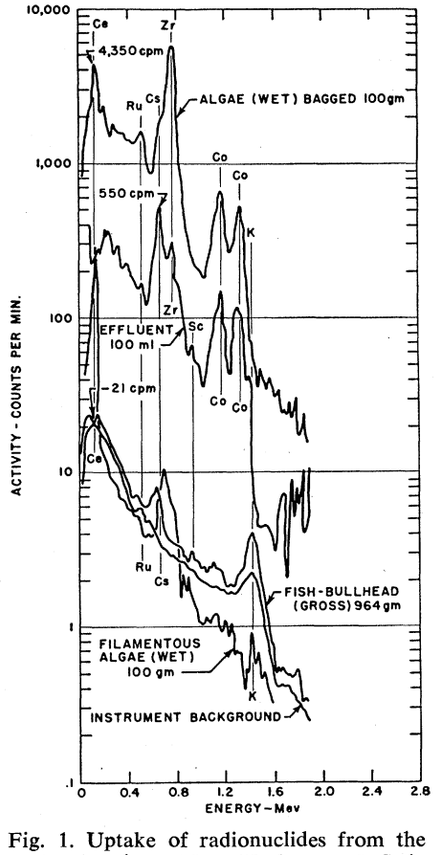

Fig. 1. Uptake of radionuclides from the Mohawk River, New York, at 17°C in September 1959 after 72 hours, by *Pithophora* in perforated polyethylene bags, compared with activity of fish (bullhead) and an indigenous filamentous green alga, *Cladophora*, taken from the river. Uptake of these radionuclides by *Pithophora* was nonmetabolic, since the alga had been killed with chlorine.