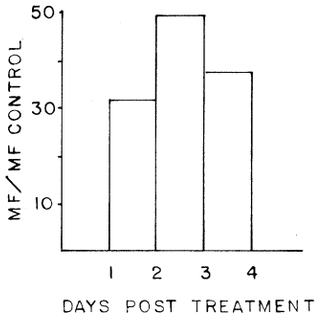

Fig. 1. Mutagenic activity detected with *S. typhimurium* (TA1538) in urine samples of a single patient treated with niridazole. The patient received one dose (25 mg/kg), and three 24-hour samples were collected. Urine samples were not concentrated, and 0.1 ml of urine was added per plate.

were taken from the orbital sinus with a sterile Pasteur pipet and mixed with heparin. Usually 0.2 to 0.3 ml was removed from the sinuses of ten animals for each time period. In order to recover the urine, ten mice were placed in a metabolism unit that separates the urine from the feces. The urine was allowed to pass through a membrane filter as it was collected and was kept at 0°C. Blood and urine samples were added to 2.0 ml of molten agar at 45°C containing the tester strain. The mixture was then poured over plates of minimal agar and incubated for 48 hours; the plates were then scored for mutant colonies. Evidence of a mutagenic effect was found in both blood and urine of mice treated with one dose, 200 mg/kg, of niridazole; activity in the urine was high initially but disappeared after 4 hours, whereas activity lasted up to 8 hours in the blood. In contrast, virtually no activity could be demonstrated in either the blood or urine of mice treated with metronidazole for 4 days with doses up to 400 mg/kg, even when the blood and urine were lyophilized in an attempt to concentrate any active material.

A patient that had been treated in the United States with niridazole was located, and samples of the patient's urine were collected. In contrast to the high initial activity and successive loss of mutagenic activity in mice, activity up to 3 days after treatment (Fig. 1) was demonstrated in urine from the patient who received niridazole (25 mg/kg).

The urines of six female patients were analyzed prior to the onset of therapy and during drug treatment with metronidazole. These patients received the normal therapeutic dose of 250 mg (in tablet form) three times a day for 10 days. Urine samples were collected 1 hour after the initial dose on the day at which the sample was analyzed. Of the six subjects, no activity was found in four just prior to therapy, and minimal activity with two of them was found on the first day of treatment. However in all of the subjects tested, significant mutagenic activity was detected when the urine was sampled on day 6, 8, or 9 of therapy (Fig. 2).

Our results indicate mutagenic activity in human subjects receiving therapeutic levels of two widely used drugs. In the animal studies, activity of niridazole was detected in the host-mediated assay as well as by urine and blood analyses, whereas metronidazole was found to exhibit marginal but significant activity in the host-mediated assay only. Although both these compounds exhibited activity when directly tested against the indicator tester strain, the presence of active metabolites not detectable in vitro has yet to be determined.

It should now be possible to study commercial drugs and various chemicals in blood and urine of man to indicate the presence of mutagenic substances by procedures such as those described above.

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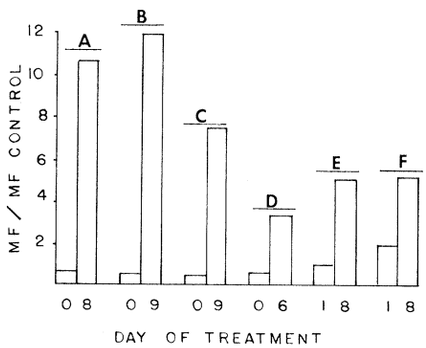

Fig. 2. Mutagenic activity detected with *S. typhimurium* (TA1535) in urine samples of six patients treated with metronidazole. Patients received 750 mg/day, and the urine was collected 1 hour after the morning dose on the day indicated. Each letter represents a separate patient, and "0" indicates a control sample taken a day prior to therapy. Urine samples were concentrated fivefold by lyophilization, and 0.1 ml of concentrated urine was added per plate.

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Manganese Encrustation of Zygospores of a *Chlamydomonas* (Chlorophyta: Volvocales)

Abstract. *In media containing normal trace-element supplements, but not in manganese-deficient media, zygospores of a new species of* Chlamydomonas *(isolated from soil) become encrusted with a dark brown mineral coating. Staining with benzidine indicates that the encrustation is rich in manganese. This has been confirmed by x-ray analysis in combination with a scanning electron microscope.*

The vegetative cell walls in several classes of algae are mineralized, usually with silica or calcium carbonate. Among the Chlorophyta the oocysts of the Charales are characteristically calcified, but in other orders with dormant zygotes (such as Volvocales) the spore walls are generally composed only of cellulose or other organic material. The genus *Chlamydomonas* comprises almost a thousand "species," many poorly described; for only a few dozen species have zygospores been reported. These diploid spores are generally green, although some become orange on maturation, and they have smooth or ornamented wall surfaces. We report here observations on an apparently new species with dark brown or black spores, mineralized with manganese (possibly MnO_2). A formal description of the species will be published elsewhere (*1*).

Pure clonal cultures were isolated from garden soil in La Jolla, California. The species is isogamous but heterothallic. Like the flagellate cells and gametes, young zygotes are green, but when illuminated for 2 to 3 days they become orange due to accumulation of secondary carotenoids. Mineralization then begins as brown plaques, which form on the outside of the zygospore wall and multiply and enlarge until they fuse to create a complete casing. Later the surface becomes increasingly verrucose, and a number of more or less cylindrical processes about 1 μm wide grow out, branching and curling, and ulti-

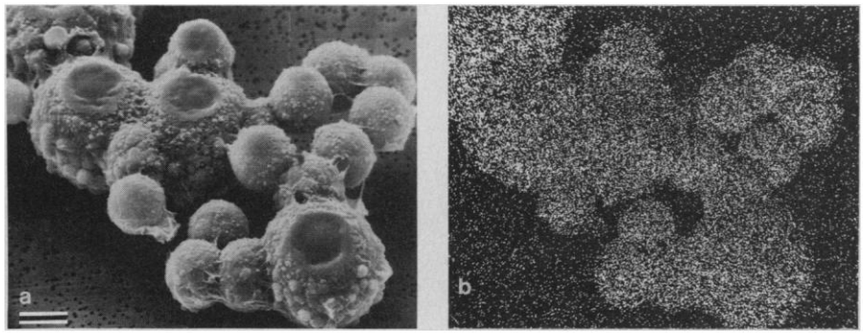

Fig. 1. (a) Scanning electron micrograph of *Chlamydomonas* spores on a Nuclepore filter. (b) X-ray scan (acceleration voltage, 20 kev) in the Mn range of the same area. Scale bar, 10 μm.

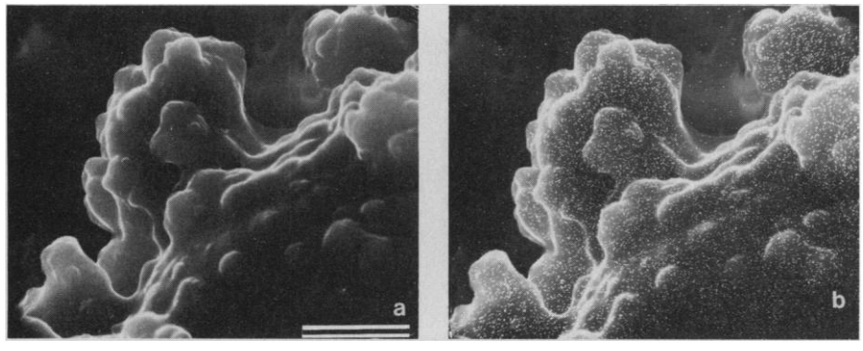

Fig. 2. (a) Scanning electron micrograph of *Chlamydomonas* cell wall encrustation (cell on Nuclepore filter). (b) Same as (a), with superimposed x-ray scan (acceleration voltage, 20 kev) in the Mn range. Scale bar, 5 μm.

mately (within 2 weeks) form a feltlike coat over most or all of the spore surface.

The encrustation is fragile. Pressure between a microscope slide and a cover slip causes the casing to crack into pieces, liberating the orange zygote. The fragility and the dark color of the casing material suggested that it might be composed largely of mineral matter. This was confirmed by physiological experiments, a chemical reaction, and x-ray analysis with a scanning electron microscope (SEM). The cells were cultured in a mineral nutrient medium containing (per liter) KNO_3 (180 mg), $MgSO_4 \cdot 7H_2O$ (50 mg), $CaCl_2$ (16 mg), NaH_2PO_4 (50 mg), vitamins B_1 (0.5 mg) and B_{12} (0.5 μg), and a mixture of trace elements comprising Fe, Mn, and B (0.5 mg) and Cu, Mo, and Zn (0.01 mg). Three or four days after gametes of the two mating types grown in this medium were mixed, brownish black spores formed at the surface of liquid cultures or on 1 percent agar media. On agar prepared with the same medium lacking trace elements, the spores developed normally but their cell walls remained thin and colorless. However, the sole addition of $MnCl_2 \cdot 4H_2O$ (10 mg/liter) to this medium resulted in the formation of black spores as before.

The cells were tested for Mn^{4+} by the benzidine reaction (*2*). The formation of benzidine blue around the mineralized zygospores, but nowhere else, indicated the presence of MnO_2 in the cell casings.

Black spores grown on agar were transferred to a membrane filter (pore size, 1 μm), fixed in 2 percent glutaraldehyde, washed, and desiccated by critical-point drying using Freon 113/13. They were then coated with carbon (for x-ray energy-dispersive analysis) or with gold/palladium (for scanning micrographs) and examined with a SEM (Cambridge model S-4) equipped with an x-ray energy-dispersive unit (Ortec DELPHI). A full x-ray spectrum from 0 to 20 kev (acceleration voltage, 20 kev) yielded two distinct peaks, corresponding to the K_α and K_β lines of Mn. Smaller peaks were identified as K_α lines of Si, P, K, and Ca. Figure 1a is a scanning electron micrograph of a group of zygospores and Fig. 1b, for the same area, is an elemental map for Mn, taken between energy-window limits of 5.73 and 6.04 kev (the Mn K_α line is at 5.89 kev). A small portion of one zygospore wall, shown in a scanning electron micrograph (Fig. 2a), was similarly subjected to an x-ray scan in the Mn range; the resulting image is shown, superimposed on the previous picture, in Fib. 2b. The topological coincidence of the dots indicates the association of Mn with the cell wall surface structures.

Studies of extracellular Mn oxidation and deposition, experimentally demonstrated in cultures of certain bacteria, fungi, and algae (*3*), may ultimately prove of importance in helping to elucidate the problem of Mn nodule formation in the deep sea.

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Double Branched Flicker Fusion Curves from the All-Rod Skate Retina

Abstract. *Electrical responses from the skate retina will only follow flicker up to frequencies of 5 hertz when intensities are below rod saturation. At greater luminances, the eye responds to rates as high as 30 hertz. As a result, a plot of critical flicker fusion as a function of intensity is a double branched curve. It seems that prolonged stimulation of skate rods, at high intensities, permits them to change their temporal response characteristics so that they follow high frequencies much as cones do in the duplex retina.*

Most vertebrates possess a duplex retina that allows them to sense changes in spatial and temporal patterns of light over an enormous range of light intensities. Rod signals mediate low-intensity (scotopic) vision, whereas the cone system subserves the upper (photopic) range of intensities. Consequently, measurements of visual acuity, critical flicker frequency, dark adaptation, and increment threshold as functions of light intensity yield curves which seem always to be divisible into two distinctly different portions. The kink in the curve marks the changeover from pre-