mil; volume, 16×10^6 km^3; sea level lowering negligible), 0.4 per mil.

3) Deep ocean cooling (2°C), 0.5 per mil.

Total of three effects: 1.7 per mil.

Is there evidence in the Arctic sediments for thick ice? Certainly the absence of foraminifera and the very low detrital sedimentation rates found for sediments deposited in the Arctic during glacial time (*12, 13*) are compatible with this idea. Thicker ice would reduce light penetration and hence plant and zooplankton production. If ablation occurred mainly at the perimeter of the cap, then any windblown detritus accumulating on the ice surface would be carried to the south by the lateral flow of the ice and released there when melting occurred. However, as the severalfold thickening of the Arctic ice cover produced by lowered temperature alone would be adequate to explain the sedimentary evidence, the sedimentary evidence cannot be used to support the existence of an ice cap.

Clearly at this point there is only evidence that thicker ice existed in the Arctic Ocean during glacial time. Direct evidence for an ice cap is lacking. The observation that the large magnitude of the observed glacial-interglacial benthic ^{18}O shift demands more ice than can be accounted for by terrestrial moraines or sea level lowering provides only a reason for giving serious thought to the possibility that thick floating ice caps existed in both polar oceans during glacial times. If so, the growth and retreat of this ice add new elements to the global dynamics of glacial-interglacial change. The ingenious Ewing-Donn theory (*14*) focused so much attention on the possibility that the Arctic was ice-free during glacial time that the other extreme, an Arctic ice cap, has received too little consideration.

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24 September 1974; revised 7 February 1975

Detection of Mutagenic Activity of Metronidazole and Niridazole in Body Fluids of Humans and Mice

Abstract. *After humans were treated at therapeutic doses with the trichomonacide metronidazole (Flagyl) and the antischistosomal agent niridazole mutagenic activity was demonstrable in their urines when tested with the histidine auxotroph of* Salmonella typhimurium. *Both compounds were active in the host-mediated assay in mice, and evidence of activity was found in the blood and urine of mice treated with niridazole but not with metronidazole.*

Streptozotocin and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) have been evaluated for mutagenic activity by both the host-mediated assay technique and by analyses of the urine and blood of treated animals (*1*). Both compounds were active in the host-mediated assay. Mutagenic activity was detected with streptozotocin in both the blood and urine of treated animals; however, activity with MNNG could not be demonstrated in urine or blood of treated mice. Urines of treated animals have also been analyzed for mutagenic activity by a variety of indicators (*2*). Of particular significance is that the urines from patients treated with cyclophosphamide, an agent used in cancer chemotherapy, exhibited mutagenic activity (*3*). We now report our evaluation of the activity of two widely used commercial drugs, metronidazole (Flagyl) and niridazole, in the host-mediated assay as well as in blood and urine of treated animals; also we now have evidence of mutagenicity in the urines of patients treated with these drugs.

Niridazole and metronidazole were both active when directly tested against the histidine auxotroph of *Salmonella typhimurium* with the use of excision-repair minus, lipopolysaccharide-deficient tester strains (*4*). It is interesting to note that these heterocyclic nitro-containing compounds reverted different tester strains, with metronidazole reverting the base substitution strain TA1535, and niridazole reverting the frameshift mutant TA1538. Niridazole was mutagenically active at much lower concentrations than metronidazole, and was inhibitory to TA1538 at 100 μg per plate. Neither compound was affected by microsomal enzymes present in liver homogenates (*5*) (Table 1).

Although both compounds were active in the host-mediated assay, metronidazole was marginally active, exhibiting only a fourfold increase after 5 days of treatment at 400 mg/kg per day, whereas niridazole gave a tenfold increase after only a single dose of 10 mg/kg (Table 2).

With treated animals, blood samples

Table 1. Evaluation of the effect of metronidazole and niridazole when these drugs were incubated with liver homogenate (LH) (microsomal enzymes) and the mutation frequency of *S. typhimurium*.

Concentration (μg per plate)	MF* compound/MF control			
	Niridazole TA 1538		Metronidazole TA 1535	
	No LH	Added LH	No LH	Added LH
0.001	0.80	1.29		
0.01	1.08	1.14		
0.1	3.90	3.23		
0.2	9.69	9.64		
2.0	Toxic	Toxic		
50			1.60	1.16
100			2.00	2.23
500			4.25	5.09

*Mutation frequency, which is the ratio of histidine$^+$ revertants to the total number of bacteria.

Table 2. The results of the host-mediated assay with niridazole and metronidazole. The mice used were Jackson Laboratory $B_6D_2F_1/J$ females, weighing 20 to 25 g. Niridazole was given for 1 day, and metronidazole was given for 5 days.

Dose (mg kg^{-1} day^{-1})	MF compound/MF control	
	Niridazole TA 1538	Metronidazole TA 1535
1.00	1.42	
5.0	5.24	
10.0	9.35	
50		1.51
100	Toxic	1.68
400		3.78

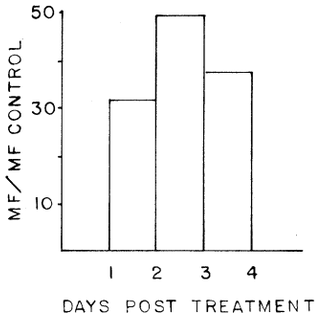

Fig. 1. Mutagenic activity detected with *S. typhimurium* (TA1538) in urine samples of a single patient treated with niridazole. The patient received one dose (25 mg/kg), and three 24-hour samples were collected. Urine samples were not concentrated, and 0.1 ml of urine was added per plate.

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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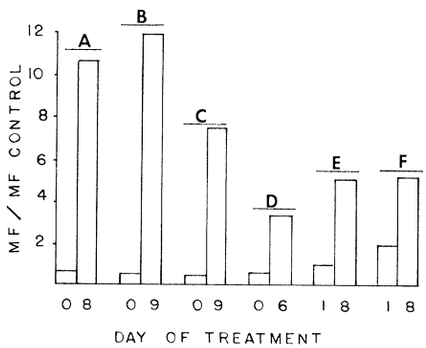

Fig. 2. Mutagenic activity detected with *S. typhimurium* (TA1535) in urine samples of six patients treated with metronidazole. Patients received 750 mg/day, and the urine was collected 1 hour after the morning dose on the day indicated. Each letter represents a separate patient, and "0" indicates a 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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6. We thank Dr. E. Bueding of Johns Hopkins University and Dr. J. Evrard of Brown University for assistance. Supported by NIH grant 1-P02 CA 13943 CAP.

25 November 1974

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-