result is found for regional ischemia by ^{31}P NMR surface coil measurements (*10*). The implication is that reducing work load and contractility helps to preserve ischemic myocardium.

Several possible extensions of the experiments reported here are foreseeable. Two or more surface coils could be used simultaneously to obtain spectra from different regions of the heart. A small coil could be placed inside the left ventricle of the perfused heart through a small incision in the left atrium to monitor the ^{31}P NMR spectrum from the interior myocardium. With a conventional transmitter coil and a small surface coil in a cross-coil configuration (*13*), one could measure other parameters such as relaxation times, diffusion coefficients, and the flow at well-defined locations in the heart and other organs. By use of surface coils, enzyme kinetic rates could be observed by saturation transfer techniques (*14*) for specific regions within the heart as a function of normal, pathological, and drug-treated states. Changes in phosphate substrate levels within localized volumes could be directly observed and related to the function of particular regions (for example, left ventricle versus left atrium) and to the contraction-relaxation cycle of the heart.

Our results, together with recent work by Grove *et al.* (*15*) on the determination of whole heart metabolism by ^{31}P NMR in vivo, indicate that surface coil NMR may eventually be used in vivo to assess the location and size of an injury or abnormality and determine the efficacy of drug treatment.

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Mutagenicity of Fly Ash Particles in *Paramecium*

Abstract. Paramecium, *a protozoan that ingests nonnutritive particulate matter, was used to determine the mutagenicity of fly ash. Heat treatment inactivated mutagens that require metabolic conversion to their active form but did not destroy all mutagenicity. Extraction of particles with hydrochloric acid, but not dimethyl sulfoxide, removed detectable mutagenic activity.*

The combustion of coal to generate electric power produces vast quantities of fly ash. Although fly ash is considered to be the most abundant industrial solid-waste product in the United States, only a few studies have been performed to evaluate the potential health effects of exposure to fly ash or its leachates. The finest fly ash fractions collected from a power plant smokestack (*1*) were found to be mutagenic in the Ames bacterial assay (*2*, *3*). Since there is a high positive correlation between carcinogenicity of substances for animals and man and mutagenic activity in the Ames test (*4*), the mutagenicity of fine fly ash particles raises concern (*2*, *3*).

A possible mode for an abatement technology was suggested by the finding that heat-treated fly ash loses detectable mutagenic activity due to the decomposition of surface mutagens (*3*, *5*). While agents identified as mutagenic by the Ames test may be presumed to be poten-

Table 1. Mutagenic effect of fly ash and heat-treated fly ash in *Paramecium*. Values not connected by the same line are significantly different from each other (Wilcoxon matched-pairs signed-ranks test, $\alpha = .05$). The data from six experiments were pooled since the control values for autogamous progeny were not significantly different. Cerophyl is the ryegrass extract used for cultivation of *Paramecium*. Induced S-9 is the Ames liver microsome fraction from rats receiving Arochlor 1254 (polychlorinated biphenyl) to activate the enzymes for conversion of promutagens to mutagenic form; uninduced S-9 is from rats receiving corn oil only (the vehicle for the Arochlor). Glass beads (1 to 3 μm) suspended in either induced or uninduced S-9 were used as a negative control for nonnutritive particles. Kaolinite was also used in one experiment, and the results were the same as those for the glass beads. Benzo[*a*]pyrene was the positive control for mutagenicity requiring induced S-9. The initial concentration of suspended fly ash was 535 μg/ml. The average number of affected progeny from treated parent cells was 20 percent higher than the average number of affected control progeny. Since one mutation would theoretically yield only 4 affected progeny in 16 autogamous progeny from a treated parent cell (*6*), the percentages, though low, reflect significant damage.

Substance	Lethal and detrimental cells (%)	Number of progeny examined
Cerophyl	1.01 ± 0.21	1568
Glass beads + uninduced S-9	1.36 ± 0.33	1904
Glass beads + induced S-9	1.41 ± 0.42	1888
Benzo[*a*]pyrene + uninduced S-9	3.2 ± 0.39	1440
Fly ash + uninduced S-9	3.7 ± 0.69	2992
Heated fly ash + uninduced S-9	3.9 ± 1.6	1728
Heated fly ash + uninduced S-9	4.6 ± 1.3	1280
Fly ash + uninduced S-9	9.3 ± 1.7	1296
Benzo[*a*]pyrene + uninduced S-9	12.5 ± 5.8	1664

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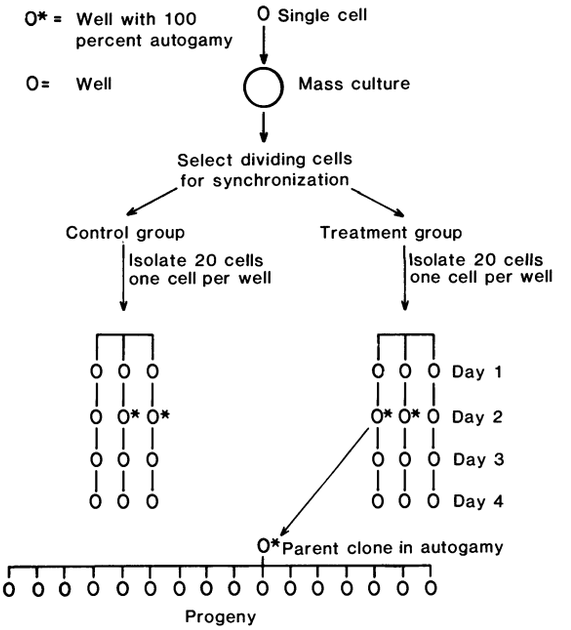

Fig. 1. *Paramecium* mutagenesis assay. Mass cultures of paramecia are derived from a single cell of known age (60 to 80 cell divisions since the last autogamy). Cells at the same stage of division are selected with a micropipette under a dissecting microscope. After 2 hours, when the cells are refractory to DNA repair (*8*), they are suspended with particles for 4 hours at 34°C and vortexed at 10-minute intervals to keep the particles in suspension. The cells are then washed by successive transfers into agent-free bacterial food, and each cell is placed into a separate well with food. The isolates multiply, and a single cell is reisolated into fresh food daily for 3 days. The remaining cells continue to multiply, starve, and undergo autogamy. When 90 to 100 percent of a sample of cells from a well containing starved cells exhibit the morphology typical of autogamy (*21*), they are used as a source for 16 autogamous progeny cells, which are distributed singly into fresh food in separate wells. After 3 days, each of the 16 wells is examined and the cells are classified as (i) viable (they have cleared the medium of bacterial food), (ii) damaged (they have not cleared the medium of food), or (iii) dead (they have died or given rise to a few moribund cells). The values for each group in an experiment were pooled, and an agent was considered damaging to DNA if the viable group was significantly smaller than the control group (*22*). The data were analyzed through pairwise comparisons of the groups. Significance was determined by placing confidence intervals on the difference of the two proportions by using the normal approximation. If the confidence interval did not capture zero the pairs were deemed significantly different. The variance of that difference was computed on the basis of a stratified random sample, in which the surviving fraction is a stratum and the sample size for each stratum is constant.

tially dangerous, negative results do not necessarily imply that they are harmless. More than one bioassay may be necessary. Protozoans offer a unique bioassay system that allows distinction between genotoxic effects on individual cells and populations of cells and represent an evolutionary link between bacteria and multicellular organisms for comparative studies of DNA-damaging agents.

Paramecium tetraurelia is especially desirable for mutagenesis studies since these cells are capable of self-fertilization (autogamy), which results in homozygosity for any recessive mutations. Thus induced damage in the parent genome is expressed in the next generation of progeny (*6*). The fraction of dead progeny or slow growers reflects the presence of lethal or detrimental mutations in the micronuclei of the parental germ line (*6–15*).

Representatives of the *Paramecium aurelia* complex have been used extensively for mutagenesis studies (*6, 7*), and a wealth of biological data is available. Mutagenicity and mechanisms for expression have been reported for many agents including *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (*6*), acridine mustard (*9*), triethylenemelamine (*9, 10*), alpha radiation (*11*), ultraviolet irradiation (*12*), benzo[*a*]pyrene after metabolic activation (*13*), methyl methanesulfonate (*13*), nitrogen mustard (*14*), and x-rays (*15*).

We used the established mutagenesis assay in *P. tetraurelia* to provide a screen for the mutagenic activity of complex mixtures (*13*) and to capitalize on the ingestion of nonnutritive particles (*16*) such as fly ash for a mutagenesis assay not requiring prior extraction. Coal combustion produces chemicals that can be absorbed by fly ash (*5, 17*) and decomposed by subsequent heat treatment (*3, 5*). Therefore we compared the mutagenicity of untreated fly ash and heat-treated fly ash (450°C for 24 hours in a sealed vial).

The *Paramecium* mutagenesis assay involves treating members of the same clone with incubation medium or test agents (Fig. 1). The ingestion of fly ash particles by this eukaryote (Fig. 2) provides a model for assaying intracellular extraction of particles and more closely mimics the phagocytosis of these particles by the pulmonary macrophages of mice (*18*) than the Ames assay, which requires particle extraction before mutagenicity testing. For inclusion in the assay, the parent cell must survive treatment and complete a minimum of seven to ten cell divisions in an agent-free medium to deplete the food and hence induce autogamy. Thus, for detection, induced damage must be heritable and sufficiently nontoxic to allow for successive cell divisions and completion of

Table 2. Mutagenicity of heat-treated fly ash extracted with HCl or DMSO. Values not connected by the same line are significantly different from each other (pairwise comparisons of proportions, $P < .05$). The concentration of fly ash particles suspended in uninduced S-9 was 1068 μg/ml. The higher than usual value for mutagenicity in the controls can be attributed to the considerable age of the clone used here [micronuclear damage increases with age (*12*)].

Substance	Lethal cells (%)	Number of progeny examined
Glass beads	3.14 ± 0.33	624
HCl-extracted particles	4.77 ± 1.25	960
DMSO-extracted particles	8.21 ± 2.00	656
Unextracted particles	14.09 ± 5.29	896

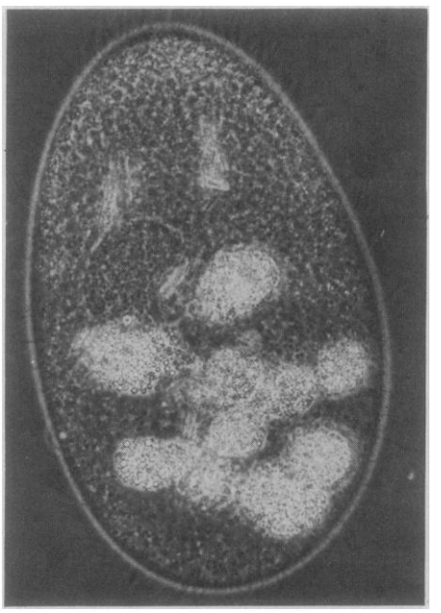

Fig. 2. Fly ash particles in *Paramecium tetraurelia*. Paramecia were exposed to Dryl's salt solution (*23*) containing fly ash. These organisms have gullets lined with membranelles and cilia that sweep in particulate material. Vacuoles are formed at the base of the oral apparatus and circulate in the cytoplasm, where digestion occurs. Undigested material is excreted by exocytosis (*24*). After 1 hour in this solution, paramecia cease ingesting particles, but ingestion is continuous when they are maintained in the nutritious liver microsomal fraction.

fertilization. To detect particle-associated promutagens, which require metabolic conversion to their mutagenic form, the S-9 microsomal fraction of liver was included (*4*). Significantly increased mortality and reduced vigor among autogamous progeny from treated parent cells were used as measures of treatment-induced damage to DNA.

Fly ash and heated fly ash were both significantly mutagenic ($P < .05$) when uninduced S-9 was added, reflecting the direct action of particle-associated mutagens (Table 1). Addition of induced S-9 increased the mutagenicity of unheated fly ash but not heated fly ash, indicating heat inactivation of indirect-acting mutagens associated with the particles. Fly ash and heated fly ash were optimally mutagenic at 535 and 1060 μg/ml, respectively. Additional evidence of mutagenicity was the appearance of morphological abnormalities including boomerang-shaped cells, bent bodies, and thin or bloated cells. Temperature-sensitive cells were also detected, predominantly among the survivors of clones that died at room temperature.

To further evaluate the nature of the heat-stable agents, the heated particles were extracted with hydrochloric acid or dimethyl sulfoxide (DMSO) before the cells were incubated with them (*19*). The HCl-extracted particles had no significant mutagenic effects, whereas the DMSO-extracted particles retained their mutagenicity (Table 2). This demonstrates that a heat-stable, aqueous acid-extractable mutagen is associated with fly ash. The solubility behavior and temperature stability are also consistent with the presence of inorganic mutagens.

Variations in the response of members of the same clone to fly ash–associated mutagens may reflect differences in susceptibility to damage. Possible causes for this include (i) differences in the threshold concentration of mutagenic particles ingested, (ii) variation in the clones' repair capability, and (iii) metabolism of the mutagenic agents to a harmless form.

Both the Ames *Salmonella* system and the *Paramecium* assay disclosed direct- and indirect-acting fly ash–associated mutagens (*2*, *3*). A major difference was the retention of mutagenic activity after heat alteration of fly ash in the protozoan assay only. Also, aqueous acid extraction did not alter mutagenesis in the Ames assay (*5*), but resulted in decreased mutagenicity in the protozoan system. This suggests protozoan sensitivity to inorganic mutagens. Although Fisher *et al.* (*5*) did not ascribe a significant component of fly ash mutagenicity to inorganic compounds, hexavalent chromium compounds, tri- and pentavalent arsenic, selenium, and *cis*-diamminedichloroplatinum II are mutagens in a variety of bacterial systems (*20*). Mammalian cell mutagenicity (or transformation) has been observed with these inorganic compounds as well as with soluble salts of cadmium, nickel, and beryllium. Thus, differences in the biological properties of fly ash in the two bioassays may reflect quantitative or qualitative differences in sensitivity.

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2-Amino-4-Phosphonobutyric Acid: A New Pharmacological Tool for Retina Research

Abstract. *Information processing in the vertebrate retina occurs in two separate channels known as* ON *and* OFF *channels. When intracellular electrophysiological recordings were obtained from the perfused retina-eyecup preparation of the mudpuppy (*Necturus maculosus*), the addition of 2-amino-4-phosphonobutyric acid to the bathing medium blocked all responses in the* ON *channel but left intact the* OFF *responses including* OFF *ganglion cell discharge. 2-Amino-4-phosphonobutyric acid blocks the light response of the* ON *bipolar cell by mimicking the endogenous photoreceptor transmitter.*

A characteristic feature of the vertebrate retina is the segregation of information processing into two separate channels. Focal light stimulation defines an ON channel that provides excitatory input to one class of ganglion cells at the onset of a light stimulus, while an OFF pathway provides excitatory input to a separate group of ganglion cells at the termination of a light flash (*1*). Both ON and OFF channels can be distinguished through many subcortical and early cortical neuronal interactions (*2*). These channels are separated at the first level of synaptic interaction where photoreceptor activity gives rise to two different types of bipolar cell responses, as shown by the polarity of the membrane potential change evoked by a focal light stimulus (*3*). Depolarizing bipolar cells provide the ON pathways, whereas hyperpolarizing bipolar cells underlie the OFF

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