Fig. 2 show that the mean absolute refractory periods of CMA neurons were significantly shorter in castrated rats after treatment with TP ($U = 5$, $P < .002$, two-tailed). Comparisons of refractory periods between the first and second experiments show no significant difference between the mean refractory period of intact (experiment 1) and testosterone-treated (experiment 2) animals ($U = 71$, $N = 12$ and 13, not significant). This experiment shows, therefore, that testosterone reverses the effect of castration observed in the first experiment.

In a third experiment we measured refractory periods of CMA neurons stimulated antidromically not from the MPH but from the VMC. Refractory periods were determined for 57 neurons from 13 intact rats and 59 neurons from 13 castrated rats. The results show no significant difference in mean refractory period between the two groups. Means and ranges are 1.23 and 0.84 to 2.11 msec for intact rats and 1.21 and 0.97 to 1.79 msec for castrated rats ($U = 81$, not significant).

All the neurons for which results are given were located in the cortical and medial nuclei of the amygdala as defined in (*13*). In accordance with anatomical evidence (*8*), the neurons driven from the MPH and VMC were encountered in the same caudal region of the CMA. The refractory period is altered, therefore, in amygdala neurons projecting via the dorsal stria to the MPH, but not in adjacent amygdala neurons projecting via the dorsal stria to the VMC. So the effect of castration is restricted to the pathway known on other grounds to be involved in the control of sexual behavior. The effect of castration must be due to the removal of testosterone, since it is reversed by testosterone injections. Testosterone must be affecting these neurons directly, since it is the refractory period that is altered, and a change in refractory period implies a membrane change.

The change in refractory period that we have demonstrated would alter the output of the pathway to high-frequency inputs. Presumably these inputs would be olfactory. This interpretation receives support from a previous study (*4*), which shows that testosterone does alter preoptic neuron responses both to electrical stimulation of the olfactory bulb and to natural sexual odors.

K. M. KENDRICK
R. F. DREWETT*
Department of Psychology, University of Durham, Durham, England

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* Reprint requests should be sent to R.F.D.

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Physical Factors Affecting the Mutagenicity of Fly Ash from a Coal-Fired Power Plant

Abstract. *The two finest, most respirable coal fly ash fractions collected from the smokestack of a power plant were more mutagenic than two coarser fractions. Mutagenicity was evaluated in the histidine-requiring bacterial strains TA 1538, TA 98, and TA 100 of* Salmonella typhimurium. *Ash samples collected from the hoppers of an electrostatic precipitator in the plant were not mutagenic. The mutagens in coal fly ash were resistant to x-ray or ultraviolet irradiation, possibly as a result of stabilization by fly ash surfaces. All mutagenic activity is lost with heating to 350°C.*

In 1977, fly ash produced and collected by coal-fired power plants was considered to be the sixth most abundant mineral in the United States (*1*). Of the 50 million metric tons of fly ash produced, 13 percent was reused commercially, predominantly as a partial replacement for cement or as fill material in construction; of the remainder, most was deposited in landfills. Electrostatic precipitators (ESP's) are used in most coal-fired power plants for in-plant collection of aerosolized particulate matter. The total mass collection efficiencies of the ESP's vary from 95 to 99.5 percent, with the lowest relative efficiency for collecting respirable, submicron-sized particles (*2*). Because vast quantities of fly ash are produced in the generation of electricity, detailed studies are required of the potential public and occupational health hazards of fly ash released from smokestacks and collected in power plants.

We have demonstrated that extracts from a stack-collected, respirable fly ash sample were mutagenic in the Ames bacterial assay (*3*); moreover, because of the correlation between mutagenicity and carcinogenicity (*4*), our study indicated that fly ash is potentially carcinogenic. We describe here physical factors affecting the mutagenicity of (i) four size-classified, stack-collected fly ash samples; (ii) one size-classified, ESP-collected sample; and (iii) one unsized, ESP-collected sample.

The stack sampling was performed at 95°C; two measurements of the stack gas temperature during the 30-day sampling period varied from 107° to 115°C (*5*). The volume median diameters (VMD's) of these four size-classified samples, fractions 1 through 4, are 20, 6.3, 3.2, and 2.2 μm, respectively. All have geometric standard deviations (σ_g) of approximately 1.8.

We also collected fly ash from the power plant's ESP hoppers (*6*). Three measurements of the temperature of the ESP's during 2 days of collection varied from 104° to 107°C. Because the ESP fly ash is predominantly composed of relatively large particles, it was aerodynamically size-classified to obtain a fraction with a VMD of 2.3 μm and $\sigma_g = 1.4$. The physical and chemical properties of the stack-collected samples and of similarly collected unsized ESP ash have been reported (*7*).

We evaluated the relative mutagenicity of serum filtrates from the four stack-collected fractions using TA-1538 because our previous studies indicated it

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to be the most sensitive of the five tester strains of *Salmonella typhimurium* studied (*3*). All four fractions were mutagenic, and the finest fractions (fractions 3 and 4) were the most mutagenic (Fig. 1). Interestingly, the finest fraction, fraction 4, appeared to be less active than fraction 3. A comparison of fraction 3 mutagenicity data from 82 replicate analyses on 18 occasions with fraction 4 data from 62 replicates on 13 occasions resulted in significantly different ($P < .001$) means of 323 and 170 his^+ revertants per plate, respectively. We also studied the effect of enzyme activity of a liver microsomal fraction (S-9) on the mutagenicity of the four fly ash fractions. Although all four fractions displayed increased mutagenicity with S-9, only fraction 3 was significantly elevated as compared to the equivalent sample without S-9. Statistical analyses of a total of 160 and 154 samples of fractions 3 and 4, respectively, indicated that the mutagenicity of fraction 3 was significantly ($P < .01$) enhanced by the addition of S-9 whereas the mutagenicity of fraction 4 did not change significantly ($P > .05$) with S-9. Because mutagens condensed on fly ash surfaces should be in higher concentrations on finer particles, as is the case for other condensed chemical forms (*8*), we suggest that the greater mutagenicity of fraction 3 compared with fraction 4 is due to relative concentrations of unidentified antimutagens (*9*).

Ten replicate analyses on two separate occasions showed that the unsized, ESP fly ash was not mutagenic to TA 1538, ± S-9. In agreement, we found no mutagenicity in extracts of the size-classified material using TA 1538, TA 98, or TA 100, ± S-9. On the basis of our mutagenicity tests, we calculate that, if there is any mutagenic activity in size-classified ESP fly ash, it must be less than 0.002 of that of the comparable stack-collected material.

Because fly ash released from the stack of the power plant would be exposed to ionizing radiation in the atmosphere and because Gibson *et al.* (*10*) reported increased mutagenicity for polynuclear aromatic hydrocarbons (PAH) irradiated with ultraviolet light (UV) and ^{60}Co, we determined the mutagenicity of fraction 3 after irradiation with either UV or x-irradiation. We chose fraction 3 because it was the most mutagenic of the fly ash samples. The UV-irradiation was performed over a 67-hour period with a mercury-vapor lamp; the sample was exposed to approximately 450 J (*11*). X-irradiation delivered a calculated dose of 150,000 R over a 7-hour period. The irradiation experiments were performed with fly ash in sealed quartz containers with either air or argon atmospheres. We evaluated the mutagenicity of triplicate samples of the serum extracts, using TA 100, TA 98, and TA 1538, ± S-9 (*12*). Ultraviolet light or x-irradiation did not significantly affect the mutagenicity of the fly ash under any of the testing conditions. Similarly, exposure of fly ash in glass flasks to ambient sunlight for 8 days did not alter the mutagenicity (*11*).

To evaluate thermal alteration of the mutagens, samples of fraction 3 were heated for 60 minutes to temperatures ranging from 50° to 350°C at 50° intervals. Throughout the heating procedure, samples were swept with filtered air. After heating, we evaluated the mutagenicity of serum filtrates, using TA 100, TA 98, or TA 1538, ± S-9 (Fig. 2). The patterns of temperature response for TA 1538 and TA 98 were similar, ± S-9; for both tester strains, a decrease in mutagenic activity was observed for temperatures of 200°C and above. Relative to unheated, room-temperature controls, the observed decreases in mutagenic activity in TA 1538 with S-9 were significant ($P < .01$) at 200° and 250°C, whereas tests without S-9 showed a significant ($P < .05$) decrease at 200°C and a suggestive decrease ($.1 < P < .2$) at 250°C. Filtrates assayed with TA 100 exhibited a temperature dependence similar to that of TA 1538 and TA 98; decreases ($P < .05$) in mutagenic activity ± S-9

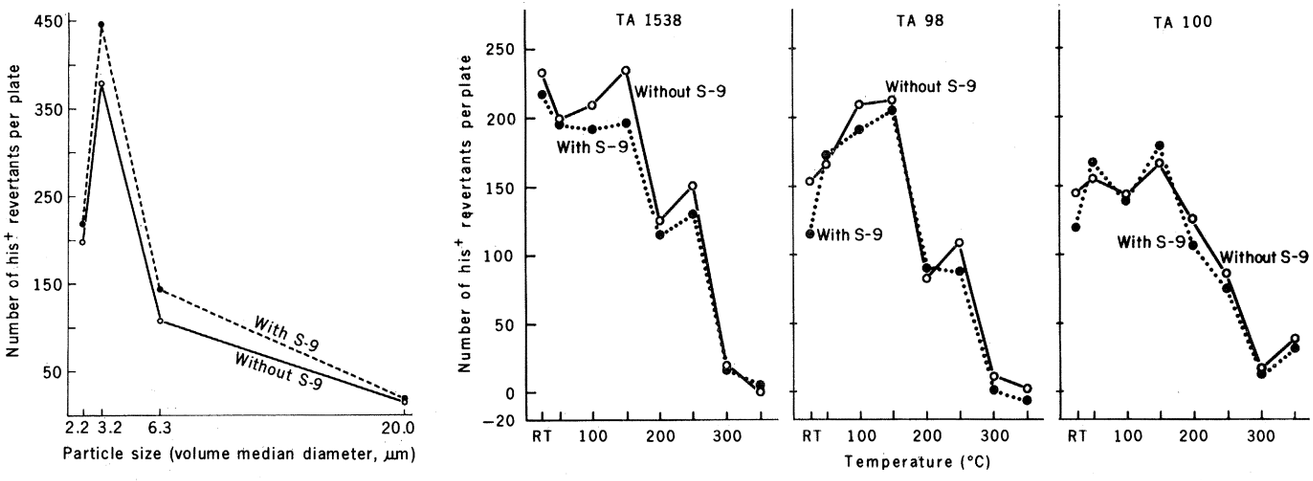

Fig. 1 (left). Size dependence of the mutagenicity of serum filtrates derived from stack-collected coal fly ash. The serum filtrates were prepared after incubation of 10 ml of horse serum with 0.80 g of fly ash for 7 days at 37°C as described in (*3*). Filtrate (100 μl) was added to 2 ml of soft, top agar before plating. The numbers of TA 1538 his^+ (histidine-synthesizing) revertants per plate are the means of ten replicate determinations minus the mean of the background revertants. The background reversion rates are the group means of the spontaneous revertants and the procedural control after determination that the means of these negative controls were not significantly different. The means (± standard error) of the background revertants were 22 (± 3) and 8 (± 1) with and without addition of the supernatant fraction of rat liver homogenate (S-9) from rats treated with polychlorinated biphenyls, respectively. Positive controls were 4-nitroquinoline-*N*-oxide without S-9 addition and 2-aminofluorene with addition of S-9. Fig. 2 (right). The effects of temperature on the mutagenicity of serum filtrates derived from size-classified, stack-collected coal fly ash (fraction 3); *RT*, room temperature. The net numbers of TA 1538, TA 98, and TA 100 his^+ revertants per plate were determined as described in Fig. 1. Three replicate plates were counted for each treatment. Controls were derived from glass microspheres (1 to 3 μm) treated in the same way as the fly ash samples. The spontaneous reversion rate and control data were not statistically different and therefore were pooled to generate the background-subtracted reversion rates. The average backgrounds for TA 1538, TA 98, and TA 100 without addition of S-9 were 16, 17, and 96 revertants per plate, respectively; those with addition of S-9 were 30, 25, and 101 revertants per plate, respectively. Methyl methanesulfonate and 4-nitroquinoline-*N*-oxide were used as positive controls for TA 100 and TA 98, respectively, without addition of S-9; 2-aminofluorene was used for both tester strains with addition of S-9.

were observed at temperatures above 200°C and 300°C, respectively. For the three bacterial strains studied, ± S-9, no mutagenic activity was observed for filtrates from fly ash heated to 350°C.

Our studies of physical factors affecting the mutagenic activity of coal fly ash extracts provide insight into the chemical properties of fly ash mutagens. The finding that the greatest activity is associated with the finest fractions of stack-collected ash is consistent with the observations of Natusch (*13*) that PAH are adsorbed on particle surfaces during cooling of the effluent stream. The observation that ESP-collected fly ash was not mutagenic (whether size-classified or not) suggests that condensation of mutagens on fly ash particles occurred after passage through the ESP but before or within the stack sampling system. Natusch and Tomkins (*14*) have predicted that a temperature near 100°C is critical for the adsorption of PAH onto fly ash. Our observations of the complete loss of mutagenic activity with experimental heating to 350°C are consistent with the hypothesis that the bulk of the mutagenic activity of the fly ash samples is associated with organic compounds. This loss of activity from stack-collected fly ash appears to begin at temperatures greater than the operating temperatures of the ESP. The temperature difference probably reflects the fact that organic compounds are chemisorbed to fly ash surfaces and therefore require higher temperatures for desorption. This hypothesis is supported by our observations that the mutagens in fly ash are resistant to photochemical decomposition upon UV- or x-ray irradiation. Similarly, Natusch and his co-workers (*8*, *15*) have observed that PAH adsorbed from the vapor phase on fly ash surfaces may be stabilized against photochemical decomposition at solar radiation wavelengths; photodecomposition occurred with irradiation of PAH as dry powders, in solution, or adsorbed onto silica, alumina, or glass.

These studies demonstrate that (i) the most respirable stack-collected fly ash samples are the most mutagenic, (ii) the ESP-collected fly ash from the same power plant is not mutagenic, (iii) the surface-associated mutagens are resistant to photodecomposition with UV- or x-ray irradiation, and (iv) the mutagenicity of fly ash is completely removed by heating to 350°C. The observation that mutagens were associated with fly ash particles when collected at temperatures below 100°C suggests a possible improvement in control technology. Fly ash could be collected at lower temperatures than normally present in ESP's. However, if such an approach were used, the industry would be confronted in 1980 with a predicted 100 million tons (*1*) of weakly mutagenic solid waste. A reasonable strategy, therefore, would consist of high-temperature, high-efficiency collection of particulate matter from the flue stream, followed by subsequent condensation and in-plant collection of volatilized organic matter.

G. L. FISHER
C. E. CHRISP
O. G. RAABE

Radiobiology Laboratory, University of California, Davis 95616

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Estimating Fatness

Part of the controversy between Frisch and Trussell (*1*) hinges on the manner in which Frisch has estimated fatness as a linear function of height and weight rather than measuring it physiologically. It appears that the random variability of the estimator has an impact on the arguments which has not been fully appreciated heretofore. Rather than actual measurements of fatness, Frisch uses the Mellits and Cheek equation (*1*):

$$\hat{TW} = -10.313 + 0.252WT\ (\text{kg}) + 0.154HT\ (\text{cm})$$

to predict a girl's total body water (TW) (assumed proportional to fatness) from her height (HT) and weight (WT) at menarche. Since this regression line has a multiple $r^2 = .97$, one might be tempted to suppose that the random variability is negligible, but this is not the case. Examination of the Mellits and Cheek data (*2*) shows that one needs to provide a band of 3.03 liters to either side of the line to cover 90 percent of the individuals in the Mellits and Cheek study. With this error band one has:

$$\hat{TW} = -10.313 + 0.252WT + 0.154HT \pm 3.03$$

and thus,

$$WT = \frac{-10.313 + 0.154HT \pm 3.03}{\hat{TW}/WT - 0.252}$$

Now, according to Frisch, the minimum required weight for the onset of menarche is given by the 10th percentile fatness line, where $\hat{TW}/WT = 0.598$, and thus we have,

$$\text{Minimum required weight} = \frac{-10.313 + 0.154HT \pm 3.03}{0.598 - 0.252}$$

From this it can be seen that about 9 kg of uncertainty is added to or subtracted from the predicted minimum required weight by the error bands. Clearly, this 9-kg variability in estimated minimum weight occurs for all heights and weights involved, rendering this procedure for determining fatness rather questionable and undermining any support these data might be thought to give to a critical fatness hypothesis.

JAXK REEVES

Statistics Department, University of California, Berkeley 94720

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