to gratings as to bars. This is a relatively small difference in sensitivity, but it is in fact about the size predicted from the spatial frequency spectra of the patterns: If a larger difference had been found it would not have been stronger evidence for spatial filtering, but evidence against it.

The greater selectivity and responsivity of cells to gratings than to bars is predictable from a consideration of them as a spatial frequency filters. A single bar of any width has a broad spatial frequency spectrum, covering virtually the whole spatial frequency range studied. Cortical cells are responsive to only a limited range of spatial frequencies, but would be expected to respond to bars of all the widths because all have in their spectra spatial frequencies within the cell's sensitivity range. Cells should thus be, as we have found, selective for spatial frequencies but not for bars (*12*).

The greater responsivity of cortical cells to gratings of the optimal spatial frequency than to bars of optimal width is also predictable from the spatial frequency selectivity of the cell. In the space domain, the RF of a cortical cell has not just a central excitatory region, but also inhibitory (antagonistic) flanks (*1*). In addition, narrowly tuned cells have further excitatory and inhibitory side bands (*8*), as would be predicted by their fine selectivity for sine waves. Both the inhibitory flanks and the additional side bands should make a grating a more effective stimulus than a bar, which excites only the RF center. Looked at in the frequency domain, a bar, with its broad spatial frequency spectrum, has much of its power at frequencies other than those to which a given cell is sensitive. It is thus less effective in driving the cell than is a grating, which has a limited frequency spectrum within the cell's sensitivity range.

Our data show that while cells in the striate cortex have been characterized as either bar and edge detectors or as cells selective for certain spatial frequencies, the latter is by far the more accurate descriptor of their behavior. Gratings are in fact the stimuli to which most cortical cells give their largest responses and to which they are the most sensitive; furthermore, all cortical cells are much more selective along the dimension of spatial frequency than they are along the dimension of bar width.

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10. If the mean level of a grating is taken as I, the peak of the wave is $I + \Delta I$ and the trough is $I - \Delta I$. Thus the contrast becomes:

$$\frac{(I + \Delta I) - (I - \Delta I)}{(I + \Delta I) + (I - \Delta I)} = \frac{2\Delta I}{2I} = \frac{\Delta I}{I}$$

This method of equating the contrast of the bars has also been used by others (*6*).
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12. Sullivan, Georgeson, and Oatley (*6*) came to similar conclusions on purely psychophysical grounds. They found that adaptation to a grating selectively adapted only nearby spatial frequencies, whereas adaptation to a bar adapted all bar widths nonselectively. P. H. Schiller, B. L. Finlay, and S. F. Volman [*J. Neurophysiol.* **39**, 1334 (1976)] also found greater selectivity to gratings than to bars, although contrasts were not matched for bars and gratings and only response functions at a single contrast were obtained.
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Nonvolatile Mutagens in Drinking Water: Production by Chlorination and Destruction by Sulfite

Abstract. *In concentrates of water produced in a laboratory simulation of a drinking water treatment process, direct-acting, nonvolatile mutagens were readily detected by means of the Ames* Salmonella *test. The mutagens were shown to be produced by the chlorination process. Treatment of the water with chloramine resulted in less mutagenic activity than treatment with free chlorine. Dechlorination of drinking water with sulfite sharply reduced the mutagenic activity. Treatment with sulfur dioxide is proposed as an effective, inexpensive method of reducing the direct-acting mutagenic activity of drinking water and of aqueous industrial effluents.*

Although there is concern over the presence of low levels of mutagens and carcinogens in drinking water (*1*), and especially over the ubiquitous appearance of chloroform after chlorination (*1, 2*), little is known about the nonvolatile organic compounds present in drinking water (*1*).

Pelon *et al.* (*3*) used the Ames *Salmonella* test (*4*) to detect low levels of direct-acting mutagens (not requiring enzymatic activation) and promutagens (requiring enzymatic activation) in unconcentrated water from the lower Mississippi River. Several subsequent studies (*5–7*) found mutagens in concentrates of several U.S. drinking waters. Glatz *et al.* (*6*) and Hooper *et al.* (*7*) suggested that in several water supplies, treatment processes such as chlorination might generate mutagenic activity. Chlorination is known to produce chloroform (*8*) in drinking water as well as mutagens in the bleaching effluents from softwood kraft pulp (*9*). The experiments we have conducted show that chlorination produces nonvolatile mutagens in drinking water and that treatment of chlorinated water with sulfite reduces mutagen levels significantly.

Water that had been softened with lime was taken from a municipal treatment plant, and the treatment that would have been followed there was followed in the laboratory (*10*). All samples (40 to 80 liters each) were treated in a similar manner except that several different procedures were used for chlorination and dechlorination. Organic compounds present in the water were adsorbed to the nonpolar resin, Amberlite XAD-4, according to the method of Glatz *et al.* (*6*). We used acetone and then methylene chloride (*11*) for desorption of these compounds and removed the solvent (and volatiles) by rotary evaporation of the samples to dryness. The residual organic compounds were dissolved in a volume of dimethyl sulfoxide (DMSO) equal to about 1/20,000th that of the original water. Samples of the water concentrate were assayed by the Ames *Salmonella* plate test (*4*).

We used several strains of *Salmonella typhimurium* for this test. The Ames test strain TA100 showed the highest rever-

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Table 1. The effect of dechlorination with sulfite on the mutagenic activity in drinking water. Chlorine was added to two identical volumes of lime-softened, recarbonated water. After the indicated contact time, the *p*H and chlorine residuals were measured. The dechlorinated sample received 1.1 to 1.2 times the amount of Na_2SO_3 necessary to eliminate all residual chlorine; this was about three times the stoichiometric ratio of sulfite to chlorine. The chlorine in the volume that was not dechlorinated was converted to the far less reactive species monochloramine by the addition of a twofold excess of NH_3 over residual Cl_2; this largely suppressed further mutagen production. After filtration, both samples were chloraminated to a $ClNH_2$ residual of about 3 ppm and subjected to XAD adsorption. The values for mutagen content represent slopes of linear dose response curves obtained by the plating in duplicate of at least five equally spaced doses of water concentrate corresponding to about 0- to 1.0-liter volumes prior to concentration. The 95 percent confidence intervals for the slopes and the number of plates in each case are indicated.

Date (1979)	Added Cl_2 (ppm)	Chlorination procedure				Mutagen content (revertants per liter)			
		Contact time (hour)	*p*H	Free residual (ppm)	Total residual (ppm)	Not dechlorinated		Dechlorinated	
						Slope	*N*	Slope	*N*
21 March	2.6	1.0	8.2	0.1	0.7	1291 ± 148	14	753 ± 76	15
5 April	5.9	1.0	7.5	0.55	1.1	1318 ± 209	18	583 ± 118	18
10 April	5.3	1.0	8.3	0.35	1.25	678 ± 82	18	333 ± 157	16
19 June	2.6	0.5	8.7	0.4	0.9	365 ± 38	18	65 ± 35	14

sion rate with the water concentrates; strains TA98, 1538, 1535, and 1537 were less responsive. All strains produced fewer revertants in the presence of S-9 (the supernatant from Aroclor-induced rat liver homogenized and centrifuged at 9000*g*). The sharpest reduction in reversion rate, about 70 percent, was observed with TA100. These responses are similar to those reported by Loper *et al.* (*5*). The results reported below were obtained with strain TA100 without S-9. Linear dose responses were always observed.

In our experiments, unchlorinated water appeared to be devoid of mutagens. Chloramine-treated (chloraminated) water, however, contained mutagenic activity; water chlorinated with Cl_2 (free chlorine) showed even greater mutagenic activity (see Fig. 1) (*12*).

The controls in these experiments consisted of volumes of distilled water treated and chlorinated in the same way as the samples of experimental water. We found that the mutagenic activities of the drinking water samples were two to ten times (average: five times) that of the controls (*13*). Most of the mutagenic activity in the drinking water appeared to be a result of chlorination of the water constituents and did not appear to result from mutagens preexisting in the chlorine or from reactions of the chlorine with the experimental apparatus.

After correcting for adventitious mutagenic activity, we found in all our experiments that, despite changes over time in the raw water and differences in treatment, chlorination with free chlorine produced higher amounts of mutagens than chloramination; no mutagens were detected in water that had not been chlorinated (*14*). In our system, chlorination was responsible for nonvolatile mutagen production.

Although for the purposes of quantitation the mutagenic activity of a distilled water control may be subtracted from the corresponding value for drinking water, we investigated the source of the mutagenic activity appearing in the controls. Some of this activity may result from chlorination of the XAD resin (*15*). We therefore used sodium sulfite to dechlorinate samples of water produced either in the laboratory or in the municipal treatment plant, before passing them through the XAD resin. Whereas chlorinated distilled water yielded an average of 20 percent of the mutagenic activity of chlorinated drinking water, dechlorination of drinking water with sulfite caused a 50 to 80 percent reduction in mutagenic activity. Thus, more activity is removed by sulfite treatment than may be attributed to chlorination of the XAD resin. Ultimate mutagens and carcinogens are electrophiles (*16*); nucleophiles, such as sulfite, would be expected to attack and destroy electrophilic chemical groups. That sulfite reacts with alkyl chlorides and epoxides to form sulfonic acids is well established (*17*).

To assess the effect of sulfite in a treatment process for drinking water that was modeled on a laboratory scale, we subjected samples of water in the laboratory

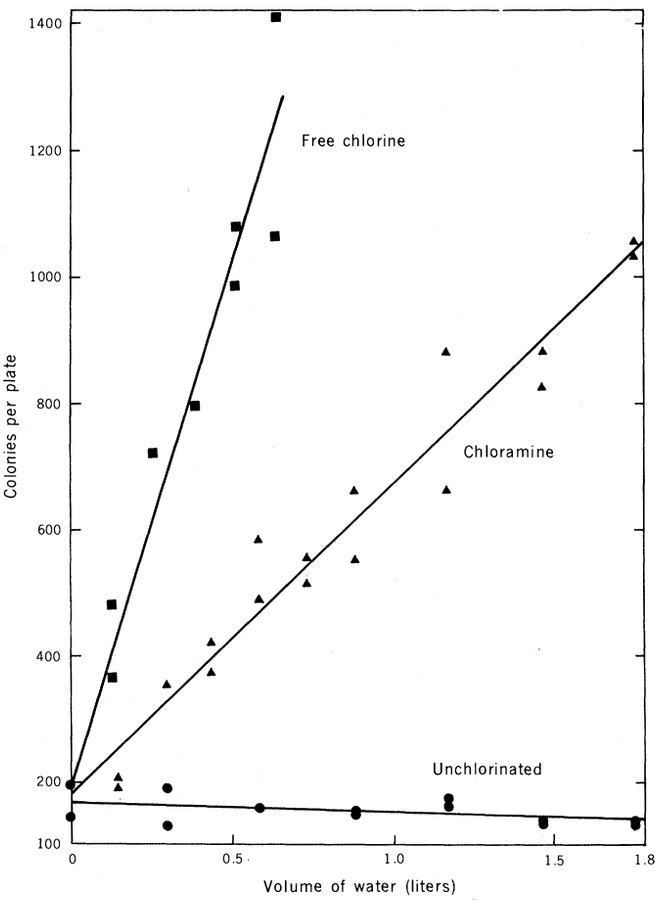

Fig. 1. Response of strain TA100 in the Ames assay to concentrates of drinking water. The abscissa shows the volume prior to concentration. This experiment was done 27 September 1978 when the organic carbon content of the raw water was 20 mg/liter and the color was 78 color units. The free chlorine sample received 4.2 ppm of Cl_2; the concentration of the final residual was 0.7 ppm. The chloramine sample received sufficient NH_3 and then Cl_2 to produce a monochloramine concentration of 4.8 ppm (as Cl_2); the concentration of the final residual was 2.0 ppm. The contact time was 4.5 hours, the temperature 17°C, and the *p*H 7.7 to 7.8. Each dose was plated in duplicate; the points represent individual plates.

to chlorination with free chlorine for 1 hour, dechlorination with Na_2SO_3 for 3 hours, and then postchlorination with monochloramine. The dechlorinated samples showed significantly lower mutagenic activity than those that were not dechlorinated (see Table 1).

The mutagens in the drinking water samples are unlikely to be trihalomethanes (*18*); their identities remain unknown. Therefore, it is not possible to determine their potential risk to health. Nevertheless, dechlorination of drinking water with SO_2 is an established treatment procedure that may not add greatly to treatment costs (*19*). While the effectiveness of the method would have to be tested on a plant scale, we suggest that the content of direct-acting mutagens in drinking water would be reduced if the water was subjected to dechlorination with SO_2 and then chloramination before being distributed to the consumer. We also suggest that SO_2 or sulfite might be used to destroy direct-acting mutagens in aqueous industrial effluents.

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10. The source of raw water was the upper Mississippi River. The water was treated at the plant as follows. Lime was added to raise the *p*H and thus cause precipitation of Ca^{2+} and Mg^{2+} (softening); alum was added simultaneously to facilitate sedimentation; CO_2 was added to lower the *p*H; and powdered activated carbon was added to control taste and odor. At this stage in the process we withdrew large volumes of water that we placed in 55-gallon (210-liter) drums or 20-gallon (76-liter) polyethylene barrels. We then modeled on a laboratory scale the subsequent plant treatment. The water was chlorinated by the addition of chlorine water or by the addition of NH_4OH and then chlorine water, and then coagulated with alum for 4 hours. The water was then filtered rapidly through a bed of sand and anthracite. Treated water was eluted through a 100-ml bed volume of XAD-4 at flow rates ranging from 2 to 10 liters per hour.
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12. These experiments were conducted from September to December 1978 when the color of the raw water changed from 91 to 29 color units and nonvolatile total organic carbon decreased from 22 to 6 mg/liter. We added enough chlorine to establish either a free residual of > 0.2 part per million (ppm) of Cl_2 after 1 hour or a chloramine residual of > 2 ppm after 4 hours. Measured residuals were 0.1 to 1.5 ppm (free chlorine) and 2 to 4 ppm (chloramine). Added chlorine concentrations were 4 to 8 ppm (free residual chlorination) and 4.5 to 7 ppm as Cl_2 (chloramination). The contact time before filtration ranged from 2 to 7 hours and the temperature range was 1° to 25°C. The chlorination *p*H varied from 7.3 to 8.5. For experiments in which comparisons were made in parallel (see Fig. 1) contact time, temperature, and *p*H were kept essentially the same.

Mutagenic activity was as follows: water treated with free chlorine produced 500 to 2000 revertants per liter; with chloramine, 120 to 500 revertants per liter; with no chlorine, slight negative slope due to DMSO. Although no systematic investigation was made, it seemed that lower mutagenic activity was associated with the lower organic content of the raw water and lower temperature found in winter, and with lower amounts of added chlorine, shorter contact times, and higher *p*H.
13. The amount of Cl_2 or chloramine added to distilled water was adjusted so that the final residual was the same as in the corresponding drinking water. The mutagenic activity in these control samples was as follows: samples treated with free chlorine produced 170 to 330 revertants per liter and samples treated with chloramine, 30 to 110 revertants per liter. Control values were 10 percent of the higher values produced for drinking water obtained in September and up to 50 percent of the lower values for drinking water obtained in December [see (*12*)].
14. Any materials that do not bind to XAD resins will not be detected.
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18. Aside from the loss of volatiles during rotary evaporation, the *Salmonella* strains used in the Ames test do not respond to chloroform or carbon tetrachloride or to other common trihalomethanes unless the tests are conducted in sealed desiccators [V. F. Simon, K. Kauhanen, R. G. Tardiff, in *Progress in Genetic Toxicology*, D. Scott *et al.*, Eds. (Elsevier/North-Holland, Amsterdam, 1977), p. 240].
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