145 to 170 minutes after injection following single and repeated amphetamine: 3.1 ± 1.0 versus 3.3 ± 1.3 (Mann-Whitney U test, $P > .05$).

18. Locomotor activity 170 to 200 minutes after injection on days 1 and 14: 49.4 ± 12.5 versus 80.1 ± 20.3 crossovers (two-tailed t-test for related groups, $P > .05$).
19. The decreased duration of the behavioral response during long-term amphetamine treatment cannot be accounted for by an accelerated metabolism of amphetamine, since we found that whole brain amphetamine concentrations were higher 3 hours after drug administration in long-term than in control rats (saline: 0.172 ± 0.014 μg per gram of tissue (wet weight); 31st amphetamine injection: 0.264 ± 0.048 μg/g. This difference in brain amphetamine concentrations disappeared by 8 days after the end of the long-term treatment.
20. D. S. Bell, *Arch. Gen. Psychiatry* **29**, 35 (1973).
21. The plasma half-life of amphetamine in humans is 7 to 14 hours in subjects with acidic urine and is markedly longer (18 to 34 hours) in subjects with alkaline urine [E. Anggard, L. E. Jonsson, A. L. Hogmark, L. M. Gunne, *Clin. Pharmacol. Ther.* **14**, 870 (1973)].
22. Supported in part by PHS grants DA-01568-04, MH-30914-02, and AA-07129-03, and NIMH research scientist award MH-70183-06. We thank Smithkline & French Laboratories for providing us with *d*-amphetamine sulfate.

4 October 1979

Mutagenicity of a New Hair Dye Ingredient: 4-Ethoxy-*m*-phenylenediamine

Abstract. *An ingredient recently introduced in hair dyes, 4-ethoxy-*m*-phenylenediamine, is mutagenic in histidine-requiring strains of* Salmonella typhimurium. *Its mutagenic activity is similar to that of the hair dye ingredient it apparently replaced, 4-methoxy-*m*-phenylenediamine.*

Most hair dyes are complex mixtures of chemicals including aromatic amines (*1*). Two aromatic amines that had been widely used in commercial hair dyes were removed from these products by the manufacturers following reports of their carcinogenicity in mammals. 2,4-Toluenediamine (TDA; 2,4-diaminotoluene; 4-methyl-*m*-phenylenediamine) (Fig. 1, structure **1**) was voluntarily removed after it was reported to be carcinogenic when fed to rats (*2*). However, the closely related chemical 4-methoxy-*m*-phenylenediamine (MMPD; 2,4-diaminoanisole) (Fig. 1, structure **2**) continued to be used. The carcinogenicity of MMPD when fed to rats and mice has recently been demonstrated (*3*), and the Food and Drug Administration has promulgated a regulation requiring a warning label on hair dyes containing MMPD (*4*). The major hair dye manufacturers in the United States have now removed this chemical from their products.

At least one hair dye manufacturer has simultaneously introduced another closely related chemical, 4-ethoxy-*m*-phenylenediamine (EMPD) (Fig. 1, structure **3**), presumably as a replacement for MMPD (*5*). Since EMPD, the third chemical in the series of 4-substituted *m*-phenylenediamine hair dye ingredients, has not been evaluated for its carcinogenic potential, we tested it for mutagenicity in *Salmonella typhimurium*. Mutagenicity in histidine-requiring strains of this bacterial species can be rapidly determined by the *Salmonella* plate assay described by Ames *et al.* (*6*). This assay measures the ability of chemicals to induce mutations to histidine independence in these bacteria. It is widely used to screen organic chemicals for potential carcinogenicity (*7*). Both TDA and MMPD are mutagenic in *S. typhimurium* (*8*).

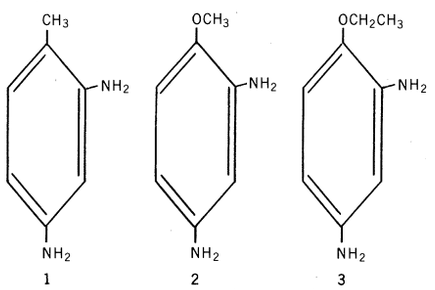

Fig. 1. Structures of TDA (**1**), MMPD (**2**), and EMPD (**3**).

The data in Fig. 2 show that EMPD is mutagenic in *S. typhimurium* strains TA98 and TA1537 in the presence of a metabolic activation system derived from rat liver. These bacterial strains contain frameshift mutations that make them dependent on histidine (*6*). The mutagenic activity of EMPD was similar to that of MMPD. In the frameshift strain TA1538 the mutagenic activity of both EMPD and MMPD was similar to that observed in strain TA98 if the results are expressed as a ratio of increase over controls (data not shown). EMPD was not mutagenic to base pair substitution strains TA1535 or TA100 when tested at doses from 30 to 10,000 μg per plate, although toxicity was evident at the highest dose tested. We conclude that EMPD is a frameshift mutagen in *S. typhimurium*.

It has been suggested that mutagenic potency in the most sensitive strain of *S. typhimurium* in the Ames test may be correlated quantitatively with carcinogenic potency in mammals (*9*). However, currently available information is not sufficient to warrant any conclusion concerning the carcinogenic potency of EMPD on the basis of its mutagenic effect on bacteria alone. Thus one cannot conclude from the data reported here that EMPD will have the same strength as a mammalian carcinogen as MMPD. There is, however, no basis for believing that exposure to EMPD is any less hazardous than exposure to an equal quantity of MMPD. The fact that both MMPD and TDA can also induce heritable mutations (sex-linked recessive lethals) in *Drosophila melanogaster* (*10*) raises the possibility that these chemicals may induce heritable genetic damage in addition to cancer in exposed mammals, including humans.

Mutagencity in bacteria is generally not considered to be a sufficient basis for taking regulatory action against a chemical already in commercial use. Results from cancer tests in animals in vivo, which usually require more than 2 years to initiate, execute, and evaluate, are needed before governmental agencies in the United States and most other countries will regulate a chemical as a carcinogen (*11*). More than 4 years elapsed after the mutagenicity of MMPD was reported (*8*) before appropriate carcinogenicity testing could be completed (*3*) and regulatory action taken (*4*). Similarly, it

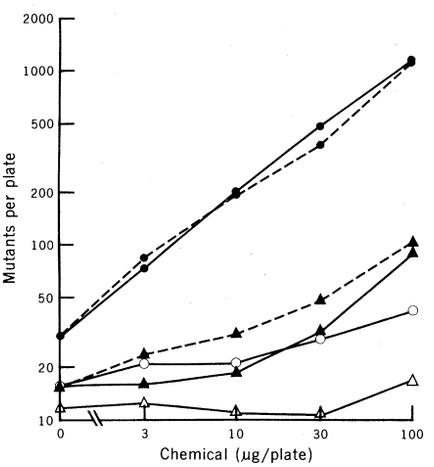

Fig. 2. Mutagenicity of EMPD sulfate and MMPD sulfate to *S. typhimurium* strains TA98 and TA1537. The plate assay described by Ames *et al.* (*6*) was performed with 50 μl of Aroclor 1254-induced rat liver S-9 per plate. The test chemicals were dissolved in dimethyl sulfoxide. The points plotted represent the means of three replicate plates. Solid lines, EMPD sulfate; dashed lines, MMPD sulfate; circles, TA98; triangles, TA1537; solid symbols, with S-9; open symbols, without S-9.

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will take several years to complete carcinogenicity studies of EMPD in vivo and to enact any appropriate regulations.

The close structural similarity of EMPD to the carcinogenic and mutagenic chemicals TDA and MMPD, as well as the results reported here, imply that EMPD should have been subjected to thorough evaluation for skin absorption, carcinogenicity, and mutagenicity in a variety of test systems before it was used in hair dyes. The use of the then-untested MMPD after the removal of the carcinogen TDA from hair dyes, and the recent introduction of untested EMPD after the carcinogen MMPD was removed from these products, underscore the importance of considering possible relationships between chemical structure and biological effects before exposing consumers to new chemicals.

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References and Notes

1. J. F. Corbett, *Cosmet. Toiletries* **91**, 21 (December 1976).
2. N. Ito, Y. Hiasa, Y. Konishi, M. Marugami, *Cancer Res.* **29**, 1137 (1969).
3. National Cancer Institute, *Bioassay of 2,4-Diaminoanisole Sulfate for Possible Carcinogenecity* (Carcinogenesis Technical Report No. 84) (Report PB279940/LLC, National Technical Information Service, Springfield, Va., 1978); J. M. Ward, S. F. Stinson, J. F. Hardisty, B. Y. Cockrell, D. W. Hayden, *J. Natl. Cancer Inst.* **62**, 1067 (1979).
4. *Fed. Regis.* **44**, 59509 (1979).
5. In the United States, ingredients of hair dyes are listed on the boxes containing these products.
6. B. N. Ames, J. McCann, E. Yamasaki, *Mutat. Res.* **31**, 347 (1975).
7. Studies on the correlation between mutagenicity in *S. typhimurium* and carcinogenicity in mammals include: J. McCann, E. Choi, E. Yamasaki, B. N. Ames, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 5135 (1975); J. McCann and B. N. Ames, *ibid.* **73**, 950 (1976); B. Commoner, A. J. Vithayathil, J. I. Henry, J. C. Gold, M. J. Reding, *Reliability of Bacterial Mutagenesis Techniques to Disguish Carcinogenic and Noncarcinogenic Chemicals* (Environmental Protection Agency Report EPA-600/1-76-022) (Report PB259934/LLC, National Technical Information Service, Springfield, Va., 1976); T. Sugimura, S. Sato, M. Nagao, T. Yahagi, T. Matsushima, Y. Seino, M. Takeuchi, T. Kawachi, in *Fundamentals in Cancer Prevention*, P. N. Magee *et al.*, Eds. (University Park Press, Baltimore, 1976), p. 191; I. F. H. Purchase, E. Longstaff, J. Ashby, J. A Styles, D. Anderson, P. A. Lefevre, F. R. Westwood, *Br. J. Cancer* **37**, 873 (1978); and V. F. Simmon, *J. Natl. Cancer Inst.* **62**, 893 (1979).
8. B. N. Ames, H. O. Kammen, E. Yamasaki, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 2423 (1975).
9. M. Meselson and K. Russell, in *Origins of Human Cancer*, H. H. Hiatt, J. D. Watson, J. A. Winsten, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1977), p. 1473.
10. W. G. H. Blijleven, *Mutat. Res.* **48**, 181 (1977).
11. An exception is the Ministry of Health of Italy, which has banned several ingredients of hair dyes, including MMPD, on the basis of their mutagenicity to *S. typhimurium* [*Gazz. Uffic. Repub. Ital.* (No. 166, 25 June 1976), p. 5025].
12. We thank the Revlon Research Center, Inc., for their generous gifts of MMPD sulfate and EMPD sulfate; Dr. B. N. Ames for the *S. typhimurium* strains used in this work; and L. Katzenstein of *Consumer Reports* for bringing the use of EMPD in hair dyes to the attention of the Food and Drug Administration.

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Adapting to Two Orientations: Disinhibition in a Visual Aftereffect

Abstract. *The tilt aftereffect of adapting to two different orientations simultaneously is weaker than the aftereffect of adapting to the more effective of the two orientations alone. This finding is consistent with explanations of orientational aftereffects in terms of lateral inhibition between cortical orientation detectors, but not with explanations in terms of neural "fatigue" from excitation.*

Visual contours that differ in retinal orientation may interact and produce distortions in their apparent orientation. For example, a line tilted 10° clockwise from the vertical induces an approximate 2° to 4° apparent counterclockwise tilt in a vertical test line when it is presented simultaneously with and adjacent to the test line (tilt illusion, orientation contrast), or when it is inspected prior to the test line (tilt aftereffect); the magnitude of the perceived orientation shifts varies with the angle between the interacting lines (*1*). Such contour interactions have been suggested to be manifestations of lateral inhibition between orientation detectors in the human visual cortex (*2–5*), a form of inhibition for which there is good neurophysiological evidence in the visual cortex of the cat (*6*). The hypothesis of orientation-specific inhibition seems uncontroversial when applied to simultaneous psychophysical contour interactions, but the hypothesis that aftereffects of spatial adaptation are the result of inhibition rather than of neural "fatigue" following prolonged excitation (*7*) is debated (*8, 9*). We have obtained evidence for the role of inhibition in the tilt aftereffect by adopting Carpenter and Blakemore's (*4*) disinhibition paradigm. They showed that the orientation contrast induced by, for example, a 10° clockwise inducing line may be canceled (rather than increased) by a second clockwise inducing line. In our experiment, the subject adapted to two orientations simultaneously.

The stimuli (inset in Fig. 1) were black lines, 0.03° wide and 1.3° long, viewed binocularly in a modified tachistoscope (Scientific Prototype N-1000). The background luminance was approximately 40 cd/m^2, and the contrast between the line and the background, defined as $(L_{max} - L_{min})/(L_{max} + L_{min})$, where L is luminance, was about 0.3. The adapting pattern consisted (except for the control condition) of two lines: A_1 was fixed at 12° clockwise tilt, the orientation of A_2 was varied between 6° and 60° clockwise tilt (*10*). The test pattern consisted of a vertical test line (T) and a variable micrometer-controlled comparison line (C). The subject's task was to set C parallel to the apparent orientation of T. The orientation of C was read to the nearest 0.07°.

To generate an aftereffect, the subject viewed the adapting pattern for 2 minutes, moving his eyes along a horizontal fixation bar to avoid confounding with afterimages. This initial adaptation period was followed by a sequence of 1.5-second presentations of the test pattern and 10-second readaptation periods; this sequence was continued until five settings were made. The experimental sessions opened with five parallel settings of C and T without previous adaptation; this null position was rechecked before each new adapting condition.

Figure 1 shows results for two subjects adjusted to a common baseline. Except when A_1 and A_2 were superimposed (actually a second run of the control condition), the aftereffects of adapting to two orientations were weaker than the aftereffect of adapting to A_1 alone. The reduction was most pronounced when A_1 and A_2 formed an angle of 10° to 15°; as the angle between the two adapting lines grew, the aftereffect gradually returned to the baseline, but it did not fully regain its strength at the largest angle tested. Thus, the angular function of the reduction effect was similar to the angular function of the aftereffect itself (*1, 10*).

These results follow directly from the hypothesis that the tilt aftereffect is an aftereffect of lateral inhibition between orientation detectors (*3*). During the adaptation phase the inhibitory signals from neurons optimally excited by orientation A_1 to the vertical and near-vertical detecting neurons are reduced by themselves being inhibited by neurons optimally excited by orientation A_2; consequently the aftereffect decreases, since it depends on the amount of inhibition received by the vertical and near-vertical detecting neurons during adaptation.

The results are, on the other hand, dif-

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