creased tyrosine hydroxylase activity, and decreased norepinephrine turnover (*14*).

At weaning, a time when amino acid transport into brain is reduced, we have observed alterations in serotonin receptor binding (*15*) and in tyrosine hydroxylase activity in norepinephrine and dopamine areas of the brain (*16*). Thus, the sparing of the brain in undernutrition does not extend to all aspects of amino acid metabolism.

The biochemical changes that we observed in undernourished rats may represent important processes underlying the aberrations in brain function and behavior seen in adult animals and humans exposed to malnutrition early in life (*17*).

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References and Notes

1. S. Zamenhof, E. van Marthens, F. L. Margolis, *Science* **160**, 322 (1968); I. Fish and M. Winick, *Exp. Neurol.* **25**, 534 (1969); M. Winick and A. Nobel, *J. Nutr.* **89**, 300 (1966); J. Dobbing, in *Applied Neurochemistry*, A. N. Davison and J. Dobbing, Eds. (Davis, Philadelphia, 1968), p. 287; E. M. Widdowson and R. A. McCance, *Proc. R. Soc. London Ser. B* **158**, 329 (1963).
2. H. H. Donaldson, *J. Nerv. Ment. Dis.* **38**, 259 (1911).
3. The rats were from Holtzman Co., Madison, Wis.
4. R. H. Barnes, E. Kwong, L. Morrissey, L. Vilhjalmsdottir, D. A. Levitsky, *J. Nutr.* **103**, 273 (1973).
5. A. J. Mueller and W. M. Cox, *J. Biol. Chem.* **119**, LXXII (1937).
6. H. L. Vis, in *Caloric Deficiencies and Protein Deficiencies*, R. A. McCance and E. M. Widdowson, Eds. (Little, Brown, Boston, 1968), p. 119.
7. S. Samuels and I. Fish, *Anal. Biochem.* **87**, 447 (1978); ——, L. S. Freedman, *Neurochem. Res.* **3**, 619 (1978). Transport rates were determined by measuring total tissue accumulataion of each labeled amino acid at 2.5 to 12.5 minutes after injection, during which time net influx was linear. The slope of the regression line indicated the transport rate. The tissue value at each time point was calculated from the mathematically derived mean plasma specific activity and the total tissue radioactivity for each animal. The rates obtained by this method are comparable to those reported with the constant infusion technique. Rates of amino acid incorporation into protein were similarly obtained.
8. The free amino acid concentrations on sulfosalicylic acid extracts of pooled samples of plasma and whole brain were:

Amino acid	Plasma (μmole/ml)		Brain (μmole/g)	
	Control	Experimental	Control	Experimental
Tyrosine	0.099	0.066	0.079	0.114
Lysine	0.635	0.410	0.466	0.383

The analyses were kindly performed by P. Norton on a Beckman amino acid analyzer in the laboratory of S. Snyderman and C. Sansaricq.
9. M. Miller, J. P. Leahy, W. C. Stern, P. J. Morgane, O. Resnick, *Exp. Neurol.* **57**, 142 (1977); W. C. Stern, M. Miller, W. B. Forbes, J. P. Leahy, P. J. Morgane, O. Resnick, *Brain Res. Bull.* **1**, 27 (1976); M. Miller, J. P. Leahy, F. McConville, P. J. Morgane, O. Resnick, *ibid.* **2**, 189 (1977).
10. K. D. Neame, in *Applied Neurochemistry*, A. N. Davison and J. Dobbing, Eds. (Davis, Philadelphia, 1968), p. 119; H. N. Christensen, *Biological Transport* (Benjamin, Reading, Mass., ed. 2, 1975).
11. S. Roberts and B. S. Morelos, *J. Neurochem.* **12**, 373 (1965); H. N. Munro, in *Mammalian Protein Metabolism*, H. N. Munro, Ed. (Academic Press, New York, 1970), vol. 4, p. 299.
12. J. W. T. Dickerson and S. K. Pao, *Biol. Neonate* **25**, 114 (1975).
13. M. K. Roach, J. Corbin, W. Pennington, *J. Neurochem.* **22**, 521 (1974); A. J. Patel, D. J. Atkinson, R. Balazs, *Dev. Psychobiol.* **5**, 453 (1975).
14. W. J. Shoemaker and R. J. Wurtman, *Science* **171**, 1017 (1971); C. Lee and R. Dubos, *J. Exp. Med.* **136**, 1931 (1972); F. Sereni, N. Principi, L. Peretti, L. P. Sereni, *Biol. Neonat.* **10**, 254 (1966); R. J. Hernandez, *Biol. Neonate* **30**, 181 (1976); W. J. Shoemaker and R. J. Wurtman, *J. Nutr.* **103**, 1537 (1973).
15. J. J. Afflito, E. Friedman, L. S. Freedman, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **38**, 439 (1979).
16. L. S. Freedman and L. E. Morgano, in preparation.
17. S. Frankova and R. H. Barnes, *J. Nutr.* **96**, 485 (1968); C. T. Randt and B. Derby, *Arch. Neurol.* **28**, 167 (1973); D. A. Levitsky and R. H. Barnes, *Nature (London)* **225**, 468 (1970); E. Pollitt and C. Thompson, in *Nutrition and the Brain*, R. J. Wurtman and J. J. Wurtman, Eds. (Raven, New York, 1977), vol. 2, p. 261.
18. C. T. Randt, S. Samuels, I. Fish, *Pharmacol. Biochem. Behav.* **4**, 689 (1976).
19. This work was supported by NIH grant HD 10309 to L.S.F. We thank J. Dancis and C. T. Randt for continued advice and support of this research.

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Multiple Daily Amphetamine Administration: Behavioral and Neurochemical Alterations

Abstract. *In rats, multiple daily amphetamine injections (2.5 milligrams per kilogram of body weight, injected subcutaneously every 4 hours for 5 days) resulted in a progressive augmentation in response, characterized by a more rapid onset and an increased magnitude of stereotypy. By contrast, offset times of both the stereotypy and the poststereotypy hyperactivity periods were markedly shortened. When the animals were retested with the same dose of amphetamine 8 days after the long-term treatment was discontinued, the time of offset of the stereotypy and hyperactivity phases had recovered to values found with short-term amphetamine treatment, whereas the more rapid onset of stereotypy persisted. Brain monoamine and amphetamine concentrations and tyrosine hydroxylase activity were determined in comparably treated rats at times corresponding to the behavioral observations. The behavioral data indicate that enhanced responsiveness to amphetamine following its repeated administration may contribute to the development of amphetamine psychosis.*

We have previously shown that repeated single daily injections of *d*-amphetamine in rats augment some features of the amphetamine response (*1*-*3*). Similar results have since been obtained in other species (*4*) and with repeated injections of other psychomotor stimulants (*5*). In humans, long-term administration of psychostimulants induces a schizophrenialike psychosis (*6*). On the basis of the behavioral augmentation produced in animals, we suggested that the heightened responsivity to amphetamine might be involved in the development of amphetamine psychosis (*1*-*3*). However, because amphetamine psychosis is most frequently associated with multiple daily drug administrations (*6*), development of a useful animal behavior model of this psychosis may require a more sustained level of amphetamine intoxication. In this regard, it has recently been reported that after 3 days of continuous amphetamine infusion in rats, motor stereotypies are replaced by increased social behaviors, such as fleeing and fighting (*7*). These results suggest that enhanced responsiveness to amphetamine may not be implicated in amphetamine psychosis. Therefore, we have extended our previous studies by characterizing the changes in behavior and in monoamine systems that occur with multiple daily injections of amphetamine in rats.

Male Wistar rats (325 to 375 g), obtained from Hilltop Laboratories, were housed individually in sound-attentuating activity chambers for 2 days before receiving 30 successive, subcutaneous injections of either saline or *d*-amphetamine sulfate (2.5 mg of free base per kilogram of body weight) at 4-hour intervals

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beginning at 10 a.m. This treatment was followed 4 hours and again 8 days later by an injection of *d*-amphetamine (2.5 mg/kg) in all animals. The experimental chambers, which housed the animals throughout the study, were brightly lighted from 6 a.m. to 6 p.m. and dimly lighted the other 12 hours; food and water were freely available.

Each animal's locomotor activity was measured automatically as crossovers from one quadrant of the chamber to another and as rearings detected by contact with touchplates set 12.7 cm above the floor. Both measures were continuously monitored throughout the study with a computer (Nova 1200) (*8*). Viewing lenses in each experimental chamber and a closed-circuit videotape system permitted us to observe the animals without disturbing them. Stereotyped behaviors, including sniffing, repetitive movements of the head and limbs, and oral stereotypies (chewing, licking, and biting), were assessed at 10-minute intervals for 3 hours after both the 31st and the retest amphetamine injections. The duration and intensity of these behaviors (*9*) were scored by trained observers who were unaware of the treatment conditions.

Additional groups of rats, also treated with multiple daily injections of either saline or *d*-amphetamine (2.5 mg/kg), were killed at times corresponding to the behavioral observations. Monoamine and amphetamine concentrations, as well as tyrosine hydroxylase activity, were subsequently measured in selected brain regions or in whole brain (*10*).

After the first injection of *d*-amphetamine, the rats exhibited a multiphasic response pattern (Figs. 1 and 2) consisting of early and late periods during which increased locomotion was the predominant response and an intermediate stereotypy phase during which locomotion was absent and repetitive movements of the head or limbs, or both, were displayed continuously. Preliminary treatment with multiple daily injections of saline did not significantly alter this response pattern. By the second amphetamine administration, and continuing throughout the course of the multiple daily injections, the latency to onset of repetitive head and limb movements was markedly decreased (*11*) and the peak stereotypy score significantly elevated (*12*). However, in addition to these effects, which resemble those we have observed with repeated single daily administrations of amphetamine (*1-3*), injections at 4-hour intervals resulted in a progressive decrease in the durations of both the stereotypy phase (*13*) and the subsequent hyperactivity interval (*14*).

Multiple daily injections of amphetamine produced no apparent qualitative changes in either the stereotypy or the hyperactivity phase of the amphetamine response. When locomotor activity subsided, the rats engaged in brief episodes of eating and grooming, followed by sleep lasting until the next injection. Injections given at other times of the day resulted in a response pattern similar to that produced by the 10 a.m. injections. Alterations in rearing activity paralleled those observed for crossovers. The response to saline administered 4 and 24 hours after the 31st amphetamine injection did not indicate a conditioned locomotor or stereotypy response. These results are consistent with our previous findings that conditioning does not account for the behavioral augmentation induced by repeated amphetamine injections (*1-3*).

The more rapid onset (*15*) and increased magnitude (*16*) of stereotypy

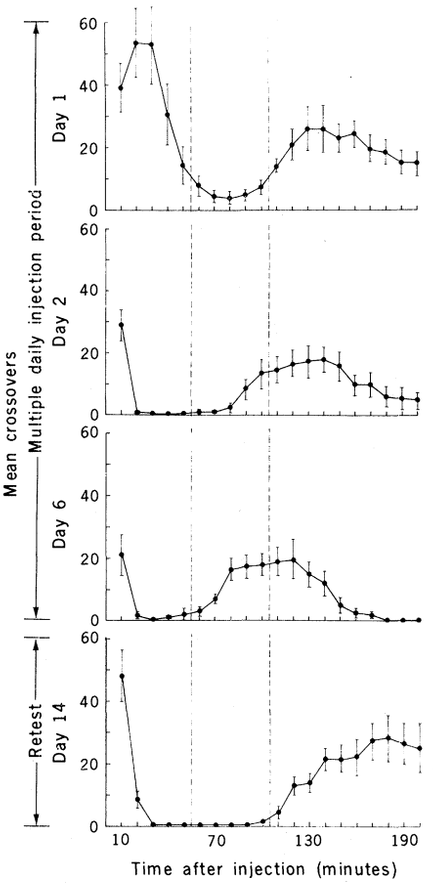

Fig. 1. Mean crossovers (± standard errors) during successive 10-minute intervals following the 10 a.m. *d*-amphetamine injection on days 1, 2, and 6, and again 8 days after the discontinuation of long-term treatment (retest day). Dashed lines indicate the period of focused stereotypy produced by the first amphetamine injection. $N \geq 11$ in each group.

were still apparent when the rats were retested with *d*-amphetamine (2.5 mg/kg) 8 days after the long-term treatment ended; the offset times of both the stereotypy (*17*) and the poststereotypy hyperactivity (*18*) periods no longer occurred earlier than those observed with single amphetamine administration, however (Figs. 1 and 2).

Multiple daily injections of *d*-amphetamine did not produce consistent changes in regional brain serotonin concentrations. By contrast, 4 hours after the 30th amphetamine injection, the norepinephrine concentration was significantly reduced in the hippocampus [saline, 0.21 ± 0.03 μg/g; amphetamine, 0.10 ± 0.02 μg/g; t (10) = 3.05, $P < .02$] and in the hypothalamus [saline, 1.09 ± 0.14 μg/g; amphetamine, 0.46 ± 0.05 μg/g; t (13) = 4.24, $P < .001$]; caudate dopamine concentration was also significantly decreased [saline, 11.38 ± 1.13 μg/g; amphetamine, 7.83 ± 0.61 μg/g; t (15) = 2.76, $P < .02$]. By 8 days after the last amphetamine injection, however, the concentration of catecholamines in these areas was no longer significantly different from the control values. Thus the changes in catecholamine levels parallel those occurring in the offsets of the amphetamine-induced stereotypy and locomotion phases. Therefore, the behavioral augmentation resulting from repeated amphetamine administration may have been restricted to the early portion of the response because the reduced concentrations of norepinephrine or dopamine were insufficient to sustain the enhanced responsiveness to amphetamine (*19*). The mechanisms responsible for the behavioral augmentation remain to be elucidated.

This study shows that multiple daily injections of amphetamine produce a more rapid onset and an increased magnitude of stereotyped behavior. These results are consistent with our previous findings (*1-3*) and indicate that behavioral augmentation, at least during the initial portion of the amphetamine response, can occur over a wide range of doses and of intervals between successive injections. Thus, as we have suggested, the enhanced responsiveness to amphetamine resulting from its repeated administration may contribute to the development of amphetamine psychosis.

The minimal dosage schedule required to induce amphetamine psychosis is not known, because systematic dose- and time-response data are not available in humans. For example, the relative contributions of dose level and treatment du-

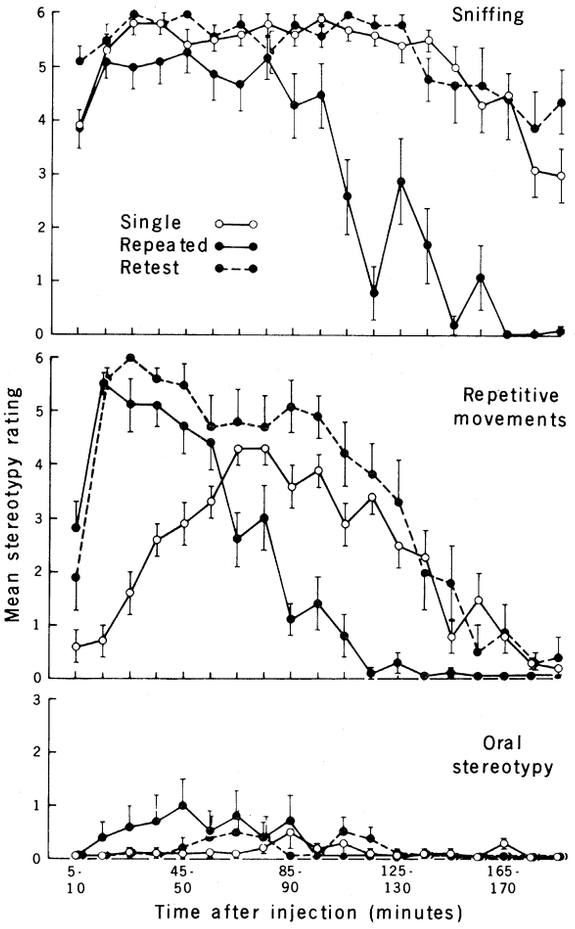

Fig. 2. Mean stereotypy scores (*11*) during successive 10-minute intervals after the injection of *d*-amphetamine (2.5 mg/kg) either 4 hours after long-term preliminary treatment with saline (single amphetamine group) or *d*-amphetamine (repeated amphetamine group), or 8 days after the long-term preliminary treatment with amphetamine was discontinued (retest group). $N \geq 11$ in each group.

ration are difficult to determine because escalating dosage regimens are frequently used. In addition, amphetamine abusers have been used as subjects in most clinical studies, further complicating interpretation of results. In fact, although amphetamine psychosis is usually associated with long-term drug administration, similar psychotic symptoms, in the presence of a clear sensorium, have been induced in some individuals after short-term administration of moderate to high doses of the drug (*20*). It is possible, therefore, that multiple injections of amphetamine lead to psychotic symptoms when a critical dose is achieved rather than as a consequence of the duration of amphetamine intoxication. Even in the absence of an escalating dosage regimen, if interadministration intervals are short, brain amphetamine concentrations would increase progressively because of the relatively long half-life of amphetamine in humans (*21*). Furthermore, as we have shown in rats, repeated administration produces an enhanced response to amphetamine, even when successive administrations are separated by relatively long intervals. Since we have not observed any apparent qualitative changes in the behavioral response to amphetamine with repeated administration in rats, it is conceivable that the behaviors that characterize the acute response to amphetamine represent an appropriate animal model of amphetamine psychosis.

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References and Notes

1. D. S. Segal, *Adv. Biochem. Psychopharmacol.* **13**, 247 (1975).
2. ——— and A. J. Mandell, *Pharmacol. Biochem. Behav.* **2**, 249 (1974).
3. D. S. Segal and D. S. Janowsky, in *Psychopharmacology: A Generation of Progress*, M. A. Lipton, A. DiMascio, K. F. Killam, Eds. (Raven, New York, 1975), p. 1113.
4. P. H. Short and L. Shuster, *Psychopharmacologia* **48**, 59 (1976); H. L. Klawans, P. Crossett, N. Dana, *Adv. Neurol.* **9**, 105 (1975); H. L. Klawans and D. L. Margolin, *Arch. Gen. Psychiatry* **32**, 725 (1975); D. S. Garver, R. F. Schlemmer, Jr., J. W. Maas, J. M. Davis, *Am. J. Psychiatry* **132**, 33 (1975); K. L. Preston, R. F. Schlemmer, Jr., D. S. Garver, J. A. Jackson, J. P. Bederka, J. M. Davis, *Neurosci. Abstr.* **2**, 853 (1976).
5. R. G. Browne and D. S. Segal, *Pharmacol. Biochem. Behav.* **6**, 545 (1977); J. S. Stripling and E. H. Ellinwood, Jr., *Exp. Neurol.* **54**, 546 (1977); M. M. Kilbey and E. H. Ellinwood, Jr., in *Cocaine and Other Stimulants*, M. M. Kilbey and E. H. Ellinwood, Jr., Eds. (Plenum, New York, 1977), p. 409; R. M. Post, in *ibid.*, p. 353; *Am. J. Psychiatry* **132**, 225 (1975); ——— and H. Rose, *Nature (London)* **260**, 731 (1976); R. M. Post, P. T. Kopanda, K. T. Black, *Biol. Psychiatry* **11**, 403 (1976); R. L. Borison, H. S. Havdala, B. I. Diamond, *Life Sci.* **21**, 117 (1977).
6. B. Angrist and S. Gershon, *Biol. Psychiatry* **2**, 95 (1970); D. S. Bell, *Br. J. Psychiatry* **111**, 701 (1965); D. S. Bell, *Arch. Gen. Psychiatry* **29**, 35 (1973); P. H. Connell, *Amphetamine Psychosis* (Maudsley Monographs No. 5, Oxford Univ. Press, London, 1958); J. D. Griffith, J. Cavanaugh, J. Held, J. Oates, *Arch. Gen. Psychiatry* **26**, 97 (1972); G. Rylander, *Psychiatr. Neurol. Neurochir.* **75**, 203 (1972); S. H. Snyder, *Am. J. Psychiatry* **130**, 61 (1973); ———, S. P. Banerjee, H. I. Yamamura, D. Greenberg, *Science* **184**, 1243 (1974); A. Sudilovsky, in *Aminergic Hypothesis of Behavior: Reality or Cliche?*, B. K. Bernard, Ed. (NIDA Monograph Series No. 3, Government Printing Office, Washington, D.C., 1975).
7. G. Ellison, M. Eison, H. Huberman, *Psychopharmacology* **56**, 293 (1978); ———, F. Daniel, *Science* **201**, 276 (1978).
8. The behavioral chambers and data recording system are described in detail in (*1*).
9. Within a 30-second sampling interval, the duration of each behavior was scored as 1 (discontinuous) or 2 (continuous); intensity was rated as 1 (mild), 2 (moderate), or 3 (intense). For analysis of results, the duration and intensity scores for each behavior at each time interval were multiplied to yield a single value. The highest score assigned to biting, chewing, or licking was used as the oral stereotypy score for that interval.
10. The rats were killed by decapitation. The caudate nucleus, hippocampus, and hypothalamus were rapidly dissected by the method of D. S. Segal and R. Kuczenski [*Brain Res.* **68**, 261 (1974)], frozen quickly in liquid nitrogen, and stored at −60°C for later assay. Norepinephrine, dopamine, and serotonin were measured as micrograms per gram of tissue (wet weight) in the same tissue sample according to a modification of the radioenzymatic catecholamine assay of C. F. Saller and M. J. Zigmond [*Neurosci. Abstr.* **3**, 321 (1977)] and W. J. Shoemaker, M. Schlumpf, B. R. Clark, L. Anderson, and F. E. Bloom [in *Catecholamines: Basic and Clinical Frontiers*, E. Usdin, I. Kopin, J. Barchas, Eds. (Pergamon, New York, 1979), vol. 1] and of the fluorimetric serotonin assay of G. Curzon and A. R. Green [*Br. J. Pharmacol.* **39**, 653 (1970)] and S. B. Weinberger, S. Knapp, and A. J. Mandell [*Life Sci.* **22**, 1595 (1978)]. Synaptosomal and soluble tyrosine hydroxylase were assayed according to R. Kuczenski and D. S. Segal [*J. Neurochem.* **22**, 1039 (1974)]; amphetamine concentrations in whole brain were measured by a modification of the radiometric method of J. J. Freeman and F. Sulser [*Neuropharmacology* **13**, 187 (1974)].
11. Locomotor activity 20 to 40 minutes after injection on days 1 and 2: 137.3 ± 31.1 versus 0.9 ± 0.3 crossovers (two-tailed *t*-test for related groups, $P < .001$). Repetitive movement 5 to 20 minutes after injection following single and repeated amphetamine: 1.3 ± 0.4 versus 8.3 ± 0.5 (Mann-Whitney U test, $P < .002$).
12. Repetitive movement 65 to 90 minutes after injection (single amphetamine group) and 15 to 40 minutes after injection (repeated amphetamine group): 12.1 ± 0.7 versus 15.7 ± 1.0 (Mann-Whitney U test, $P < .02$).
13. Locomotor activity 80 to 100 minutes after injection on days 1 and 6: 16.3 ± 4.9 versus 52.0 ± 9.8 crossovers (two-tailed *t*-test for related groups, $P < .01$). Repetitive movement 85 to 140 minutes after injection following single and repeated amphetamine: 18.5 ± 1.6 versus 3.7 ± 1.0 (Mann-Whitney U test, $P < .002$).
14. Locomotor activity 160 to 200 minutes after injection on days 1 and 6: 92.5 ± 18.6 versus 5.1 ± 2.1 crossovers (two-tailed *t*-test for related groups, $P < .001$).
15. Locomotor activity 20 to 50 minutes after injection on days 1 and 14: 171.3 ± 42.2 versus 9.1 ± 2.8 crossovers (two-tailed *t*-test for related groups, $P < .01$). Repetitive movement 5 to 20 minutes after injection following single and repeated amphetamine: 1.3 ± 0.4 versus 7.5 ± 0.7 (Mann-Whitney U test, $P < .002$).
16. Repetitive movement 65 to 90 minutes after injection (single amphetamine group) and 15 to 40 minutes after injection (repeated amphetamine group): 12.1 ± 0.7 versus 17.3 ± 0.4 (Mann-Whitney U test, $P < .002$).
17. Locomotor activity 110 to 120 minutes after injection on days 1 and 14: 34.7 ± 9.1 versus 14.1 ± 3.2 crossovers (two-tailed *t*-test for related groups, $P < .05$). Repetitive movement

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.

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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*).

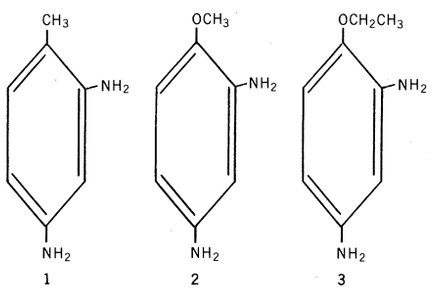

Fig. 1. Structures of TDA (**1**), MMPD (**2**), and EMPD (**3**).

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

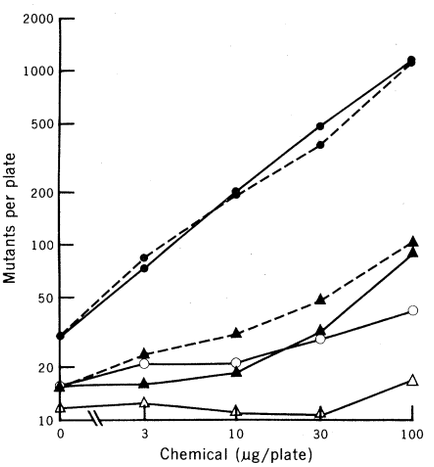

Fig. 2. Mutagenicity of EMPD sulfate and MMPD sulfate to *S. typhimurium* strains TA98 and TA1537. The plate assay described by Ames *et al.* (*6*) was performed with 50 μl of Aroclor 1254-induced rat liver S-9 per plate. The test chemicals were dissolved in dimethyl sulfoxide. The points plotted represent 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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