such as that observed by others (*11*), and an increase in the prevalence of micronuclei. Since it has been shown that lymphocytes of normal individuals will undergo three or four divisional cycles during 72 hours of culture (*12*), the observed micronuclei might indicate that there is an increased anaphase lag with or without chromosomal breakage during the preceding cell divisions in vitro. Anaphase lag, formation of hypodiploid cells, and alterations of DNA content were also observed in cultures of human lung explants exposed to marihuana smoke (*13*). Tetrahydrocannabinol in 3 to 9 μM concentration inhibits the growth of tetrahymena by reducing DNA and RNA synthesis (*14*).

Further studies are required to elucidate the exact mechanism by which marihuana products might affect DNA synthesis and the genetic equilibrium of T (thymus derived) lymphocyte population.

GABRIEL G. NAHAS
NICOLE SUCIU-FOCA
JEAN-PIERRE ARMAND
AKIRA MORISHIMA

Departments of Anesthesiology, Surgery, and *Pediatrics, College of Physicians & Surgeons of Columbia University, New York 10032*

References and Notes

1. G. G. Nahas, D. Zagury, I. W. Schwartz, M. D. Nagel, *Nature (Lond.)* **243**, 407 (1973).
2. N. Suciu-Foca, J. Buda, T. Thiem, T. Suciu, *Transplantation* **14**, 711 (1972).
3. N. Suciu-Foca, J. Buda, J. McManus, T. Thiem, K. Reemtsma, *Cancer Res.*, in press.
4. N. Suciu-Foca, J. Buda, J. McManus, K. Reemtsma, *Excerpta Med. Int. Congr. Ser. No. 275* (1973), p. 119.
5. M. E. Weksler, *Clin. Res.* **31**, 590 (1973).
6. A. R. Cheema and E. A. Hersh, *Cancer Res.* **28**, 851 (1971).
7. H. Huber, D. Pastner, P. Dietrich, H. Braunsteiner, *Clin. Exp. Immunol.* **5**, 75 (1969).
8. D. S. Kreuz and J. Axelrod, *Science* **179**, 391 (1973).
9. P. Beaconfield, J. Ginsburg, R. Rainbury, *N. Engl. J. Med.* **287**, 209 (1972); L. Vachon, M. Y. Fitzgerald, N. H. Solliday, I. A. Gould, E. A. Gaensler, *ibid* **288**, 985 (1973).
10. E. B. Raftery and A. M. Denman, *Br. Med. J.* **2**, 452 (1973); J. W. Hadden, E. M. Hadden, E. Middletown, *Cell. Immunol.* **1**, 583 (1971).
11. M. A. Stenchever, T. J. Kunysz, M. A. Allen, *Am. J. Obstet. Gynecol.*, in press.
12. N. Kamata, A. Morishima, J. H. Tjio, *Clin. Immunol. Jap.* **2**, 657 (1971).
13. C. Leuchtenberger, R. Leuchtenberger, A. Schneider, *Nature (Lond.)* **241**, 137 (1973); C. Leuchtenberger, R. Leuchtenberger, U. Ritter, *ibid.* **242**, 403 (1973).
14. A. M. Zimmerman and D. K. McClean, in *Drugs and the Cell Cycle*, A. M. Zimmerman, G. M. Padilla, I. L. Cameron, Eds. (Academic Press, New York, 1973), pp. 67–94.
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Amphetamine in Human Plasma: A Sensitive and Specific Enzymatic Assay

Abstract. *A sensitive and specific enzymatic-isotopic method of determining plasma amphetamine concentrations in man is described. The assay is based on the transfer of the tritiated methyl group of* S-*adenosyl*-L-[*methyl-*^3H]*methionine to amphetamine in the presence of a partially purified* N-*methyltransferase from rabbit lung. With this assay as little as 10 nanograms of amphetamine per milliliter of plasma can be accurately determined. The concentrations of* d- *and* l-*amphetamine in the plasma after 20 to 30 milligrams of the drug had been ingested by human subjects are reported.*

Amphetamine is a potent sympathomimetic amine widely abused for its central stimulant effects and used clinically in the treatment of hyperactive children (*1*), obesity (*2*), and narcolepsy (*3*). Amphetamine also produces a psychosis that has been a useful model for the study of schizophrenia (*4*).

The lack of a sensitive, specific, and reproducible assay for amphetamine in plasma has hampered efforts to establish therapeutic dosages, to understand tolerance and the different potencies and effects of *d*- and *l*-amphetamine, and to detect abusers of the drug. Several assay methods have been proposed (*5*), but none has, as yet, become fully accepted.

We now describe an enzymatic assay for amphetamine in plasma such that 10 ng of amphetamine per milliliter of plasma can be accurately and reproducibly measured. The assay is based on the *N*-methylation of amphetamine to form radioactive methamphetamine, by means of an *N*-methyltransferase from rabbit lung and *S*-adenosyl-L-[methyl-^{3}H]methionine (^{3}H-SAME) as methyl donor. Sensitivity and specificity are achieved by extraction into solvents and drying at high temperatures.

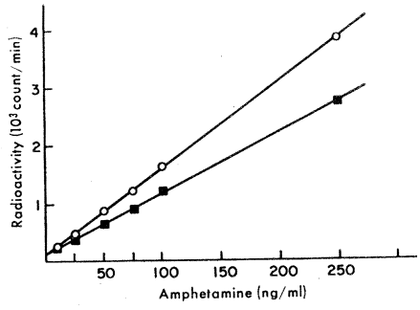

Fig. 1. Standard curves for *d*- (■) and *l*- (○) amphetamine added to plasma. Results are expressed as counts per minute produced per nanogram of amphetamine per milliliter of plasma. The blank values for amphetamine-free plasma were 60 to 150 counts per minute.

Blood samples were collected in heparinized syringes or in ACD-containing Vacutainers and were centrifuged at 4°C for 10 minutes at about 5000 rev/min. (Plasma samples can be stored at −15°C for at least 1 month without loss of amphetamine.) Samples (4 ml) of plasma were transferred to a 45-ml glass-stoppered centrifuge tube, adjusted to *p*H 11 with 50 μl of 5*N* NAOH, and shaken with 16 ml of pentane (*6*) for 30 minutes at about 6°C to avoid evaporation. The tubes were centrifuged at 1500 rev/min for 10 minutes, the amphetamine was returned to the aqueous phase by transferring 15 ml of the pentane extract to another 45-ml glass-stoppered centrifuge tube containing 0.6 ml of 0.2 m*M* HCl and shaking for 15 minutes. After centrifugation, the acid phase was frozen with acetone and Dry Ice and the pentane was decanted. It is important that residual pentane be removed; normally this is done by leaving the centrifuge tubes containing the acid extracts in the cold room overnight. The acid phases were then divided into two 0.25-ml fractions and transferred to 13-ml glass-stoppered centrifuge tubes. The *p*H was adjusted to 8.6 with 25 μl of 0.01*M* tris buffer (*p*H 8.6), and 75 μl of lung enzyme plus 1.25 μc (0.156 nmole) of ^{3}H-SAME (*7*) were added. This mixture was incubated at 37°C for 90 minutes, and 0.5 ml of 0.5*M* borate buffer (*p*H 10) plus 2.5 μg (25 μl) of *dl*-methamphetamine hydrochloride were added. A mixture (6 ml) of heptane and isoamyl alcohol (98.5 : 1.5, by volume) was then added and the [^{3}H]methamphetamine was extracted into the organic phase by shaking for 10 minutes and centrifuging at 1500 rev/min. The following shaking and centrifuging steps were carried out in the same manner. The organic extract was then washed with an additional 0.5 ml of borate buffer, following which the [^{3}H]methamphetamine was extracted into 0.5 ml of 0.1*M*

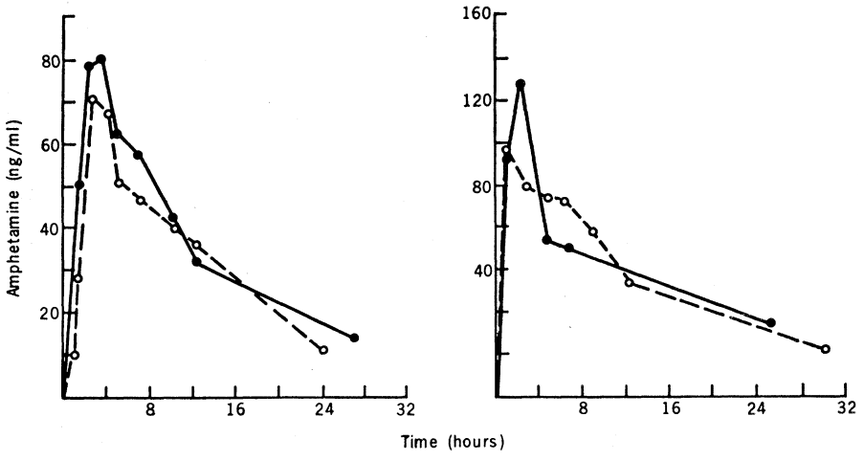

Fig. 2. Amphetamine in the plasma of human subjects after they had ingested *d*- (●) or *l*- (○) amphetamine. (A) *d*- and *l*-Amphetamine in plasma after 0.23 mg/kg (free base); (B) after 0.5 mg/kg. The results are expressed as nanograms of amphetamine per milliliter of plasma at various times after ingestion of the drug. Differences in the standard curves of the isomers were taken into account in obtaining the concentration of *l*- and *d*-amphetamine in blood.

HCl. The acid phase was readjusted to *p*H 10 with 1 ml of borate buffer, and the [^{3}H]methamphetamine was again extracted into another 6-ml portion of the heptane–isoamyl alcohol mixture. A portion (5 ml) of the organic phase was transferred to a counting vial containing 2 ml of the heptane–isoamyl alcohol mixture and 2 mg (100 μl) of *dl*-methamphetamine hydrochloride, and dried at 80°C for about 12 hours. An additional 20 mg of methamphetamine hydrochloride plus 1 ml of ethanol was then added, and the vial was dried at 125°C for 24 hours. Toluene phosphor (10 ml) and ethanol (1 ml) were finally added for counting in a liquid scintillation counter. For each group of plasmas assayed a standard curve was made by adding amphetamine to plasma obtained from the blood bank.

The *N*-methyltransferase was prepared from a 40 to 60 percent ammonium sulfate precipitate of the supernatant obtained by centrifuging at 50,000*g* the homogenate of the rabbit lung (*8*). The precipitate was dialyzed for 24 hours, and the enzyme, which was stored at −15°C in 1- to 5-ml portions, was stable for several months if not thawed and refrozen.

The combination of the extraction and drying procedures provides the assay with sensitivity, specificity, and reproducibility. The sensitivity and linearity of the assay for *d*- and *l*-amphetamine are shown in Fig. 1; the assay was linear from 10 ng/ml to at least 1 μg/ml, and *l*-amphetamine values were 30 percent higher than those of *d*-amphetamine. The blank for amphetamine-free plasma was 60 to 150 count/min; 10 ng of *d*-amphetamine per milliliter of plasma gave values twice that of the blank. Under the conditions of the assay plasma does not contain endogenous substances contributing to the blank. However, the blank doubled in a month because of slow deterioration of the ^{3}H-SAME. The latter compound was stored at 4°C; it should not be refrozen.

The recovery of amphetamine added to plasma was 45 percent, but inhibition of the lung enzyme by plasma and pentane reduced the overall recovery to 25 percent (*9*). Plasma from different subjects gave an identical degree of inhibition. Variability in the recovery of amphetamine added to plasma was eliminated by the drying procedures. The error in reproducibility of the assay was 5 to 10 percent for duplicate samples.

Specificity of the assay was shown in three ways. The closely related substrates, phenylethylamine and methamphetamine, had less than 5 percent the sensitivity of amphetamine when added to plasma and assayed, while tryptamine had less than 1 percent. Thin-layer chromatograms of apparent amphetamine isolated from plasma of subjects receiving the drug and authentic amphetamine added to blood bank plasma and treated as above had single radioactive peaks with identical migration as authentic [^{3}H]methamphetamine on three solvent systems (*10*). Also, partition coefficients of the apparent and authentic [^{3}H]methamphetamine between the heptane–1.5 percent isoamyl alcohol and aqueous phases of varying *p*H were identical (*11*).

d- and *l*-Amphetamine sulfate were given orally to four patients in single doses of 20 to 30 mg (free base), and their concentrations in plasma were assayed. Results are shown in Fig. 2 for two patients after various time intervals Peak levels of *d*- and *l*-amphetamine were reached in 1 to 3 hours and slowly declined with an apparent half-life of 10 to 13 hours (Fig. 2).

This assay technique is potentially applicable to other endogenous substances and drugs that can be *N*-methylated by the lung enzyme (*8*).

DAVID S. KREUZ*
Pharmacology-Toxicology Program, National Institute of General Medical Sciences, Bethesda, Maryland 20014
JULIUS AXELROD
Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, Maryland 20014

References and Notes

1. P. Wender, *Minimal Brain Dysfunction in Children* (Wiley, New York, 1971); B. G. Winsberg, I. Bialer, S. Kupitz, J. Tobias, *Am. J. Psychiatry* **128**, 109 (1972); L. E. Arnold, V. Kirilcuk, S. A. Corson, E. Corson, *ibid.* **130**, 165 (1973).
2. W. Modell, *J. Am. Med. Assoc.* **173**, 1131 (1960).
3. M. Prinzmital and W. Bloomberg, *ibid.* **105**, 2051 (1935).
4. J. D. Griffith, J. H. Cavanaugh, J. Held, J. A. Oates, in *Amphetamines and Related Compounds*, E. Costa and S. Garattini, Eds. (Raven, New York, 1970), p. 897; S. H. Snyder, *Arch. Gen. Psychiatry* **27**, 169 (1972).
5. E. Anggard, L. M. Gunne, F. Niklasson, *Scand. J. Clin. Lab. Invest.* **26**, 137 (1970); P. Lebish, B. S. Finkle, J. W. Brackett, *Clin. Chem.* **16**, 195 (1970); N. Narasimhashari and P. Vouros, *Anal. Biochem.* **45**, 154 (1972).
6. Pentane (Spectroquality) was obtained from Aldrich Chemical Co., Milwaukee, Wisconsin.
7. *S*-adenosyl-L-[methyl-^{3}H]methionine (8.02 c/mmole) was obtained from New England Nuclear Corp., Boston, Massachusetts.
8. J. Axelrod, *J. Pharmacol. Exp. Ther.* **138**, 28 (1962). Rabbit lung (type 1, young) was obtained frozen from Pel-Freez Biologicals, Rogers, Arkansas. The *N*-methyltransferase preparation had a protein concentration of 25 mg/ml, or about 20 unit/ml, with *d*-amphetamine as a substrate. One unit of enzyme activity is the amount of enzyme that forms 1 nmole of product per milligram of protein per hour.
9. Redistillation of the pentane and HCl did not reduce the inhibition, nor did heating remove possible traces of residual organic solvent.
10. The thin-layer chromatography systems were: acetone, ammonia (99 : 1, on silica), R_F 0.35; chloroform, methanol (1 : 1, on silica), R_F 0.71; butanol, acetic acid, water (4 : 1 : 1, on cellulose), R_F 0.40.
11. The distribution between the 6-ml heptane–1.5 percent isoamyl alcohol and the 0.5-ml aqueous phase of varying *p*H was the same for assayed plasma samples from patients having received amphetamine and for authentic amphetamine added to plasma and assayed enzymatically.

* Present address: Payne-Whitney Clinic, New York Hospital, 525 East 68 Street, New York 10021.

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