References and Notes

1. A. L. Byzov and Yu. A. Trifonov, *Vision Res.* **8**, 817 (1968).
2. J. Del Castillo and B. Katz, *Prog. Biophys. Biophys. Chem.* **6**, 121 (1956); F. Colomo and S. D. Erulkar, *Fed. Proc.* **29**, 391 (1970).
3. J. E. Dowling and H. Ripps, *Nature (Lond.)* **242**, 101 (1973).
4. G. L. Walls, *The Vertebrate Eye and Its Adaptive Radiations* (Hafner, New York, 1963).
5. A. Lasansky, *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* **262**, 365 (1971).
6. The composition of the medium, in millimoles per 1000 cm^3 of water, was Na^+, 128; K^+, 2.6; Ca^{2+}, 2; Mg^{2+}, 2; Cl^-, 112; HCO_3^-, 20 [S. R. Fleisey, *Fed. Proc.* **27**, 287 (1968)].
7. D. A. Baylor and M. G. F. Fuortes, *J. Physiol. (Lond.)* **207**, 77 (1970).
8. L. Cervetto and E. F. MacNichol, Jr., *Science* **178**, 767 (1972).
9. Two separate effects of calcium on the nervous system have been described, one facilitating the release of transmitter (*2*) and the other depressing the excitability of nerve fibers and nerve cells [B. Frankenhaeuser and A. L. Hodgkin, *J. Physiol. (Lond.)* **137**, 218 (1957); J. S. Kelly, K. Krnjevic, G. Somjen, *J. Neurobiol.* **2**, 197 (1969); G. E. Dambach and S. D. Erulkar, *J. Physiol. (Lond.)* **228**, 799 (1973)]. In addition, calcium ions have been shown to reduce the dark current of rat photoreceptors [S. Yoshikami and W. A. Hagins, *Biophys. Soc. Annu. Meet. Abstr.* **11**, 47a (1971)]. In *Bufo marinus* an excess of calcium has been reported to produce a hyperpolarization of photoreceptors and to reduce the response to light [L. H. Pinto and J. E. Brown, *Biol. Bull.* **143**, 473 (1972)].
10. D. A. Baylor, M. G. F. Fuortes, P. M. O'Bryan, *J. Physiol. (Lond.)* **214**, 265 (1971).
11. The experiments with high magnesium were started under the sponsorship of E. F. MacNichol at the National Institute of Neurological Diseases and Stroke, Bethesda, Maryland, while L.C. was on a visiting fellowship. We thank V. Alpigiani, M. Benvenuti, G. Bottaro, B. Margheritti, and M. Morelli for their valuable technical help.

5 July 1973; revised 24 September 1973

Inhibition of Cellular Mediated Immunity in Marihuana Smokers

Abstract. *The cellular mediated immunity of 51 young chronic marihuana smokers, as evaluated by the lymphocyte response in vitro to allogeneic cells and to phytohemagglutinin, was significantly decreased and similar to that of patients in whom impairment of T (thymus derived) cell immunity is known to occur. This inhibition of blastogenesis might be related to an impairment of DNA synthesis.*

It has been previously reported (*1*) that delta-9-tetrahydrocannabinol (Δ^9-THC), a psychoactive substance of *Cannabis*, when administered to rodents alters their cellular mediated immune responsiveness, and it was suggested that similar changes might also occur in man. In our study the mixed lymphocyte culture (MLC) and phytohemagglutinin (PHA) responsiveness of 51 marihuana smokers, 16 to 35 years old (median age 22), were studied. Only subjects who had used *Cannabis* products (at the exclusion of other drugs) at least once a week (average four times a week) for at least 1 year (average 4 years) were selected for this investigation.

Eighty-one healthy volunteers, 20 to 72 years of age (median age 44) were used as controls. Purified lymphocyte suspensions were prepared from fresh samples of venous blood by the Ficoll-Isopaque density gradient method (*2*). A microculture system was used for screening of cellular responsiveness (*3*). For the MLC test, 1×10^5 responding cells were incubated, per well, with 2×10^5 stimulating cells pooled from a panel of ten donors, phenotypically different [allogeneic cells in which 25 different HL-A specificities were represented (*4*)].

For the PHA test, 2×10^5 responding cells were incubated per well with 1 μg of purified PHA. The medium used was RPMI 1640 with penicillin, streptomycin, and glutamine, to which 25 percent autologous serum was added.

Results are summarized in Table 2 and compared with data obtained in 60 patients with cancer, 20 patients with uremia, and 24 renal allograft recipients with iatrogenically induced immunosuppression. The mean values registered in the group of marihuana users were significantly lower than those of the normal, but much older, control group. Since an inverse correlation exists between cellular immunity, as reflected by in vitro lymphocyte blastogenesis and aging (*5*), results obtained in the group of marihuana smokers may be interpreted as being indicative of cellular hyporesponsiveness. Supporting this conclusion is the close similarity between the depressed MLC and PHA responsiveness of marihuana users and that of cancer (*6*), uremia (*7*), and immunosuppressed transplant patients in whom impairment of T (thymus derived) cell immunity is known to occur. Furthermore, we observed that in vitro inhibition of PHA-induced blastogenesis of normal human lymphocytes started with 1.6 μM THC and was complete with 20 μM.

The major psychologically active constituent of *Cannabis sativa* is Δ^9-THC. This substance, as well as its metabolites, is insoluble in H_2O, but is very fat soluble, and has a half-life of several days in tissues where it might exert a cumulative and pharmacological effect (*8*). Such an effect might be related in a still unknown way to the depressed cellular immune response in vitro of chronic marihuana smokers. The effect of THC on adrenergic receptors (*9*) might also play a role in its immunosuppressive activity, as was suggested for other drugs administered continuously over a long period (*10*).

This inhibition of blastogenesis might result from an impairment of DNA synthesis. One of us (A.M.) sampled lymphocytes from four marihuana smokers, cultivated the cells for 72 hours, and then observed a decreased number of cells during the period of DNA synthesis (S period of the cell cycle). There was also an increased incidence of chromosomal breakages,

Table 1. Comparative cellular mediated immunity of normal subjects, marihuana smokers, and patients with impairment of T cell immunity. The in vitro blastogenic response of lymphocytes was studied by the MLC and the PHA tests. The incorporation rate of [^{3}H]thymidine of the T lymphocytes is given in counts per minute ± the standard error.

Subjects	MLC		PHA	
	No. tested	[^{3}H]Thymidine incorporated (count/min)	No. tested	[^{3}H]Thymidine incorporated (count/min)
Normal controls	81	26400 ± 200	81	23250 ± 210
Cancer patients				
Primary tumors	16	14894 ± 792	16	17501 ± 124
Regional spread	23	15816 ± 420	23	13345 ± 540
Distant spread	21	8968 ± 459	21	10516 ± 580
Uremic patients	26	12001 ± 272		
Transplant patients*	24	12307 ± 357		
Marihuana smokers†	34	15679 ± 499	51	13779 ± 169

* After 1 to 4 years of immunosuppressive therapy. † At least 1 year, at least once a week; no other drug taken.

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.
15. Supported in part by the Philippe Foundation, a gift from H. G. Doll, State of New York Department of Health Kidney Disease Institute research grant C-48408, and NIH grant GM-09069-11. We thank G. Theim for technical assistance.

16 July 1973; revised 9 October 1973

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.

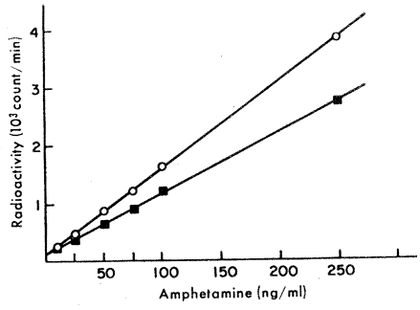

Fig. 1. Standard curves for *d*- (■) and *l*- (○) amphetamine added to plasma. Results are expressed as counts per minute produced per nanogram of amphetamine per milliliter of plasma. The blank values for amphetamine-free plasma were 60 to 150 counts per minute.

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*