in all six rabbits. However, CBD in doses of 10, 15 and 20 mg/kg did not produce convulsions in the rabbits we tested. These findings suggest that CBN, and not CBD, has stimulant properties in our paradigm and may explain the observation that CBD has greater potential for anticonvulsant activity than CBN or Δ^9THC (*11*).

We also tested the effects of cannabicyclol and cannabichromene, two naturally occurring constituents of marijuana (Table 1). Cannabichromene (8.0 mg/kg) produced no convulsions or other observable effects in either of the rabbits tested. When cannabicyclol (8.0 mg/kg) was given to rabbit No. 3 no convulsions or observable change occurred. However, approximately 7 minutes after rabbit No. 8 had been given cannabicyclol (8.0 mg/kg) the rabbit began to sprawl, then went into a brief convulsive-like thrashing, and died. A necropsy of this rabbit showed that death was due to a pulmonary hemorrhage which may explain this unusual finding. Finally, rabbit Nos. 81 through 86, 89, and 90 did not convulse with relatively high doses of the following hallucinogens: lysergic acid diethylamide (100 μg/kg), mescaline (40.0 mg/kg), psilocybin (3.0 mg/kg), phencyclidine (2.0 mg/kg), and methamphetamine (0.5 mg/kg), suggesting that convulsions are specific to the cannabinoids.

A preliminary study in our laboratory (*2*) suggested that tolerance may develop to the convulsant inducing properties of Δ^9THC in this population of rabbits. Therefore, a more extensive study of the long-term effects of Δ^9THC was undertaken. We gave five rabbits one injection per day of 0.5 mg/kg Δ^9THC until no convulsion occurred. The following and subsequent days (if necessary) the dose of Δ^9THC was doubled until a convulsion was again elicited. This higher dose then was given daily until the rabbit again became tolerant to the cannabinoid induced convulsion. As shown in Table 2, although there was some individual variation, all rabbits exhibited behavioral tolerance at Δ^9THC doses of 0.5 mg/kg. An increase in dose to 1.0 mg/kg was sufficient to elicit behavioral convulsions in four out of the five rabbits, and each of these four rabbits became tolerant to this higher dose. One tolerant rabbit (No. 3) exhibited convulsions after an increase of dosage to 2.0 mg/kg, and as with the other four subjects, tolerance subsequently was demonstrated with this higher dose. Two days after becoming tolerant to convulsions with the highest dose, rabbit Nos. 3 and 33 were injected with 0.5 mg of Δ^9THC per kilogram in an effort to reinstate seizures, but neither convulsed. One week after becoming tolerant to convulsions with the last administered dose of Δ^9THC, each subject was injected with 0.5 mg/kg. In all five rabbits, convulsions were again elicited, indicating that tolerance had been lost.

These findings suggest the occurrence of an animal model that is uniquely and differentially sensitive to the (extreme) stimulant action of cannabinoids. This is especially interesting because CBD, which perhaps has the greatest anticonvulsant potential in experimental animals, did not elicit convulsions. These findings suggest that a model for testing the effects of marijuana, its congeners, and potential antagonists might be provided by this population of New Zealand White rabbits.

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References and Notes

1. P. Consroe, B. Jones, H. Laird, J. Reinking, in *The Therapeutic Potential of Marihuana*, S. Cohen and R. C. Stillman, Eds. (Plenum, New York, 1976), p. 363.
2. P. F. Consroe, B. C. Jones, L. Chin, *Pharmacol. Biochem. Behav.* **3**, 173 (1975).
3. P. Consroe, B. Jones, H. Laird, *Ann. N.Y. Acad. Sci.*, in press.
4. W. Hohenboken and G. Nellhaus, *J. Hered.* **61**, 107 (1970).
5. L. E. Hollister, *Experientia* **29**, 825 (1973).
6. B. Zitko, J. Howes, R. Razdan, B. Dalzell, H. Dalzell, J. Sheehan, H. Pars, *Science* **177**, 442 (1972).
7. L. Lemberger, J. Axelrod, I. Kopin, *Pharmacol. Rev.* **23**, 371 (1971).
8. L. Lemberger and A. Rubin, *Life Sci.* **17**, 1637 (1975).
9. M. Willinsky, S. de Carolis, V. Longo, *Psychopharmacologia* **31**, 365 (1973).
10. R. Karler, W. Cely, S. Turkanis, *Life Sci.* **13**, 1527 (1973).
11. S. Turkanis, W. Cely, D. Olsen, R. Karler, *Res. Commun. Chem. Pathol. Pharmacol.* **8**, 231 (1974).
12. This research was supported by grant 9 R01-DA 01448 from the National Institute of Drug Abuse (NIDA) of the Alcohol, Drug Abuse and Mental Health Administration. We thank M. Braude of the NIDA for the supply of cannabinoids and for her encouragement, and H. Pars of Sharps Associates for providing SP-111A and gamma-morpholinobutyric acid hydrobromide.

7 June 1976; revised 19 July 1976

Nitrous Oxide "Analgesia": Resemblance to Opiate Action

Abstract. *Nitrous oxide produced a dose-related "analgesia" in mice (median effective dose, 55 percent). The analgesia was evaluated by means of a phenylquinone writhing test. Narcotic antagonists or chronic morphinization reduced nitrous oxide analgesia. Either nitrous oxide releases an endogenous analgesic or narcotic antagonists have analgesic antagonist properties heretofore unappreciated.*

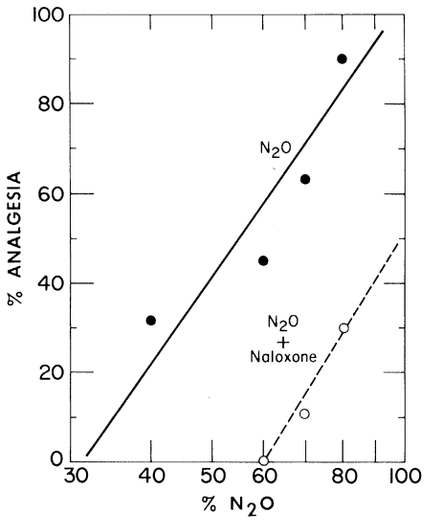
Nitrous oxide has a long history of use as a euphoriant and analgesic (*1*). The purpose of our study was to characterize the nature of nitrous oxide analgesia. To estimate the "analgesia" in mice we used the phenylquinone writhing test (*2*). Mice were injected intraperitoneally with phenylquinone and placed in a clear plastic enclosure and exposed to mixtures of nitrous oxide and oxygen (*3*).

Nitrous oxide produces an "analgesic" response in mice, that is, an inhibition of writhing. This analgesia was dose-related with 50 percent inhibition of writhing occurring in the presence of 55 percent nitrous oxide (Fig. 1). Naloxone hydrochloride (5 mg/kg, subcutaneously) administered immediately before the phenylquinone had no significant effect on writhing. However, as can be seen in Fig. 1, naloxone administration did reduce the analgesic efficacy of nitrous oxide. In additional experiments, the average analgesia produced by 80 percent nitrous oxide in six groups of mice (five mice per group) was 84 ± 6 percent (Fig. 2). Naloxone reduced the analgesia caused by 80 percent nitrous oxide to 37 ± 4 percent. With 60 percent nitrous oxide, analgesia averaged 54 ± 6 percent, whereas 60 percent nitrous oxide plus naloxone produced only 12 ± 11 percent analgesia. Lower doses of naloxone were not effective in reversing nitrous oxide analgesia.

Naltrexone is also a narcotic antagonist (*4*), and this drug also antagonized nitrous oxide analgesia. If naltrexone was given alone in a dose of 5 mg/kg subcutaneously, it had no effect on the phenylquinone-induced writhing. It did, however, reduce the analgesia produced by 70 percent nitrous oxide from 64 percent to 21 percent, and also reduced the analgesic effects of 60 percent nitrous oxide from 45 percent to 12 percent.

The analgesic effects of nitrous oxide were also reduced in mice that had received morphine for several days prior to the test. In these experiments, morphine hydrochloride (*5*) was given subcutaneously in a dose of 30 mg/kg twice on day 1; 50 mg/kg twice on day 2; and 60 mg/kg three times on day 3. A paired group of mice received only saline injections and served as controls. On day 4, no morphine was given and the mice were tested for analgesia.

Both the saline- and morphine-injected mice reacted to the phenylquinone in a similar manner. Two groups of saline-treated mice writhed a total of 53 and 57 times, whereas the morphine-treated mice writhed 60 and 56 times. Thus, no



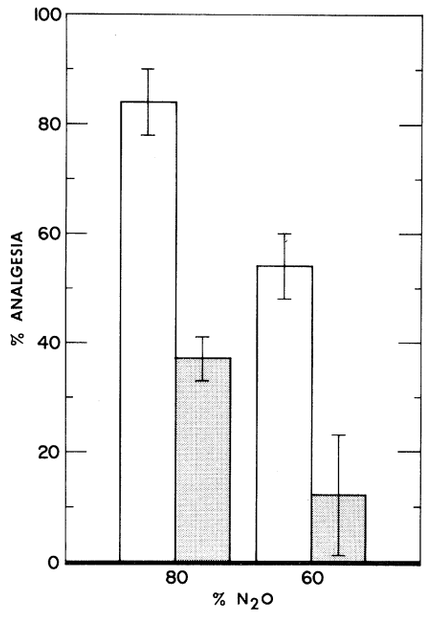


Fig. 1 (left). Analgesic action of nitrous oxide. Groups of mice, five animals per group, were injected intraperitoneally with *p*-phenylquinone and the analgesia was determined (*3*) in the presence of nitrous oxide or nitrous oxide plus naloxone (5 mg/kg subcutaneously). The narcotic antagonist was injected just prior to the exposure to nitrous oxide. The lines were fit to the data points by a method of least squares. Fig. 2 (right). Effect of naloxone on the analgesic action of nitrous oxide. Results are the means ± the standard error of three to six groups of mice, five mice per group. The open bars represent the response to nitrous oxide and the closed bars represent the response to nitrous oxide plus naloxone. The results were statistically significant, $P < .05$.

residual morphine from the previous day's injection was present to interfere with the test for analgesia. Nitrous oxide analgesia, as measured by the inhibition of writhing, was almost totally abolished in the mice which had received morphine over a 4-day period. Eighty percent nitrous oxide produced only 16 percent analgesia in two groups of mice (range, 0 to 35 percent). In contrast, 80 percent nitrous oxide produced 93 percent inhibition of writhing in saline-injected mice.

Although nitrous oxide is widely used as an analgesic, its mechanism of action is not understood and has been difficult to study because nitrous oxide is gaseous. The phenylquinone writhing method described for examining the "analgesic" effect of nitrous oxide is sensitive and simple; it should also lend itself to the study of other gases that might produce analgesia. Even though nitrous oxide is an analgesic in man, the analgesic action of nitrous oxide in animals as measured by other methods such as the hot-plate or tail-flick tests would be important to study. However, these tests are not easily accomplished with gaseous drugs. The effective analgesic dose of nitrous oxide in the writhing test in mice is in the range of that used as an analgesic in man (*6*). Since both naloxone and naltrexone reduced the analgesic effect of nitrous oxide, one conclusion of these studies is that narcotic antagonists diminish the analgesia produced by nitrous oxide. It should be noted, however, that the doses of the narcotic antagonists required for antagonism of nitrous oxide are higher than those required for antagonism of morphine.

Nitrous oxide, in the concentrations used, did not cause anesthesia in mice. One matter which concerned us was whether ataxia produced by nitrous oxide would interfere with the analgesic test. Since ataxia is present when nitrous oxide is used in man, this effect in mice was not unexpected, nor is it a sufficient reason to disqualify the test. Ataxia did not interfere with the writhing test since, in the presence of naloxone or in mice that had received morphine over a long period, maximal writhing response could be measured even in the presence of up to 80 percent nitrous oxide.

In addition to the use of narcotic antagonists to examine molecular mechanisms of "analgesia" which may be defined as opiate-like, an alternate approach is to examine the response in subjects or animals that have been exposed to narcotics. Mice that received morphine for 3 days prior to nitrous oxide exposure displayed tolerance to the "analgesic" effect of nitrous oxide. Whether mice made tolerant to morphine by pellet implantation show even greater tolerance to nitrous oxide analgesia remains to be determined. A patient who was highly dependent on pentazocine (*7*), requiring ten times the average daily dose, showed no withdrawal symptoms when a 50 percent nitrous oxide–oxygen mixture was substituted for pentazocine. Moreover, with continuous use of nitrous oxide, tolerance to its analgesic effect developed.

In conclusion, the analgesia produced by nitrous oxide in the phenylquinone writhing studies in mice has several features in common with the action of opiates and one possible explanation for its mechanism could be that nitrous oxide releases or potentiates an endogenous analgesic or opiate of the type recently proposed (*8*). Alternatively, the narcotic antagonists may have analgesic antagonist properties heretofore unappreciated.

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References and Notes

1. P. J. Cohen, in *The Pharmacological Basis of Therapeutics*, L. Goodman and A. Gilman, Eds. (Macmillan, New York, 1975), p. 53.
2. E. Siegmund, R. Cadmus, G. Lu, *Proc. Soc. Exp. Biol. Med.* **95**, 729 (1957); L. C. Hendershot and J. Forsaith, *J. Pharmacol. Exp. Ther.* **125**, 237 (1959); B. Berkowitz and S. Spector, *Science* **178**, 1290 (1972).
3. Phenylquinone (phenyl-*p*-benzoquinone, Eastman Kodak) was made up as a 0.02 percent solution in 5 percent ethanol and injected intraperitoneally into groups of mice, five animals per group, in a dose of 0.01 ml/g. The mice weighed 20 to 25 g and were CF-1 males obtained from Charles River Breeders, Wilmington, Massachusetts. After injection, animals were placed in small wire cages in a clear plastic box (volume, 14 liters) with a close-fitting cover and gas inlet and outlet ports at either end. Gas mixtures were delivered from calibrated flowmeters at a total flow of 10 liters per minute. The oxygen concentration of exhaust gas was measured with an oxygen analyzer and the difference from 100 percent served as a check on the nitrous oxide concentration in the box. All results are based on the number of writhes observed during the 5-minute interval from 10 to 15 minutes after injection of phenylquinone. Approximately 7 minutes were needed for the gas in the box to reach the delivered concentration. The percentage of analgesia was calculated as: 100 × (control writhes − treatment writhes)/control writhes.
4. H. Blumberg, H. B. Dayton, P. S. Wolf, *Toxicol. Appl. Pharmacol.* **10**, 406 (1967); R. B. Resnick, J. Valarka, A. Freedman, M. Thomas, *Am. J. Psychiatry* **131**, 646 (1974); W. Martin, D. Jasinski, P. Mansky, *Arch. Gen. Psychiatry* **28**, 784 (1973).
5. Mice injected with increasing doses of morphine over several days exhibit both dependence [J. Marshall and D. G. Grahame-Smith, *J. Pharmacol. Exp. Ther.* **173**, 634 (1971)] and tolerance (E. L. Way, University of California, San Francisco, personal communication). Mice injected with this schedule of morphine exhibited dependence and withdrawal jumping if naloxone was injected on day 4.
6. H. L. Price, in *The Pharmacological Basis of Therapeutics*, L. Goodman and A. Gilman, Eds. (Macmillan, New York, 1975), p. 82.
7. B. J. Kripke and H. B. Hechtman, *Anesth. Analg. Curr. Res.* **51**, 520 (1972).
8. J. Hughes, *Brain Res.* **88**, 295 (1975); G. Pasternak, R. Goodman, S. H. Snyder, *Life Sci.* **16**, 1771 (1975); H. Teschemacher, K. E. Opheim, B. M. Cox, A. Goldstein, *ibid.*, p. 1771; J. Belluzzi, N. Grant, R. Garsky, D. Sarantukis, C. D. Wise, L. Stein, *Nature (London)* **260**, 625 (1976); G. Pasternak, R. Simantov, S. Snyder, *Mol. Pharmacol.* **12**, 504 (1976).
9. Supported in part by NIH grants GM-00056 and GM-09065 (for A.D.F. and S.H.N.).

29 June 1976