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7. Thin sections were prepared as follows: the cells were cultured in basal salt medium with 0.02 percent yeast extract and colloidal sulfur; thin sections fixed with glutaraldehyde and osmium tetroxide were examined in an electron microscope (Hitachi HU-11B).
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10. In tolerance studies, molybdenum was supplied as $Na_2MoO_4 \cdot 2H_2O$. Manometric tolerance studies were performed on a differential respirometer (Gilson).
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13. The samples were prepared for scanning electron microscopy as follows: molybdenite fines from inoculated acid media placed on aluminum specimen stubs were air-dried and metallized with a mixture of gold and palladium (60 : 40) in vacuum to a thickness of roughly 150 Å. Observations were made in a scanning electron microscope (Hitachi HHS-2R) operated at 20 kv and in a scanning electron microscope (Cambridge Stereoscan Mark II) fitted with a nondispersive x-ray analyzer (Princeton Gamma-Tech) operated at an accelerating potential of 20 kv. L. E. Murr, *Electron Optical Applications in Materials Science* (McGraw-Hill, New York, 1970).

14. Research at the New Mexico Bureau of Mines and Mineral Resources was supported in part by U.S. Bureau of Mines grant GO122123. We thank Dr. J. V. Scaletti, University of New Mexico Medical School, for preparing the transmission electron micrograph.

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Lack of Tolerance to Δ^9-Tetrahydrocannabinol in Chimpanzees

Abstract. *Five chimpanzees were given Δ^9-tetrahydrocannabinol (Δ^9THC): 1.0 milligram per kilogram of body weight for 21 days and 4.0 milligrams per kilogram of body weight for 42 days. Although accuracy and speed of performance on a delayed matching-to-sample task were significantly affected by both doses, tolerance to Δ^9THC did not develop. No long-term behavioral effects of Δ^9THC were observed after termination of the drug regimens.*

Although the short-term (acute) effects of marihuana have been determined, the long-term effects of marihuana have not been well defined (*1*). For example, the issue of whether marihuana tolerance develops in man has not been settled experimentally, and some discrepancy still exists regarding the effects of repeated marihuana administrations in nonhuman species. In those animal experiments where the effects of marihuana on unlearned behavior or on learned shock-avoidance behavior have been studied, no consensus as to the occurrence of marihuana tolerance has been reached (*2, 3*).

In contrast, the development of a marked tolerance to the effects of marihuana on simple behavioral tasks maintained by appetitive rewards has been consistently demonstrated in a wide range of animal species (*2, 4, 5*). Most of these behavioral tasks have involved situations of the "go, no-go" type in which delivery of reward is made dependent on the repeated occurrence, as compared to the nonoccurrence, of a simple learned response. We now report a lack of tolerance in the chimpanzee to the behavioral effects of Δ^9-*trans*-tetrahydrocannabinol (Δ^9THC), the major active constituent of marihuana, on a conditional discrimination task which makes reward dependent on correct choice responses.

The effects of repeated Δ^9THC administrations on short-term memory have not been studied in animals or man. We chose the delayed matching to sample task for our chimpanzee experiments since this task has proved useful for the study of conceptual processes and short-term memory in nonhuman primates, as well as for the study of short-term effects of psychotropic drugs (*6*). In addition, both the delayed matching to sample performance of nonhuman primates and human short-term memory have been shown to be impaired by Δ^9THC (*7, 8*).

Each of the three male and two female adult chimpanzees had extensive behavioral and Δ^9THC histories (*5, 8–10*) but had not been given drugs for approximately 3 weeks prior to the experiments reported below. The indoor area of the chimpanzee's living cage was modified to hold a stimulus-response panel containing three horizontally aligned choice-response keys and a sample response key located above the middle choice key. A delayed matching-to-sample trial was initiated by illuminating the sample response key with one of three white geometric forms (Δ, $\times$, or $-$) or one of three colors (green, red, or blue). The chimpanzee was required to make ten consecutive responses to the sample key after which the sample stimulus was turned off and a 20-second delay period was initiated. After the delay period, each of the three choice keys was simultaneously illuminated with a different choice stimulus. Although the positions of the choice stimuli were determined randomly, the choice stimuli for a given trial were from the same stimulus dimension as the sample stimulus for that trial. A response to the choice stimulus, which was the same as the previously presented sample stimulus, produced a reward consisting of a 1-g banana pellet (Ciba) and terminated the trial. A response to either of the two incorrect choice stimuli simply terminated the trial. Successive trials were separated by 15 seconds. Each daily experimental session consisted of 100 trials or 85 minutes, whichever came first.

After the behavior of individual chimpanzees appeared stable over seven consecutive sessions, the chimpanzees were given six control sessions in which the drug was not given, 21 consecutive Δ^9THC sessions at 1.0 mg/kg, and 21 recovery sessions in which no drug was given. Finally, 42 consecutive Δ^9THC sessions at 4.0 mg/kg were followed by 33 recovery sessions during which no drug was given. The Δ^9THC (*11*) was orally administered 2.5 hours before each drug session in a vehicle consisting of water, corn syrup, and orange extract. The drug vehicle alone was administered 2.5 hours before each session in which no drug was given.

Since no systematic differences were obtained between color and form stimuli, the percentage of correct matching responses was plotted as an average for color and form trials (Fig. 1). Separate *t*-tests indicated that the initial Δ^9THC doses of 1.0 mg/kg and 4.0 mg/kg produced significant ($P < .01$) decreases in matching accuracy as compared to the immediately preceding sessions without the drug. The magnitudes of these performance decrements were apparently not dose related. More importantly, no significant decrease in the drug effect on matching accuracy was observed during either long-term drug regimen. Recovery after the drug was more rapid after termination of the 1.0 mg/kg dose. In fact, a significant ($P < .05$) decrease in matching

accuracy was obtained during the first three sessions after the 4.0 mg/kg dose.

The distributions of response latencies were highly skewed. Accordingly, the latencies of responding to the sample and choice stimuli were converted to response speeds by taking the reciprocal of the response latencies (Fig. 2). Choice response speed was not substantially affected by either dose. In contrast, at 1.0 mg/kg, Δ^9THC produced an increase in the speed of responding to the sample stimulus for all 21 sessions of repeated dosing. Recovery from this drug-produced increase in sample response speed was rapid, occurring within the first three sessions after administration of the drug was stopped. In contrast, a *t*-test between the first and last series of sessions at 4.0 mg/kg indicated that this dose had produced a significant ($P < .05$) decrease in sample response speed for the 42 consecutive days of drug administration. Slower sample response speeds persisted for 15 sessions after the administration of the 4.0 mg/kg regimen was stopped.

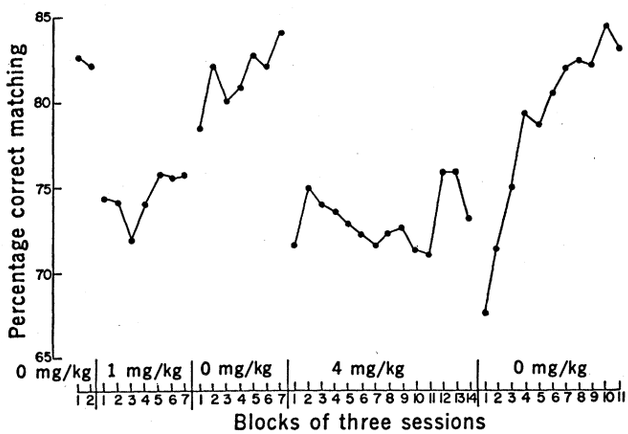
Dose related effects were also observed on the number of delayed matching-to-sample trials completed each session by the chimpanzees. Under the 1.0 mg/kg dose all chimpanzees completed the maximum number of 100 trials each session, whereas under the 4.0 mg/kg dose, three of the five chimpanzees did not always complete 100 trials during a session. This cessation of response was possibly related to a reduction in motivation since two of these three chimpanzees did not always consume the food given them after the administration of the 4.0 mg/kg dose. Termination of the 4.0 mg/kg regimen was initially accompanied by a still higher incidence of cessation of response and a reduction in appetite. Complete recovery of the behavior similar to that of the baseline control was achieved during succeeding sessions after the drug was stopped, and no other behavorial symptoms of drug withdrawal were observed after termination of either regimen.

The data indicate a lack of tolerance to Δ^9THC effects on delayed matching-to-sample performance in chimpanzees. Although both doses of Δ^9THC produced a significant impairment in matching accuracy, there was no reduction in the magnitude of this effect during successive drug administrations. Repeated exposure to 1.0 mg/kg likewise did not reduce the effectiveness of this dose in producing an increase in response speed to the sample stimulus. In fact, the one cumulative effect of the drug, produced on sample response speed by 4.0 mg/kg, was suggestive of a toxic effect of Δ^9THC. In this context, it should be noted that significant behavioral effects on behavior controlled by a reinforcement schedule can be obtained in chimpanzees at doses as low as 0.2 mg/kg (*10, 12*).

The sessions after administration of the drug provided data relevant to possible long-term effects of repeated exposure to a marihuana compound. Since, after termination of drug administration, all behavioral measures eventually returned to those (control) before the drug was given, we conclude that Δ^9THC did not produce permanent behavioral effects. Nevertheless, an overall decrement in performance, relative to performance under the drug, did occur immediately after the 4.0 mg/kg regimen. Unfortunately, the mechanism of action underlying this temporary behavioral decrement after administration of the drug could not be determined from our experiment.

The lack of development of tolerance as measured by our behavioral task is at odds with results obtained in other animal studies in which, under simpler learning tasks, tolerance to Δ^9THC was evident (*2, 4*). Schuster, Dockens and Woods (*13*) have hypothesized that learning or drug-behavior interactions may largely determine the development of tolerance to psychotropic drugs. In accord with this hypothesis, Ferraro (*14*) has presented evidence that tolerance to the behavioral effects of marihuana compounds is more likely when an organism can make behavioral adjustments that serve to counteract drug-produced decrements in performance. Under simple reinforcement schedule baselines which require a "go, no-go" type of response, an organism can counteract the effects of marihuana by making compensatory changes in the ongoing rate of response. In those instances where no compensatory behaviors are possible or where marihuana produces an improvement in performance, the occurrence of tolerance would be less likely.

Such a compensatory response hypothesis need not exclude the possibility that, in some situations, pharmacodynamic factors may primarily or even exclusively determine the development of marihuana tolerance (*15*). However, it is conceivable that toler-



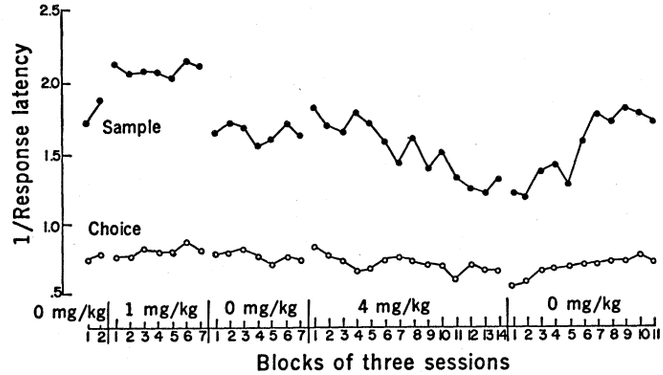


Fig. 1 (left). Mean percentage of correct 20-second delayed matching-to-sample responses for five chimpanzees across blocks of three experimental sessions. Fig. 2 (right). Mean sample and choice response speeds under a 20-second delayed matching-to-sample task for five chimpanzees across blocks of three sessions. Response speeds were obtained by taking the reciprocal of the response latency to the onset of either the sample or choice stimuli.

ance was not observed under the discrimination task used here primarily because no compensatory responses were readily available. The delayed matching-to-sample task incorporates a memory component not present in simpler behavioral tasks. Several studies have demonstrated that short-term memory in both animals and humans is disrupted by short-term administrations of Δ^9THC (*7, 8*). To the extent that memory impairment is not easily overcome by high probability compensatory behaviors, our failure to obtain tolerance may be considered as an instance which supports the importance of drug-behavior interactions in the development of marihuana tolerance.

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Epstein-Barr Virus: Detection of Genome in Somatic Cell Hybrids of Burkitt Lymphoblastoid Cells

Abstract. *Somatic cell hybrids of Burkitt lymphoblastoid cells, from which Epstein-Barr virus can be recovered, were examined for the presence of virus DNA by DNA-RNA hybridization. Four clones of hybrid cells, each negative for virus antigens by immunofluorescence, contained virus DNA in varying genomic equivalents. The number of virus genome equivalents increased in the hybrid cells after induction of virus with iododeoxyuridine.*

The Epstein-Barr virus (EBV) and EBV-specific antigens have been observed in several lymphoblastoid cell lines (*1*). There are lymphoblastoid cell lines that do not contain either virus particles detected by electron microscopy or EBV-specific antigens detected by immunofluorescence. When cells from one of these lymphoblastoid cell lines, as well as cells from an EBV-positive cell line, were treated with 5-bromodeoxyuridine, EBV-specific antigens and virus were induced in both lines (*2*).

The Burkitt lymphoblastoid cell lines EB_3 and P3J-HR-1 (HR-1) can be hybridized to mouse and human cells, respectively (*3*). After D98/HR-1 (a somatic cell hybrid of HR-1 cells and D98, a human sternal marrow cell line) was exposed to 5-iododeoxyuridine (IdU), EBV-specific antigens were detected by immunofluorescence and virus particles were found by electron microscopy (*4*).

The technique of DNA-RNA hybridization on nitrocellulose filters can be used to detect integrated virus DNA associated with host cell DNA. Studies with both DNA-DNA and DNA-RNA hybridization have shown that Burkitt lymphoblastoid cells containing EBV antigens and so-called "normal" lymphoblastoid cell lines (without EBV antigens) contain virus DNA in varying amounts (*5*). We report here that DNA of EBV was detected in somatic cell hybrids of Burkitt lymphoblastoid cells, whereas no evidence of EBV in the hybrid cells was found either by immunofluorescence or electron microscopy until after treatment with IdU.

In each experiment, cells were grown in HAT selective medium (*3, 6*) or in Eagle's medium. Cells from five 250-ml plastic tissue culture flasks were pooled in saline buffered with tris-(hydroxymethyl)aminomethane (*p*H 9). Clones 1, 2, 3, and 8 of the hybrid cells as well as the parental monolayer cell type, D98, were tested in the assay. Clone 1 of the hybrid cells was grown for 3 days in Eagle's medium containing IdU (60 μg/ml), the medium was then replaced with normal Eagle's medium, the cells were grown for an additional 7 days, and the cells were examined for EBV genomes. The procedures for the preparation of DNA from EBV for use as template, the preparation of RNA complementary to virus DNA, and the determination of the number of genome equivalents per cell from DNA-RNA hybridization have been described (*5*).

Clones 1, 2, 3, and 8 of D98/HR-1

Table 1. DNA-RNA hybridization tests. An amount of cRNA (RNA complementary to DNA of Epstein-Barr virus) containing 1.5 $\times 10^5$ count/min was used in each test. The amount of radioactivity bound to DNA of D98 cells (159 count/min) was subtracted from the other values before the number of genome equivalents was estimated. Cell lines not described in the text are Raji, a Burkitt lymphoblastoid cell line that was negative for virus by immunofluorescence, and Hep-2, a cell line not associated with Burkitt's lymphoma.

DNA on filter	cRNA hybridized (count/min per 50 μg of DNA)	Genome equivalents per cell (No.)
D98	159	0
HR-1	21,282	610
Raji	2,411	60
D98/HR-1 clone:		
1	562	11
2	299	4
3	829	18
8	709	15
1, treated with IdU	3,499	97
Hep-2	148	0