ter addition of choleragen in culture (*10*). Since the evidence presented here and in previous studies suggests that cyclic AMP is the signal for SNAT induction in the pineal organ, it seems likely that changes in phosphodiesterase activity can play a key role in determining the magnitude of this and other cellular responses to β-receptor stimulation in the pineal body, by modifying the half-life of the intracellular mediator, cyclic AMP. Phosphodiesterase activity appears to be induced by high levels of cyclic AMP, and to decline when cyclic AMP levels decline, with a short lag period. These changes may play a part in the well-documented phenomena of sub- and supersensitivity in the pineal body (*11*). After periods of maximal receptor stimulation, phosphodiesterase activity will be increased, thereby decreasing the half-life of newly formed cyclic AMP; but after periods of receptor inactivity, phosphodiesterase activity will be low, thereby increasing the half-life of newly formed cyclic AMP. However, differences in [^{3}H]alprenolol binding to sub- and supersensitive pineals have recently been demonstrated (*12*), which suggests that there may be several mechanisms participating in these phenomena.

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Phytohemagglutinin-Induced Lymphocyte Transformation in Humans Receiving Δ^9-Tetrahydrocannabinol

Abstract. *Eight otherwise healthy male chronic marijuana smokers were hospitalized for a period of 30 days. Initially they received placebo, then a sustained dose of 210 milligrams of Δ^9-tetrahydrocannabinol (Δ^9-THC) per day for 18 days, followed by placebo. Lymphocyte responses to phytohemagglutinin were examined during each of these periods. Neither the daily ingestion of marijuana extract containing 210 milligrams of Δ^9-THC for 18 days nor the history of chronic marijuana smoking had a depressive effect on the lymphocyte responses of these subjects to phytohemagglutinin.*

A controversy currently exists regarding the status of cell-mediated immunity in otherwise healthy marijuana smokers. Nahas *et al.* (*1*) reported that in vitro blastogenic responses to phytohemagglutinin (PHA) and allogeneic cells in chronic marijuana smokers were depressed by 40 percent, bringing them to levels seen in patients with cancer or uremia or transplant recipients with iatrogenic immunosuppression. Although this work has not been directly confirmed, Gupta *et al.* (*2*) found a 5 percent mean decrease in the rosette-forming capacity of lymphocytes from chronic marijuana smokers, supporting the concept of a T-cell defect. By contrast, Silverstein and Lessin (*3*) noted no difference between chronic marijuana users and normal controls as evaluated by in vivo response to skin sensitization with 2,4-dinitrochlorobenzene, a technique for determining the functional integrity of cell-mediated immunity. Most recently White and coworkers (*4*) have reported that they could find no significant difference between the blastogenic responses of lymphocytes from long-term marijuana smokers and matched control subjects in response to the mitogens PHA and pokeweed. This is in direct conflict with the earlier report by Nahas *et al.* (*1*), underscoring the need for further investigation of this subject. A recent report indicates that oral delta-9-tetrahydrocannabinol (Δ^9-THC) is an effective antiemetic in patients receiving cancer chemotherapy (*5*). The use of Δ^9-THC in these patients would be undesirable if it contributed significantly to immune suppression.

A major problem in interpretation of these studies has been the experimental variation introduced by the use of chronic marijuana smokers who smoke material of unknown potency at unspecified times in a nonregulated manner. The purpose of this study has been to compare the PHA-induced blastogenesis of normal human subjects and subjects receiving a known quantity of Δ^9-THC, the major psychoactive component of marijuana, at scheduled time intervals under carefully controlled hospital conditions.

Eight male volunteers between the ages of 21 and 30 were selected for this study. They were part of an extensive study of many marijuana effects. All were regular marijuana smokers (mean frequency of 13.5 marijuana cigarettes per week for an average duration of 3.5 years). They denied the regular use of drugs (other than alcohol and tobacco), particularly narcotics, barbiturates, and amphetamines. A complete medical history and physical examination confirmed their good mental and physical health. Subjects were asked to refrain from all drug usage for 1 week prior to admission to the Clinical Research Ward of Langley Porter Neuropsychiatric Institute in San Francisco, California. They were then admitted in groups of two and kept under constant observation for the 30 days of the hospitalization period.

Each subject received a capsule containing either placebo (0.2 ml of ethanol) or drug (a crude marijuana extract in 0.2 ml of ethanol, recently assayed for Δ^9-THC content) (*6*) every 4 hours during the entire hospitalization period. Placebo was given in a double blind situation to all subjects for the first 6 days, during which time baseline data were acquired. Then rapidly increasing doses of drug were given until a maximum of 210 mg of Δ^9-THC per day in divided 30-mg doses was reached. This dose level was maintained for 18 days, followed by an abrupt switch to placebo for the remaining 4 days of hospitalization. In addition to the oral doses, all subjects smoked a 1-g marijuana cigarette just before and on the last day of prolonged oral administration. Blood samples were drawn for lymphocyte cultures during the initial placebo period just before the drug was started, at the end of the period of prolonged drug administration, and at the end of the final placebo period. The control subjects were aged 21 to 50 years and were recruited from laboratory staff and friends. Most had never used cannabis and none had any in the previous 6 months. They all were in excellent health.

Blood samples were drawn with 10

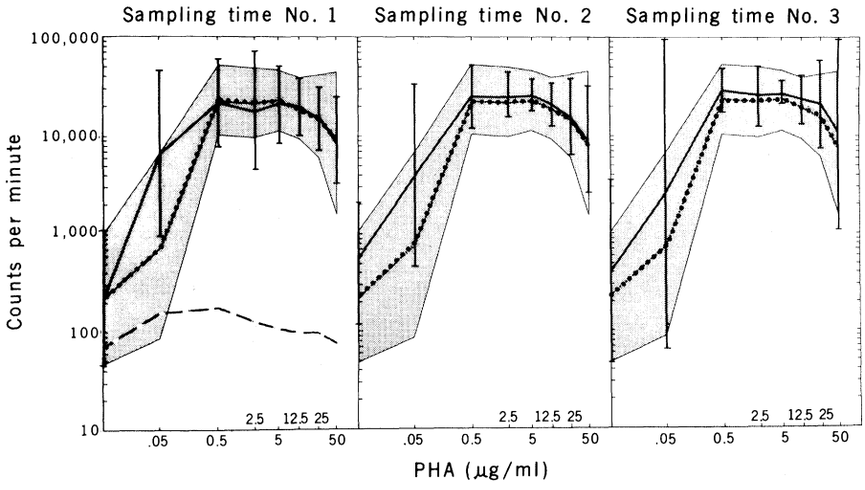

Fig. 1. The lymphocyte responses to phytohemagglutinin for eight patients on the drug protocol. Sampling time No. 1 is at the end of the initial placebo period, sampling time No. 2 is at the end of the period during which the subjects received 210 mg of Δ^9-THC per day, and sampling time No. 3 is after rapid withdrawal of the drug. In each graph the dotted line represents the mean, and the shaded area ± 2 standard deviations (S.D.), for normal control lymphocytes tested simultaneously with the subjects' lymphocytes (N = 15). The solid line is the mean for the subject group with the vertical bars representing ± 2 S.D. The dashed line in the lower part of the first graph is the PHA dose-response curve of lymphocytes from a renal transplant patient on immunosuppressive drugs.

units of preservative-free heparin per milliliter and processed according to the method of Mangi and Kantor (*7*) for short-term storage of human lymphocytes prior to in vitro stimulation. Subject and control samples drawn at the same time were coded, packed together in the same insulated cartons with no refrigerant, and shipped via air express to Ann Arbor for the lymphocyte studies. Cultures were established within 24 hours of venipuncture. Lymphocyte suspensions were prepared by separation on a Ficoll-Hypaque gradient (density 1.077) and lymphocyte incubations were established by a modification of the microculture system described by Thurman *et al.* (*8*). Each culture contained 1×10^5 lymphocytes in 0.2 ml of medium 199 (Grand Island Biological) containing penicillin, streptomycin, amphotericin B, and glutamine plus 25 percent serum, either autologous or homologous. Dose-response curves to PHA (phytohemagglutinin-P, Difco) employed triplicate cultures at seven doses ranging from 0.05 to 50.0 μg/ml. The cultures were incubated at 100 percent humidity, 37°C, 97 percent air, and 3 percent CO_2 for 66 hours, at which time 0.2 μc of [^{3}H]thymidine, specific activity 25 c/mmole (Nuclear Dynamics), was added. After an additional 6 hours of incubation the cultures were harvested with a multiple automated sample harvester and counted in a scintillation counter. Lymphocyte blastogenesis was measured by the incorporation of [^{3}H]thymidine into DNA and expressed as the average number of counts per minute per culture. Dose-response curves of the subjects and controls at each sampling time were prepared by using geometric statistics (*9*).

These results are shown in Fig. 1. The dose-response curves of six subjects for whom complete data were available for all three time intervals were compared by analysis of variance to the dose-response curves of six contemporaneous controls. The controls were selected randomly from a pool of 15 controls (*10*). There was a statistically significant ($P < .05$) higher unstimulated background count at times 2 and 3 during and shortly after high doses of Δ^9-THC. There was a statistically significant increased response to PHA at all doses during and immediately after high doses of Δ^9-THC ($P < .0005$), but this effect was abolished if the data were normalized to account for the higher background counts of the subjects. At all time periods the response of the subjects to a very low (0.05 μg/ml) dose of PHA is greater ($P < .05$) than that of normal controls. This increased response persists when the data are normalized to account for the higher background counts. The analysis clearly indicates that at very low doses of PHA the subjects had enhanced responses. The responses to PHA in total are statistically increased in the subject group, although we are unwilling to assign any biologic significance to the small differences observed. The results obtained on lymphocytes from a kidney transplant patient whose blood was coded and shipped along with the subjects' lymphocytes are included in the graph to illustrate the magnitude of suppression of lymphocyte response seen with potent immunosuppressive agents.

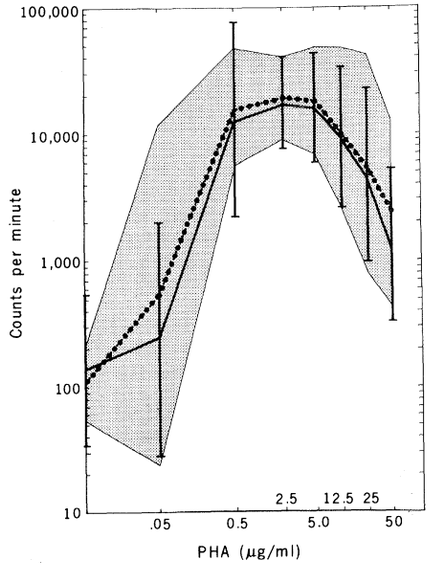

Fig. 2. Lymphocyte responses to phytohemagglutinin (PHA) in seven chronic marijuana smokers. The dotted line represents the mean and the shaded area ± 2 S.D. for normal controls in this laboratory. The solid line represents the mean and the vertical bars ± 2 S.D. for chronic marijuana smokers whose lymphocytes were studied 8 to 72 hours after smoking marijuana.

No differences in the blastogenic response were observed when the subjects' lymphocytes were incubated in homologous type AB$^+$ serum as compared to autologous serum. Furthermore, in experiments not shown, no alteration of the response of normal lymphocytes was seen when serum from patients receiving 210 mg of Δ^9-THC per day was added to the supporting medium. We conclude from these observations that (i) these chronic marijuana smokers had evidence of an increased lymphocyte response to a suboptimal stimulating dose of PHA; (ii) no evidence of depression of lymphocyte responses to PHA could be ascribed to their prior marijuana use or to oral Δ^9-THC administration; and (iii) the serum of these subjects did not inhibit the blastogenic response to PHA of normal lymphocytes.

In the absence of a sensitive and practical assay for Δ^9-THC serum levels, and because our data are from subjects receiving oral cannabis rather than smoking marijuana, the following observations are provided to confirm that adequate drug absorption does occur via this route. The usual cardiovascular effects following a single marijuana cigarette are tachycardia and peripheral vasodilata-

tion (*11*). After continuous administration of Δ^9-THC, however, the initial tachycardia was followed in some subjects by bradycardia. In this group of subjects the mean pulse rate was 60 per minute for the first 5 days (placebo period), rose to 66 per minute on the first day of cannabis administration, and dropped to a low of 54.3 per minute after 12 days of drug administration. Other cardiovascular effects consistent with Δ^9-THC intoxication (*12*) included a fall in mean systolic blood pressure of 14 mm-Hg, a fall in mean diastolic blood pressure of 17 mm-Hg, and an associated weight gain which averaged 4.54 kg per subject during the first 16 days of drug administration. The weight gain, due to fluid retention, was lost within 48 hours of stopping drug administration. Typical marijuana effects were also noted by changes in electroencephalograms, autonomic nervous system, perceptual motor tasks, endocrine system functions, and ward behavior. The participants reported subjective feelings of intoxication during the period of drug administration. Other experiments with similar subjects have shown that Δ^9-THC given orally produces a near complete cross-tolerance to single acute doses of smoked marijuana administered at various points during the oral dosage schedule (*12*).

An additional seven chronic marijuana smokers were studied. This group consisted of males and females aged 20 to 26 years. The group averaged 4.7 marijuana cigarettes per week with a mean duration of smoking of 4.6 years. The blood samples were drawn 8 to 72 hours after the last use of marijuana. Controls consisted of non-drug-using laboratory personnel in the same age range. Control and experimental lymphocytes were cultured at the same time. As indicated in Fig. 2, the mean PHA responses of marijuana smokers fell well within the normal range. These results are in agreement with those found in the hospitalized group and with those of a similar study reported by White and co-workers (*4*).

In summary, in otherwise healthy chronic marijuana smokers, eight of whom were observed under controlled conditions and given pharmacologic amounts of Δ^9-THC, normal lymphocyte responses to PHA were observed.

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Aspartate: Distinct Receptors on *Aplysia* Neurons

Abstract. Aplysia *neurons have specific aspartate receptors that are distinct from those to glutamate. In some cells, aspartate selectively increases the membrane permeability to chloride, giving rise to a hyperpolarization, while on other cells it increases the permeability to sodium, causing a depolarization. There are also specific receptors for* L-*glutamate which mediate sodium, chloride, or potassium conductance increases, and another class of receptors activated by both glutamate and aspartate.*

While L-glutamate has been regarded as a putative excitatory neurotransmitter in both vertebrates and invertebrates, L-aspartate has been reported only to act like glutamate (*1, 2*). However, recent experiments in lobster muscle and the mammalian central nervous system have suggested that aspartate may have a distinct role as a neurotransmitter (*3*). Both aspartate and glutamate are involved in a number of cellular processes such as metabolism, protein formation, and intracellular osmotic and ionic regulation. Aspartate has been found to be a major anion in excitatory, inhibitory, and sensory nerve tissues in the lobster and crab (*4*).

In the gastropod mollusk *Aplysia*, glutamate (*5, 6*) and aspartate (*6*) have been detected in both ganglia and single nerve cells. Glutamate is uniformly distributed, while aspartate concentrations vary over a fourfold range among individual neurons. In contrast, hemolymph, muscle, and connective tissue have significantly lower concentrations. In a number of gastropod mollusks, aspartate has been reported to act like glutamate, which can cause hyperpolarizing or depolarizing responses, or both (*2, 7, 8*). Whether aspartate should be considered a putative neurotransmitter depends not only on its presence in nervous tissue and its ability to cause voltage and conductance changes, but also on the demonstration of aspartate receptors that are specific and distinct from those for other amino acids. We have found specific receptors for aspartate which evoke either depolarizing or hyperpolarizing responses.

Abdominal, cerebral, pleural, and buccal ganglia of *Aplysia californica* or *Aplysia dactylomela* were removed from the animal and pinned to a Sylgard (Dow Corning) layer in a Lucite chamber and perfused with artificial seawater containing 150 m*M* Mg^{2+} at room temperature (20° to 24°C). The connective tissue capsule was slit with a razor blade to expose the cell bodies. Neurons were penetrated with two independent micropipettes filled with 2*M* potassium acetate and having resistances of about 10 megohms. Recording was performed as before (*9*); one pipette was used to record membrane potential and the other to pass current pulses or d-c current for measurement of membrane resistance and reversal potentials. Drugs were applied by iontophoresis with a five-barreled extracellular micropipette. The iontophoretic control unit was designed to pass the same total charge through each of the five barrels, and would automatically vary pulse duration to achieve constant total charge (*10*). L-Glutamic acid and L-aspartic acid were dissolved in distilled water at concentrations of 1*M* and titrated to *p*H 8 with NaOH. Both were passed as anions, while acetylcholine chloride (ACh), 2*M*, was also iontophoresed as a cation. The resistance of each drug barrel was more than 20 megohms. When there was any question of the specificity of the iontophoretic response, two controls were included: (i) reversing the