Kinetic studies (*7*) have also shown that an increasing concentration of the sugar is more effective than a stable or falling concentration, again giving this parameter qualities similar to those elicited by release of insulin stimulated by D-glucose (*8*). Finally, a selective stimulation of the release of insulin by the α anomer of D-glucose, compared to the β anomer, has been found (*9*), corroborating the impression that the beta cell membrane has unique qualities in its recognition of α-D-glucose concentration.

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4. The proportion of α and β anomer was conveniently analyzed by gas-liquid chromatography (GLC) of the per(trimethylsilyl) derivatives obtained by treatment with a mixture (3 : 1 : 9) of hexamethyldisilazane, chlorotrimethylsilane, and pyridine [Sylon HTP (Supelco)] for 30 minutes at room temperature, and direct injection on a Perkin-Elmer 900 gas chromatograph equipped with a stainless steel column (0.3 by 152 cm) containing 0.1 percent OV-17 on GLC 110 (120 to 140 mesh) (Supelco) at a temperature of 80°C and programmed for a rise of 10°C per minute; injection block temperature, 200° to 210°C; the per(trimethylsilyl) derivative of the α anomer appeared at 7.1 minutes from the solvent peak and that of the β anomer at 7.9 minutes.
5. Melting point 133° to 135°C; $[\alpha]_D^{25}$ - + 21° (3 minutes) → + 66° (7 hours) (concentration, 0.2 percent methanol); in (*6*): m.p. 133° to 135°C, $[\alpha]_D$ - + 28° → + 68° (methanol).
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Mitogen-Induced Blastogenic Responses of Lymphocytes from Marihuana Smokers

Abstract. *Blastogenic responses in vitro to phytohemagglutinin and pokeweed mitogen were examined in microcultures of peripheral blood lymphocytes from a group of 12 healthy, long-term marihuana smokers and a group of matched control subjects. With either mitogen, no significant difference in cellular incorporation of [^3H]thymidine was noted between the groups. These results were interpreted to indicate that the functional status of blood lymphocytes was not altered by long-term smoking of marihuana.*

The influence of long-term marihuana use on health is not yet clearly defined although evidence to document its potential hazards and deleterious effects on basic cellular mechanisms is accumulating. The report by Nahas and co-workers (*1*) that blastogenic responses in vitro to phytohemagglutinin and allogeneic lymphocytes were depressed in lymphocytes of long-term marihuana smokers is especially provocative. Their data indicated that the depressed responses were comparable to those of cancer and uremia patients and of transplant recipients undergoing immunosuppressive therapy. The impression gained from these data that long-term marihuana use may impair the expression of cell-mediated immunity and thus render the host more susceptible to disease is still unvalidated; the results of related studies are conflicting (*2*) and, in fact, the observations of Nahas *et al.* have not yet been confirmed directly. For these reasons, we examined the functional status of thymus-dependent (T) and -independent (B) lymphocytes from long-term marihuana smokers. However, because lymphocyte blastogenic responses have been reported to be depressed by upper respiratory infections (*3*) and by inadequate nutrition (*4*), only confirmed healthy individuals were selected for study. We report here that mitogen-induced blastogenic responses of lymphocytes from healthy, long-term marihuana smokers do not differ from those of matched control subjects.

Table 1. Mitogen-induced blastogenic responses of lymphocytes from marihuana smokers and matched control subjects; S.D., standard deviation.

Experiment	Radioactivity (dpm) per culture	
	Smokers	Controls
	Phytohemagglutinin	
1	216,418	197,306
2	163,746	167,027
3	208,781	181,150
4	155,362	163,708
5	186,119	191,547
6	128,834	125,983
7	158,440	129,687
8	202,630	202,241
9	245,436	184,572
10	221,013	141,866
11	90,166	161,611
12	168,784	147,758
Mean ± S.D.	178,811 (43,486)	166,205 (25,903)
	Pokeweed	
1	141,448	100,540
2	163,225	153,372
3	99,984	110,029
4	94,467	120,627
5	167,983	150,436
6	107,180	173,707
7	75,893	99,772
8	126,051	90,498
9	76,932	86,072
10	86,691	107,214
11	90,587	101,932
12	115,015	106,852
Mean ± S.D.	112,121 (31,535)	116,754 (27,585)

A group of 12 individuals, ranging in age from 19 to 32, who had smoked marihuana at least once per week for the previous year (average 3.4 times per week for 4.8 years) was studied. The group consisted of one black and ten white males and one white female. At the time of study, all admitted that they were still smoking marihuana and that they had smoked at least once during the preceding 48 hours. Twelve other individuals who never had smoked marihuana and denied other forms of drug abuse were selected for study as age-, sex-, and racially matched controls. All study subjects were not receiving medication at the time of study and were in good health, as judged from a detailed medical history; the values for complete blood counts, erythrocyte sedimentation, total serum protein, and serum albumin were normal. In addition, all individuals in both groups showed normal values for serum glutamic-oxaloacetic transaminase, glutamic-pyruvic transaminase, alkaline phosphatase, and bilirubin and were negative on testing for hepatitis B antigen. The normal liver function tests reasonably excluded

the possibility of subclinical hepatitis—a condition known to depress cell-mediated responses (*5*)—and indirectly indicated that the subjects were not using drugs, at least those that cause abnormal liver enzyme levels (*6*).

The functional status of T and B lymphocytes was determined in vitro by their respective blastogenic responses to phytohemagglutinin-P (PHA) (Difco Laboratories) and pokeweed mitogen (PWM) (Grand Island Biological) in a microculture system similar to that described by Thurman and associates (*7*). A marihuana smoker and an appropriately matched control subject were studied within the same experiment. Lymphocyte suspensions were prepared from fresh, heparinized, peripheral blood by separation in a Hypaque-Ficoll gradient. Approximately 2×10^5 lymphocytes were cultured in 0.2 ml of RPMI 1640 medium (Grand Island Biological) containing 20 percent autologous plasma. Triplicate cultures were stimulated with 0.4 μg of either PHA or PWM; unstimulated cultures served as controls. The cultures were incubated at 37°C for 3 days when each was treated with 1 μc of [^{3}H]-thymidine (New England Nuclear) and harvested 4 hours later. Lymphocyte blastogenesis was measured by assay of cellular incorporation of radioactivity and was expressed as the average number of disintegrations per minute (dpm) per culture. The methods described by Croxton (*8*) were used for paired and unpaired *t*-tests of the significance of differences in blastogenic responses between the marihuana smokers and the matched control subjects.

The comparison of mitogen-induced blastogenic responses of lymphocytes from marihuana smokers and matched control subjects is shown in Table 1. Statistical analysis of the data by both the paired and unpaired *t*-tests confirmed that there were no significant differences ($P > .10$) in the responses to either mitogen between the groups. There also was no significant difference in [^{3}H]thymidine incorporation in the unstimulated control cultures; the respective mean values for marihuana smokers and control subjects were 735 and 737 dpm.

These results indicate that long-term marihuana smoking had no significant effect on the functional status of T and B lymphocytes and are consistent with recent evidence suggesting that chronic marihuana smokers have unimpaired immune response capabilities. They have been found to develop and exhibit delayed-type hypersensitivity responses to 2,4-dinitrochlorobenzene in the same manner as healthy nonsmokers (*9*) and even to develop humoral antibody reactivity against *Cannabis* extracts (*10*).

Our findings, however, differ completely from those of Nahas and his co-workers (*2*) who described depressed in vitro blastogenic responses in lymphocytes of marihuana smokers. Although the disagreement cannot be explained at present, it is possible that our study populations were not comparable, other than on the basis of marihuana use. They did not describe the health status of their subjects, and there was no indication in their report that they attempted to exclude subclinically ill subjects from their study, as we purposely did. Another variable to consider is the time elapsed between blood sampling and when the subjects last smoked marihuana. Because plasma levels of Δ^9-tetrahydrocannabinol, the putative active component of marihuana, reach a peak within 15 minutes after smoking and decrease rapidly thereafter (*11*), it is possible that impaired lymphocyte responses may be detectable only within a relatively short period after smoking. Even though our study subjects admitted to smoking at least once within 48 hours before study, it is likely that only a few had smoked within the 12 hours immediately preceding study. Nahas and his associates did not include this information in their report so that this possibility also remains to be evaluated. Obviously, similar immunologic studies of other populations of marihuana smokers appear necessary to clarify these divergent observations.

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Hot Hydrogen in Prebiological and Interstellar Chemistry

I was interested to read the recent report by Hong *et al.* (*1*) on the production of amino acids and gas-phase organic compounds from the ultraviolet irradiation of simple gases, with hot hydrogen atoms used as the principal energy conversion agent. Although the earlier work of Sagan and Khare (*2*) on the subject is mentioned, it is not apparent from (*1*) that the initiating mechanism of Hong *et al.*—the production of hot hydrogen atoms by ultraviolet photodissociation of such long-wavelength photon acceptors as H_2S—is the same as ours, that their quantum yields for amino acid production of several times 10^{-5} are the same as ours, and even that the specific amino acids produced in comparable experiments are the same as ours. The great importance of such experiments is that they employ the long-wavelength part of the solar ultraviolet spectrum, where most of the energy available for organic synthesis resides. Although CH_3SH and C_2H_5OH probably were not abundant constituents of the primitive atmosphere of the earth, H_2S and HCHO, used in our experiments, probably were in fair abundance. Our results suggest that the product of energy flux and efficiency is, of all known energy sources, highest for long-wavelength ultraviolet prebiological organic synthesis. The claim by Hong *et al.* that one-carbon-atom precursors rather than two-carbon-atom precursors are adequate is of interest and increases the overall yields, since the primitive ratio of CH_4 to C_2H_6, for example, is likely to be