duced no tumors, the high plating efficiency of the tumor cells was like that of the Balb/3T3 cells, and the morphology of the tumor cells in culture was similar to that of Balb/3T3 cells.

There are now two pieces of evidence that the Balb/3T3 cell is a vascular endothelial cell: It looks like an endothelial cell by scanning electron microscopy (*7*), and it forms tumors that resemble those derived from vascular endothelial cells. The evidence is further supported by the resemblance in morphology and behavior between endothelial cells in vivo and Balb/3T3 cells in vitro. Endothelial cells forming the walls of capillaries appear as a tightly adherent monolayer of nondividing polygonal cells that are activated to grow by wounding and that do not grow as a multilayer, since this would tend to occlude the lumen of the capillary.

It appears that the in vitro properties of the Balb/3T3 cloned line of probable endothelial cells should not be used as the standard for nontumorigenic mouse cells, not only because the cells represent just one of dozens of embryonic cell types, but also because they are neoplastic when inoculated subcutaneously attached to a solid substrate.

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Anomeric Specificity of 3-O-Methyl-D-glucopyranose against Alloxan Diabetes

Abstract. *The individual α and β anomers of the nonmetabolized glucose analog 3-O-methyl-D-glucopyranose (3MG) were studied as protective agents against the alloxan toxicity to pancreatic beta cells in an in vivo rat model. The α 3MG provides greater protection than either the β or the equilibrated compound, as indicated by plasma glucose concentrations 24 hours after the experiment. This specificity suggests that the beta cell membrane is extremely stereospecific, and that glucose or 3MG provide protection against alloxan injury directly by an interaction with the cell membrane and not by subsequent metabolism of the protecting compound.*

It has been reported (*1*) that the α anomer of D-glucose provides better protection than the β anomer does against the toxic effects of alloxan on pancreatic beta cells in fasted rats. These data suggested a highly stereospecific protection site, but did not exclude an effect that might be secondary to a selective transport or metabolism of the α anomer as compared to the β anomer. 3-*O*-Methyl-D-glucopyranose (3MG) is not metabolized in rats (*2*) and several laboratories have shown that it is able to provide protection against alloxan toxicity (*3*).

Commercial crystalline 3-*O*-methyl-D-glucose (Sigma) was analyzed and found to be in the α-D-pyranose form (98 percent α- and 2 percent β-D-pyranose) (*4*). In water solution, it underwent a mutarotation similar to that of α-D-glucose to give an equilibrium mixture of 32 percent of α and 68 percent of β anomer. Removal of the α anomer from the equilibrium mixture (*5*) gave the pure β anomer (96 percent β- and 4 percent α-D-pyranose form) (*6*). Thus, the biological activity of the α and β anomers and of the equilibrium mixture could be compared.

As shown in Table 1, at a dose of 0.28 mmole, α-3MG exerted protection against alloxan toxicity, near normal plasma glucose concentrations being observed 24 hours after alloxan administration, but β-3MG was less effective. These studies confirm that the protective site involves a highly stereospecific conformation of the carbohydrate molecule and does not result from a metabolite.

Table 1. Effect of α and β anomers of 3-*O*-methyl-D-glucopyranose (3MG) against alloxan diabetes. Male rats weighing approximately 200 g were fasted for 24 hours. The crystalline α or β anomers (0.28 mmole) rapidly dissolved in 0.5 ml of saline solution, or a solution of the equilibrium mixture (68 percent of β and 32 percent of α, total 0.28 mmole, in 0.5 ml of saline), were infused over 60 seconds, followed in 4 seconds by a rapid infusion (0.5 ml) of alloxan (40 mg/kg). The animals were fed freely and plasma glucose was determined 24 hours later to express the degree of alloxan diabetes. Control animals receiving neither alloxan nor 3MG had a mean plasma glucose concentration of 164 ± 2 mg/100 ml (N = 21). S.E.M., standard error of the mean; N, number of animals.

Substances injected	Plasma glucose (mg/100 ml)	
	Mean ± S.E.M.	N
Alloxan alone	431 ± 17	6
α-3MG and alloxan	195 ± 26*	6
β-3MG and alloxan	359 ± 25	6
Equilibrium mixture and alloxan	305 ± 47	6

* $P < .005$; Student's t-test comparing α versus β.

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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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