Effect of 6-Mercaptopurine on Induction of α-Glucosidase in Candida.* (27973)

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The antifungal antibiotic cycloheximide and the antitumor drug 6-mercaptopurine both inhibit antibody formation(1,2) and growth of microbes(3,4) and mammalian cells(5,6). Moreover, both agents act by suppressing nucleic acid synthesis(7,8). Because cycloheximide prevents induced enzyme formation in yeast(9), it was postulated that 6-mercaptopurine would act in a similar manner. The test system chosen for this study was the maltose-induced α-glucosidase of *Candida stellatoidea*. Enzymic adaptation was retarded by 6-mercaptopurine; this inhibition was substantially alleviated by adenine and hypoxanthine.

Methods. *C. stellatoidea* was grown in complex maltose or glucose medium, and prepared for manometric studies as described previously(10). Cell-free preparations were made by the quick shear method, employing a piston-type cell press(11). Enzyme inductions were carried out aerobically with washed glucose-grown cells in a milieu containing maltose, arginine and KH_2PO_4(12).

Results. As much as 100 μg 6-mercaptopurine/ml of complex glucose medium did not retard growth of *C. stellatoidea*. Because several substances are known to antagonize the growth inhibiting action of 6-mercaptopurine (13,14), constituents of the complex medium may have protected the yeast cells. Therefore, a minimal medium containing 40 g glucose, 2 g NH_4Cl, 1 g KH_2PO_4, 0.5 g $MgSO_4$ and 2 μg biotin per liter of deionized water was devised. Growth of *C. stellatoidea* in the minimal medium was suppressed by 50 μg 6-mercaptopurine/ml (Fig. 1).

C. stellatoidea grown in glucose medium and maltose medium was tested for power to ferment these sugars in the presence of 50 μg purine/ml. The following purines did not significantly affect carbon dioxide evolution from glucose by washed glucose-grown cells or from maltose and glucose by washed maltose-grown cells: purine (free base), 6-methylpurine, inosine, 8-azaguanine, 6-methylaminopurine and 6-mercaptopurine (Table I). Adenine and hypoxanthine stimulated fermentation by 10-20%. In induction experiments, purine (free base), inosine, 6-methylaminopurine and 8-azaguanine did not substantially alter the course of induction whereas adenine and hypoxanthine stimulated induction, and 6-methylpurine and 6-mercaptopurine retarded induction (Table II). The inhibitory

TABLE I. Effect of 6-Mercaptopurine on Sugar Utilization.

	μl CO_2/hr			
	Glucose		Maltose	
Organism	—	6MP	—	6MP
Maltose-grown cells				
whole "	750	705	600	610
cell-free	1410	1460	1440	1470
Glucose-grown cells				
whole "	900	900	39	40

Carbon dioxide evolution was measured manometrically with 1 ml of washed cells (3×10^8/ml) or cell-free material, 100 mg sugar, 2 ml of 0.067 M KH_2PO_4, 50 μg 6-mercaptopurine/ml when indicated, and a nitrogen atmosphere.

TABLE II. Effect of Purines on Induction.

Supplement	6MP	μl CO_2/hr	% inhibition
—	—	270	
—	+	131	51
adenine	—	342	
"	+	288	16
hypoxanthine	—	355	
"	+	290	18
xanthine	—	288	
"	+	180	37
6-methylpurine	—	53	80

Glucose-grown cells were harvested, washed and diluted to 10^8 yeast/ml; the suspension containing cells, 30 mg maltose/ml and 100 μg arginine/ml was incubated aerobically for 4 hours. Purines, including 6-mercaptopurine (6MP) were added, when indicated, to a concentration of 50 μg/ml. Maltose utilization was measured manometrically with a nitrogen atmosphere.

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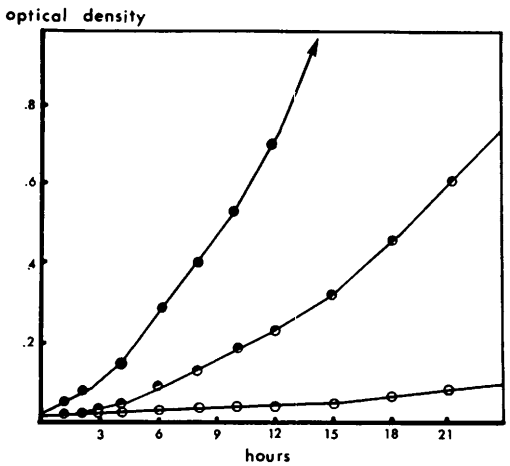

FIG. 1. Growth response of *C. stellatoidea* in complex glucose medium with up to 100 μg 6-mercaptopurine/ml (●), in minimal medium with (○) or without (◓) 50 μg 6-mercaptopurine/ml. Turbidity was measured at 625 mμ.

action of 6-mercaptopurine was partially overcome by adenine and hypoxanthine and less effectively by xanthine.

Discussion. The structure of 6-mercaptopurine resembles those of the 6-substituted purines adenine and hypoxanthine; evidence for competitive inhibition comes from the ability of adenine and hypoxanthine to restore growth and inductive capacity to 6-mercaptopurine inhibited cells. The ribotide, however, rather than the free purine, may be the active form(15,16). Both adenine and hypoxanthine are precursors for adenosine triphosphate, which also protects cells from the deleterious effects of 6-mercaptopurine.

Summary. The antitumor agent 6-mercaptopurine suppresses growth and induced enzyme formation by *Candida stellatoidea.* These inhibitory effects are substantially reversed by adenine and hypoxanthine.

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Effect of Dietary Fat on the Disposition of Cholesterol-4-C^{14} in Rats.* (27974)

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The hypocholesteremic effect of dietary unsaturated fat has stimulated interest in the fate of cholesterol disappearing from the serum. Hellman and coworkers(1) and Gordon *et al.*(2) found in humans that the cholesterol which disappeared from the serum after feeding unsaturated fats was excreted in the feces in some form. Bronte-Stewart *et al.*(3) reported that a fall in serum cholesterol level attributable to changes in dietary fat was accompanied by an increase of bile acids in feces. In rats fed diets contain-

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