Ca^{2+}. This indicates that membrane Ca^{2+} permeability has a significant effect on the resting membrane potential of the fibres in these preparations. Ruthenium red inhibits synaptosomal membrane Ca^{2+} flux[23]. Treatment of 2 dorsal root preparations with ruthenium red (1 µM) depressed responses to CHEB irreversibly and in 2 preparations treated with 5 µM ruthenium red CHEB-induced responses were abolished with no recovery up to 60 min after washout of the dye. Responses induced by kainate were unaffected by ruthenium red treatment (figure, b). Treatment of dorsal root preparations with 10 mM, but not 1 mM, Mg^{2+} had a similar selective blocking action against responses induced by CHEB.

In conclusion, the results show that the convulsant properties of the barbiturate analogues listed in the table are clearly related to their potencies as direct excitants and that this excitation probably results from an influx of Ca^{2+}. Convulsant barbiturates have been shown to inhibit a Ca^{2+}-dependent ATPase, believed to represent a Ca^{2+} pump, in synaptosomal membranes[24].

Inhibition of such a membrane Ca^{2+} pump might explain the Ca^{2+} dependence of the depolarizing action of these compounds. Since neurotransmitter release is dependent on Ca^{2+} influx[25], transmitter release from nerve terminals might be stimulated in the presence of CHEB, or other barbiturate convulsants. The convulsant action probably results from this effect in combination with excitation resulting from direct depolarization of neurones[12].

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Methyl cellosolve-induced sensitization of mice to bacterial endotoxin

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Summary. Treatment of mice with a sublethal dose of methyl cellosolve renders the animals 64-fold more sensitive to the toxic effects of bacterial endotoxin.

In the course of studies on drug-endotoxin interactions in mice, methyl cellosolve was used to solubilize certain hydrophobic drugs. It soon became clear that the solubilizing agent itself increased the lethality of endotoxin and this report will describe the magnitude and conditions of the sensitization due to methyl cellosolve.

Materials and methods. Male, NMRI strain mice weighing between 18 and 22 g were injected i.v. with various doses of *S. marcescens* endotoxin suspended in 0.25 ml deionized water. Immediately following the endotoxin, mice received i.v. injections (0.25 ml) 1 M methyl cellosolve or 1 M propylene glycol. Evaluation of the LD_{50} was based on the 7-day mortality and was calculated by probit analysis of the dose[3].

Methyl cellosolve (ethylene glycol monomethyl ether) was purchased from Fisher Scientific Company, Fair Lawn, NJ, and propylene glycol was obtained from Mayco Industries Inc., Philadelphia, Pa. The *S. marcescens* endotoxin (lot No. 508948) was a Boivin preparation obtained from Difco Laboratories, Detroit, MI.

Observations and discussion. Mice receiving endotoxin alone exhibited an LD_{50} of 17 mg/kg (table). Treatment of mice with 1 M propylene glycol, in addition to endotoxin, did not change the sensitivity of the mice to endotoxin as shown by the 16 mg/kg LD_{50} in these animals. However, mice treated with endotoxin, followed immediately by 1 M methyl cellosolve (i.v.), showed a reduction in the LD_{50} to 0.25 mg/kg. This represents a 64-fold sensitization in the methyl cellosolve-treated animals. The injection of 0.25 ml of 1 M methyl cellosolve represents a dose of 950 mg/kg on a weight basis, and this amount of methyl cellosolve caused no deaths when injected into 20 mice.

Further characterization of the drug-endotoxin interaction was undertaken by modifying the route of administration and the time interval between endotoxin and methyl cellosolve treatment. The data in the table show that i.p.

injection of methyl cellosolve also sensitized the mice to endotoxin. If the methyl cellosolve and endotoxin were preincubated (15 min, 37 °C) prior to injection, the sensitization still occurred. However, methyl cellosolve administered 4 h prior to lipopolysaccharide failed to sensitize the mice. Methyl cellosolve treatment 4 h after the endotoxin increased sensitivity to endotoxin to approximately the same degree as the simultaneous injection.

Effect of methyl cellosolve on endotoxin lethality in mice

Treatment	No. of mice	Endotoxin[a] LD_{50}	95% (confidence limits)
Endotoxin	122	17	(15 -20)
Propylene glycol + endotoxin	125	16	(14 -19)
Methyl cellosolve + endotoxin	105	0.25	(0.19-0.33)[c]
Methyl cellosolve (i.p.)[b] + endotoxin	82	1.5	(1.0 -2.2)[c]
Methyl cellosolve preincubated with endotoxin	65	1.6	(1.2 -2.2)[c]
Methyl cellosolve 4 h before endotoxin	82	15	(12 -20)
Methyl cellosolve 4 h after endotoxin	44	0.87	(0.49-1.5)[c]

[a] Values are expressed as mg endotoxin per kg. [b] Methyl cellosolve was injected i.p. in this group. All other groups received i.v. injection of drug and endotoxin. [c] Significantly different from propylene glycol control at $p < 0.01$.

Methyl cellosolve is a volatile, organic liquid which has a LD_{50} in mice of 2150 mg/kg[4]. There have been a number of reports of methyl cellosolve intoxication in humans [5-7] and the symptoms include metabolic acidosis, which may play a role in sensitization to endotoxin. Another possibility is that methyl cellosolve may inhibit RNA or protein synthesis and thereby increase the lethality of endotoxin. Further studies to clarify the mechanism by which this agent sensitizes mice to endotoxin may prove to be useful in characterizing the toxic mechanism of endotoxin action.

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Potentiation of the inhibition of xanthine oxidase by a combination of 6-mercaptopurine and 6-thioguanine

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Summary. Carcinostatic agents, 6-mercaptopurine and 6-thioguanine inhibited the in vitro and in vivo activity of the enzyme xanthine oxidase (xanthine-oxygen oxidoreductase, E.C. 1.2.3.2.). Simultaneous addition of a mixture of the 2 antimetabolites produced a synergistic effect on the inhibition of the enzyme activity.

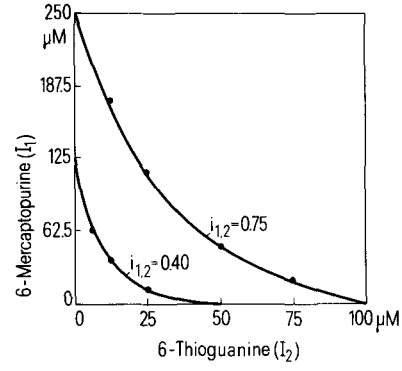
Various purine analogs, including 6-mercaptopurine and 6-thioguanine which are active as carcinostatic agents, have been shown to act as potent inhibitors of xanthine oxidase (xanthine-oxygen oxidoreductase, EC 1.2.3.2.)[3-6]. 6-Mercaptopurine is also a substrate for this enzyme[7] and consequently its pharmacological efficacy and its toxicity are related to the activity of xanthine oxidase. However, this enzyme is not involved in the metabolic degradation of 6-thioguanine[8].

The current emphasis in cancer chemotherapy is on the use of combinations of drugs, taking into account the mode of action of the agents, their synergistic properties and the part of the cell cycle on which they act. The fact that 6-thioguanine and 6-mercaptopurine are both used for the treatment of leukemias[9] prompted us to study the interaction of the combination of the 2 antimetabolites on the activity of xanthine oxidase.

The in vitro studies on milk xanthine oxidase revealed a definite synergistic effect of the combination of the 2 drugs on the inhibition of the enzyme activity. In an attempt to understand further the effect of simultaneous administration of the 2 drugs on tissue xanthine oxidase activity, the response of this enzyme to the in vivo administration of the combination of 6-thioguanine and 6-mercaptopurine was studied in the liver of rats.

Xanthine, 6-mercaptopurine, 6-thioguanine and calcium phosphate gel were purchased from Sigma Chemical Co. (St. Louis, MO, USA). 2,3,5-Triphenyltetrozolium chloride was obtained from British Drug House (London). All other reagents were of A.R. Grade or the purest grade commercially obtainable.

Milk xanthine oxidase was prepared and purified by the method of Massey et al.[10]. The specific activity was deter-



Isobologram for simultaneously acting inhibitors, 6-mercaptopurine (I_1) and 6-thioguanine (I_2) on xanthine oxidase.