without strict isolation facilities and known virus-free mice.

Summary. Mouse hepatitis viruses were readily isolated, by a variety of technics, from pooled feces of weanling mice of the majority of colonies tested. The infection was highly contagious to contacts from a non-infected mouse stock. Evidence is presented that contact infection with hepatitis viruses produced high mortality in infant mice of the non-infected stock.

We are pleased to acknowledge the cooperation of Dr. Virginia J. Evans, Nat. Cancer Inst., who provided the mouse liver cell line, and the technical assistance of Mr. Theodore Catchings.

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Antagonistic Relationship Between *myo*-Inositol and 2-*O*,*C*-Methylene-*myo*-Inositol in Animals.* (27981)

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(Introduced by A. E. Harper)
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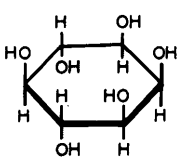
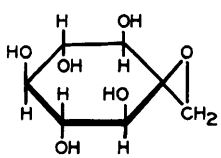
Recent researches have provided information about the reactions by which *myo*-inositol (I) is incorporated into phospholipids in animal tissues(1), or is converted to well-known intermediates of carbohydrate metabolism(2), but the physiological significance of these processes is not clear. Since investigations of this kind, and the discovery of additional metabolic pathways, are facilitated by the availability of metabolic antagonists, it seemed of interest to search for an anti-inositol active in animals. On testing a number of cyclitols, some of which had been shown to be antagonists of *myo*-inositol in certain microorganisms(3), we found that 2-*O*,*C*-methylene-*myo*-inositol (II) was toxic to mice and rats. Our observations on the effects of this compound, and on the suppression of these effects by *myo*-inositol, are reported here.



myo-INOSITOL
I



2-O,C-METHYLENE-
myo-INOSITOL
II

Materials and methods. 2-*O*,*C*-Methylene-*myo*-inositol, which hereafter will be designated as *myo* methylene oxide, was prepared from *myo*-inosose-2(4) essentially as described by Posternak(5).† Compounds were administered as aqueous solutions by intraperitoneal injection.

Male weanling mice (Taconic strain, Rolfsmeyer Farms) and male weanling rats (Holtz-

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† Posternak referred to the compound as "oxyde de méthylène-penta-oxy-cyclohexane." The name 2-*O*,*C*-methylene-*myo*-inositol describes the stereochemical configuration, and is consistent with the cyclitol nomenclature currently used in the U. S.

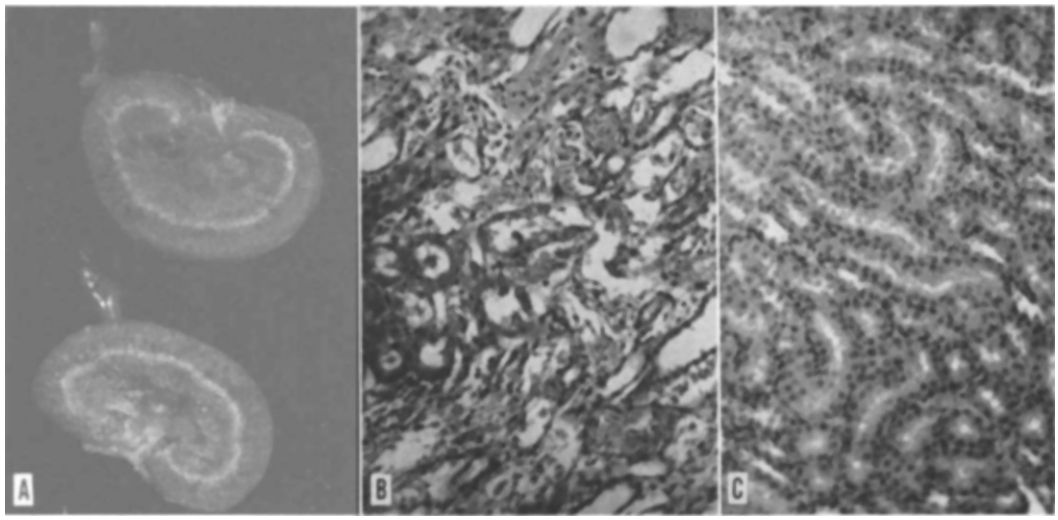

FIG. 1. Effect of *myo* methylene oxide on the kidney, and reversal of the effect by *myo*-inositol. Rats were injected with 150 mg/kg *myo* methylene oxide, and sacrificed 48 hr later. The kidneys were fixed immediately in 10% neutral buffered formalin; sections were stained with hematoxylin and eosin. A) Whole kidney bisected longitudinally; B) section of juxtamedullary region; C) same, normal kidney (no treatment).

man Co.) were maintained in the departmental animal room for several days before being put on experiment. A low-inositol synthetic diet was used, but is unnecessary. All the effects described are produced equally well in animals fed natural rations.

Results. Four groups of 10 mice each received *myo* methylene oxide in doses of 100, 125, 135 and 150 mg per kg body weight for the respective groups, and were observed for 5 days, by which time all survivors had resumed growth. A plot of dose *vs* per cent mortality according to the method of Litchfield and Wilcoxon(6) gave a figure of 123 mg per kg for the LD_{50} (single dose, intraperitoneal) in mice. Observations on rats showed that the figure is lower for these animals, probably about 70 mg per kg. Doses of 150 mg per kg in mice and 100 mg per kg in rats were almost invariably fatal; these were the quantities administered when a minimal lethal dose (MLD) is specified in the following sections.

The chronic toxicity of *myo* methylene oxide in mice was studied on a limited scale by administering the compound daily for 12 days at 20, 30, and 40 mg per kg. Four mice were used at each dosage level. None of the animals died. Those receiving 20 and 30 mg per kg per day appeared normal, but gained only two-thirds as much as the saline-injected controls. At 40 mg per kg per day the growth depression was severe and there were obvious toxicity symptoms.

Animals receiving single doses of 1-1.5 times the MLD of *myo* methylene oxide usually remained normal in appearance for 20-24 hours. They then became progressively weaker, and developed labored breathing, hyperactive tendon reflexes, and muscle twitching. Stupor and finally coma with periodic breathing were followed by death, usually in 36-54 hours. Occasionally, animals survived 5-8 days. At autopsy, the kidneys were usually pale, and when hemisectioned showed clearly demarked rings in the juxtamedullary region (Fig. 1A). Pale stomachs, and spleens much reduced in size, were seen in mice which had survived several days. Histological examination of brain, liver, kidneys, testes, intestine, lungs, heart, pancreas, and spleen revealed no additional abnormalities, but showed that the white rings in the kidneys were due to necrosis of the epithelial cells (Fig. 1B).

The necrotic lesion could first be observed in rats about 8 hours after injection of an MLD of the methylene oxide, and it did not spread appreciably during the remaining period of the animals' survival. No expansion of the necrotic area resulted from repeated administration of sublethal doses, or from

TABLE I. Effect of *myo*-Inositol Given with an MLD of *myo* Methylene Oxide.*

Inositol (mg/kg)	Inositol: methylene oxide	Deaths in 14 days	Avg wt change in 14 days (g)
2500	16.7	0	+ 7.7
2000	13.3	0	+ 7.5
1500	10.0	0	+ 6.6
1000	6.7	0	+ 6.4
500	3.3	1	— 3.7
300	2.0	3	—
0†	—	0	+10.1

* Four male mice (15-20 g) were used in each group. The compounds were given simultaneously as a single injection; the dosage of methylene oxide was 150 mg/kg.

† Received 1 ml of saline, no cyclitols.

single injections of 3 to 6 times the minimal lethal dose. At still higher dosages, the animals died within 2 hours, without developing the gross symptoms described above. In these cases there were no detectable changes in the kidney tissue.

Protection against the toxic effects of *myo* methylene oxide was afforded by sizeable doses of *myo*-inositol administered shortly before or simultaneously with the methylene oxide, but very large injections of *myo*-inositol after 0.5 hour, or repeated smaller injections beginning at 1 hour were without effect. Quantitative data on the protection against mortality are shown in Tables I and II. Complete prevention of the kidney necrosis, when an MLD of methylene oxide was given, was achieved with 1000 mg per kg of *myo*-inositol.

The nature of the symptoms produced by lethal doses of *myo* methylene oxide, together with the finding of severe kidney damage,

TABLE II. Effect of Varying Doses of *myo* Methylene Oxide and *myo*-Inositol.*

Inositol (mg/kg)	Methylene oxide (mg/kg)	Inositol: methylene oxide	Deaths in 14 days	Avg wt change in 14 days (g)
340	170	2	0	+ 2
1200	300	4	1	+ 3
3000	300	10	0	+ 7.5
1200	600	2	3	—
2400	600	4	2	— 1
6000	600	10	1	— 2
0†	0	—	0	+10.5

* Three male mice (15-20 g) were used in each group. The compounds were given simultaneously as a single injection.

† Received 1 ml of saline, no cyclitols.

suggested that the treated animals were suffering from uremia. Analyses (Table III) verified this and also showed that the elevation of blood urea caused by 60 mg per kg of methylene oxide (in rats) was completely suppressed by a 10-fold amount of *myo*-inositol. A graded, partial response was obtained with smaller amounts.

To test whether the antagonism is a specific property of *myo*-inositol, 5 other cyclitols, known to be non-toxic at the dosage employed, and 5 open-chain polyalcohols were tried as protective agents. Sequoyitol, L-inositol, D-inositol, (+)-pinitol and *myo*-inosamine-2

TABLE III. Effect of *myo* Methylene Oxide on Blood Urea Nitrogen and Blood Creatinine in Rats.*

Methylene oxide (mg/kg)	Sampling time (hr post inj)	Urea nitrogen (mg/100 ml)	Creatinine (mg/100 ml)
0	—	14	.8
20	48	13	—
60	58	142	3.6
100	49	210	—
150	19	90	—

* One rat was used for each value. Blood was taken by heart puncture. Urea nitrogen was determined in a Technicon Autoanalyzer(7); creatinine by the method of Peters(8).

were the cyclitols used;‡ the open-chain compounds were ribitol (adonitol), D-arabitol, D-glucitol (sorbitol), D-mannitol and galactitol (dulcitol). All were given intraperitoneally to mice at 5 g/kg, simultaneously with an MLD of *myo* methylene oxide (3 animals per compound). None of the compounds delayed the onset of toxicity symptoms and death, or had an effect on the associated kidney necrosis.

Discussion. *Myo* methylene oxide was shown by Schopfer and Posternak to oppose the growth-stimulating effect of *myo*-inositol in the yeast *Schizosaccharomyces pombe* and the mold *Neurospora crassa* (inositolless mutant)(3). The present observations demonstrate that the antagonism between the 2 compounds extends to animal systems. In the microorganisms, the antagonism is typical in that the amount of methylene oxide required for 50% inhibition of growth is many times

‡ For the structural formulas of these compounds, see ref. 9.

the amount of inositol present. However, the claim that this antagonism is of the competitive type was not rigorously established, since the antagonist : inositol ratio giving half-maximal growth was not determined over a wide range. In mice and rats the protective action of the normal metabolite is observed only when it is provided in larger amounts than the antagonist; also it appears that as the dose of methylene oxide is increased, protection is maintained only by an increase in ratio of inositol to antagonist. On this basis the antagonism between *myo* methylene oxide and *myo*-inositol in animals is of the non-competitive type as defined by Woolley(10), and is perhaps to be compared with the antagonism between Dicumarol® and vit. K.

That *myo* methylene oxide produces a necrotic lesion in the kidney cortex is perhaps not surprising, since such lesions are caused by many toxic agents. The necrosis is unusual, however, in that it remains narrowly confined to the juxtamedullary region under a wide variety of dosage regimens. The evidence suggests that kidney failure is the primary cause of death from doses of 1-1.5 times the MLD, but some contribution from other effects is indicated by the fact that survival time of the treated animals is usually slightly less than average survival time after bilateral nephrectomy(11,12).

A logical interpretation of the injurious effect of a *myo*-inositol antagonist on the kidney would be that *myo*-inositol has a particularly critical role in kidney metabolism. Such an inference is not yet warranted, however. *Myo* methylene oxide is a 1,2-epoxide, which places it in the class of monofunctional alkylating agents. It is not unlikely that this property, rather than its cyclitol nature, is primarily responsible for the toxic action of the compound. Presumably the fact that it is a cyclitol, differing only slightly from *myo*-inositol in configuration, is the basis for the antagonism of its action by the inositol.

Investigations of the effects of *myo* methylene oxide on *myo*-inositol metabolism will be reported elsewhere.

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Degradation of Insulin by Tissue Extracts of Normal and Nephrotic Rats.* (27982)

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Insulin requirements are decreased in some patients following onset of diabetic glomerulosclerosis(1,2,3). Analogously, induction of nephrotoxic serum nephrosis in rats with alloxan or pancreatectomy diabetes diminishes blood and urinary glucose(4,5,6). We(6) have shown that I^{131} insulin injected intravenously disappears from the blood more

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