were pathognomonic only of RV3. The distinctive lesions of MHV were regularly lacking. Other types of MHV have not been studied in this connection.

*Summary.* A passaged strain of reovirus type 3 (oncolytic virus of Nelson and Tarnowski) was established in weaned Swiss mice and maintained for 52 weeks by liver transfer in the absence of ascites tumor cells. The only pathologic response was the presence of peritoneal fluid in trace amounts during the first week. Pathogenicity was not altered by prior injection of *Eperythrozoon coccoides*.

Active virus was recovered from the liver and brain through the 2nd week after injection. It was present in heart's blood and intestinal washings only during the first week but was detectable in peritoneal washings for at least 18 weeks. The virus was identified by oncolysis in the presence of tumor cells, the response in infant mice, and the cytopathic reaction in HeLa cell cultures. Interference with the development of a particular murine hepatitis virus by reovirus 3 was observed in nurslings injected with a mixture of the 2 viruses.

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## Enhanced Methylglyoxal Toxicity in Oxythiamine Induced Thiamine Deficiency in Mice.* (29460)

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In contrast to earlier reports, recent studies have indicated that thiamine deficient animals do not excrete methylglyoxal(1,2). However, it has been shown that methylglyoxal can accumulate *in vitro* when the tissues of thiamine deficient animals are incubated with fructose-diphosphate(3). Hence it is possible that a transient, localized accumulation of methylglyoxal in certain tissues may be a contributing factor in the thiamine deficiency syndrome in animals.

Although the administration of methylglyoxal has not been shown to produce the characteristic symptoms of thiamine deficiency in normal animals, it does exhibit considerable toxicity. One might expect greater toxicity from methylglyoxal administered to a thiamine deficient animal if such material accumulates during deficiency or if oxidative degradation is inhibited. In the present study, the toxicity of methylglyoxal was determined in mice made deficient by dietary lack of thiamine as well as by the use of thiamine antagonists. The urine, brain and muscles of normal and deficient mice were also analyzed for the presence of methylglyoxal.

*Materials and methods.* Male Swiss-Webster mice weighing 18-22 g were either pretreated with pyrithiamine or oxythiamine, or maintained on a commercial thiamine deficient diet† for 10 days. Methylglyoxal solu-

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TABLE I. Toxicity of Intraperitoneal Methylglyoxal in Mice.

| Pretreatment | No. of mice | Time after injection: 24 hr — Methylglyoxal $LD_{50}$ (mg/kg) | 24 hr — 95% confidence limits | 72 hr — Methylglyoxal $LD_{50}$ (mg/kg) | 72 hr — 95% confidence limits |
|---|---|---|---|---|---|
| None | 100 | 290 | 232–362 | 260 | 211–319 |
| Oxythiamine† | 36 | 150* | 96–232 | 140* | 82–231 |
| Pyrithiamine‡ | 30 | 410 | 230–705 | 215 | 153–301 |
| Thiamine deficient diet§ | 60 | 450 | 321–630 | 240 | 190–302 |

* Significantly different from control.

† 250 mg/kg subcutaneously in 3 divided doses over an 8 hr period one day prior to methylglyoxal injection.

‡ 50 mg/kg subcutaneously in 3 divided doses over an 8 hr period one day prior to methylglyoxal injection.

§ Deficient diet for 10 days prior to methylglyoxal injection.

tions were prepared daily by dilution of a 25% stock solution and injected intraperitoneally in doses ranging from 50 to 600 mg/kg. Calculation of the $LD_{50}$ and the 95% confidence limits was done by the method of Litchfield and Wilcoxon(4). The method of Liang was used for determination of methylglyoxal(2). Analyses were made on samples of brain, gastrocnemius muscle and urine from mice showing gross symptoms of thiamine deficiency, *i.e.*, 7 days after a single injection of 1.25 mg of pyrithiamine, after 7 daily injections of 1 mg of oxythiamine or after 28 days maintenance on a thiamine deficient diet. Urine was collected for a 12-hour period prior to killing the thiamine deficient mice and overnight from mice that had received methylglyoxal injections. One ml of a saturated solution of 2-4 dinitrophenylhydrazine in 2 N HCl was added to each collection vessel in order to stabilize any methylglyoxal excreted.

*Results.* The results of the toxicity studies with methylglyoxal are shown in Table I. The intraperitoneal $LD_{50}$ of methylglyoxal in control mice was found to be 290 mg/kg at 24 hours. Administration of 250 mg/kg of oxythiamine one day prior to injection of methylglyoxal significantly reduced the $LD_{50}$ of methylglyoxal to 150 mg/kg at 24 hours and 140 mg/kg at 72 hours. Neither the pyrithiamine pretreatment nor the maintenance on a thiamine deficient diet significantly altered the $LD_{50}$ of methylglyoxal.

The immediate cause of death from methylglyoxal administration appeared to be dose dependent. Doses of 400 mg/kg and over produced death in 20-30% of the mice within 10 minutes after injection. These animals salivated profusely, convulsed and apparently died a cardiovascular death. At doses of less than 400 mg/kg of methylglyoxal, the animals did not die until after 12 hours or more. Methylglyoxal at these lower doses acted as a depressant in mice. Gross necropsy of the mice revealed only a mild peri ıitis present at the injection site.

Although we were able to detect as little as 25 μg of exogenous methylglyoxal added to urine containing 2-4 dinitrophenylhydrazine, we were unable to detect it in the urine of any thiamine deficient mice. Nor were we able to detect it in the urine of normal mice that had been given intraperitoneal injections of 125 mg/kg of methylglyoxal. We also could not detect it in the brain or gastrocnemius muscles of any of our mice.

*Discussion.* It was evident from daily weighings that our mice did not show a normal weight gain after 10 days on the thiamine deficient diet. Methylglyoxal toxicity was determined at this early stage of deficiency so that the factors of starvation and general debilitation which became more evident with prolonged deficiency would not contribute greatly to the toxicity. The observation that methylglyoxal was not more toxic in thiamine deficient mice than in controls is in agreement with the work of Haynes and Weiss on moderately deficient rats(5). Carbohydrate metabolism is apparently not markedly disturbed at this early stage of thiamine deficiency(6), and methylglyoxal can be normally degraded.

The increased toxicity of methylglyoxal in oxythiamine treated mice as compared to pyrithiamine treated ones may be dependent on the known differences in action of these antagonists. Pyrithiamine inhibits the phosphorylation of thiamine. When administered to animals it causes a variable decrease in thiamine pyrophosphate concentration in certain tissues. Oxythiamine, however, is itself phosphorylated and then acts as a competitor with thiamine pyrophosphate for active enzyme sites(7). Jones(6) has shown that neither a thiamine deficient diet nor the addition of thiamine antagonists to the diet markedly inhibited pyruvate decarboxylation. However, he did note that oxythiamine had a specific, though limited, inhibiting effect on pyruvate decarboxylation. It has been shown that oxythiamine is a more potent inhibitor of lactic dehydrogenase than pyrithiamine (8). It is thus reasonable to expect that administration of a large dose of oxythiamine would more profoundly alter carbohydrate metabolism than an equivalent dose of pyrithiamine. Since methylglyoxal is oxidized *via* lactic acid, an alteration in carbohydrate metabolism resulting from previously administered oxythiamine would be likely to enhance its toxicity.

The failure to detect methylglyoxal in the brain, muscle or urine of mice made thiamine deficient by dietary means is in agreement with the observations of Liang(2) on rats. Our inability to detect it in the oxythiamine treated mice was somewhat disappointing in view of the toxicity studies. However, Drummond has shown that even in markedly deficient animals, the level of glyoxalase activity may not be reduced sufficiently to prevent its oxidation to lactic acid(1).

*Summary.* Intraperitoneal injection of methylglyoxal was toxic to mice, the $LD_{50}$ in normal mice after 24 hours being 290 mg/kg. The cause of death with doses of 400 mg/kg and above appeared to differ from that produced by lower doses. Neither pretreatment with a thiamine deficient diet nor pyrithiamine significantly affected the toxicity of methylglyoxal. However, pretreatment with oxythiamine lowered the $LD_{50}$ of methylglyoxal to 150 mg/kg. Methylglyoxal was not detected in the urine of normal mice given methylglyoxal, nor in the urine, brain or muscle of mice treated with pyrithiamine or oxythiamine or in mice fed a thiamine deficient diet.

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## The Neuraminidase of Parainfluenza Virus (Type 2).* (29461)

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The ability of the influenza viruses to elute from erythrocytes appears to be dependent upon the presence of active neuraminidase in the viral particle(1,2,3,4). The parainfluenza viruses are also able to agglu-

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