tailed data on purification of some anti-fungal conjugates are presented. Ethodin extraction of serum antibody preparatory to conjugation and gel filtration further expedites the process.

We are indebted to Sophia M. Cohen and Roy W. Stevens for data on viral and bacterial conjugates, respectively, and to Harold W. Doppel for instruction in preparation and use of the Sephadex column. Milton Allen performed the numerous protein determinations. The figure was designed by Frank C. Reed.

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Received September 5, 1961. P.S.E.B.M., 1962, v109.

Effect of Vitamin B_6 And Magnesium on Renal Deposition of Calcium Oxalate Induced by Ethylene Glycol Administration.* (27115)

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It has recently been estimated(1) that 40-60 deaths occur each year in the United States from ingestion of ethylene glycol, commonly used as anti-freeze and an industrial solvent. Acute ethylene glycol toxicity is characterized by central nervous system depression, hemolysis and renal tubular necrosis. In chronic ethylene glycol poisoning kidney damage is caused by deposition of calcium oxalate since ingested ethylene glycol is partially oxidized to oxalic acid. Recent studies from this laboratory(2,3,4) have associated the endogenous production of oxalic acid accompanied by renal calcium oxalate deposition with the state of Vit. B_6 nutriture. Diets high in magnesium have been found to protect against deposition of calcium oxalate in the kidneys of Vit. B_6 deficient rats even though urinary oxalate levels were not decreased(5). In the present study the effect of Vit. B_6 and magnesium on the metabolism of ethylene glycol and renal deposition of calcium oxalate derived from ingested ethylene glycol have been studied.

Materials. In these experiments male Charles River rats were used. The basal diet fed contained (expressed as %): casein 15, sucrose 74.45, glycine 3, Jones and Foster Salts(6) minus its magnesium and calcium salts 2.25, corn oil 4, cod liver oil 1, and choline 0.3. Each 100 g of diet was supplemented with the following (in mg): thiamine 0.4, riboflavin 0.8, niacin 4, Ca pantothenate 2, folic acid 0.1, menadione 0.1, biotin 0.02, and Vit. B_{12} 0.005. Pyridoxine HCl, MgO, and $CaCO_3$ were added to the basal diets as required.

Anatomic study—methods. In this experiment 4 groups of 6 weanling rats were fed *ad libitum* the basal diet supplemented with 1.5 g $CaCO_3$ per 100 g. Two-tenths or 20 mg of pyridoxine HCl and 40 or 400 mg of Mg as MgO were also added per 100 g of diet. Water was provided by a 0.25% ethyl-

* Supported in part by grants-in-aid from Nat. Inst. of Arthritis and Metab. and Fund for Research and Training, Dept. of Nutrition, Harvard School of Public Health.

TABLE I. Influence of Dietary Vitamin B_6 and Magnesium on Renal Lesions Induced in Rats by Ethylene Glycol.*

B_6, mg %	.2	.2	20	20
Mg, mg %	40	400	40	400
Wt gain, g†	70 ±9	87 ±9	95 ±9	127 ±10
Ca oxalate lesions				
Parenchymal deposition	6	0	3	0
Extent of deposits	Tr-4+	0	0-1+	0
Papillary apical incrustations	4	0	2	0
Ureteral concretions	1	0	0	0
Hydroureter	2	0	0	0
Gross hematuria	1	0	0	0

* Six weanling rats/group were maintained for 4 wk on drinking water containing 0.25% ethylene glycol.

† Values include stand. error of mean.

ene glycol solution. After 4 weeks on this regimen the rats were sacrificed for morphologic study. The kidneys and lower urinary tracts were examined under magnification and dissected as previously described(4). The amount of parenchymal calcium oxalate deposition was graded visually on a 1-4+ basis. Small papillary apical incrustations were also seen, similar to these found as solitary lesions in Vit. B_6 deficient rats(4), and it is presumed that dislodgement of these incrustations may give rise to the ureteral concretions and hydroureter seen in the present experiment. Formalin-fixed, paraffin-embedded sections were cut at 5 μ and stained with alcian blue-hematoxylin-eosin for histologic study.

Anatomic study—results. Excessive dietary Vit. B_6 imparts partial protection against the renal lesions of chronic ethylene glycol administration (Table I). Excessive dietary magnesium is more effective in this regard, and under the conditions of this experiment resulted in complete protection against renal lesions. Chronic ethylene glycol intoxication results in decreased weight gain. Excessive supplements of either dietary factor tend to increase growth while the presence of both results in a marked growth response, the latter animals having essentially normal body weights.

There was very close agreement between the gross quantitative grading of renal calcium oxalate and that seen histologically. Thus, no oxalate was found in either of the groups receiving high levels of dietary magnesium or in 3 animals noted as grossly negative from the group maintained on high Vit. B_6 and "normal" magnesium intake. When present, oxalate crystals were intratubular, being most prominent within the cortex but also occurring within the medulla, including the formation of exteriorized papillary apical deposits as noted grossly. Occasional instances of epithelialized calcium oxalate deposits were seen in the calyceal fornix or ureteral epithelium. Epithelialization of intratubular calcium oxalate was minimal. The epithelium of tubules containing crystals was frequently compressed or non-apparent. Frequently these and adjacent tubules had lost their normal eosinophilia or were even basophilic in staining reaction. Moderate dilatation of tubules was seen especially in the medulla. Acute inflammation and mitoses in epithelial cells were virtually absent, though in unpublished experiments with higher ethylene glycol intakes, these features have been prominent. Tubular epithelial changes similar to the above were also seen in both groups of animals receiving high Vit. B_6 supplements and in the absence of oxalate crystals. Associated with these changes were interstitial accumulations of lymphocytes and plasma cells. The significance of these changes is not apparent. Oxalate crystals were not seen in any of the other viscera. The bones were not examined histologically.

Metabolic studies—methods. In view of these results, 3 separate experiments were undertaken to determine whether the action of high levels of Vit. B_6 and magnesium in protecting against the renal deposition of calcium oxalate were due to an effect on oxidation of ethylene glycol. In each experiment groups of 5 or 6 rats were fed the basal diet plus 1.5 g $CaCO_3$/100 g of diet. In the first of these 3 experiments rats approximately 60 g in weight were fed this diet plus 40 mg % of magnesium and either 0 or 0.4 mg % pyridoxine. In the second experiment weanling rats received 40 mg % of magnesium and no

TABLE II. The Effect of Vitamin B_6 and Magnesium on the Metabolism of Ethylene Glycol.

Mg, mg %	B_6, mg %	No. of rats	Avg wt	% inj. ethylene glycol recovered as CO_2 1 hr	4 hr	
40	—	5	82	.9 ± .1†	10.2 ± .6	$p < .001$
40	0.4	5	103	2.5 ± .3	19.0 ± 1.3	
40	—	6	57		8.7 ± .8	$p < .01$
40	+*	6	59		15.5 ± 2.0	
40	0.4	5	74		22.4 ± 1.3	
400	0.4	5	85		23.8 ± 1.5	

* Vitamin B_6-deficient rats inj. with 25 mg of pyridoxine HCl 24 hr before ethylene glycol administration.

† Values include stand. error of the mean.

Vit. B_6 in their diets until 24 hours before the end of the experimental period at which time half of the animals were injected intraperitoneally with 25 mg of pyridoxine. In the third experiment, weanling rats were fed diets containing 0.4 mg % pyridoxine and either 40 or 400 mg % of magnesium. After 2 weeks on these 3 regimens each rat was injected intraperitoneally with 54 μg of ethylene-1, 2-C^{14} glycol (containing 2 μc of radioactivity) per 100 g of body weight and per cent of ethylene glycol oxidized over a 4 hour period was determined by measuring expired radioactive CO_2. Since ethylene glycol is a polyhydroxy alcohol, the possible effect of Vit. B_6 on the oxidation of glycerol was also studied. Twelve weanling rats were fed the Vit. B_6 deficient basal diet plus 1.5 g $CaCO_2$ and 40 mg of Mg/100 g and after 13 days half the rats were injected intraperitoneally with 25 mg of pyridoxine. Twenty-four hours later all the rats were injected intraperitoneally with 2 μc of glycerol-1, 3-C^{14} per 100 g of body weight.

Metabolic studies—results. The results of the first 3 experiments are shown in Table II. These data clearly show that under the conditions of these experiments Vit. B_6 but not magnesium has a marked effect in accelerating *in vivo* oxidation of ethylene glycol. In the last study, the per cent of injected glycerol recovered as radioactive CO_2 during the next 3 hours after injection did not differ statistically between the 2 groups, being 62 ± 0.9% for those rats receiving pyridoxine and 59 ± 1.5% for those deficient in Vit. B_6.

Discussion. There have been few studies of the metabolism of ethylene glycol. Pohl (7) and Mayer(8) administered ethylene glycol to rabbits and produced marked oxaluria. Mayer(8) also observed glycollic acid in rabbit urine following ethylene glycol administration. Dakin(9) reported that ethylene glycol, glyoxylic acid and glycollic acid all give rise to oxaluria in animals. In more recent times Ratner *et al.*(10) have described enzyme systems which catalyze the oxidation of glycine to glyoxylic acid and glyoxylic acid to oxalic acid. Weinhouse has reported(11) conversion of glycollic and glyoxylic acids to oxalic acid and glycine. It seems probable that ethylene glycol is oxidized to oxalate by way of glycollic and glyoxylic acids. However, it has been shown(12) that the main metabolic product of ethylene glycol in animals is CO_2. Oxidation to oxalic acid is a relatively minor reaction. In *in vitro* studies glyoxylate yielded oxalate only when present in relatively high concentrations(11). It has been suggested(2) that inhibition of transaminase activity in Vit. B_6 deficiency could result in an accumulation of glyoxylic acid with increased oxalate formation. The results obtained in this work are consistent with this reasoning.

Oxidation of ethylene glycol to CO_2 was inhibited by Vit. B_6 deficiency and feeding of large amounts of Vit. B_6 provided partial protection against renal oxalate deposition induced by ethylene glycol ingestion. Nevertheless, definitive studies of the way in which B_6 deficiency affects the conversion of ethylene glycol to oxalate are needed.

The role of magnesium in protecting against calcium oxalate deposition appears to be quite different from that of Vit. B_6. There

was no significant difference in the rate of oxidation of ethylyene glycol to CO_2 in rats fed the 2 levels of magnesium used in this work. Hammarsten(13) has reported that the hyperoxaluria which occurs following administration of ethylene glycol to rats is not affected by dietary magnesium levels. It is possible that in experiments reported here as well as in our previous studies(5) magnesium may protect against the renal deposition of oxalate by altering the solvent characteristics of urine.

In this and many other laboratories rats are routinely fed diets containing 40 mg % of magnesium. This level of magnesium and 0.2 mg % pyridoxine HCl in otherwise complete diets permits maximal growth. Nevertheless, in these experiments the provision of additional Vit. B_6 and/or magnesium provided a greater degree of resistance to chronic ethylene glycol toxicity. This suggests a basis for a possible course of therapy in cases of human ethylene glycol poisoning, in which survival is prolonged beyond the initial phase of central nervous system depression and in which the renal lesions become operative. Animal experiments are in progress to study the value of magnesium and Vit. B_6 in the treatment of such renal oxalate deposits once established.

Summary. Oral administration of ethylene glycol to rats and the resultant renal deposition of calcium oxalate were studied in terms of dietary levels of Vit. B_6 and magnesium. Under the conditions of these experiments partial protection against oxalate deposition was achieved with excessive dietary Vit. B_6 while complete protection resulted when large amounts of MgO were fed. The *in vivo* oxidation of ethylene glycol to CO_2 was found to be related to amount of Vit. B_6 administered. Dietary magnesium levels, however, did not affect oxidation of ethylene glycol and it is suggested that dietary magnesium protects against renal deposition of calcium oxalate by altering the solvent characteristics of urine.

The authors wish to thank T. P. Faherty for the histologic preparations.

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Received September 5, 1961. P.S.E.B.M., 1962, v109.

Effect of Cysteine and Pyridoxine on Taurine Excretion of Male Rats.* (27116)

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The decrease in urinary excretion of taurine in Vit. B_6 deficiency(1) has been attributed, in part, to the decrease in level of the pyridoxal phosphate enzyme, cysteinesulfinic

* This investigation was supported in part by funds appropriated under the Research and Marketing Act of 1946 as part of Western Regional Exp. Station Project W-57, U.S.D.A.