tration of 320 mg of quinine carbonate can serve to detect the presence or absence of free gastric hydrochloric acid without subjecting the individual to intubation. Under proper conditions with this dose of quinine carbonate the differentiation between a low and at least a normal acid secretor seems feasible merely by assaying the 2-hour urine for quinine by the simple semi-quantitative technic previously described(4,7).

Experimentation with larger doses of quinine carbonate, if tolerated, or with other comparable insoluble salts with exogenous indicator ions, may make it possible to differentiate approximately between normal and excessive output of hydrochloric acid per unit of time. It is obvious that the time of urine collection can be changed to include the night excretion if desirable, since in achlorhydric and gastrectomized patients practically no difference was noted in the amount of quinine excreted in the 2-, 5- and 8-hour urine collections.

Summary. 1. The rationale of the use of insoluble salts with special exogenous indicator ions to determine gastric acidity without intubation has been described. 2. Theoretical consideration and *in vitro* and *in vivo* experiments have demonstrated that by determining the amount of quinine in the first 2-hour urine excretion after the oral administration of 320 mg of quinine carbonate, the presence of free hydrochloric acid from a low to a normal or higher range or its absence can be estimated without subjecting the individual to intubation. 3. Further experimentation with larger doses of quinine carbonate, if tolerated, or with comparable insoluble salts with various exogenous indicator ions, may make it possible to differentiate approximately between normal and high gastric hydrochloric acid secretion without intubation.

The authors wish to express their appreciation for the technical aid given by Miss Elizabeth Plumb and Mrs. Elizabeth Arrowsmith.

1. Segal, H. L., Miller, L. L., and Morton, J. J., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 218.

2. Segal, H. L., Miller, L. L., Morton, J. J., and Young, H. Y., *Gastroenterology,* 1950, v16, 380.

3. Segal, H. L., *Med. Clinics of N. A.,* 1951, v35, 593.

4. Segal, H. L., Miller, L. L., and Morton, J. J., *J. Nat. Cancer Inst.,* 1953, v13, 1079.

5. Malach, M., and Banks, B. M., *New Eng. J. Med.,* 1952, v247, 880.

6. Flood, C. A., Jones, B., Rotton, W. M., and Schwartz, H., *Gastroenterology,* 1953, (April issue).

7. Segal, H. L., *J. N. Y. Acad. Sci.,* in press.

8. Maurer, W., and Zimmer, A., *Münchener Med. Wochenschrift,* 1952, v94, 1072.

9. Kelsey, F. E., and Geiling, E. M. R., *J. Pharm. and Exp. Therap.,* 1942, v75, 183.

Received May 20, 1953. P.S.E.B.M., 1953, v83.

Molybdenum Toxicity in *Lactobacillus leichmannii.* (20392)

LOUISE J. DANIEL AND LOUISE F. GRAY. (Introduced by L. A. Maynard)

From the Department of Biochemistry and Nutrition, Cornell University, and the U. S. Plant, Soil and Nutrition Laboratory, Bureau of Plant Industry, Soils and Agricultural Engineering, A.R.A., Ithaca, N. Y.

Molybdenum has been shown to be toxic for cattle(1-3), rats(4-6), and rabbits(7). Neilands, Strong and Elvehjem(4) found that whole liver substance overcame molybdenum toxicity in rats on an alcohol-extracted casein diet. Since this diet would be somewhat deficient in vit. B_{12}, it was thought that this vitamin might be involved in the correction produced by whole liver. A microorganism requiring vit. B_{12}, *Lactobacillus leichmannii* 4797, was chosen to expedite a study to determine if molybdenum produced a growth inhibition reversible by vit. B_{12}.

Experimental. Lactobacillus leichmannii 4797 was obtained from the American Type Culture Collection. The medium used and the procedure followed was that described by Peeler, Yacowitz and Norris(8), with the

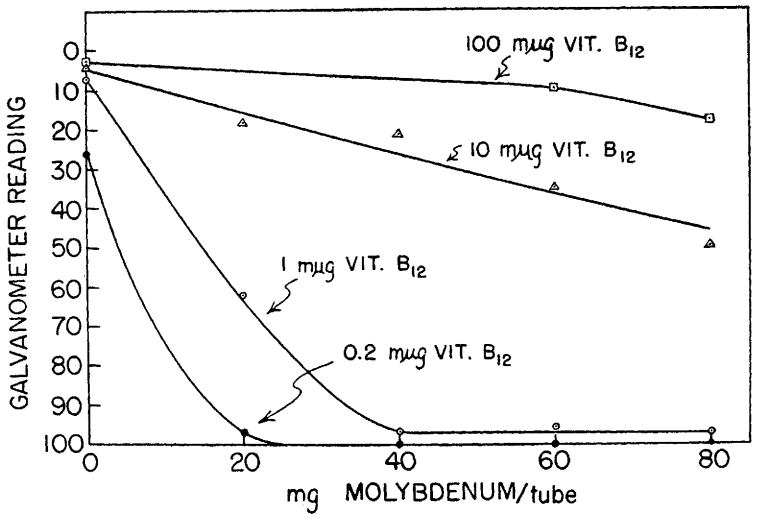

FIG. 1. Inhibition of growth of *Lactobacillus leichmannii* by molybdenum, and its reversal by vit. B_{12}. An 18-hr turbidimetric assay was used; a galvanometer reading of 100 represents no growth.

modifications noted. Each assay tube contained a final volume of 10 ml. When sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$) was added to the medium, a yellow color was produced on steaming. The intensity of this color varied with the amount of molybdenum present. By adding each component of the basal medium separately to the molybdate solution, cysteine was found to be responsible for the color produced on steaming at pH 5.5, the pH of the original medium. As the pH was increased, less color was produced. However, pH 6.0 was the highest at which good growth of the organism could be obtained. Henceforth, the medium was modified by omitting cysteine, adjusting all supplements and the medium to pH 6.0, and by adding the molybdate solution after Seitz filtration to the sterile tubes. Either a 16-20-hour turbidimetric or a 40-48-hour titrimetric assay was employed.

Results. The first experiment was designed to establish the levels of molybdenum required to inhibit the growth of *Lactobacillus leichmannii*. Four levels of molybdenum were used, *i.e.*, 20, 40, 60 and 80 mg per tube in combination with several levels of vit. B_{12}. The results of this study are given in Fig. 1. Large amounts of molybdenum were required to inhibit the growth of this organism under the conditions stated, and relatively small quantities of vit. B_{12} prevented the inhibition. The relationship between molybdenum and vit. B_{12} appears to be competitive in nature, since as more molybdenum was added more vit. B_{12} was required to reverse its effect. Cobalt included at levels equivalent to the cobalt added as vit. B_{12} had no effect on molybdenum inhibition. Thus, the action of vit. B_{12} was apparently not due to cobalt *per se*.

The effect of cysteine on molybdenum toxicity was next investigated because of the earlier observation that cysteine and molybdate produced a yellow colored solution indicating possible complex formation.[9] Levels of molybdenum, vit. B_{12} and cysteine were used in various combinations. Data representative of some of the results obtained are presented in Fig. 2. The addition of cysteine effectively reduced the inhibition caused by molybdenum. Methionine and cystine were then tried in combination with vit. B_{12} to learn if these compounds would have a similar effect; neither amino acid counteracted the inhibition caused by molybdenum.

Since cysteine is a reducing agent, it was of interest to determine if other reducing agents would decrease this inhibition by molybdenum. Ascorbic acid, sodium thioglycollate, glutathione and ferrous sulfate were tried. Only glutathione responded in a manner similar to

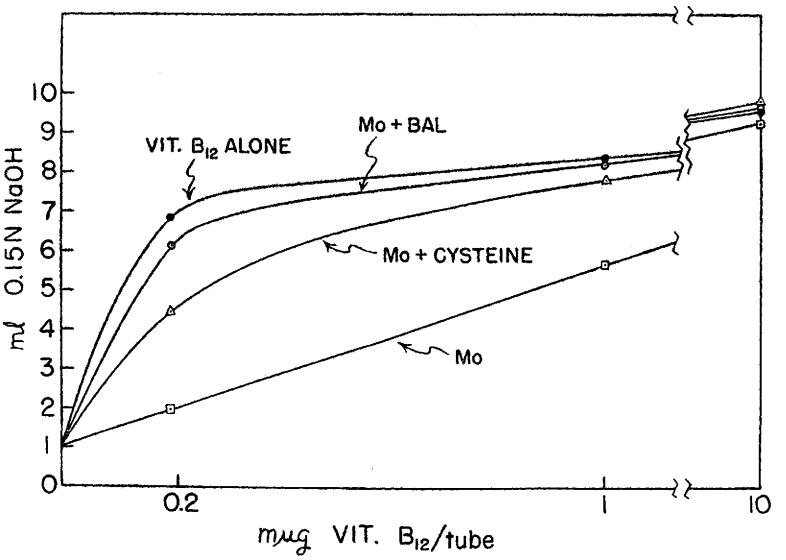

FIG. 2. Effect of cysteine and BAL on inhibition of *Lactobacillus leichmannii* by molybdenum at various concentrations of vit. B_{12}. A 41-hr titrimetric assay was employed. Molybdenum was added at 20 mg/tube, cysteine at 10 mg/tube, and BAL at 10 mg/tube. Molybdenum plus cysteine values have been corrected for growth stimulation caused by cysteine itself.

cysteine. The reducing property *per se* was apparently not the requirement for preventing molybdenum toxicity. The fact that both effective substances are sulfhydryl compounds suggested the study of 2,3-dimercaptopropanol, British antilewisite (BAL). A titrimetric assay was conducted in this instance because BAL produced a deep red color when added to the medium. The data for the BAL study are given in Fig. 2. BAL at 10 mg per tube was as effective as cysteine at 20 mg per tube in reversing the molybdenum inhibition.

Discussion. The mechanism by which molybdenum inhibits the growth of *Lactobacillus leichmannii* is unknown. Two possible explanations are: 1) The integrity of the sulfhydryl groups of certain enzymes may be destroyed. 2) Molybdenum may catalyze the hydrolysis of organic phosphate bonds of some essential metabolites.

The action of molybdenum on the sulfhydryl enzymes could be explained by the formation of chelates or mercaptides, or by the oxidation of the sulfhydryl group to the disulfide bond. The latter explanation is probably not valid, since reducing agents *per se* did not counteract the inhibition.

On the other hand, molybdenum has been shown to increase the hydrolysis of organic phosphate bonds(9,10), especially the high-energy bonds of adenosine triphosphate, acetyl phosphate, and creatine phosphate(9). Thus, molybdenum may decrease the effective concentration of compounds containing high energy phosphate bonds and hence inhibit growth.

The protective effect of cysteine, glutathione and BAL was probably due to the formation of mercaptides with molybdenum, thus reducing the amount of this metal available for its toxic action. Thiol compounds have long been known to reverse the inhibitory effect of mercaptide-forming agents on sulfhydryl enzymes. Barron and Kalnitsky(11) have found that in metal poisoning of succinoxidase, BAL was decidedly better than glutathione in reducing the inhibition. Since dithiols form more stable mercaptides than do monothiols, this would explain the greater effectiveness of BAL over cysteine or glutathione in reversing molybdenum toxicity.

The function of vit. B_{12} in reducing the inhibition of molybdenum is less readily explained. Several possible explanations are as follows: 1) Vit. B_{12} may be a part of a prosthetic group of an enzyme that is joined to the

protein through a sulfhydryl group. 2) This vitamin may be maintaining the sulfhydryl enzymes in a reduced state as proposed by Dubnoff(12,13). 3) Vit. B_{12} may be concerned with the maintenance or production of high-energy phosphate bonds.

Summary. 1. Molybdenum at relatively high concentrations inhibited the growth of *Lactobacillus leichmannii.* 2. This inhibition was prevented or reduced by additions of vit. B_{12}, cysteine, glutathione or BAL. 3. Reducing agents *per se* had no effect on the inhibition. 4. The possible interactions of molybdenum, vit. B_{12}, and sulfhydryl compounds on the growth of the microorganism have been discussed.

1. Ferguson, W. S., Lewis, A. H., and Watson, S. J., *J. Agr. Sci.,* 1943, v33, 44.

2. Britten, J. W., and Goss, H., *J. Am. Vet. Med. Assn.,* 1946, v108, 176.

3. Comar, C. L., Davis, G. K., and Singer, L., *J. Biol. Chem.,* 1948, v174, 905.

4. Neilands, J. B., Strong, F. M., and Elvehjem, C. A., *J. Biol. Chem.,* 1948, v172, 431.

5. Gray, L. F., and Ellis, G. H., *J. Nutrition,* 1950, v40, 441.

6. Comar, C. L., Singer, L., and Davis, G. K., *J. Biol. Chem.,* 1949, v180, 913.

7. Arrington, L. R., and Davis, G. K., *J. Animal Sci.,* 1952, v11, 756.

8. Peeler, H. T., Yacowitz, H., and Norris, L. C., PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 515.

9. Weil-Malherbe, H., and Green, R. H., *Biochem. J.,* 1951, v49, 286.

10. Lutwak, L., and Sacks, J., *J. Biol. Chem.,* 1953, v200, 565.

11. Barron, E. S. G., and Kalnitsky, G., *Biochem. J.,* 1947, v41, 346.

12. Dubnoff, J. W., *Arch. Biochem.,* 1950, v27, 466.

13. ———, *Arch. Biochem. Biophys.,* 1952, v37, 37.

Received May 21, 1953. P.S.E.B.M., 1953, v83.

Leptospiral Macroscopic Agglutinating Antigens (20393)

WARREN G. HOAG, WILLIAM S. GOCHENOUR, JR., AND ROBERT H. YAGER.
From the Veterinary Division, Army Medical Service Graduate School, Washington, D. C.

The laboriousness of microscopic agglutination technics for the demonstration of leptospiral antibodies has severely limited their use in the laboratory diagnosis of leptospirosis. The search for simpler and more rapid methods, and for more stable antigens, has led to the development of a number of leptospiral macroscopic agglutinating antigens(1-4), but experience with antigens of these types has not been wholly satisfactory. Their sensitivity, stability, and specificity were not of the same order as that of the more widely used microscopic agglutinating antigens(5,6). Further, visible reactions were often granular in character and difficult to detect. Dye precipitation in stained antigens frequently confused interpretation. The potential usefulness of leptospiral macroscopic agglutinating antigens, however, warranted further studies directed toward overcoming these deficiencies. The influence of electrolytes upon stability of bacterial suspensions, and upon antigen-antibody reactions, has repeatedly been demonstrated(7-9). In particular, the sensitivity of bacterial agglutinating antigens has been shown to be markedly affected by changes in pH and ionic strength. Information about the various optimal conditions for leptospiral agglutinating reactions has not been reported in the literature available to the authors, so that initial studies were directed toward their determination. The adsorption of leptospiral agglutinating antigens onto inert particles had likewise not previously been reported, and it appeared reasonable to determine whether or not this procedure might result in the formation of more readily discernible aggregates in the agglutination reaction.

Macroscopic agglutinating antigens were prepared from nine leptospiral serotypes. The sensitivity, specificity, and stability of these antigens were investigated with immune sera prepared in rabbits and with sera from cases of human, equine, bovine, porcine, and canine leptospirosis.

Methods. 1) *Propagation of Leptospires.*