developed essentially complete cross-resistance to each other and to chlortetracycline. No cross-resistance or increases in sensitivity to penicillin, streptomycin, bacitracin, polymyxin, neomycin or erythromycin developed as a result of the increases in resistance to the tetracyclines. Three strains of coliform bacilli each showed significant cross-resistance to chloramphenicol following subcultures in either tetracycline or oxytetracycline, but similar cross-resistance was not observed in 5 gram-positive coccal strains.

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Prevention of Urinary Calculi Formation in Mink by Alteration of Urinary pH.* (20779)

W. L. LEOSCHKE AND C. A. ELVEHJEM.

From the Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison.

Chemical analyses of mink urinary calculi (1) have shown that the main component is magnesium ammonium phosphate hexahydrate ($MgNH_4PO_4 \cdot 6H_2O$). Small amounts of calcium phosphate and magnesium phosphate may also be present. Magnesium ammonium phosphate calculi are a type of calculi known to form in an alkaline urine. *In vitro* studies(2) have shown that magnesium ammonium phosphate is quite soluble in water when the pH is reduced to 6.0 or less. However, there is a sharp drop in the solubility of this compound when the pH range is 6.0 to 7.5. The pH of urine from mink on ranch diets varies from 5.5 to 7.5(3,4). It appears that the pH of the urine is an important factor in the formation of urinary calculi in mink.

The alteration of urinary pH by dietary means may be a logical method to attack the urinary calculi disease problem in mink. A practical and inexpensive means of acidifying the urine is the addition of the chemical ammonium chloride (NH_4Cl) to the diet. Studies on the use of ammonium chloride for the prevention of urinary calculi in mink are presented in this report.

Experimental. Mink were placed in individual metabolism cages and were given the following ranch diet: commercial mink cereal,† 25%; horsemeat (with bone), 60%; and horse liver, 15%. During the ammonium chloride feeding period (Experimental Period

TABLE I. Alteration of Mink Urinary pH by Ammonium Chloride.

Exp. period	Diet	Urinary pH* Avg	Range
I	Ranch diet	6.4	6.0–6.8
II	Ranch diet + 1 g NH_4Cl/mink/day	5.7	5.5–5.9

* Avg of 5 different 24-hr urine collections from 17 mink.

† Federal Xtra Mink Mix.

* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

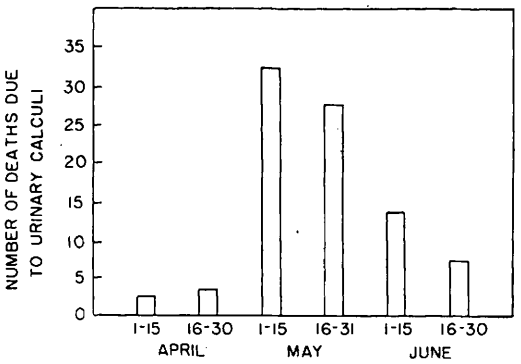

FIG. 1. Typical losses of pregnant mink due to urinary calculi on a mink ranch during the spring of 1952.

II) 1 g of ammonium chloride (U.S.P. grade) was added to each mink's daily ration with thorough mixing. The mink urine samples were collected under toluene. Immediately after each 24-hour urine collection period the pH of the urine was measured with a glass electrode pH meter.

The *results* are shown in Table I. The addition of ammonium chloride at a level of 1 g per mink per day lowers the average pH of the urine from 6.4 to 5.7. With ammonium chloride in the diet the range of urinary pH among individual animals is from 5.5 to 5.9.

The incidence of urinary calculi in mink is largely confined to two distinct periods of the year. In the spring calculi are found primarily in female mink during pregnancy or soon after whelping of the kits. In the summer months calculi occur mainly in young male kits. Fig. 1 shows typical losses of pregnant mink due to urinary calculi on a mink ranch during the spring of 1952. Within a period of 3 months 84 out of the 2,000 pregnant mink on the ranch were lost with urinary calculi. From Fig. 1 it is seen that the critical period of urinary calculi formation in female mink is during the months of April, May and June. The acidification of mink urine during this critical period by dietary ammonium chloride might be a means of preventing urinary calculi formation in the mink.

An experiment designed to study the effect of dietary ammonium chloride on the formation of urinary calculi in mink was set up in the spring of 1952. Six hundred Royal Pastel female mink (bred in March) were divided into 2 experimental groups. The 400 mink in Group I received the regular ranch diet. The 200 mink in Group II received the regular ranch diet plus 1 g of ammonium chloride per mink per day (200 g of ammonium chloride added daily to their portion of the ranch ration). The ammonium chloride was added to the diet of Group II from April 22 to May 27. From the data shown in Table II it is evident that the addition of ammonium chloride to the mink's diet at a level of 1 g per mink per day will prevent the formation of urinary calculi. By July 1, 16 out of the 400 mink in Group I on the ranch diet had died with urinary calculi. Of the 200 mink in Group II on the ranch diet plus ammonium chloride, only one mink had died with urinary calculi. This mink died only 3 days after the beginning of the experiment. This would indicate that the calculi had already been formed in the bladder of the mink. There were no indications of toxicity of ammonium chloride to the mink.

In the spring of 1953 the use of ammonium chloride for the prevention of urinary calculi in mink was extended to include over 50,000 mink on 100 mink ranches in the United States and Canada. In order to determine the minimum level of ammonium chloride in the diet required to prevent the formation of urinary calculi, a ½ g level as well as the 1 g level of the chemical was tested. Preliminary pH studies (6 mink) had shown that a level of ½ g of ammonium chloride per mink per day would lower the urinary pH from 6.3 to 5.8. Ten thousand mink received ammonium chloride at a level of 1 g per mink per day and 40,000 mink received the chemical at a level of ½ g per mink per day. Because it

TABLE II. Effect of Dietary Ammonium Chloride on Formation of Urinary Calculi in Mink.

Exp. group	No. of mink	Diet	No. of deaths due to urinary calculi
I	400	Ranch diet	16
II	200	Ranch diet + 1 g NH_4Cl/mink/day	1*

* This mink died 3 days after start of exp.

was impractical for the mink ranchers to divide their mink into separate groups for control measures, whole ranches were placed on the ammonium chloride feeding program. The experimental period was from April 10 to June 1, 1953. Mink ranches feeding ammonium chloride at the 1 g level had small losses of mink with urinary calculi (less than .2%). Over half of those ranches which fed the ½ g level of the chemical had some significant losses of mink with urinary calculi (.5-3.0%).

Discussion. The addition of ammonium chloride to the diet of the mink at a level of 1 g per mink per day is a practical and effective means of preventing the formation of urinary calculi in mink. The feeding of ammonium chloride at a level of ½ g per mink per day has been found to be ineffective in preventing urinary calculi formation on many mink ranches. It should be emphasized that the ammonium chloride regimen for the control of urinary calculi disease in mink is only a prophylactic measure. The urinary pH produced by the recommended level of ammonium chloride feeding is not low enough for rapid dissolution of the mink calculi. Although small urinary calculi may be dissolved, the animal may die before the larger calculi have been dissolved by the slightly acid urine. Partially dissolved calculi have been observed in mink from mink ranches starting the ammonium chloride feeding program late in May, *i.e.*, towards the end of the critical period of urinary calculi formation in mink.

The effectiveness of the ammonium chloride feeding program in preventing calculi formation in mink emphasizes the importance of urinary pH in urinary calculi formation. Urinary pH may be altered by dietary as well as by chemical means. It may be possible to control urinary calculi disease in mink by dietary management. The low incidence of urinary calculi on ranches feeding high levels of fish would indicate that control of urinary calculi in mink by dietary management may be feasible.

Summary. The formation of magnesium ammonium phosphate ($MgNH_4PO_4 \cdot 6H_2O$) urinary calculi in mink can be prevented by the addition to the mink's diet of 1 g of ammonium chloride (NH_4Cl) per mink per day. A level of ½ g of NH_4Cl per mink per day has been found to be ineffective in preventing urinary calculi formation in mink.

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Localization of Cu^{64} in Serum Fractions Following Oral Administration: An Alteration in Wilson's Disease. (20780)

A. G. BEARN AND HENRY G. KUNKEL.

From the Hospital of The Rockefeller Institute for Medical Research, New York.

Previous work(1,2) has shown that the circulating serum iron is bound specifically to a β_1 globulin and that under normal conditions no binding occurs with any of the other plasma components. Experiments *in vitro,* however, have shown that this metal combining globulin will also form complexes with copper and zinc(2,3), and it was initially suggested that copper may be transported in the circulation attached to the β_1 metal combining globulin. More recently Holmberg and Laurell(4) have shown that copper in human serum is largely bound to a protein with a mobility of an α_2 globulin. This protein has been named ceruloplasmin and has been shown to exhibit oxidase activity towards a variety of substrates, the