

gether, they are not conjugated *in vivo* to form the Aminonucleoside in any significant amount.

The striking specificity of structural configuration that is required for production of this drug-induced nephrosis suggests an enzymatic mechanism of action of Aminonucleoside. Evidence to support this contention is being sought.

Summary. Of 5 structural analogues of Puromycin that were investigated, only one, 6-dimethyl-amino-purine-3-amino-d-riboside, produced a nephrotic syndrome in rats. The specific structural configuration necessary to induce this syndrome is discussed.

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Influence of Dietary Chlortetracycline on Incidence of Urinary Calculi in Sheep.* (23901)

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The development of urinary calculi in sheep and other animals has been associated with many of the commonly used feedstuffs(1,2), with vitamin deficiencies(3,4), with unbalances of minerals, particularly of calcium and magnesium(5,6) and of potassium and phosphate (7,8), and with insufficient water intake and other factors. The exact cause of calculi formation in sheep, however, is unknown and only a few materials are known to affect the course of calculi development. Elam, Ham and Schneider(8) reported the reduction of urinary calculi in high potassium acid phosphate diets by the use of 10% NaCl in the diet. Loeschke and Elvehjem(9) noted that the addition of 1 g of NH_4Cl daily to the diets of mink reduced the incidence of calculi.

In an experiment designed to study the effect of chlortetracycline on the utilization by lambs of nonprotein nitrogen (urea) and in

diets in different physical states (meal or pelleted), a varying incidence of urinary calculi was noted. This variation in the incidence of calculi with the dietary treatment and its possible implications are the subject of this report.

Procedure. Two hundred seventy-six fine wool wether feeder lambs (averaging 55 lb) were divided by weight into 7 lots of 39-40 lambs per lot. They were self-fed a total mixed diet. The general experimental outline is indicated with results in Table I. Basic constituents of experimental diets were ground milo, molasses, ground alfalfa, and cottonseed hulls. The protein or nitrogen supplements, *i.e.*, cottonseed meal (CSM) or urea, were added at the expense of the milo and cottonseed hulls (footnotes, Table I). The cottonseed meal diets with and without chlortetracycline in the meal or pelleted states were fed to 4 groups of lambs. Pelleted urea diets with and without chlortetracycline were fed to 2 groups of lambs and the low protein diet without antibiotic was fed to the seventh group of lambs. The calculated total digesti-

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TABLE I. Death Losses from Urinary Calculi and Incidence of Calculi in Surviving Lambs.

Group No.	Nitrogen supplement	Physical state	Chlortetracycline, mg/lb feed	No. lambs	Deaths, calculi	Incidence calculi, surviving lambs, %	Avg calculi, surviving lambs, mg/kidney
I	CSM*	Meal	0	40	7	11	3
II			10	40	1	11	2
III	"	Pelleted	0	39	9	44	246
IV			10	39	4	24	5
V	Urea†	"	0	39	2	26	5
VI			10	39	0	6	1
VII	None‡	"	0	40	3	4	1

* Diet composition: 10% cottonseed meal, 45% ground milo, 10% molasses, 8% ground alfalfa, 27% ground cottonseed hulls.

† Diet composition: 1.1% urea, 52% ground milo, 10% molasses, 8% alfalfa, 29% cottonseed hulls.

‡ Diet composition: 52% ground milo, 10% molasses, 8% alfalfa, 30% cottonseed hulls.

ble nutrient contents were equivalent in all diets. Crude protein (N x 6.25) was 11% in the supplemented diets and 8% in the low protein diet. Average Ca and P contents were 0.40% and 0.27%, respectively, in the cottonseed meal diets and 0.43% and 0.22%, respectively, in the urea and low protein diets. When used, chlortetracycline was added to the feed at a level of 10 mg per lb feed as a crude material (Aurofac) containing the equivalent of 1.8 g chlortetracycline • HCl per lb of crude material. Water and salt were available *ad libitum*. All lambs which died during the experiment were autopsied and the entire urinary tract of each was examined for presence of urinary calculi. Upon slaughter of the remaining lambs at termination of a 98-day experiment, one kidney was removed from each of 146 randomly selected carcasses and the kidney was inspected for presence of urinary calculi.

Results. There were 26 deaths due to urinary calculi during the 98-day trial. Calculi were found variably in kidneys, ureters, and bladder, but complete or partial urethral blockage occurred in every instance. When the urethra was stopped completely, apparent infection occurred at site of blockage and the urethra ruptured allowing urine to flow into the body cavity and resulted in death due to uremia within a few days. In several cases the urethra was not completely blocked but urination was impaired and the animal showed symptoms of extreme discomfort, anorexia, and loss of weight. Some animals living 4-5 weeks died as a result of secondary diseases

such as pneumonia or diarrhea, but urinary calculi was definitely diagnosed in each case.

Variation in incidence of deaths caused by calculi with various treatments is shown in Table I. Death losses due to urinary calculi were reduced significantly ($p < 0.01$) among lambs receiving chlortetracycline in the diet.

At end of experiment, no visible symptoms of urinary calculi were shown by remaining animals but upon examination of kidneys taken from carcasses of slaughtered surviving lambs, calculi were found in 18% of the kidneys. Accretions in most lambs ranged from about 1 mg to 90 mg, but 4 lambs which received pelleted cottonseed meal diet without chlortetracycline showed extensive accumulation of calculi or hydronephrosis with from 370 mg to 4.19 g of calculi in the kidney. The incidence of calculi in surviving lambs and the extent of calculi development (calculated as average calculi/kidney in all examined surviving lambs) tend to support the conclusion which may be drawn from death losses that chlortetracycline reduces development of urinary calculi.

Death losses were significantly fewer with the urea supplemented and low protein diets than with cottonseed meal supplemented diets ($p < .01$). There were no death losses with the urea plus chlortetracycline supplemented diet. Also of importance is that deaths from urinary calculi within the urea containing diets (Groups V and VI) occurred after 80 days while those on cottonseed meal diets began occurring after 30 days on feed.

A greater quantity of renal calculi was found in lambs receiving pelleted cottonseed meal diet than in lambs receiving the same diet in the meal state. Pelleted feeds thus appeared to be more inductive to renal calculi. The 4 death losses in the group receiving pelleted cottonseed meal diet containing chlortetracycline may be indicative of some decrease in effectiveness of chlortetracycline in a pelleted feed, but an analysis of the chlortetracycline in this diet indicated an activity of only 3.8 mg/lb of feed. Since a 10 mg level was added to the feed, it is conceivable that chlortetracycline may be deactivated in the pelleting process where heat and steam are used. The marked reduction of calculi formation in the kidney suggests that chlortetracycline at even this lower level was effective in reduction of renal calculi formation.

The main mineral components of the calculi observed were Ca, 8%; Mg, 5.3%; P, 15%; and carbonate, 18%. The renal calculi were small white particles which in the more severe cases were sedimented together to form easily crushable stones which ranged to a size of over one-half inch in diameter. The occurrence of 1 to more than 4 g of calculi in the pelvis of the kidney of several lambs and an enlargement of the ureter as the stones moved downward suggested that the formation of calculi occurred principally in the pelvis of the kidney.

Discussion. The reduction of incidence of urinary calculi by dietary inclusion of chlortetracycline may point to a new physiological effect of antibiotics in the ruminant.

As to the mechanism by which chlortetracycline may have affected the course of calculi development, a number of possibilities are evident. A hypothesis may be applicable that the antibiotic tends to reduce minor kidney infections thus reducing potential nuclei for calculi formation. The dietary chlortetracycline may maintain a more healthy state in the kidney and thus more effectively regulate the rate of renal reabsorption and excretion of mineral elements. The antibiotic may also reduce the numbers of bacteria producing urease in the kidney and thereby prevent a calculogenic increase in pH of the urine.

Indirect effects, which may be mediated

through the action of rumen or other gastrointestinal tract bacteria, on the physiology and metabolism of sheep are also possible methods of inhibition of calculi formation. Addition of chlortetracycline to the diets of lambs has been shown to increase the efficiency of feed utilization as well as affecting the incidence of bacterial induced gastro-intestinal disorders such as enterotoxemia and diarrhea coincident with placing lambs on a self-fed ration for fattening purposes(12). The intestinal absorption of Ca in chicks can be influenced by dietary antibiotics(10,11) and a similar effect may have occurred in the sheep with consequent inhibitory effects on the development of calculi. Mineral balance studies or analyses of serum Ca and P, however, have not been carried out under the conditions of the present experiment. The apparent aggravation of the development of urinary calculi by pelleting the feed may be further suggestive of an involvement of the activity of rumen microorganisms in the course of calculi development although the mechanism of such involvement is difficult to visualize at this point.

The lower incidence of urinary calculi in the urea supplemented diets may lead to wider interpretation to the observations that urinary calculi are reduced in mink(9) and other animals by the administration of NH_4Cl . The principal intermediate in the metabolism of urea by rumen microorganisms is $(\text{NH}_4)_2\text{CO}_3$. However, the low level of death losses and low incidence of renal calculi with the low protein diet (Group VII) would indicate that the urea had little effect upon urinary calculi and that other basic feed components contain the calculogenic factors.

Summary. Two hundred seventy-six wether feeder lambs were divided into 7 lots for 98-day fattening period. The animals were self-fed total mixed ground milo-molasses-alfalfa-cottonseed hull diets calculated to contain the same total digestive nutrients with urea or cottonseed meal as a source of supplementary nitrogen. Twenty-six deaths from urinary calculi occurred. There were significantly fewer death losses in groups of lambs fed diets containing 10 mg chlortetracycline/lb of feed than in lambs receiving control diets. Also,

fewer deaths occurred among lambs fed diets containing urea than among those receiving cottonseed meal as a source of nitrogen. Observations of calculi in the kidney of 146 of the remaining lambs at slaughter revealed a lower incidence and less calculi in those lambs having received chlortetracycline in the diet. The possibility of a physiological effect of dietary chlortetracycline on the reduction of renal calculi formation and subsequent death from urinary calculi is suggested.

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Effect of Intrinsic Factor Concentrates on Vitamin B₁₂ Receptor Protein of Tissues.* (23902)

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Reports from this laboratory indicate that intrinsic factor (IF) containing materials stimulate certain serum proteins to combine with Vit. B₁₂(1) and IF concentrates stimulate uptake of radio-active Vit. B₁₂ by slices of rat liver or kidney(2). The data presented here demonstrate that IF containing materials stimulate receptor proteins to combine with Vit. B₁₂. These proteins are distributed in all tissues thus far tested. Furthermore, the data offer support to the view that IF stimulates uptake of Vit. B₁₂ in slices by first being absorbed into the cell.

Methods and materials. Wistar strain, adult rats grown on stock diet were used. The animals were decapitated and organs to be studied quickly removed and chilled on cracked ice. Homogenates, 10%, were prepared in buffer by a Potter-Elvehjem homogenizer. Incubations, 1 hr, were carried out in standard Warburg apparatus at 37°. The

main chamber contained 1 ml of homogenate, intrinsic factor concentrate (IFC)[†] dissolved in buffer(3) and buffer to make a volume of 2.6 ml. The side arm contained 0.4 ml of cobalt₆₀ labeled Vit. B₁₂[‡] in buffer which contained 30 mμg of Vit. B₁₂ and approximately 0.05 μc of radioactivity. The final volume was 3 ml. The system was aerated 5 min. with 20% O₂, 5% CO₂, and 75% N₂; after allowing a 5 min. period for temperature equilibration, the vitamin was tipped-in from side arm. The experiment was terminated by aspirating contents of each flask into a pipette and transferring to test tube. To each test tube was added 60 mg charcoal, suspended in one ml of buffer. The charcoal quickly adsorbs the free Vit. B₁₂ and stops the reaction. All tubes from each experiment were shaken

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[†] Intrinsic factor concentrate was supplied by Abbott Laboratories, North Chicago, Ill. Its biological potency was established as 5 mg/day, using urinary excretion test as means of assay.

[‡] Radioactive vit. B₁₂ was generously supplied by Merck and Co.