cytopathogenic agent to strain RPL 12 virus is provided by results of serum neutralization tests. Antibodies produced in chickens with RPL 12 virus readily neutralized the tissue cultured virus in the same range as did the specific antibodies to tissue cultured virus. A significant fact is that antiserum to a virus of similar origin and biological characteristics, but propagated *in vitro* at another laboratory, neutralized our tissue cultured virus at high dilutions.

Summary. A cytopathogenic agent isolated from RPL 12 in chicken embryo liver tissue cultures is reported. The virus produces a marked CPE on liver parenchymal cells. Virus serially passed in tissue culture, when inoculated into susceptible chicks, produced visceral lymphomatosis. Neutralization tests with antiserum against RPL 12 indicate a relationship of tissue culture virus with RPL 12; however, positive identity of the cytopathogenic agent with the virus causing visceral lymphomatosis has not been established.

1. Furth, J., Breedis, C., *Arch. Path.*, 1937, v24, 281.
2. Doljanski, L., Pikovski, M., *Can. Res.*, 1941, v1, 205.
3. Doljanski, L., *ibid.*, 1942, v2, 626.
4. Lo, W. H. Y., Gey, G. O., Shapras, P., *Bull. Johns Hopkins Hosp.*, 1955, v97, 248.
5. Manaker, R. A., Groupé, V., *Virology*, 1956, v2, 839.
6. Fontes, A. K., Burmester, B. R., Iseler, P. E., *Poultry Sci. Assn. Proc.*, 1957.
7. Sharpless, G. R., Defendi, V., Cox, H. R., *ibid.*,
8. Burmester, B. R., Gentry, R. F., *Poultry Sci.*, 1955, v35, 17.
9. Gentry, R. F., Burmester, B. R., *ibid.*, 1955, v34, 669.
10. Youngner, J., PROC. SOC. EXP. BIOL. AND MED., 1954, v85, 202.
11. Morgan, J. F., Morton, H. J., Parker, R. C., *ibid.*, 1950, v73, 1.
12. Burmester, B. R., Walter, W. G., Fontes, A. K., *Poultry Sci.*, 1957, v36, 79.

Received February 6, 1958. P.S.E.B.M., 1958, v97.

Structural Analogues of Puromycin in Production of Experimental Nephrosis in Rats.* (23900)

BENJAMIN A. BOROWSKY, DAVID M. KESSNER AND LILLIAN RECANT
(Introduced by Robert E. Shank)
Nutrition Research Laboratory, Dept. of Preventive Medicine, Washington University School of Medicine, St. Louis, Mo.

Sherman, Taylor, and Bond(1) first demonstrated that the antibiotic 6-dimethylamino-9 - (3′-p-methoxy-L-phenylalanylamino-B-d-ribofuransoyl) purine (Puromycin) obtained from *Streptomyces alboniger,* has a nephrotoxic effect in rats. Subsequently, it was shown by Frenk and Antonowicz(2), that the derivative of Puromycin, 6-dimethyl-aminopurine-3′-amino - d - riboside (Aminonucleoside), produced a typical nephrotic syndrome. Feigelson, Drake, and Recant(3) described the syndrome in detail. The present work was undertaken to determine the structural configuration of these compounds essential for production of the nephrotic syndrome. To achieve this end, a variety of structural analogues of Puromycin was studied.

Materials and methods. Fifty-five male Sprague-Dawley rats, weighing 80-100 g, were divided into 10 experimental groups of 4 animals, and one control group of 15 animals. Each group was treated with daily subcutaneous injections of 0.3 ml of 0.017 M solution of one of the following: Aminonucleoside, Puromycin, 3-amino - d - ribose, 6-dimethyl-amino-purine, and 6-dimethyl-adenosine. 3′-amino-adenosine was administered in dosages of 0.3 ml of 0.017 M, 0.0085 M, 0.0034 M, and 0.0017 M solutions.† An additional group re-

* Supported by research grant from St. Louis Heart Assn.

† We are indebted to Lederle Laboratories, who generously supplied these compounds.

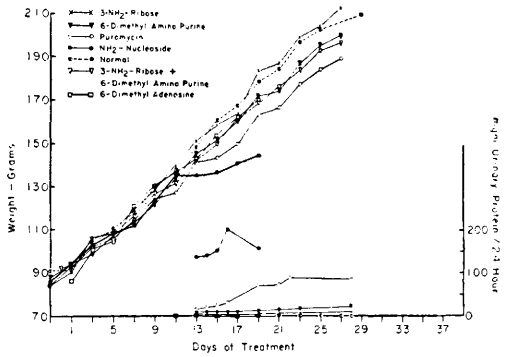

FIG. 1. Weight and proteinuria curves in animals treated with Puromycin analogs.

ceived simultaneous injections of 3-amino-d-ribose and 6-dimethyl-aminopurine. Control animals received no injections since previous controls injected daily with saline or distilled water showed no abnormalities. Animals were maintained on Purina Laboratory Chow and drinking water *ad libitum,* and housed in individual metabolism cages. Observations ranged from 10 to 30 days. Body weights were recorded every second day. To assess activity of the drugs, those constituents of serum and urine characteristically altered in nephrosis were measured. 24-hour urinary outputs were collected and urinary proteins determined by the Esbach method(4). At conclusion of each experiment, the rats were sacrificed by decapitation and blood collected in heparinized tubes. Serum cholesterol was determined on these samples by the method of Saifer and Kammerer(5); total serum protein and albumin and globulin fractions were determined by the micro-method of Lowry, Rosebrough, Farr, and Randall(6), using Howe fractionation technic(7). Kidneys were fixed in Bouin's solution for histologic study.

Results. As seen in Fig. 1, each group of animals showed a weight curve that paralleled the normal, with exception of those being treated with Aminonucleoside. The weight curve of Aminonucleoside-treated animals followed the normal for first 11 days of injection, but at this point the curve became almost flat for 4 days, after which it began to rise again. The secondary phase of weight gain occurred when the animals manifested the accumulation of ascites. The weight curve of Puromycin-treated animals, although somewhat lower than the normal curve, remained essentially parallel to it, and showed no distinct plateau.

Significant proteinuria became manifest in the Aminonucleoside group when growth reached a plateau. Animals receiving Puromycin showed a slowly progressing proteinuria beginning on thirteenth day and reaching a maximum on twenty-second day; the level of proteinuria reached its peak, therefore, at a much later date than in the Aminonucleoside group, and the maximal level was considerably lower. None of the other groups studied showed any significant proteinuria.

As to serum constituents (Table I) it can be seen that only Aminonucleoside animals developed nephrosis as manifested by elevated serum cholesterol, decreased total serum proteins and marked hypoalbuminemia. Serum chemical changes in the Puromycin group were in the same direction, but of much lesser degree. All other animals had normal serum cholesterol and protein values. Table I also reveals that all animals treated with Aminonucleoside had severe ascites at necropsy, whereas animals treated with other drugs had ascites. Microscopic examination of kidneys of the Aminonucleoside animals showed Periodic-Acid-Schiff positive casts, tubular dilatation and widening of Bowman's space; there were no structural changes in the glomeruli. No abnormalities were noted in kidneys of Puromycin-treated animals.

3′-amino-adenosine, although differing from the Aminonucleoside only by absence of dimethyl-substitution on the amino group in position 6 of purine ring (Fig. 3), proved to be considerably more toxic in terms of growth and survival. Animals which received this drug in a dosage equivalent to that used of other drugs showed a rapid loss of weight and died within a few days (Fig. 2). Those which received half this dose also showed rapid weight loss. Indeed, animals treated with only one-tenth the full dose showed average weight gain of only 20 g in 10 days, as compared to 50 g by normal rats. There was no evidence of nephrosis in any animal treated with this compound, *i.e.,* no proteinuria, ascites, hypercholesterolemia, or hypoalbuminemia. Although total serum proteins were moderately depressed, this may be attributed to their extreme state of cachexia. Both gross

TABLE I. Mean Concentration of Certain Constituents of Serum.

Exp. groups	Cholesterol, mg %	Total serum proteins	Albumin	Globulin	Ascites, 0 - ++++
		g %			
Aminonucleoside*	868	3.1	.91	2.19	++++
Puromycin	280	3.75	1.5	2.25	0
6-Dimethylaminopurine	98.5	4.4	2.3	2.1	0
3-Amino ribose hydrochloride	100	4.4	2.3	2.1	0
3'-Amino-3'-deoxyadenosine	103	3.8	2.3	1.5	0
6-Dimethylaminopurine + 3-amino ribose hydrochloride	79.4	5.1	2.6	2.5	0
6-Dimethyl adenosine	99.4	5.2	2.5	2.7	0
Controls	100.9	4.8	2.7	2.1	0

* All compounds were used as 0.017 M solutions except 3'-amino-3'-deoxyadenosine, which was 0.0085 M.

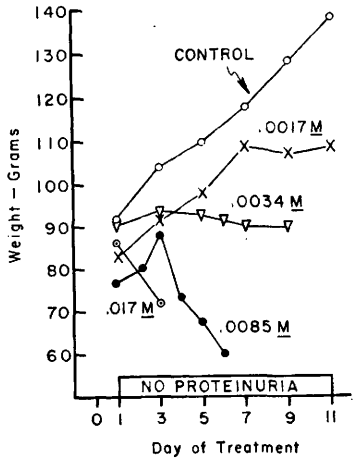

FIG. 2. 3'-amino-adenosine treated animals.

and microscopic examination of kidneys and other major organs failed to reveal any abnormalities.

Discussion. Structural analogues of Puromycin illustrated in Fig. 3 have been tested with reference to their ability to induce the nephrotic syndrome in rats. It is apparent that only 2 of them, Aminonucleoside and Puromycin, are active in this respect (Table I). The milder degree and delay in time of onset of nephrosis, which results from Puromycin as compared to Aminonucleoside, may possibly be related to the complex side chain binding the amino group of Puromycin (Fig. 3). It seems reasonable to assume that before Puromycin is fully effective in production of nephrosis, the side chain must be cleaved from the molecule leaving free Aminonucleoside.

Aminonucleoside differs from the naturally occurring metabolite, adenosine, in 2 respects: 1) presence of a dimethyl substitution on the amino group attached to carbon 6 of the purine ring, and 2) substitution of an amino group on carbon 3 of the ribose moiety. The fact that neither 6-dimethyl-adenosine nor 3'-amino-adenosine, each of which contains one but not the other of these two substitutions, can induce nephrosis, indicates that presence of both substitutions is essential for induction of the syndrome. It is of interest that neither 6-dimethyl-adenine nor 3-amino-ribose, when administered separately or simultaneously, were nephrotoxic. This indicates that although the purine and pentose components of the Aminonucleoside molecule are injected to-

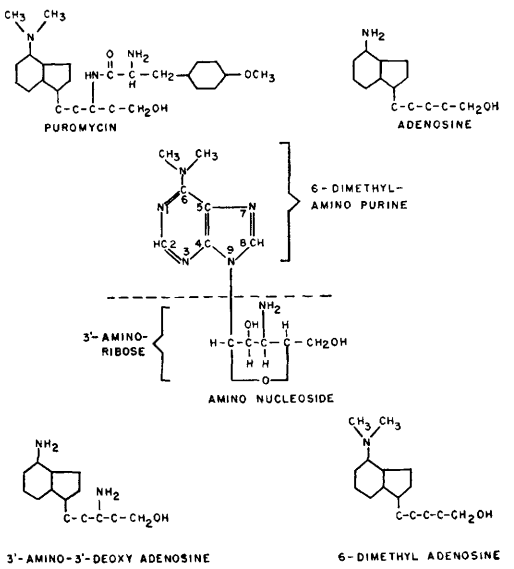

FIG. 3. Chemical structure of Puromycin and its analogs.

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.

1. Sherman, J. F., Taylor, D. J., Bond, H. W., *Antibiotics Annual* 1954-55, p757, *Medical Encyclopedia,* N. Y. City.

2. Frenk, S., Antonowicz, J. M., Craig, J. M., Metcoff, J., PROC. SOC. EXP. BIOL. AND MED., 1955, v89, 424.

3. Feigelson, E. B., Drake, J. W., Recant, L., *J. Lab. and Clin. Med.,* 1957, v50, 437.

4. Ham, T. H., *A Syllabus of Laboratory Examination in Clinical Diagnosis,* 1950, Harvard University Press, Cambridge, Mass.

5. Saifer, A., Kammerer, O. F., *J. Biol. Chem.,* 1946, v164, 657.

6. Lowry, O. H., Rosebrough, N. J., Farr, L. A., Randall, R. J., *ibid.,* 1951, v193, 265.

7. Howe, P. E., *ibid.,* 1921, v49, 109.

Received February 14, 1958. P.S.E.B.M., 1958, v97.

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

LEONARD V. PACKETT, JR., T. D. WATKINS, JR., AND H. O. KUNKEL
Department of Animal Husbandry, Texas Agricultural Experiment Station, College Station

The development of urinary calculi in sheep and other animals has been associated with many of the commonly used feedstuffs(1,2), with vitamin deficiences(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-

* This investigation was supported in part by grant from American Cyanamid Co., Pearl River, N. Y. Appreciation is expressed to Dr. F. T. Lynd and C. H. Bridges, Dept. of Veterinary Pathology, A. & M. College of Texas, for assistance in autopsies.