corresponding groups in Table I (unpublished data).

The function of tetrahydrofolic acid in the metabolism of the formimino group of FGA has been delineated by Tabor and Wyngarden (9). No such role has been described for Vit. B_{12}. From the data in this paper it seems possible that Vit. B_{12} affects utilization of FGA in some way in addition to that involving the metabolism of methionine. The 1% level of methionine did not cause FGA excretion of the Vit. B_{12} deficient chicks to stay at the same low level as that of Vit. B_{12} supplemented chicks when histidine was fed at the highest levels. Only 0.3% supplemental methionine is required to support normal growth of B_{12} deficient chicks with this diet(10). Secondly, the chicks that were severely deficient in methionine did not excrete large amounts of FGA until Vit. B_{12} was omitted from the diet. Perhaps either methionine or Vit. B_{12} or both modify the cofactor role of folic acid in the metabolism of the formimino group by the chick in a manner like that demonstrated in the rat by Noronha and Silverman(11).

Summary. Day old chicks were fed a diet borderline in methionine for 3 to 4 weeks. They excreted large amounts of formiminoglutamic acid (FGA), a metabolite of histidine, when either folic acid or Vit. B_{12} was omitted from the diet. FGA was excreted in very small amounts by control chicks fed the complete diet. Incorporation of supplemental methionine at 1% of the diet caused a marked reduction in FGA excretion of Vit. B_{12} deficient chicks and a moderate reduction in the folic acid deficient chicks. Further elevation of dietary histidine and continuation of the supplemental methionine resulted in increased FGA excretion which was relatively greater in the folic acid deficient than in the Vit. B_{12} deficient group. Only on the last day of the experiment was the excretion elevated for the control group. Chicks fed diets severely deficient in methionine did not excrete large amounts of FGA until Vit. B_{12} was omitted.

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Inhibition of Aminonucleoside Nephrosis in Rats. II. Effect of Nucleic Acid Precursors and L-triiodothyronine.* (27042)

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The mechanism by which the aminonucleoside (P-A) of puromycin induces the classical nephrotic syndrome in rats is still unknown. Since adenine, a primary precursor of RNA in rat, has been shown partially to block the nephrotoxicity of P-A(1,2), it seemed worthwhile to evaluate the protective action of other RNA and DNA intermediates.

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Methods. Female rats, descendants of the

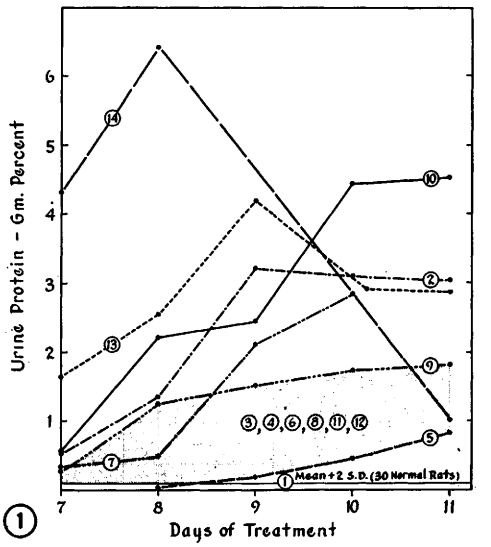
Holtzman strain, weighing 200±20 g were housed in individual metabolism cages in a temperature-controlled room (76° ±2°F) and maintained on Fox Chow and water *ad lib.* They were injected in the evening with an aqueous solution or suspension of P-A and nucleic acid intermediates as shown in Table I. Each was injected at a separate site subcutaneously, and P-A was always injected 15 to 30 minutes after the intermediate. In order to minimize sloughing at site of injection, the pH of each injected intermediate was adjusted to 6 to 8 with 1 N NaOH or phosphate buffer unless otherwise specified. The unsterilized preparations were kept frozen until they were ready for injection at which time they were thawed to a liquid state. The amount to be injected was removed and brought to body temperature, and the bulk of the material was immediately refrozen. Urine was collected daily beginning on the 7th day of injection and protein concentrations were determined by the method of Shevsky and Stafford as modified by McKay(3).

In a separate experiment 2.5 μg of L-triiodothyronine (T-3) was administered subcutaneously into 6 rats daily for 20 days. Beginning on the 8th day P-A was also administered as shown in Fig. 2.

Results. The details regarding the dosage schedule of the nucleic acid intermediates are listed in Table I and their effect on protein excretion is shown in Fig. 1. L-Glutamine (curve 13) and D-glucosamine (curve 14) appeared to cause earlier and more severe proteinuria than P-A alone (curve 2); however, this effect was not sustained. Uridine (curve 10) had no important influence initially, but protein excretion on the 10th and 11th days exceeded that of any other group. The remainder of the nucleic acid intermediates including guanine, adenylic acid, 4-amino-5-imidazole carboxamide, D-ribose, orotic acid, 2,6-diaminopurine, and thymine appeared to decrease the severity of the proteinuria and are shown grouped together in the shaded area of Fig. 1.

Rats treated with T-3 developed proteinuria 2 days earlier and excreted protein in much greater quantity than animals treated with P-A alone (Fig. 2).

Discussion. The demonstration that adenine



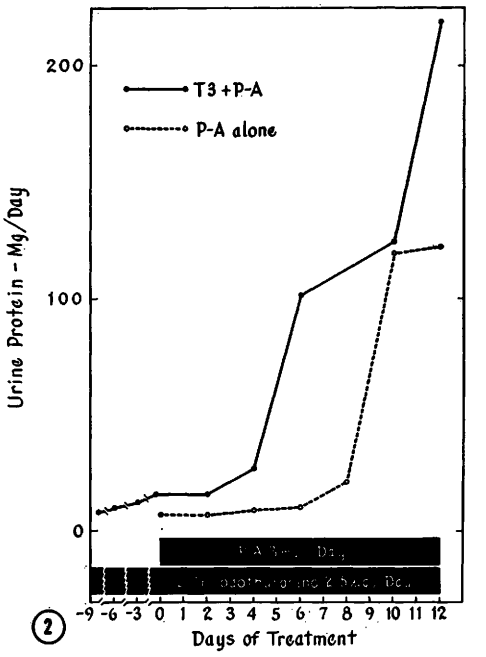


FIG. 1. Effect of nucleic acid intermediates on P-A induced proteinuria. Numbers refer to compounds listed in Table I.

FIG. 2. Enhancement of P-A induced proteinuria by pretreatment with L-triiodothyronine.

afforded partial protection against P-A induced nephrosis(1,2) suggested that the nephrotoxic agent competed successfully with nucleic acid intermediates for polynucleotide or nucleoprotein synthesis. Glutamine contributes 3 nitrogen atoms to position 1, 3, and 9

TABLE I. Dose Schedule of Nucleic Acid Intermediates Administered with P-A.

Intermediate	No. of rats	I† concent. %	No. daily doses	Mole† ratio, I/P-A
1. Control	30			—
PURINES				
2. P-A*	16	.5	10	—
3. P-A + guanine HCl	4	2.0	10	7/1
4. P-A + disodium guanylate	4	7.0	10	8/1
5. P-A + 2,6-diaminopurine sulphate	4	1.0	10	4/1
6. P-A + inosine	4	2.0	10	5/1‡
7. P-A + adenylic acid	4	7.0	10	10/1
PYRIMIDINES				
8. P-A + orotic acid	4	1.0	10	10/1
9. P-A + thymine	4	1.0	14	10/1
10. P-A + uridine	4	2.0	14	5/1
OTHER				
11. P-A + 4-amino-5-imidazole carboxamide	4	2.0	10	12/1
12. P-A + ribose	4	2.0	10	13/1
13. P-A + glutamine	4	1.25	10	5/1
14. P-A + D-glucosamine	4	2.0	10	5/1

* Aminonucleoside standard dose = 1.5 mg/100 g body wt/day.
† I = Intermediate. ‡ 2.5/1 on day 3.

of the purine ring(4) and, therefore, this compound might be expected to accelerate the synthesis of adenine, which is the most important nucleic acid precursor in the rat(5). However, the fact that glutamine accentuated the proteinuria argues against such a mechanism. Based on the assumption that P-A might be incorporated into mucoprotein, a more likely explanation for the unfavorable effect of both glutamine and glucosamine is the fact that hyaluronic acid which is present in cellular basement membrane is synthesized from glucosamine, which in turn derives its amino group from glutamine(6,7). It is interesting that glucosamine and P-A are aminosugar and aminosugar derivative, respectively. Replacement of the aminosugar moiety in P-A with a normal sugar (D-ribose) has been shown to prevent its nephrotoxic action(8). Except for uridine, the other nucleic acid intermediates (i.e. thymine, guanine, adenylic acid, 4-amino-5-imidazole carboxamide, D-ribose, orotic acid, 2,6-diaminopurine, and inosine) reduced the toxicity of P-A.

Thyroxine has been shown to result in uncoupling of oxidative phosphorylation(9) and in swelling of rat kidney mitochondria accompanied by increased oxidation of DPNH(10). These effects, which may be related, are believed to be secondary to increased permeability of mitochondrial membrane to substrate. ATP can prevent swelling caused by thyroxine(11). The kidney is also uniquely different from other organs in having a very high rate of oxygen consumption(12) and the ability to convert thyroxine to triiodothyronine(13). It is not surprising, therefore, that the presumed increased metabolic rate following T-3 administration was accompanied by early and more severe proteinuria. The accumulation of interstitial fluid rich in mucopolysaccharide which occurs in absence of thyroxine has been pointed out by Watson and Pearce(14) and by Ludwig et al(15). The mechanisms of action of thyroid hormones have been thoroughly discussed recently(16).

Summary. The aminonucleoside of puromycin (P-A), which produces the nephrotic syndrome in rats, was administered simultaneously with various nucleic acid intermediates. L-glutamine, D-glucosamine, and uridine intensified the nephrotoxicity, whereas guanine, adenylic acid, 4-amino-5-imidazole carboxamide, D-ribose, orotic acid, 2,6-diaminopurine, inosine, and thymine appeared to ameliorate the condition. Rats primed with L-triiodothyronine (T-3) developed proteinuria earlier than rats treated with P-A alone.

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Mechanism of Action of Staphylococcal Toxin in Rabbits.* (27043)

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Introduction. Bacterial exotoxins are known to inflict serious damage on specific systems. Thus, the alpha toxin of the Welch bacillus contains a red-cell lecithinase; the diphtheria toxin produces a myocardial lesion; and the exotoxin of the Staphylococcus when given intravenously causes renal necrosis. Bacterial exotoxins, like endotoxins, also can cause peripheral vascular collapse. The endotoxins do so when the reticulo-endothelial system (R.E.S.) loses its capacity to destroy them, so that they remain free in the circulation to inflict vascular damage(1). If exotoxins can injure the R.E. system, the shock and death from exotoxins might be brought about by development of a secondary endotoxemia, the source of which are the endotoxins continuously entering the circulation from the intestine(2).

Some of the experiments to be reported below were done to see whether the death caused by staphylococcal toxin is due to loss of R.E.S. function. Other experiments were done to see whether the renal injury which is a constant feature of the pathology at death from this exotoxin is involved in the death.

Materials & Methods. Adult albino rabbits (wt 2kg) were used throughout. Two separate batches of staphylococcal toxin† were used. The MLD/100 of a 1:10 dilution in saline was determined by administering increasing volumes into the ear veins of rabbits until the amount necessary to kill all rabbits within 24 hours was reached. For the first batch of toxin this MLD/100 was 1.6ml/kg, and for the second batch 2.5ml/kg. Gross postmortem examination was performed, but only the kidney was studied histologically‡.

To exclude contamination of the exotoxin with endotoxin each batch of exotoxin was extracted by the Boivin method(6), and found to contain no endotoxin on assay by the chick embryo technic(7).

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† Staphylococcal toxin, #42925-199 (Wood #46) preserved in 0.01% thiomerosal, Lederle Lab., Pearl River, N. Y.

‡ The effect of intravenously administered Staphylococcal toxin on the kidney is well established(3,4,5) and consists of diffuse cortical necrosis and focal medullary hemorrhagic necrosis. Other findings at death in about ½ the animals in a series include scattered petechial hemorrhages among the viscera.