Inhibition of Aminonucleoside Nephrosis by Adenine.* (24556)

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Repeated injection of 6-dimethylamino-9-(3′-amino-3′-deoxy-β-D-ribofuranosyl) purine, (hereinafter referred to as aminonucleoside)†, a derivative of the antibiotic, Puromycin, produces a disorder in rats characterized by hyperlipemia, azotemia, hypoproteinemia and proteinuria(1-5). This disorder resembles the nephrotic disease of children and the experimental disease produced in rats by injection of a rabbit anti-rat kidney serum(6-9). Several metabolic manifestations of the heteronephrotoxic type of nephritis have been reported. These include: (a) impaired phosphorylation during anaerobic glycolysis using kidney slices(10), (b) grossly normal carbohydrate metabolism(11), (c) primary protein loss followed by an increased protein turnover, increased utilization of carbohydrate and accelerated amino acid metabolism (12), (d) mobilization of body fat following the primary protein loss(13). Since nephrosis is induced by minute amounts of aminonucleoside, and as it is related structurally to adenine and adenosine, it seemed likely that this compound could be acting as a metabolic antagonist in some phase of purine metabolism including reactions involving ATP. The aminonucleoside has an anti-trypanosomal effect in mice infected with *Trypanosoma equiperdium*(14). This effect is partially reversed by a number of synthetic purine derivatives and by adenine but not by adenosine. Additionally, the aminonucleoside is effective against other organisms, and various purines serve to reverse its growth inhibiting activity(15). As the compound appears to be a metabolic antagonist whose activity is reversed by purines in lower forms, it seemed desirable to determine whether it acted similarly in mammalian species. The experiments to be described were designed to determine the effect of adenine and adenosine on the production of the aminonucleoside-induced nephrotic syndrome in the rat, with the hope that the information gained would be of value in elucidation of the basic etiology of heteronephrotoxic kidney disease and lipoid nephrosis of children.

Materials and methods. 58 female Sherman strain rats ranging 70 to 123 g were divided into 5 groups. Each rat received the following compounds by subcutaneous injection in amounts indicated/g of body weight/day. Those in Group 1 received 0.015 mg of aminonucleoside. Those in Group 2 received 0.015 mg of aminonucleoside plus 0.056 mg of adenine suspension. Those in Group 3 received 0.015 mg of aminonucleoside plus 0.8 mg of adenosine suspension. Those in Group 4 received 0.056 mg of adenine suspension and those in Group 5 received 0.8 mg of adenosine suspension. The animals in Groups 2 and 3 received adenine or adenosine just prior to the aminonucleoside at a separate subcutaneous site. The animals were held for 14 or 15 days during which time they were given 10 injections, the last on day of sacrifice and the day preceding sacrifice. On final day of experiment urines were collected, tested for presence of protein by heat and acetic acid test and the results gauged on a 0 to 4+ scale. The animals were anesthetized and a midline laparotomy incision was made. When present, the amount of ascites was measured by withdrawing as much of fluid as possible into a small graduate. The animals were killed by exsanguination and total serum lipids determined by the turbidometric method of de la Huerga *et al.*(16). Kidney samples were taken for sectioning and staining with H&E and PAS. The sections to

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TABLE I. Comparison of Mean Values of Ascites, Proteinuria and Total Serum Lipids in Rats Which Received Aminonucleoside with and without Addition of a Purine Derivative.

Group	Compound(s) inj.	No. of rats	ml of ascites	% with 3+ or 4+ proteinuria	mg % total serum lipid
1	Aminonucleoside	11	13	91	1005
2	" + adenine	12	0	25	592
3	" + adenosine	12	7	92	750
4	Adenine	11	0	0	300
5	Adenosine	12	0	17	286
	None	12			338

be stained with H&E were cut at 7 μ and those to be stained with PAS were cut at 4-5 μ. Comparative statistical studies were made between appropriate groups. The *t* test was used to compare data for lipid levels and amounts of ascitic fluid. A value was considered to be statistically significant when its P value was .05 or less. The chi square test was used to evaluate the significance of proteinuria data taking into account the 5 values ranging from 0 to 4+.

Results. Comparisons of data obtained from each group of rats and from 12 normal animals of similar weight are shown in Tables I and II. When compared to results obtained with rats which received aminonucleoside alone, concomitant administration of adenine with the aminonucleoside 1) prevented accumulation of ascitic fluid, 2) resulted in a highly significant decrease in number of animals with 3+ or 4+ proteinuria and 3) led to markedly lower serum lipid levels. Concomitant administration of larger amounts of the more soluble adenosine (over 8 times as much adenosine as adenine on a molar basis) with the aminonucleoside, was not followed by similar manifestations of reversal of toxicity of the aminonucleoside. At the level of adenine administered (molar ratio of adenine to aminonucleoside 8:1) reversal of toxicity was not complete. However, it did not seem feasible to increase the molar ratio of adenine to aminonucleoside, since in preliminary experiments adimnistration of larger amounts of adenine alone led to toxic manifestations. Neither adenosine nor adenine were totally innocuous compounds at the levels used, inasmuch as administration of either compound was followed by a significant change in degree of proteinuria. Administration of adenine alone did not cause 3+ or 4+ proteinuria but was followed by more than the expected incidence of 1+ and 2+ reactions.

TABLE II. Statistical Comparison of Data.

Group	Groups compared	Total serum lipids Diff. ± S.E.	Total serum lipids P value	Proteinuria P value
1	Aminonucleoside			
2	Aminonucleoside + adenine	423 ± 123	<.01	<.01
2	Aminonucleoside + adenine			
4	Adenine	289 ± 71	<.01	>.05
1	Aminonucleoside			
3	Aminonucleoside + adenosine	255 ± 129	>.05	>.05
3	Aminonucleoside + adenosine			
5	Adenosine	470 ± 82	<.01	<.01

Serum lipid levels of animals which received aminonucleoside plus adenine were significantly lower than those of animals which received only aminonucleoside. Levels in animals which received both compounds however, were significantly higher than those in animals which received adenine alone. Thus the aminonucleoside effect was not totally blocked. Lipid levels in serum of animals treated with adenine alone, were not significantly different from those of normal animals.

Administration of adenosine with aminonucleoside did not result in significant differences in lipid levels from those of animals which received aminonucleoside alone. It is interesting that injection of adenosine alone resulted in significantly lower lipid values than those observed in normal animals.

Administration of aminonucleoside produced an injury of the basement membrane,

manifested by a deposit of PAS positive material. Either adenine or adenosine also caused early changes in basement membrane characterized by splitting and fraying. The appearance of basement membrane was not altered by addition of either adenine or adenosine to aminonucleoside. The PAS positive material deposited in the glomerulus did not appear as dense as that seen in the nephrotoxic nephritis, however, the tubular lumina contained prominent PAS positive casts.

Discussion. The data herein presented provide evidence that the mode of action of aminonucleoside in production of the nephrotic syndrome in the rat is that of an antimetabolite. Reversal of toxicity of the compound by adenine would implicate a block in some phase of nucleotide or nucleic acid metabolism. The question is thus raised as to whether other types of nephrotoxic disease may have a similar etiology, *i.e.*, deranged nucleic acid or nucleotide metabolism. In a yeast system it has been observed(17) that aminonucleoside inhibited ATP formation. The negative results obtained with adenosine do not necessarily exclude the possibility of a defect in the phosphorylation mechanism. Adenosine deaminase has been found to be very active in mammalian tissues(18) and it is possible that rapid conversion of adenosine to inosine might account for its lack of effect in the present study. Brown *et al.*(19) noted greater incorporation of exogenous adenine than of adenosine into the nucleic acids of the rat and proposed that the route for incorporation of the free base is different from that of nucleotides synthesized *de novo*. Buchanan (20) has related that aminonucleoside does not inhibit any of the enzyme systems involved in the *de novo* synthesis of purine derivatives.

The intraglomerular deposits of PAS positive material seen here corroborate the findings of Frenk *et al.*(1) but are at variance with the report of Fisher *et al.*(4). In the experiments of the latter workers injections of the aminonucleoside were stopped on day 9-11 and the animals were held until day 14. The possibility exists that in the experiments of Fisher the PAS positive material had been present at the end of injection period and had decreased in amount in the 3-5 day period immediately prior to sacrifice. This would be in accord with the observations of Frenk *et al.* (1) that structural alterations are reversible in animals receiving 5-20 consecutive daily injections of the aminonucleoside. It should also be noted in considering the results of these two authors and those presented here, that 3 different strains of rats were used. Thus genetic differences in susceptibility of the mucopolysaccharides of the basement membrane to injury could have been responsible for the discrepancy in results. The fact that morphological alleviation of the disease lagged behind the functional improvement may have been due to the masking effect of the adenine which itself gave rise to early basement membrane changes.

Summary. Studies were made of the effects of administration of adenine and adenosine on nephrosis of rats induced by injection of aminonucleoside obtained from Puromycin. Adenine partially reversed nephrotoxic activity of the aminonucleoside, while adenosine did not. These findings have been interpreted as evidence that aminonucleoside acts as an antimetabolic agent in some phase of nucleotide or nucleic acid metabolism in production of the nephrotic state in the rat.

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Inhibition of Experimental Tumors by Orthophenylenediamine (OPDA). (24557)

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The presence in animals of certain tumors has been observed to provide either biochemical protection or liability in terms of survival time for the host when challenged with a lethal dose of specific chemical agents such as the phenylenediamines(1,2). The Cloudman S91 mouse melanoma protects its host against the acute toxicity of paraphenylenediamine (PPDA) whereas the same tumor is a disadvantage to the host when the challenging compound is orthophenylenediamine (OPDA). With the above experimental design this phenomenon extended to other combinations of tumor and compound variously to the advantage, disadvantage, or neither for the host. In possible correlation, it was also observed that PPDA combines with certain pigmented tumor constitutents *in vitro* as determined by oxygen-consuming reactions in the Warburg apparatus when added to melanoma tissue, urine, blood plasma, or pure dihydroxyphenylalanine (DOPA)(3,4,5). Further pursuit of these phenomena led to chemotherapeutic testing of phenylenediamines against melanoma and other tumors. The results with the ortho isomer, which is the least toxic and most effective, are reported here with a variety of transplanted mouse and hamster experimental tumors.

Materials and methods. Several inbred and hybrid strains of mice were employed for carrying the tumor types studied. Most melanoma experiments utilized the Cloudman S91 (6) although the Harding-Passey(7), the Fortner hamster melanoma(8), and several non-melanoma tumors were used for comparative purposes. The transplanted hamster melanoma is designated as Melanotic Melanoma No. 2 (H.M.M. #2) and originated "spontaneously" in a Syrian golden hamster. The histological and biological characteristics have been described(8,9). The Cloudman S91 mouse melanoma was carried in both male and female DBA/2 hybrid mice. The Harding-Passey melanoma and the Ehrlich car-

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