conditions, did not report qualitatively comparable dose reduction. From a comparison of the exposure conditions in the two series of experiments, it appears most likely that the differences between Therkelsen's findings and those of the present study are a reflection of the relatively small amount of potentially protective agent present during irradiation in the former experiments. This is supported by our finding and that of Vos *et al.*[3] that the degree of protection is correlated with concentration of protective agent present during irradiation.

Although the parameter used to determine radioprotective effect differed in the experiments of the Dutch group,[3, 4] the sequence of steps immediately preceding and during irradiation was similar to that in the present study; and among the DRF's these workers reported were ~1·8 (4 mM MEA), ~3·3 (16 mM MEA), and ~3·8 (32 mM MEA). These findings are strikingly and almost quantitatively comparable with the present data.

It is conceivable that MEA exerts its radioprotective effect by depleting the cellular environment of oxygen. While it is true that in our experiments (Fig. 1), 10 mM MEA (0·2 mmole per bottle) protected the cells in the presence of about 2 mmoles oxygen (per bottle), the oxygen concentration in the medium in the immediate cellular environment might nevertheless have been sharply reduced. Our data relative to MEA protection during irradiation under anoxic conditions are limited, but it may be seen (Fig. 2) that a combination of anoxic conditions and MEA provided greater radioprotection than did either procedure alone. In confirmation of Vergroesen *et al.*,[4] and Kohn and Gunter,[7] this finding demonstrates that the radiation protection exerted by cysteamine probably cannot be ascribed to a condition of anoxia resulting from simple oxidation of the added chemical agent.

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Departments of Pharmacology and Radiology,
Stanford University School of Medicine,
Palo Alto, Calif., U.S.A.

J. D. Gaines
C. B. Harbury
L. Aronow
R. F. Kallman

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Aminonucleoside of puromycin: Elimination of nephrotoxicity by acetylation of the aminoribose moiety

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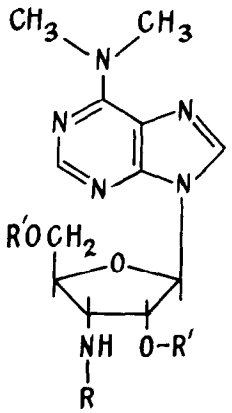
Puromycin,[1, 2] its aminonucleoside (I, Fig. 1; hereafter abbreviated PA) as well as some benzylidine[3] and amino acid analogs[4] have been shown to possess antitumor activity against the mouse mammary adenocarcinoma and certain strains of mouse leukemia. Puromycin itself is less active in this respect than PA; indeed, its antitumor as well as its trypanocidal properties[5] may well reside in the aminonucleoside portion of the molecule Clinical trials against a spectrum of human tumors have been reported for both puromycin[6] and for PA,[7, 8] but were not furtner pursued in the absence of beneficial results, and especially with the appearance of nephrotoxic manifestations in the form of excessive

proteinuria on administration of the latter. PA nephrotoxicity has also been observed in rhesus monkeys[9] and in rats,[10] but not in mice, guinea pigs, or rabbits,[11] while proteinuria alone was inducible in dogs without the appearance of edema, ascites, and hyperlipemia as regularly observed in monkeys and rats. The nephrotic syndrome produced in the rat mimics so closely the syndrome that is clinically observed in humans that PA has since been utilized extensively for the experimental induction of this disease.[12, 13] In this connection, Borowsky *et al.*,[13] have speculated that puromycin is less nephrotoxic than PA owing to the necessity for hydrolysis of the former to the latter *in vivo*.



I: $R = R' = H$
II: $R = -COCH_3$; $R' = H$
III: $R = R' = -COCH_3$

FIG. 1. Aminonucleoside of puromycin and its acetylated derivatives.

The current interest in enzyme-activated drugs and, in particular, the esterase-activated acetyl derivatives of purine and pyrimidine nucleoside antimetabolites such as polyacetylated psicofuranine,[14] azauridine,[15] and 5-halo-2′-deoxyuridines[16, 17] and their reportedly facilitated absorption from the gastrointestinal tract prompted us to compare the nephrotoxicity of N$^{3'}$-monoacetyl-PA (II) and of triacetyl-PA (III) by the subcutaneous and oral routes.

METHODS

The acetylated derivatives of PA were prepared by the procedure of Baker *et al.*[18] PA, N$^{3'}$-monoacetyl-PA and triacetyl-PA were administered daily to female albino rats (Holtzman) according to the schedule of Table 1. The compounds were made up as 0·017 M aqueous solutions or comparable suspensions and administered in equivalent molar doses, the oral dose being always twice the parenteral dose. Triacetyl-PA was ground to a fine powder and homogenized in water containing 0·05% Tween-20, and the stock suspension thoroughly mixed by vigorous shaking just before use. Rats were housed in individual metabolism cages and were allowed water and commercial fox chow *ad libitum*. Urines from individual animals were collected on alternate days and their protein content determined by the sedimentation method of Shevsky and Stafford as modified by MacKay.[19] All rats were screened before the experiment and those with proteinuria greater than 10 mg/day were eliminated. At the end of the treatment all rats were autopsied, and the kidneys were examined grossly and histologically.

RESULTS AND DISCUSSION

To our surprise, neither N$^{3'}$-monoacetyl-PA nor triacetyl-PA was nephrotoxic by parenteral or oral administration, whereas equimolar doses of PA induced the classic nephrotic syndrome within two weeks (Table 1). In the case of monoacetyl-PA, daily subcutaneous administration for a total of

83 days, equivalent to 6 times the standard dose regimen for PA, failed to elicit any toxic manifestations. Urine protein excretion remained normal throughout, and at autopsy all the organs of these animals appeared grossly and histologically normal. In contrast, the PA animals were clinically nephrotic, and histologically their kidneys showed the changes typical of this condition. Other investigators have shown that puromycin in similar daily doses produced mild proteinuria (<100 mg/day) which was slightly delayed in onset.[13]

TABLE 1. COMPARATIVE NEPHROTOXICITY OF AMINONUCLEOSIDE AND ITS ACETYLATED DERIVATIVES

Compound	Route of admin.	No. of rats and wt. range (g)	Dose (mg/rat/day)	Days treated	Total dose (mg)	Total dose (mmole)	Max. urine protein* (mg/day/rat)	Nephrosis
PA	S.C.	20 (180–220)	3·0	14	42	0·14	300†	+
	Oral‡	6 (195–306)	6·0	14	84	0·29	188§	+
$N^{3'}$-Monoacetyl-PA	S.C.	4 (190–235)	3·4	83	282	0·84	12	0
	Oral	5 (190–235)	6·9	26	179	0·53	7·3	0
Triacetyl-PA	S.C.	4 (195–225)	4·3	37	159	0·39	10	0
	Oral	6 (142–161)	8·6	25	215	0·51	9·7	0

* Maximal levels (averaged for each group) during treatment period. Average protein excretion by screened control rats always fell below 10 mg/day in more than 100 controls observed to date.
† Day 11.
‡ By gavage.
§ Day 13.

The effect of these acetylated PA derivatives on replication of tumor cells was tested in an *in-vitro* system. Whereas acetylation of the aminoribose moiety of PA eliminated nephrotoxicity, cytotoxicity appeared to be retained. Thus, at a concentration of $1·0 \times 10^{-4}$ M, $N^{3'}$-monoacetyl-PA inhibited the growth of the ascites form of the F-66 mouse mammary tumor in tissue culture by 24%, whereas triacetyl-PA had very little effect. PA at the same concentration inhibited growth by 47%. At $1·0 \times 10^{-2}$ M all three compounds completely arrested cell growth and gave rise to the appearance of 16, 60, and 46% of nonviable cells, respectively, with monoacetyl-PA, triacetyl-PA, and PA. These results indicate that PA in its altered acetylated forms should be reinvestigated at the clinical level as a carcinostatic, amoebicidal or trypanocidal agent. The selectivity of action introduced by this slight modification of the PA molecule can readily be rationalized on the basis of current concepts in chemical pharmacology; however, any explanations offered must remain conjectures until knowledge is gained of the physiological disposition of these compounds, particularly the comparative metabolic fates of the acetyl groups in the amide and ester linkages (Fig. 1). Such studies with the aid of ^{14}C-labeled derivatives are in progress.

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Medical Research Laboratories,
Veterans Administration Hospital;
and the Departments of Medicine and of
Pharmaceutical Chemistry,
University of Minnesota,
Minneapolis, Minn., U.S.A.

C. S. ALEXANDER
H. T. NAGASAWA

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Sex difference in murine sensitivity to several nitrogen mustards

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IN THE course of study of the protective effect of the mercaptoalkylamines against nitrogen mustard,[1] consistent sex differences in protection were observed. Further evaluation of our data suggested that these differences actually resulted from an underlying sex difference in murine sensitivity to several alkylating agents. To test this possibility, dose–per cent effect curves were obtained for four nitrogen mustards: HN_2 (nitrogen mustard), CQM (chloroquine mustard), PAM (L-phenylalanine mustard), and CTX (cyclophosphoamide mustard). The toxicity tests were performed on DBA/2 × C3H F_1 hybrid mice,[2] with single doses of drug administered i.p. in 0·5 ml of 0·15 M NaCl. Dose–response curves were based on at least two experiments, involving 5 doses each and using 10 male or female mice weighing 20 ± 2 g. Results were scored after 30 days.

Data showing the ED_{16}, ED_{50}, and ED_{84} values, as well as the 5% confidence limits for the ED_{50} values, are given in Table 1. The ED values were obtained by means of a log–probit plot (Codex 3128 log–probability paper) according to the method of Litchfield and Wilcoxon;[3] analyses for homogeneity, parallelism, and potency differences were also performed according to the procedures described by these authors. The data were homogeneous and did not depart from parallelism for the four pairs of male–female dose–response curves.

Although Table 1 shows that at all levels male animals are more sensitive to β-chloroethyl alkylating agents, only with PAM and CTX are these differences significant. Accordingly, the potency ratio (PR) and its 5% confidence limits were calculated,[3] along with similar data for the slopes (SR) of the dose–response curve. The data of Table 2 show that at its upper and lower confidence limits (5%), CTX is 1·5–1 times as toxic to males as to females; for PAM the relative potency for males ranges from 2·10–1·10 times that for females.