Further Studies on Renal Toxicity of PGA.* (20684)

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The occurrence of renal lesions in guinea pigs given folic acid (pteroylglutamic acid, PGA) parenterally has been reported previously from this laboratory(1). The present investigation was undertaken to study the possibility that photolytic products of PGA(2,3) might have been present in the solutions of PGA used and have produced these renal lesions. In addition, it was planned to test the impression that vit. C enhanced the resistance of the guinea pig to this form of renal injury.

Methods. The scorbutigenic diet and the experimental routine have been described(1). The two preparations of PGA used for injections were prepared from crystalline PGA‡ as follows: 1) Crystalline PGA was recrystallized twice from hot water, dissolved in 0.2 M phosphate buffer of pH 7.5 in a concentration of 8 mg/ml and kept in the dark. This PGA was recrystallized a third time every other day during the course of the experiment. Analyses for diazotizable amine(4) revealed a concentration of less than 0.013 mg/ml (equivalent to 0.16% PABA) 2 days after the final recrystallization. This solution will be referred to as recrystallized PGA. 2) A solution of PGA containing 8 mg/ml in the same buffer was exposed to sunlight in a clear glass bottle for several days before injections were begun and then kept in the dark. Diazotizable amine was present in this solution at a concentration of 12 mg % (1.5% as PABA). This preparation will be called irradiated PGA. Following a 10-day conditioning period on the scorbutigenic diet supplemented with 5 mg of ascorbic acid daily, the guinea pigs were divided into experimental groups receiving daily subcutaneous injections into the abdominal wall of 1 ml of a PGA preparation (8 mg PGA) or control injections of 1 ml of the phosphate buffer. Half the animals in each group were given 2 mg of ascorbic acid daily and this vitamin was withheld from the remainder. These guinea pigs weighed 350-450 g, and all but 3 were females. After 10 daily injections the surviving animals were killed by a blow on the head, their kidneys removed, weighed, and sections fixed in Bouin's fixative were stained with hematoxylin and eosin.

Results. Gross pathological examination revealed changes suggestive of scurvy in only one of the animals fed the scorbutigenic diet without supplementary vit. C. Scarring and hematomata were present in the abdominal walls of the animals receiving PGA in either form. An apparent increase in size of the kidneys and mottling of the surface were frequently noted in both groups receiving PGA. The number of animals surviving for 10 days, their change in weight, the weight of their kidneys, and the incidence of the microscopic changes(1) previously reported are given in Table I.

All of the animals receiving either recrystallized or irradiated PGA had microscopic renal lesions.§ The lesions produced by the irradiated PGA could not be differentiated from those produced by recrystallized PGA. No differences in the severity of the microscopic changes, weight changes or kidney weights could be attributed to the presence or absence of ascorbic acid in the diet. The animals receiving either solution of PGA lost more weight than the control group ($P < 0.05$) and their kidneys were heavier on both an absolute ($P < .001$) and relative basis ($P < .01$). The

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TABLE I. Comparison of Renal Effects in the Guinea Pig of Recrystallized and Irradiated PGA.

	Ascorbic acid, mg/day	No. of 10-day survivors/No. in group	% incidence microscopic renal lesions	Change in body wt in 10 days	Kidney wt of 10-day survivors, %/body wt	Kidney wt of all animals at death
				(g)		(g)
Controls	2	5/5	0	+ 1 ± 18	.89 ± .04	2.62 ± .15
	0	5/5	0	— 3 ± 8	1.01 ± .03	2.88 ± .09
Irradiated PGA, 8 mg/day	2	8/10	100	—32 ± 18	1.38 ± .16	3.34 ± .10
	0	7/10	100	—29 ± 19	1.15 ± .10	3.40 ± .16
Recrystallized PGA, 8 mg/day	2	6/10	100	—22 ± 12	1.29 ± .13	3.62 ± .19
	0	6/10	100	—40 ± 14	1.41 ± .14	3.52 ± .18

TABLE II. Effectiveness of Minimal Dosages of PGA in Production of Renal Lesions in the Guinea Pig.

PGA, mg/day	Renal lesions per No. of animals	6-day wt change, g
.5	2/5	+15 ± 5
1.0	1/5	+16 ± 7
2.0	1/5	— 2 ± 8

greater mortality noted in the group receiving the recrystallized PGA was not significant.

An attempt was made to determine the minimal subcutaneous dose PGA which would produce these lesions. Groups of 5 guinea pigs weighing 250-300 g received the scorbutigenic diet without added ascorbic acid and were each given 0.5, 1, or 2 mg of recrystallized PGA subcutaneously daily for 6 days and killed on the seventh day. Results are shown in Table II. Although 4 animals showed the renal lesions, they were not as pronounced as those noted in the previous experiment. The differences between the weight changes of the 3 groups were not significant. These findings indicate that the renal lesions appear regularly only when amounts of PGA greater than 2 mg per day for 6 days are administered, and that the lesions are observed regularly when the dose is 8 mg daily for 10 days. The minimal effective dose in reversing the hydroxyphenyluria in the scorbutic tyrosine-fed guinea pig lies between 2.0 and 5.0 mg of PGA per day(5). These doses of PGA, approximately 8-20 mg per kg of body weight, correspond to the amounts effective in reversing the tyrosyluria of scorbutic infants(6) or in producing hypertension in rats(7). Similar renal lesions were observed in association with the latter effect(7), although no changes in kidney weight were found. Blood pressure measurements have not been made on guinea pigs treated with PGA.

Summary. 1. The photolytic oxidation products of PGA could not be implicated in the production in guinea pigs of renal lesions by large parenteral doses of PGA. 2. These lesions were uniformly produced in guinea pigs by the daily administration of 8 mg of PGA (20 mg of PGA/kg) for periods of 6-10 days, and they occurred in occasional animals at a daily dosage of 2 mg (8 mg/kg). 3. Ascorbic acid did not appear to be a factor in the production of the lesions.

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