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Effect of Pteroylglutamic Acid on Weight and "Alkaline" Phosphatase of Kidneys of the Mouse.

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Kidney injury in guinea pigs and rabbits treated with pterolyglutamic acid (PGA) was first reported by Harned and coworkers, although PGA when given orally to rats was not harmful as judged by the normal diuretic test of Lipschitz.[1] However, these authors found a frequent precipitation of the PGA in the renal *tubuli* of the damaged kidneys. No other reports on kidney injury produced by PGA have been published so far.[2]

In the course of a toxicity study of PGA, one of us was able to find an enlargement of the kidneys in all the mice injected with PGA, even with doses far below the toxic level.[3] It has been observed that the weight of the kidneys increases markedly probably due to the precipitation of the insoluble PGA in the renal *tubuli*, which is able to induce some modifications on the enzyme content of the tissue. It was therefore of interest to investigate whether the phosphatase of the kidney was affected by injected PGA. Our first results showed that the "alkaline" phosphatase decreases even when small doses of PGA are administered subcutaneously (0.5 mg/kg). The increase of weight seems not to be due to an apparent retention of water but probably to a lowering of the excreted solids through the urine. In the present paper, the changes of the weight and of the "alkaline" phosphatase content of the kidneys after administration of low and high doses of PGA will be reported.

Material and methods. Male white mice of a homogeneous stock were used throughout this study. The animals were fasted 16 hours before autopsy and killed by exsanguination. The kidneys were immediately removed, weighed and homogenized in a mortar with distilled water. The kidneys homogenate was diluted with 40 ml of distilled water per g of tissue, according to Kochakian and Fox.[4] The samples were filtered to remove connective tissue and a final 10-fold dilution was made. King & Armstrong's method using disodium phenylphosphate as a substrate was used with slight modifications.[5] Blank determinations were run simultaneously, the incubation period being omitted. The results were expressed in units of "alkaline" phosphatase activity, one unit being equivalent to 1 mg of phenol liberated by hydrolysis. The disodium phenylphosphate solution was prepared each 2 days and stored in the ice box. In our experience older solutions give higher blanks and erratic values.

The age of the mice being a factor influencing the phosphatase content of the kidney, as showed by Kochakian and Fox, mice were selected having the same limit of age and weight and submitted to a standard diet. Adult mice weighing 20 to 22 g (65 to 90 days old) were used. Normal kidney weight and "alkaline" phosphatase values from 54 well fed mice are summarized in Table I.

Different groups of mice were injected subcutaneously with graded doses of PGA and animals killed 24 hours after the injection. The LD_{50} for this drug given intraperitoneally was found to be 600 mg/kg.[1] In our experience the dose of 375 mg/kg killed 50% of the animals when injected subcutaneously. Therefore, we used doses far below this limit to avoid acute intoxication. No death occurred in all the mice studied except for

[1] Harned, B. K., Cunningham, R. W., Smith, H. D., and Clark, M. C., *New York Acad. Sci.*, 1946, **48**, 289.

[2] Jukes, T. H., and Stokstad, E. L. R., *Physiol. Rev.*, 1948, **28**, 51.

[3] Villela, G. G., *Arch. Biochem.*, 1947, **15**, 157.

[4] Kochakian, C. D., and Fox, P., *J. Biol. Chem.*, 1944, **153**, 669.

[5] King, E. J., Micro-Analysis in Medical Biochemistry, London, 1946, J. and A. Churchill, Ltd., p. 57.

TABLE I.
Average Values for Normal Mice and Mice Injected with PGA.

No. of mice	PGA mg/kg	Wt of kidneys, mg	σ	S.E.	Alkaline phosphatase in units					
					Total		S.E.	per g		S.E.
31	0.5-5.0	292	14.3	3.97	104	21.5	5.96	367	75.9	2.10
57	25-500	416	11.2	1.67	61.5	17.3	2.58	146	14.5	2.16
54	Controls	269	29.4	13.36	127	21.3	9.68	469	19.4	8.81

σ = standard deviation = $\sqrt{\frac{\Sigma(x-m)^2}{n-1}}$ S.E. = standard error = $\frac{\sigma}{\sqrt{n}}$

those few injected with the dose up to 375 mg/kg.

Since a single dose of 0.5 mg/kg has only a slight effect, we tried repeated doses to know if a cumulative effect could be obtained. Five mice weighing 20-21 g were injected daily with 0.5 mg/kg until a total amount of 5 mg/kg was attained. The average weight of the kidneys was 331 mg and the "alkaline" phosphatase values were respectively 100 and 312 units for the total kidneys and per g of tissue. These results show that repeated doses of PGA produce a cumulative effect probably due to the slow rate of PGA excretion through the kidneys. The increase of weight of the kidneys is not related to the water content since the average mean values for the normal and injected mice were not significant. In one experiment 20 normal mice were used and 20 others were injected with 0.5 mg/kg to 25 mg/kg; the results for the first group showed 76.3 $\pm$ 0.3% of water and for the second group 75.4 $\pm$ 0.8%.

The inspection of the Table I demonstrates the effect of PGA on the weight and "alkaline" phosphatase of the kidneys. The difference between the means of the injected and non-injected animals is higher than twice the standard error of the mean for all groups analysed. These results are therefore statistically significant.[6] Even if the group injected with small doses is considered a significant value is obtained.

Summary. PGA when administered subcutaneously to normal mice in a single injection, produces after 24 hr an effective increase of the total weight and a decrease of the "alkaline" phosphatase of the kidneys. Normal values based on 54 mice are reported and compared with those obtained from 88 mice injected with graded doses of PGA (0.5 mg/kg to 500 mg/kg of body weight). The "alkaline" phosphatase was determined by a slight modification of the colorimetric method of King and Armstrong using disodium phenylphosphate as a substrate. The retention and precipitation of PGA in the renal *tubuli*, could be responsible for the increased weight and decreased phosphatase activity of the kidneys. Further experiments to determine the mechanism of action of PGA on kidney phosphatase are in progress and will be reported in the near future.

[6] Fisher, R. A., Statistical Methods for Research Workers, London, 1934, Oliver and Boyd, p. 112.