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Kidney Alkaline Phosphatase of Rats Following Alloxan Induced Diabetes and Acute Hypo- and Hyperglycemia.

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It has been assumed that kidney phosphatase hydrolyzes a phosphate ester of glucose during the reabsorption of sugar from the glomerular filtrate. The following experiments were undertaken to determine whether the quantity of alkaline phosphatase of the kidney cortex, located principally in the proximal convoluted tubules, could be altered by varying the amount of glucose available in the tubules for reabsorption. Alterations in the glucose concentration of the body fluids, and presumably of the glomerular filtrates, were achieved in 3 ways.

One group of rats was made diabetic with alloxan and studied at intervals up to 6 weeks after administration of the drug; another group was made hypoglycemic with insulin; and a third group was made hyperglycemic by glucose administration.

The alkaline phosphatase activity was determined on an extract of renal cortical tissue, and the kidneys were examined for alkaline phosphatase content histochemically. A control series of rats was similarly studied.

Experimental procedure and methods. Rats of 4 different strains (Long-Evans, Hisaw, Wistar, and mixed) and of both sexes were used. No variation attributable to these factors was found. Blood sugar analyses by the micromethod of Folin[1] were made on many of the animals at the time they were sacrificed. In addition, determinations of reducing substances in the urine were carried out on most of the diabetic rats and on those given large amounts of glucose.

Seven rats constituted the control series. The experimental animals comprised 3 groups: *Group 1—Rats with alloxan diabetes.* Seven rats were given one dose of 200 mg/kg of alloxan intraperitoneally. Subsequently, some were maintained on 2 to 3 units of insulin daily up to 3 to 4 days before the conclusion of the experiments. The others were given no insulin. *Group 2—Hypoglycemic rats.* Two rats were given 6 units per kilo of regular insulin and 5 units per kilo of protamine insulin. The doses were repeated after 3½ hours. The animals went into collapse 4 hours after the first dose and frequent hypoglycemic convulsions followed. The experiment was terminated 2 to 3 hours later. *Group 3—Hyperglycemic rats.* Four rats were given 7.5 g of glucose over a 12-hour period by subcutaneous injections at 2-hour intervals of a 10% solution of glucose in modified Ringer's solution (composition: 100 ml of water, 10 g glucose, KCl 0.5 mM, $CaCl_2$ 0.1 mM, $NaHCO_3$ 2.5 mM, NaCl 8.0 mM).

Method for determining alkaline phosphatase activity. The activity of the alkaline phosphatase of the kidney cortex was determined on an extract of the dried tissue, and the results were expressed as milligrams of phosphorus hydrolyzed per hour per g of dried kidney weight. β-glycero-phosphate was used as the substrate, without magnesium according to Bodansky's method,[2] and also with magnesium added as activator.

Preparation of the extract. The method of Fischer[3] was adopted with some modification as appears below. Fresh renal cortex was snipped into small pieces and dried overnight in a vacuum desiccator over NaOH. The tissue was then pulverized in a mortar and an aliquot dried in an oven at 110°C

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1 Folin, O., and Svedberg, A., *J. Biol. Chem.*, 1930, **88**, 85.

2 Bodansky, A., *J. Biol. Chem.*, 1933, **101**, 93.

3 Fischer, Clary J., unpublished data.

TABLE I.
Kidney Alkaline Phosphatase Determinations in Normal Rats and in Alloxan Diabetes. Results Expressed as mg Phosphorus Released per Gram Tissue Solids per Hour.

Normals		Alloxan diabetics			
Rat No.*	mg P†	Rat No.*	Time after alloxan, days	Blood sugar, mg %	mg P†
1a	26	8a	7	—	2.0
2	25	8b	7	—	1.7
3	17	9a	11	—	5.1
4a	62	10	12	500	1.9
4b	66	11‡	21	—	27-48
5a	33-45	12‡	21	602	10-14
5b	47-66	13‡	21	612	12-21
6	22-34	14a	42	764	12-17
7	27-45	14b	42	764	13-17

* Nos. with *a* or *b* indicate assays of individual kidneys. Other numbers refer to pooled kidney analyses.

† Where 2 figures are given, the first is the result obtained without added Mg; the second with added Mg. Where only one figure is given, no Mg was added.

‡ Animals 11, 12, and 13 received no insulin. Others of this alloxan-treated group received 2-3 units per day until 3-4 days before animals were sacrificed for analysis.

TABLE II.
Rat Kidney Alkaline Phosphatase Determinations in Experimental Hypo- and Hyperglycemia Expressed as mg P Released per g Tissue Solids per Hour.

Hypoglycemics			Hyperglycemics		
Rat No.*	Blood sugar, mg %	mg P†	Rat No.*	Blood sugar, mg %	mg P†
15a	33	26-50	17a	800	25-45
15b	33	32-49	17b	800	23-38
16	40	26-41	18	1024	23-37
			19	888	23
			20a	812	27-40
			20b	812	27

* and † have the same meaning indicated in Table I.

to provide data for calculating the dry weight of the portion extracted. Aliquots weighing 30 to 80 mg were extracted for 24 hours at 4°C in 5 ml distilled water. The extract used for phosphatase assay was freed of the residue by centrifugation.

Incubation of enzyme with substrate. The substrate consisted of sodium β-glycero-phosphate (concentration 0.5%)[4] at a pH of 8.6 with and without the addition of 0.009 M $MgCl_2$. The incubation of the filtrate with the substrate and the determination of the inorganic phosphorus liberated were carried out according to the procedure described by Shwachman.[4] In the present study, the amount of phosphorus hydrolyzed was related to the dry weight of the tissue.

The average variation in phosphorus liberated by different aliquots of the same enzyme sample for 13 consecutive pairs of samples was 4%; the range for all but one was 0-6%, the exception being 14%. The average variation between the extracts of the 2 kidneys when determined separately was 11.9% for 7 pairs, with a range of 0.8 to 25%. The values of phosphatase activity obtained with added magnesium plotted against the values without magnesium showed a linear relationship, corresponding to the

$$\text{ratio } \frac{\text{activity with Mg}}{\text{activity without Mg}} = 1.7.$$

Histochemical methods employed. After fixation of the tissues in cold 80% alcohol and subsequent imbedding in paraffin, the

4 Shwachman, H., *J. Ped.*, 1941, **19**, 38.

sections were incubated at 37°C in glycerophosphate at pH 9.4 for 3 and 6 hours according to Dempsey and Deane's modification of Gomori's method.[5] Hematoxylin eosin preparations were also made from the same blocks.

Results. The results on the normal and alloxan diabetic rats are presented in Table I, and those on the hypo- and hyperglycemic rats in Table II. The normal rat kidneys released an average of 36 mg P per g of tissue per hour. When the abnormally high results on the kidneys of rat No. 4 were excluded, the average was 28 without Mg, and 47 with Mg. All but one of the alloxan diabetic rats had abnormally low phosphatase activity. Those sacrificed 7 to 12 days after alloxan administration had activities of 5 or less; 3 of the 4 rats allowed recovery periods of 21 to 42 days had activities of 10-13 without Mg, and 14-21 with Mg. Rat No. 11 had a normal phosphatase activity 21 days after the alloxan administration. All of the rats whose blood was analyzed showed marked hyperglycemia.

Neither hypoglycemia nor hyperglycemia, maintained for the relatively short periods of our experiments, had any demonstrable effect on the phosphatase activity of the kidneys (Table II).

There were no qualitative histochemical differences in alkaline glycero-phosphatase between the kidneys of normal and of acutely hyperglycemic and hypoglycemic animals. However, in the rats given alloxan 1 to 6 weeks before autopsy, there was a greatly reduced amount of phosphatase in the epithelium of groups of proximal convoluted tubules in which the cells were flatter and less eosinophilic than average. These tubules were particularly numerous in the subcapsular region and unquestionably represented regenerated tubules that had replaced those damaged by alloxan. In addition, small formless intratubular masses were present in the medulla. These masses were stained by hematoxylin and were frequently covered by epithelium. They appeared to consist of necrotic and calcified clumps of epithelium and were more common in the kidneys of rats killed soon after receiving the dose of alloxan than in rats killed later.

A recent paper[6] reports a drop in the phosphatase content of the kidney in 6 to 72 hours after administration of alloxan, which it ascribes to renal damage. Bennett and Behrens[7] have shown that the elevation in non-protein nitrogen following alloxan is roughly proportional to the dose, is independent of hyperglycemia, is not altered by insulin, and may persist for as long as 31 to 34 days after administration of the drug; and further, that it is correlated with the "histopathological" changes in the kidneys. Breedis, Florey and Furth[8] have reported that, following administration of a nephrotoxic agent, such as uranium nitrate, the reappearance of phosphatase in the regenerating tubules is slow, not attaining a normal value in 25 days. In the present study, 1 of 3 rats exhibited a normal amount of phosphatase after 21 days, a fourth examined after 42 days still showed a low phosphatase activity.

Summary. 1. The alkaline phosphatase activity of the rat kidney, which reflects principally the enzyme content of the proximal convoluted tubules, could not be changed from the normal by varying the amount of glucose for reabsorption over periods of 6 to 12 hours.

2. The kidneys of rats suffering from chronic alloxan diabetes as a rule contained less than normal amounts of alkaline phosphatase. The lowest values were obtained when first examined one week after the dose of alloxan, but recovery of a normal content was not complete when the last examination was performed after 42 days. The proximal convoluted tubule epithelium that had regenerated after the alloxan injury was thus observed to be slow in acquiring its normal enzyme content.

[5] Dempsey, E. W., and Deane, H. W., *J. Cell. Comp. Physiol.*, 1946, **27**, 159.

[6] Menten, M. L., Janouch, M., PROC. SOC. EXP. BIOL. AND MED., 1946, **63**, 33.

[7] Bennett, L. L., and Behrens, T., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 5.

[8] Breedis, C., Florey, C. M., and Furth, J., *Arch. Path.*, 1943, **36**, 402.