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STUDIES IN EXPERIMENTAL DIABETES.

PART IV.

TISSUE PHOSPHATASE ACTIVITY IN PROTECTED AND IN ALLOXAN-DIABETIC RATS.

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INTRODUCTION.

CANTOR *et al.* (1947, 1948) found increased serum alkaline-phosphatase activity in alloxan diabetic rats. Significant increase of alkaline phosphatase activity of liver in well-established alloxan diabetic rat was observed by Drobkin and Marsh (1947). Decreased alkaline phosphatase activity of the kidney in alloxan diabetic rat was observed by Menten and Janouch (1946), Burgen and Lorch (1947), Baunting (1948), Soulairac (1948) and by Arvy and Gabe (1949). In pancreatectomized diabetic rats decreased alkaline-phosphatase activity of kidney was observed by Cardeza *et al.* (1949). The above findings show that diabetogenic action of alloxan does not affect the alkaline phosphatase activity in different tissues in the same way. Potter and Simonson (1951) had shown that an inhibitor which completely inhibits an enzyme in one tissue may not affect the enzyme in other tissues to the same extent. Glutathione (Lazarow, 1946), glucose (Sen and Bhattacharya, 1952), and sodium-β-glycerophosphate (Mukherjee *et al.*, 1955) have been shown to prevent the diabetogenic action of alloxan. Further, glutathione (Thanhauser *et al.*, 1937) and sodium-β-glycero-phosphate (Velazquez *et al.*, 1496) have been shown to cause the lowering of serum-phosphate activity. With a view to see whether alteration, if any, of the alkaline-phosphate activity occurs in the tissues of alloxan diabetic rats and of the rats protected from the diabetogenic action of alloxan by previous administration of glucose and glycerophosphate, the present work was taken up.

Materials and methods.

Adult albino rats, of Central Drug Research Institute colony, approximately of same weight, were selected. The animals were kept under observation on stock diet for 12 days. Overnight fasting blood sugar was estimated in all the animals. Individual animals were kept in separate metabolic cage during experiment. Alloxan, 40 mg./kg., was injected intravenously to one group of animals. Sodium-β-glycerophosphate, 2 g./kg., and glucose 1 g./kg., both in 25 per cent solution were injected to two separate groups of animals. Some of the animals from each of the above two groups were kept as control. To the rest of the glucose and glycerophosphate-administered animals were given alloxan, 40 mg./kg. intravenously at the end of 3 minutes after glucose and at the end of 5 minutes after sodium-β-glycerophosphate. All the injections were given in overnight fasting condition. Blood for sugar- and alkaline-phosphatase estimation was collected from tail vein and by heart puncture, respectively. The animals were then sacrificed by decapitation and exsanguination in batches, at the end of 18 hours and 7 days of experiment. Kidney, liver and pancreas were dissected out and a weighed portion of each of the tissues was extracted according to the method of Shinowara *et al.* (1942). Blood sugar was estimated by Folin and Wu (1920) micromethod. Alkaline-phosphatase activity in serum and tissue extract was estimated by the method of King and Armstrong (1934). Tissues were examined histochemically for alkaline phosphatase by the method of Gomori (1946).

Results.

Table 1.

Alkaline-phosphatase activity in the tissues and blood of normal rats and of rats after injection of glucose and sodium-β-glycerophosphate.

Number of animals.		Phosphatase activity in K and A unit*.				Blood sugar in mg. per 100 c.c.,
		Per g. of wet tissue:			Per 100 c.c. of serum.	
		Kidney.	Liver.	Pancreas.		
12	Normal ...	80±2·5	0·81±0·0006	3·78±0·17	114±4·8	136±3·3
6	18 hour after glucose	82±1·3	0·80±0·0004	3·58±0·18	110±2·6	130±4·6
6	18 hour after G.P.†	53±0·32	0·79±0·004	2·21±0·42	83±3·8	150±2·7

* K and A units=King and Armstrong units. †G. P.=Sodium-β-glycerophosphate.

Eighteen hour after administration of glucose alone, practically no change of phosphatase activity from normal is observed in the tissues studied. But 18 hours after administration of sodium-β-glycerophosphate the serum, kidney and pancreatic-phosphatase activity diminishes significantly, liver phosphatase activity remains almost the same as normal.

TABLE II.

Alkaline-phosphatase activity in the tissues and blood of rats after alloxan injection.

Number of animals.		PHOSPHATASE ACTIVITY IN K AND A UNITS*:				Blood-sugar mg. per 100 c.c.
		Per g. of wet tissue.			Per 100 c.c. of serum.	
		Kidney.	Liver.	Pancreas.		
12	18 hours after alloxan	50·89±3·8	1·74±0·07	3·58±0·22	65±2·4	424±41·2
10	7 days after alloxan	78·89±0·94	1·63±0·05	3·34±0·31	124·±2·6	540±19·3

Eighteen hours after alloxan administration kidney and serum-phosphatase activity show marked fall; on the other hand, liver-phosphatase activity is significantly increased above normal. Pancreatic-phosphatase activity does not show any appreciable change from normal. On the 7th day after alloxan, the serum-phosphatase activity exceeds the normal level and kidney-phosphatase activity returns almost to normal level, whereas liver-phosphatase activity is maintained almost at the same high level as observed at 18 hours after alloxan. No significant change from normal is observed in pancreatic-phosphatase activity.

TABLE III.

Alkaline phosphatase activity in tissues and blood of rats protected from diabetogenic action of alloxan.

Number of animals.		PHOSPHATASE ACTIVITY IN K AND A UNITS:*				Blood-sugar in mg. per 100 c.c.
		Per g. of wet tissue.			Per 100 c.c. of serum.	
		Kidney.	Liver.	Pancreas.		
12	Protected by glucose 18 hours after	42·69±1·6	1·11±0·06	2·29±0·09	80·6±1·52	110·±6·04
14	Protected by G.P† 18 hours after	45·45±1·6	1·15±0·05	1·37±0·17	90·0±1·6	150±3·7

*K & A units = King and Armstrong units. †GP = Sodium-β-glycerophosphate.

Results of Table III show that maximum diminution of kidney phosphatase occurs at the end of 18 hours in both glucose- and glycerophosphate-protected animals. Pancreatic-phosphatase activity is also significantly diminished (being more marked in glycerophosphate-protected animals). Serum-phosphatase value also remains lower than normal. Liver-phosphatase activity though remains higher than in normal shows lower value than that of 18 hours after administration of alloxan (Table II).

The results of 7 days after glucose and glycerophosphate administration and in protected animals do not differ materially from those of normal and, therefore, have not been shown separately.

The results of histological studies of phosphatase activity in different tissues are parallel to the values obtained by biochemical technique. As no significant difference could be detected in the phosphatase activity of liver and pancreas of different groups of animals, in the photomicrographs, these figures are not reproduced. Significant difference is evident in the photomicrographs of the kidney which have been represented in Plate XXVIII.

Discussion.

Alloxan, which is excreted through the kidney, has been shown by Menten and Janouch (*loc. cit.*) to exert toxic action on the kidney phosphatase enzyme. Baunting (*loc. cit.*) observed that the return of kidney-phosphatase activity is delayed after alloxan by more than seven weeks. But though in our experiments kidney-phosphatase activity remains lower than normal, it shows a tendency to return towards normal by the end of 7 days. This difference between the observations of Baunting and those recorded in this Laboratory is probably due to the difference in the route of administration of alloxan. Baunting used subcutaneous route, while our observation has been made on intravenous administration. The excretion of alloxan will be delayed when administered subcutaneously than when administered intravenously. Therefore, the toxic action of alloxan on subcutaneous administration will be prolonged on kidney phosphate enzyme and consequently its return to normal level will be delayed. In glucose and glycero-phosphate-protected animals though the lowering of kidney phosphatase activity is enhanced by the end of 18 hours, the return to normal is completed by 7 days. Glycerophosphate alone has been found to causel owering of kidney phosphatase activity in normal rats. This finding, though interesting, cannot be explained without further study.

Increased liver-phosphatase activity in well-established alloxan diabetes has been observed by Drabkin and Marsh (*loc. cit.*). Though in the present experiment the liver phosphatase activity in protected animals remains at a slightly higher level at the end of 18 hours as compared to normal, yet it is at a much lower level than that of alloxan-diabetic animals. The return of liver-phosphatase activity in protected animals to normal is completed within 7 days. This shows that glucose and glycerophosphate, though could not completely prevent the action of alloxan on liver phosphatase, arrest its action within 18 hours and bring down the liver phosphatase activity to normal within 7 days.

Liver is an important organ being necessary for maintaining a normal blood sugar level and for this an equilibrium between the process of phosphorylation and dephosphorylation is essential. In all types of diabetes, it is found that there is an increased dephosphorylation due to increased phosphatase activity. From our data it is evident that alloxan by increasing the phosphatase activity also acts in a similar way. On the contrary, protective substances, such as glucose and sodium-β-glycerophosphate by arresting the rise of phosphatase activity prevent the increase of blood sugar level.

Serum-phosphatase activity in alloxan diabetic rats shows an initial fall at the end of 18 hours, but at the end of 7 days it exceeds the normal level. This initial fall and subsequent rise in serum-phosphatase activity after alloxan has also previously been observed by Cantor Tuba and Capsey (1947).

FIG. A. Section of kidney from a normal animal showing alkaline-phosphatase activity. ×30.

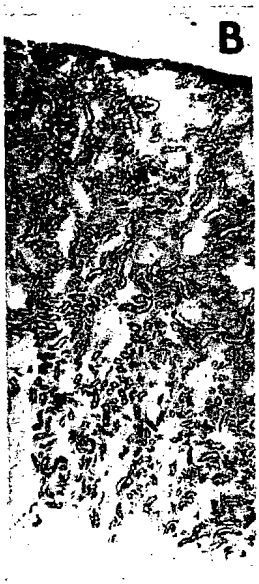

FIG. B. Section of kidney from an animal sacrificed 18 hours after a diabetogenic dose of alloxan. Considerable inhibition of phosphatase activity is evident. ×30.

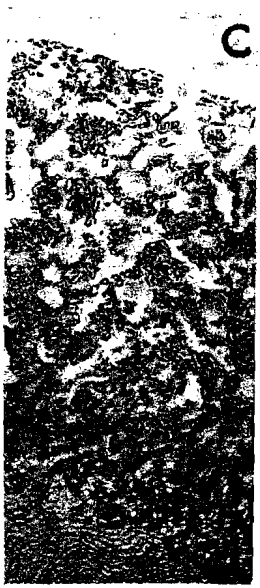

FIG. C. Section of kidney from an animal sacrificed 7 days after a diabetogenic dose of alloxan, showing slight increase of alkaline-phosphatase activity as compared to Fig. B. ×30.

FIG. D. Section of kidney from a glucose-protected animal sacrificed 18 hours after the experiment, demonstrating maximum inhibition of phosphatase activity. ×30

FIG. E. Section of kindey from a sodium-β-glycerophosphate protected animal, sacrificed 18 hours after the experiment showing inhibition of alkaline-phosphatase activity, lesser in magnitude compared to Fig. D. ×30.

In the present work, it has been observed, that in sodium-β-glycerophosphate administered normal and in glucose and sodium-β-glycerophosphate protected animals serum phosphatase activity is significantly inhibited. Sodium-β-glycerophosphate not only inhibits phosphatase activity but it causes hypoglycemia in rabbits (Fernendez and Aguilar, 1950). According to them this hypoglycemia is due to increased glycolysis resulting from an increased transport of phosphate group. Therefore, it appears, that inhibition of phosphatase activity may augment phosphorylation to produce a desired physiological effect. Insulin, which produces blood sugar level (possibly one of the mechanism being increased rate of phosphorylation) has been shown by Drabkin and Marsh (*loc. cit.*) and Cantor, Tuba and Capsey (*loc. cit.*) also to inhibit phosphatase activity.

At present we cannot fully explain the significance of the alteration in the pancreatic-phosphatase activity as obtained from the data. It is, however, apparent that the protective agents cause a definite inhibition of the phosphatase enzyme in the pancreas, whereas no such inhibition is observed in case of alloxan-diabetic animals. Although no definite conclusion can be arrived at present as to how sodium-β-glycerophosphate and glucose prevent the diabetogenic effect of alloxan, it is apparent that by a generalized reduction of phosphatase activity, metabolic phosphorylation may be enhanced indirectly leading to prevention of diabetes from the action of alloxan. Further work is in progress to study the hexokinase activity under similar experimental conditions as stated in this paper to see whether any relationship, exists between it and phosphatase activity, and which can be correlated to the mechanism of such protection.

Summary.

1. Alkaline-phosphatase activity in kidney, liver, pancreas and serum has been studied in alloxan diabetic rats and in rats protected from diabetogenic action of alloxan by administration of glucose and sodium-β-glycerophosphate prior to alloxan.

2. Lowering of phosphatase activity of kidney and serum, increase in phosphatase activity of liver and no change of pancreatic-phosphatase activity occur in alloxan diabetic animals at the end of 18 hours after alloxan. By the end of 7 days, kidney-phosphatase activity approaches normal level, serum-phosphatase activity exceeds the normal level and liver-phosphatase activity maintains the same high level as at the end of eighteen hours after alloxan. No change occurs in pancreatic phosphatic activity.

3. In 'protected' animals, alkaline-phosphatase activity of kidney, serum and pancreas remains lower than normal at the end of 18 hours. Though liver-phosphatase activity remained higher than normal, it is still lower than that of diabetic animals at the end of same period. By the end of 7 days the phosphatase activity in all the tissues of protected animals returns to normal.

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