EFFECT OF ALLOXAN-DIABETES ON THE GLUCOSE-ATP PHOSPHOTRANSFERASE ACTIVITY OF ADIPOSE TISSUE

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Renold (1965) and Crofford & Renold (1965) have recently reviewed the evidence which shows that the rate-limiting step in glucose utilization by adipose tissue is the entry of glucose into the adipose cell. It appears that the phosphorylating capacity of this tissue is ordinarily in excess of the rate at which glucose can be supplied to the intracellular space. On the other hand Katzen & Schimke (1965) have drawn attention to the existence of multiple types of hexokinase in a number of tissues, including epididymal fat pad. One of these types, designated by these authors hexokinase type II, is present in high concentration in those tissues which show a marked insulin effect, whereas those tissues which do not show this effect contain largely type I. Further, they have shown that the proportions of the different hexokinase vary with the physiological state and, in particular, that hexokinase II decreases in adipose tissue on starvation.

Glucose utilization is strongly depressed in adipose tissue of diabetic rats (Hausberger et al., 1954; Milstein, 1956; see Masoro, 1962). This may be due to a failure of the transport mechanism or to a decline of the glucose-ATP phosphotransferase. In the light of the above findings it seemed important to investigate the total glucose phosphorylating capacity of this

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Table 1. EFFECT OF ALLOXAN-DIABETES ON THE GLUCOSE-ATP PHOSPHOTRANSFERASE ACTIVITY OF ADIPOSE TISSUE

	Control rats	Alloxan-diabetic rats	Fisher's 'P'
No. of animals	7	15	
Final body weight (g)	235±45	230±40	
Epididymal fat pad weight (mg)	862±65	258±59	<0.001
Protein content of epididymal fat pad (mg)	9.2±0.9	4.7±0.7	<0.001
Glucose-ATP phosphotransferase			
Units*/fat pad	210±45	50±10	0.002
Units*/mg protein	22± 3	12± 1	0.006

* The results are expressed as mμ moles glucose-6-phosphate formed/min at 25° either per epididymal fat pad or per mg protein contained in the supernatant fraction after centrifugation at 100,000g for 45 mins. The enzymes were estimated by following the rate of formation of NADPH at 340 mμ in the presence of excess purified glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, the optical density readings being halved to correct the results to mμ moles glucose-6-phosphate formed. The activity was determined essentially according to the method of Sharma, Manjeshwar & Weinhouse (1963) with a final glucose concentration of 10mM because of the relatively high k_m of adipose tissue hexokinase (DiPietro, 1963; Katzen & Schimke, 1965). The results are given as means ± S.E.M.; Fisher's 'P' values are given and are regarded as significant if 'P' is less than 0.05. The rats, male albinos, were used 21 days after the induction of diabetes by a single subcutaneous injection of 20 mg alloxan/100g body weight.

tissue and the relative distribution of this activity between hexokinase types $\underline{\overline{I}}$ and $\underline{II}$ using starch gel electrophoresis as described by Katzen & Schimke (1965).

The total glucose-ATP phosphotransferase activity was measured using a dialysed high speed supernatant preparation essentially according to the method of Sharma, Manjeshwar & Weinhouse (1963). The total glucose phosphorylating capacity per epididymal fat pad was decreased in the diabetic rats to a quarter of the control values whereas the protein content had

fallen to half. Thus, the total glucose-ATP phosphotransferase activity was significantly lower in the diabetic animals when considered either as activity per fat pad or per mg. tissue protein (see Table 1).

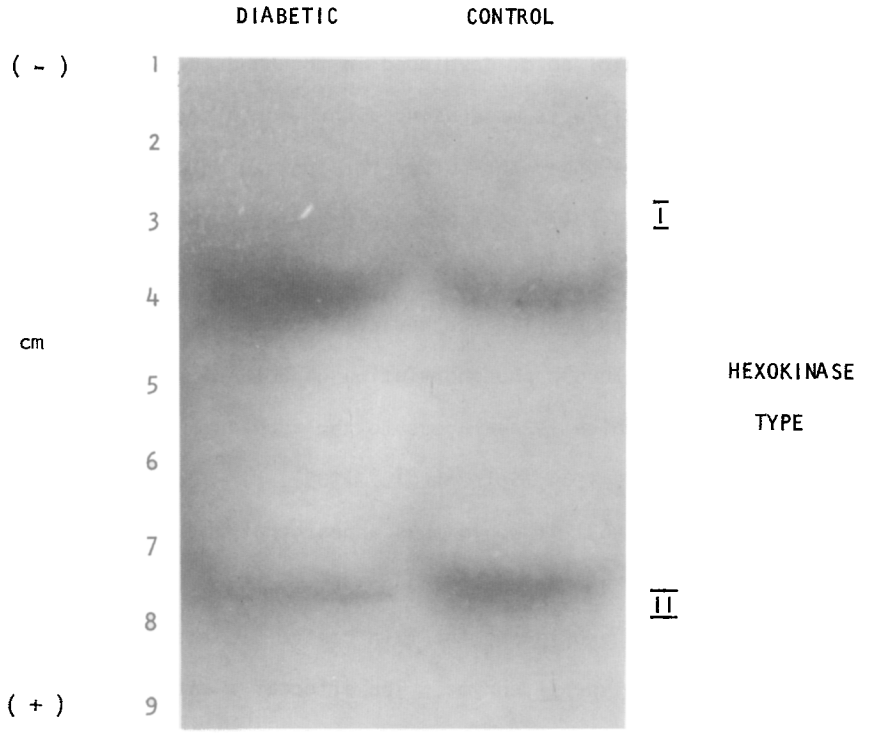

Fig. 1. Starch gel electrophoresis of hexokinase activity in extracts of epididymal fat pad from normal and alloxan-diabetic rats.

Horizontal starch gel electrophoresis (Smithies, 1955) was carried out at 2° for 16 hr. using 10% starch gels and a discontinuous buffer system (gel buffer, 0.02 M sodium barbitone buffer pH 8.6 containing 1 x 10^{-3} M Na_2 EDTA and 5 x 10^{-3} M mercaptoethanol; electrode buffer, 0.06 M sodium barbitone buffer pH 8.6 containing 1.7 x 10^{-3} Na_2 EDTA and 5 x 10^{-3} M mercaptoethanol). Gels were sliced and stained as described by Katzen & Schimke (1965), using a glucose concentration of 10 mM. Epididymal fat pads from control or diabetic rats were homogenised in a medium containing 150 mM KCl/5 mM $MgCl_2$/5 mM EDTA/10 mM mercaptoethanol and adjusted to pH 7.4 with $KHCO_3$. These preparations were centrifuged at 100,000 g. average for 30 min. The supernatant fraction was dialysed against the same extracting medium for 1 hr. and this preparation used for enzyme determination and electrophoresis. The volume of medium used for homogenising was adjusted so that the final protein concentration of the control and diabetic tissue extracts were approximately the same, these were 7.0 and 10.0 mg. protein/ml. extracts respectively. The protein was applied to the starch gel by means of a filter paper pad (Whatman No. 17), approximately 0.1 ml. of each extract was used.

The dialysed high speed supernatant fraction from epididymal adipose tissue was used for the separation of the glucose-ATP phosphotransferase enzymes by starch gel electrophoresis essentially as described by Katzen & Schimke (1965). As shown in Fig. 1, there was a greater loss of hexokinase type II than of hexokinase type I in the diabetic rats. Measurements of the intensity of color developed in the bands corresponding to hexokinase type I and II were made using a Chromoscan (Joyce, Lobel & Co.). Comparison of the areas under the curves for the two enzymes showed that while the control had a ratio of 1:0.8 for hexokinase type I to hexokinase type II, this ratio was 1:0.5 for the alloxan diabetic rats.

The present results provide further evidence that adipose tissue has an enzyme system for glucose phosphorylation capable of responding to physiological change which is analagous to the adaptable glucokinase system of liver (Moore et al., 1964; Sols et al., 1965). In accord with the views of Katzen & Schimke (1965) it would thus appear that adipose tissue may adapt to physiological and hormonal changes by modification of the proportions of type I and type II hexokinase, the main control in the case of diabetes being exerted via the type II enzyme. The effector agent could then well be the insulin level since type II also decreased in starvation (Moore et al., 1964; Katzen & Schimke, 1965) and diabetes and starvation have in common a marked decrease of insulin secretion. Such a mechanism has, in fact, been proposed by Sols et al., (1965) for the control of liver glucokinase.

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