EFFECT OF INSULIN ON PYRUVATE CARBOXYLASE IN ALLOXAN DIABETIC ANIMALS

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Increased gluconeogenesis in experimental diabetes and after cortisol administration is coupled with an accelerated incorporation of CO_2 and pyruvate into glucose (ASHMORE et al, 1956; WAGLE and ASHMORE, 1961). The first indications that gluconeogenesis from pyruvate is controled at the level pyruvate-oxalacetate came from studies of WAGLE and ASHMORE (1963) and HENNING et al in this laboratory (1963): incorporation of $C^{14}O_2$ into oxalacetate in the presence of pyruvate and ATP could be shown to be sevenfold increased in homogenates of diabetic livers (WAGLE and ASHMORE, 1963). Cortisol administration to rats caused a simultaneous rise of glycogen, glucose-6-phosphate and pyruvate carboxylase in liver (HENNING et al, 1963). More recently WAGLE (1964) showed that the latter enzyme is also elevated in diabetic liver.

In this paper quantitative data for pyruvate carboxylase activities in livers of normal and alloxan-diabetic rats are presented. Results about the influence of insulin upon the levels of this enzyme are reported.

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MATERIAL AND METHODS

Male Wistar albino rats were fed ad libitum on rat pellets (Altromin GmbH, Lage/Lippe). Livers were removed in light ether anaesthesia and stored at -15° C for 1-2 days. Extracts were prepared and assayed for pyruvate carboxylase as described by HENNING and SEUBERT (1964). Alloxan diabetes was produced by a single injection of alloxan at a dosage of 175 mg per kg in 48 hours fasted rats. They were used after 1-2 weeks when they displayed severe polyuria, and glucose levels from 220-600 mg per 100 ml. Insulin treated animals were used 14-16 hours after a single injection of 8 units long acting insulin (Depot-Insulin, Farbwerke Hoechst, Germany). This dosage proved to be necessary to bring blood sugar levels down to normal (60-130 mg per 100 ml). Blood glucose was determined by the method of KEILIN and HARTREE (1945).

RESULTS AND DISCUSSION

In table I the levels of pyruvate carboxylase in livers of normal, diabetic and insulin treated diabetic animals are summarized. The activities of the enzyme in diabetic animals are significantly elevated compared to controls ($p < 0,001$) and reach levels which also have been observed after cortisol treatment (HENNING et al, 1963). Insulin treatment brings the levels down to normal ($p < 0.001$).

These results keep in line with observations on other enzymes involved in gluconeogenesis. Repression of elevated enzyme levels by insulin in diabetes has been reported for glucose-6-phosphatase and fructose-1,6-diphosphatase (ASHMORE

and WEBER, 1959). The lowered extent of gluconeogenesis from glycine after insulin administration to diabetic animals is in accord with these observations (NADKARNI and CHITNIS,1963).

Table 1. Pyruvate carboxylase in livers of normal, diabetic and insulin treated diabetic rats

Controls		Diabetic		Insulin treated Diabetic	
sp.act.**	units/g* rat liver	sp.act.	units/g rat livers	sp.act.	units/g rat liver
25	3.5	45	7.45	20	2.87
30	3.6	55	5.95	35	3.25
38	4.45	68	7.8	27	3.0
40	3.7	64	6.77	21	2.0
34	3.5	50	5.45	27	1.7
21	2.33	53	5.4	34	3.25
28	2.0	45	5.9	33	3.15+
21	1.5	74	6.4	29	1.9 +
$\bar{X}$ 29±7	3.07±1.0	57±11	6.4±0.95	28±6	2.64±0.64

*One enzyme unit catalyzes the carboxylation of one μM pyruvate to oxalacetate per minute under the conditions as described by HENNING and SEUBERT (1964)

**Specific activities are expressed in mμM/min/mg protein.

+ Blood sugar levels have been lowered only from 456 to 250 and 415 to 217 mg per 100 ml, respectively.

Reduced hyperglycaemia in adrenalectomized and hypophysectomized rats compared to controls after treatment with anti-insulinserum suggests that other endocrine factors than insulin might be involved in the observed changes (STERN et al, 1963). In case that adrenal corticoid steroids are involved the early response of pyruvate carboxylase to cortisol (HENNING et

al, 1963) would favor this enzyme as one of the primary acting factors which regulate the rate of gluconeogenesis from pyruvate in diabetes. In addition a regulatory effect of acetyl-CoA as activator of pyruvate carboxylase has to be considered (UTTER and KEECH, 1963). As WIELAND and WEISS (1963) have shown this substrate is also elevated in livers of diabetic rats. The effect of insulin upon the levels of acetyl-CoA in diabetic animals is under investigation.

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