

## Insulin Clearance by the Isolated Perfused Livers of Insulin Deficient Rats

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*Summary.* The ability of the liver to metabolise insulin in response to varying blood insulin levels was studied using normally fed, starved and streptozotocin diabetic rats. Insulin clearance by the livers of fasted and diabetic rats was significantly increased compared with normal fed controls. Administration of insulin to diabetic animals prior to liver perfusion, or an increase

in the concentration of the hormone in the perfusion medium used for normal livers resulted in a significant decrease in clearance. The percentage uptake of insulin during single passage through the livers of starved and diabetic animals was significantly greater than normal.

*Key words:* Diabetes, insulin, starvation, liver.

The liver has been shown by many workers to be a major organ of insulin degradation in the body, [9, 19, 11, 16]. However, the physiological importance of this in total body insulin metabolism remains to be clarified. It has been previously demonstrated that the liver can alter its rate of degradation of corticosteroids in response to varying blood levels, [17, 22], and an autoregulatory negative feed-back mechanism has been postulated. The present investigation was undertaken to study the response of the liver to fluctuations in blood insulin levels.

### Materials and Methods

Male albino rats of Wistar strain weighing 150–200 g were used throughout and the isolated intact rat liver perfusion technique of Miller *et al.* [8] and Schimassek [14], as modified by Hems *et al.* [4], was employed. Each insulin clearance experiment was preceded by a control perfusion period of 15 min. Pork insulin (Novo, 10 times recrystallised, Lot. No. S23267) of potency 25 I.U. per milligramme was then added to the perfusate to give a final concentration of 200 microunits/ml. unless otherwise stated in the protocol. A further 6 min perfusion was allowed for equilibration [15], after which the first of a series of approximately 0.5 ml samples was taken directly from the hepatic effluent. Further samples were taken at 3, 6, 9, 14, 19, 24, and 34 min. Samples were immediately centrifuged to remove red blood cells and stored frozen at  $-20^{\circ}\text{C}$ . Insulin in the samples and in the plasma of experimental animals was measured by the radioimmunoassay technique of Yalow and Berson, [21] as modified by Herbert *et al.* [5] and Buchanan and McCarroll [2].

### Calculation of Results

The results were calculated as the percentage of maximal (initial) concentration determined 6 min after the insulin had been added to the perfusate and the

half life was determined from the linear regression equation of insulin concentration against time. No correction was made for the error introduced by the decreasing perfusate volume, as this was less than 5%.

### Experimental Protocol 1

a) One group of animals was made insulin deficient by starvation for 72 h, but allowed free access to water. At the end of the starvation period isolated liver perfusion was carried out. The livers of fully fed litter mates acting as controls were also perfused.

b) A second group of animals was made insulin deficient by intravenous injection of streptozotocin (65 mg/kg body weight) dissolved immediately beforehand in a citrate-phosphate buffer pH 4.5. At the same time rats of similar weight were put aside to act as controls. Both groups of animals were maintained on normal diets for periods varying from 2 to 6 weeks and then their livers were perfused in the fed state. Plasma glucose at the time of perfusion was measured by a glucose-oxidase method on an Auto Analyzer.

Insulin deficiency in both starved and diabetic animals was confirmed by plasma insulin estimations made at the time of perfusion.

### Experimental Protocol 2

A second group of streptozotocin-treated animals was given Protamine Zinc Insulin 10 I.U. subcutaneously twice daily for 48 h, after which time liver perfusion was carried out.

### Experimental Protocol 3

Liver perfusion studies were carried out with 1000 microunits of insulin per ml of perfusate in 8 normally fed rats.

### Experimental Protocol 4

The "capture" mechanism of the liver. Perfusion medium without added insulin was allowed to circulate

through the livers of 3 groups of rats for 15 min as follows:

- a) 5 fed rats;
- b) 5 rats which had been starved for 72 h;
- c) 5 rats which had been made diabetic by injection of streptozotocin (65 mg/kg body weight.) After this equilibration period identical perfusate to which 200 microunits of insulin per ml had been added was allowed a single passage through the livers; samples of insulin-containing perfusate were taken before and afterwards.

The effect on hepatic insulin half life of insulin administration to diabetic rats is seen in Table 2. There is a highly significant increase in insulin  $t_{1/2}$  in treated animals as compared with controls.

Table 3 shows the effect on hepatic insulin half life of increasing the concentration of insulin in the perfusion medium of normally fed rat livers. Again the insulin half life is significantly increased with increase in substrate concentration.

Table 4 shows the percentage uptake of insulin by fed, starved and streptozotocin treated livers during

Table 1. Hepatic insulin half-life in controls and insulin deficient rats. ( $M \pm SEM$ )

Group	No. of rats	Hepatic insulin $t_{1/2}$ min.	Significance of difference in half-life between control and test groups
1. Starved 72 h	7	$8.35 \pm 0.80$	$t = 7.6771$
2. Fed Control	10	$18.1 \pm 0.81$	$p < 0.0005$
3. Streptozotocin Treated	19	$8.9 \pm 0.46$	$t = 5.7453$
4. Control	19	$15.29 \pm 1.0$	$p < 0.0005$

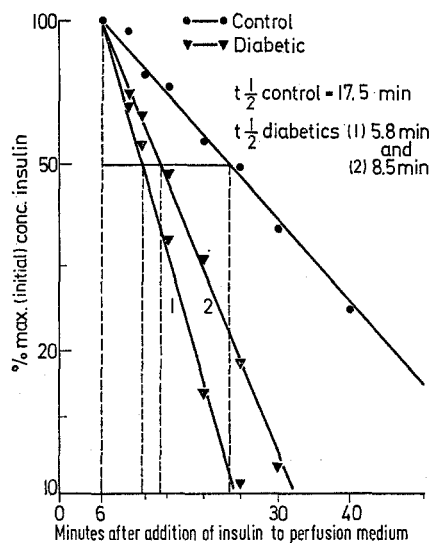


Fig. 1. Insulin decay curves for diabetic animals and control

### Results

Streptozotocin administered to rats in a dose of 65 mg/kg body weight invariably produced a severe diabetic state with wasting, polyuria and gross hyperglycaemia.

Table 1 shows the hepatic insulin  $t_{1/2}$  in starved animals, streptozotocin treated rats and normally fed controls. It will be seen that  $t_{1/2}$  is significantly shorter in the insulin deficient animals than in controls.

Fig. 1 shows two individual decay curves for streptozotocin-treated animals as compared with a control.

single passage of perfusate. There was a significantly greater uptake by the livers of both insulin deficient groups as compared with normal.

### Discussion

Insulin clearance by the livers of insulin deficient rats, whether induced by starvation or the administration of streptozotocin, was found to be significantly greater than that by controls (Table 1). These findings agree with those of others using either liver slices [10], the intact perfused liver of the alloxanised bullfrog [20], or the isolated perfused liver of the rat [7]. It has also been found that insulin clearance by the normal liver decreased significantly as the concentration of hormone in the perfusion medium was increased [7]. This observation has been confirmed in the present study. (Table 3). We have shown a similar effect following the administration of insulin to diabetic animals *in vivo* (Table 2). However, earlier work showed a decrease of "insulinase" activity in liver extracts and homogenates of liver tissue from fasted rats [1] and very recently a group using similar techniques also found a decrease in insulin degradation in insulin deficient tissue which, reverted to control levels when the concentration of hormone increased [18]. Based on these observations it is argued that a physiologically important, auto-regulatory system for insulin degradation exists in the liver [18].

The conflict in the published data can possibly be explained by the difference in the preparations used by the different workers. This is all the more likely in view of the fact that Mirsky [9 and 10] obtained two sets of diametrically opposed results by merely chang-

ing the experimental preparation. When tissue extracts or homogenates are used only enzymatic activity is studied, whereas with tissue slices or with isolated organ perfusion systems, as in the present investigation, the intact cell is studied. At least two other steps are, therefore, necessarily interposed between the union of substrate and enzyme, namely uptake or "capture" of substrate by the cells, and transcellular penetration by the hormone to reach the reaction site. The liver has been shown to remove 20 to 50% of insulin during the

for example, to the concept of renal tubular maximum (T<sub>m</sub>), or else they are actually functionally inhibited. The limitations of our method do not allow us to draw any conclusions regarding the final enzymatic step in the breakdown of insulin.

An increased rate of insulin clearance in starvation seems reasonable if one accepts the hypothesis that the insulin-fuel inter-relationship in starvation determines blood glucose levels, and is mediated via the pancreatic-hepatic axis, [3]. Accordingly, with a diminished

Table 2. *Hepatic insulin half-life in controls and insulin-treated diabetic rats ( $M \pm SEM$ )*

Group	No. of rats	Hepatic insulin min.	Significance of difference in half-life between control and treated group
1. Diabetic treated with P.Z.I.	7	35.35 ± 3.5	$t = 5.5759$
2. Control	7	17.73 ± 0.65	$p < 0.0005$

Table 3. *Hepatic insulin half-life in the normal liver in the presence of increased insulin concentration ( $M \pm SEM$ )*

Amount of insulin in perfusion medium	No. of rats	Hepatic insulin half-life min.	Significance of difference in half-life between control and test group
1. 200 uU/ml.	10	18.10 ± 1.2	$t = 11.3415$
2. 1000 uU/ml.	8	37.59 ± 2.86	$p < 0.0005$

Table 4. *Percentage uptake of insulin by fed, starved and diabetic livers on single passage ( $M \pm SEM$ )*

Group	No. of	Percentage uptake of insulin	Significance of difference in uptake between control and test groups
1. Control	5	22.2 ± 3.3	$t = 2.0670$
2. Starved	5	42.4 ± 9.1	$p < 0.05$
3. Diabetic	5	38.87 ± 2.2	$t = 4.5055$ $p < 0.0025$

first single passage through it, indicating its vast capacity for hormone uptake [6, 13]. It was concluded from studies on the "capture" mechanism of the liver that the ultimate rate limiting step in the hepatic breakdown of insulin might be its transfer from the site of "capture" to the degrading enzyme system [11]. It seems clear that intact cell preparations are unsuited to the study of enzymatic degradation, because of the interposition of these two interfering factors.

Our clearance findings favour the suggestion that "capture" or uptake of insulin by binding sites and transcellular transport of hormone, both of which are dependent on the presence of the intact cell, are more active in the insulin deficient liver. The significantly greater percentage uptake of insulin by starved and diabetic livers during a single transhepatic passage lends further support to the suggestion. Conversely, at high concentrations of substrate, either the capacity of these mechanisms is exceeded in a manner similar,

fuel supply there is decreased requirement for circulating insulin and a need for greater insulin clearance by the liver. In diabetes mellitus, however, where maximum conservation of circulating hormone might be expected, this mechanism appears inappropriate. It may possibly contribute to often massive insulin requirements observed clinically in cases of diabetic ketosis.

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