Decreased Activity of 3-Hydroxybutyrate Dehydrogenase in Diabetic Liver Mitochondria (35668)

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According to Boveris *et al.* (1), pancreatectomy decreased the rate of oxidation of 3-hydroxybutyrate by isolated rat liver mitochondria. This observation was consistent with a previous one by Schäefer and Nägel (2) showing that insulin administration to normal rats increased the activity of 3-hydroxybutyrate dehydrogenase (D-3-hydroxybutyrate:NAD oxidoreductase; EC 1.1.1.30) in liver mitochondria (LM-HBDH). The purpose of the present study was first, to confirm the eventual diminution of LM-HBDH after pancreatectomy, which was implicit in Boveris *et al.* (1) result, and second, to establish whether the growth hormone secretion of the hypophysis affects the activity of LM-HBDH in parallel with the action on carbohydrate metabolism, antagonistic to that of insulin [Houssay (3)]. On these grounds, we investigated the effect of pancreatectomy, hypophysectomy, insulin, and growth hormone on LM-HBDH in the dog. The metabolic state of animals was monitored by measuring blood sugar, ketone bodies, and free fatty acids in plasma.

Materials and Methods. Male and female dogs weighing 8–12 kg were used. The animals were separated into six experimental groups namely, (I) normal dogs after laparotomy (N); (II) depancreatized dogs, 2 days after operation ($\overline{P}$); (III) same as ($\overline{P}$) treated with insulin for 3 days after pancreatectomy ($\overline{P}$ + I); (IV) hypophysectomized dogs, 8 days after operation ($\overline{H}$); (V) same as ($\overline{H}$) depancreatized, 2 days after pancreatectomy ($\overline{H}$ + $\overline{P}$) and (VI) same as ($\overline{H}$ + $\overline{P}$) injected with growth hormone for 2 days after pancreatectomy ($\overline{H}$ + $\overline{P}$ + GH). Insulin (protamin-zinc, Lilly) was injected subcutaneously (1.0 IU/kg/day). Growth hormone (bovine; NIH-GH-B12) dissolved in saline was injected intraperitoneally (1 mg/kg/day). Surgical operations were performed under anesthesia (33 mg of sodium pentobarbital/kg). Mitochondria were isolated from samples of liver tissue (about 3 g), as described by Boime *et al.* (4) and were subjected (3 times) to freezing and thawing before measuring enzyme activities. LM-HBDH (5), protein in mitochondrial suspensions (6), blood sugar (7), total ketone bodies (8, 9), acetoacetate (8, 9), and free fatty acids in plasma (10) were determined as described in the respective references. 3-Hydroxybutyrate concentration was calculated by subtracting acetoacetate from total ketone bodies value.

Results and Discussion. Table I summarizes the results obtained: (a) Pancreatectomy reduced about 50% the activity of LM-HBDH, in good agreement with the observations of Boveris *et al.* (1). At the same time, blood alterations clearly showed the appearance of the diabetic state (Expt. II). (b) Treatment with insulin restored the level of LM-HBDH and the blood parameters to the normal values. (Expt. III). Although the number of observations was limited, the difference with respect to the $\overline{P}$-control is significant and in good agreement with the results of Schäefer and Nägel (2) in the rat. (c) Hypophysectomy decreased, to some extent, the level of LM-HBDH, but the variation was at the limit of significance; blood parameters were scarcely affected (Expt. IV. (d) Pancreatectomy after hypophysectomy produced a decrease of LM-HBDH activity, but the blood parameters were less affected than in depancreatized, normal dogs

(Expt. V). (e) Administration of growth hormone to hypophysectomized-depancreatized (Houssay) dogs produced the appearance of a typical diabetes as shown by the increase of blood sugar, free fatty acids, and ketone bodies in plasma but did not significantly affect the level of LM-HBDH (Expt. VI).

The results summarized in Table I lead to the conclusion that no clear cut antagonism occurred between insulin and the pituitary growth hormone at the level of LM-HBDH. This conclusion is supported by several facts. First, the diminution of enzyme activity after pancreatectomy was not reversed by removal of the pituitary gland, in contrast to the effect of insulin. Second, hypophysectomy did not increase the level of LM-HBDH as may be expected from the opposite actions of insulin and growth hormone on carbohydrate metabolism. Third, the injection of growth hormone to the Houssay dogs did not affect the level of LM-HBDH, in spite of the metabolic alterations that followed the hormone injection.

LM-HBDH is located in the mitochondrial cristae (11) and its high activity establishes an equilibrium between the 3-hydroxybutyrate–acetoacetate and the NADH–NAD^+ redox pairs (12, 13). The decrease in the plasma [3-hydroxybutyrate]/[acetoacetate] ratio in Expts. II and VI would reflect a decrease in the intramitochondrial [NADH]/[NAD^+] ratio in the diabetic state. A similar decrease in the ratio of the reduced vs oxidized substrates of LM-HBDH was observed in frozen liver of alloxan diabetic rats by Williamson *et al.* (14).

Summary. Pancreatectomy reversibly decreased the activity of 3-hydroxybutyrate dehydrogenase of dog liver mitochondria, while hypophysectomy or injection of growth hormone to hypophysectomized–depancreatized dogs did not significantly affect the enzyme level in parallel with the metabolic alterations induced by those conditions.

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