195. Influence of Insulin and Glucagon on the Cholesterol Synthesis in Rat Liver *in vivo* Evidence against a Rate Limiting Function of the HMG-Coenzyme A Reductase

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Summary

Physiological doses of insulin and glucagon in the range of 0.5–2.5 m unit and 0.05–2 μg respectively per 100 g body weight stimulate first and inhibit afterwards the cholesterol synthesis from labeled acetate in rat liver *in vivo*. It could be shown that these effects are not caused by a regulating influence of the HMG-coenzyme A reductase.

Reported results on the influence of insulin on the cholesterogenesis in rat liver are controversial. With liver slices of alloxan-diabetic rats, an unchanged [1] or an increased [2] [3] incorporation of labeled acetate into cholesterol was observed. Whereas the addition of insulin to liver homogenate resulted in a decrease of the incorporation of labeled acetate into cholesterol [5], no effect of insulin on the cholesterogenesis in perfused liver was seen [4]. Recently a stimulation of the β-hydroxy-β-methylglutaryl (HMG)-coenzym A reductase (E.C.1.1.1.34) of the liver after injection of insulin was reported thereby pointing to a stimulation of the cholesterol synthesis. Glucagon as such showed no effect, but injected simultaneously with insulin, it prevented the stimulation of the enzyme [6]. In all the reported experiments, the amounts of insulin and glucagon used exceeded the effective dose on the blood glucose level by more than a thousand times. The purpose of the

Table. *Influence of insulin and glucagon on the incorporation of labeled acetate into liver cholesterol* (percent of the controls)

	Time after injection and dose per 100 g body weight					
	3 h	6 h	19 h	21 h	22 h	25 h
Insulin	2.5 m unit 104%	2.5 m unit 177%	0.5 m unit 205%			1 m unit 50%
Glucagon	0.2 μg 115%	2 μg 148%		0.5 μg 83%	0.05 μg 46%	0.05 μg 52%

Each figure represents the average value of 4–5 rats after injection of 20–30 μCi 1-[^{14}C]sodium-acetate per rat.

present investigation was to study the influence of physiological doses of insulin and glucagon on the cholesterol synthesis in rat liver *in vivo*.

Results. – The table shows that insulin stimulates the incorporation of labeled acetate into cholesterol reaching a maximal value several hours after the injection of the hormone. This stimulation is followed by a significant inhibition of the cholesterol synthesis after a period of about 24 hours. Glucagon shows a similar effect, but the initial stimulation of the cholesterol synthesis seems less pronounced.

Discussion. – The observed stimulation and inhibition of the cholesterol synthesis by insulin and glucagon cannot be due to a rate limiting function of the HMG-coenzyme A reductase. A stimulating effect of insulin on this enzyme was only observed with doses of at least 3 units per 100 g body weight, its duration being inferior to 6 hours [6], whereas the incorporation of labeled acetate into cholesterol was influenced with doses in the range of 0.5–2.5 m units over a period of more than 24 hours. 200 µg of glucagon per 100 g body weight had no effect on HMG-coenzyme A reductase (6) whereas 0.05–2 µg were effective on the incorporation of acetate into cholesterol. It can be argued that the inhibition subsequent to stimulation could be caused by a reduced food intake as a consequence of the hormone injections. This possibility could be excluded by comparing body and liver weight of the rats. This ratio was not affected by the hormone injections, whereas by a fasting period of only a few hours this ratio is significantly changed by a more rapid weight loss of the liver than of the body (unpublished results). The interferences of a diurnal rhythm, whose existence has, however, been seriously put to doubt recently (7), was excluded by injecting the labeled acetate at the same time of the day for both control and experimental groups.

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Experimental procedure. – *Animals and materials.* Rats of a *Wistar-Glaxo* strain with an average weight of about 200 g were used. 1-[^{14}C]sodium-acetate (0.67 mCi/mg) from *Radiochemical Centre Amersham* was injected intraperitoneally in 0.1 ml H_2O at 8.30 a.m. The rats were sacrificed 1 h after the injection of the labeled acetate. Insulin (*Fluka* AG, Buchs SG, Switzerland) and glucagon (*Eli Lilly* & Co., Indianapolis USA) were injected intraperitoneally, dissolved in 0.1 ml H_2O.

Isolation procedure. The unsaponifiable of the pooled liver of groups of 4–5 rats was submitted to TLC. (silica gel) and the cholesterol extracted for radioactivity measurements. Details of the procedure are described elsewhere [8].

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