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PRELIMINARY COMMUNICATIONS

Stimulation by Insulin of the Incorporation of U^{14}C-Glucose into Lipids Released by the Liver

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Summary. In a new incubation system, liver slices from rat have been shown to incorporate glucose into lipids, which are released into the incubation medium. This synthesis was stimulated by insulin with a proportionally more marked increase in esterified fatty acids than in free fatty acids.

In order to determine, *in vitro*, what is the action of insulin on the fate of exogenous glucose entering the liver, we have measured the release of labelled lipids by liver slices incubated with radioactive glucose (U^{14}C-glucose).

Methods

The liver slices were studied in the "perifusion" apparatus described previously [4]. Liver from 100 g fed male rats were used throughout. The incubation medium used was a modified Krebs-Ringer bicarbonate buffer (K^+ 70 mM, Na^+ 78.1 mM), pH 7.4, containing bovine serum albumin 2% w/v and glucose. Gas phase: $O_2 : CO_2$ (95 : 5). The constant flow rate was 0.2 ml/min. Insulin, when used was added as a single injection through a T-tube at the base of the incubation chamber (100 mU insulin in 0.1 ml buffer in 1 min). The effluent was collected and its lipids extracted [2].

An aliquot of the washed heptane phase was dried and the total lipids were counted with toluene PPO-POPOP in a Packard liquid scintillation counter (Model 3320). Another aliquot was extracted with alkali-ethanol [1] to separate the free fatty acids (FFA) from the other lipids which were then saponified. The non-saponifiable lipids (mostly cholesterol) accounted for less than 2% of the total ^{14}C lipids and were not included in the results. The saponifiable lipids or esterified fatty acids (EFA) were mainly tri-, di- and mono-glyceride. Phospholipids accounted for about 3% of the total ^{14}C lipids as shown by thin layer chromatography.

Table 1. *Basal lipid production by rat liver fragments from glucose (U^{14}C-glucose) over 5 h*

Basal lipid production in serial perifusion samples[a] (μAtC/g FFD wt/min × 10^5)							
Fraction (No)	μAt	Fraction (No)	μAt	Fraction (No)	μAt	Fraction (No)	μAt
(1)	3.7	(6)	1.1	(11)	1.3	(16)	1.1
(2)	1.8	(7)	0.9	(12)	1.2	(17)	1.4
(3)	1.3	(8)	1.0	(13)	1.2	(18)	1.3
(4)	1.2	(9)	1.0	(14)	1.2	(19)	1.6
(5)	1.0	(10)	1.0	(15)	1.1	(20)	2.1

[a] 15 min fractions at flow rate 2.6 ± 0.2 of a perifusion alb-KRB buffer containing glucose 2.5 mM (0.15 mCi/mM).

Table 2. *Basal rates of FFA and EFA production by liver fragments from glucose[a] (U^{14}C-glucose) and their response to added insulin*

Perfusion additions	(Fraction) (No)	Incorporation into lipid (μAtC/g FFD wt/min)			Ratios: —		
		TL	FFA	EFA	FFA:TL	EFA:TL	EFA:FFA
—	(1)	1.35	1.08	0.08	0.80	0.06	0.07
—	(2)	0.94	0.68	0.08	0.72	0.09	0.12
—	(3)	0.70	0.53	0.05	0.76	0.06	0.09
—	(4)	0.65	0.47	0.06	0.72	0.08	0.13
—	(5)	0.74	0.53	0.06	0.71	0.09	0.11
—	(6)	0.94	0.64	0.06	0.68	0.06	0.09
Insulin 100 mU	(7)	2.87	1.09	1.03	0.34	0.36	0.94
—	(8)	2.53	1.06	0.96	0.42	0.38	0.91
—	(9)	1.20	0.91	0.13	0.76	0.11	0.14

[a] 30 min fractions at flow rate 5.1 ml ± S.D. 0.12 of a perifusion alk-KRB buffer containing glucose 0.16 mM (3.1 mCi/mM).

The liver tissue lipids were also extracted [3], and the dried tissues were weighed (fat-free dry weight). This value was used for calculation of the results. All results are expressed as micro-atoms glucose carbon incorporated into lipids/g fat-free dry weight/minute (μAtC/g FFD wt/min).

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Preliminary results and Discussion

After an initially higher rate of lipid production, this preparation gives a reasonably stable output of total lipids for 5 h as shown in a representative experiment (Table 1). This stability was repeatedly found in various experiments and for many hours. Furthermore, the proportional incorporation into FFA and EFA is even more stable (Table 2).

When insulin was injected (fraction 7, Table 2) a sharp effect was noted, almost confined to the two periods (60 min) after its injection. Incorporation into both FFA and EFA was increased, but the stimulation of EFA production was proportionally more marked. Similar results were obtained in 3 different experiments, although the magnitude of the effect did vary.

Further experiments are underway to define this effect more precisely and also the relevance of the glucose concentration in the medium. These results would suggest that insulin has an effect on the liver's production of EFA, which is mostly triglyceride, though they give no clue to its site of action. It may increase the production of triglyceride by increasing the availability of glycerophosphate, by affecting a hormone-sensitive lipase or by promoting the lipoprotein formation. Some other experiments [5, 6] have also hinted that insulin may have such an action.

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