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Effect of glucose on carbohydrate synthesis from alanine or lactate in hepatocytes from starved rats

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In hepatocytes from starved rats, 10mM-glucose suppressed the incorporation of 2mM labelled alanine into glucose + glycogen by more than 40%, whereas no inhibition was observed with labelled lactate as substrate. Addition of glycerol instead of glucose did not show this inhibition. The inhibitory effect could also be demonstrated in label-free experiments.

Uncertainty exists on the regulation of gluconeogenesis by glucose. In perfusion studies of livers from starved rats, Haft (1967) and Exton & Park (1967) failed to observe any inhibitory effect of glucose on gluconeogenesis from lactate. Seglen (1974) similarly found that gluconeogenesis from lactate, pyruvate or fructose was not subject to end-point inhibition by physiological concentrations of glucose in hepatocytes from starved rats. However, in hepatocytes of fed rats, glucose inhibited the incorporation of [U-^{14}C]lactate (Claus *et al.*, 1975) as well as of [U-^{14}C]pyruvate (Feliu *et al.*, 1976) into glucose + glycogen. Ruderman & Herrera (1968) showed in a perfusion study with livers from starved rats that incorporation of labelled alanine into glucose + glycogen was decreased when the glucose concentration in the medium was raised.

As all these experiments were done under somewhat different experimental conditions, yielding apparently conflicting results, we have re-investigated the effect of glucose on gluconeogenesis from different substrates with hepatocytes from starved rats. A preliminary report has appeared (Solanki *et al.*, 1980).

Experimental

Livers of 24h-starved male Wistar rats (180–250g) were used for the isolation of hepatocytes as described earlier (Walter *et al.*, 1976). Yields varied between 6×10^8 and 8×10^8 cells per liver. The cells were approx. 90% viable as judged by Trypan Blue exclusion.

For an experiment, 10×10^6–20×10^6 cells were incubated in 25ml Erlenmeyer flasks in a shaking water bath at 37°C in 3ml of Krebs bicarbonate buffer in an atmosphere of O_2/CO_2 (19:1) (Nyfeler & Walter, 1979). The cells were preincubated for 20min, thereafter substrates and effectors were added and the incubation was continued for 60min or as indicated in the legends.

The glucose content in supernatants obtained after centrifugation of the cell suspensions (150*g* for 5min) was measured by the hexokinase method (Bergmeyer *et al.*, 1974). Glycogen was isolated and measured by standard procedures (Good *et al.*, 1933; Walaas & Walaas, 1950). When radioactivity in glycogen was to be determined, a sample of the glycogen was hydrolysed by H_2SO_4 (Good *et al.*, 1933) and the hydrolysate was applied to filter-paper strips, dried overnight and counted for radioactivity. For the measurement of conversion of U-^{14}C-labelled substrates into glucose or into glucose + glycogen, the methods of Claus *et al.* (1975) were used. For lactate measurements, the incubations were stopped with 0.5ml of 7.5% (w/v) $HClO_4$, the precipitate was washed with 3×1ml of 2.5% $HClO_4$ and the combined supernatants were neutralized and used for the enzymic measurement of lactate (Gutmann & Wahlefeld, 1974).

Incorporation of U-^{14}C-labelled substrates into glucose, glucose + glycogen or glycogen was expressed as μmol of substrates converted/g of liver. The number of μmol incorporated was obtained by dividing the total radioactivity incorporated into the hexoses by the specific radioactivity of added uniformly labelled substrate. All the results are expressed per g of liver wet wt., corresponding to 125×10^6 cells (Weibel *et al.*, 1969; Wheatley, 1972).

Chemicals were of the highest grade available. Bovine albumin (fraction V) was purchased from Miles (Cavenago, Brianza, Italy), purified as described by Chen (1967), dialysed first against 0.15M-NaCl and then against water.

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Results and discussion

As shown in Table 1, 10mM-glucose inhibited the incorporation of [U-^{14}C]alanine into glucose + glycogen by 43%, whereas no effect was observed on the incorporation of [U-^{14}C]lactate into carbohydrates. These results suggest that gluconeogenesis from alanine is subject to end-point inhibition by glucose.

The effects of different glucose concentrations on the incorporation of labelled substrates into glucose + glycogen are shown in Fig. 1. The onset of inhibition of [^{14}C]alanine incorporation was within the physiological range of glucose concentrations, since practically no effect was observed at 5mM-glucose, whereas addition of 10mM-glucose already caused more than 40% inhibition. At 20mM- and 30mM-glucose, gluconeogenesis from alanine was inhibited by 60 and 68% respectively. In contrast, gluconeogenesis from lactate was affected only

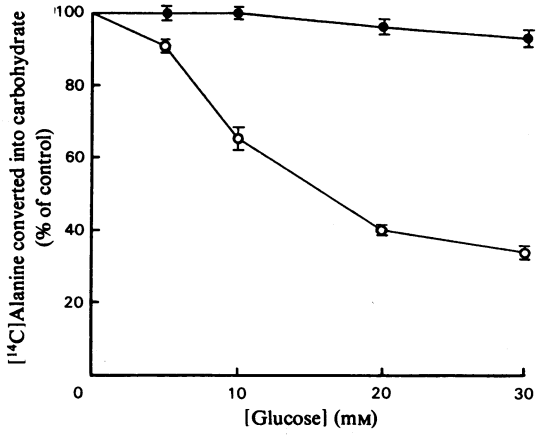

Fig. 1. *Effect of different glucose concentrations on the incorporation of U-^{14}C-labelled substrates into carbohydrates*

Hepatocytes were incubated with 10mM-[U-^{14}C]-alanine (○) and 9.1mM-[U-^{14}C]lactate (+0.9mM-pyruvate) (●). The points represent means ± S.E.M. for five incubations with the same cell preparation. Control values represent the conversion of the substrate into glucose + glycogen and were for alanine 13.00 ± 0.47 and for lactate + pyruvate 40.26 ± 0.31 μmol/h per g of liver in the absence of glucose.

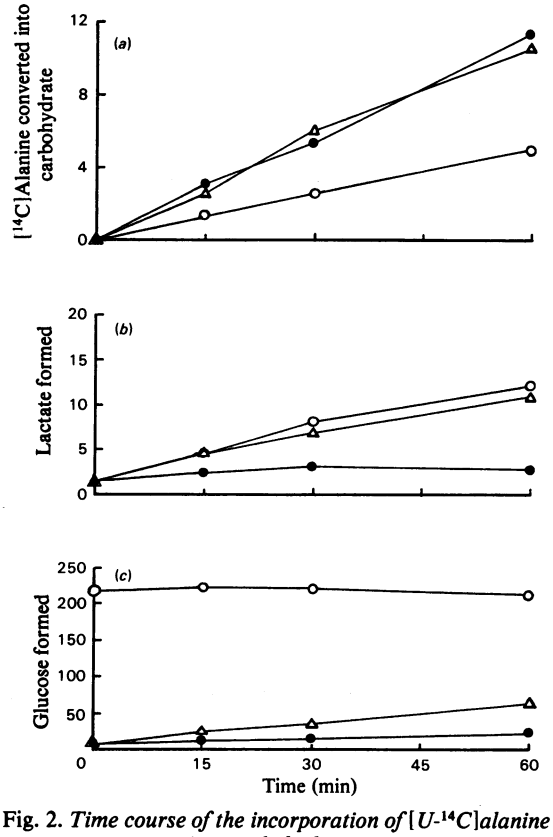

Fig. 2. *Time course of the incorporation of [U-^{14}C]alanine into carbohydrates*

Hepatocytes were incubated with 2mM-[U-^{14}C]alanine (●), with added 10mM-glucose (○) or with added 10mM-glycerol (△), and the following measurements were carried out: (*a*) alanine converted into glucose + glycogen; (*b*) lactate formed; (*c*) glucose formed. All values are μmol/g of liver. Each value is the mean of eight incubations from two different cell preparations.

Table 1. *Effect of glucose on the conversion of U-^{14}C-labelled substrates into carbohydrates*

Hepatocytes were incubated with 2mM-[U-^{14}C]alanine or 4.5mM-[U-^{14}C]lactate (+0.5mM-pyruvate) in the absence and presence of 10mM-glucose. Results are means ± S.E.M. for the number of cell preparations shown in parentheses. Student's *t* test was performed on unpaired data; *b* versus *a*, $P < 0.001$.

Additions	^{14}C-labelled substrate incorporated into glucose + glycogen (μmol/h per g of liver)
Alanine	9.88 ± 0.42[a] (14)
Alanine + glucose	5.69 ± 0.43[b] (14)
Lactate + pyruvate	26.26 ± 0.72 (6)
Lactate + pyruvate + glucose	27.76 ± 1.69 (6)

slightly at these glucose concentrations. When the alanine concentration was varied between 2 and 10mM in the presence or absence of 10mM-glucose, the incorporation of ^{14}C into carbohydrates was found to be inhibited by 50–60% independently of the alanine concentration (results not shown).

In further experiments in which the incorporation into glucose and glycogen was separately measured, it was found that the major portion of the label was incorporated into glucose. In the presence of 2mM-[U-^{14}C]alanine, 9.07 ± 0.53 ($n = 5$) μmol of [^{14}C]alanine/h per g of liver was incorporated into glucose, and only 0.24 ± 0.02 μmol/h per g into glycogen. On addition of glucose, 4.4 ± 0.04 μmol/h per g went into glucose and 0.33 ± 0.02 μmol/h per g into glycogen.

As shown in the time study in Fig. 2, the inhibitory effect of glucose could be observed as early as 15min after its addition. In contrast with glucose, addition of 10mM-glycerol did not inhibit the incorporation of label from alanine into glucose, even though glucose production was increased. In experiments not shown, we furthermore observed that glucose analogues, such as 3-*O*-methylglucose and 2-deoxyglucose, did not suppress gluconeogenesis from alanine to any extent.

Our results on the incorporation of label into carbohydrates are all based on the initial specific radioactivity of the substrates added. Addition of glucose caused lactate production, and the resulting dilution of the C_3 compounds could then lead to an apparent inhibition of the incorporation into glucose. The results in Table 2, however, clearly show that the extent of the inhibition by glucose is independent of the dilution of the lactate pool.

In the experiments summarized in Table 3, net changes of glucose and glycogen were measured in incubations with unlabelled lactate, pyruvate or alanine. The results show that also here addition of glucose suppresses the net production of glucose + glycogen in the presence of alanine to a much greater extent than in the presence of lactate + pyruvate. In contrast with the experiment with [^{14}C]lactate (Table 1, Fig. 1), gluconeogenesis from unlabelled lactate is somewhat inhibited by glucose.

Table 2. *Effect of glucose and glycerol on the incorporation of [U-^{14}C]alanine into carbohydrates*

In all incubations 2mM-[U-^{14}C]alanine was present. Results are means ± S.E.M. for five incubations from the same cell preparation.

Additions (mM)		Products (μmol/h per g of liver)	
Glucose	Glycerol	[^{14}C]Alanine converted into glucose + glycogen	Lactate found
—	—	10.79 ± 0.09	0.40 ± 0.15
10	—	6.52 ± 0.17	4.93 ± 0.18
10	5	7.00 ± 0.17	6.67 ± 0.17
10	10	7.46 ± 0.07	9.31 ± 0.29
15	—	4.28 ± 0.06	9.16 ± 0.40
—	5	10.77 ± 0.17	2.61 ± 0.26
—	10	9.33 ± 0.13	7.20 ± 0.38

Table 3. *Net glucose production in incubations with alanine, lactate + pyruvate and glucose*

All substances were added after a 20min preincubation period, and the incubation was continued further for 90min. All values are means ± S.E.M. of the numbers of experiments shown in parentheses with different cell preparations. Student's *t* test was performed on unpaired data, except for the glucose value in the experiment with added glucose, where paired data were used. *P* values always refer to the respective controls without added C_3 compounds. *b* versus *a*, *d* versus *c*, *e* versus *f*, $P < 0.001$; *g* versus *e*, $P < 0.025$. Control in experiments with glucose: owing to the differing amounts of cells used in each experiment (6×10^6–19×10^6 cells), the initial values of μmol/g of liver greatly varied. The subsequent results in this column are therefore expressed as the mean of the differences between the experiments with glucose alone (controls) and those with glucose plus C_3 compounds.

Additions (mM)	Glucose (μmol/90min per g of liver)	Glycogen (μmol/90min per g of liver)
None (control)	11.9 ± 1.0 (16)[a]	0.9 ± 0.1 (16)
Alanine (10)	43.6 ± 3.5 (10)[b]	1.4 ± 0.2 (10)
Lactate (9) + pyruvate (1)	67.6 ± 5.5 (10)[b]	1.0 ± 0.2 (10)
Glucose (10)	Control[c] (see the legend)	2.9 ± 0.6 (17)[e]
Glucose (10) + alanine (10)	$+1.1 \pm 4.7$ (10)	9.8 ± 1.9 (10)[f]
Glucose (10) + lactate (9) + pyruvate (1)	$+38.1 \pm 6.1$ (10)[d]	5.5 ± 1.3 (10)[g]

From the results presented, it can be concluded that the liver possesses a feedback mechanism for gluconeogenesis by glucose with alanine as substrate, which is independent of hormonal influences.

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