Glucose-dependent induction of acetyl-CoA carboxylase in rat hepatocyte cultures

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The carbohydrate-dependent long-term regulation of acetyl-CoA carboxylase was studied in primary hepatocyte cultures from adult rats. (1) The enzyme activity was increased 2-fold either by elevation of the glucose concentration to 20 mM or by enhancement of the insulin concentration to 0.1 μ M. Simultaneous increases in glucose and insulin resulted in a 5-fold increase in the enzyme activity. (2) As shown by immunochemical titration, the enhancement of the enzyme activity was due to an increase in the enzyme protein. (3) Incorporation of [35 S]methionine and immuno-precipitation of the enzyme revealed that the increase in enzyme protein was due to an increased rate of enzyme synthesis. The rate of enzyme degradation remained essentially unchanged. (4) The glucose- and insulin-dependent induction of acetyl-CoA carboxylase was prevented by the addition of α -amanitin (10 μ M) or cordycepin (10 μ M), indicating a transcriptional regulation of the enzyme level. (5) The parallel induction of acetyl-CoA carboxylase and of ATP citrate lyase indicates a co-ordinate long-term regulation of lipogenic enzymes.

Acetyl-CoA carboxylase (EC 6.4.1.2), the first enzyme in the biosynthesis of fatty acids, can be regarded as the rate-limiting enzyme of this pathway (Lane *et al.*, 1974; Volpe & Vagelos, 1976). The long-term regulation of acetyl-CoA carboxylase is effected by the alteration of enzyme synthesis and degradation (Majerus *et al.*, 1968; Nakanishi & Numa, 1970). The enzyme is decreased in diabetes mellitus as well as by starvation. Insulin therapy (Nepokroeff *et al.*, 1974) or re-feeding of a carbohydrate-rich fat-free diet are followed by a drastic increase of acetyl-CoA carboxylase and of other lipogenic enzymes above the normal range (Nakanishi & Numa, 1970; Kornacker & Lowenstein, 1965). Yet it is difficult to discern by experiments *in vivo* whether glucose induces the enzyme directly or via secondary effects mediated by hormonal or nervous signals. By using the less complex system of cultured rat hepatocytes it was demonstrated in a previous paper that the acetyl-CoA carboxylase activity was significantly increased in the presence of high glucose concentrations, even in the absence of insulin (Katz & Ick, 1981). The present investigation was performed to characterize this enhancement.

It is shown by immunochemical titration that

the glucose-dependent enhancement of the enzyme activity was due to a corresponding elevation of the enzyme protein. This was effected by enhanced enzyme synthesis rather than by altered enzyme degradation.

Experimental

Materials

Malate dehydrogenase, coenzymes, culture medium 199 and foetal-calf serum were obtained from Boehringer Mannheim. Collagenase type II, penicillin and streptomycin sulphate were supplied by Sigma. Bovine insulin, glucagon, dexamethasone, 3,3',5-tri-iodo-L-thyronine, phenylmethanesulphonyl fluoride, cycloheximide, cordycepin, oleic acid, poly(ethylene glycol), glucose-free medium 199, methionine-free minimum essential medium and bovine serum albumin were obtained from Serva. Aprotinin was supplied by Bayer. L-[35 S]Methionine and NaH 14 CO $_3$ were purchased from New England Nuclear. Sepharose 2B was obtained from Pharmacia. DEAE-cellulose (DE 52) was supplied by Whatman. α -Amanitin was given by Dr. H. Faulstich, Heidelberg. All other chemicals were reagent grade and supplied by Merck. Minisart sterile filters were a gift from

Sartorius. Albumin was defatted as described by Chen (1967); a complex of oleic acid and albumin was prepared as described by Garland *et al.* (1964).

Cell culture

Hepatocytes were prepared from fed male Wistar rats (150–200g; Winkelmann, Paderborn, Germany) and cultured on Falcon tissue-culture dishes as described previously (Katz & Giffhorn, 1983). After the initial plating period in the presence of 5% (v/v) foetal-calf serum, the cultures were maintained in serum-free medium 199 containing defatted serum albumin (2g/l), and basic concentrations of glucose (5.5mM), insulin (0.5nM) and dexamethasone (1nM). This standard medium was changed every 24h. The induction of lipogenic enzymes was initiated after a change of medium in 24h cultures by increasing the glucose and insulin concentrations; other hormones were added simultaneously as indicated. Inhibitors of protein synthesis were added 1h before glucose and insulin were increased.

The rates of the specific and the general protein synthesis were measured by incorporation of L-[³⁵S]methionine (80Ci/mol) into the immunoprecipitable acetyl-CoA carboxylase and into the trichloroacetic acid-precipitable protein respectively (Mans & Novelli, 1960). Protein synthesis was measured during the induction period in the presence of 70% (v/v) methionine-free minimum essential medium and 30% (v/v) medium 199, with a final methionine concentration of 30μM. [³⁵S]-Methionine was added 8h after enhancement of the glucose and insulin concentrations. The cultures were harvested another 8h later.

Enzyme degradation was measured during the induction period. Between 4h and 24h of culture, hepatocytes were maintained in the presence of [³⁵S]methionine (80Ci/mol) in 70% methionine-free minimum essential medium and 30% medium 199 with basic hormone and glucose concentrations. After removal of the radioactive label and three washings with medium 199, the cells were cultured under standard and inducing conditions. The decrease of radioactivity in acetyl-CoA carboxylase and in ATP citrate lyase was measured immunochemically up to 72h of culture. Reutilization of [³⁵S]methionine released by proteolysis was not detectable, owing to the dilution by unlabelled methionine to a final specific radioactivity of less than 0.2 Ci/mol.

Enzyme assay

After washing with iso-osmotic solution containing 130mM-NaCl, 30mM-KCl and 5mM-Tris/HCl, pH7.4, the cell monolayer was collected and homogenized as described by Katz & Giffhorn (1983). The acetyl-CoA carboxylase activity was

determined in the supernatant after centrifugation at 12000g for 10min as described by Majerus *et al.* (1968) and Witters *et al.* (1979), with the following modifications. After preincubation of the supernatant for 30min at 37°C in the presence of citrate (5mM), MgCl₂ (5mM), 2-mercaptoethanol (1.25mM), defatted albumin (1g/l) and Tris/HCl (100mM), pH7.3, the enzyme reaction was started by addition of ATP (2.8mM), acetyl-CoA (0.15mM) and KH¹⁴CO₃ (15.6mM; 0.2Ci/mol). Samples were removed and the reaction was stopped by addition of HClO₄ (0.25M) at the beginning as well as after 1 and 2min of reaction. The acid supernatant was dried overnight and dissolved in 200μl of water and 2ml of scintillation fluid (Bray, 1960) for counting. The other enzyme activities were measured spectrophotometrically at 30°C in a 100000g supernatant. The ATP citrate lyase and the lactate dehydrogenase activities were determined as described by Katz & Giffhorn (1983). The 3-hydroxyacyl-CoA dehydrogenase activity was measured as described by Morrison *et al.* (1965) in the supernatant after freezing and thawing of the homogenate in order to disrupt the mitochondria.

Preparation of antibodies

Rat liver acetyl-CoA carboxylase was purified to homogeneity as described by Alam & Srere (1980; T. Alam & P. A. Srere, personal communication). The livers of starved and re-fed rats were homogenized in 3vol. of 250mM-sucrose/1mM-EDTA/15mM-2-mercaptoethanol at 2°C. After centrifugation for 1h at 15000g (NH₄)₂SO₄ was slowly added to the filtered supernatant at 2°C to a final concentration of 19.4g/100ml. The precipitate was collected by centrifugation for 30min at 15000g and dissolved in 100mM-potassium phosphate (pH7.0) 50mM-potassium citrate/1mM-EDTA/15mM-2-mercaptoethanol. After dialysis for 12h against the same buffer and removal of insoluble protein, the enzyme was precipitated by addition of poly(ethylene glycol) to a final concentration of 5% (w/v). After stirring for 6h at 20°C, the precipitate was collected by centrifugation for 10min at 27000g and dissolved at 37°C in the same buffer without poly(ethylene glycol). This precipitation was repeated, and the dissolved precipitate was subjected to gel chromatography on Sepharose 2B at 2°C. The equilibration and elution were performed with 50mM-potassium phosphate (pH7.0)/20mM-potassium citrate/1mM-EDTA/5mM-dithioerythritol/10% glycerol (v/v). The homogeneity of the acetyl-CoA carboxylase fraction was checked by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis (Laemmli, 1970). This enzyme preparation was used for immunization of rabbits. The immunoglobulin-G fraction of the antiserum

obtained was isolated by precipitation with 1.2M- Na_2SO_4 /0.1M-potassium phosphate, pH 7.4. The specificity of the antibody was checked by double immunodiffusion (Ouchterlony, 1948), which showed a single precipitation line between the antibody and rat liver cytosol. Purification of ATP citrate lyase and preparation of a specific antibody were performed as described by Katz & Giffhorn (1983).

Immunological determinations

The immunotitration of the acetyl-CoA carboxylase activity was performed by preincubation of samples of the 100000g supernatant with increasing amounts of the specific antibody. After incubation for 30 min at 30°C and for 4 h at 2°C, the immunoprecipitate was removed by centrifugation for 30 min at 13000g. The soluble enzyme activity was tested in the supernatant.

For determination of the rate of synthesis and degradation of acetyl-CoA carboxylase and ATP citrate lyase, the immunoprecipitations were performed with a 100000g supernatant, which was partially purified by chromatography on a small column of DEAE-cellulose as described by Kitajima *et al.* (1975). After elution, a 2–3-fold excess of unlabelled acetyl-CoA carboxylase or ATP citrate lyase was added as carrier to the radioactive protein, and the precipitation was performed with

double the amount of antibody necessary for total precipitation of the unlabelled carrier. After incubation for 30 min at 30°C and 12 h at 2°C in the presence of phenylmethanesulphonyl fluoride (1 mM) and aprotinin (125 units/ml), the immunoprecipitate was sedimented by centrifugation at 13000g for 30 min and washed three times with 150 mM-NaCl/5 mM-EDTA/50 mM-Tris/HCl, pH 7.4. The final pellet was dissolved as described by Laemmli (1970) and counted for radioactivity in scintillation fluid (Bray, 1960). A sample of the dissolved immunoprecipitate was analysed by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis and fluorography. The specificity of immunoprecipitation was demonstrated by the occurrence of radioactivity exclusively in the band of the corresponding enzyme (Fig. 1).

The statistical evaluation was performed by Student's *t* test.

Results

Metabolite- and hormone-dependent regulation

After an initial decrease during day 1 of culture, the acetyl-CoA carboxylase activity remained essentially constant during the following days under basic culture conditions. At this time, increasing the glucose concentration up to 20 mM resulted in an increase of the enzyme activity within 24 h up to 200% even in the absence of insulin (Fig. 2). Simultaneous addition of basic or high insulin concentrations led to a further increase in the glucose-dependent enhancement of the acetyl-CoA carboxylase activity up to 500%, as described in detail by Katz & Ick (1981).

Increasing the basic concentration of dexamethasone to 0.1 μM or addition of tri-iodothyronine (10 μM) did not significantly alter either the basic activity or the glucose- or insulin-dependent increase (Table 1). Glucagon (0.1 μM) did not influence the basic enzyme activity, but it decreased the glucose + insulin-dependent enhancement significantly (Table 1). Since in the presence of high glucose concentrations the enhanced formation of lactate resulted in a slight acidification of the culture medium, the influence of pH on the acetyl-CoA carboxylase activity was studied. No alteration of the acetyl-CoA carboxylase activity was observed between pH 7.20 and 7.60 (results not shown).

The specificity of the glucose- and insulin-dependent enhancements were tested by comparing the activities of the lipogenic enzymes acetyl-CoA carboxylase and ATP citrate lyase with the activities of the fatty acid-degrading enzyme 3-hydroxyacyl-CoA dehydrogenase and of the non-inducible enzyme lactate dehydrogenase. Both lipogenic enzyme activities were increased in the

Fig. 1. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of ^{35}S -labelled acetyl-CoA carboxylase isolated by immunoprecipitation

Acetyl-CoA carboxylase was precipitated by a specific antibody from hepatocytes cultured in the presence of ^{35}S -methionine. The immunoprecipitate was dissolved and subjected to sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. The radioactivity was determined by fluorography on Kodak X-Omat AR 5 film and scanning of the gel.

Table 1. *Effect of hormones on the glucose- and insulin-dependent induction of the acetyl-CoA carboxylase*
Hepatocytes were cultured under standard conditions. Hormones and glucose were added when the medium of 24h cultures was changed. The enzyme activity was determined 24h later. Values are means $\pm$ S.E.M. from three to seven experiments with two to three determinations. Significant differences from corresponding cultures without addition of glucagon, dexamethasone or tri-iodothyronine are indicated by * $P < 0.01$.

Addition (final concn.)	Acetyl-CoA carboxylase ($\mu\text{mol/min per g of DNA}$)			
	—	Insulin (0.1 μM)	Glucose (20mM)	Insulin (0.1 μM) + glucose (20mM)
—	62 $\pm$ 7	98 $\pm$ 6	129 $\pm$ 15	188 $\pm$ 18
Dexamethasone (0.1 μM)	77 $\pm$ 15	113 $\pm$ 24	139 $\pm$ 24	215 $\pm$ 22
Tri-iodothyronine (10 μM)	89 $\pm$ 15	96 $\pm$ 10	121 $\pm$ 13	210 $\pm$ 10
Glucagon (0.1 μM)	68 $\pm$ 4	87 $\pm$ 8	100 $\pm$ 8	115 $\pm$ 5*

Fig. 2. *Glucose- and insulin-dependent enhancement of the acetyl-CoA carboxylase activity*

After the first 24h under standard culture conditions, the hepatocytes were provided with glucose- and insulin-free standard medium for up to 24h more. Glucose and insulin were added as indicated. Values are means $\pm$ S.E.M. for two to nine experiments.

presence of high concentrations of glucose, insulin and insulin plus glucose, whereas the activities of the other two enzymes remained essentially unchanged. Oleate (2mM) as an alternative energy substrate instead of 20mM-glucose did not alter any of the enzyme activities.

Fig. 3. *Immunochemical titration of acetyl-CoA carboxylase in hepatocyte cultures*

Cultures were maintained under standard conditions (●) or in the presence of 0.1 μM -insulin (○), 20mM-glucose (△) and 0.1 μM -insulin plus 20mM-glucose (□). Samples (100 μl) of 100000g supernatant were incubated with increasing amounts of a specific immunoglobulin preparation (1.1mg of protein/ml of homogenization buffer) for 30min at 30°C and 4h at 2°C. The volume was adjusted to 150 μl by addition of homogenization buffer. The enzyme activity was determined in the supernatant after sedimentation of the immunoprecipitate at 13000g for 30min.

Enzyme synthesis and degradation

The titration of acetyl-CoA carboxylase activity by a specific antibody was performed in order to determine whether the increase of enzyme activity was due to an activation (for review; see Kim,

Table 2. *Effect of glucose and insulin on the activity and synthesis of acetyl-CoA carboxylase (ACC) and ATP citrate lyase (ACL)*
The enzyme induction was initiated by the addition of glucose and insulin to 24h cultures with methionine-poor medium. [³⁵S]Methionine (80 Ci/mol) was added 8h later. The enzyme activity and the incorporation of the radioactive label into immunoprecipitated enzymes [d.p.m. (ACC), d.p.m. (ACL)] and into trichloroacetic acid precipitable cytosolic protein [d.p.m. (cytosol. protein)] were determined after a further incubation for 8h. Values are means ± S.E.M. for three to five determinations. Significant differences from cultures without additions are indicated by **P* < 0.02.

Addition (final concn.)	Acetyl-CoA carboxylase		ATP citrate lyase	
	Enzyme activity (μmol/min per g of DNA)	Relative incorporation of [³⁵ S]methionine [d.p.m. (ACC) × 1000] [d.p.m. (cytosol. protein)]	Enzyme activity (μmol/min per g of DNA)	Relative incorporation of [³⁵ S]methionine [d.p.m. (ACL) × 1000] [d.p.m. (cytosol. protein)]
—	67 ± 8	2.21 ± 0.29	148 ± 18	4.49 ± 0.65
Insulin (0.1 μM)	74 ± 6	2.53 ± 0.20	185 ± 1	5.05 ± 0.74
Glucose (20mM)	90 ± 12	3.14 ± 0.14	206 ± 18	5.45 ± 0.02
Insulin (0.1 μM) + glucose (20mM)	123 ± 17*	4.41 ± 0.40*	228 ± 12*	6.71 ± 0.21*

1983) or to an increase in the enzyme protein. The immunochemical titration of acetyl-CoA carboxylase revealed a linear correlation between the enzyme activity and the amount of antibody necessary for the complete precipitation of the enzyme (Fig. 3). The parallelism of the titration curves demonstrates that the specific activity of acetyl-CoA carboxylase was identical under all conditions. Thus the enzyme activity was increased by an increase in amount of the enzyme protein rather than by allosteric activation or interconversion. A corresponding regulation was observed for ATP citrate lyase (Katz & Giffhorn, 1983).

The mechanism of the glucose- and insulin-dependent enhancements was examined by incorporation of [³⁵S]methionine into acetyl-CoA carboxylase, ATP citrate lyase and cytosolic protein (Table 2). The elevation of glucose and insulin concentrations specifically increased the incorporation of [³⁵S]methionine into the two lipogenic enzymes.

In addition to the relative rates of enzyme synthesis, the rates of enzyme degradation were measured by radioimmunochemical determination. After pulse labelling with [³⁵S]methionine, the decay of the radioactive label in acetyl-CoA carboxylase and in ATP citrate lyase was followed in the presence of basic and enhanced glucose and insulin concentrations. The half-time (*t*₁) of degradation of acetyl-CoA carboxylase was 39h under standard culture conditions and 38h in the presence of 0.1 μM-insulin; it was slightly decreased to 36h, in the presence of 20mM-glucose and 20mM-glucose + 0.1 μM-insulin (Fig. 4). A similar alteration of the half-life was observed for ATP citrate lyase, which was 48h under basic culture conditions, 42h in the presence of 20mM-glucose and 41h in the presence of 20mM-glucose + 0.1 μM-insulin.

In order to distinguish whether the induction of acetyl-CoA carboxylase and of ATP citrate lyase was due to a transcriptional or to a translational regulation, the effect of different inhibitors of protein synthesis was studied (Table 3). The induction of both enzymes was prevented in the presence of 2.5 μM-cycloheximide. At this concentration the overall protein synthesis was inhibited by 74%. In contrast with effects of higher concentrations of the inhibitor (Katz & Ick, 1981; Katz & Giffhorn, 1983), the morphology of cultured hepatocytes was not significantly impaired for 24h under this condition. Moreover, the enzyme induction was prevented by α-amanitin and cordycepin (each 10 μM). This pattern of inhibition suggests that the observed induction was due to a transcriptional rather than to a translational regulation.

Fig. 4. Lack of effect of glucose, insulin and glucose plus insulin on the degradation of acetyl-CoA carboxylase. Hepatocytes were maintained in the presence of [^{35}S]methionine between 4 h and 24 h of culture as described in the Experimental section. After removal of the radioactive label, the cells were cultured under standard conditions (a), or in the presence of $0.1\ \mu\text{M}$ -insulin (b), 20 mM -glucose (c) or $0.1\ \mu\text{M}$ -insulin plus 20 mM -glucose (d) for up to 48 h more. Acetyl-CoA carboxylase was isolated by immunoprecipitation. Values are means for three to four single determinations in two representative experiments.

Table 3. Inhibition of glucose- and insulin-dependent induction by inhibitors of protein synthesis. Inhibitors of protein synthesis were added with the change of medium to 24 h cultures. After 1 h preincubation, glucose and insulin were added. The cells were harvested 16 h later. Values are means $\pm$ S.E.M. for five to eight determinations. Significant differences from the corresponding cultures with basic glucose and insulin concentrations are indicated by * $P < 0.001$.

Addition (final concn.)	Acetyl-CoA carboxylase ($\mu\text{mol/min per g of DNA}$)			ATP citrate lyase ($\mu\text{mol/min per g of DNA}$)		
	—	Glucose (20 mM)	Glucose (20 mM) + insulin ($0.1\ \mu\text{M}$)	—	Glucose (20 mM)	Glucose (20 mM) + insulin ($0.1\ \mu\text{M}$)
—	76 ± 8	$134 \pm 7^*$	$178 \pm 16^*$	175 ± 18	$271 \pm 25^*$	$442 \pm 17^*$
Cycloheximide ($2.5\ \mu\text{M}$)	66 ± 8	75 ± 18	41 ± 4	189 ± 16	188 ± 14	176 ± 8
α -Amanitin ($10\ \mu\text{M}$)	67 ± 5	71 ± 12	42 ± 9	167 ± 15	187 ± 17	192 ± 6
Cordycepin ($10\ \mu\text{M}$)	53 ± 11	60 ± 12	72 ± 18	152 ± 11	153 ± 11	174 ± 18

Discussion

Enzyme synthesis and degradation

The present study shows glucose- and insulin-dependent increases in the acetyl-CoA carboxylase activity in primary cultures of rat hepatocytes. It was demonstrated that the carbohydrate- and the hormone-dependent enhancements of the enzyme activity were due to an increased amount of the

enzyme protein. The enhancement of enzyme protein was effected by a specific increase of the rate of enzyme synthesis rather than by decreased enzyme degradation. This is in line with the described induction of acetyl-CoA carboxylase and of other lipogenic enzymes observed in starved rats during re-feeding (Majerus & Kilburn, 1969; Nakanishi & Numa, 1970; Gibson *et al.*, 1972). An enhancement of the acetyl-CoA carboxylase

owing to decreased enzyme degradation was observed exclusively in obese mice, but not in normal animals (Nakanishi & Numa, 1971).

The half-life of acetyl-CoA carboxylase as determined in primary hepatocyte cultures in the present study (39 h) was higher than that observed in an established hepatocyte cell line [28 h (Kitajima *et al.*, 1975)]. However, it was smaller than the half-life in intact rats [48 h (Majerus & Kilburn, 1969); 59 h (Nakanishi & Numa, 1970)]. This may be explained by the difficulty of excluding *in vivo* the reutilization of a radioactive label, which leads to an overestimation of the half-life (Kitajima *et al.*, 1975). On the other hand, the half-life of ATP citrate lyase (48 h) was higher than that observed in the intact animal [27 h (Gibson *et al.*, 1972)] or in hepatocytes cultured on collagen gels [18 h (Spence *et al.*, 1979)]. This difference may be due to the use of different strains of rats as well as to different experimental or culture conditions.

The prevention of induction of acetyl-CoA carboxylase and ATP citrate lyase by α -amanitin, an inhibitor of RNA polymerase II (Faustich, 1981), as well as by cordycepin indicates that the glucose- and the glucose + insulin-dependent long-term regulation was effected at the transcriptional level. This is in line with the observed increase in polyribosomes synthesizing acetyl-CoA carboxylase during re-feeding of starved rats (Nakanishi *et al.*, 1976; Finkelstein *et al.*, 1979). Although translational regulation by glucose or insulin is unlikely, it cannot be excluded definitively; the amount of mRNA might be so drastically decreased during long-lasting inhibition of transcription that different rates of translation might be no longer detectable. Further studies will be necessary to quantify the specific mRNA during the induction of acetyl-CoA carboxylase described here.

Co-ordinate regulation of lipogenic enzymes

The specific induction of acetyl-CoA carboxylase and ATP citrate lyase in hepatocyte cultures by glucose and insulin suggests that there is a common pattern for the long-term regulation. This may include the regulation of other lipogenic enzymes, which exhibit a similar carbohydrate- and hormone-dependent regulation in cultured hepatocytes (Spence & Pitot, 1982). Both studies demonstrate a co-ordinate regulation of lipogenic enzymes, as was proposed previously from experiments *in vivo* (Gibson *et al.*, 1972; Finkelstein *et al.*, 1979).

Effect of hormones on glucose-dependent induction

Increasing the dexamethasone concentration or addition of tri-iodothyronine did not significantly increase the basic acetyl-CoA carboxylase activity or the glucose- or insulin-dependent induction.

Thus a permissive effect, as demonstrated for the insulin-dependent induction of glucokinase (Katz *et al.*, 1979; Schudt, 1979) and tyrosine aminotransferase (Michalopoulos *et al.*, 1978) or the glucagon-dependent induction of phosphoenolpyruvate carboxykinase (Probst & Jungermann, 1983) was not detectable. However, since both hormones exhibit long-lasting effects on cellular metabolism (Spence *et al.*, 1979), it cannot be excluded from the present results that a deficiency of both hormones for several days would impair the enzyme induction.

The diminution of the glucose-dependent induction by glucagon suggests that this induction was mediated by a glycolytic metabolite rather than by glucose itself. The formation of a hypothetical inducing metabolite from glucose could be increased by insulin and decreased by glucagon. This hypothesis is supported furthermore by the observation that the effects of glucose and insulin were co-operative rather than additive: increasing either glucose or insulin alone resulted in a 100% increase of acetyl-CoA carboxylase, whereas simultaneous increases in glucose plus insulin resulted in a 400% increase. However, further studies will be necessary to discover the hypothetical inducing metabolite and to elucidate whether insulin induces the lipogenic enzymes directly in addition to its effect on the metabolite-dependent regulation.

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