Regulation of Phosphoenolpyruvate Carboxykinase (GTP) in Adipose Tissue *in vivo* by Glucocorticoids and Insulin

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1. The regulation of the synthesis of phosphoenolpyruvate carboxykinase (GTP) (EC 4.1.1.32) in epididymal adipose tissue, liver and kidney *in vivo* was studied immunohistochemically. 2. Phosphoenolpyruvate carboxykinase (GTP) synthesis in adipose tissue is increased by starvation, diabetes and noradrenaline, and decreased by re-feeding and insulin. These changes were also seen in adrenalectomized rats and are qualitatively similar to those observed for the liver enzyme. This indicates the involvement of cyclic AMP as an inducer and insulin as a de-inducer in the regulation of phosphoenolpyruvate carboxykinase (GTP) in both tissues. (Induction and de-induction are defined as selective increase and decrease respectively in the rate of enzyme synthesis, regardless of the mechanism involved.) 3. Adrenalectomy had little effect on phosphoenolpyruvate carboxykinase (GTP) synthesis in liver and kidney, but increased the synthesis rate of the adipose-tissue enzyme. Starvation and adrenalectomy had additive effects in increasing the synthesis rate of adipose-tissue phosphoenolpyruvate carboxykinase (GTP). In adrenalectomized diabetic rats glucocorticoids increased phosphoenolpyruvate carboxykinase (GTP) synthesis in liver and kidney while decreasing enzyme synthesis in adipose tissue. De-induction of adipose tissue phosphoenolpyruvate carboxykinase (GTP) is therefore regulated independently by glucocorticoids and insulin. 4. Although liver, kidney and adipose-tissue phosphoenolpyruvate carboxykinases (GTP) are seemingly identical, there is an apparent tissue-specific differentiation in regulatory systems for the enzyme.

Phosphoenolpyruvate carboxykinase (GTP) (EC 4.1.1.32) is present in the liver, kidney cortex and epididymal adipose tissue of the rat. Its physiological function in all these three tissues was described previously (Scrutton & Utter, 1968; Iynedjian *et al.*, 1975; Reshef *et al.*, 1972; Hopgood *et al.*, 1973). In each of these tissues the cytosolic forms of the enzyme share similar catalytic and immunological properties (Ballard & Hanson, 1969; Longshaw & Pogson, 1972; Hopgood *et al.*, 1973; Iynedjian *et al.*, 1975), yet the hormonal regulation of their activities *in vivo* varies. This phenomenon indicates an apparent differentiation not only in the physiological regulation of phosphoenolpyruvate carboxykinase activity, but also in the mechanism of hormone action.

The glucocorticoids are known to affect the activity of phosphoenolpyruvate carboxykinase in all three tissues. In the liver they increase the activity and synthesis rate of the enzyme slightly, compared with cyclic AMP, and their effect is most apparent in

diabetic animals or in liver-derived cells (Gunn *et al.*, 1975*a,b*). Under most conditions *in vivo*, the effect of glucocorticoids on hepatic phosphoenolpyruvate carboxykinase is masked by enhanced insulin release, which subsequently decreases the rate of enzyme synthesis (Gunn *et al.*, 1975*a*). In contrast both the activity and synthesis rate of the enzyme in the renal cortex are markedly increased by glucocorticoids (Iynedjian *et al.*, 1975; Gunn *et al.*, 1975*a*) and do not appear to be directly affected by insulin (Kamm *et al.*, 1974; Iynedjian *et al.*, 1975).

Insulin and glucocorticoids are also important in the regulation of phosphoenolpyruvate carboxykinase in adipose tissue. Adrenalectomy, starvation and diabetes all cause an increase in enzyme activity in this tissue, whereas injection of either insulin or glucocorticoid into adrenalectomized-diabetic animals decreases the activity of phosphoenolpyruvate carboxykinase (Reshef *et al.*, 1969*a,b*, 1970). Therefore, in contrast with both the liver and kidney enzyme, phosphoenolpyruvate carboxykinase activity in adipose tissue appears to be decreased by both

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insulin and glucocorticoid acting independently. Hopgood *et al.* (1973) have shown that, when starved animals are re-fed, there is a decrease in the synthesis rate of adipose tissue phosphoenolpyruvate carboxykinase. This is circumstantial evidence indicating that insulin regulates this enzyme, as it does the liver enzyme, through changes in synthesis rate. In the present paper the separate effects of insulin and glucocorticoids on the synthesis rate of phosphoenolpyruvate carboxykinase in adipose tissue, liver and kidney have been investigated more thoroughly *in vivo* in adrenalectomized and diabetic animals.

Experimental

Male Wistar rats (6–8 weeks old) were used throughout the experiments. Adrenalectomized animals were maintained on 1% (w/v) NaCl and used 4–6 days after the operation. Diabetes was induced by subcutaneous injection of 18.5 mg of alloxan/100 g body wt. Only animals exhibiting marked glucosuria were used in the study. Bilateral adrenalectomy was performed on diabetic rats 3 days after alloxan treatment and the adrenalectomized–diabetic animals were used 3 or 4 days later provided that a high glucosuria was still evident.

Cortisol 21-phosphate (disodium salt) and noradrenaline were from Sigma Chemical Co., St. Louis, MO, U.S.A., and triamcinolone acetonide (9 α -fluoro-11 β ,21-dihydroxy-16 α ,17 α -isopropylidenedioxypregna-1,4-diene-3,20-dione) from E.R. Squibb and Sons, New York, NY, U.S.A. Glucagon-free insulin was the gift of Eli Lilly and Co., Indianapolis, IN, U.S.A. The L-[4-³H]leucine (2.1–2.8 Ci/mmol) was from the Nuclear Research Centre, Negev, Beer-Sheva, Israel, and NaH¹⁴CO₃ (2–10 mCi/mmol) was from The Radiochemical Centre, Amersham, Bucks, U.K.

Liver, kidney and adipose-tissue cytosolic phosphoenolpyruvate carboxykinases are immunochemically identical (Ballard & Hanson, 1969; Hopgood *et al.*, 1973; Iynedjian *et al.*, 1975) and the same procedure was used for the immunoprecipitation of the enzyme from all three tissues. Specific antibodies to phosphoenolpyruvate carboxykinase from the cytosol of rat liver were produced in goats as described by Hopgood *et al.* (1973). To measure the synthesis rate of the enzyme, rats were injected with 100 μ Ci of [³H]leucine intraperitoneally and killed 30 min later. The liver, kidneys and epididymal adipose tissues were removed and homogenized in 0.25 M-sucrose, and a cytosol fraction was prepared by centrifugation at 100000g for 45 min. The activity of phosphoenolpyruvate carboxykinase was determined for each cytosol fraction (Ballard & Hanson, 1969). One unit of enzyme activity catalyses the fixation of 1 μ mol of NaH¹⁴CO₃/min at 37°C. Radioactivity incorporated into phosphoenolpyruvate carboxykinase, isolated as an antigen–antibody precipitate, and into total

cytosol protein was determined as described (Hopgood *et al.*, 1973). Synthesis rates for phosphoenolpyruvate carboxykinase are expressed both as the radioactivity incorporated into the enzyme/30 min per mg of tissue and as the percentage of cytosol protein synthesis.

Results

Phosphoenolpyruvate carboxykinase activity in the epididymal adipose tissue of the rat is increased by injection of adrenaline or noradrenaline into intact animals, increased by bilateral adrenalectomy, and decreased by injection of cortisol or triamcinolone (Reshef *et al.*, 1970; Reshef & Hanson, 1972). These changes are due, at least in part, to changes in the synthesis rate of the enzyme (Table 1). Further, these effects of noradrenaline and glucocorticoids on phosphoenolpyruvate carboxykinase are not due to large alterations in the rate of cytosol protein synthesis, for although adrenalectomy increased the incorporation of leucine into adipose-tissue proteins, the effect on the enzyme was greater (Table 1).

This increase in the activity and synthesis rate of adipose-tissue phosphoenolpyruvate carboxykinase in adrenalectomized rats is not reflected by complementary changes in either the activity of the hepatic enzyme (Reshef *et al.*, 1969a,b, 1970) or the activity and synthesis rate of the renal enzyme (Iynedjian *et al.*, 1975). Starvation of intact rats, however, increases both the activity and the synthesis rate of the enzyme in all three tissues (Hopgood *et al.*, 1973; Tilghman *et al.*, 1974; Gunn *et al.*, 1975a). Therefore a comparison of the effects of glucocorticoids on the activity and synthesis rate of phosphoenolpyruvate carboxykinase in all three tissues in both adrenalectomized and starved animals might more clearly define the tissue differences in the regulation of the enzyme by glucocorticoids (Table 2).

As found previously (Reshef *et al.*, 1969a,b), the activity of phosphoenolpyruvate carboxykinase in adipose tissue is increased by starvation and adrenalectomy and further increased by a combination of both treatments. These changes are accompanied by comparable increases in the synthesis rate of the enzyme (cf. Tables 1 and 2). Under all three conditions both the activity and synthesis rate of phosphoenolpyruvate carboxykinase may then be decreased by injection of triamcinolone (Table 2). Again there was little change in the synthesis rate of adipose-tissue cytosol proteins (results not shown). Although triamcinolone tended to decrease the rate of protein synthesis in adipose tissue (see Table 1), the specific effect on phosphoenolpyruvate carboxykinase was greater, as is evident from the decrease in the relative rate of enzyme synthesis. There was extreme variability in the total incorporation of [³H]leucine into adipose-tissue protein, a situation possibly caused by

Table 1. *Effect of noradrenaline and glucocorticoids on the synthesis rate of phosphoenolpyruvate carboxykinase in adipose tissue*

Fed rats were injected subcutaneously with noradrenaline (0.2 mg/100 g body wt.) and killed 5 h later. Fed adrenalectomized rats were injected intraperitoneally with cortisol (5 mg/100 g body wt.) and killed 10 h later. At 0.5 h before death all animals were injected with 100 μ Ci of [3 H]leucine. Phosphoenolpyruvate carboxykinase was assayed and the synthesis rate determined as described in the Experimental section. Values are means $\pm$ S.E.M. of the numbers of observations in parentheses.

Treatment	Phosphoenolpyruvate carboxykinase (munits/g fresh wt.)	10 ⁻³ $\times$ Radioactivity incorporated (d.p.m./g of tissue)		Incorporation into phosphoenolpyruvate carboxykinase (%)
		Cytosol protein	Phosphoenolpyruvate carboxykinase	
None (3)	62 $\pm$ 6	293 $\pm$ 4	2.68 $\pm$ 0.28	0.95 $\pm$ 0.15
Noradrenaline (6)	157 $\pm$ 35	293 $\pm$ 67	6.51 $\pm$ 0.89	2.73 $\pm$ 0.26
Adrenalectomy (3)	327 $\pm$ 50	472 $\pm$ 50	10.32 $\pm$ 1.93	2.16 $\pm$ 0.15
Adrenalectomy + cortisol (3)	209 $\pm$ 10	276 $\pm$ 75	2.70 $\pm$ 1.22	1.07 $\pm$ 0.25

Table 2. *Tissue differences in the response of phosphoenolpyruvate carboxykinase activity and synthesis rate to adrenalectomy and starvation*

Fed and 24 h-starved rats were injected subcutaneously with triamcinolone (5 mg/100 g body wt.) at time zero, with 100 μ Ci of [3 H]leucine at 9.5 h and were killed at 10 h. Phosphoenolpyruvate carboxykinase activity (units/g fresh wt.) was assayed and the relative synthesis rate of the enzyme (%) determined as described in the Experimental section. Values are the means $\pm$ S.E.M. of three or more observations. N.D., not determined (see Gunn *et al.*, 1975a).

Treatment	Phosphoenolpyruvate carboxykinase in					
	Adipose tissue		Liver		Kidney	
	Activity (units/g fresh wt.)	Relative synthesis rate (%)	Activity (units/g fresh wt.)	Relative synthesis rate (%)	Activity (units/g fresh wt.)	Relative synthesis rate (%)
Adrenalectomy	0.31 $\pm$ 0.02	1.99 $\pm$ 0.42	4.34 $\pm$ 0.43	1.88 $\pm$ 0.15	4.77 $\pm$ 0.52	1.88 $\pm$ 0.21
+ triamcinolone	0.09 $\pm$ 0.03	1.28 $\pm$ 0.20	3.75 $\pm$ 0.27	0.74 $\pm$ 0.16	12.42 $\pm$ 0.89	6.59 $\pm$ 0.22
Starvation	0.21 $\pm$ 0.06	1.90 $\pm$ 0.29	10.43 $\pm$ 0.23	2.39 $\pm$ 0.24	N.D.	N.D.
+ triamcinolone	0.07 $\pm$ 0.01	0.46 $\pm$ 0.13	8.67 $\pm$ 0.52	1.13 $\pm$ 0.03	N.D.	N.D.
Adrenalectomy + starvation	0.45 $\pm$ 0.11	4.89 $\pm$ 0.90	11.05 $\pm$ 0.51	2.78 $\pm$ 0.14	14.16 $\pm$ 0.67	5.55 $\pm$ 0.29
+ triamcinolone	0.23 $\pm$ 0.05	2.44 $\pm$ 0.50	11.35 $\pm$ 1.11	2.31 $\pm$ 0.17	17.33 $\pm$ 1.30	8.82 $\pm$ 0.53

direct absorption of radioactive leucine into the tissue. For this reason, the changes in leucine incorporation into total tissue phosphoenolpyruvate carboxykinase do not readily show the trends that become apparent when the results are expressed as percentage of cytosol protein synthesis.

In marked contrast with its effect on adipose-tissue enzyme, triamcinolone increases both the activity and synthesis rate of renal phosphoenolpyruvate carboxykinase in intact (Iynedjian *et al.*, 1975; Gunn *et al.*, 1975a) as well as adrenalectomized rats (Table 2). Hepatic enzyme activity and synthesis rate, on the other hand, although increased by starvation, were little affected by adrenalectomy and decreased after injection of triamcinolone. We have previously reported (Gunn *et al.*, 1975a) that administration of

glucocorticoids results in insulin release and that this is the probable cause of the decrease in the rate of hepatic enzyme synthesis seen in both fed and starved animals. Insulin given directly to diabetic rats, or the release of insulin mediated either by re-feeding starved animals or by triamcinolone injection, produces a rapid fall in the rate of hepatic phosphoenolpyruvate carboxykinase synthesis (Hopgood *et al.*, 1973; Tilghman *et al.*, 1974; Gunn *et al.*, 1975a). However, this effect did not appear to be as marked for the liver enzyme in starved adrenalectomized rats (Table 2). Since the activity and synthesis rate of adipose-tissue phosphoenolpyruvate carboxykinase is also decreased by insulin, re-feeding and triamcinolone (Reshef *et al.*, 1970; Hopgood *et al.*, 1973; Tables 1 and 2), we used starved, adrenalectomized rats to

Table 3. *Effect of re-feeding on phosphoenolpyruvate carboxykinase synthesis in the adipose tissue and liver of adrenalectomized rats*

Adrenalectomized rats were re-fed after 21 h starvation. At 2.5 h later the animals were injected with 100 μ Ci of [3 H]leucine and were killed at 3 h. Re-fed animals (+) are compared with rats starved for 24 h (–), Phosphoenolpyruvate carboxykinase was assayed and the synthesis rate determined as described in the Experimental section. Values are the means $\pm$ S.E.M. of the number of observations in parentheses.

Tissue	Re-feeding	Phosphoenolpyruvate carboxykinase (units/g fresh wt.)	$10^3 \times$ Radioactivity incorporated (d.p.m./g of tissue)		Incorporation into phosphoenolpyruvate carboxykinase (%)
			Cytosol protein	Phosphoenolpyruvate carboxykinase	
Adipose (2)	–	0.32	778	24.7	3.2
Adipose (2)	+	0.39	1212	22.0	1.8
Liver (5)	–	12.30 $\pm$ 0.32	1132 $\pm$ 95	37.8 $\pm$ 19.3	3.30 $\pm$ 0.29
Liver (5)	+	10.32 $\pm$ 0.65	896 $\pm$ 29	11.7 $\pm$ 4.7	1.09 $\pm$ 0.18

Table 4. *Effect of insulin on phosphoenolpyruvate carboxykinase synthesis in the adipose tissue and liver of diabetic rats*

Diabetic rats were injected subcutaneously with 5 units of insulin/100 g body wt. (+) at time zero, with 100 μ Ci of [3 H]leucine at 1.5 h and were killed at 2 h. Phosphoenolpyruvate carboxykinase was assayed and the synthesis rate determined as described in the Experimental section. Values are the means $\pm$ S.E.M. of the number of observations in parentheses.

Tissue	Insulin	Phosphoenolpyruvate carboxykinase (units/g fresh wt.)	$10^{-3} \times$ Radioactivity incorporated (d.p.m./g of tissue)		Incorporation into phosphoenolpyruvate carboxykinase (%)
			Cytosol protein	Phosphoenolpyruvate carboxykinase	
Adipose (3)	–	0.29 $\pm$ 0.08	76 $\pm$ 19	1.14 $\pm$ 0.27	1.57 $\pm$ 0.28
Adipose (3)	+	0.24 $\pm$ 0.05	243 $\pm$ 40	3.14 $\pm$ 0.21	1.28 $\pm$ 0.17
Liver (6)	–	13.40 $\pm$ 0.60	585 $\pm$ 77	23.2 $\pm$ 2.1	3.99 $\pm$ 0.21
Liver (6)	+	10.54 $\pm$ 0.59	281 $\pm$ 120	1.41 $\pm$ 0.41	0.47 $\pm$ 0.12

investigate the possibility of a glucocorticoid–insulin interaction being involved in the response of the hepatic and adipose tissue enzymes to re-feeding (Table 3).

Re-feeding starved adrenalectomized rats for 3 h (Table 3) resulted in a decreased rate of hepatic phosphoenolpyruvate carboxykinase synthesis, although this was not as marked as that seen previously (Hopgood *et al.*, 1973; Tilghman *et al.*, 1974) in intact animals. Similarly, the relative synthesis rate of adipose-tissue phosphoenolpyruvate carboxykinase was also decreased in re-fed adrenalectomized rats (Table 3), but as in intact animals the effect was slow in comparison with the liver (Hopgood *et al.*, 1973). The difference in the response of the liver enzyme to re-feeding and glucocorticoids in starved intact or adrenalectomized rats might reflect the amount of insulin secreted during the different treatments. Compared with the liver enzyme, the slower response of adipose-tissue phosphoenolpyruvate carboxykinase to re-feeding might reflect differences in insulin concentration between the portal vein and peripheral vessels. Insulin injection into diabetic rats (Table 4) causes a rapid decrease in the synthesis rate of hepatic

phosphoenolpyruvate carboxykinase, but has little effect, over the first 2 h of treatment, on the relative synthesis rate of the adipose-tissue enzyme.

The data in Tables 3 and 4 therefore indicate that phosphoenolpyruvate carboxykinase in both adipose tissue and liver is de-induced by insulin, that de-induction is slow in adipose tissue compared with liver, and that this effect of insulin *in vivo* is separate and independent of any direct effect of the glucocorticoids. On the other hand, the possibility exists that the action of glucocorticoids to decrease the synthesis rate of adipose-tissue phosphoenolpyruvate carboxykinase is mediated via insulin release (Gunn *et al.*, 1975a). However, the difference in the response of the enzyme in adipose tissue, liver and kidney to adrenalectomy and triamcinolone injection (Table 2) indicates that, in contrast with the enzyme in the other two tissues, glucocorticoids act in the absence of insulin to decrease the synthesis rate of adipose-tissue phosphoenolpyruvate carboxykinase.

These alternatives are resolved in Table 5. Triamcinolone injection into adrenalectomized diabetic rats increased the activity and synthesis rate of phosphoenolpyruvate carboxykinase in both the liver and

Table 5. Tissue differences in the response to triamcinolone of phosphoenolpyruvate carboxykinase synthesis in adrenalectomized diabetic rats

Adrenalectomized diabetic rats were injected subcutaneously with triamcinolone (5 mg/100 g body wt.) and were killed 10 or 24 h later. At 0.5 h before death all animals were injected with 100 μ Ci [3 H]leucine. Phosphoenolpyruvate carboxykinase was assayed and the synthesis rate determined as described in the Experimental section. Values are the means $\pm$ S.E.M. of the number of observations in parentheses.

Tissue	Time after triamcinolone (h)	Phosphoenolpyruvate carboxykinase (units/g fresh wt.)	10 ⁻³ $\times$ Radioactivity incorporated (d.p.m./g of tissue)		Incorporation into phosphoenolpyruvate carboxykinase (%)
			Cytosol protein	Phosphoenolpyruvate carboxykinase	
Adipose	0 (8)	0.40 $\pm$ 0.03	422 $\pm$ 48	6.68 $\pm$ 0.88	1.84 $\pm$ 0.09
	10 (3)	0.15 $\pm$ 0.03	303 $\pm$ 26	4.27 $\pm$ 0.43	1.40 $\pm$ 0.29
	24 (9)	0.05 $\pm$ 0.01	299 $\pm$ 48	2.99 $\pm$ 0.54	1.09 $\pm$ 0.20
Liver	0 (16)	6.90 $\pm$ 0.61	1026 $\pm$ 58	19.3 $\pm$ 2.2	2.04 $\pm$ 0.22
	10 (8)	6.63 $\pm$ 0.65	975 $\pm$ 74	21.3 $\pm$ 2.1	2.15 $\pm$ 0.15
	24 (13)	9.44 $\pm$ 0.55	1216 $\pm$ 152	29.8 $\pm$ 3.5	3.11 $\pm$ 0.49
Kidney	0 (16)	7.04 $\pm$ 0.69	599 $\pm$ 59	16.1 $\pm$ 1.9	3.16 $\pm$ 0.40
	10 (8)	11.34 $\pm$ 1.03	499 $\pm$ 38	33.3 $\pm$ 3.2	7.26 $\pm$ 0.27
	24 (13)	17.29 $\pm$ 1.66	634 $\pm$ 79	33.4 $\pm$ 2.5	6.19 $\pm$ 0.56

the kidney. However, the activity and synthesis rate of the adipose-tissue enzyme was decreased under those conditions. Thus in contrast with the enzyme in liver and kidney, the synthesis rate of phosphoenolpyruvate carboxykinase in adipose tissue is decreased by both insulin and glucocorticoids apparently acting independently.

In some cases the decrease in phosphoenolpyruvate carboxykinase activity in adipose tissue caused by triamcinolone was greater than the decrease in synthesis rate of the enzyme. The possibility that this was due to antigenically active, but catalytically inactive, enzyme was ruled out by immunotitration studies, which showed that the decrease in cytosol enzyme activity after triamcinolone treatment paralleled the decrease in cytosol antigenicity (O. Meyuhas, unpublished work). Technical difficulties prevent an accurate determination of degradation rate in adipose tissue *in vivo* (see Hopgood *et al.*, 1973). Therefore the effect of glucocorticoids on the apparent degradation rate of phosphoenolpyruvate carboxykinase was investigated in adipose-tissue organ culture and is reported in the accompanying paper (Meyuhas *et al.*, 1976).

Discussion

The cytosolic forms of phosphoenolpyruvate carboxykinase in adipose tissue, liver and kidney share similar physical, catalytic and immunochemical properties (Ballard & Hanson, 1969; Longshaw & Pogson, 1972; Hopgood *et al.*, 1973; Iynedjian *et al.*, 1975; Meyuhas *et al.*, 1976). This indicates that the primary structure of the enzyme might be identical in all three tissues, and further, that the organ-specific response of the enzyme might reside in the systems

that control the tissue content of the enzyme. Consequently a comparative study of the regulation of the enzyme in the three tissues may shed some light on the differentiation of regulatory systems in the different organs. Here we show that the activity and synthesis rate of adipose-tissue phosphoenolpyruvate carboxykinase is increased by starvation, diabetes, adrenalectomy and noradrenaline, and decreased by re-feeding starved animals, insulin and glucocorticoids. These data, in addition to those previously reported from this laboratory for the enzyme in liver and kidney as well as adipose tissue, enable certain statements to be made about the tissue-specific regulation of the enzyme.

The regulation of phosphoenolpyruvate carboxykinase activity in adipose tissue, liver and kidney shares certain features common to all three tissues. Thus enzyme activity and synthesis rate are increased in diabetic and starved animals (Hopgood *et al.*, 1973; Tilghman *et al.*, 1974, 1975; Gunn *et al.*, 1975a,b; Tables 1, 2 and 4). Characteristically, however, the regulation of phosphoenolpyruvate carboxykinase under these conditions, at least in the liver and kidney, is mediated by different primary effectors. Hepatic enzyme activity and synthesis rate are increased by glucagon, acting through cyclic AMP, and decreased by insulin (Tilghman *et al.*, 1974, 1975; Gunn *et al.*, 1975a,b), whereas the activity and synthesis rate of renal phosphoenolpyruvate carboxykinase are not directly affected by these effectors, but by the acid-base status of the animal (Gunn *et al.*, 1975a; Iynedjian *et al.*, 1975).

Evidence for a direct involvement of insulin in the regulation of adipose-tissue and liver cytosol phosphoenolpyruvate carboxykinase comes from experiments in isolated cell systems (Meyuhas *et al.*, 1976;

Tilghman *et al.*, 1975). In both cases insulin de-induced the synthesis of the enzyme. Also *in vivo*, the activity and synthesis of liver and adipose-tissue enzymes are decreased by injecting insulin to diabetic rats or by re-feeding starved animals (Reshef *et al.*, 1970; Hopgood *et al.*, 1973; Tilghman *et al.*, 1974; Tables 3 and 4). However, in the liver the rate of de-induction of phosphoenolpyruvate carboxykinase synthesis exceeds that of the adipose tissue enzyme by about an order of magnitude (Hopgood *et al.*, 1973; Tables 3 and 4). Whether this observation has any tissue-specific regulatory significance remains to be resolved.

The other striking feature is the tissue-specific response of cytosol phosphoenolpyruvate carboxykinase in all three tissues to glucocorticoids. Thus the activity and synthesis of both kidney and liver enzymes are increased by triamcinolone (Table 5; Gunn *et al.*, 1975a; Iynedjian *et al.*, 1975), but the effect on the liver enzyme is small and apparent only in diabetic rats (cf. Tables 2 and 5; Gunn *et al.*, 1975a). Moreover removal of corticosteroids, by adrenalectomy of intact rats, has little effect on the response of the two enzymes to starvation and is only apparent in diabetic rats.

In contrast, the activity and synthesis of adipose-tissue phosphoenolpyruvate carboxykinase are markedly decreased by glucocorticoids and increased by adrenalectomy. They are independent of insulin release as is indicated by the glucocorticoid effect on diabetic rats (Table 5) and by a direct effect of these hormones on adipose-tissue organ culture (Meyuhas *et al.*, 1976). This tissue-specific response of adipose tissue to glucocorticoids may be associated with a decreased protein synthesis caused by the hormone in this tissue. Measurements of leucine concentration in the amino

acid pool of adipose tissue (Table 6) enable one to determine its true specific radioactivity and hence the amount incorporated into cytosol proteins. We note that the tissue concentration of free leucine is not significantly changed by triamcinolone treatment of adrenalectomized diabetic rats. Since the radioactivity in the trichloroacetic acid-soluble fraction is also not affected (results not shown), and hence the specific radioactivity of tissue leucine, it implies that corticosteroids decrease the synthesis of total cytosol proteins in adipose tissue. This is not the only metabolic activity so affected by corticosteroids in adipose tissue. Decreased glucose transport (Munck, 1962) and a decreased incorporation of [³H]uridine (O. Meyuhas & L. Reshef, unpublished work) have been demonstrated in adipose tissues *in vitro*. The decreased synthesis of adipose-tissue phosphoenolpyruvate carboxykinase caused by glucocorticoids is therefore probably a resultant expression of one or all of these effects. Why the response of this enzyme to glucocorticoids is more sensitive than that of other cytosol proteins (as is evident from the change in the relative rate of its synthesis) remains to be resolved.

The data reported here therefore indicate that adipose-tissue phosphoenolpyruvate carboxykinase, in contrast with the enzyme in liver and kidney, is de-induced by both glucocorticoids and insulin acting independently of one another. Enhanced insulin release after triamcinolone injection into non-diabetic animals will therefore lead to the more rapid de-induction of adipose-tissue phosphoenolpyruvate carboxykinase, evident in comparing Tables 2 and 5, whereas a combination of adrenalectomy and starvation leads to additive effects in terms of increased enzyme activity and synthesis rate (Table 2).

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Table 6. Effect of triamcinolone on leucine concentration in amino acid pools of adipose tissue from adrenalectomized diabetic rats

Adrenalectomized diabetic rats were injected subcutaneously with triamcinolone (5 mg/100 g body wt) and killed 24 h later. Non-injected adrenalectomized diabetic rats served as controls. Two batches of adipose tissue from untreated rats and two batches of rats treated with triamcinolone were frozen by liquid air and homogenized with 5% (w/v) trichloroacetic acid in 0.05 M-sodium citrate buffer, pH 2.2, and analysed on a Beckman model 120B amino acid analyser according to the makers' instructions. Numbers in parentheses indicate the number of animals used for each batch.

Triamcinolone	Free leucine ($\mu\text{mol/g}$ of tissue)
-(3)	0.024
-(4)	0.028
+(4)	0.044
+(4)	0.024

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