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Glucocorticoids and the Regulation of Phosphoenolpyruvate Carboxykinase (Guanosine Triphosphate) in the Rat

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The effect of glucocorticoids on the synthesis and degradation of phosphoenolpyruvate carboxykinase (GTP) (EC 4.1.1.32) in rat liver and kidney *in vivo* was studied immunochemically. The glucocorticoid analogue triamcinolone (9α-fluoro-11β,21-dihydroxy-16α,17α-isopropylidenedioxypregna-1,4-diene-3,20-dione) increased the synthesis rate of the kidney enzyme in fed rats, but did not further increase the high synthesis rate of this enzyme in starved animals. Both triamcinolone and cortisol decreased the synthesis rate of hepatic phosphoenolpyruvate carboxykinase (GTP) in fed and starved rats, but were without effect on the degradation rate of the enzyme. This effect of triamcinolone in liver was reversed by injection of dibutyryl cyclic AMP. However, in diabetic animals glucocorticoids increased the synthesis rate of hepatic phosphoenolpyruvate carboxykinase (GTP). Triamcinolone administration to starved rats *in vivo* is shown to cause an increase in the portal blood concentrations of insulin and glucose. Since the physiological de-inducer of liver phosphoenolpyruvate carboxykinase (GTP) is insulin, this is the probable cause of the decrease in the synthesis rate of the hepatic enzyme noted when glucocorticoids are administered to non-diabetic animals.

There is confusion in the literature over the effect of glucocorticoid administration on hepatic phosphoenolpyruvate carboxykinase (GTP) (EC 4.1.1.32) activity in the rat. Several papers (Shrago *et al.*, 1963; Foster *et al.*, 1966; Wicks *et al.*, 1969; Huttner *et al.*, 1974; Krone *et al.*, 1974) report that single or multiple injections of cortisone, cortisol or triamcinolone§ into intact fed or adrenalectomized fed rats cause an increase in hepatic enzyme activity. Similarly in Reuber H35 hepatoma cells in culture, physiological concentrations of glucocorticoids increase phosphoenolpyruvate carboxykinase activity (Barnett & Wicks, 1971; Wicks *et al.*, 1974). Longshaw *et al.* (1972) show that, whereas a single injection of triamcinolone abolishes the normal rhythm of the liver enzyme, multiple injections increase enzyme activity. However, Reshef *et al.* (1969) find that triamcinolone has no effect in fed animals. Further, in starved rats, glucocorticoids decrease (Ray *et al.*, 1964; Foster *et al.*, 1966; Reshef *et al.*, 1969) whereas in diabetic animals they increase (Reshef *et al.*, 1970; Exton *et al.*, 1973) hepatic phosphoenolpyruvate carboxykinase activity. Such conflicting results apparently argue against a direct role (Wicks & McKibbin, 1972; Wicks *et al.*, 1974) for the glucocorticoids in the regulation of this enzyme.

One possible interpretation is that the effect of glucocorticoids on hepatic phosphoenolpyruvate carboxykinase is secondary to their primary role in regulating other metabolic systems. This is partially supported by the observations that, although the activity of renal phosphoenolpyruvate carboxykinase is markedly elevated by a single injection of triamcinolone (Longshaw & Pogson, 1972; Longshaw *et al.*, 1972), enzyme activity from adipose tissue is decreased by glucocorticoids (Reshef *et al.*, 1969, 1970; Reshef & Hanson, 1972). Clarification of the role of the glucocorticoids in the regulation of hepatic phosphoenolpyruvate carboxykinase might be obtained by determining rates of synthesis and degradation of the enzyme *in vivo*, since this provides a more sensitive assay than activity measurement alone. Accordingly, in this paper we have determined, immunochemically, the effect of triamcinolone on the synthesis of the kidney enzyme and the synthesis and degradation of the liver enzyme. The results of these studies indicate that a glucocorticoid–insulin interaction might be involved in the regulation of hepatic phosphoenolpyruvate carboxykinase *in vivo*.

§ Abbreviation: Triamcinolone (acetonide), 9α-fluoro-11β,21-dihydroxy-16α,17α-isopropylidenedioxypregna-1,4-diene-3,20-dione.

Experimental

Materials

Cortisol-21 phosphate (disodium salt) and dibutyryl cyclic AMP (sodium salt) were obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A., and triamcinolone acetonide was from E. R. Squibb and Sons, New York, N.Y., U.S.A. The L-[4,5-^{3}H]leucine (30–50Ci/mmol) and $NaH^{14}CO_3$ (2–10mCi/mmol) were purchased from New England Nuclear, Boston, Mass., U.S.A. and NADH, IDP, phosphoenolpyruvate and malate dehydrogenase (NAD^+) (EC 1.1.1.37) were from Boehringer (Mannheim), New York, N.Y., U.S.A. Specific antibodies to phosphoenolpyruvate carboxykinase from the cytosol of rat liver were produced in goats as described by Hopgood *et al.* (1973).

Animals

Male Wistar rats (150–170g) were fed on standard laboratory chow and maintained on a cycle of alternating 12h periods of darkness and light with the light period starting at 07:00h. To minimize diurnal variation the experiments were started between 07:00 and 09:00h except for the 18h point in Fig. 4 in which case the animals were injected with triamcinolone at 15:00h and killed 18h later. Diabetes was induced by the subcutaneous injection into starved rats of 7.5mg of alloxan per 100g body weight. The animals were used 5–7 days later when marked glucosuria had developed; when they were killed they had blood glucose values in excess of 400mg/100ml.

Immunoprecipitation of phosphoenolpyruvate carboxykinase

Kidney and liver cytosolic phosphoenolpyruvate carboxykinases are immunologically identical (Longshaw & Pogson, 1972) and the same procedure was used for the immunoprecipitation of the enzyme from both tissues. In experiments measuring synthesis of the enzyme, rats were injected with 200μCi of [4,5-^{3}H]leucine intraperitoneally and killed 30min later. The livers and kidneys were removed and homogenized in 0.25M-sucrose and a cytosol fraction was prepared by centrifugation at 105000*g* for 40min. The activity of phosphoenolpyruvate carboxykinase was determined for each cytosol fraction by a modification (Ballard & Hanson, 1969) of the method of Chang & Lane (1966). One unit of enzyme activity catalyses the fixation of 1μmol of $NaH^{14}CO_3$/min at 37°C. The radioactivity incorporated into phosphoenolpyruvate carboxykinase isolated as an antigen–antibody complex and into total cytosol protein was determined as described previously (Ballard & Hopgood, 1973; Hopgood *et al.*, 1973; Tilghman *et al.*, 1974). The pulse-labelling period of 30min used in the synthesis experiments is less than 10% of the reported half-life of phosphoenolpyruvate carboxykinase (Shrago *et al.*, 1963; Reshef *et al.*, 1970; Treadow & Khairallah, 1972; Ballard & Hopgood, 1973; Hopgood *et al.*, 1973; Hanson *et al.*, 1973). Thus any error arising from protein turnover involved in measuring enzyme synthesis will be negligible. Synthesis rates for phosphoenolpyruvate carboxykinase are expressed both as the radioactivity incorporated into the enzyme/30min per mg of tissue and as the percentage incorporation relative to cytosol protein. The latter method allows for variability in the amount of label injected. Also, when synthesis is expressed as a relative rate, any changes in the precursor leucine pool will not influence the results provided that total cytosol proteins and phosphoenolpyruvate carboxykinase are synthesized from the same pool of amino acids.

In the degradation experiment, starved rats were injected with 200μCi of [4,5-^{3}H]leucine followed by a chase of 12.5μmol of unlabelled L-leucine 1h later. The animals were killed 1, 7 and 18h after the injection of radioactive leucine, and the extent of labelling of the enzyme and cytosol protein was determined as indicated above. The degradation rate of phosphoenolpyruvate carboxykinase is measured as the rate of isotope loss after the enzyme pool has been labelled with radioactive L-leucine. Data are expressed as the relative amount present compared with radioactivity in total cytosol proteins. The rate of degradation of phosphoenolpyruvate carboxykinase will therefore be underestimated because of the turnover of all proteins. However, provided that the turnover of the enzyme is rapid compared with that of the average for protein, the error will not be large.

Insulin and glucose determinations

Serum insulin was assayed by the method of Soeldner & Slone (1965) and blood glucose by the method of Hill & Kessler (1961).

Results

Hepatic phosphoenolpyruvate carboxykinase is responsive to induction by injected dibutyryl cyclic AMP (Reshef & Hanson, 1972; Wicks *et al.*, 1972; Hanson *et al.*, 1973; Tilghman *et al.*, 1974) as shown by an increased activity and rate of enzyme synthesis 2h after injection (Table 1). Triamcinolone, however, markedly decreased the relative synthesis rate of hepatic phosphoenolpyruvate carboxykinase 7h after injection into fed rats (Table 1). This change was not due to a large alteration in the rate of cytosol protein synthesis, for although triamcinolone slightly depressed the incorporation of leucine into liver cytosol proteins, the effect of the glucocorticoid on phosphoenolpyruvate carboxykinase was greater

Table 1. *Effect of dibutyryl cyclic AMP, triamcinolone and cortisol on hepatic phosphoenolpyruvate carboxykinase synthesis in fed rats*

Triamcinolone was injected intraperitoneally at a dose of 5mg/100g body weight at time zero. Cortisol was given intraperitoneally at 2.5mg/100g at 0 and 3.5h and dibutyryl cyclic AMP, 2.5mg/animal, at 5 and 6h. All animals were injected with [^{3}H]leucine at 6.5h and killed at 7h. Phosphoenolpyruvate carboxykinase was assayed and the synthesis rate determined as described in the Experimental section. The data are presented as the means ± S.E.M. of the numbers of observations in parentheses.

Treatment	Phosphoenolpyruvate carboxykinase (units/g fresh wt.)	Radioactivity incorporated (c.p.m./mg of liver)		Incorporation into phosphoenolpyruvate carboxykinase (%)
		Cytosol protein	Phosphoenolpyruvate carboxykinase	
None (10)	4.05±0.29	669±91	6.83±1.11	1.04±0.12
Dibutyryl cyclic AMP (6)	6.15±0.23	437±45	10.2 ±1.0	2.34±0.31
Triamcinolone (6)	4.57±0.28	533±32	1.49±0.30	0.28±0.06
Cortisol (3)	4.80±0.44	854±262	3.43±0.87	0.42±0.04

Table 2. *Effect of dibutyryl cyclic AMP, triamcinolone and cortisol on renal phosphoenolpyruvate carboxykinase synthesis in fed rats*

Experimental details are given in the legend to Table 1 and in the Experimental section.

Treatment	Phosphoenolpyruvate carboxykinase (units/g fresh wt.)	Radioactivity incorporated (c.p.m./mg of kidney)		Incorporation into phosphoenolpyruvate carboxykinase (%)
		Cytosol protein	Phosphoenolpyruvate carboxykinase	
None (8)	3.93±0.26	357±40	4.76±1.17	1.32±0.21
Dibutyryl cyclic AMP (6)	3.67±0.39	221±14	2.53±0.16	1.17±0.11
Triamcinolone (6)	7.11±0.47	240±18	9.67±1.93	3.92±0.65
Cortisol (3)	5.94±0.39	338±15	14.1 ±4.4	3.94±0.25

(Table 1). The renal enzyme was not affected by dibutyryl cyclic AMP and was increased in activity by triamcinolone owing to a 2–3-fold increase in its relative rate of synthesis (Table 2). This effect of the glucocorticoid was expected on the basis of published observations (Longshaw & Pogson, 1972; Longshaw *et al.*, 1972). There was little change in the synthesis rate of renal cytosol proteins indicating that the effect of triamcinolone was specific to phosphoenolpyruvate carboxykinase.

There are a number of possible explanations for the decreased rate of hepatic phosphoenolpyruvate carboxykinase synthesis found in fed rats (Table 1). Triamcinolone is not a naturally occurring glucocorticoid and is relatively long-lived *in vivo* (Florin *et al.*, 1961). Therefore its effect on the hepatic enzyme might not be shared by other glucocorticoids. However, cortisol phosphate (Table 1) gave quantitatively similar results. It increased the relative synthesis rate of the kidney enzyme and decreased the relative synthesis rate of liver phosphoenolpyruvate carboxykinase. Again these changes in the relative rate of enzyme synthesis were not due to gross changes in total cytosol protein synthesis (Table 1). A dose–response curve (Fig. 1) also indicates that 0.1mg of triamcinolone per 100g body weight effectively lowers the relative synthesis rate of hepatic phosphoenolpyruvate carboxykinase 7h after injection into fed rats. This dose–response curve is similar to that reported by us for the effect of triamcinolone on hepatic enzyme activity in adrenalectomized starved rats (Reshef *et al.*, 1969) and suggests that the amount of hormone administered in Table 1 was not excessive.

Foster *et al.* (1966) reported that, after a single injection of cortisol or triamcinolone into fed intact or adrenalectomized rats, hepatic phosphoenolpyruvate carboxykinase activity increased for 4h then decreased to the control value by 8h. Assuming no increase in the degradation rate, these data might reflect an initial increase followed, between 4 and 8h, by a marked decrease in the synthesis rate for the enzyme. To test this possible interpretation of our results, we followed the time-course of the effect of triamcinolone on the liver and kidney enzymes in fed rats (Fig. 2). In both tissues, triamcinolone had no effect on the relative synthesis rate of phosphoenolpyruvate carboxykinase over the first 3h. Thereafter

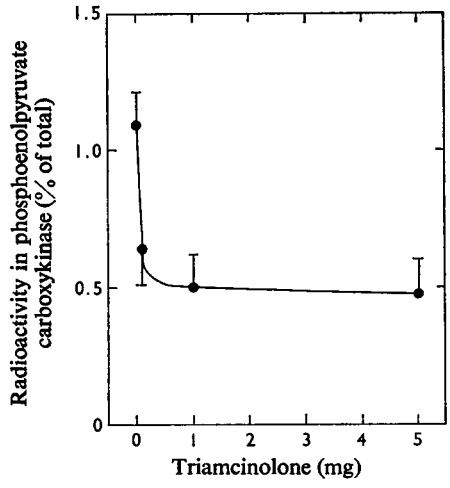

Fig. 1. *Dose-response to triamcinolone of the relative synthesis rate of hepatic phosphoenolpyruvate carboxykinase*

Fed rats were injected intraperitoneally with triamcinolone at time zero, with 200μCi of [^{3}H]leucine at 6.5h, and killed at 7h. The dose of steroid is given in mg/100g body weight. The synthesis rate was determined as described in the text. Each point is the mean $\pm$ S.E.M. of six observations.

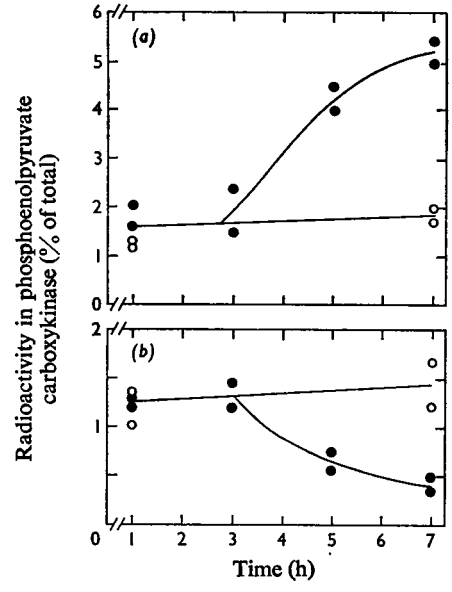

Fig. 2. *Time-course of the effect of triamcinolone on the relative synthesis rate of phosphoenolpyruvate carboxykinase in liver and kidney*

Fed rats were injected intraperitoneally with 5mg of triamcinolone/100g body weight at time zero and killed at the times shown. At 0.5h before death they also received 200μCi of [^{3}H]leucine intraperitoneally. For further experimental details see the text. (*a*) Relative synthesis rate of the kidney enzyme; (*b*) relative synthesis rate of the liver enzyme, for controls (○) and triamcinolone (●). Each point represents the mean of a duplicate determination on one animal.

there was a progressive increase in the relative synthesis rate of kidney phosphoenolpyruvate carboxykinase and a progressive decrease in this parameter for the liver enzyme.

Two factors that are important in regulating the concentration of hepatic phosphoenolpyruvate carboxykinase are cyclic AMP (Table 1) and insulin; cyclic AMP increases and insulin decreases the synthesis rate and activity of the enzyme (Wicks & McKibbin, 1972; Wicks *et al.*, 1972; Hanson *et al.*, 1973; Tilghman *et al.*, 1974; Wicks *et al.*, 1974). It seemed possible that glucocorticoids decrease the synthesis rate of hepatic phosphoenolpyruvate carboxykinase either by antagonizing cyclic AMP within the hepatocyte or by increasing the concentrations and/or effectiveness of insulin. Since the concentration of cyclic AMP in the rat liver is increased by starvation and diabetes (Jefferson *et al.*, 1968; Exton *et al.*, 1971, 1973; Park & Exton, 1972), the effect of triamcinolone on the synthesis rate of the enzyme was investigated under these conditions.

Triamcinolone was found to be as effective in lowering the synthesis rate of hepatic phosphoenolpyruvate carboxykinase in starved (Table 3) as in fed animals, but had no further stimulatory effect on the elevated rate of synthesis noted in the kidney of starved rats (Table 4). A single injection of triamcinolone effectively blocks (Table 3, Expt. B) the normal increase in hepatic phosphoenolpyruvate carboxykinase activity and synthesis rate produced by starving for 24h (i.e. fed animals were injected with triamcinolone and then starved for 24h). Again the effects of the glucocorticoid and starvation were not additive in increasing the synthesis of kidney phosphoenolpyruvate carboxykinase (Table 4, Expt. B). It is also clear that, in both the liver and kidney, it is specific protein synthesis that is being effected by triamcinolone rather than synthesis of total cytosol proteins. However, if the concentration of cyclic AMP is further increased by injection of the dibutyryl analogue (Table 3, Expt. C), the decrease in synthesis rate of hepatic phosphoenolpyruvate carboxykinase is overcome with a resultant increase in enzyme activity and synthesis rate. In this experiment, dibutyryl cyclic AMP was injected 5 and 6h after the glucocorticoid by which time the relative synthesis rate of the hepatic enzyme would have declined to about one-half the untreated value (Fig. 2). This result is similar to our previous report that dibutyryl

Table 3. *Effect of triamcinolone on hepatic phosphoenolpyruvate carboxykinase synthesis in starved rats*

In Expts. A and C, rats starved overnight were injected intraperitoneally with triamcinolone (5mg/100g) at time zero and, in Expt. C, with dibutyryl cyclic AMP (2.5mg/animal) at 5 and 6h. The animals were given [^{3}H]leucine at 6.5h and the activity and synthesis rate of phosphoenolpyruvate carboxykinase determined as described in the Experimental section. In Expt. B, fed rats were injected with triamcinolone (5mg/100g) and were then starved for 24h before receiving [^{3}H]leucine. Data are means ± S.E.M. for the number of observations in parentheses.

	Treatment	Phosphoenolpyruvate carboxykinase (units/g fresh wt.)	Radioactivity incorporated (c.p.m./mg of liver)		Incorporation into phosphoenolpyruvate carboxykinase (%)
			Cytosol protein	Phosphoenolpyruvate carboxykinase	
Expt. A	None (3)	5.38±0.17	1425±71	29.2±0.2	2.06±0.11
	Triamcinolone (3)	4.68±0.54	1162±186	9.85±2.34	0.86±0.16
Expt. B	None (3)	7.27±0.21	1074±18	18.4±2.3	1.72±0.15
	Triamcinolone (3)	3.83±0.27	1046±23	7.00±0.32	0.67±0.04
Expt. C	Triamcinolone (3)	9.94±0.28	1216±101	15.3±2.4	1.28±0.28
	Triamcinolone + dibutyryl cyclic AMP (3)	9.06±0.47	1383±52	36.5±4.1	2.63±0.26

Table 4. *Effect of triamcinolone on renal phosphoenolpyruvate carboxykinase synthesis in starved rats*

Experimental details are given in the legend to Table 3 and in the Experimental section.

	Treatment	Phosphoenolpyruvate carboxykinase (units/g fresh wt.)	Radioactivity incorporated (c.p.m./mg of kidney)		Incorporation into phosphoenolpyruvate carboxykinase (%)
			Cytosol protein	Phosphoenolpyruvate carboxykinase	
Expt. A	None (3)	10.38±0.24	515±30	25.6±0.5	5.01±0.37
	Triamcinolone (3)	9.92±0.38	360±68	19.0±1.6	5.26±0.97
Expt. B	None (3)	12.39±0.73	498±11	24.4±1.5	4.92±0.29
	Triamcinolone (3)	15.96±0.38	537±11	25.8±1.5	4.81±0.18

Table 5. *Effect of triamcinolone on hepatic phosphoenolpyruvate carboxykinase synthesis in diabetic rats*

Triamcinolone (5mg/100g) was injected intraperitoneally at time zero and [^{3}H]leucine at 6.5h. Phosphoenolpyruvate carboxykinase was assayed and the synthesis rate determined as described in the Experimental section. Data are means ± S.E.M. of the number of observations in parentheses.

Treatment	Phosphoenolpyruvate carboxykinase (units/g fresh wt.)	Radioactivity incorporated (c.p.m./mg of liver)		Incorporation into phosphoenolpyruvate carboxykinase (%)
		Cytosol protein	Phosphoenolpyruvate carboxykinase	
None (8)	10.74±1.29	418±58	12.3±1.8	2.95±0.14
Triamcinolone (12)	13.84±1.37	515±127	20.8±6.6	4.02±0.19

cyclic AMP prevents the decrease in hepatic phosphoenolpyruvate carboxykinase synthesis normally seen when diabetic rats are treated with insulin (Tilghman *et al.*, 1974).

Since insulin and cyclic AMP are major regulators of the liver enzyme and insulin is known to decrease hepatic cyclic AMP concentrations (Park & Exton, 1972; Park *et al.*, 1972), it is possible that insulin release in response to glucocorticoids might be a key factor in the marked decrease in the rate of hepatic phosphoenolpyruvate carboxykinase synthesis. In partial support of this hypothesis we find (Table 5) that injection of triamcinolone into diabetic rats further increased, rather than decreased, the high synthesis rate of the liver enzyme. These data on starved and diabetic animals supports previous

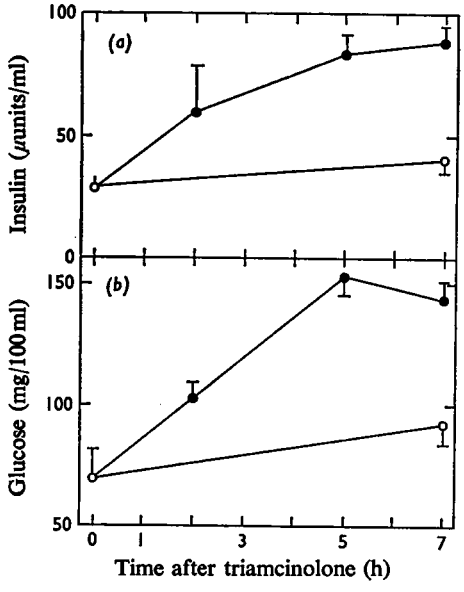

Fig. 3. *Time-course of the effect of triamcinolone on portal blood glucose and insulin concentrations*

Starved rats (○) were injected intraperitoneally with 5mg of triamcinolone/100g body weight (●) at time zero. At the time-points shown, the rats were anaesthetized and approx. 4ml of blood was withdrawn from the portal vein. Each point is the mean ± S.E.M. of four observations for serum insulin (*a*) and blood glucose (*b*).

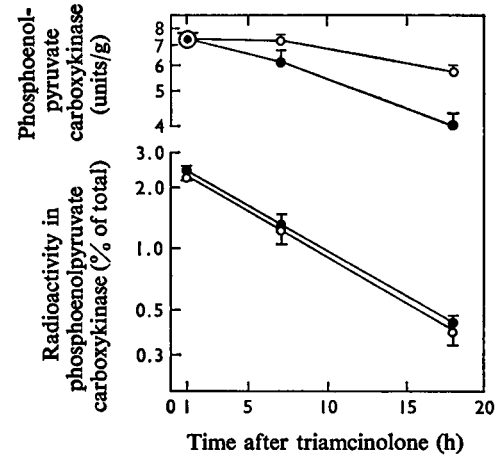

Fig. 4. *Effect of triamcinolone on the activity and relative degradation rate of hepatic phosphoenolpyruvate carboxykinase in starved rats*

Starved rats were injected intraperitoneally with 0.9% NaCl (○) or 5mg of triamcinolone/100g body weight (●) and 200μCi of [^{3}H]leucine. At 1h later they were killed (1h points) or given 12.5μmol of L-leucine and killed at 7 and 18h. The animals were starved for the duration of the experiment. Radioactivity in phosphoenolpyruvate carboxykinase is expressed as a percentage of the total radioactivity in cytosol proteins (see the Experimental section). Values are means ± S.E.M. of duplicate determinations of four animals.

observations of changes in enzyme activity (Ray *et al.*, 1964; Foster *et al.*, 1966; Reshef *et al.*, 1969, 1970; Exton *et al.*, 1973).

Direct effects of a single hormone are most readily distinguished from interactions with other hormones in an isolated system. We have shown (Gunn *et al.*, 1975; Tilghman *et al.*, 1975) that the relative synthesis rate of phosphoenolpyruvate carboxykinase in Reuber H35 cells is increased by dexamethasone and dibutyryl cyclic AMP, although the effect of the glucocorticoid is small compared with the cyclic nucleotide. Thus, in the absence of insulin, either *in vivo* or in Reuber H35 cells in culture, glucocorticoids are able to increase the relative synthesis rate of phosphoenolpyruvate carboxykinase.

However, in normal animals glucocorticoids are known to elicit insulin release (Cambell *et al.*, 1966; Malaisse *et al.*, 1967; Owen & Cahill, 1973; Curry & Bennett, 1973; Altszuler *et al.*, 1974). Therefore, in non-diabetic animals, if triamcinolone is affecting hepatic phosphoenolpyruvate carboxykinase via insulin, an increase in portal blood insulin should occur at the same time or before the decrease in relative synthesis rate of the enzyme (Fig. 2). Also, there should be a concomitant increase in blood glucose concentration since this is a necessary requirement for insulin release. The data in Fig. 3 clearly demonstrate that a single injection of triamcinolone increases the portal blood concentrations of insulin and glucose in starved rats. Further, these changes precede the decrease in the synthesis rate of hepatic phosphoenolpyruvate carboxykinase (Fig. 2).

Although triamcinolone decreases the synthesis rate of the liver enzyme, it does not always lower the activity of the enzyme within 7h (Tables 1 and 3). Thus the steroid (or insulin) might be decreasing both the rates of synthesis and degradation of the enzyme. Fig. 4 shows that triamcinolone has no effect on the relative degradation rate of hepatic phosphoenolpyruvate carboxykinase. The labelling in cytosol proteins remained approximately constant. The calculated half-lives for the radioactive enzyme under these conditions were 6.7 and 6.9h respectively for the control and steroid-treated animals, which agree with the value of approx. 6h reported in the literature (Shrago *et al.*, 1963; Reshef *et al.*, 1970; Treadow & Khairallah, 1972; Ballard & Hopgood, 1973; Hopgood *et al.*, 1973). Since the synthesis rate of the enzyme is not depressed

until approx. 4h after the administration of triamcinolone (Fig. 2) and the degradation rate is unaffected, the activity as measured over 7h may not be correspondingly decreased. Over a longer time-course, triamcinolone significantly decreased the activity of the liver enzyme (Fig. 4) presumably because of the marked decrease in synthesis.

Discussion

A number of investigators have reported that the injection of glucocorticoids into intact rats causes an elevation in hepatic phosphoenolpyruvate carboxykinase activity (Shrago *et al.*, 1963; Foster *et al.*, 1966; Wicks *et al.*, 1969; Longshaw & Pogson, 1972; Huttner *et al.*, 1974; Krone *et al.*, 1974). In our own studies (Reshef *et al.*, 1969, 1970) we have not noted such an increase unless the animals were first made diabetic. Exton *et al.* (1973) also demonstrate an induction of the enzyme by glucocorticoids, but again in diabetic adrenalectomized rats. In the present study we report that the injection of triamcinolone or cortisol into fed or starved rats has no effect on the degradation rate but actually decreases the rate of enzyme synthesis. From the results of these and a number of other studies carried out in our laboratory, it is our contention that glucocorticoids are not as important in the primary regulation of hepatic phosphoenolpyruvate carboxykinase as are cyclic AMP and insulin. We are proposing, on the basis of the present work, that the glucocorticoids will increase hepatic phosphoenolpyruvate carboxykinase only in the absence of insulin and that the injection of glucocorticoids into intact animals results in insulin secretion, thereby actually decreasing the synthesis rate of the enzyme.

Hepatic phosphoenolpyruvate carboxykinase activity is increased in starvation and diabetes (Shrago *et al.*, 1963; Foster *et al.*, 1966) conditions associated with an increased hepatic cyclic AMP concentration (Jefferson *et al.*, 1968; Exton *et al.*, 1971, 1973; Park & Exton, 1972), and by administration of glucagon or dibutyryl cylic AMP (Shrago *et al.*, 1963; Wicks *et al.*, 1969; Reshef & Hanson, 1972). Such changes (Tables 1, 3 and 5) are due to an increase in the synthesis rate for the enzyme (Wicks *et al.*, 1972; Hopgood *et al.*, 1973; Hanson *et al.*, 1973; Tilghman *et al.*, 1974). Re-feeding or insulin treatment of diabetic rats produces a decrease in enzyme activity (Shrago *et al.*, 1963; Foster *et al.*, 1966) through a rapid fall (half-time 40min) in synthesis rate (Hopgood *et al.*, 1973; Tilghman *et al.*, 1974) and in both cases this is blocked by dibutyryl cyclic AMP (Tilghman *et al.*, 1974). Insulin also antagonizes the stimulatory effect of the cyclic nucleotide on phosphoenolpyruvate carboxykinase activity and synthesis rate in Reuber H35 cells in culture (Barnett & Wicks, 1971; Wicks *et al.*, 1974; Gunn *et al.*, 1975; Tilghman *et al.*, 1975). Thus a high concentration of insulin effectively counteracts the stimulatory effect of cyclic AMP on enzyme synthesis.

The analogy between these experiments with cyclic AMP and insulin and those reported here leads us to conclude that insulin is mediating the glucocorticoid effect on the synthesis rate of hepatic phosphoenolpyruvate carboxykinase in normal animals. In support of this we have shown that triamcinolone evokes insulin release *in vivo*, even in starved animals (Fig. 3) and that this precedes the decrease in the synthesis rate of hepatic phosphoenolpyruvate carboxykinase (Fig. 2). In keeping with the antagonistic actions of cyclic AMP and insulin on the enzyme, subsequent injection of dibutyryl cyclic AMP prevents this depressive effect of triamcinolone on enzyme synthesis (Table 3). Further circumstantial evidence is provided by the opposite response to glucocorticoids of hepatic phosphoenolpyruvate carboxykinase in diabetic animals (Table 5) and by the response of the renal enzyme in fed animals. As shown in Table 2, the synthesis rate of renal phosphoenolpyruvate carboxykinase is markedly increased by the administration of glucocorticoids. The kidney enzyme is not directly affected by insulin, being responsive instead to insulin-induced changes in the acid–base status of the animal (Longshaw & Pogson, 1972; Longshaw *et al.*, 1972; Kamm *et al.*, 1974). Therefore an increased pancreatic output of insulin after glucocorticoid injection would not result in a decreased rate of enzyme synthesis but rather an induction due to the steroid. It is probable that much of the confusion in the literature over the effects of glucocorticoids on hepatic phosphoenolpyruvate carboxykinase *in vivo* revolves around the use of diabetic or non-diabetic animals as well as the efficacy with which different glucocorticoids are able, either directly or indirectly, to evoke insulin release.

Glucocorticoids increase the rate of hepatic gluconeogenesis both by altering the supply of glucose precursors and by changing the rate of glucose synthesis from these substrates (Russell, 1955; Renold *et al.*, 1956; Ray *et al.*, 1964; Dunn *et al.*, 1969). In diabetic animals this is accompanied by an increase in the activity (Reshef *et al.*, 1970; Exton *et al.*, 1973) and synthesis rate (Table 5) of phosphoenolpyruvate carboxykinase. In normal animals, however, before these changes in the enzyme occur, the increase in blood glucose and insulin promoted by triamcinolone (Fig. 3) will produce a decrease in gluconeogenesis (Park & Exton, 1972; Park *et al.*, 1972) and a decrease in the synthesis rate of the enzyme (Fig. 2). Similarly, the ability of glucocorticoids to induce hepatic glycogen deposition largely depends on insulin secretion (Kreutner & Goldberg, 1967; Nichols & Goldberg, 1972; Exton *et al.*, 1973).

An important question which remains unanswered

by this study is what is the mechanism whereby glucocorticoids increase the activity of hepatic phosphoenolpyruvate carboxykinase when insulin is not present? It is clear from studies with Reuber H35 cells that glucocorticoids can increase both the activity (Barnett & Wicks, 1971; Wicks *et al.*, 1974) and the synthesis rate (Gunn *et al.*, 1975) of the enzyme, a situation analogous to their effect in the diabetic animal *in vivo* (Table 5). Wicks and co-workers (Wicks & McKibbin, 1972; Wicks *et al.*, 1974) have suggested that glucocorticoids induce the enzyme at the level of transcription whereas cyclic AMP acts at some post-transcriptional step. Evidence also points to a transcriptional effect of glucocorticoids in the stimulation of renal phosphoenolpyruvate carboxykinase (Iynedjian *et al.*, 1975). However, definitive proof of this mechanism awaits the isolation and quantification of specific mRNA coding for the enzyme. If glucocorticoids are major regulators of hepatic phosphoenolpyruvate carboxykinase, then enzyme activity should be decreased in adrenalectomized animals. This does not occur. Enzyme activity is the same as in intact animals and the normal starved response is not impaired (Shrago *et al.*, 1963; Ray *et al.*, 1964; Foster *et al.*, 1966; Reshef *et al.*, 1969). This suggests that the glucocorticoids might not be required to maintain hepatic phosphoenolpyruvate carboxykinase at normal values and that they may play some secondary role in the regulation of the hepatic enzyme.

The mechanism whereby glucocorticoids alter the rate of gluconeogenesis in mammalian liver is also not clear. Currently they are thought to interact with cyclic AMP in a so-called 'permissive' way, that is, they augment the action of cyclic AMP. Thus the stimulation of gluconeogenesis by glucagon, adrenaline or cyclic AMP is decreased in the perfused livers of adrenalectomized rats, even though these compounds cause an increase in the intracellular content of cyclic AMP. Rates of glucose synthesis can be restored to normal, however, by treatment with glucocorticoids either administered to the animal *in vivo* or included in the perfusion medium (Friedmann *et al.*, 1967; Park & Exton, 1972; Exton *et al.*, 1973). In agreement with these findings, the response of phosphoenolpyruvate carboxykinase to dibutyryl cyclic AMP is decreased in the livers of adrenalectomized rats (Wicks *et al.*, 1974; Krone *et al.*, 1974). It is possible, therefore, that the 'permissive' effect of glucocorticoids on gluconeogenesis, as well as the stimulation of hepatic phosphoenolpyruvate carboxykinase synthesis which occurs *in vivo* in the absence of insulin and in Reuber H35 cells, reflect some general modulation of cyclic AMP action by the glucocorticoids.

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