The effects of stress and injury on the activity of phosphoenolpyruvate carboxykinase in the liver of the rat

John G. ROSE and Dennis F. HEATH

MRC Trauma Unit, Stopford Building, University of Manchester, Oxford Road, Manchester M13 9PT, U.K.

The effects of stress (diethyl ether anaesthesia for 4–8 min, or intravenous injection of 0.05 ml of a dimethyl sulphoxide/water mixture) and of a scald injury given under ether anaesthesia on hepatic PEPCK (phosphoenolpyruvate carboxykinase, EC 4.1.1.32) were studied in the post-absorptive rat. Injury raised PEPCK activity by about 70% in 2 h and by over 100% in 4 h, over three times as fast as in animals that had only been handled (controls). The two stresses, both of types commonly imposed in animal experiments, had almost as much effect as injury for the first 2 h, although much less thereafter. The roles of sympathetic stimulation and corticosterone in mediating these rises were studied by using $\alpha\beta$ -blockers and trilostane respectively as inhibitors. (Trilostane only decreased corticosterone concentrations to a little above control values.) The ether-induced increase was somewhat decreased by $\alpha\beta$ -blockade, but was only eliminated by combined $\alpha\beta$ -blockade and trilostane. After injury, however, PEPCK synthesis was unaffected by either $\alpha\beta$ -blockade or trilostane, although it was decreased by their combined action; and it seems that either corticosterone or sympathetic stimulation was sufficient to stimulate PEPCK synthesis maximally. Stimulation by corticosterone was much greater than reported previously by others, for reasons that are discussed. Sympathetic stimulation may have been mediated by glucagon and cyclic AMP, since injury raised portal glucagon concentrations, and stress and injury raised those of hepatic cyclic AMP. PEPCK synthesis was, however, stimulated despite increases in portal insulin concentration, and was not related to the [insulin]/[glucagon] ratio. Thus stress and injury over-rode normal control mechanisms.

INTRODUCTION

The work described below was originally undertaken to see whether the increase in glucose labelling from [^{14}C]pyruvate that is observable by 2 h after injury in the rat (Ashby *et al.*, 1965) was accompanied by any increase in the maximal activity in the key gluconeogenic enzyme, hepatic PEPCK. As shown below, injury did increase it considerably in the period 1.5–5.5 h after withdrawal of food. The emphasis here, however, is on the mechanisms involved, and the finding that an increase in PEPCK activity is a stress response also brought about by a short period of diethyl ether anaesthesia or intravenous injection of a DMSO/water mixture.

In order to explain the findings, measurements were made of the circulating hormones corticosterone, glucagon and insulin, and of hepatic cyclic AMP and plasma glucose and lactate. The effects of sympathetic stimulation were deduced from those of $\alpha\beta$ -adrenergic blockade. In some experiments the concentrations of corticosterone, the main corticosteroid in the rat, were held close to normal by an inhibitor of adrenal steroidogenesis, trilostane (Barton & Little, 1978).

MATERIALS AND METHODS

Animal experiments

Rats. Male Wistar rats of the Porton strain were housed for 3 weeks before use in a room at 18–22 °C, lit from 07:00 to 19:00 h, and were fed on Labsure CRM nuts (The Christopher Hill Group, Poole, Dorset, U.K.),

containing about 17.5% (w/w) of protein. Food was removed at 09:00 h on the day of experiment, when rats were taken from the holding room to the laboratory and weighed (range 230–260 g). The stress of such ‘handling’ alters liver metabolism (Heath *et al.*, 1971) and increases catecholamines transiently (Kvetňanský, 1980; Da Prada *et al.*, 1980), and experiments were not started for at least 1 h to allow these effects to subside. Ambient temperatures during experiments were 20–22 °C, except for rats under $\alpha\beta$ -adrenergic blockade, for which it had to be 2 °C higher to maintain core temperatures in uninjured animals. After scald injury the core temperature falls, to about 33 °C by 4 h.

Stress. Three stresses were used: diethyl ether anaesthesia, intravenous injection of DMSO/water, and scald injury, all at 10:30 h. ‘Controls’ were those rats that had only been handled at about 09:00 h. Ether anaesthesia was for 4–8 min. DMSO/water (5:1, v/v; 0.05 ml/rat) was injected via a tail vein over a 30 s period, since rapid injection visibly disturbed the rats. Scald injury (dorsal, full-thickness scald of 20% of the body surface) was carried out under ether anaesthesia as described by Arturson (1961). Scald injury therefore always involved stress from ether, and, when DMSO was used as a vehicle for drugs, stress from DMSO also. The effects of injury were expected to predominate, since, although it is not painful, owing to the destruction of the nerve endings, it produces most of the effects of a severe injury on glucose metabolism (Heath, 1985b).

Some rats, those from which specimens of liver for

cyclic AMP measurements or portal blood were to be taken, were anaesthetized a second time later for the purpose and killed under anaesthesia.

Adrenergic blockade and inhibition of corticosterone synthesis. Trilostane was given to us by Sterling-Winthrop, Guildford, Surrey, U.K., the α -blocker, phentolamine mesylate, by Ciba, Horsham, Sussex, U.K., and the β -blocker, propranolol hydrochloride, by I.C.I., Macclesfield, Cheshire, U.K.

The antagonists and trilostane had to be given intravenously 10–15 min before induction of anaesthesia to inhibit the stimulatory effects of ether. Additional trilostane had to be given subcutaneously during the anaesthesia for its effects to be maintained for 4 h. Trilostane for intravenous injection was dissolved in DMSO, to which water (1:5, v/v) was added immediately before use. Solutions injected intravenously were as follows (per rat): trilostane, 0.04 ml of 25 mg/ml; combined adrenergic blockers, 0.1 ml of an aqueous solution containing phentolamine mesylate (2.5 mg/ml) and propranolol hydrochloride (25 mg/ml); combined trilostane and adrenergic blockers, 0.05 ml of DMSO/water containing trilostane (20 mg/ml) and twice the above concentrations of the blockers. A few experiments were carried out in which blockers alone were given in DMSO/water (0.05 ml/rat). Subcutaneous injection of trilostane was into each thigh (0.1 ml of 50 mg/ml in arachis oil/ethanol, 9:1, v/v).

Collection of blood and liver specimens. This was carried out after the rats had been decapitated, except when measurements were to be made in portal blood or (for cyclic AMP) in liver, when rats were anaesthetized (see above). Blood collection was confined to the first 2 ml from injured rats, in which the blood volume is decreased, and to 3 ml from the rest.

Analytical methods

Instrumentation and chemicals. Radioactivities were measured with scintillation spectrometers, model 2450 for β -activity and model 5130 for γ -activity (Packard Instrument Co., Reading, Berks., U.K., who also provided the scintillation cocktails); spectrophotometric measurements were made with a recording spectrophotometer (SP8-200; Pye-Unicam, Cambridge, U.K.) and fluorimetric measurements with a recording fluorimeter (MPF-43A; Perkin-Elmer, Beaconsfield, Bucks., U.K.). For centrifugation during cyclic AMP measurements a micro-centrifuge (Burkard 320a) was adapted by its suppliers (Coomber and Son, Stockport, Cheshire, U.K.) to provide an 18-place swing-out rotor for use at 12 000 g.

Enzymes, coenzymes and the kit for measurement of glucose were from B.C.L. (Lewes, Sussex, U.K.); corticosterone was from Sigma Chemical Co. (Poole, Dorset, U.K.); radiochemicals and the radioimmunoassay kit for cyclic AMP were from Amersham International (Amersham, Bucks., U.K.); and rat standard insulin, aprotinin and the radioimmunochemical kit for glucagon assay were from Novo Research Institute (Basingstoke, Hants., U.K.). The antiserum for measurements of rat insulin was from Miles-Yeda (Miles Laboratories, Slough, Bucks., U.K.); and ethanol used in the glucagon assay was from BDH (Poole, Dorset, U.K.).

Enzyme activities. A liver specimen (1 g) was macerated with an ILA homogenizer (Scientific Instrument Centre Ltd., Liverpool, U.K.) at 0 °C in 15 ml of buffer (pH 7.5, containing 0.25 M-sucrose, 5 mM-Tris/HCl, 1 mM-dithiothreitol and 1 mM-EDTA). Measurements were made on the supernatant after centrifugation (12000 g for 8 min at 0–4 °C). PEPCK was measured by the method of Seubert & Huth (1965), as modified by Pogson & Smith (1975), the method of Czok & Lamprecht (1974) being used for PEP.

Hormones, messengers and other compounds. Glucagon and cyclic AMP, the latter in a HClO_4 extract of freeze-clamped liver, were measured according to the instructions with the kits referred to above, except that ^{125}I measurements were made in a γ -counter, and glucagon extracts were dried in a stream of N_2 instead of in a desiccator. Insulin was measured by a single-antibody technique (Barton & Passingham, 1980), corticosterone as described by Nicholson & Van Loon (1973), plasma lactate as described by Heath & Rose (1985), and plasma glucose according to the instructions with the kit.

Statistics. Results are given as means $\pm$ S.E.M. (n), except that, when the scatter was great, the S.E.M. range calculated from the log-normal distribution is given. Comparisons are made by Student's unpaired t test unless stated otherwise.

RESULTS

Maximal PEPCK activities

All activities are given per g wet wt. of liver. Activity rose steadily in handled rats by about 0.25 $\mu\text{mol/min per g}$ per h, to reach 9.0 ± 0.4 (8) $\mu\text{mol/min per g}$ after 24 h starvation. Effects of treatment are assessed in relation to this steady increase. Intravenous injection of saline (0.9% NaCl; 0.1 ml/rat) was without effect. Other results are shown in Table 1.

Scald injury trebled the rate of increase of PEPCK activity, so that by 4 h activity was doubled and was over 70% of that after 24 h starvation. The rate of increase was not lessened by $\alpha\beta$ -adrenergic blockade or by trilostane, but was considerably decreased by their combined action ($P < 0.001$).

A brief period of ether anaesthesia stimulated PEPCK activity nearly as much as did injury for the first 2 h, after which there was only a slow rise, similar to that in the handled controls. The increase was decreased by $\alpha\beta$ -adrenergic blockade ($P < 0.001$, t test), and more so by a combination of blockade and treatment with trilostane.

It was, however, notable that for the first 2 h activity was increased nearly as much by the intravenous injection of the DMSO/water vehicle for trilostane as by ether, although significantly less than by scald injury ($P < 0.001$). Thus, although the three treatments could be graded in order of severity as scald > ether anaesthesia > vehicle, their effects on PEPCK were similar for the first 2 h.

Glucose, cyclic AMP and hormones

Corticosterone concentrations in controls were mostly below 0.4 $\mu\text{mol/l}$ of plasma, with geometric means under 0.3 $\mu\text{mol/l}$. Scald injury produced a sustained increase, as

Table 1. Effects of scald injury, ether anaesthesia and DMSO injection on PEPCK and cyclic AMP in liver and corticosterone and glucose in plasma

Compounds were measured as described in the Materials and methods section. Times were measured from 10:30 h. ('Handled' rats were handled at 09:00–09:15 h.) When the stated stress was intravenous injection of DMSO, the rats were not given ether. When DMSO was used as a vehicle, this is indicated by an asterisk against the 'stress'; these animals received two stresses, that stated and DMSO injection. Measurement of cyclic AMP involved ether anaesthesia when the liver specimen was taken in addition to the stated stress. The values for corticosterone concentration at 0.5 h in ether-stressed rats were from B. J. Passingham (personal communication). Abbreviations: $\alpha\beta$ -B, dual ($\alpha\beta$) adrenergic blockade; Tril, trilostane-treated.

Time (h)	Stress	Treatment	PEPCK ($\mu\text{mol}/\text{min per g}$) (mean $\pm$ S.E.M.)	Corticosterone ($\mu\text{mol}/\text{l}$ of plasma) (S.E. range)	Cyclic AMP (pmol/g of liver) (mean $\pm$ S.E.M.)	Glucose (mmol/l of plasma) (mean $\pm$ S.E.M.)
0.0	Handled	None	2.97 $\pm$ 0.09 (12)	0.51–0.65 (12)	643 $\pm$ 35 (11)	8.33 $\pm$ 0.16 (12)
0.25	Handled	$\alpha\beta$ -B	—	1.49–1.75 (6)	—	6.85 $\pm$ 0.09 (6)
	DMSO	None	—	0.88–1.12 (6)	—	8.66 $\pm$ 0.14 (6)
	DMSO	$\alpha\beta$ -B + Tril	—	0.99–1.11 (6)	—	7.76 $\pm$ 0.18 (6)
	Scald*	Tril	—	0.52–0.73 (6)	—	14.47 $\pm$ 0.56 (6)
0.5	Ether	None	—	1.20–1.58 (8)	764 $\pm$ 25 (6)	8.31 $\pm$ 0.22 (5)
	Scald	None	—	—	890 $\pm$ 38 (6)	—
1.0	Handled	None	3.11 $\pm$ 0.15 (6)	0.19–0.26 (3)	—	8.14 $\pm$ 0.19 (3)
	Ether	None	—	—	683 $\pm$ 16 (22)	—
	Ether	$\alpha\beta$ -B	—	—	566 $\pm$ 27 (6)	—
	Scald	None	3.58 $\pm$ 0.17 (5)	1.71, 1.93	908 $\pm$ 39 (6)	12.36 $\pm$ 0.77 (6)
2.0	Handled	None	3.81 $\pm$ 0.17 (22)	0.17–0.22 (20)	663 $\pm$ 36 (3)	8.21 $\pm$ 0.07 (19)
	Handled	$\alpha\beta$ -B	4.30 $\pm$ 0.33 (5)	0.17–0.40 (4)	—	8.20 $\pm$ 0.04 (4)
	DMSO	None	4.53 $\pm$ 0.32 (6)	0.33–0.46 (6)	—	7.80 $\pm$ 0.18 (6)
	Ether	None	4.93 $\pm$ 0.09 (41)	0.31–0.42 (25)	—	9.12 $\pm$ 0.18 (26)
	Ether	$\alpha\beta$ -B	4.52 $\pm$ 0.15 (11)	0.23–0.41 (11)	563 $\pm$ 28 (4)	8.15 $\pm$ 0.18 (11)
	Ether*	$\alpha\beta$ -B + Tril	4.13 $\pm$ 0.22 (6)	0.61–0.64 (6)	—	7.74 $\pm$ 0.12 (6)
	Scald	None	5.24 $\pm$ 0.19 (25)	1.59–1.71 (25)	—	13.71 $\pm$ 0.58 (27)
	Scald	$\alpha\beta$ -B	—	—	653 $\pm$ 25 (4)	—
	Scald*	Tril	4.55 $\pm$ 0.34 (6)	0.46–0.53 (6)	—	12.05 $\pm$ 0.30 (6)
4.0	Handled	None	4.06 $\pm$ 0.18 (14)	0.26–0.33 (18)	649 (1)	8.05 $\pm$ 0.06 (18)
	DMSO	None	5.05 $\pm$ 0.19 (5)	0.40–0.50 (5)	—	7.80 $\pm$ 0.21 (5)
	Ether	None	5.24 $\pm$ 0.24 (12)	0.21–0.27 (17)	655 $\pm$ 15 (17)	8.42 $\pm$ 0.08 (15)
	Ether	$\alpha\beta$ -B	5.55 $\pm$ 0.16 (5)	0.47–0.70 (7)	—	8.13 $\pm$ 0.10 (7)
	Ether*	DMSO	—	—	668 $\pm$ 51 (5)	—
	Scald	None	6.48 $\pm$ 0.16 (18)	1.43–1.52 (21)	1093 $\pm$ 46 (10)	11.33 $\pm$ 0.59 (27)
	Scald*	$\alpha\beta$ -B	6.48 $\pm$ 0.32 (6)	1.77–1.93 (13)	—	9.74 $\pm$ 0.26 (13)
	Scald*	Tril	6.33 $\pm$ 0.25 (6)	0.57–0.67 (6)	—	9.48 $\pm$ 0.23 (6)
	Scald*	$\alpha\beta$ -B + Tril	5.15 $\pm$ 0.29 (12)	0.77–0.85 (14)	741 $\pm$ 39 (5)	9.42 $\pm$ 0.26 (14)

found previously (Barton, 1977). Ether anaesthesia and injection of DMSO/water also raised corticosterone concentrations considerably by 0.25–0.5 h, but values had returned nearly to normal by 2 h. Adrenergic blockade initially increased corticosterone concentrations, as found in dogs by Wolfe *et al.* (1981), but this effect had worn off by 2 h. Trilostane held corticosterone concentrations down after injury, but rarely to within the control range.

Scald injury greatly raised hepatic cyclic AMP concentrations. Ether had a small effect, which was only significant ($P < 0.001$) at 0.5 h, after which values returned to those in 'handled' rats. Since liver specimens were taken under ether anaesthesia, there is doubt as to the true baseline value, and that under $\alpha\beta$ -blockade may be a better estimate. The concentrations of cyclic AMP were, however, all consistent with the view that they depended on sympathetic stimulation and glucagon concentration, in that they were raised by injury or ether and decreased by $\alpha\beta$ -blockade.

As expected, plasma glucose concentrations reflected

those of hepatic cyclic AMP. The usual marked rise in glucose concentration after injury was suppressed more slowly by trilostane than in the experiments by Barton & Little (1978). By 4 h after injury, however, suppression by $\alpha\beta$ -blockade, by trilostane and by both together was very similar in extent, although not complete; the glucose concentrations were about 1.5 $\mu\text{mol}/\text{ml}$ greater than those in rats that had only been handled.

Portal glucagon concentrations (Table 2) were probably raised by the ether anaesthesia under which specimens of portal blood were taken, since concentrations were less in specimens taken under pentobarbitone anaesthesia, and, at least in the dog, the latter are close to those in the conscious animal (Ishida *et al.*, 1983). Even compared with the values under ether anaesthesia, however, scald injury caused a big, sustained, increase that was not significantly affected by trilostane but was prevented by $\alpha\beta$ -blockade, either alone or with trilostane. Thus the well-known sympathetic stimulation of glucagon release (e.g. Unger & Orci, 1976) accounted for most of the rise in glucagon concentration after injury.

Table 2. Effects of stress on portal glucagon and [insulin]/[glucagon] molar ratios

Portal blood was taken immediately after induction of anaesthesia by ether, or, as indicated by †, Sagatal (pentobarbitone sodium; 70 mg/kg given intraperitoneally). Asterisks denote treatments that involved injection with DMSO. Times given are those after the original stress. Standard-error ranges are shown assuming that the statistical distribution was log-normal, except that, when only two measurements were made, individual values are shown.

Time (h)	Stress	Treatment	Glucagon (pmol/l of plasma)		[Insulin]/[glucagon]	
0.0	Handled	None	183–206	(4)	–	
	Handled†	None	43–56	(7)	29–47	(5)
1.0	Ether	None	116–136	(14)	6.0–9.0	(3)
	Scald	None	290–427	(6)	2.4,	3.8
4.0	Ether	None	110–140	(6)	5.0–13.3	(3)
	Ether*	DMSO	85–109	(7)	12.2–19.0	(5)
	Scald	None	331–497	(6)	2.9–6.3	(4)
	Scald*	$\alpha\beta$ -B	140–176	(9)	5.9–7.5	(9)
	Scald*	Tril	265–362	(5)	2.0–4.0	(5)
	Scald*	$\alpha\beta$ -B + Tril	113–143	(13)	4.6–5.1	(8)

Scald injury raised portal insulin and glucagon concentrations much more than did ether anaesthesia; the [insulin]/[glucagon] ratios, however, were similar.

Plasma lactate and hepatic glycogen concentrations

Plasma lactate concentrations fell in each group during the 4 h period of study. The lowest values were in injured rats at 4 h 0.97 ± 0.07 (6) mmol/l of plasma, against 1.76 ± 0.10 (11) mmol/l in handled rats at the start of the period.

Acid-soluble glycogen concentrations, measured by the method of Keppler & Decker (1974) in the extracts made for determination of cyclic AMP, fell from 55 ± 3 mg/g shortly after ether anaesthesia to 44 ± 3 mg/g 4 h after anaesthesia and to 34 ± 2 mg/g 4 h after injury. The decrease after injury was significantly greater than that after ether alone ($P < 0.001$).

DISCUSSION

The effects of injury on hormones and glucose (Tables 1 and 2) were typical of those seen shortly after any severe injury in many species, including man (Gann & Lilly, 1984), except for the increase in insulin discussed below. The effects of diethyl ether anaesthesia and injection of DMSO/water were less and much more transient, although consistent with other reports (reviewed by Seitz *et al.*, 1973) that ether anaesthesia increases corticosteroid release and sympathetic activity.

The marked hyperglycaemia and 'insulin resistance' (see below) associated with injury are well-developed by 2 h, but are not induced by a short period of ether anaesthesia. Neither can, therefore, be attributed to the increase in PEPCK, which, up to 2 h, was as great after

anaesthesia as after injury. Again, the increased incorporation of pyruvate label into glucose after injury referred to in the Introduction (Ashby *et al.*, 1965) cannot be attributed to the action of ether, since the controls in these experiments had been treated with ether. It is, however, unknown whether a short period of anaesthesia can increase incorporation of pyruvate label, since comparable experiments on unanaesthetized animals have not been done.

Changes in PEPCK activity are attributed to changes in its synthesis rate (Tilghman *et al.*, 1976), and glucagon activity is treated as an aspect of sympathetic stimulation, since this controlled glucagon concentration. It should be borne in mind, however, that, whereas $\alpha\beta$ -blockade presumably antagonized sympathetic stimulation almost completely, trilostane did not eliminate the effects of corticosterone, but merely decreased its concentrations nearly to normal. Any permissive effects (Krone *et al.*, 1976) would therefore have remained.

Sympathetic activity is well known to stimulate PEPCK synthesis, and 2.5 h immobilization stress increased PEPCK activity (Németh, 1978) as much as was found 2 h after injury. It was, however, surprising that for 2 h the effects of apparently much smaller stresses, DMSO injection and ether anaesthesia, were nearly as great as those of injury, and that after injury the increase was maintained despite $\alpha\beta$ -blockade, and was apparently unaffected by the observed increase in insulin concentrations.

After stress and injury the increases in PEPCK in rats under $\alpha\beta$ -blockade have to be attributed at least partly to the action of corticosterone. First, they were much decreased by trilostane treatment. Secondly, 2 h after ether anaesthesia PEPCK activities were roughly correlated with corticosterone concentrations ($P < 0.05$, linear correlation). Thirdly, 4 h after injury a similar correlation existed in rats given trilostane as well as $\alpha\beta$ -blockers ($P = 0.06$). [Close correlations were not to be expected, since PEPCK synthesis is likely to depend on the impulse of corticosterone (the area under its concentration–time curve), which may not be closely related to its terminal concentration.] In the injured rat, indeed, either the sympathetic stimulation or the increase in corticosterone concentration was, in the absence of the other, sufficient to stimulate PEPCK synthesis maximally, since on its own neither $\alpha\beta$ -blockade nor trilostane affected the increase in enzyme activity measured at 4 h. Thus our results do not support the usual view that PEPCK synthesis is much more sensitive to sympathetic stimulation and glucagon than to corticosteroids.

Much smaller effects of corticosteroids are usually seen in adult rats, except when insulin output is inhibited (Tilghman *et al.*, 1976). We took care to avoid disturbing the circadian rhythm, and confined our study to the period within a few hours after withdrawal of food, when the activities of gluconeogenic enzymes, including PEPCK, are increasing rapidly (Guder & Schmidt, 1976; Jungermann & Sasse, 1978). Other findings can be explained by different experimental conditions. Thus later in the day, when both baseline PEPCK and corticosterone concentrations were higher, immobilization no longer raised PEPCK activity as it did in the morning (Németh, 1978). Again, in rats that appear to have had continuous access to food, high doses of cortisol only increased PEPCK by 40–60% in 4 h; but this was followed by an almost equally rapid fall (Lardy *et al.*,

1964; Foster *et al.*, 1966). Thus the effects of feeding may have opposed those of cortisol throughout.

The ether-induced rise in corticosterone concentration, although transient, was enough to raise PEPCK activity in rats under $\alpha\beta$ -blockade from 3.81 (handled rats) to 4.52 $\mu\text{mol/min per g}$ ($P < 0.01$). Raised sympathetic activity was, however, also needed to raise PEPCK activities at 2 h to values close to those after injury.

Since scald injury was known to raise plasma insulin concentrations rapidly by a factor of about 4 (Frayn, 1975, 1976), we carried out only a few measurements, mainly to see whether treatment by $\alpha\beta$ -blockade + trilostane, which decreased PEPCK synthesis, altered the [insulin]/[glucagon] ratio. Its values were very similar in all four groups studied 4 h after injury (Table 2), when, it appears, the ratio did not control the rate of PEPCK synthesis, contrary to findings under other conditions (Krone *et al.*, 1976). The increases in insulin concentration after injury were proportionately as great as those found by Frayn (1975, 1976), and, it is plain, did not much inhibit the synthesis of PEPCK. Trilostane is known to lower insulin concentrations after injury, presumably via the decrease in glucose concentration (Barton & Passingham, 1980), and both trilostane and $\alpha\beta$ -blockade decreased them in our experiments, though less than did $\alpha\beta$ -blockade + trilostane ($P = 0.05$, $P < 0.05$ and $P < 0.01$ respectively). The only change in insulin associated with a change in the rate of PEPCK synthesis was the last, where low insulin accompanied a low synthetic rate, contrary to expectation.

Thus stress-stimulated PEPCK synthesis is mediated by corticosteroids and sympathetic stimulation, and inhibition by insulin and control by the [insulin]/[glucagon] ratio are over-ridden. We have no explanation to offer for these findings.

Two hypotheses are implausible. Shrago *et al.* (1963) found that PEPCK synthesis could be stimulated by lactate; but in our experiments plasma lactate concentrations fell during the experiments and were lowest 4 h after injury. A switch of parenchymal cells towards gluconeogenesis, as during starvation (Jungermann & Sasse, 1978), for which the decrease in liver glycogen may be taken as evidence, also seems unlikely to account for the results, since forced exercise that decreased liver glycogen concentrations below those in rats 4 h after injury did not stimulate PEPCK synthesis (Sanchez-Medina *et al.*, 1971). There is also other evidence from the labelling of liver glutamate by [^{14}C]pyruvate that injury decreases the proportion of gluconeogenic cells in the parenchyma (Heath, 1973, 1985a).

The increase in PEPCK despite raised insulin concentrations appears to be one of a number of ill-understood responses to stress and injury that are often referred to as 'insulin resistance' (reviews by Heath, 1981; 1985b), some features of which can be reproduced by infusing cortisol, adrenaline and glucagon (Eigler *et al.*, 1979; Shamoon *et al.*, 1981). Glucose uptake by insulin-dependent tissues is decreased until glucose and insulin concentrations have risen considerably, while hepatic output of glucose is maintained against these concentrations. In the injured rat trilostane restores muscle uptake to normal (Barton & Passingham, 1980), whereas, as shown here, it does not affect hepatic PEPCK synthesis.

Whatever the mechanism, however, it is plain that stresses likely to be involved in common procedures used

in animal experiments can alter the activity of PEPCK by a readily measurable amount.

We thank all those firms who gave us pharmacological agents, and Mr. B. J. Passingham for carrying out some of the corticosterone measurements.

REFERENCES

- Arturson, G. (1961) *Acta Chir. Scand. Suppl.* **274**, 1–135
- Ashby, M. M., Heath, D. F. & Stoner, H. B. (1965) *J. Physiol. (London)* **179**, 193–237
- Barton, R. N. (1977) *J. Endocrinol.* **73**, 347–353
- Barton, R. N. & Little, R. A. (1978) *J. Endocrinol.* **76**, 293–302
- Barton, R. N. & Passingham, B. J. (1980) *J. Endocrinol.* **86**, 363–370
- Czok, R. & Lamprecht, W. (1974) in *Methods of Enzymatic analysis* (Bergmeyer, H. U., ed), 2nd edn., vol. 3, pp. 1446–1451. Academic Press, New York
- Da Prada, M., Pieri, L. & Picotti, G. B. (1980) *Catecholamines Stress: Recent Adv.; Dev. Neurosci. (Amsterdam)* **8**, 231–236
- Eigler, N., Saccà, L. & Sherwin, R. S. (1979) *J. Clin. Invest.* **63**, 114–123
- Foster, D. O., Ray, P. D. & Lardy, H. A. (1966) *Biochemistry* **5**, 555–562
- Frayn, K. N. (1975) *Eur. J. Clin. Invest.* **5**, 331–337
- Frayn, K. N. (1976) *Br. J. Exp. Pathol.* **57**, 316–320
- Gann, D. S. & Lilly, M. P. (1984) *Multiple Trauma: Prog. Crit. Care Med.* **1**, 15–47
- Guder, W. G. & Schmidt, U. (1976) *Hoppe-Seyler's Z. Physiol. Chem.* **357**, 1793–1800
- Heath, D. F. (1973) *Neurohumoral Metabolic Aspects of Injury: Adv. Exp. Biol.* **33**, 271–276
- Heath, D. F. (1981) *Homeostasis in Injury and Shock: Adv. Physiol. Sci.* **26**, 63–70
- Heath, D. F. (1985a) *Br. Med. Bull.* **41**, 240–245
- Heath, D. F. (1985b) in *The Scientific Basis of the Care of the Critically Ill* (Little, R. A. & Frayn, K. N., eds), Manchester University Press, in the press.
- Heath, D. F. & Rose, J. G. (1985) *Biochem. J.* **227**, 851–867
- Heath, D. F., George, D. R. & Rose, J. G. (1971) *Biochem. J.* **125**, 765–771
- Ishida, T., Lewis, R. M., Hartley, C. J., Entman, M. L. & Field, J. B. (1983) *Endocrinology (Baltimore)* **112**, 1098–1109
- Jungermann, K. & Sasse, D. (1978) *Trends Biochem. Sci.* **3**, 198–202
- Keppler, D. & Decker, K. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed), 2nd edn., vol. 3, pp. 1127–1131, Academic Press, New York
- Krone, W., Huttner, W. B., Seitz, H. J. & Tarnowski, W. (1976) *Biochim. Biophys. Acta* **437**, 62–70
- Kvetňanský, R. (1980) *Catecholamines Stress: Recent Adv.; Dev. Neurosci. (Amsterdam)* **8**, 7–18
- Lardy, H. A., Foster, D. O., Shrago, E. & Ray, P. D. (1964) *Adv. Enzyme Regul.* **2**, 39–47
- Németh, S. (1978) *Endokrinologie* **72**, 223–230
- Nicholson, W. E. & Van Loon, G. R. (1973) *J. Lab. Clin. Med.* **81**, 803–808
- Pogson, C. I. & Smith, S. A. (1975) *Biochem. J.* **152**, 401–408
- Sanchez-Medina, F., Sanchez-Urrutia, L., Medina, J. M. & Mayor, F. (1971) *FEBS Lett.* **19**, 128–130
- Seitz, H. J., Faupel, R. P., Kampf, S. C. & Tarnowski, W. (1973) *Arch. Biochem. Biophys.* **158**, 12–18
- Seubert, W. & Huth, W. (1965) *Biochem. Z.* **343**, 176–191
- Shamoon, H., Hendler, R. & Sherwin, R. S. (1981) *J. Clin. Endocrinol. Metab.* **52**, 1235–1241
- Shrago, E., Lardy, H. A., Nordlie, R. C. & Foster, D. O. (1963) *J. Biol. Chem.* **238**, 3188–3192

Tilghman, S. M., Hanson, R. W. & Ballard, F. J. (1976) in Gluconeogenesis, Its Regulation in Mammalian Species (Hanson, R. W. & Mehlman, M. A., eds), pp. 47–91, J. Wiley and Sons, New York

Unger, R. H. & Orci, L. (1976) *Physiol. Rev.* **56**, 778–826

Wolfe, R. R., Durkot, M. J. & Burke, J. F. (1981) Homeostasis in Injury and Shock: *Adv. Physiol. Sci.* **26**, 151–158

Received 26 June 1985/19 August 1985, accepted 10 September 1985