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THE ROLES OF GLUCAGON, INSULIN AND GLUCOCORTICOID HORMONES IN THE EFFECTS OF SUB-LETHAL DOSES OF ENDOTOXIN ON GLUCOSE HOMEOSTASIS IN RATS

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Abstract—(1) The effects of sub-lethal doses of endotoxin on plasma glucose, glucagon, insulin, glucocorticoids and non-esterified fatty acids (NEFA) were determined in rats. (2) Endotoxin did not change the plasma concentration of glucocorticoids, but blocked the effects of elevated glucocorticoid hormone concentrations on both plasma glucose and hepatic tryptophan dioxygenase activity. (3) Endotoxin increased the plasma concentrations of glucose, glucagon and insulin in rats with basal glucocorticoid concentrations, and changed the observed relationships between glucose, glucagon and insulin in a manner consistent with an increased sensitivity of glucagon secretion to lowered glucose concentrations. (4) At the highest dose of endotoxin used, 20 mg/kg over 6 hr, a substantial decrease (>7-fold) in the insulin/glucagon ratio provides evidence for changes in basal (as opposed to hormone-stimulated) glucose production and/or utilisation *in vivo*.

A marked disturbance in carbohydrate metabolism is one of the characteristics of the syndrome of gram-negative septic shock, which is thought to be caused by endotoxins derived from the cell wall of gram-negative bacteria [1, 2]. Changes in the plasma concentration of many substrates and hormones have been reported to occur with lethal doses (LD_{50}–LD_{100}) of endotoxin in various mammalian species, used as experimental models of septic shock in man [3–8]. These studies have shown that lethal doses of endotoxin cause a transient hyperglycaemia followed by a profound hypoglycaemia. It has been suggested [3] that the metabolic disturbances caused by endotoxin, and in particular this hypoglycaemia, may play an important role in the pathogenesis of septic shock. Several authors have reported that endotoxin administration causes changes in the plasma concentrations of glucagon and insulin [4, 6–12]; there is considerable disagreement, however, as to the direction and magnitude of these effects. There is also disagreement as to the importance of the changes in plasma insulin concentrations, which have been suggested by some workers [8, 10] to have a primary role in the hypoglycaemia of endotoxic shock. Changes in the concentration of or sensitivity to endogenous glucocorticoid hormones could play a role in the metabolic disturbances of endotoxaemia, because exogenous glucocorticoids can be protective in endotoxic and septic shock [13, 14], and because the effects of these exogenous steroids are blocked (at least in mice) by prior administration of endotoxin (reviewed in [15]).

In order to identify the primary effects of bacterial endotoxin on carbohydrate metabolism we have carried out a systematic study of the effects of sub-lethal doses of endotoxin on glucose homeostasis. We have determined the effects of 5 or 20 mg/kg *Salmonella typhimurium* endotoxin on plasma glucose, glucagon, insulin and glucocorticoids in fasted rats over 6 hr, under conditions in which glucocorticoid hormone concentrations were either elevated (protocol A) or basal (protocol B); these experiments permit an assessment of the role of glucocorticoids in the effects of endotoxin. The changes in plasma glucose, glucagon and insulin caused by endotoxin were also studied in experiments on streptozotocin-diabetic rats; this allowed us to examine the role of insulin. Finally, experiments were carried out on rats pretreated with low doses (1 or 2 mg/kg) of endotoxin overnight, to allow the attainment of a steady-state condition; the hypoglycaemic action of tryptophan was then used to obtain a range of plasma glucose, glucagon and insulin concentrations, in order to study the changes in the relationships between these parameters in the presence of endotoxin.

MATERIALS AND METHODS

Animals. Male Sprague–Dawley rats, 200–250 g in weight, were used throughout and, except where specified, were fed *ad libitum* on CRM diet (Labsure, C. Hill Group, Dorset, U.K.).

Experimental protocols

A. *(Series* 1, *Table* 1 *and Fig.* 1*).* Rats were starved overnight from 1600 hr. At 1000 hr (0 hr) the rats were lightly anaesthetised with ether and a small volume of blood (about 0.1 ml) was taken from the tail. The rats then received an injection of pyrogen-free NaCl (0.9% w/v; 1 ml/kg body weight, i.p.) containing 0 (controls), 5 or 20 mg endotoxin/ml. At 1, 2, 4 and 6 hr following this injection the rats were

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lightly anaesthetised and blood samples were taken from the tail. At 6 hr blood samples were also taken from the inferior vena cava; in some experiments samples of liver were taken.

B. *(Series 2 and 3, Table 1).* Rats were starved overnight from 1600 hr. At 1000 hr (0 hr) the rats received an injection of pyrogen-free NaCl (0.9% w/v; 1 ml/kg body weight, i.p.) containing 0 or 5 (Series 2), or 0 or 20 (Series 3) mg endotoxin/ml. The rats were left undisturbed until 6 hr after the saline or endotoxin injection; they were then anaesthetised and samples of inferior vena cava blood, and of liver, were taken.

It should be noted that the treatment of rats in Protocols A and B at 0 hr is identical; the 0 hr values of plasma glucose and glucocorticoid concentration and of hepatic tryptophan dioxygenase activity presented in the Results section under either Protocol A or B should therefore be relevant to both protocols.

C. *(Table 2, Figs 2 and 3).* Rats received an injection i.p. of pyrogen-free NaCl (0.9% w/v; 1 ml/kg body weight, i.p.) containing 0 (controls), 1 or 2 mg endotoxin/ml at 1600 hr and were then fasted overnight. The lower dose of endotoxin was used to avoid any fatalities following tryptophan injection: rats pretreated with 5 mg/kg endotoxin die with very low plasma glucose 2–4 hr following the injection of tryptophan (data not shown). At 1000 hr the following day rats were lightly anaesthetised and either blood samples were taken (0 hr, Table 2), or L-tryptophan (200 mg/kg) was administered i.p., or they were left untreated. After recovering from the anaesthesia, the rats were left undisturbed for various times (1.5, 3, 4.5 or 6 hr); blood samples were then taken.

Endotoxin was prepared fresh daily, in pyrogen-free saline (Sodium Chloride Injection B.P., from Antigen Ltd., Roscrea, Ireland). L-Tryptophan was prepared as a microcrystalline suspension (40 mg/ml) in 0.9% (w/v) NaCl, 0.1% (w/v) Tween 80, by heating the suspension until all was dissolved, and then allowing the solution to cool while stirring. Acute diabetes was induced by intravenous injection of streptozotocin (80 mg/kg, prepared freshly in pyrogen-free saline). The rats were used after 48 hr, and were all diabetic as assessed by a fasting plasma glucose concentration above 10 mM.

Preparation of samples. Routinely, blood samples were taken into an heparinised syringe from the inferior vena cava under ether anaesthesia. In some experiments, multiple blood samples were taken from the tail under ether anaesthesia, and then at the final time point blood was also taken from the inferior vena cava. The plasma glucose in samples obtained by the two methods were not significantly different.

Plasma was obtained from the chilled, heparinised blood samples by centrifugation at 12,000 *g* for 1 min and stored at −80° for subsequent assay of glucose, non-esterified fatty acids, and glucocorticoids. Plasma samples for assay of immunoreactive glucagon and insulin were obtained by mixing 1.4 ml of blood with 0.08 ml of Trasylol (15,000 kI.U./ml), EDTA (30 mg/ml) and centrifuging as before. The plasma was then stored at −80° for subsequent assay, in glass tubes for glucagon and in plastic tubes for insulin assay.

Liver samples were taken under ether anaesthesia, freeze-clamped in liquid nitrogen, and stored at −80°.

Assays. Glucose was routinely determined using glucose oxidase and peroxidase [16]. Some samples from experiments using tryptophan were also checked by a hexokinase/glucose-6-phosphate dehydrogenase assay [17] to eliminate the possibility that tryptophan or its metabolites might interfere with the glucose oxidase assay; no differences were observed between results obtained with the two assays. For the determination of glycogen content, samples of liver or muscle were solubilised in boiling 30% (w/v) KOH; the glycogen was precipitated by addition of 0.2 volumes of saturated Na_2SO_4 and 3 volumes of ethanol and incubation overnight at 4°, and was then assayed as glucose after hydrolysis with glucosidase [18].

Non-esterified fatty acids (NEFA) were determined in chloroform extracts of plasma as described by Duncombe [19], using oleic acid as standard. Plasma glucocorticoid hormone concentrations were determined as corticosterone, by displacement of [1α,2α-^{3}H]corticosterone from corticosteroid-binding globulin, essentially as described in ref. [20]; this assay determines the sum of the concentrations of the glucocorticoid hormones, because the corticosteroid-binding globulin has an approximately equal affinity for all the major endogenous glucocorticoid hormones. Insulin and glucagon concentrations were determined in plasma samples by radioimmunoassay as previously described [21–23].

Tryptophan dioxygenase (L-tryptophan-O_2 2,3-oxidoreductase, EC 1.13.11.11) was assayed in homogenates prepared from samples of liver, as described in ref. [24].

Results are expressed as means ± SEM. The equality of sample variances was assessed by variance-ratio *F*-tests. When variances were equal, Student's *t*-test was used to assess the significance of differences in means; when variances were unequal the significance test described by Bailey [25] was used.

Sources of materials. The endotoxin used was a lipopolysaccharide preparation, extracted from *Salmonella typhimurium* by a phenolic extraction procedure, and obtained from Sigma Chemical Co. Ltd., Poole, Dorset, U.K. [1α,2α(*n*)-^{3}H]corticosterone and [^{125}I]insulin were from Amersham International plc, Amersham, Buckinghamshire, U.K. and [^{125}I]glucagon from New England Corp., South Manston, U.K. Streptozotocin was a gift from Dr D. C. N. Earl, ICI Pharmaceuticals Division, Macclesfield, Cheshire, U.K. Other chemicals were of analytical grade, or the purest grade available, and were from Sigma Chemical Co., B.D.H. Chemicals Ltd. (Speke, Liverpool, U.K.) or BCL Ltd. (Lewes, East Sussex, U.K.).

RESULTS

None of the rats treated with endotoxin (1 or 2 mg/kg over 24 hr with concurrent fasting, 5 or 20 mg/kg over 6 hr following 18 hr fasting) died during the

Table 1. The effects of endotoxin on plasma glucose and glucocorticoids, and on hepatic tryptophan dioxygenase

		Protocol A (multiple samples) Series 1		Protocol B (single samples) Series 2	Series 3	
A. Plasma glucose (mM)						
Control	0 hr	5.6 ± 0.19				
	6 hr	8.2 ± 0.43	(12)	5.4 ± 0.25 (8)	6.0 ± 0.18	(8)
5 mg/kg endotoxin	0 hr	5.2 ± 0.28				
	6 hr	5.9 ± 0.60‡	(12)	6.7 ± 0.33 (8)**		
20 mg/kg endotoxin	0 hr	6.2 ± 0.50				
	6 hr	6.6 ± 0.20‡	(4)		7.0 ± 0.37*	(8)
B. Plasma glucocorticoids (nM)						
Control	0 hr				46 ± 7.5	(8)
	6 hr	187 ± 5.0	(4)		81 ± 12.9	(8)
20 mg/kg endotoxin	6 hr	191 ± 2.3	(4)		117± 16.3	(8)
C. Hepatic tryptophan dioxygenase (mU/g liver)						
Control	0 hr				21 ± 4.2	(8)
	6 hr	92 ± 9.1	(8)		18 ± 2.2	(8)
5 mg/kg endotoxin	6 hr	32 ± 1.2‡	(4)			
20 mg/kg endotoxin	6 hr	35 ± 3.8‡	(4)		29 ± 2.8**	(8)

0, 5 or 20 mg endotoxin/kg was administered (i.p.) to fasted rats, and blood and liver samples were taken, as described in the methods, protocols A and B. The results presented are means ± SEM from the numbers of rats given in parentheses. Significance levels for the effects of endotoxin vs time-matched controls are: *$P < 0.05$, **$P < 0.005$ for a significant increase; †$P < 0.05$, ‡$P < 0.005$ for a significant decrease.

course of the experiments. Glycogen is not an important factor in these experiments with overnight-starved rats since, after such a fast, liver glycogen has decreased from 310 ± 31 (3) to 2 ± 0.4 (8) and muscle glycogen has decreased from 67 ± 29 (3) to 4 ± 1.6 (8) (all values are μmole glucose per g tissue, means ± SEM from the numbers of rats shown in brackets).

The effects of endotoxin on plasma glucose and glucocorticoids concentrations

In rats with basal plasma glucocorticoid concentrations (protocols B and C), endotoxin administration caused a modest but significant increase in plasma glucose (Table 1A, series 2 and 3 and Table 2). In rats from which multiple blood samples had been taken (protocol A), plasma glucocorticoid concentrations approximately doubled over 6 hr (Table 1B). In these rats the plasma glucose rose over the 6-hr period (Fig. 1 and Table 1A), perhaps in response to the increased glucocorticoids. Endotoxin abolished this effect on plasma glucose concentration, after an initial hyperglycaemia (Fig. 1); at 6 hr the plasma glucose concentration of these rats (series 1) was not significantly different from that of endotoxin-treated rats with basal glucocorticoid concentrations (Table 1A, series 3).

Livers of rats with elevated plasma glucocorticoid concentrations (protocol A) also had a greater activity of tryptophan dioxygenase, an enzyme which is known to be induced by glucocorticoids. After 6 hr these rats have activities of tryptophan dioxygenase 4–5-fold higher than at 0 hr, or than that in rats with basal glucocorticoid concentrations (Table 1C; protocol B). Endotoxin administration prevents this increase in activity; Table 1C shows that endotoxin-treated rats have similar tryptophan dioxygenase activities whether they have elevated (series 1) or basal (series 3) glucocorticoid concentrations.

The effects of endotoxin on plasma glucagon and insulin concentrations

In order to investigate in detail the effects of endotoxin on the relationships between plasma glucose, glucagon and insulin concentrations, rats were pretreated with endotoxin overnight (protocol C) to allow them to reach a steady-state over a 6-hr period of tryptophan-induced hypoglycaemia. That the concentrations of glucose, glucagon and insulin did not

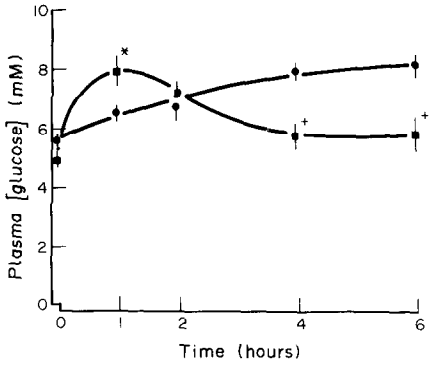

Fig. 1. The effects of endotoxin on plasma glucose concentration in rats with elevated plasma glucocorticoid concentrations. The experiment was carried out as described in the Methods, protocol A. ● control; ■ 5 mg/kg endotoxin at 0 hr. The results shown are means ± SEM from determination on 12 rats in each group. Significance levels vs controls: *$P < 0.05$, +$P < 0.005$.

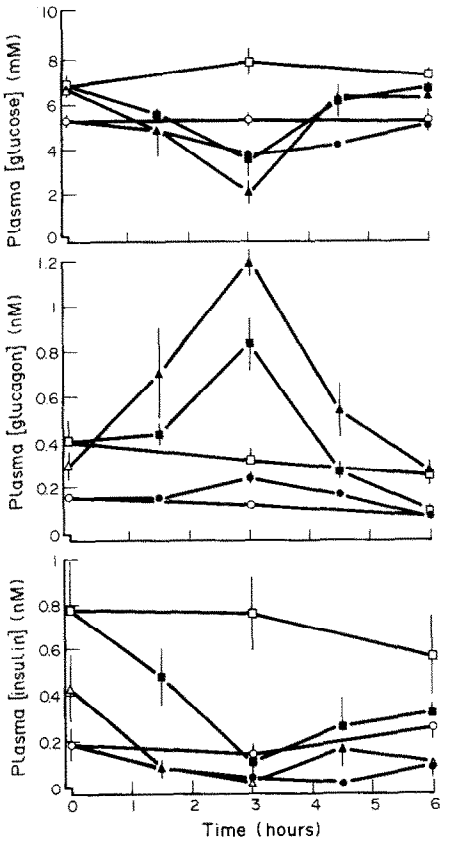

Fig. 2. The effects of tryptophan on plasma glucose, glucagon and insulin concentrations in control and endotoxin-treated rats. As described in the Methods, (protocol C) rats were fasted and given 0 (○●), 1 (□■) or 2 (△▲) mg/kg of endotoxin i.p. at 1600 hr. At 1000 hr the following day (0 hr) rats were either given L-tryptophan (200 mg/kg) (filled symbols), or left untreated (controls, open symbols); at the indicated times samples of blood were taken. Each point is a mean ± SEM from determinations on plasma samples from six rats; some SEM bars are within the symbols.

change over this 6-hr period in rats not treated with tryptophan is shown in Fig. 2. Treatment of rats overnight with 1 or 2 mg/kg endotoxin caused a significant increase in the plasma concentrations of both glucagon and insulin compared with those in control rats (Table 2, Fig. 2). Endotoxin also caused a decrease in concentrations of NEFA, but had no significant effect on plasma glucocorticoid concentrations.

The effects of tryptophan administration to these starved and endotoxin-treated starved rats were then determined over 6 hr. Tryptophan caused a transient decrease in plasma glucose concentrations in control rats; this decrease was moderate but significant (Fig. 2). The endotoxin-pretreated rats had higher initial plasma glucose concentrations, but responded more dramatically to tryptophan (Table 2, Fig. 2): the effects of tryptophan on plasma glucose at 3 hr as a percentage of control were 29 ± 4% (no endotoxin), 54 ± 10%, $P < 0.05$ (1 mg/kg) and 73 ± 7%, $P < 0.001$ (2 mg/kg). Figure 2 also shows the effects of tryptophan on plasma glucagon and insulin concentrations in the three groups of rats: in all three groups the hypoglycaemia was associated with increased plasma glucagon and decreased plasma insulin concentrations. The relationships between glucose and insulin or glucagon, revealed by the tryptophan-induced hypoglycaemia over 6 hr show that whereas endotoxin has caused no clearcut systematic change in the relationship between plasma concentrations of glucose and insulin, it has substantially increased both the slope and the intercept of the line correlating the plasma concentrations of glucose and glucagon.

The effect of these changes on the relationship between the plasma glucose concentration and the molar insulin:glucagon ratio is shown in Fig. 3, in which the data obtained with individual rats have been plotted; the change of ordinate unit in this figure allows the clear representation of widely varying insulin/glucagon ratios (approximately 0.01–10.00). This figure shows that the position of the inflexion point of the line relating the glucose concentration to the insulin:glucagon ratio has been shifted to the right of the graph, by about 2 mM glucose, upon pre-treatment of rats with endotoxin (2 mg/kg). The data obtained with rats pretreated with endotoxin (1 mg/kg) have not been shown for the sake of clarity, but are consistent with a line intermediate between the two shown; this line has an inflexion point at 6.6 mM glucose, compared with 5.1 mM (control rats) and 7.0 mM (endotoxin 2 mg/kg).

Tryptophan had no significant effects on plasma glucocorticoids in these experiments (results not shown).

The effects of endotoxin (5 mg/kg over 6 hr) on

Table 2. The effects of endotoxin on plasma glucose, glucagon, insulin, NEFA and glucocorticoids

	Endotoxin dose (mg/kg)				
	0	1		2	
Glucose (mM)	5.2 ± 0.29	6.8 ± 0.51	($P < 0.05$)	6.6 ± 0.49	($P < 0.05$)
Glucagon (pM)	163 ± 20	406 ± 96	($P < 0.05$)	298 ± 64	($P < 0.05$)
Insulin (pM)	189 ± 68	777 ± 220	($P < 0.05$)	424 ± 147	n.s.
Non-esterified fatty acids (mM)	0.73 ± 0.060	0.59 ± 0.075	n.s.	0.51 ± 0.058	($P < 0.05$)
Glucocorticoids (nM)	72 ± 10.2	60 ± 4.8	n.s.	67 ± 14.3	n.s.

Rats were fasted from 1600 hr, and the indicated dose of endotoxin administered (i.p.) at that time; blood samples were taken at 1000 hr the following day (protocol C). The results shown are mean plasma concentrations ±SEM, from determinations on six rats in each group. The significance levels shown are for an increase or decrease vs the control group (zero endotoxin).

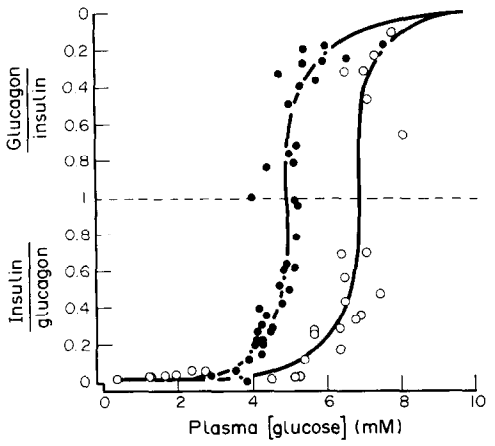

Fig. 3. The relationship between the molar insulin: glucagon ratio and the plasma glucose concentration in control and endotoxin-treated rats. The data are from the experiment described in Fig. 2. Data from individual rats are plotted: ● control; ○ 2 mg/kg endotoxin treated.

plasma concentrations of glucose, glucagon, insulin, NEFA and glucocorticoids were also determined (protocol B) in overnight-starved, acutely-diabetic rats. Endotoxin caused no significant change in the concentrations of insulin, NEFA or glucocorticoids in these diabetic rats. The plasma glucagon concentration was elevated moderately (control 173 ± 17.5 pM, endotoxin 383 ± 64.9 pM, means ± SEM, each from six rats; $P < 0.02$), and in the absence of a compensating elevation in plasma insulin, the plasma glucose concentration rose substantially (control 25.7 ± 1.68 mM, endotoxin 37.3 ± 2.35 mM, $P < 0.005$).

The molar insulin:glucagon ratios of control rats and of rats given endotoxin at doses of 1 mg/kg or 2 mg/kg (Table 2; protocol C) fell within the range insulin/glucagon = 0.5 to glucagon/insulin = 0.5. However, at the highest dose of endotoxin used in this study (20 mg/kg over 6 hr, protocol B), the insulin/glucagon ratio was much lower: control 1.38 ± 0.173, endotoxin 0.18 ± 0.045 (means ± SEM from 7 and 8 rats respectively, $P < 0.001$); this change was attributable to an increase in plasma glucagon (control 161 ± 10 pM, endotoxin 1126 ± 39 pM, means ± SEM) rather than a decrease in plasma insulin (control 223 ± 33 pM, endotoxin 193 ± 46 pM, means ± SEM).

DISCUSSION

Lethal doses of endotoxin cause hypoglycaemia [3, 7, 11, 28]. In contrast, sub-lethal doses, however, either have no effect on [12, 29] or increase [7] blood glucose concentrations. This is confirmed by the present findings.

Hyperglycaemia can be a result of stress mediated by glucocorticoids. However, the results of Tables 1 and 2 show that this is not the mechanism for endotoxin action in these experiments.

Previous work, mainly with mice, has demonstrated that endotoxin blocks the changes in blood glucose, and induction of the enzymes tryptophan dioxygenase, phosphoenolpyruvate carboxykinase (EC 4.1.1.32) and glycogen synthase (EC 2.4.1.11), brought about by exogenous glucocorticoids (reviewed in [30]); this may be mediated by the macrophage-derived glucocorticoid-antagonising factor [31]. The present work demonstrates for the first time that this phenomenon also applies to the effects of physiological concentrations of endogenous glucocorticoids in rats. The absence of any decreases in either plasma glucose or hepatic tryptophan dioxygenase on administration of low doses of endotoxin to rats with basal plasma glucocorticoids, demonstrates that this effect is limited to antagonism of the effects of *elevated* concentrations of such hormones.

The effect of tryptophan in fasted rats *in vivo* has been shown to be on the rate of production of glucose (i.e. gluconeogenesis), at least partly by inhibition of phosphoenolpyruvate carboxykinase [26, 27]. The lines shown in Fig. 3 were thus generated by inhibition of basal gluconeogenesis.

The results obtained in the absence of tryptophan (Table 2, Fig. 2) confirm other reports which suggest that plasma concentrations of insulin and/or glucagon are elevated in rats and dogs in endotoxic shock [4, 6, 8, 11]. It is relevant to note that this pattern, of elevated plasma concentrations of glucose, glucagon and insulin with no change in plasma glucocorticoid concentration, has also been reported to occur in man in sepsis [32, 33].

The results shown in Fig. 3 demonstrate that endotoxin administration causes a clear shift in the relationship between the molar insulin:glucagon ratio and the plasma glucose concentration. We have assumed that endotoxin does not alter hepatic clearance of insulin or glucagon [4, 12].

Overall the data are consistent with the hypothesis that endotoxin, at these doses, causes an increase in the plasma glucagon concentration because of increased pancreatic glucagon secretion; this should cause an increase in the plasma concentration of glucose and therefore an increase in that of insulin.

Data obtained from studies with diabetic rats (see Results and refs [7, 29]) are entirely consistent with this supposition.

Decreases in basal gluconeogenesis, or increases in basal glucose utilisation, would shift the plasma glucose concentration and the plasma insulin: glucagon ratio away from the inflexion point (Fig. 3). The highest dose of endotoxin used in this study caused a substantial decrease in the insulin/glucagon ratio; this suggests that in these rats the plasma glucose concentration has been significantly depressed below the inflexion point, even though it is higher than that in control rats (Table 1, series 3). This observation implies that there is inhibition of basal gluconeogenesis and/or stimulation of basal glucose utilisation *in vivo* in rats treated with larger doses of endotoxin; this effect may underlie the hypoglycaemia of lethal endotoxic shock.

Unfortunately, studies of glucose turnover *in vivo* cannot determine whether this effect of endotoxin is on basal glucose production or utilisation, because the elevated plasma glucagon and insulin concentrations will affect glucose turnover indepen-

dently of such effects. However, at doses equivalent to the LD_{50}, endotoxin has been reported to decrease maximal rates of basal gluconeogenesis from lactate measured subsequently in both isolated perfused liver [34] and isolated liver cells [28, 35]. We have obtained similar results using a low dose of endotoxin (<10% lethality): we observe a substantial decrease in basal gluconeogenesis by isolated rat liver cells (results not shown). It is unclear whether the endotoxin-induced resistance to glucocorticoid hormones has a role in the observed impairment of hepatic gluconeogenesis; glucocorticoids are thought to have a role in stimulating hepatic gluconeogenesis (reviewed in [36]).

CONCLUDING REMARKS

The data presented show that endotoxin affects carbohydrate metabolism in three ways:

(a) by increasing the rate of glucagon secretion;
(b) by antagonising the effects of elevated plasma glucocorticoid concentrations;
(c) by inhibiting basal gluconeogenesis and/or stimulating basal glucose utilisation (at higher doses of endotoxin).

These results provide no support for the view that changes in the plasma concentrations of insulin or glucagon play an important causative role in the development of hypoglycaemia in lethal endotoxic shock [8, 10]; indeed, the effect on the plasma glucagon concentration observed both in the present and in other studies [4, 6, 11, 12] will play an important role in resisting the onset of hypoglycaemia. Since leucocyte mediators have been reported to have both glucocorticoid-antagonising and glucagon secretion-stimulating activities [31, 37], it is possible that such mediators, elicited from leucocytes by endotoxin, may produce *in vivo* all of the effects on glucose homeostasis observed in this study.

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