Differential Effects of Tryptophan on Glucose Synthesis in Rats and Guinea Pigs

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1. Tryptophan inhibition of gluconeogenesis in isolated rat liver cells is characterized by a 20 min lag period before linear rates of glucose output are attained. 2. Half-maximal inhibition of gluconeogenesis in isolated rat hepatocytes is produced by approx. 0.1 mM-tryptophan. 3. Tryptophan inhibits gluconeogenesis from all substrates giving rise to oxaloacetate, but stimulates glycerol-fuelled glucose production. 4. Gluconeogenesis in guinea-pig hepatocytes is insensitive to tryptophan. 5. Changes in metabolite concentrations in rat liver cells are consistent with a locus of inhibition at the step catalysed by phosphoenolpyruvate carboxykinase. 6. Inhibition of gluconeogenesis persists in cells from rats pretreated with tryptophan *in vivo*. 7. Tryptophan has no effect on urea production from alanine, but decreases [1-¹⁴C]palmitate oxidation to ¹⁴CO₂ and is associated with an increased [hydroxybutyrate]/[acetoacetate] ratio. 8. These results are discussed with reference to the control of gluconeogenesis in various species.

The administration of tryptophan to rats at pharmacological doses is associated with a variable hypoglycaemia (Gullino *et al.*, 1955; Mirsky *et al.*, 1956; McDaniel *et al.*, 1973; Smith & Pogson, 1977), inhibition of hepatic gluconeogenesis (Ray *et al.*, 1966; Oravec & Sourkes, 1967; McDaniel *et al.*, 1972; Smith & Pogson, 1977), increased fatty acid synthesis (Sakurai *et al.*, 1973, 1974; Miyazawa *et al.*, 1975), increased ribosomal aggregation (Wunner *et al.*, 1966; Baglia *et al.*, 1968; Sidransky *et al.*, 1968), and changes in the total activities of specific enzymes (Greengard *et al.*, 1963; Rosen & Milholland, 1963; Cihak *et al.*, 1973, 1975; Ballard & Hopgood, 1973).

Lardy's group (Veneziale *et al.*, 1967) have shown that, in the isolated perfused liver, gluconeogenesis remains sensitive to inhibition by tryptophan in the perfusion medium. Inhibitory effects were also observed with a number of tryptophan metabolites of the 'kynurenine' pathway (Veneziale *et al.*, 1967). It was concluded that the action of tryptophan was attributable to the formation and intracellular accumulation of quinolinate. This is supported by the correlation between the extent of diminution of glucose synthesis and quinolinate accumulation (McDaniel *et al.*, 1973; Miyazawa *et al.*, 1975).

Tryptophan (Ui *et al.*, 1973; Meijer *et al.*, 1975; Crow *et al.*, 1978), quinolinate (Williamson *et al.*, 1971; Söling & Kleineke, 1976) and the quinolinate analogue 3-mercaptopycolinate (DiTullio *et al.*, 1974; Blackshear *et al.*, 1975) have all been used

effectively to inhibit gluconeogenesis both *in vivo* and *in vitro*. Alvarez & Ray (1974) have, however, claimed that neither tryptophan nor quinolinate is effective in diabetic rats. The ineffectiveness of tryptophan at least is underlined by the failure of quinolinate to accumulate in the livers of diabetic animals (McDaniel *et al.*, 1973). In a previous paper (Elliott *et al.*, 1977), however, we reported that quinolinate remains as potent an inhibitor in diabetic as in normal starved liver cells.

The metabolism and effects of tryptophan are somewhat variable between groups of animals of the same species (Knott *et al.*, 1977; Smith & Pogson, 1977) and are clearly distinct in liver and kidney (De Castro *et al.*, 1957; Pogson *et al.*, 1975). Enzyme studies indicate that more profound differences may occur between species (Badawy & Evans, 1974; Yeh & Brown, 1977). It is clearly established that the pathways of gluconeogenesis and the associated regulatory events differ from species to species (Hanson, 1974; Elliott & Pogson, 1977a). A study of the effects of tryptophan on hepatic gluconeogenesis in species other than the rat is thus of some interest.

Most investigations involving tryptophan have used high concentrations of the amino acid (Ray *et al.*, 1966; Oravec & Sourkes, 1967), so that it has not been clear whether the effects so observed are truly of physiological significance. Failure to follow the full time course of inhibition may also have led to underestimates of the magnitude of the effect (Cornell *et al.*, 1974; Crow *et al.*, 1978). A report (Wittman, 1976) that the absence of dietary tryptophan affects carbohydrate tolerance suggests, however, the possibility that physiological concentrations of tryptophan

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may play a role in the control of glucose metabolism *in vivo*.

In the present paper we have examined the concentration-dependence of the tryptophan-induced inhibition of gluconeogenesis in rat hepatocytes, the persistence of this phenomenon in diabetic animals, and contrast these effects with those in guinea-pig liver cells.

Some of these data have been presented in preliminary form elsewhere (Elliott *et al.*, 1976b).

Materials and Methods

Animals

Male Sprague-Dawley CSE/ASH strain rats (200–250 g) and Dunkin-Hartley guinea pigs (300–400 g) were used throughout. All animals were, unless otherwise stated, deprived of food for 48 h before cell preparation. In some experiments, hepatocytes were prepared from 48 h-starved rats pretreated for 45 min with L-tryptophan (750 mg/kg body wt.) (Smith & Pogson, 1977).

Three types of diabetic rat were used. First, animals were injected intravenously with a solution of alloxan (60 mg/kg body wt.) in 0.9% (w/v) NaCl. These animals were used after 48 h. Secondly, animals were similarly treated, but received 60 mg of streptozotocin in 0.9% NaCl/kg body wt. Thirdly, animals were injected intravenously with streptozotocin (50 mg/kg body wt.) and were used after 10–12 days. All diabetic animals were fed *ad libitum* (no. 1 maintenance diet; Cooper Nutritional Products, Witham, Essex, U.K.). Diabetes was confirmed by glycosuria (Clinistix; Ames Co., Stoke Poges, Slough, Berks., U.K.) and subsequent measurement of plasma glucose.

Chemicals

L-Tryptophan, inulin, palmitic acid (sodium salt), glucose oxidase (type II), peroxidase and gluconeogenic substrates were from Sigma (London) Chemical Co., Poole, Dorset, U.K. Alloxan and butyl-PBD[5-(4-biphenyl)-2-(4-t-butylphenyl)-1-oxa-3,4-diazole] were from Koch-Light Laboratories, Colnbrook, Bucks., U.K. Norit GSX was from Norit Clydesdale Co., Glasgow, Scotland, U.K., and PCS solubilizer from Hopkin and Williams, Chadwell Heath, Essex, U.K. Bovine serum albumin was obtained as a 20% (w/v) solution from Armour Pharmaceutical Co., Eastbourne, East Sussex, U.K., and was freed of fatty acids and other materials by the method of Chen (1967). Radiochemicals came from The Radiochemical Centre, Amersham, Bucks., U.K. Streptozotocin was a gift from Dr. D. C. N. Earl, I.C.I. Ltd., Alderley Park, Cheshire, U.K.

All other enzymes and biochemicals were the products of Boehringer Corp. (London) Ltd., Lewes, Sussex, U.K. Other basic chemicals were of the purest grade available from standard suppliers.

$1\text{-}^{14}\text{C}$ -labelled and unlabelled palmitate were associated with serum albumin in incubation media as described by Garland & Randle (1964).

Liver cell preparation

Isolated liver cells were prepared from rats and guinea pigs by procedures detailed elsewhere (Elliott *et al.*, 1976a; Elliott & Pogson, 1977b; Smith, 1977). Cell viability was determined by measuring the linear rate of glucose production obtained 30–90 min after addition of 10 mM-L-lactate (final concn.) to cell incubations. Batches of cells that failed to synthesize glucose at rates greater than 160 nmol/h per mg dry wt. (rat) or 100 nmol/h per mg dry wt. (guinea pig) were discarded.

Cell incubations

(i) *Standard incubations.* This procedure was followed generally, with the exceptions detailed below.

Portions (0.3 ml) of isolated liver parenchymal-cell suspensions (containing 4–8 mg dry wt. of tissue) were added to 1.7 ml of buffer (Krebs & Henseleit, 1932), supplemented with 2% (w/v) bovine serum albumin and gassed with O_2/CO_2 (19:1), in 20 ml silicone-treated glass liquid-scintillation vials. The vials were stoppered with Suba-Seals (Laboratory Apparatus and Glassblowing Co., Dewsbury, Manchester, U.K.). Incubations were carried out under an atmosphere of O_2/CO_2 (19:1) at 37°C; the vials were shaken at 100 oscillations/min in Dubnoff-type shaking water baths (Mickle Engineering Ltd., Gomshall, Surrey, U.K.). Substrates were added by syringe injection after a preincubation period of 40 min, and glucose-synthesis rates were determined between 30 and 90 min thereafter; rates so measured were linear with time throughout. Incubations were performed in triplicate and were terminated by addition of 0.2 ml of 2M-HClO₄. Precipitated protein was removed by centrifugation (1200g; 5 min), and portions of the supernatant were neutralized with an appropriate volume of 0.5M-triethanolamine/HCl, pH 7.4, containing 2M-KOH. Glucose was determined in the supernatant after removal of KClO₄ by centrifugation (1200g; 5 min).

(ii) *Incubation conditions for the determination of cellular metabolite concentrations.* The experimental procedure was as above, except that 5 ml portions of cell suspensions (approx. 60–90 mg dry wt. of tissue) were added to 10 ml of buffer [as in (i) above] in 100 ml silicone-treated glass conical flasks, stoppered with rubber bungs. L-Lactate (final concn. 10 mM) was

added after 40 min preincubation, and L-tryptophan (final concn. 0.5 mM) a further 30 min later. At various times after this cells were rapidly separated from incubation medium in the larger size of centrifuge tube described by Hems *et al.* (1975). A portion of each supernatant was acidified with 0.1 vol. of 2M-HClO₄; after centrifugation (1200g; 5 min), both cell and medium fractions were neutralized as above. Acetoacetate, pyruvate and 2-oxoglutarate were assayed immediately; other metabolites were assayed in fractions stored at -20°C.

Contamination of the cell pellet with incubation medium was calculated from the 'carry-down' of [³H]inulin (sp. radioactivity 0.25 µCi/mg), which was added to incubations immediately before cell separation. Appropriate corrections are included in the relevant metabolite concentrations in the text.

(iii) *Procedure for measurement of fatty acid oxidation.* Conditions were as described under (i) above with the following exceptions: (a) 10 ml conical flasks (Kontes Glass Co., Vineland, NJ, U.S.A.) with disposable centre wells and rubber stoppers were used throughout; (b) incubations were terminated by injection of 0.2 ml of 2M-HClO₄ into each flask. Phenethylamine/methanol (1:1, v/v; 0.25 ml) was injected into the well, which contained a piece of folded filter paper. Both additions were made through a septum in the stopper. All flasks were then shaken at 75 oscillations/min at room temperature (18–20°C) for a further 1 h to ensure complete absorption of CO₂. Centre well and contents were transferred to scintillation vials and radioactivity was determined with scintillator as previously described (Pogson & Smith, 1975).

Assay procedures

The following compounds were measured by standard procedures: lactate (Gutmann & Wahlefeld, 1974a), pyruvate and phosphoenolpyruvate (Czok & Lamprecht, 1974), 3-hydroxybutyrate (Williamson & Mellanby, 1974), acetoacetate (Mellanby & Williamson, 1974), ATP (Jaworek *et al.*, 1974a), ADP and AMP (Jaworek *et al.*, 1974b), malate (Gutmann & Wahlefeld, 1974b), aspartate (Bergmeyer *et al.*, 1974b), citrate (Dagley, 1974), 2-oxoglutarate (Bergmeyer & Bernt, 1974), urea (Fawcett & Scott, 1960) and 3':5'-cyclic AMP (Tovey *et al.*, 1974).

Blood glucose concentrations were measured with glucose oxidase and peroxidase (Krebs *et al.*, 1963). Since many compounds, including tryptophan and 5-hydroxytryptamine, are potent inhibitors of chromogen formation in this assay (Nelson & Huggins, 1974), glucose in incubations was determined with hexokinase and glucose 6-phosphate dehydrogenase (Bergmeyer *et al.*, 1974a).

Results and Discussion

Effects of tryptophan on rates of gluconeogenesis

It has become accepted that tryptophan itself does not inhibit hepatic gluconeogenesis directly, but requires to be converted into an active metabolite for such inhibition to become apparent. It is likely, therefore, that the onset of inhibition after addition of tryptophan will follow the rate of formation of the 'active' inhibitor. It follows equally that the true effectiveness of tryptophan in this respect may only be gauged after the establishment of steady-state conditions with the cell.

When tryptophan is added to cells from rat liver during the steady-state phase of gluconeogenesis from lactate, there is little immediate effect, and inhibition develops only after some 20 min of further incubation (Fig. 1a). Inhibition becomes constant from approx. 30 min onwards.

Linear rates of glucose production with all substrates are obtained when cells have been preincubated with tryptophan (at all concentrations used) for

Fig. 1. Time course of glucose production from lactate in rat and guinea-pig liver cell suspensions

Cells were preincubated for 40 min before addition of lactate (final concn. 10 mM). After a further 30 min, tryptophan (0.5 mM) was added where appropriate (zero time). All values are means $\pm$ S.E.M. (three cell preparations). (a) Rat; (b) guinea pig. ●, Lactate alone; ■, lactate plus tryptophan.

40 min. In contrast with previous reports (Ray *et al.*, 1966; Cornell *et al.*, 1974) we find that 50% inhibition of gluconeogenesis from lactate is obtained with approx. 0.1 mM-tryptophan. This concentration is within the range found in blood plasma (Knott & Curzon, 1972; Knott *et al.*, 1977), and suggests that the inhibition may indeed be of physiological importance. Cells from rats rendered acutely diabetic with alloxan or streptozotocin remained sensitive to tryptophan, although somewhat higher concentrations of the amino acid were required for inhibition comparable with that in 48 h-starved cells. This sensitivity to tryptophan is apparently at variance with a previous report (Alvarez & Ray, 1974). The two sets of results are, however, reconcilable, at least in part, in that gluconeogenesis in hepatocytes from chronically diabetic rats (conditions similar to those used by Alvarez & Ray, 1974) appears to be considerably more resistant to tryptophan than that in cells from acutely diabetic rats (Table 1). The differences between the acute and chronic conditions may be related to the relatively slow changes in the activities of certain enzymes of tryptophan metabolism (Ikeda *et al.*, 1965).

Hepatocytes from 48 h-starved guinea pigs are clearly different in their sensitivity to tryptophan. Addition of tryptophan to cell suspensions metabolizing lactate (Fig. 1b) or preincubation with tryptophan before addition of substrate is wholly without effect. Similar results are obtained over the whole range of concentrations examined (Table 1).

The effect of tryptophan on gluconeogenesis from a range of substrates in both rat and guinea-pig hepatocytes is shown in Table 2. The extent of inhibition is difficult to define precisely (see Cornell

et al., 1974). This is because it is not possible readily to assess the contribution to glucose production from endogenous materials in the presence of added substrates. Results have therefore been calculated in two ways: (i) assuming that exogenous substrates suppress endogenous activity, and (ii) assuming that the two rates (endogenous plus exogenous) are additive. Since tryptophan has no apparent effect on endogenous gluconeogenesis, the lack of precision is greatest with those compounds that are poor precursors. Rates obtained with glycerol, and perhaps with fructose, in rat cells are stimulated by tryptophan. The mechanism of this effect is unknown. With guinea-pig hepatocytes, tryptophan again has no significant inhibitory effect with any of the substrates tested, although the rate obtained with glycerol is again stimulated.

Effects of tryptophan on intracellular metabolites

The concentrations of intracellular metabolites in rat liver cells incubated with and without tryptophan are given in Table 3. At 90 min after tryptophan addition, significant increases are found in cell contents of malate and aspartate; with the decrease in phosphoenolpyruvate, these changes are consistent with inhibition of phosphoenolpyruvate carboxykinase (Ray *et al.*, 1966; Smith & Pogson, 1977). Although there is no significant change in the cytosolic redox state $[\text{lactate}]/[\text{pyruvate}]$: control 7.2 ± 0.5 ; plus tryptophan 8.6 ± 2.8 (three observations; $\pm$ S.E.M.), and no change in the contents of total ketone bodies, tryptophan increases the mitochondrial $[\text{NADH}]/[\text{NAD}^+]$ ratio $[\text{hydroxybutyrate}]/[\text{acetoacetate}]$: control 0.49 ± 0.05 ; plus tryptophan,

Table 1. *Effect of L-tryptophan on glucose production from L-lactate in hepatocytes from rats and guinea pigs*
Incubations were as described in the Materials and Methods section. Cells were preincubated for 40 min with or without added tryptophan before addition of L-lactate (10 mM final concn.). Rates of gluconeogenesis are uncorrected for endogenous glucose production and are either single determinations or means $\pm$ S.E.M. with the numbers of cell preparations in parentheses. The plasma glucose concentrations (mM) were 58 ± 9 (3), 29.0 and 34.9 for the acute alloxan-diabetic, acute streptozotocin-diabetic and chronic streptozotocin-diabetic rats respectively.

[Tryptophan] (mM)	Glucose production (nmol/h per mg dry wt.)				
	Rat				Guinea pig 48 h-starved
	48 h-starved	Alloxan-diabetic (acute)	Streptozotocin-diabetic (acute)	Streptozotocin-diabetic (chronic)	
0	217±16 (11)	395±14 (3)	557	321	138±9 (7)
0.05	208±7 (3)	—	561	—	141±11 (8)
0.1	138±10 (4)	272±9 (3)	482	295	137±11 (6)
0.25	50±15 (6)	—	407	—	152±17 (4)
0.5	24±6 (8)	85±21 (3)	230	258	150±11 (8)
0.75	15±4 (3)	—	—	—	—
1.0	14±5 (3)	61±21 (3)	—	237	141±20 (3)
2.0	—	—	—	159	—
5.0	—	—	—	—	149±13 (4)

Table 2. *Effect of L-tryptophan on glucose production from various precursors in hepatocytes from rats and guinea pigs* Incubations were as described in the Materials and Methods section. Cells were preincubated with L-tryptophan (0.5 mM) for 40 min before addition of substrate. Rates of glucose synthesis were determined between 30 and 90 min after addition of substrate (except fructose, which was from 0 to 30 min). Rates are expressed in two ways: uncorrected, in which no allowance has been made for endogenous production, and corrected, in which endogenous production has been subtracted. (Rates of glucose production with and without added tryptophan agreed within 10%.) All values are the means for two similar experiments.

Substrate (10 mM)	Glucose production					
	Rat			Guinea pig		
	Rate without tryptophan (nmol/h per mg dry wt.)	Rate with tryptophan (% of uninhibited rate)		Rate without tryptophan (nmol/h per mg dry wt.)	Rate with tryptophan (% of uninhibited rate)	
		Corrected	Uncorrected		Corrected	Uncorrected
None	17	0	100	7	0	100
Lactate	197	5	14	137	124	127
Alanine	102	6	25	64	91	93
Serine	43	9	30	42	108	110
Proline	45	28	55	—	—	—
Propionate	69	47	59	108	107	111
Pyruvate	179	29	37	117	100	100
Asparagine	41	0	16	—	—	—
Glutamine	144	0	15	—	—	—
Glycerol	132	168	161	49	133	143
Fructose	660	105	105	290	109	110

Table 3. *Effect of L-tryptophan on intracellular metabolites in rat and guinea-pig hepatocytes*

Incubations were as described in the Materials and Methods section. Metabolite contents were measured 90 min after the addition of 0.5 mM-tryptophan and are expressed as means $\pm$ S.E.M. (three cell preparations). Cells were preincubated for 40 min before addition of lactate, and then for a further 30 min before addition of tryptophan. Rat cell suspensions contained, in addition, 1 mM-palmitate throughout. * $P < 0.05$ (versus corresponding controls) (Student's t test).

Metabolite	Metabolite contents (nmol/mg dry wt.)			
	Rat		Guinea pig	
	Control	Plus tryptophan	Control	Plus tryptophan
Lactate	10.5 $\pm$ 0.1	14.7 $\pm$ 2.5	8.5 $\pm$ 1.5	9.0 $\pm$ 3.0
Pyruvate	1.47 $\pm$ 0.10	2.00 $\pm$ 0.50	0.53 $\pm$ 0.05	0.79 $\pm$ 0.14
2-Oxoglutarate	1.62 $\pm$ 0.16	1.90 $\pm$ 0.23	3.0 $\pm$ 0.3	1.9 $\pm$ 0.3
Phosphoenolpyruvate	0.25 $\pm$ 0.05	0.06 $\pm$ 0.02	—	—
Malate	1.20 $\pm$ 0.20	4.00 $\pm$ 0.80*	1.5 $\pm$ 0.1	1.6 $\pm$ 0.3
Aspartate	1.10 $\pm$ 0.10	4.20 $\pm$ 1.00	0.9 $\pm$ 0.2	0.7 $\pm$ 0.2
Citrate	2.40 $\pm$ 0.70	4.10 $\pm$ 1.30	—	—
Acetoacetate	2.30 $\pm$ 0.38	1.60 $\pm$ 0.21	0.67 $\pm$ 0.19	0.86 $\pm$ 0.11
3-Hydroxybutyrate	1.15 $\pm$ 0.29	2.10 $\pm$ 0.16	0.58 $\pm$ 0.04	0.38 $\pm$ 0.10
ATP	8.70 $\pm$ 0.90	11.2 $\pm$ 0.90	14.7 $\pm$ 1.5	10.7 $\pm$ 2.1
ADP	2.90 $\pm$ 0.50	2.50 $\pm$ 0.30	2.3 $\pm$ 0.4	2.2 $\pm$ 0.1
AMP	0.70 $\pm$ 0.20	0.61 $\pm$ 0.20	0.67 $\pm$ 0.25	0.51 $\pm$ 0.06
3':5'-Cyclic AMP	0.0008 $\pm$ 0.0001	0.0008 $\pm$ 0.0001	—	—

1.36 $\pm$ 0.24 ($P < 0.05$; three observations)). There are no similar significant changes in metabolite contents of guinea-pig cells. The degree of correlation between metabolite changes and the onset of inhibition is seen by comparison of the time courses (Figs. 1a and 2).

Effect of tryptophan administration in vitro on subsequent gluconeogenesis

We have investigated whether the effects of tryptophan added to liver cells *in vitro* also occur in the

Fig. 2. Time course of metabolite changes in rat hepatocytes after tryptophan addition

The conditions were as described in the Materials and Methods section and the legend to Table 3. Tryptophan (0.5 mM) was added at zero time. All values are means $\pm$ S.E.M. (three cell preparations), and are expressed as percentages of metabolite contents in control incubations. *P* (versus control): * <0.05 ; ** <0.01 ; *** <0.001 (Student's *t* test). $\circ$, 3-Hydroxybutyrate; $\bullet$, aspartate; ∇ , acetoacetate; $\blacktriangle$, malate; $\blacksquare$, phosphoenolpyruvate.

whole animal. In these experiments, rats were injected with tryptophan 45 min before the beginning of cell preparation. The plasma concentration of tryptophan at the start of perfusion was between 2.5 and 3.5 mM (Smith & Pogson, 1977). Perfusion and incubation media, however, contained no added tryptophan, and the liver itself was thoroughly washed *in situ* with Krebs-Henseleit bicarbonate buffer to prevent contamination with any amino acid remaining in the peritoneal cavity. The rates of gluconeogenesis indicate the persistence of inhibition for more than 100 min. These effects are attributable to the accumulation of intracellular tryptophan metabolites resistant to 'wash-out' during cell preparation and incubations (Table 4). Glucose production from glycerol is stimulated (cf. Table 2) to such an extent that glycerol becomes a better precursor than either lactate or pyruvate.

Effects of tryptophan on other metabolic processes in rat liver cells

The changes in the ratio of ketone bodies in rat liver-cell incubations with tryptophan prompted us to investigate the effects on fatty acid oxidation. The results are presented in Table 5. As expected, lactate stimulated palmitate oxidation (Exton *et al.*, 1972; Fröhlich & Wieland, 1972), and this was inhibited by some 40% by 0.5 mM-tryptophan. Although inhibition of the oxidation of palmitate alone failed to reach significance, there was a clear increase in the

Table 4. Effect of pretreatment with L-tryptophan *in vivo* on gluconeogenic capacity of isolated rat hepatocytes. Cells were prepared from 48 h-starved rats or from similar animals pretreated for 45 min with L-tryptophan (750 mg/kg. body wt.). Substrates (10 mM final concn.) were added at the beginning of incubation, and glucose production was measured between 60 and 90 min thereafter. Rates were linear with time over this period. Results are means $\pm$ S.E.M. for three cell preparations.

Substrate	Glucose (nmol/h per mg dry wt)		<i>P</i> (versus control)
	Control	Plus tryptophan	
None	19 $\pm$ 7	12 $\pm$ 4	—
Pyruvate	208 $\pm$ 7	111 $\pm$ 20	<0.01
Lactate	238 $\pm$ 12	127 $\pm$ 29	<0.05
Glycerol	136 $\pm$ 6	172 $\pm$ 6	<0.02

ratio [hydroxybutyrate]/[acetoacetate] with tryptophan. In the presence of lactate and tryptophan, total ketone bodies were increased, contrasting with a previous report that ketogenesis is inhibited under similar conditions (Bässler & Brinkrolf, 1971).

Addition of tryptophan to cell suspensions with lactate did not affect O_2 uptake over substantial incubation periods. Furthermore, tryptophan had no effect on urea production from 10 mM-alanine, although the expected inhibition of gluconeogenesis was evident in the same experiments (results not shown).

General discussion

Apart from considerations of availability, the rat has been widely used as a model system for gluconeogenesis because of its dietary similarities to man. This use has been questioned, however, because the intracellular distribution of phosphoenolpyruvate carboxykinase in the rat is generally atypical, and, in particular, unlike that in man (Hanson, 1974). Although the disposition of this enzyme in other species, including the guinea pig (Elliott & Pogson, 1977a), resembles that in man more closely, it is now apparent that there are important differences (e.g. effects of fatty acids, inducibility of enzyme activities) in the regulatory characteristics of glucose production between even closely related species. The clear difference in the effects of tryptophan between rat and guinea-pig liver cells further highlights this point. Since gluconeogenesis in rat cells is sensitive to tryptophan at concentrations in the physiological range, this parameter may merit serious consideration in the selection of model systems. Although human volunteers exhibit a transitory hypoglycaemia when given tryptophan (Ajdukiewicz *et al.*, 1968;

Table 5. *Effect of L-tryptophan on fatty acid oxidation in rat liver cells*

Cells were preincubated for 40 min before addition of lactate. Where relevant, [$1\text{-}^{14}\text{C}$]palmitate and tryptophan were added at the start of the preincubation period. The rates for palmitate oxidation and glucose production were obtained between 30 and 90 min after lactate addition. Values for ketone-body production represent accumulation over the 130 min preincubation plus incubation period. Results are duplicates or means $\pm$ S.E.M. for three cell preparations.

* $P < 0.05$ [versus appropriate control value (Student's t test)].

Addition	Final concn. (mM)	Palmitate oxidation to CO_2 (nmol oxidized/h per mg dry wt.)	Glucose output (nmol/h per mg dry wt.)	Acetoacetate (nmol/mg dry wt.)	3-Hydroxybutyrate (nmol/mg dry wt.)	Total ketone bodies (nmol/mg dry wt.)	[Hydroxybutyrate]/[acetoacetate] ratio
None	—	—	23 $\pm$ 5	136 $\pm$ 13	29 $\pm$ 12	155 $\pm$ 7	0.22
Lactate	10	—	259 $\pm$ 39	79 $\pm$ 12	40 $\pm$ 17	106 $\pm$ 4	0.60
Tryptophan	0.5	—	23 $\pm$ 3	71 $\pm$ 12	67 $\pm$ 29	116 $\pm$ 25	1.1
Lactate	10	—	18 $\pm$ 7*	63 $\pm$ 17	63 $\pm$ 25	105 $\pm$ 4	1.4
Tryptophan	0.5						
Palmitate	1.0	6.6 $\pm$ 0.7	24 $\pm$ 5	154 $\pm$ 26	491 $\pm$ 30	601 $\pm$ 23	3.1 $\pm$ 0.7
Palmitate	1.0	4.7 $\pm$ 0.4	6 $\pm$ 3*	96 $\pm$ 13	597 $\pm$ 37	693 $\pm$ 35	6.6 $\pm$ 1.1*
Tryptophan	0.5						
Palmitate	1.0	16.2 $\pm$ 2.0	420 $\pm$ 43	169 $\pm$ 16	227 $\pm$ 15	391 $\pm$ 6	1.4 $\pm$ 0.2
Lactate	10						
Palmitate	1.0	8.8 $\pm$ 0.9*	24 $\pm$ 14*	84 $\pm$ 3*	397 $\pm$ 33*	481 $\pm$ 19*	5.2 $\pm$ 1.3*
Lactate	10						
Tryptophan	0.5						

Fürst *et al.*, 1971), this response does not necessarily reflect inhibited gluconeogenesis.

Under all conditions tested in the present experiments, gluconeogenesis in rat liver cells was inhibited by tryptophan. We have reported elsewhere (Elliott *et al.*, 1977) that such cells are sensitive also to quinolinate, a suggested mediator of this effect (Veneziale *et al.*, 1967). Since gluconeogenesis in guinea-pig hepatocytes is also sensitive, albeit less so, to quinolinate, the explanation for the ineffectiveness of tryptophan may be in species differences in the metabolism of tryptophan through the kynurenine pathway, and in the failure of quinolinate to accumulate in guinea-pig liver.

In rats, death after tryptophan injection is associated with profound hypoglycaemia (Mirsky *et al.*, 1956; Smith & Pogson, 1977). The present data suggest that the greater toxicity of tryptophan in guinea pigs (Hvitfelt & Santti, 1972) cannot be simply attributed to direct effects on gluconeogenic flux.

Changes in hepatocyte metabolite concentrations after tryptophan addition are consistent with a block of phosphoenolpyruvate carboxykinase activity. Our results agree with those of Sakurai *et al.* (1974) in that there was no evidence of changes in adenine nucleotide contents (cf. Hirata *et al.*, 1967). A second site of action for tryptophan, as proposed by Spydevold *et al.* (1974), may be required to explain the changes both in fatty acid oxidation and in fatty acid synthesis (Sakurai *et al.*, 1974). At the present time,

however, the data in the present paper and elsewhere do not justify detailed speculation as to the molecular mechanisms involved.

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