Tryptophan and the control of triglyceride and carbohydrate metabolism in the rat

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1. Hepatic fatty acid synthesis, measured in vivo using ^3H_2O, was increased by a single dose of L-tryptophan (50 mg/kg body-weight) to both fed and fasted rats and by a supplement of tryptophan to the diet (2·5 g/kg diet for 7 d) when the rats were killed midway through the feeding period.

2. Additional dietary tryptophan was hypotriglyceridaemic in normal rats but exacerbated the hypertriglyceridaemia in rats when lipoprotein clearance was impaired 24 h after an injection of Triton WR 1339 (Chromatography Services Co., Birkenhead, Cheshire).

3. The effects of tryptophan on hepatic fatty acid synthesis and the concentration of serum triglyceride were not directly related to the action of the amino acid on gluconeogenesis. A lack of correlation between inhibition of gluconeogenesis and enhancement of lipogenesis was confirmed using mercaptopicolinic acid, a specific inhibitor of phosphoenolpyruvate carboxykinase (*EC* 4.1.1.32).

4. DL-Tryptophan itself did not provide a significant contribution of substrate to the total rate of lipogenesis. Other possible explanations for the activity of tryptophan noted in the present experiments are discussed.

5. In conclusion, moderate intakes of tryptophan affect fatty acid and triglyceride metabolism under physiological conditions and it is proposed that the amino acid may be involved in the control of lipid metabolism in a variety of metabolic states.

L-Tryptophan has a number of metabolic effects besides its role as rate-limiting amino acid in protein synthesis and polysome function. For example, several groups of workers have commented on the hypoglycaemic activity of large doses of tryptophan to fasted rats (McDaniel *et al.* 1973; Smith & Pogson, 1977) and a single dose of the amino acid is known to stimulate hepatic fatty acid synthesis in both fasted (Sakurai *et al.* 1974) and fed rats (Miyazawa *et al.* 1975). However, an enhancement of lipogenesis in rats seems inconsistent with the observed hypotriglyceridaemic activity for tryptophan in human nephrotic hyperlipidaemia (Schapel *et al.* 1974) although the metabolic states are, of course, very different. It was the purpose of the present experiments to investigate in more detail some of the effects of relatively moderate supplements of tryptophan on intermediary metabolism in the rat under physiological conditions. In particular, we have attempted to reconcile the various effects of tryptophan on triglyceride and fatty acid (FA) metabolism and to determine the relative importance of the effects on lipid and carbohydrate levels.

MATERIALS AND METHODS

Animals and diet. Male rats of the CFY strain (initial body-weight 80–100 g) were obtained from Carworth (Europe) Ltd, Alconbury, Hunts. Animals were fed *ad lib.* on a stock pelleted diet (Oxoid Breeding Diet; Lillico and Sons, Wonham Mill, Betchworth, Surrey) for 7 d, after which they were allocated to experimental groups (eight rats/group, four

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rats/cage) so that the mean body-weight of each group was the same. Where applicable, body-weight was recorded throughout the experimental period.

Biological measurements. The synthesis of FA in liver and small intestine, in vivo, was measured by the method of Fears & Morgan (1976). FA synthesis in fat pads, in vivo, was measured by procedures similar to those used for the other tissues. The incorporation of radioactivity from DL-[benzene ring U-^{14}C]tryptophan into FA was determined by procedures similar to those described for the incorporation of ^3H_2O. The incorporation of radioactivity into protein was determined by the method of Mans & Novelli (1961). For the measurement of radioactivity as tryptophan, and metabolites other than lipid and protein, 1 vol. tissue homogenate (200 g/l 0·16 M saline (9 g sodium chloride/l)) was mixed with 2 vol. 0·6 M-trichloroacetic acid and the precipitate was washed with 2 vol. 0·6 M-trichloroacetic acid. The supernatant fractions were combined and extracted three times with 5 vol. diethyl ether. Radioactivity in the aqueous phase was measured. Total triacylglycerol (TG) in serum was measured using the Technicon Instruments Corp. (1965) AutoAnalyzer method. Liver TG was measured using an AutoAnalyzer (Technicon Instruments Corp., Basingstoke, Hants) on the chloroform phase of total lipid extract prepared by the method of Folch *et al.* (1957) as modified by Bligh & Dyer (1959). Total free fatty acid (FFA) in the serum was measured by a combination of the methods of Duncombe (1963) and Itaya & Ui (1965). Total FFA in the liver was measured using a similar method on the chloroform phase of total lipid extract. Liver glycogen was measured by the method of Montgomery (1957). Blood glucose was measured by the method of Trinder (1969). For the determination of erythrocyte enzyme activities, red cells were separated from whole blood and washed with 1 vol. saline. The cells were haemolysed by the addition of 2 vol. distilled H_2O and then freezing. The activities of L-alanine aminotransferase (*EC* 2.6.1.2) and L-aspartate aminotransferase (*EC* 2.6.1.1) were determined using Boehringer Mannheim test combinations (The Boehringer Corporation (London) Ltd, Uxbridge Road, London W5 2TZ). The oxidation of [1-^{14}C]palmitic acid was measured by the method of Ontko & Jackson (1964) using a liver homogenate 100 g/l 0·25 mM-sucrose–1 mM-EDTA. Serum chylomicrons, very-low-density and low-density lipoproteins were precipitated using 0·066 M-manganese chloride and heparin (200 u.s.p. units/ml) (Burstein & Scholnick, 1973). The concentration of TG in the chloroform phase of the total lipid extract of the precipitate was measured using an AutoAnalyzer.

Statistical analysis. Statistical analyses were done using Student's *t* test.

RESULTS

Acute effects of a single dose of trypotphan

The lipogenic response to a single dose of L-tryptophan, calculated to be approximately equivalent to 20% of the normal daily intake, was similar in rats fed *ad lib.* and in rats fasted for 24 h before dosing (Table 1). The synthesis of FA, 2 h after the intraperitoneal dose of tryptophan, was increased without any change in the concentration of liver TG. Serum TG were slightly increased in concentration. As expected, both hepatic FA synthesis and the concentration of serum triglyceride were higher in fed than in fasted rats.

The stimulatory effect on FA synthesis in vivo is in contrast to the inhibitory effect observed in vitro. Tryptophan and its metabolites, kynurenic and xanthurenic acids, reduced [1-^{14}C]acetate incorporation into FA in liver slices taken from rats maintained on the control diet for at least 2 weeks. At 0·5 mM lipogenesis in vitro was inhibited by 76, 72 and 75% respectively.

In the fasted rats, the concentration of liver glycogen, already low, was markedly reduced

Table 1. *Effect of a single intraperitoneal dose of tryptophan (50 mg/kg body-weight) on serum and liver lipids and lipogenesis in fed and fasted rats*

(Mean values with their standard errors for eight rats/treatment)

Treatment...	Control		Tryptophan	
	Mean	SE	Mean	SE
24 h-fasted:				
Serum lipids (mmol/l)				
Triglyceride	0·55	0·08	0·77	0·13
Free fatty acid	0·45	0·12	0·46	0·10
Fatty acid synthesis†	158	22	227	11*
Liver glycogen (g/kg)	15	2·7	4·0	1·7**
Liver free fatty acid (g/kg)	3·3	0·3	3·8	1·0
Fed:				
Serum triglyceride (mmol/l)	0·69	0·04	0·82	0·08
Fatty acid synthesis†	270	16	532	50**
Liver triglyceride (g/kg)	4·4	0·3	4·5	0·3

Significantly different from corresponding control value: $*P < 0.01$, $** P < 0.001$.
† Fatty acid synthesized (μg/h per g tissue), measured by incorporation of ^3H from ^3H_2O.

by tryptophan (Table 1) but a small decrease in blood glucose was not statistically significant. There was no change in the FFA concentration in serum or liver.

Effects of supplementary dietary tryptophan

To determine whether the acute stimulatory effect of tryptophan on lipogenesis was maintained when additional tryptophan was included in the diet, rats were fed for 7 d on a stock diet supplemented so as to give approximately twice the control intake of tyrptophan. Under these conditions, tryptophan did not influence the body-weight gain or the food intake. The response to additional tryptophan depended on the time of day when the rats were killed (Table 2). At midnight, that is during the feeding period when lipogenesis is maximal, but not at midday, tryptophan increased FA synthesis in the liver but not at other important body sites, for example, ileum and epididymal adipose tissue. We found no effect of dietary supplements (2·5 g/kg) of two other amino acids: DL-alanine and L-lysine hydrochloride on hepatic lipogenesis.

The concentration of serum TG was significantly reduced by tryptophan, whether the rats were killed at midday or midnight, there was no effect on the concentration of TG or glycogen in the liver. The activities of the erythrocyte enzymes L-alanine aminotransferase and L-aspartate aminotransferase were measured as indices of pyridoxine status (Beaton & Cheney, 1965) but were not affected by the additional tryptophan.

The effect of dietary tryptophan on TG metabolism was investigated further in rats given an intraperitoneal dose of Triton WR 1339 (Chromatography Services Co., Birkenhead, Cheshire), a non-ionic detergent which inhibits the clearance of serum TG by the enzyme lipoprotein lipase (*EC* 3.1.1.3) (Scanu, 1965). As before, dietary tryptophan alone reduced serum TG with no effect on liver TG or body-weight gain (Table 3). As expected, Triton WR 1339 given 24 h before the end of the experiment increased both serum TG and the concentration of heparin-precipitable lipoproteins (chylomicrons, very-low-density and low-density lipoproteins). The combination of Triton WR 1339 plus dietary tryptophan induced an even greater elevation of serum TG and of the heparin-precipitable lipoproteins than did Triton WR 1339 alone, suggesting that the previous hypotriglyceridaemic activity of tryptophan was mediated by an enhancement of lipoprotein lipase activity.

Table 2. *Effect of supplementary dietary tryptophan (2·5 g/kg diet) for 7 d on serum and liver triglyceride and lipogenesis in rats killed at the mid-points of the light and dark periods*

(Rats were maintained on either a day-light-dependent lighting cycle or a reversed-light cycle (light period 16.00–04.00 hours). Rats were adapted to the appropriate lighting cycle for 14 d before the addition of tryptophan to the diet. Rats were killed between 10.00 and 12.00 hours. Mean values with their standard errors for eight rats/treatment)

Period of sampling...	Dark				Light			
Treatment...	Control		Tryptophan		Control		Tryptophan	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Body-wt gain (g/rat per experimental period)	47·8	3·1	47·6	1·5	40·1	4·5	45·5	3·9
Relative liver weight (g/kg body-wt)	0·41	0·02	0·45	0·02	0·44	0·01	0·45	0·03
Serum triglyceride (mmol/l)	1·16	0·15	0·76	0·10*	0·76	0·07	0·55	0·04*
Liver triglyceride (g/kg)	4·6	0·4	4·2	0·3	4·3	0·5	4·0	0·3
Liver glycogen (g/kg)	40	4·1	40	5·3	—	—	—	—
Tissue fatty acid synthesis†								
Liver	243	21	315	24**	140	24	124	14
Ileum	74	7	87	8	—	—	—	—
Epididymal adipose tissue	119	30	104	35	—	—	—	—
Erythrocyte enzymes‡								
L-alanine aminotransferase	—	—	—	—	131	6·4	148	16
L-aspartate aminotransferase	—	—	—	—	475	28	452	6·7

Significantly different from corresponding control value: * $P < 0·05$, ** $P < 0·02$.
† Fatty acid synthesized (μg/h per g tissue), measured by incorporation of ^3H from ^3H_2O.
‡ Enzyme activities (U/l blood).

Table 3. *Effects of supplementary dietary tryptophan (2·5 g/kg diet) for 7 d on serum and liver triglyceride in rats given Triton WR 1339 24 h before killing*

(Rats received an intraperitoneal dose of Triton WR 1339 (500 mg/kg body-weight) in saline (9 g sodium chloride/l) (2 ml/kg body-weight) or saline (2 ml/kg body-weight) alone, 24 h before the end of the experiment. Mean values with their standard errors for eight rats/treatment)

Treatment...	Saline-control		Triton WR 1339-control		Saline-tryptophan		Triton WR 1339-tryptophan	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Body-wt gain (g/rat per experimental period)	51·1	3·0	53·1	2·8	53·1	3·3	51·5	2·3
Relative liver weight (g/kg body-wt)	0·47	0·01	0·46	0·02	0·48	0·02	0·45	0·01
Serum triglyceride (mmol/l)	1·30	0·09	7·69	0·84**	0·99	0·10*	11·25	0·84***
Liver triglyceride (g/kg)†	3·9	—	3·9	—	4·0	—	4·3	—
Serum very low-density plus low-density lipoproteins (arbitrary units)	0·35	0·02	0·83	0·08**	0·34	0·02	1·05	0·09***

Significantly different from saline-control: * $P < 0·05$, ** $P < 0·001$.
Significantly different from Triton WR 1339-control: *** $P < 0·05$.
† Samples pooled from each rat in the group.

Metabolism of radio-labelled DL-*tryptophan*

Tryptophan is known to be metabolized to acetyl-CoA (Nishizuka *et al.* 1970) and might itself provide the substrate for an increase in FA synthesis. This possibility was examined using radio-labelled DL-tryptophan (Table 4). At 2 h after a single intraperitoneal dose of DL-[benzene ring U-^{14}C]tryptophan to fed rats, radioactivity was found in the lipid fraction

Table 4. *Incorporation of radioactivity 2 h after an intraperitoneal dose of* DL-[*benzene ring U-*^{14}*C*]*tryptophan* (8 *μCi*, 450 *mg/kg body-weight*) *to fed rats*

(Mean values with their standard errors for five rats)

Tissue...	Liver		Epididymal fat pad		Ileum	
	Mean	SE	Mean	SE	Mean	SE
Radioactivity (disintegrations/ min per g tissue)						
Fatty acid	581	96	31	4·9	105	15
Protein	5220	488	280	66	2800	586
Amino acids	12223	1986	888	130	5964	1093

of liver, small intestine and adipose tissue in addition to the protein and amino acid fractions. If the assumption is made that the specific radioactivity of free tryptophan in the liver and other tissues is equivalent to the specific radioactivity in the dose then the contribution made by acetyl-CoA from tryptophan to total FA synthesis is calculated to be of the order of less than 1%. Because of the contribution made by endogenous tryptophan, the specific radioactivity in the body will be less than that in the dose. Hence the rate of lipogenesis from radiolabelled tryptophan is underestimated. However, the dose of tryptophan administered was large in relation to the total body pool of the free amino acid so it seems unlikely that tryptophan could make a significant contribution to the substrate for lipogenesis.

Effects of a single dose of mercaptopicolinic acid

To investigate the possibility that the effect of tryptophan on lipid metabolism might be related to an inhibition of gluconeogenesis, the effect of mercaptopicolinic acid (SK&F 32488; Smith, Kline and French Laboratories Ltd), a standard inhibitor of phosphoenolpyruvate carboxykinase (*EC* 4.1.1.32) (Di Tullio *et al.* 1974; Blackshear *et al.* 1975), on lipid metabolism was examined in detail.

In the first experiment with mercaptopicolinic acid, a single oral dose to fasted rats did appear to increase FA synthesis in the liver after 2 h but not in the ileum or epididymal adipose tissue (Table 5). However, the increase in lipogenesis was insufficient to account for the large increments observed in the concentration of liver and serum TG. In a second experiment, we studied the influence of mercaptopicolinic acid on the oxidation of FFA in fasted rats. After 2 h the production of carbon dioxide and ketone bodies from [1-^{14}C]palmitate ex vivo was markedly inhibited with a concomitant increase in the concentration of hepatic FFA. Mercaptopicolinic acid lowered blood glucose in fasted rats in both experiments although the change was more marked in the second study.

In contrast to the effects of tryptophan and in contrast to its effects on fasted rats, a single dose of mercaptopicolinic acid had negligible effect on carbohydrate and lipid metabolism in fed rats.

DISCUSSION

Our studies have confirmed previous observations (Sakurai *et al.* 1974; Miyazawa *et al.* 1975) of an enhanced rate of hepatic FA synthesis after a single dose of L-tryptophan and have noted a similar action after supplementation of the diet with tryptophan when the rats were killed during the feeding period. The observation that FA synthesis was not elevated when the rats were killed in the post-absorptive state suggests that the stimulus is of limited duration. In addition, however, in contrast to the response to a single intraperitoneal dose, supplementary dietary tryptophan reduced the concentration of serum TG, whether or not

Table 5. *Effect of a single oral dose of mercaptopicolinic acid (125 mg/kg body-weight) on serum and liver triglyceride tissue lipogenesis and blood glucose in fed and fasted rats*

(Mean values with their standard errors for eight rats/treatment)

Treatment...	Control		Mercaptopicolinic acid	
	Mean	SE	Mean	SE
24 h-fasted: Expt 1				
Serum triglyceride (mmol/l)	0·27	0·02	0·35	0·02*
Liver triglyceride (g/kg)	3·1	0·3	4·7	0·6*
Blood glucose (mmol/l)	3·9	0·3	2·8	0·5
Tissue fatty acid synthesis†				
Liver	132	7	378	33***
Ileum	391	34	478	50
Epididymal adipose tissue	203	36	207	29
Expt 2				
Serum lipids (mmol/l)				
Triglyceride	0·53	0·07	1·08	0·17**
Free fatty acid	0·71	0·04	0·65	0·03
Liver free fatty acid (g/kg)	10·3	2·3	14·3	1·5
Blood glucose (mmol/l)	4·0	0·17	1·5	0·14***
Palmitate catabolism‡				
CO_2	99·2	16·3	48·9	4·0**
Acetoacetate	155	30·2	63·1	4·9**
Fed:				
Serum triglyceride (mmol/l)	0·74	0·02	0·81	0·04
Blood glucose (mmol/l)	5·8	0·16	5·5	0·18
Liver fatty acid synthesis†	241	23	254	9
Liver glycogen (g/kg)	51·8	2·6	46·5	3·2

Significantly different from corresponding control value: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
† Fatty acid synthesized (μg/h per g tissue), measured by incorporation of ^3H from ^3H_2O.
‡ Free fatty acid oxidation (nmol/h per g tissue).

FA synthesis was stimulated. The reduction in serum triglyceride cannot be attributed to an impaired lipoprotein output from the liver because we observed no increase in liver lipid, although other workers have noted an effect at a much higher dose of tryptophan (Hirata *et al.* 1967).

The effect of the amino acid on lipid metabolism seems more general than the effect on carbohydrate metabolism, which was confined to fasted rats. The hypoglycaemic action of tryptophan, and the metabolite quinolinic acid, has been attributed to an inhibition of gluconeogenesis at phosphoenolypruvate carboxykinase (Rosen & Nichol, 1964; McDaniel *et al.* 1973). It has been suggested (Miyazawa *et al.* 1975) that the tryptophan-induced increase in FA synthesis is a result *inter alia* of the activation of the lipogenic enzyme acetyl-CoA carboxylase (*EC* 6.4.1.2) by citrate whose accumulation is dependent upon the intramitochondrial concentration of oxaloacetate which increases because of the inhibition of phosphoenolpyruvate carboxykinase. In general, there is no doubt that an inverse correlation exists between the rates of gluconeogenesis and lipogenesis in a variety of metabolic states (Young *et al.* 1964; Sellers *et al.* 1974; Reed & Tarver, 1975). In our experiments, however, an effect of tryptophan on FA synthesis was observed even when carbohydrate metabolism appeared unaffected and the lack of association between lipogenesis and gluconeogenesis is underlined by the results obtained with mercaptopicolinic acid, which was without demonstrable effect in fed rats.

Our results also exclude other possible explanations for the effect of tryptophan on FA synthesis. Although the catabolism of tryptophan does provide acetyl-CoA as substrate for

lipogenesis, the flow of C2 units is too small to contribute significantly to the synthesis of lipid. Neither can the results be attributed to pyridoxine deficiency (Wittman, 1976) arising from increased utilization of pyridoxal phosphate by the tryptophan catabolic pathway. No impairment of pyridoxine status was observed and the diet used, Oxoid Breeding Diet, supplies twice the recommended intake of pyridoxine for normal rats.

The action of tryptophan in the liver might seem to be inconsistent with the serum TG-lowering effect but the results from the Triton experiment suggest an additional activation of lipoprotein lipase (*EC* 3.1.1.3). The ability of tryptophan to lower serum TG might be explained by metabolism to nicotinic acid, a known hypolipidaemic agent, which is believed to increase the activity of lipoprotein lipase (Nikkila, 1971). However, we found that nicotinic acid, at a dose of 100 mg/kg body-weight in the diet for 7 d, was not hypotriglyceridaemic. This dose was considered to provide an intake equivalent to the concentration produced endogenously from the supplementary tryptophan provided in our experiments (Harris & Kodicek, 1950).

We consider that the explanation of our findings for tryptophan is as follows. Tryptophan stimulates insulin secretion (Floyd *et al.* 1966; Ajdukiewicz *et al.* 1968) and the sensitivity of rats to tryptophan is dependent on the presence of functional pancreatic β cells (Smith & Pogson, 1977). Insulin increases the activities of hepatic FA synthetase (Lakshmanan *et al.* 1972) and adipose tissue lipoprotein lipase (Wing *et al.* 1966). An effect by tryptophan on insulin levels has physiological relevance because tryptophan and its metabolite 5-hydroxytryptamine are known to reduce physical activity in the rat (Taylor, 1976). As food intake was unaffected in our experiments a greater proportion of dietary energy was available for storage as carcass lipid, this process being mediated by increases in the activities of the enzymes of hepatic FA synthesis and of adipose tissue lipoprotein lipase.

Although our results have been obtained at relatively high dietary intakes of tryptophan, a more moderate intake of the amino acid may also participate in the control of intermediary metabolism. At least some of the effects noted in our rat experiments are produced by small supplements of tryptophan to man (Schapel *et al.* 1974). A role in carbohydrate metabolism has been emphasized by previous workers but the present results suggest that the influence of tryptophan and its metabolites on lipid metabolism is more general and might explain a number of observations. For example, the diurnal rhythm in the rate of hepatic lipogenesis can be correlated with the diurnal variation in plasma tryptophan (Fernstrom *et al.* 1971). Elevated rates of FA synthesis are found in those metabolic states characterized by an increase in tryptophan catabolism along the nicotinic acid pathway, as after oral contraceptive therapy (Horwitt *et al.* 1975). Finally, the effects on lipid metabolism of agents such as clofibrate have been attributed to their actions on the metabolism of tryptophan (Sirtori *et al.* 1977).

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