The Effects of Diet, Lipolysis and Limb Ischaemia on the Distribution of Plasma Tryptophan in the Rat

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A non-linear relationship between the plasma non-esterified fatty acid concentration and the percentage of free plasma tryptophan was found in rats in different nutritional states, although non-esterified fatty acids are not the only factors determining the percentage of free tryptophan. This relationship was not seen in rats injured by limb ischaemia. The effect of drugs causing rapid increases in the plasma non-esterified fatty acid concentration was also studied. Isoprenaline decreased the total plasma tryptophan concentration. Dichloroisoprenaline caused a sustained increase in the plasma non-esterified fatty acid concentration which was accompanied by an increase in the concentration of free plasma tryptophan and followed by a fall in the concentration of total tryptophan. The loss of tryptophan from the plasma was attributed to an altered distribution of tryptophan in the extracellular space rather than to increased metabolism. This interpretation was supported by determinations of the irreversible disposal rate of plasma tryptophan which in uninjured rats was unaffected by the concentration of free plasma tryptophan. In the injured rats this rate was unaltered during limb ischaemia but was decreased after removal of the tourniquets; increased competition for tissue entry by other neutral amino acids and the fall in body temperature could be factors in this fall.

Tryptophan differs from other plasma amino acids in being partially bound to albumin. It is the fraction which remains free in the plasma which passes readily through the walls of the exchange vessels to be taken up by the tissues and used in protein synthesis, kynurenine formation and the synthesis of 5-hydroxytryptamine. At the present time the last-named pathway, although quantitatively minor, is receiving much attention since 5-hydroxytryptamine is an important neurotransmitter in the central nervous system where its metabolism is affected by many types of acute stress and trauma, as well as by the more chronic affective states. Our interest arose out of work on the role of the serotonergic pathways in the cerebral response to trauma (Stoner & Elson, 1971). Since cerebral 5-hydroxytryptamine synthesis is influenced by the free tryptophan concentration in the plasma (Fernstrom & Wurtman, 1972; Tagliamonte *et al.*, 1973) investigation of the changes in the latter in normal and pathological states was required, particularly with regard to the effect of changes in the concentration of plasma non-esterified fatty acids which have an important effect on the binding of tryptophan to plasma albumin as discussed in the preceding paper (Cunningham *et al.*, 1975).

Whereas a non-linear relationship between the percentage of free tryptophan in the plasma and the plasma non-esterified fatty acid concentration was found in normal rats in different nutritional states,

this relationship was not seen when the plasma non-esterified fatty acid concentration was altered by the stress of injury. When rapid changes in the plasma non-esterified fatty acid concentration were produced by drugs more complicated changes in tryptophan distribution occurred. These findings necessarily required a study of the irreversible disposal (Shipley *et al.*, 1967) of tryptophan in the whole animal in relation to the free tryptophan concentration in the plasma.

Materials and Methods

Materials

Chemicals. In general AR grade chemicals were used. The sources of certain additional chemicals were as follows. Ninhydrin hydrate, dithiothreitol and L-tryptophan ('A' grade; Calbiochem, San Diego, Calif., U.S.A.); L-[methylene- ^{14}C]tryptophan (54 mCi/mmol; The Radiochemical Centre, Amersham, Bucks., U.K.); palmitic acid [Sigma (London) Chemical Co., London S.W.6, U.K.]; methanol (Aristar grade; British Drug Houses, Poole, Dorset, U.K.); (–)-isoprenaline bitartrate (Wyeth, Taplow, Berks, U.K.); dichloroisoprenaline hydrochloride (Aldrich, Milwaukee, Wis., U.S.A.); Dowex 50W (X8) resin (Bio-Rad, Richmond, Calif., U.S.A.); Insta-gel scintillation fluid and [^{14}C]toluene (Packard Instrument Co. Inc., Downers Grove, Ill., U.S.A.).

Animals. Male albino rats of the Porton strain weighing between 197 and 278 g (mean, 238 g) were used. They were fed on M.R.C. diet 41B (Bruce & Parkes, 1956) and had been kept since weaning at an environmental temperature of 18–22°C with controlled lighting giving 12h (07:00–19:00h) light per day. All the experiments were carried out at this environmental temperature. Intravenous injections were given into a tail vein. Blood was obtained from the severed neck vessels after guillotining and heparin was added as an anticoagulant. When necessary, blood from similarly treated rats was pooled to provide sufficient plasma for the various estimations. Haematocrit values were obtained with a Hawkesly micro-haematocrit centrifuge. Plasma was separated by centrifugation at 2000g at 10°C. Ultrafiltrates of plasma were obtained by centrifugation at 450g at 15°C for 20min in 'Centriflo' membrane cones CF 50 (Amicon Corp., Lexington, Mass., U.S.A.). These membranes proved unsuitable for use with rat serum.

To obtain plasma with different non-esterified fatty acid concentrations the rats were killed at different times of day or after 24h of starvation. Rats were also killed 30min after the oral administration of 5.0ml of 20% glucose or at intervals after the subcutaneous injection of 200 µg of (–)-isoprenaline (base)/kg or the intravenous injection of 7mg of dichloroisoprenaline hydrochloride/kg. Blood from injured rats was obtained during and after 4h bilateral hind-limb ischaemia produced by Rosenthal's (1943) method. The tourniquets were applied during ether anaesthesia of about 3min duration at about 10:00h. The controls were similarly anaesthetized at the same time of day.

For the radioactivity experiments 50 µCi of [¹⁴C]tryptophan was dissolved in 0.3ml of 0.2M-Sörenson phosphate buffer, pH 7.0 (Umbreit *et al.*, 1957), diluted to 2.0ml with rat serum and injected intravenously in a dose of 0.1 ml/100g body wt. After a preliminary experiment the sampling times were chosen by the method of Corney & Heath (1970).

Methods

Analytical techniques. Tryptophan was as a routine determined by the method of Denkla & Dewey (1967), 20 µl of plasma (duplicate samples) or 0.2ml of ultrafiltrate being treated with 1.8ml of 10% (w/v) trichloroacetic acid. The deleterious effect of exposure to light described by Lehman (1971) was overcome by adding a freshly prepared solution of FeCl₃ to the formaldehyde solution and mixing this with the sample immediately before boiling. In the radioactivity experiments 0.5ml of plasma was treated with 5.0ml of 5% (v/v) HClO₄, and the supernatant was separated by centrifugation at 2000g at 10°C. Tryptophan was isolated from the

supernatant by column chromatography and measured with ninhydrin as described by Gaitonde (1974). Duplicate samples of the column eluate were used in the final determination and for the measurement of radioactivity. The latter was done with routine scintillation counting equipment with Insta-gel as the scintillation fluid and [¹⁴C]toluene as internal standard, and the results were normalized to a dose of 6×10^6 d.p.m./100g body wt. Comparing the two methods the total tryptophan concentrations in three plasma samples were 0.168, 0.170 and 0.107 µmol/ml by the Denkla & Dewey (1967) method and 0.147, 0.154 and 0.104 µmol/ml by Gaitonde's (1974) method.

Plasma non-esterified fatty acid concentrations were measured as described by Cunningham (1973) except that the concentration of Thymol Blue in propan-2-ol was 100mg/litre. Total plasma protein was measured by the method of Robinson & Hogden (1940) as modified by Aldridge (1957).

Mathematical techniques. Where possible the results have been expressed as the mean ± standard error (S.E.M.). Statistical comparisons of means have been made by the Student's *t* test as modified by Fisher (1934) for small samples.

Steady-state conditions were assumed in the radioactivity experiments and the irreversible disposal rates for plasma tryptophan calculated from the areas to infinity under specific radioactivity-time curves by the methods of Corney & Heath (1970) and Heath & Barton (1973). Because of the rapid exchange of label between free and bound tryptophan the plasma tryptophan may be considered as a single pool in these experiments. An advantage of the methods used is that they allow a model-free determination of the rate of irreversible disposal of total plasma tryptophan. In these experiments 'irreversible' refers to labelled tryptophan which is lost from the plasma and which does not return to the plasma during the experiment (40min). Our aim was to examine the relationship between the irreversible disposal rate of total plasma tryptophan and the free concentration.

Results and Discussion

Uninjured rats

The total tryptophan concentration in the plasma of the uninjured rats (Table 1) was highest shortly after the end of feeding and then fell as expected (Fernstrom & Wurtman, 1971). At 09:30h after overnight starvation the concentration was about the same as in fed rats at that time and fell similarly during the next few hours. In contrast, the concentration of free tryptophan in the plasma rose markedly during starvation. In post-absorptive (Heath & Threlfall, 1968) rats at 14:00h the concentration was

Table 1. Total tryptophan concentrations in plasma of uninjured rats at different times of day and of injured rats at the end of 4 h bilateral hind-limb ischaemia (14:00 h) and 1.5 h after removal of the tourniquets (15:30 h)

Numbers of samples are shown in parentheses. The values are means $\pm$ S.E.M.

Time of day (h)	Total tryptophan ($\mu\text{mol/ml}$)	
	Uninjured	Injured
07:30	0.134 $\pm$ 0.008 (14)	
09:30	0.119 $\pm$ 0.008 (21)	
14:00	0.103 $\pm$ 0.003† (44)	0.105 $\pm$ 0.003 (36)
15:30	0.112 $\pm$ 0.005* (12)	0.110 $\pm$ 0.002 (54)
09:30‡	0.119 $\pm$ 0.004 (17)	—
14:00‡	0.104 $\pm$ 0.003† (29)	0.096 $\pm$ 0.007 (6)

* Significantly different from 07:30 h value ($P < 0.05$).

† Significantly different from 07:30 h value ($P < 0.01$).

‡ Starved during previous night.

0.0050 $\pm$ 0.0002 $\mu\text{mol/ml}$ ($n = 35$) and after 24 h of starvation it had risen to 0.0101 $\pm$ 0.0006 $\mu\text{mol/ml}$ ($n = 28$). The free tryptophan concentrations in this paper are lower than would be found *in vivo* since the plasma was ultrafiltered at 15°C. The influence of temperature on the percentage of free tryptophan was examined with four samples of rat plasma. In the temperature range 10–30°C the percentage free tryptophan increased 0.09 $\pm$ 0.01 per °C rise in temperature.

The effect of starvation on the concentration of free tryptophan confirmed the results of Knott & Curzon (1972) and raises the question of the role in these changes of the plasma non-esterified fatty acids which are also albumin-bound and increased by starvation. The percentage free tryptophan and the concentration of non-esterified fatty acids in the plasma were compared in uninjured rats in nutritional states corresponding to the times shown in Table 1. To increase the number of rats with low plasma non-esterified fatty acid concentrations 12 rats were given 1 g of glucose by stomach tube as a single bolus of a 20% solution at times between 09:30 and 11:00 h and killed 30 min later. The total plasma tryptophan concentration in these rats was 0.104 $\pm$ 0.004 $\mu\text{mol/ml}$. Including these rats the plasma non-esterified fatty acid concentration in the uninjured animals ranged between 0.27 and 1.52 $\mu\text{mol/ml}$. The total tryptophan concentration was not correlated with the plasma non-esterified fatty acid concentration but the free tryptophan was (Fig. 1). As expected from the experiments *in vitro* (Cunningham *et al.*, 1975) in the plasma non-esterified fatty acid concen-

Fig. 1. Percentage of free tryptophan and non-esterified fatty acid concentration in rat plasma

Each point represents one sample; □, from fed rats at 07:30 h; ●, from fed rats at 09:30 h; ▲, from rats given glucose (see the text); ○, from post-absorptive rats at 14:00 h; ■, from rats at 09:30 h after starving overnight; ▽, from rats at 14:00 h after starving overnight.

tration range, 0.27–0.6 $\mu\text{mol/ml}$, there was no correlation between an increase in the non-esterified fatty acid concentration and the percentage of free tryptophan, but when the non-esterified fatty acid concentration exceeded 0.6 $\mu\text{mol/ml}$ the percentage of free tryptophan increased markedly and progressively.

However, *in vivo*, albumin bound tryptophan more tightly than the sample of rat albumin used for the experiments *in vitro* so that quantitative agreement between the percentages of free tryptophan found and those predicted was poor. Although the plasma non-esterified fatty acid concentration may be the most important factor in determining the percentage of free tryptophan in the plasma of normal rats the large variations in the percentage at the higher non-esterified fatty acid concentrations (Fig. 1) imply that other factors are also involved. From the data presented by Bender & Bamji (1974) a similar non-linear relationship might also exist in man.

Effect of (–)-isoprenaline

To increase the number of plasma samples with high non-esterified fatty acid concentrations nine rats were injected with 200 μg of isoprenaline/kg body wt. (subcutaneously) to stimulate lipolysis, and killed 20 min later. A rise in the plasma non-esterified fatty acid concentration to 0.95 $\pm$ 0.03 $\mu\text{mol/ml}$ was associated with a rise in the percentage of free plasma tryptophan to between 6.8 and 25.0%, but the

Fig. 2. Total free tryptophan and non-esterified fatty acid concentrations in the plasma of rats given 7 mg of dichloroisoprenaline hydrochloride/kg body wt. intravenously

Each point represents the mean, and the S.E.M. is indicated by the vertical line through the point. ○, Non-esterified fatty acid; ●, total tryptophan; ■, free tryptophan.

Time after dichloroisoprenaline injection (min)	0	4	8	15	30	45
No. of samples of total tryptophan and non-esterified fatty acid	12	8	11	8	12	12
% free tryptophan (mean $\pm$ S.E.M.)	4.5 ± 0.3	13.4 ± 3.3	18.6 ± 4.5	13.5 ± 4.1	11.8 ± 2.7	14.7 ± 3.0
No. of samples	7	6	5	4	6	6

principal effect was a marked fall in the total tryptophan content of the plasma to $0.061 \pm 0.004 \mu\text{mol/ml}$ (cf. Table 1). These rats were not included in Fig. 1. Two mechanisms which could lead to such a fall were considered; displacement of tryptophan from the bound plasma pool followed by a redistribution of tryptophan to extravascular pools (Smith & Lakatos, 1971), and/or a consequent increase in the rate at which tryptophan was being removed by the tissues. The effect on plasma tryptophan of a rapid and sustained rise in the plasma non-esterified fatty acid concentration after the injection of dichloroisoprenaline was therefore investigated.

Effect of dichloroisoprenaline

Although dichloroisoprenaline is a β -receptor-blockade agent it has a marked agonist action in the rat and causes prolonged lipolysis (see Stoner & Little, 1969). After the injection of 7 mg of dichloroisoprenaline hydrochloride/kg body wt. (intravenously) in post-absorptive rats, the plasma non-esterified fatty acid concentration reached a maximum after 4 min and the high concentration was maintained for over 45 min (Fig. 2). The free tryptophan concentration also rose to a peak at 4 min, being 2.73 times greater than the initial value. This was followed by a fall in the total tryptophan concentration which

Table 2. *Plasma non-esterified fatty acid and free tryptophan concentrations in injured rats*

Numbers of samples are shown in parentheses. The values are means $\pm$ S.E.M. Differences between post-absorptive rats were significant at * $P < 0.01$, ** $P < 0.001$. Differences between post-absorptive and starved rats at the end of limb ischaemia were not significant.

	Concentration ($\mu\text{mol/ml}$ of plasma)	
	Non-esterified fatty acid	Free tryptophan
End of limb ischaemia		
Post-absorptive rats	0.84 ± 0.02 (17)	0.0048 ± 0.0003 (35)
Starved rats	0.93 ± 0.04 (6)	0.0054 ± 0.0007 (6)
1.5h after limb ischaemia		
Post-absorptive rats	$0.53 \pm 0.01^{**}$ (36)	$0.0062 \pm 0.0003^*$ (50)

Fig. 3. *Percentage of free tryptophan and non-esterified fatty acid concentrations in the plasma of post-absorptive (○) and starved (△) rats at the end of 4h bilateral hind-limb ischaemia and of post-absorptive (●) rats 1.5h after such a period of limb ischaemia*

The points represent separate samples.

was practically complete 8min after the injection. The percentage of free tryptophan remained high (see legend to Fig. 2); its concentration fell but had not returned to its initial value by the end of the experiment. It would have done so had the rate of utilization of tryptophan increased with the rise in the concentration of free tryptophan, assuming a constant inflow. The findings suggested that the rate of utilization in the whole rat was not affected by the concentration of free tryptophan in the plasma and this conclusion was supported by measurements of the irreversible disposal rate at different free tryptophan concentrations (see below). Assuming a plasma volume of $4.3 \text{ ml}/100 \text{ g}$ body wt, about $0.132 \mu\text{mol}$ of tryptophan left the plasma after the injection of

dichloroisoprenaline and it is likely that this represented a reversible redistribution of tryptophan to extravascular spaces.

It was noted that the rise in the percentage of free tryptophan associated with the rise in plasma non-esterified fatty acid concentration after dichloroisoprenaline (Fig. 2) was much higher than expected (Fig. 1). This effect is not understood and may reflect effects of dichloroisoprenaline on the binding of tryptophan, either directly or indirectly, in addition to those caused via an increased non-esterified fatty acid concentration. Neither isoprenaline nor dichloroisoprenaline altered the total plasma protein concentration.

Effect of bilateral hind-limb ischaemia

As used in these experiments 4h bilateral hind-limb ischaemia is a serious injury with an 85% mortality rate in the rat. In post-absorptive rats the average survival time is about 12h and this is shortened if the rats are starved before the injury (Threlfall & Stoner, 1954; Stoner, 1970). There is a sustained increase in lipolysis from the time of application of the tourniquets which is reflected in an increase in the plasma non-esterified fatty acid concentration during the limb ischaemia with a return to normal or lower concentrations when the tourniquets are removed and the perfusion of the fat-depots is decreased (Stoner, 1962; Stoner & Matthews, 1967; Heath & Stoner, 1968). In the present experiments rats were examined at the end of 4h bilateral hind-limb ischaemia and 1.5h after removal of the tourniquets and the expected changes in plasma non-esterified fatty acid concentration found (Table 2).

Limb ischaemia did not alter the total concentration of tryptophan in the plasma (Table 1) and the differences in the free tryptophan concentrations

Table 3. *Specific radioactivity of plasma tryptophan at intervals after the intravenous injection of [14 C]tryptophan*
Results are given as means $\pm$ S.E.M. for a dose of 6×10^6 d.p.m./100 g body wt. Numbers of rats are shown in parentheses.

Time after injection (min)	$10^{-4} \times$ Specific radioactivity (d.p.m./ μ mol)			
	Post-absorptive uninjured	24h-starved uninjured	End of 4h limb ischaemia	1.5h after limb ischaemia
2	228.1 $\pm$ 11.6 (5)	169.0 $\pm$ 6.1 (6)	242.0 $\pm$ 16.1 (4)	343.7 $\pm$ 5.1 (4)
7	122.6 $\pm$ 4.6 (6)	103.6 $\pm$ 5.6 (6)	124.4 $\pm$ 5.7 (5)	151.1 $\pm$ 7.5 (5)
17	68.76 $\pm$ 4.31 (5)	50.44 $\pm$ 3.74 (6)	70.52 $\pm$ 2.30 (4)	87.86 $\pm$ 2.49 (4)
40	20.97 $\pm$ 1.87 (5)	25.61 $\pm$ 1.59 (5)	25.12 $\pm$ 2.64 (4)	39.24 $\pm$ 2.14 (4)

Table 4. *Rate of irreversible disposal (R) of plasma tryptophan in post-absorptive, 24h-starved uninjured rats and post-absorptive rats at the end of 4h bilateral hind-limb ischaemia and 1.5h after removal of the tourniquets at the end of this period of ischaemia*

Numbers of samples are shown in parentheses. d.f. = degrees of freedom. Values are means $\pm$ S.E.M.

State	Plasma tryptophan (μ mol/ml)		Irreversible disposal rate (μ mol/min per 100g)
	Total	Free	
Post-absorptive uninjured	0.103 $\pm$ 0.004 (24)	0.0056 $\pm$ 0.0003 (18)	0.165 $\pm$ 0.005 d.f. 17
24h-starved uninjured	0.106 $\pm$ 0.003 (23)	0.0100 $\pm$ 0.0006* (22)	0.173 $\pm$ 0.005 d.f. 19
End of 4h limb ischaemia	0.098 $\pm$ 0.003 (18)	0.0037 $\pm$ 0.0002* (18)	0.152 $\pm$ 0.005 d.f. 14
1.5h after limb ischaemia	0.103 $\pm$ 0.003 (18)	0.0048 $\pm$ 0.0003 (16)	0.106 $\pm$ 0.002* d.f. 14

* $P < 0.001$, in comparison with uninjured post-absorptive rats.

during and after limb ischaemia were not those expected from the differences in the plasma non-esterified fatty acid concentrations (Table 2). Consequently when the percentage free tryptophan was plotted against the plasma non-esterified fatty acid concentration (Fig. 3) there was no clear correlation between them. These results show that in pathological states it may not always be possible to forecast changes in the concentration of free plasma tryptophan from those in the concentration of plasma non-esterified fatty acids. Decreased binding of tryptophan to albumin in the injured rat may be due to competition from other compounds entering the plasma in abnormal quantities. Increased binding in the presence of high concentrations of non-esterified fatty acids is more difficult to explain. The variability in the concentration of free tryptophan at the same plasma non-esterified fatty acid concentrations and the differences in the amount of binding *in vitro* and *in vivo* suggest that the transport of tryptophan in blood is not yet completely understood.

Plasma tryptophan turnover

The rate of disappearance of [14 C]tryptophan from the plasma after its intravenous injection as a single dose was determined in four groups: (1) uninjured post-absorptive rats; (2) uninjured 24h-starved rats at the same time of day (14:00h); (3) post-absorptive rats during the last 40 min of 4h bilateral hind-limb ischaemia; (4) post-absorptive rats about 1.5h after such a period of limb ischaemia. The results are shown in Table 3. The rate of disappearance in uninjured post-absorptive rats was similar to that found by Tyce *et al.* (1967) using intravenous doses of non-radioactive tryptophan.

The total radioactivity of the plasma was measured in addition to that of the isolated tryptophan. Up to 17 min after the injection of [14 C]tryptophan practically all the radioactivity was due to the tracer. After 40 min 41.5 $\pm$ 2.9% of the plasma radioactivity was in the form of tryptophan in the uninjured post-absorptive rats. The percentages in the other

groups were as follows: starved uninjured rats 53.2 ± 1.9 ($P < 0.01$); post-absorptive rats at the end of limb ischaemia 31.3 ± 2.8 ($P < 0.05$); 1.5 h later 37.4 ± 1.4 . The P values refer to comparisons with the uninjured post-absorptive rats. No attempt was made to identify the non-tryptophan components.

Irreversible disposal rates (R), calculated from the areas to infinity under specific radioactivity-time curves, are shown in Table 4 with the concentrations of total and free plasma tryptophan in the same rats. The free tryptophan concentrations in both groups of injured rats in Table 4 were significantly less ($P < 0.01$) than those in Table 2 although bearing the same relationship to one another, i.e. the concentration was significantly higher ($P < 0.01$) after removal of the tourniquets than before. The reason for this difference was not found.

Despite large differences in the plasma concentrations of free tryptophan in post-absorptive and starved uninjured rats and rats at the end of 4 h bilateral hind-limb ischaemia, R did not vary significantly. Although the mean values of R appeared to be positively correlated with the mean plasma free tryptophan concentrations with the few degrees of freedom available, the correlation coefficient (0.931) was not significant. Hence, the rate of irreversible disposal of tryptophan is not greatly affected by changes in the concentration of free tryptophan in the plasma. A full interpretation of R is not possible since we cannot describe the anatomical limits of the pool from which this tryptophan is lost. It presumably includes the plasma and interstitial fluid, but in some tissues it could extend intracellularly to the first enzyme in the metabolism of tryptophan.

The initial fall in the specific radioactivity of the total plasma tryptophan is mainly due to the distribution of the tracer in the body spaces. This was faster in the starved rats (Table 3) where the specific radioactivity was significantly lower than in the post-absorptive rats at 2 ($P < 0.01$) and 7 ($P < 0.05$) min after the injection of [^{14}C]tryptophan. This can be attributed to the increase in the exchangeable pool of free plasma tryptophan (Table 4). These results, with the lack of response of R to an increase in the free plasma tryptophan concentration, emphasize the importance of changes in the distribution of tryptophan within the body in explaining the effects of drugs, etc., which affect, directly or indirectly, the binding of tryptophan to albumin.

In rats 1.5 h after removal of the tourniquets [^{14}C]tryptophan disappeared from the plasma more slowly than in the other groups (Table 3) and R was significantly decreased (Table 4). At this point in the response to injury the changes could not be explained by poor tissue perfusion or tissue hypoxia (Stoner, 1970; Threlfall, 1970; Barton, 1971). The fall in core temperature which occurs when the tourniquets are

removed (Stoner, 1970) could be a contributing factor. The core temperature of these rats would be about 35°C at the time of the experiments. It would have been technically difficult to try to assess the significance of this factor as raising the ambient temperature to the thermoneutral zone to prevent this fall greatly shortens the survival time (Haist, 1960), and this would make it difficult to obtain a 40 min run in steady-state conditions. A further reason for the decrease in R could be increased competition for entry into the cells by other neutral amino acids (Fernstrom & Wurtman, 1972), since proteolysis in the muscles of the hind limb is increased by ischaemia so that when the tourniquets are removed amino acids enter the circulation and increase the total α -amino N concentration in the plasma (Hoar & Haist, 1944; Stoner & Green, 1948).

In summary, the main effect of changes in plasma non-esterified fatty acid concentration is to alter the distribution of tryptophan in the body rather than to alter its total rate of metabolism. Changes in the plasma free tryptophan concentration could still affect the rate of disposal by a minor pathway.

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References

- Aldridge, W. N. (1957) *Biochem. J.* **67**, 423–431
- Barton, R. N. (1971) *Clin. Sci.* **40**, 463–477
- Bender, D. A. & Bamji, A. N. (1974) *J. Neurochem.* **22**, 805–809
- Bruce, H. M. & Parkes, A. S. (1956) *J. Anim. Tech. Ass.* **7**, 54
- Corney, P. L. & Heath, D. F. (1970) *J. Appl. Physiol.* **28**, 672–674
- Cunningham, V. J. (1973) *Biochem. J.* **136**, 545–550
- Cunningham, V. J., Hay, L. & Stoner, H. B. (1975) *Biochem. J.* **146**, 653–658
- Denkla, W. D. & Dewey, H. K. (1967) *J. Lab. Clin. Med.* **69**, 160–169
- Fernstrom, J. D. & Wurtman, R. J. (1971) *Science* **174**, 1023–1025
- Fernstrom, J. D. & Wurtman, R. J. (1972) *Science* **178**, 414–416
- Fisher, R. A. (1934) *Statistical Methods for Research Workers*, 5th edn., pp. 121, 158, Oliver and Boyd, London
- Gaitonde, M. K. (1974) *Biochem. J.* **139**, 625–631
- Haist, R. E. (1960) in *The Biochemical Response to Injury* (Stoner, H. B. & Threlfall, C. J., ed.), pp. 313–340, Blackwell, Oxford
- Heath, D. F. & Barton, R. N. (1973) *Biochem. J.* **136**, 503–518
- Heath, D. F. & Stoner, H. B. (1968) *Brit. J. Exp. Pathol.* **49**, 160–169
- Heath, D. F. & Threlfall, C. J. (1968) *Biochem. J.* **110**, 337–362
- Hoar, W. S. & Haist, R. E. (1944) *J. Biol. Chem.* **154**, 331–338

- Knott, P. J. & Curzon, G. (1972) *Nature (London)* **239**, 452-453
- Lehman, J. (1971) *Scand. J. Clin. Lab. Invest.* **28**, 49-55
- Robinson, H. W. & Hodgen, C. G. (1940) *J. Biol. Chem.* **135**, 707-731
- Rosenthal, S. M. (1943) *Pub. Health Rep.* **58**, 1429-1436
- Shipley, R. A., Chudzik, E. B., Gibbons, A. P., Jongedyk, K. & Brummond, D. O. (1967) *Amer. J. Physiol.* **213**, 1149-1158
- Smith, H. G. & Lakatos, C. (1971) *J. Pharm. Pharmacol.* **23**, 180-189
- Stoner, H. B. (1962) *Brit. J. Exp. Pathol.* **43**, 556-563
- Stoner, H. B. (1970) in *Energy Metabolism in Trauma: Ciba Found. Symp.* (Porter, R. & Knight, J., eds.), pp. 1-22, Churchill, London
- Stoner, H. B. & Elson, P. M. (1971) *J. Neurochem.* **18**, 1837-1846
- Stoner, H. B. & Green, H. N. (1948) *Brit. J. Exp. Pathol.* **29**, 121-132
- Stoner, H. B. & Little, R. A. (1969) *Brit. J. Exp. Pathol.* **50**, 107-124
- Stoner, H. B. & Matthews, J. (1967) *Brit. J. Exp. Pathol.* **48**, 58-65
- Tagliamonte, A., Biggio, G., Vargiu, L. & Gessa, G. L. (1973) *Life Sci.* **12**, part II, 277-287
- Threlfall, C. J. (1970) in *Energy Metabolism in Trauma: Ciba Found. Symp.* (Porter, R. & Knight, J., eds.), pp. 127-144, Churchill, London
- Threlfall, C. J. & Stoner, H. B. (1954) *Quart. J. Exp. Physiol.* **39**, 1-9
- Tyce, G. M., Flock, E. V., Owen, C. A., Stobie, G. H. C. & David, C. (1967) *Biochem. Pharmacol.* **16**, 979-992
- Umbreit, W. W., Burris, R. H. & Stauffer, J. F. (1957) *Manometric Techniques*, p. 168, Burgess, Minneapolis