Uptake and Metabolism of Tryptophan by the Isolated Perfused Guinea-Pig Mammary Gland

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Tryptophan uptake by the isolated perfused lactating guinea-pig mammary gland was $46.5 \pm 4.6 \mu\text{g/h}$ per g. Results of absorption studies and the use of [methylene- ^{14}C]tryptophan suggest that tryptophan is one of the group of amino acids that are transferred almost quantitatively from blood plasma to milk protein.

Previous studies of the mammary absorption of amino acids in relation to milk-protein secretion (see Mepham, 1976) have excluded tryptophan because of difficulties associated with its accurate determination by the chromatographic procedure used for other amino acids. In attempting to define the extent to which the availability of different amino acids to the mammary gland is rate-limiting for protein synthesis this has been a serious omission. Not only is tryptophan an essential amino acid, but it is also unique in showing an appreciable extent of binding to plasma proteins, a characteristic that could impose a further rate-limiting step in the sequence of events involved in its cellular utilization.

This paper reports data on the uptake of tryptophan by the isolated perfused guinea-pig mammary gland (Davis & Mepham, 1974). In two experiments [methylene- ^{14}C]tryptophan was added to the perfusate to assess the extent of its metabolism within the gland.

Experimental

Blood sampling

Guinea pigs (approx. 700 g) of the Dunkin-Hartley strain (from the Joint Animal Breeding Unit, Sutton Bonington, Loughborough, Leics., U.K.) were anaesthetized and heparin-treated by intraperitoneal injections of sodium pentobarbitone (35 mg/kg body wt.; Nembutal from Abbott Laboratories, Sittingbourne, Kent, U.K.) and heparin (500 i.u.; from Boots Co., Nottingham, U.K.) respectively. Blood was obtained by cardiac puncture and centrifuged at 1500 g for 30 min at 4°C. The plasma was removed and stored at -20°C until required for analysis. All blood samples were taken between 10:00 and 12:00 h to minimize the possible

effects of diurnal variation in concentration (see Wurtman, 1970).

Perfusion experiments

The method of Davis & Mepham (1974) was used; 14 perfusions were performed with glands from animals at peak lactation (5–8 days *post partum*). Instead of the arteriovenous-difference method used previously (Davis & Mepham, 1974, 1976; Mepham *et al.*, 1976), a different procedure, the 'serial sampling' method, was used for the measurement of substrate uptake by the gland. The latter method requires the sampling of arterial perfusate only, and a knowledge of the rate of substrate infusion. Thus uptake of a substrate

$$= \frac{(C_{t_0} \times V_{t_0}) - (C_t \times V_t) + Q_{t-t_0}}{t - t_0}$$

where C is perfusate concn. of substrate, V is plasma volume, t_0 is beginning of period of uptake measurement and t is the end of period, Q_{t-t_0} is weight of substrate infused into the perfusate in the period t_0 to t . Uptake so determined is thus the mean for a period, in contrast with the arteriovenous-difference method, which gives an estimate of uptake at a particular time. The serial sampling method gives results that do not differ significantly from those obtained with the arteriovenous-difference method, but show a smaller degree of variation about the mean (Davis, 1974). Results were calculated by using the ICL 1906 computer at the Cripps Computing Centre, University of Nottingham. Uptake values are expressed as $\mu\text{g/h}$ per g of tissue.

Hydrolysis of tissue protein

The method of Spies (1967) was used. This involves use of the enzyme Pronase (ex *Streptomyces griseus*; from Koch-Light Laboratories, Colnbrook, Bucks., U.K.). Samples of gland tissue (0.1 g) were incubated

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with 10ml of Pronase solution (1%, w/w, in 0.1M-sodium phosphate buffer, pH7.5) at 40°C for 24h. The suspensions were centrifuged at 1500g for 10min and the supernatants stored at -20°C until analysis.

Assay of tryptophan

Tryptophan was assayed by the spectrofluorimetric method of Denckla & Dewey (1967), as modified by Lehmann (1971). Free (i.e. non-protein-bound) tryptophan was measured by prior centrifugation of 0.5ml samples in membrane cones (Centriflo 224-CF 50; from Amicon Corp., Lexington, MA, U.S.A.) for 5min at 1500g. Since the method of Denckla & Dewey (1967) assays total tryptophan, protein-bound tryptophan was derived by difference. The ratio bound/free was unaffected by centrifugation for periods of 5–30min.

Radioactivity assays

L-[methylene-¹⁴C]Tryptophan (>45mCi/mmol) was obtained from The Radiochemical Centre, Amersham, Bucks., U.K.; the specific radioactivities of ninhydrin-positive compounds were measured with a continuous-flow scintillation counter linked to an automatic amino acid analyser, as described by Davis & Mepham (1976). Radioactivity in CO₂ evolved during perfusions was measured as described by Davis & Mepham (1976), except that the time-course of ¹⁴CO₂ evolution was estimated by sampling from the NaOH traps at 5min intervals after radioisotope administration.

Results and Discussion

Tryptophan concentration in guinea-pig blood plasma

In perfusion experiments, attempts are made to infuse substrates into the perfusate at rates sufficient to maintain physiological concentrations in the plasma. Very limited data were available on tryptophan concentrations in guinea pigs, namely 6.2 µg/ml, of which 78% was protein-bound (Fuller & Roush, 1973; data from a single animal of unspecified sex,

age and dietary status). Thus it was first necessary to determine the blood concentrations in lactating animals. For comparative purposes, analyses were also performed on blood from non-lactating females and males. The data (Table 1) show that the concentrations were in all cases greater than that quoted by Fuller & Roush (1973).

Statistical analysis of the data by using Student's *t* test shows no significant difference between total tryptophan concentrations in plasma of non-lactating females and males, but a highly significant difference between non-lactating and lactating females ($P < 0.001$) and between males and lactating females ($P < 0.01$). The percentage of protein-bound tryptophan did not differ significantly between the three groups of animals.

Comparable data for other amino acids are not available, but the decrease in plasma amino acid concentrations during lactation compared with the non-lactating state has been reported in other species (e.g. cows; Halfpenny *et al.*, 1969).

Mean tryptophan concentrations in the perfusate plasma ranged from 5 to 50 µg/ml in the 14 experiments. In six experiments, measurements were made of the concentration of free tryptophan. There was no significant difference between the percentage determined, 6.2 ± 0.2 (S.E.M.)%, and those measured *in vivo*.

Tryptophan uptake during perfusions

In six of the 14 perfusion experiments, the mean tryptophan concentrations were in the physiological range, 5.1–18.2 µg/ml. The mean mammary uptake for these six perfusions was 46.5 ± 3.2 µg/h per g. The 'percentage extraction' of tryptophan from the plasma, i.e. the proportion of the tryptophan supplied to the gland in the perfusate that was absorbed by the gland, showed a variation between experiments of 4.2–19.6%. There was a significant inverse linear relationship between the percentage extraction and the plasma concentration ($P < 0.01$). Since free tryptophan accounted for about 6% of the total, in those perfusions in which the percentage extraction was greater than this value rapid dissociation of protein-bound tryptophan is indicated.

In the remaining eight experiments the perfusate concentrations were greater than physiological values (20–50 µg/ml). Nevertheless the mean mammary uptake in these experiments (41.7 ± 3.2 µg/h per g) was not significantly different from that in perfusions in which the tryptophan concentrations were normal. Moreover, linear regression analysis of data from all 14 perfusions revealed no significant correlation between perfusate tryptophan concentration and mammary uptake. Thus, as for other amino acids (Davis, 1974; Davis & Mepham, 1974), tryptophan uptake was unaffected by variation in arterial concentrations over a wide range.

Table 1. Tryptophan concentrations in guinea-pig blood plasma

The values in parentheses represent the numbers of values used to calculate the means.

Animal	Total tryptophan concentration (µg/ml $\pm$ S.E.M.)	Free tryptophan (% $\pm$ S.E.M.)
Males	16.12 ± 1.09 (5)	6.48 ± 0.53
Non-lactating females	18.00 ± 1.09 (5)	7.12 ± 0.82
Lactating females	9.53 ± 0.90 (9)	7.32 ± 0.52

Davis (1974) reported a highly significant correlation between the uptakes of four essential amino acids, namely methionine, histidine, phenylalanine and tyrosine, and their respective concentrations in guinea-pig milk protein. On this basis, an index of milk-protein synthesis has been derived, i.e. the 'predicted rate of protein synthesis' (PRPS), which is based on the uptakes of these four amino acids and their concentrations in milk protein (Davis, 1974). To calculate the PRPS for tryptophan, the value of 2.26 g/100 ml for the tryptophan content of guinea-pig casein (C. B. E. Whitly, unpublished work; measured in this laboratory by using Pronase hydrolysis) was used, since casein accounts for 90% of total milk protein at this stage of lactation (Mephram & Beck, 1973). On this basis, the mean PRPS for tryptophan in the 14 experiments was 1.93 ± 0.11 mg of milk protein/h per g. This value compares favourably with the mean PRPS of the four amino acids quoted for the same experiments, i.e. 1.96 ± 0.19 (S.E.M.) mg/h per g.

Metabolism of [methylene- ^{14}C]tryptophan

In Expts. 7 and 8, in which the mean tryptophan concentrations were 23.5 and 23.9 $\mu\text{g/ml}$, two single-pulse injections of [methylene- ^{14}C]tryptophan were made into the perfusate reservoir ($2 \times 2.5 \mu\text{Ci}$ in each perfusion), the second injection being administered 1 h after the first.

During the perfusions, evolved CO_2 was trapped in NaOH and subsequently assayed for radioactivity. At the end of the experiments radioactivity was assayed in the perfusate and gland tissue. Since milk was not collected during these experiments radioactivity incorporated into milk protein is included in the data for tissue analysis. The distribution of radioactivity at the end of the experiments is shown in Table 2.

In Expt. 7, 50 mg of spermidine was added to the perfusate at the same time as the second radioisotope injection, since the effect of spermidine on tryptophan metabolism was also being investigated (Peters & Mephram, 1976).

In both perfusions, over 95% of the radioactivity recovered was in tryptophan present in the gland tissue. Radioactivity was detected in only two other compounds, namely CO_2 and an unidentified compound in the plasma, which was eluted from the amino acid analyser column just before the ammonia peak. In both experiments about 3% of the injected radioactivity was recovered in this unidentified compound. The maximal radioactivity in the perfusate was reached within 30 min in perfusion 7 and within 60 min in perfusion 8, and the subsequent constancy of its specific radioactivity indicates that it was not re-utilized by the gland.

Recovery of label in CO_2 in Expt. 8 (0.17%) and before spermidine injection in Expt. 7 (0.05%) was very slight, but the administration of spermidine increased the extent of incorporation of label into CO_2 by 15-fold. We have previously reported that spermidine induces a marked stimulation of tryptophan uptake by the perfused gland (Peters & Mephram, 1976), and it seems reasonable to suppose that the elevated intracellular concentration of tryptophan was the cause of its increased catabolism. The time-course of $^{14}\text{CO}_2$ evolution showed a very rapid rate of tryptophan catabolism, the maximum specific radioactivity being reached within 10 min of [^{14}C]tryptophan injection and having fallen to negligible values within 30 min.

Categorization of tryptophan

The above results indicate that tryptophan is a member of the category of essential amino acids, which also includes methionine, histidine, phenyl-

Table 2. Details of [methylene- ^{14}C]tryptophan experiments

[methylene- ^{14}C]Tryptophan ($2.5 \mu\text{Ci}$) was injected twice in each experiment. At the end of both experiments, radioactivity was detected in tryptophan, an unidentified compound in the perfusate and in CO_2 (for details see the text).

	Expt. no. ... 7		8	
Radioactivity added (μCi)	2×2.5		2×2.5	
Time added (min)	90		95	
	150		155	
Duration of expt. (min)	245		245	
Total recovered radioactivity (μCi)	4.14 (82.8%)		4.40 (87.9%)	
Distribution of radioactivity	(μCi)	(% of recovered radioactivity)	(μCi)	(% of recovered radioactivity)
Perfusate				
Tryptophan	2.09	50.80	1.62	36.90
Unidentified compound	0.12	2.90	0.17	3.86
Gland tryptophan	1.90	45.90	2.60	59.20
CO_2 before second injection	0.0022	0.05	0.0050	0.11
at end of experiment	0.03	0.72	0.0086	0.17

alanine and tyrosine, the mammary uptakes of which are apparently used almost exclusively for milk-protein synthesis. The suggestion that the supply of this category of essential amino acids may be limiting for milk-protein synthesis (Mephram, 1976) must consequently be modified to include tryptophan.

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