Metabolism of L-[U-¹⁴C]Valine, L-[U-¹⁴C]Leucine, L-[U-¹⁴C]Histidine and L-[U-¹⁴C]Phenylalanine by the Isolated Perfused Lactating Guinea-Pig Mammary Gland

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1. The incorporation of L-[U-¹⁴C]valine, L-[U-¹⁴C]leucine, L-[U-¹⁴C]histidine and L-[U-¹⁴C]phenylalanine into casein secreted during perfusion of isolated guinea-pig mammary glands was demonstrated. 2. The extent of incorporation of label into casein residues was consistent with their being derived from free amino acids of the perfusate plasma. 3. The mean transit time of the amino acids from perfusate into secreted casein was approx. 100 min. 4. Whereas radioactive histidine and phenylalanine were incorporated solely into milk protein, radioactivity from [U-¹⁴C]valine was also transferred to CO₂ and to an unidentified plasma component, and from [U-¹⁴C]leucine to plasma glutamic acid. 5. Evidence from experiments with [U-¹⁴C]phenylalanine suggests that, as in rats, but in contrast with ruminant species, guinea-pig mammary tissue does not possess phenylalanine hydroxylase activity. 6. The results are discussed in relation to the possible role of essential amino acid catabolism in the control of milk-protein synthesis.

Little is known of the ability of lactating mammary tissue to metabolize essential amino acids along pathways other than that of protein synthesis. By contrast, the synthesis of non-essential amino acids, which are subsequently incorporated into milk proteins, has been investigated in a number of laboratories (see Mephram, 1971).

Data obtained from studies on the balance between amino acid uptake by the mammary gland and output of the corresponding residues in milk protein (see Mephram, 1971, 1976; Bickerstaffe *et al.*, 1974; Derrig *et al.*, 1974) have indicated that arginine, valine, leucine, isoleucine, threonine, histidine and lysine are absorbed by goat and cow mammary tissue in amounts that are frequently in excess of their output in milk protein, an observation that suggests that these amino acids are metabolized along routes other than direct incorporation into protein. The utilization of arginine as a precursor of proline in isolated perfused goat and cow glands (see Mephram, 1971), and of spermidine in cultured mouse mammary explants (Oka & Perry, 1974), has been established. Moreover, the ability of bovine mammary tissue to catabolize valine (Derrig *et al.*, 1973) and sheep mammary-tissue threonine (Verbeke *et al.*, 1972) has also been demonstrated. Sheep mammary tissue possesses phenylalanine hydroxylase activity (Verbeke *et al.*, 1972), a property also exhibited by

bovine, but not rat, mammary cells cultured *in vitro* (Schingoethe *et al.*, 1967).

Experiments with the isolated perfused guinea-pig mammary gland (Davis, 1974; Davis & Mephram, 1974) have indicated that essential amino acids fall into two groups, namely those which show a fairly uniform plasma uptake/milk output ratio and those that show a larger and more variable ratio. It was the purpose of the present study to investigate the mammary metabolism of essential amino acids to (i) test the hypothesis that the uptake of the former group (exemplified by phenylalanine and histidine) may be used as an index of the rate of milk-protein synthesis, and (ii) assess the extent of utilization of amino acids in the latter group (exemplified by valine and leucine) for roles other than direct incorporation into protein.

Experimental

Radiochemicals

L-[U-¹⁴C]Valine, L-[U-¹⁴C]leucine, L-[U-¹⁴C]histidine and L-[U-¹⁴C]phenylalanine (all 25 mCi/mmol) were from The Radiochemical Centre, Amersham, Bucks., U.K.

Perfusion technique

The method, origin and status of the animals used were essentially those described by Davis & Mephram (1974). Eight experiments were performed.

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In each case about 5 μ Ci of the radioactive amino acid to be examined were added to the perfusate in 0.5 ml of 0.9% NaCl, after 50–100 min of perfusion.

Two experiments were carried out with each radioactive amino acid. Arterial and venous perfusates were sampled at approx. 20 min intervals and milk was collected via a teat cannula at 30–60 min intervals after injections of 1–5 munits of oxytocin (Pitocin; Parke-Davis Co., Hounslow, London, U.K.).

Analytical methods

Amino acid analysis. Plasma samples were prepared for amino acid analysis as described previously (Davis & Mepham, 1974). Amino acid analysis was carried out with an automatic amino acid analyser of the type described by Spackman *et al.* (1958), manufactured by Locarte Co., London W12 9RT, U.K., by using a single-column system with lithium citrate buffers.

Radioactivity assays. (a) Determination of the specific radioactivity of 14 C-labelled amino acids in casein hydrolysates and perfusate plasma samples was done by using a Coruflo scintillation counter (Tracerlab S.A., Mechelen, Belgium). The eluate from the chromatograph column of the amino acid analyser was passed directly through a U-shaped glass flow cell (1.5 ml capacity) packed with beads (100 μ m diam.) of plastic scintillant (type NE102A; Nuclear Enterprises, Edinburgh, Scotland, U.K.), before being returned to the analyser for the routine analysis of amino acids. The flow cell was situated between two photomultiplier tubes whose output was fed to a rate-meter. In order to obtain a continuous record of radioactivity present in the eluate the output from the rate-meter was shunted with a 27 k Ω resistance to give 5 mV at full-scale deflection, and this output was then fed to the chart recorder of the amino acid analyser, providing a radioactivity trace running parallel to the analytical (mass) trace.

The absolute efficiency of the scintillation counter was not determined. However, the lowest amount of radioactivity (14 C) capable of quantification by triangulation was equivalent to about 1000 d.p.m. The background count rate varied from 20 to 30 c.p.m. The scintillation counter was calibrated by adding a standard quantity of radioactive amino acid (about 40 nCi) to the routine amino acid standard solution. The use of the flow scintillation counter did not interfere significantly with amino acid resolution.

(b) Residual radioactivity in the glands after perfusion was determined by oxidation of 100 mg samples of wet tissue in a Packard Tri-Carb sample oxidizer (type 306; Packard Instrument Co., Downers Grove, IL 60515, U.S.A.).

(c) Oxidation of [14 C]valine during two perfusions was measured by trapping evolved 14 CO₂ by drawing the gas outflow from the oxygenator through two

wash bottles in series, each containing 250 ml of 1 M-NaOH. At the end of each experiment 1 ml samples were transferred to scintillation vials containing 2.5 ml of water and 15 ml of scintillation fluid (0.4 g of 2,5-diphenyloxazole/100 ml of toluene + 50 ml of Triton X-100). Vials were then counted for radioactivity in an automatic liquid-scintillation counter (Corumatic 200; Tracerlab); counting efficiency was determined by the external-standard channels-ratio method with 226 Ra as the external standard source.

Casein analysis. Casein in milk samples obtained during perfusion was isolated by the method of Rowland (1938). The casein pellet obtained was dissolved in 15 ml of 6 M-HCl/formic acid (1:1, v/v) and hydrolysed by refluxing for 24 h. The hydrolysate was evaporated to dryness in a rotary evaporator, washed with water and re-evaporated three times to remove excess of HCl, and finally dissolved in 2 ml of lithium citrate buffer for analysis as described above.

Calculation of precursor-product relationships. The proportion of product (i.e. amino acid residue in casein) derived from each 14 C-labelled precursor was measured by comparison of the areas under the specific-radioactivity decay curve of the amino acid in the perfusate plasma and the area defined by the corresponding casein specific-radioactivity incorporation curve (see Zilversmit *et al.*, 1943; Barry, 1961). Areas were measured by weighing tracings of each curve. For the casein-incorporation curve each graph was extrapolated to zero radioactivity at 400 min after the addition of radioactivity to the perfusate. This time was derived from the most extensively defined incorporation curve (see Fig. 1). With this procedure, exact equivalence between the plasma specific-radioactivity and casein incorporation specific-radioactivity curves indicates that all the amino acid residues in the secreted casein were derived from the same free amino acid in the plasma. However, a lower casein incorporation specific-radioactivity curve may also be produced by the presence of pre-formed milk in the gland, and is not necessarily indicative of an alternative precursor. Indeed, since all the amino acids studied were in the essential category, this explanation may be discounted.

Results

Viability of the perfused gland preparation

The evaluation of the perfusion technique has been described previously (Davis & Mepham, 1974). Relevant details of the eight experiments are shown in Table 1. Most experiments were of 4–5 h duration and milk was collected on all but two occasions (where problems were encountered with positioning of the teat cannula within the teat sinus). Milk yield during the other six perfusions varied from 0.44 to 0.99 g/h (Table 1).

Table 1. *Details of perfusion experiments*

Perfusion experiments were conducted as described by Davis & Mephram (1974). A single-pulse injection of radioisotope was given after 50–100 min of perfusion. For details of procedures see the text. Mean milk yield was calculated excluding the first milk sample, which contained pre-formed milk. n.d., Not detected.

Expt. no.	Tracer amino acid	Duration* of expt. (min)	Gland wet wt. (g)	Mean perfusate flow rate (ml/min)	Mean milk yield (g/h)	Substrate infusion rate (ml/100min)
1	L-[U- ¹⁴ C]Valine	278 (200)	12.3	12.8	0.65	7.3
2	L-[U- ¹⁴ C]Valine	291 (173)	12.2	23.3	0.85	7.8
3	L-[U- ¹⁴ C]Leucine	275 (178)	15.6	17.5	0.49	8.7
4	L-[U- ¹⁴ C]Leucine	131 (80)	12.3	19.4	n.d.	8.8
5	L-[U- ¹⁴ C]Histidine	285 (205)	13.7	16.7	0.44	8.5
6	L-[U- ¹⁴ C]Histidine	279 (180)	12.8	24.5	0.82	7.8
7	L-[U- ¹⁴ C]Phenylalanine	351 (274)	12.4	19.1	0.99	8.6
8	L-[U- ¹⁴ C]Phenylalanine	264 (181)	12.3	14.6	n.d.	7.3

* Numbers in parentheses indicate the duration of perfusion after the addition of radioisotope to the perfusate.

Fig. 1. *Incorporation of L-[U-¹⁴C]phenylalanine into casein secreted during Expt. 7*

L-[U-¹⁴C]Phenylalanine (4μCi) was added to the perfusate after 77 min. Sp. radioactivity of phenylalanine in plasma (○) and in casein (■). The histogram shows the rate of milk secretion during the perfusion.

The uptake of glucose, amino acids and acetate during perfusion experiments has been reported elsewhere (Davis & Mephram, 1974; Mephram *et al.*, 1976). The correlation of amino acid uptake with the rate of infusion of exogenous substrates has been confirmed with more extensive data now available. However, the possibility of tryptophan being a causal factor in this relationship, as suggested by Davis & Mephram (1974), has not been confirmed (Davis, 1974).

Barry (1961) showed that in experiments in which lactating goats received intravenous injections of ¹⁴C-labelled essential amino acids, 1.5–2 h elapsed between administration of the label and the attainment of maximum specific radioactivity in the corresponding residues of the secreted milk protein. Results for the perfused guinea-pig gland show a

comparable transit time, i.e. approx. 100 min in each experiment. The time-course of radioactive labelling in Expt. 7 is illustrated in Fig. 1, and the data for the other experiments are given in Table 2. There was no significant difference between the arterial and venous plasma specific radioactivity of the injected amino acid in any experiment.

Measurement of total recovery of administered radioisotope were precluded by the fact that insufficiently large milk samples were available to measure recovery in milk protein, and also by the absence of data on incorporation of label into CO₂ in all but two experiments. However, in the experiments with [¹⁴C]valine, in which CO₂ was collected, it has been calculated that 30.4 and 38.4%, in Expts. 1 and 2 respectively, of the injected label was present in milk.

Table 2. *Specific radioactivity of amino acids in plasma and casein during perfusion*

Numbers in parentheses for plasma samples indicate time of sampling (min) and for casein samples the median time during a milk-collection period.

Perfusate plasma samples

Expt. no. ...	Specific radioactivity ($\mu\text{Ci}/\text{mg}$)						
	1	2	3	4	5	6	8
	2.59 (78)	2.75 (108)	4.77 (97)	2.08 (51)	12.80 (80)	6.26 (99)	8.30 (84)
	1.97 (86)	1.25 (120)	1.88 (113)	1.69 (59)	8.60 (86)	2.58 (109)	4.56 (94)
	1.61 (94)	0.30 (169)	0.91 (126)	0.91 (75)	3.23 (105)	1.46 (131)	3.57 (102)
	0.84 (110)	0.13 (190)	0.23 (167)	0.41 (90)	1.05 (126)	0.58 (158)	1.67 (118)
	0.36 (134)	0.08 (210)	0.12 (201)	0.15 (110)	0.49 (146)	0.42 (180)	0.80 (143)
	0.18 (158)	0.08 (228)		0.10 (131)	0.20 (181)	0.31 (199)	0.45 (167)
	0.10 (184)	0.02 (247)			0.14 (200)	0.19 (216)	0.30 (192)
	0.07 (209)				0.10 (229)	0.14 (237)	0.23 (217)
	0.03 (256)					0.11 (256)	0.20 (235)
							0.16 (264)

Casein samples

Expt. no. ...	Specific radioactivity ($\mu\text{Ci}/\text{mg}$)						
	1	2	3	4*	5	6	8*
	0.26 (111)	0.05 (128)	0.02 (97)		0.57 (117)	0.08 (117)	
	0.39 (173)	0.33 (175)	0.15 (159)		0.79 (199)	0.52 (163)	
	0.40 (220)	0.41 (216)	0.19 (219)		0.59 (242)	0.76 (205)	
	0.32 (254)	0.31 (254)	0.14 (264)		0.50 (271)	0.67 (243)	
						0.60 (270)	

* No milk was collected in these experiments.

Calculation of radioisotope recovery in casein, by using the amino acid composition of casein quoted by Davis & Mepham (1974) and assuming that the milk casein content was 6.2 g/100 ml of milk [derived from the data of Mepham & Beck (1973) on the composition of guinea-pig milk *in vivo*], gives values of 45.5 and 39.2% respectively. Thus, although the agreement was good in Expt. 2, there was a marked discrepancy in Expt. 1, which is probably attributable to the progressive decline in milk protein concentration observed in some perfusion experiments (Davis, 1974).

Metabolism of L-[U- ^{14}C]valine

In Expts. 1 and 2, 5 μCi of L-[U- ^{14}C]valine was added to the perfusate after 78 and 108 min respectively. The areas under the plasma specific-radioactivity decay curve and casein incorporation curve were equal in Expt. 1, whereas in Expt. 2 the area under the casein incorporation curve was 91% of that under the plasma curve. Thus it appears that most of the valine residues in casein secreted during perfusion were derived from free valine in the perfusate.

In Expts. 1 and 2, 6.0 and 5.6% respectively of the added radioactivity was recovered in CO_2 , and on amino acid analysis a significant incorporation was

Fig. 2. *Incorporation of radioactivity into an unidentified component of plasma during Expts. 1 and 2, in which L-[U- ^{14}C]valine was added to the perfusate*

The graphs show the radioactivity of the unidentified compound, expressed as nCi/ml of plasma, during the two perfusions. ▲, ●, Arterial plasma; △, ○, venous plasma. ▲, △, Expt. 1; ●, ○, Expt. 2.

observed into a compound that was rapidly eluted from the chromatograph column.

The production of isobutyrate and β -hydroxyisobutyrate from valine by lactating bovine mammary

tissue has been reported (Derrig *et al.*, 1973) and it seems likely that the valine metabolite found in the perfusate is one of these compounds, or, possibly, 2-oxoisovalerate. The time-course of the appearance of the metabolite in both experiments is shown in Fig. 2. Its rapid appearance and the subsequent maintenance of a constant specific radioactivity in the perfusate suggest that it was not utilized by the mammary gland after formation, and the marked negative arteriovenous differences of radioactivity on the ascending limb of the output curve indicate its formation by the gland rather than within the perfusate. The accumulation of radioisotope in this compound in both experiments corresponded to about 8% of the added radioactivity.

Metabolism of L-[U-¹⁴C]leucine

Two experiments were carried out with L-[U-¹⁴C]-leucine added to the perfusate. In Expt. 3, 5 μ Ci was injected into the perfusate after 97 min of perfusion. The area of the casein incorporation curve was 82% of the plasma curve. No radioactivity was detected in any other compound in casein or perfusate in this experiment.

In Expt. 4, 5 μ Ci was added to the perfusate after 51 min, but unfortunately no milk was collected for analysis. However, radioactivity was detected in perfusate glutamate residues, and to a lesser extent in glutamine and proline. The appearance of these metabolites in this experiment only may have been related to the efficacy of perfusion, as the gland became oedematous and perfusate flow rate fell after 2 h, whereas in Expt. 3 the gland appeared normal on terminal inspection.

The maximum specific radioactivity of glutamate in the perfusate was 0.06 nCi/ μ g, the initial specific radioactivity of leucine being 2.08 nCi/ μ g. There was a consistent increase in glutamate specific radioactivity across the gland (Table 3), indicative of the formation of glutamate from leucine within the tissue rather than the perfusate.

No assessment was made of the extent of leucine oxidation during perfusion in either experiment, but as leucine can be metabolized to glutamate, and the latter is oxidized by the guinea-pig mammary gland during perfusion (Davis, 1974), it seems likely that leucine carbon can contribute to the output of CO₂ in the guinea pig.

Metabolism of L-[U-¹⁴C]histidine

In Expts. 5 and 6, 5.0 and 3.4 μ Ci respectively of L-[U-¹⁴C]histidine were added to the perfusate. In Expt. 6 the areas under the casein incorporation and plasma specific-radioactivity curves were equal, whereas in Expt. 5 the former area was 83% of the latter. No significant radioactivity was detected in any other compound in the tissue, perfusate, or in casein.

In view of these findings it seems probable that all the histidine residues in casein secreted during perfusion were derived from perfusate histidine, and that no significant catabolism of histidine by guinea-pig mammary tissue occurs.

Metabolism of L-[U-¹⁴C]phenylalanine

In Expts. 7 and 8 L-[U-¹⁴C]phenylalanine was added to the perfusate after 77 min (4.0 μ Ci) and 83 min (5.0 μ Ci) respectively. Milk was obtained in Expt. 7 only, and the plasma specific-radioactivity decay curve and casein specific-radioactivity incorporation curve are shown in Fig. 1. The areas under the two curves were in poor agreement, the casein incorporation curve having only 56% of the area of the plasma curve, but radioactivity was not detected in any compound other than phenylalanine in casein, mammary tissue or perfusate. The most probable explanation of the poor agreement between the curve areas is the presence of large amounts of pre-formed milk in the gland, since the milk yield was higher in this than in any other perfusion reported here, although the gland weights were similar (Table 1).

Table 3. Plasma glutamate specific radioactivity during Expt. 4, in which L-[U-¹⁴C]leucine was added to the perfusate

The specific radioactivity of glutamic acid was determined in arterial and venous perfusate samples taken simultaneously at the times specified.

Time of sampling after [¹⁴ C]leucine injection (min)	Perfusion sampling time (min)	Source of plasma	Sp. radioactivity of glutamate (nCi/mg)
24	75	Arterial	36
		Venous	49
39	90	Arterial	50
		Venous	60
59	110	Arterial	46
		Venous	53
80	131	Arterial	40
		Venous	45

Fig. 3. Expt. 8: effect on the mammary uptake of five amino acids produced by depletion of the perfusate tyrosine, and its re-supplementation

(a) Concentration of tyrosine ($\mu\text{g/ml}$) in the perfusate plasma. Tyrosine was omitted from the infusate solution for the period 112–212 min of perfusion. The rates of mammary uptake from the plasma of tyrosine (b), methionine (c), phenylalanine (d), histidine (e) (no data for 90–120 min) and leucine (f) are shown.

During Expt. 8 the availability of tyrosine to the gland was decreased (by omitting it from the substrate infusate), in an attempt to induce the formation of tyrosine from phenylalanine, since phenylalanine hydroxylase activity has been reported in some

species. There was a marked distinction between the effects of decreased tyrosine availability on the uptakes of phenylalanine, methionine and histidine on the one hand and of leucine, isoleucine, valine and lysine on the other. Whereas the uptakes of the former

group were decreased by over 80% during the period of tyrosine depletion, those of the latter group were decreased by less than 25% (Fig. 3; Davis, 1974). Resupplementation with tyrosine resulted in restoration of the uptakes of all these amino acids to approximately their initial values within 40 min. Examination of tissue protein hydrolysates and free tissue amino acids in the gland on termination of the experiment showed that there had been no transfer of radioactivity to tyrosine.

Discussion

On the basis of previous experiments with the isolated perfused guinea-pig mammary gland, it was proposed that the essential amino acids could be classified into two groups according to uptake, namely: (i) those whose uptake was in excess of requirements for milk protein synthesis; (ii) those whose uptake was indicative of the rate of milk protein synthesis. For the latter group, in the absence of extensive data for methionine and histidine, the uptake values of phenylalanine, tyrosine, arginine and leucine were used by Davis & Mephram (1974) to predict protein-synthesis rates during perfusion. With the more extensive data now available, the rate of protein synthesis has been quantified on the basis of methionine, tyrosine, phenylalanine and histidine uptake values (Davis, 1974) and found to be 39 ± 2.1 mg/h. However, the uptake values of the other essential amino acids studied, with the exception of arginine (i.e. threonine, valine, isoleucine, leucine and lysine), were at least 30% in excess of that required to support milk protein synthesis. Unlike the goat and sow, where arginine uptake is 2–3 times in excess of that required for milk protein synthesis (Mephram, 1971), the mammary uptake of arginine in the guinea pig is apparently not excessive, although arginase activity has been detected in one experiment where L-[U- 14 C]arginine was added to the perfusate (Davis, 1974). However, it has not been ascertained whether this activity was of perfusate or of mammary origin.

The data obtained from the experiments described here are in accord with the hypothesis proposed above. The guinea-pig mammary gland during perfusion possesses a significant catabolic potential for valine and to a lesser extent for leucine, but there were no indications that the mammary utilization of phenylalanine and histidine was along any metabolic route other than that of milk protein synthesis.

Our findings are largely in agreement with known catabolic pathways for amino acids in mammary and non-mammary tissues. Oxidation of valine by the isolated perfused goat gland has been observed (Mephram, 1971), as has the incorporation of carbon from valine into β -hydroxyisobutyrate, isobutyrate, aspartate, glutamate, glycine and alanine in bovine

mammary slices (Derrig *et al.*, 1973). These observations are consistent with the deamination of valine and the conversion of its carbon skeleton via β -hydroxyisobutyrate into succinyl-CoA (3-carboxypropionyl-CoA), with entry of the latter into the tricarboxylic acid cycle. The oxidation of leucine and the incorporation of leucine carbon into glutamate have also been observed in rat mammary slices (Abraham *et al.*, 1964), results which are consistent with the catabolism of leucine to acetyl-CoA (see Rodwell, 1969), glutamate arising by transamination of 2-oxoglutarate in the tricarboxylic acid cycle.

However, there seem to be marked differences between species with respect to phenylalanine hydroxylase activity in mammary tissue. In ruminants the designation of tyrosine as an essential amino acid with regard to milk protein synthesis may be unwarranted in view of the presence of phenylalanine hydroxylase activity in both bovine (Schingoethe *et al.*, 1967) and sheep (Verbeke *et al.*, 1972) mammary tissue, though the quantitative significance of this activity has not been established *in vivo*. Lactating mammary tissue of the rat and guinea pig does not appear to possess phenylalanine hydroxylase activity, thus justifying the designation of tyrosine as an essential amino acid.

As far as we are aware no isotopic tracer studies have been made previously on histidine metabolism by lactating mammary tissue, and it would appear that in the guinea pig histidine residues are utilized solely for milk protein synthesis.

Significance of mammary catabolism of essential amino acids

In view of the observed catabolism of certain essential amino acids, the question arises of the extent to which the catabolic pathways compete for the supply of amino acids for milk protein synthesis. In the experiment in which tyrosine availability was decreased in the perfusate, the uptake values of methionine, histidine and phenylalanine were decreased by over 80%, whereas values for other amino acids (valine, isoleucine, leucine and lysine) were decreased by only 20–25% (Davis, 1974). This observation would suggest a potential for catabolism of the latter amino acids of sufficient magnitude to constitute a possible control mechanism for milk protein synthesis. In lactating goats the oxidation of glucose and acetate accounts for 56–76% of the CO_2 production of the mammary glands in fed animals (Annison & Linzell, 1964). The source of most of the remaining 24–44% is uncertain, but our results suggest that oxidation of essential amino acids may contribute substantially to mammary CO_2 output.

The quantitative significance of our data depends on the extent to which essential amino acid catabolism is an obligatory process in the mammary gland, and

thus serves to decrease the availability of amino acids for direct incorporation into milk proteins. It is, however, clear that in the quest for amino acids limiting for milk protein synthesis, simple comparison of uptake from the plasma with output in milk protein is not a valid approach in the case of several amino acids (see Mepham, 1976). Indeed, the use of the parameter 'percentage extraction' (arteriovenous difference expressed as a percentage of arterial concentration) may well prove to be the best indicator of amino acid availability in relation to mammary requirements.

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