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Uptake of Free Plasma Amino Acids by the Lactating Cow's Udder and Amino Acid Composition of Udder Lymph

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1. Total α-amino N and the amounts of 24 ninhydrin-positive substances were determined in several samples of plasma and lymph from the cow's udder. The arteriovenous differences of these substances across the mammary glands were measured in several experiments performed on lactating cows and in one experiment on a 'dry' cow. Udder lymph obtained from live lactating cows by a lymph fistula and taken after killing lactating cows was analysed. 2. The concentrations of the individual free amino acids in udder lymph obtained from the live cow were similar to those found in cow's plasma. The concentrations of many amino acids in udder lymph taken immediately after death were two- to four-fold higher than those of the corresponding amino acids in udder lymph obtained from the live cow. 3. Most amino acids of the blood showed a considerable decrease in concentration by passage across the lactating mammary gland. Ornithine, a non-casein amino acid, showed arteriovenous differences of up to 60% of the arterial plasma concentration. No substantial amino acid uptake by the udder could be demonstrated in the experiment on the non-lactating cow. 4. The arteriovenous differences obtained for arginine, glutamine, isoleucine, leucine, lysine, valine, threonine and histidine were probably large enough to provide all the respective amino acid residues in milk protein. 5. The uptake of aspartic acid, asparagine, glutamic acid, serine and proline by the lactating cow's udder was not sufficient to account for all these respective amino acid residues found in milk protein.

It is now firmly established that the free amino acids of the blood stream are the principal precursors for the milk proteins synthesized in the mammary gland.

The concentration of α-amino N in mammary venous blood of lactating cows is markedly lower than that in the arterial blood (Cary, 1920; Blackwood, 1932; Graham, 1937; Shaw & Petersen, 1938; Nikitin, 1949); ten essential amino acids were absorbed from the blood during perfusion of the isolated cow's udder (Bouckaert, Oyaert, Peeters & Sierens, 1953). From tracer experiments it was concluded that valine, lysine and methionine of casein are derived from the free amino acids of plasma (Campbell & Work, 1952; Askonas, Campbell & Work, 1954), and not from plasma proteins (Askonas, Campbell, Godin & Work, 1955). Barry (1952) demonstrated that the goat's mammary gland takes up free lysine and tyrosine to provide the lysine and tyrosine of casein. A considerable amount of the glutamine and glutamic acid (Barry, 1956), proline (Barry, 1958) and asparagine (Sansom & Barry, 1958) are absorbed from the blood by the mammary gland.

Arteriovenous differences across the udder of four different individual amino acids were determined by Sheldon-Peters & Barry (1956) in the plasma of lactating cows. The uptake of glutamine and threonine by the lactating mammary gland corresponded to the expected value if all of the glutamine and threonine residues and some of the serine and glutamic acid residues of casein came from the blood. In the present study, Barry's work has been extended to include all the ninhydrin-positive compounds normally present in cow's plasma. We also report several analyses performed on udder lymph collected from both live and slaughtered cows.

TECHNIQUES

Sampling of blood and udder lymph. A complete chromatographic amino acid analysis was carried out on samples from one 'dry' and ten lactating animals. More details of these animals and the samples collected are given in Table 1.

When samples from the artery and mammary vein were collected, precautions were taken to avoid excitement of the cows. At 20 min. before collection, 100 mg. of Largactyl

Table 1. *Details of cows and collection of samples*

A, Samples taken from the internal iliac artery; V, samples collected from the mammary vein; L (a.s.), udder lymph collected after slaughter; L (i.v.), udder lymph collected from live animal by lymph fistula.

Cow no.	Milk yield (l./day)	Stabling	Type of samples collected	Further information
1	7	Slaughterhouse	V, L (a.s.)	
2	12	Slaughterhouse	A, V, L (a.s.)	
3	16	Slaughterhouse	A, V, L (a.s.)	
4	6	Slaughterhouse	V, L (a.s.)	
5	dry	Slaughterhouse	A, V	
6	24	Experimental Farm	A, V	
7	19	Experimental Farm	A, V	
8	22	Experimental Farm	A, V	
9	10	Veterinary School	V, L (i.v.)	Collection immediately after cannulating lymphatic fistula
10	17	Veterinary School	A, V, L (i.v.)	Collection 2 days after cannulating lymphatic fistula
11	11	Veterinary School	A, V, L (i.v.)	Collection 2 days after cannulating lymphatic fistula

Specia (a tranquillizer) was injected intravenously and a light anaesthesia was effected with 8 ml. of 2% procaine given epidurally. Food was given to divert the attention of the animal. The arterial samples were taken by a puncture of the internal iliac artery through the rectal wall, the subcutaneous abdominal vein being pierced simultaneously (Graham, Kay & McIntosh, 1936). Samples (120 ml.) were collected in flasks containing 15 mg. of heparin powder (Evans). If the sampling procedure took longer than 3·5 min., or if the cow showed symptoms of excitement, the samples were discarded.

In the first series of experiments, arteriovenous differences were investigated on slaughterhouse cows. Three cows with normal lactating udders and one 'dry' cow were selected. The lactating animals were milked, put in a quiet stable and received food and drink. Then 18 hr. later they were milked again and the milk volume was measured. Blood samples were collected 1 hr. after the second milking. Then 10 min. later the animals were shot, and the efferent lymph vessels of the udder were freed by dissection and clamped as quickly as possible. The udder was then removed and approx. 25 ml. of lymph was collected with a syringe.

In the second series of experiments, samples from the artery and mammary vein were collected from three cows in full lactation in the herds of two Experimental Stations. These animals were milked at 7 a.m. and the samples were taken 3 hr. later.

In the third series a fistula was cannulated by means of polyethylene tubing in the efferent lymph vessels of one half udder as described by Peeters, Cocquyt & De Moor (1963). The lymph flow from the fistula was very variable, the highest mean outflow rate amounting to 19 ml./min. Blood and lymph samples were collected simultaneously.

Haemoglobin was measured in whole blood as cyanhaemoglobin (Betke & Savelsberg, 1950). Samples with an arteriovenous difference in haemoglobin content exceeding 1% were not further analysed.

Deproteinization of plasma and lymph. The blood samples were immediately centrifuged and the plasma was collected. Lymph was centrifuged and the sediment removed. The proteins were precipitated by adding 5 vol. of 1% (w/v) picric acid to 1 vol. of plasma or lymph (Hamilton & Van Slyke, 1943). The filtrate was stored at −15° until analysed.

Determination of free amino acids except asparagine, glutamine and citrulline in blood plasma and lymph. This was performed on Dowex 50 (X4) columns (150 cm. × 0·9 cm.) (Moore & Stein, 1954). The sample (50 or 80 ml. of filtrate) was freed from excess of picric acid on Dowex 2 (Cl^- form) columns (2 cm. × 2 cm.). The eluate and washings were adjusted to pH 3·5 and freeze-dried. After dissolution of the residue in a small amount of water, cysteine was oxidized to cystine at pH 7 (Stein & Moore, 1954). The sample was then adjusted to pH 2 and chromatographed. Fractions (1 ml.) were collected and the amino acid concentration was determined by a modification of the ninhydrin–KCN method of Cocking & Yemm (1955).

The ninhydrin reagent was freshly prepared each day by mixing: (*a*) 15 ml. of ninhydrin solution [stock solution made by dissolving 8 g. of ninhydrin (Sigma) in 300 ml. of 2-methoxyethanol (Eastman Kodak)], (*b*) 15 ml. of 0·2 mM-KCN in 2-methoxyethanol and (*c*) 50 ml. of 1·6 M-sodium acetate buffer solution, pH 5·05.

To 1 ml. of eluate was added 2 ml. of ninhydrin reagent. The boiling time was 20 min. After cooling, the colour was diluted with 60% (v/v) ethanol and the extinction measured at 570 mμ (440 mμ for proline). The extinctions of the peak were added and divided directly by the extinction coefficient of the corresponding amino acid, giving the μmoles of amino acid present on the column. The method gave closely agreeing replicates for amino acids present in amounts of at least 0·5 μmole (S.E. ±2%). For histidine, however, the reproducibility was less and differences up to 10% between replicates were encountered.

Determination of asparagine. Asparagine was determined after alkaline hydrolysis of alternate fractions of the 'glutamine+asparagine' peak from the Dowex 50 (X4) column (Moore & Stein, 1954). The recoveries of aspartic acid and glutamic acid corresponded to 100±6% (S.E.) of the quantities of amides present in the combined peak.

Determination of citrulline. Alternate fractions of the glycine peak were combined and thiodiglycol was removed by absorbing the amino acids on Dowex 50 (H^+ form) columns (5 cm. × 0·9 cm.). After the resin bed had been washed to neutrality, citrulline and glycine were eluted with 2 N-ammonia. The eluate was evaporated to dryness, dissolved in 0·1 N-HCl (containing 0·5% of BRIJ) and analysed with diacetylmonoxime reagent (Archibald, 1944). The recovery of known amounts of citrulline (10–80 μg.) added to the peak was within ±5%.

Determination of glutamine. Glutamine was determined accurately from the difference in glutamic acid before and after hydrolysis of the free amino acids with dilute HCl.

Excess of picric acid was removed from the sample (20 ml. of filtrate) on Dowex 2 (Cl^- form) columns (3 cm. × 0·9 cm.) as described above. After evaporation of the sample to dryness, 10 ml. of 1 N-HCl was added to the residue and the solution hydrolysed under reflux for 2 hr. The sample was concentrated *in vacuo* and adjusted to pH 7·0, and the resulting glutamic acid and aspartic acid were determined on Dowex 1 columns (16 cm. × 0·9 cm.) as described by Marko (1957).

Determination of total α-amino nitrogen. In several samples obtained from cows mentioned in Table 1 and from other cows, the α-amino N content was determined directly by the manometric ninhydrin–CO_2 method described by Hamilton & Van Slyke (1943).

Identification of the amino acids. The identification of the amino acids in the column eluate was checked by paper chromatography after desalting, as described by Raafat, Verbeke & Peeters (1963). Aspartic acid and cystine, because of their low concentrations in plasma, were not tested. Urea and citrulline were identified by a specific colour reaction (Archibald, 1944). In some experiments, alternate fractions of the proline and ornithine peaks were quantitatively determined by the Chinard (1952) reaction. The results obtained for these amino acids by the Chinard method agreed closely with the values obtained with the ninhydrin reaction.

RESULTS

Total α-amino nitrogen content calculated from the chromatographic analysis compared with that determined by the manometric method. In general, the yield for α-amino N in the chromatographic procedure amounted to 90 ± 1% (S.E.M. of 16 determinations) of the value found by the manometric method. For samples collected from the same cow the ratio of the calculated α-amino N content to the value determined in the manometric procedure was constant within the limits of error (S.E. ±3%). The consistently lower yields obtained with the chromatographic procedure suggested that some material other than the common amino acids was reacting in the manometric procedure.

Free amino acids in cow's plasma and udder lymph. In total, 24 different ninhydrin-positive substances were detected on the chromatograms. As shown in Tables 2 and 3, definite concentrations of ornithine and citrulline were found in addition to the common amino acids. Small amounts of α-amino-*n*-butyric acid were always present. Tryptophan was not determined.

Different samples of plasma and lymph were analysed for α-amino N by the manometric method. The α-amino N content in arterial plasma [2·93 ± 0·24 (S.E.M. of six determinations)] was significantly ($P < 0·01$ for paired differences) higher than that found in venous plasma [2·40 ± 0·13 (S.E.M. of 14 determinations)]. The mean α-amino N content in lymph [3·02 ± 0·11 (S.E.M. of ten determinations)] was significantly higher ($P < 0·01$) than that found in venous plasma. No statistical difference existed between the α-amino N contents of arterial plasma and lymph.

Table 2 shows the mean concentration of the free amino acids found in arterial and venous plasma of lactating cows. Glycine is quantitatively present in the greatest amount, its content accounting for 20–24% of the total α-amino N. Alanine, glutamine and valine each contribute 9–13% of the total α-amino N content. Very low concentrations of aspartic acid, methionine and cystine were found. The amounts of aspartic acid and cystine found permitted only approximate values to be given for these amino acids.

Table 2. *Mean concentrations of free amino acids in samples of blood plasma*

The samples were obtained from the internal iliac artery and the mammary vein of lactating cows. The amino acid concentrations (mg./100 ml. of plasma) are given as means ± S.E.M., with the numbers of observations in parentheses.

Amino acid	Artery	Mammary vein
Taurine	0·36 ± 0·06 (7)	0·34 ± 0·05 (8)
Urea	30·7 ± 3·6 (7)	28·3 ± 3·9 (8)
Aspartic acid	0·13 ± 0·02 (7)	0·10 ± 0·01 (8)
Threonine	0·85 ± 0·07 (7)	0·56 ± 0·07 (8)
Serine	0·82 ± 0·09 (7)	0·66 ± 0·08 (8)
Glutamine	2·63 ± 0·23 (7)	2·01 ± 0·22 (8)
Asparagine	0·42 ± 0·05 (7)	0·29 ± 0·02 (8)
Proline	0·81 ± 0·11 (7)	0·60 ± 0·12 (8)
Glutamic acid	0·80 ± 0·05 (7)	0·36 ± 0·04 (8)
Citrulline	0·97 ± 0·15 (7)	0·90 ± 0·14 (8)
Glycine	3·05 ± 0·43 (7)	2·80 ± 0·44 (8)
Alanine	1·52 ± 0·09 (7)	1·44 ± 0·13 (8)
α-Amino-*n*-butyric acid	0·22 ± 0·07 (7)	0·19 ± 0·05 (8)
Valine	2·47 ± 0·29 (7)	1·81 ± 0·30 (8)
Methionine	0·21 ± 0·03 (6)	0·14 ± 0·02 (8)
Isoleucine	1·47 ± 0·17 (7)	0·91 ± 0·15 (8)
Leucine	1·70 ± 0·19 (7)	0·86 ± 0·16 (8)
Tyrosine	0·54 ± 0·04 (7)	0·27 ± 0·04 (8)
Phenylalanine	0·65 ± 0·03 (7)	0·40 ± 0·04 (8)
Ornithine	0·54 ± 0·07 (6)	0·26 ± 0·05 (7)
Lysine	0·93 ± 0·10 (7)	0·41 ± 0·09 (8)
Histidine	0·90 ± 0·05 (7)	0·65 ± 0·09 (8)
Arginine	0·72 ± 0·14 (7)	0·39 ± 0·09 (8)

Table 3. *Mean concentrations of free amino acids in udder lymph*

The samples were collected from living animals or after slaughtering the cows. The amino acid concentrations (mg./100 ml. of lymph) are given as means, with the number of observations in parentheses, and the ranges are also indicated.

Amino acid	Udder lymph cannulated from living cows	Udder lymph obtained immediately after death
Taurine	0·20 (3); 0·15– 0·26	0·77 (4); 0·50– 1·12
Urea	27·9 (3); 17·5 –44·0	27·6 (4); 23·3 –39·8
Aspartic acid	0·14 (3); 0·11– 0·16	0·55 (4); 0·41– 0·61
Asparagine	0·53 (3); 0·45– 0·59	0·85 (3); 0·72– 1·05
Threonine	1·01 (3); 0·93– 1·09	1·45 (4); 1·36– 1·54
Serine	0·73 (3); 0·67– 0·85	1·71 (4); 1·61– 1·84
Proline	0·85 (3); 0·65– 1·05	2·26 (4); 1·78– 3·43
Glutamic acid	0·62 (3); 0·41– 0·90	1·49 (3); 0·96– 2·17
Glutamine	3·74 (3); 2·98– 4·21	8·09 (2); 7·33– 8·85
Citrulline	0·95 (3); 0·40– 1·31	1·20 (4); 0·85– 1·64
Glycine	2·94 (3); 2·32– 3·59	4·72 (4); 2·84– 5·62
Alanine	1·87 (3); 1·22– 2·56	3·09 (4); 2·48– 3·91
α-Amino-*n*-butyric acid	0·11 (3); 0·10– 0·12	0·29 (2); 0·20– 0·38
Valine	3·13 (3); 2·87– 3·31	2·92 (2); 2·30– 3·87
Cystine	0·36 (1)	0·46 (3); 0·31– 0·65
Methionine	0·42 (3); 0·29– 0·54	0·61 (4); 0·47– 0·89
Isoleucine	1·65 (3); 1·58– 1·80	1·81 (4); 1·13– 2·74
Leucine	1·89 (3); 1·64– 2·19	2·94 (4); 2·54– 3·85
Tyrosine	0·65 (3); 0·47– 0·77	1·25 (4); 0·99– 1·66
Phenylalanine	0·88 (3); 0·60– 1·08	1·47 (4); 1·36– 1·65
Ornithine	0·34 (3); 0·27– 0·48	0·46 (4); 0·27– 0·77
Lysine	0·88 (3); 0·61– 1·23	1·99 (4); 1·46– 2·57
Histidine	0·99 (3); 0·58– 1·47	1·54 (4); 0·58– 2·45
Arginine	0·71 (3); 0·46– 0·97	1·89 (4); 1·49– 2·41

The mean amino acid content of udder lymph obtained from live cows or after slaughtering the animals is given in Table 3. From Tables 2 and 3 it is clear that the mean amino acid concentrations of udder lymph obtained from the living cow were of the same magnitude as those found in plasma. By contrast, the concentrations of several amino acids was increased two- to four-fold compared with those of plasma in udder lymph collected after slaughtering. Comparison of both lymphs showed that urea, valine, isoleucine, ornithine, citrulline, methionine, cystine and histidine had comparable concentrations in both. The other amino acids were present in statistically higher concentrations in lymph obtained after killing the animal.

Arteriovenous differences. The mean arteriovenous differences obtained in seven experiments on lactating cows and the arteriovenous difference obtained in one experiment on a 'dry' cow are given in Table 4. From Tables 2 and 4 it is clear that the essential amino acids, as well as glutamine, glutamic acid and the non-casein amino acid ornithine, showed a considerable fall in concentration by passage across the lactating udder. For ornithine, arteriovenous differences up to 68% of the arterial concentration were found.

The serine, proline, asparagine and alanine contents were significantly lower in mammary venous plasma than in arterial plasma, though the differences were less pronounced than those noted for the above-mentioned amino acids.

The concentration of glycine was not altered significantly by passage through the lactating udder. Owing to its high concentration in plasma, it was difficult to demonstrate convincingly a small arteriovenous difference. By contrast, the low content of aspartic acid in plasma prevented an accurate determination of its concentration. The arteriovenous difference found for citrulline was not significant. Apparently taurine, urea and α-amino-*n*-butyric acid were not absorbed by the udder.

From Table 4 it is evident that there were no pronounced positive arteriovenous differences in the experiment on the 'dry' cow. Histidine showed an appreciable negative arteriovenous difference in this experiment.

DISCUSSION

Little information is available in the literature on the composition of free amino acids in cow's plasma and udder lymph. The content of some amino acids in mammary venous plasma and in udder lymph

Table 4. *Arteriovenous differences of the free amino acids across the udder*

The observed arteriovenous differences of the ninhydrin-positive substances (mg./100 ml. of plasma) across the udders of several lactating cows (as means ± S.E.M.) and one 'dry' cow are given, with the numbers of observations in parentheses. The theoretical arteriovenous differences of the casein amino acids during lactation were calculated as described in the text. The values marked with an asterisk (*) were calculated by assuming that 60% of the aspartic acid and 50% of the glutamic acid in the acid hydrolysate of milk protein came from asparagine (Sansom & Barry, 1958) and glutamine residues respectively (Barry, 1956).

		Observed	
		Lactating	'Dry'
Amino acid	Calculated	cows	cow
Taurine	0	+0·03±0·04 (7)	−0·01
Urea	0	+1·12±1·1 (7)	−2·5
Aspartic acid	0·25*	+0·03±0·02 (7)	+0·02
Asparagine	0·37*	+0·14±0·04 (7)	+0·03
Threonine	0·39	+0·27±0·02 (7)	−0·07
Serine	0·43	+0·16±0·02 (7)	+0·03
Proline	0·76	+0·17±0·03 (7)	−0·16
Glutamic acid	0·91*	+0·43±0·04 (7)	+0·07
Glutamine	0·91*	+0·73±0·05 (7)	+0·07
Citrulline	0	+0·13±0·05 (7)	−0·04
Glycine	0·17	+0·09±0·07 (7)	−0·07
Alanine	0·30	+0·18±0·04 (7)	−0·23
α-Amino-*n*-butyric acid	0	0·00±0·02 (7)	+0·01
Valine	0·55	+0·52±0·07 (7)	−0·11
Methionine	0·20	+0·11±0·02 (6)	−0·01
Isoleucine	0·54	+0·48±0·06 (7)	+0·05
Leucine	0·82	+0·78±0·07 (7)	+0·04
Tyrosine	0·40	+0·26±0·03 (7)	−0·02
Phenylalanine	0·42	+0·26±0·03 (7)	−0·07
Ornithine	0	+0·27±0·02 (6)	−0·08
Lysine	0·66	+0·48±0·03 (7)	−0·24
Histidine	0·22	+0·21±0·06 (7)	−0·46
Arginine	0·29	+0·30±0·05 (7)	+0·17
α-Amino N (by chromatography)		+0·67±0·10 (6)	−0·10

obtained after slaughter may be compared with those obtained by Heyndrickx (1961) by paper chromatography. The concentrations he gives for alanine, glycine, leucine + isoleucine, lysine, serine, threonine, valine and urea agree well with the concentrations that we found for the same material. The values reported for tyrosine, glutamic acid and asparagine were higher than those obtained in the present work.

In general, the individual amino acid contents in udder lymph obtained from the living cow after cannulating a lymphatic fistula are similar to the concentrations found in plasma. The different samples of udder lymph collected from live cows had total α-amino N concentrations comparable with that of arterial plasma but higher than that of mammary venous plasma. This is in sharp contrast with the results obtained from udder lymph after slaughter, where different amino acids showed concentrations two- to four-fold higher than those present in the plasma. Heyndrickx (1961) also found high concentrations of amino acids in udder lymph from dead cows as compared with concentrations in the venous plasma of living animals. These findings suggest that the amino acid concentrations of udder lymph taken after slaughter are strongly influenced by post-mortem phenomena, as pointed out by Linzell (1960*b*).

We have shown that most amino acids of the blood, the non-casein amino acid ornithine included, show a considerable decrease in concentration by passage across the lactating mammary gland. The absence of any substantial amino acid uptake across the non-lactating udder indicates that these substances are absorbed to provide the amino acid residues in the milk proteins.

Important qualitative information on the origin of most milk-protein amino acids would be gained if the observed arteriovenous differences were compared with theoretical arteriovenous differences, assuming that free amino acids of the blood are the sole precursors of the amino acid residues in milk protein and that the absorbed amino acids are only used for the synthesis of milk proteins. The amount of a particular amino acid secreted in 1 vol. of milk may be calculated from its concentration in milk protein (Aa, as g./100 g.) and the milk-protein content (Mp, as μg./100 ml.). If P is the number of plasma volumes that pass the udder for the secretion of 1 vol. of milk, the theoretical arteriovenous difference (A − V) is calculated from the relation:

$$A - V = \frac{Mp}{P} \times Aa \qquad (1)$$

Although it is impossible to calculate accurately from our results such expected arteriovenous differences, a rough estimate may be obtained. This relation would not longer hold if a considerable part of the absorbed amino acids is used for catabolic processes or provide free amino acids drained into lymph or milk. From experiments on lactating goats, Linzell (1960*b*) concluded that only a small proportion (about 1%) of the milk precursors removed from the plasma appears as udder lymph. Analysis by Shahani & Sommer (1951) indicates that the free amino acid content in milk is low (maximum 1%) as compared with the milk-protein N. As the blood flow through the udder was not measured in the present experiments, we accepted for our calculations a mean value of 400 vol. of plasma for 1 vol. of milk secreted (see Folley, 1949). This value is only a rough approximation, as Linzell (1960*a*) showed that this blood/milk ratio is not fixed but varies as a function of the milk yield.

Neither the milk-protein content nor its amino acid composition was determined. The milk-protein concentration is known to vary only to a limited extent, and a mean value of 3·3 g./100 ml. was used in the present calculations. The amino acid composition in total milk protein, given by Block & Weiss (1956), was used, since at least 90% of the protein secreted is synthesized in the mammary gland.

In Table 4, the expected arteriovenous differences are compared with the mean arteriovenous differences found in the present experiments. The mean arteriovenous differences for arginine, glutamine, isoleucine, leucine, lysine, valine, histidine and threonine are of the same order as those calculated. The observed arteriovenous differences are 15–25% lower than the expected values, which may be the result of the rough estimations used for the plasma/milk ratio and the milk-protein content. The uniform behaviour of this group of amino acids, however, suggests that the mammary gland takes these substances from the blood stream to provide the respective amino acid residues in milk protein. This group of amino acids probably behaves as the essential amino acids for the lactating cow's udder.

The comparatively low arteriovenous differences obtained with proline, serine, aspartic acid, asparagine and glutamic acid suggest that the absorption of these substances by the lactating gland is inadequate to supply all the corresponding amino acid residues in milk protein. Comparison of the calculated arteriovenous differences with the mean arterial plasma concentration of the amino acids (see Tables 2 and 4) also indicates that the arterial concentrations of aspartic acid, proline, asparagine and glutamic acid would hardly suffice to provide the respective amino acid residues of milk protein.

Statistical treatment of the arteriovenous measurements led to essentially the same conclusion. Division of the arteriovenous differences of the amino acids by their corresponding content in milk protein (A−V/Aa=reduced arteriovenous difference; see eqn. 1) enabled us to analyse the results by variance analysis (Snedecor, 1951). Aspartic acid, asparagine, cystine, glycine and histidine were not included in the statistical analysis, as their arteriovenous differences were difficult to determine accurately (see the Results and Techniques sections). This analysis showed a highly significant difference ($P < 0.001$) for the values obtained for the different amino acids.

The reduced arteriovenous differences of arginine, glutamine, valine, isoleucine, leucine, lysine and threonine formed a relatively homogeneous group whose means differed significantly ($P < 0.05$) from a second group of amino acids.

This second group consisted of the non-essential amino acids proline, serine and glutamic acid.

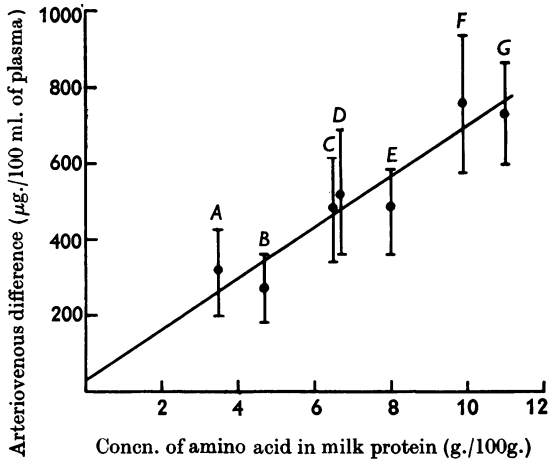

Fig. 1. Relationship between amino acid concentration in milk protein and the arteriovenous differences obtained for the 'essential amino acids' by the lactating cow's udder. *A*, Arginine; *B*, threonine; *C*, isoleucine; *D*, valine; *E*, lysine; *F*, leucine; *G*, glutamine. The line is a least-squares fit of: A−V = 32+66·4 Aa. The standard deviation of the slope is ±8·1 μg./ml. Experimental points are the means of seven arteriovenous differences. The limits are ±2 S.E.M.

Tyrosine, phenylalanine, methionine and alanine formed a third group whose members differed statistically from most amino acids of the first group and from proline in the second group ($P < 0.05$).

For the amino acids mentioned in the first group, the linear regression equation was calculated by using the amino acid concentration in milk protein in relation to the mean arteriovenous difference. This equation, whose correlation coefficient was highly significant ($r = 0.789$; $P < 0.001$), is shown graphically in Fig. 1. The intercept of this line not being statistically significant, the equation may be described by eqn. (1): thus the slope of the regression line is proportional to Mp/P. From the regression coefficient, the mean plasma/milk ratio may be calculated (33 000/66·4 = 495). This ratio, estimated from our results, compares favourably with the mean ratio found by direct methods on the goat (Linzell, 1960*a*) and by indirect methods on the cow (see Folley, 1949). This calculated ratio, which is of the right order of magnitude, serves as a test of the validity of the assumptions made in our calculations.

Our results suggest that at least a significant part of the proline, serine, glutamic acid, aspartic acid and asparagine residues in milk protein are derived from a source other than the free amino acids of the blood. From tracer experiments on lactating goats it was concluded that at least half of the proline and asparagine residues of casein were derived from the corresponding plasma amino acids (Barry, 1956;

Sansom & Barry, 1958). The arteriovenous differences of serine and glutamic acid across the lactating cow's udder appeared to be inadequate to provide the corresponding casein amino acids (Sheldon-Peters & Barry, 1956). The source of the aspartic acid and of part of the asparagine, serine, glutamic acid and proline in casein is thus not clear. Results obtained in perfusion experiments on the isolated cow's udder, however, suggested that part of the non-essential amino acids may be synthesized in the udder itself from volatile fatty acids present in the blood. After the administration of [1-^{14}C]acetate or [1-^{14}C]propionate (Verbeke, Aqvist & Peeters, 1957) to the perfusion blood, a high specific radioactivity was found in glutamic acid and aspartic acid residues (after acid hydrolysis) of casein, and appreciable radioactivity was found in serine. Alanine, glycine and proline showed only low levels of radioactivity. Also, glutamic acid and aspartic acid may function respectively as precursors of glutamine and asparagine of casein (Verbeke & Peeters, 1960). Kleiber (1958) also concluded that there is a considerable formation of some non-essential amino acids in the udder itself. From tracer experiments on lactating cows, Wood, Gillespie, Joffe, Hansen & Hardenbrook (1958) calculated that 25% of the casein serine was synthesized in the udder itself.

Part of the proline in milk protein may come indirectly from ornithine, as a result of the biochemical relationship between ornithine, proline and glutamic acid (for review see Greenberg, 1961). If a similar reaction from ornithine to proline could occur in the udder, the high arteriovenous differences for ornithine would be explained and an important source of casein proline revealed.

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