A Quantitative Assessment of the Contribution of Individual Plasma Amino Acids to the Synthesis of Milk Proteins by the Goat Mammary Gland

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1. Arteriovenous differences of plasma free amino acids across the lactating mammary glands of six goats have been measured. 2. In four experiments, measurements of blood flow, amino acid arteriovenous differences, milk yield and milk nitrogen showed that the uptake of nitrogen in the form of amino acids was sufficient to provide all the nitrogen of the milk proteins synthesized in the mammary gland. 3. In the same four experiments the uptake from the plasma and output into the milk of individual amino acids per unit time were compared. The uptakes of essential amino acids and glutamic acid were approximately equal to the corresponding output figures. The uptake of serine was consistently less than the output, and the uptake of other non-essential amino acids was very variable, in some experiments being approximately equal to the output figures and in others being considerably less. 4. As in cows, there was an uptake of ornithine in all experiments, though ornithine is absent from milk. In goats, though not in cows, the uptake of arginine was consistently greatly in excess of the requirement for arginine residues in milk protein. 5. The possible significance of the uptakes of arginine and ornithine for the synthesis of serine and other non-essential amino acids in the mammary gland is discussed. 6. The importance of clamping the external pudic vein, when sampling mammary venous blood from the caudal superficial epigastric vein, is indicated.

Ideas about the immediate blood precursors of the specific proteins of milk have undergone considerable change since the first investigation into the problem by Cary (1920). His conclusion, later corroborated by Blackwood (1932) and Lintzel (1934), that the absorption of plasma free amino acids was of an order sufficient to provide all the nitrogen of the milk proteins, was subsequently challenged by Graham (1937) and Shaw & Petersen (1938), whose results indicated that amino acid uptake was only sufficient to provide about 30% of the milk protein. Graham, Peterson, Houchin & Turner (1938) suggested that milk nitrogen was derived chiefly from globulin of the blood. All these early workers based their conclusions on the magnitude of the fall in plasma concentrations during passage through the udder of lactating cows and goats. However, largely because of the difficulty of obtaining arterial samples without disturbing the animal and of measuring the small arteriovenous difference accurately, the method fell into disrepute (Barry, 1961). Later the availability of radioactive isotopes enabled experiments to be done with labelled amino acids and plasma

proteins and, by using the single-shot technique, both Work and his colleagues and Barry (see Barry, 1961) produced evidence to show the quantitatively major importance of plasma free amino acids for the synthesis of milk protein.

However, not all the amino acids have been studied with isotopic tracers and, furthermore, the single-shot technique is not, quantitatively, of high precision, since according to Barry (1961) the proportion of a milk component derived from a plasma precursor can only be estimated with an accuracy of $\pm 30\%$. Since major improvements have been made in the arteriovenous technique, in the present work we have reverted to this method because quantitative amino acid uptake data were needed to design a substrate mixture, for infusion into the isolated perfused goat mammary gland preparation (Hardwick & Linzell, 1960), which more closely corresponds to the requirements of the gland than the mixture used hitherto.

The improvements were as follows: 1. Precautions were taken to ensure the sampling of venous blood of purely mammary origin. This was necessary because it has been shown (Linzell & Mount,

1955; Linzell, 1960a; Rasmussen, 1963, 1965), that in both cows and goats the caudal superficial epigastric vein ('milk vein') customarily used for sampling mammary venous blood carries a variable proportion of the total mammary blood and, moreover, in lactation it frequently carries venous blood from the abdomen in addition to that of purely mammary origin, the non-mammary blood in some cases doubling the blood flow through the milk vein. This fact, which seriously affects the validity of arteriovenous measurements, has not previously been recognized in determinations of amino acid uptake by this method. 2. The use of surgically prepared trained animals, so that samples could be obtained without disturbance (Linzell, 1960b). 3. The use of column chromatography for the estimation of individual amino acids in plasma and milk. 4. The measurement of the rate of mammary blood flow at the time of sampling.

It has thus been possible to compare, in the conscious undisturbed animal, the uptake from the blood with the output into the milk of amino acid nitrogen and also of individual amino acids.

METHODS

Blood sampling. Carotid arterial and mammary venous blood samples were taken from six lactating Saanen goats between 24 and 132 days of lactation, and from one 'dry' goat. All the goats, which were sampled when standing quietly on their milking stands, had been previously surgically prepared so that samples could be obtained without disturbance in the conscious animal. A carotid artery and milk vein on one side had been permanently exteriorized as skin-covered loops and the halves of the udder had been separated with skin to divide the blood vessels crossing between the right and left gland, so that blood was obtained from one gland only (Linzell, 1960b). To prevent the dilution of mammary venous blood with blood of non-mammary origin (Linzell, 1960a, 1966) the external pudic vein was manually clamped while samples were being taken and blood-flow measurements made.

Blood samples, collected into tubes containing heparin, were immediately centrifuged at 0° and the plasma was removed and stored at -20° until deproteinization.

Estimation of mammary blood flow. At the time of sampling the flow of blood in the exteriorized milk vein was measured by Fegler's thermo-dilution method as described by Linzell (1960b), with the modification that an intermittent continuous injection of cold saline was used instead of single shots (Linzell, 1966).

Preparation of plasma for analysis. To estimate recovery through the deproteinization procedure norleucine (1 μ mole/ml.) was added to blood plasma to give a final concentration about 20 μ moles/100 ml.

Proteins were precipitated by the addition of 5 vol. of 1% (w/v) picric acid (Hamilton & Van Slyke, 1943). The samples were centrifuged and excess of picric acid was removed from the supernatant by passage through a Dowex 2 (Cl⁻ form) column (Stein & Moore, 1954).

In preliminary analyses samples were brought to pH 7

and allowed to stand at room temperature for 4 hr. to promote the oxidation of cysteine to cystine (Stein & Moore, 1954), as cysteine is not detected by the method used. Since the cystine peaks obtained on the chromatogram were too small to permit measurement, this procedure was omitted in the analyses reported here.

Amino acid estimations. Plasma free amino acids were estimated by using an automatic amino acid analyser of the type described by Spackman, Stein & Moore (1958). Analyses were performed on samples equivalent to about 4 ml. of plasma, which were evaporated to dryness on a rotary evaporator and dissolved in citrate buffer, pH 2.2, before application to the columns.

Neutral and acidic amino acids were estimated by using a 150 cm. column under the conditions described by Spackman *et al.* (1958), except that the column temperature was 50° throughout. The recovery factor was calculated from the size of the norleucine peak. Basic amino acids were estimated by using a 50 cm. column, as described by Kominz (1962). The 50 cm. column also allowed estimation of tyrosine and phenylalanine, so that comparison of the sizes of these peaks on both columns enabled calculation of a recovery factor for the basic amino acids.

Under the conditions used for the 150 cm. column serine, asparagine and glutamine were eluted as a single peak. To estimate these amino acids a duplicate sample was refluxed with 2N-HCl for 3 hr. and on subsequent chromatography the amides were determined by the increases in the glutamic acid and aspartic acid peaks and serine was measured directly (Sheldon-Peters & Barry, 1956). This chromatogram served as a duplicate analysis for the other neutral amino acids. The estimation of methionine probably involved a small error for which allowance has not been made in the reported results. Methionine is known to be susceptible to oxidation to methionine sulphone and sulfoxides during the preparation of samples, and small peaks at the theoretical elution times of these methionine derivatives have consistently been observed on the chromatogram traces; but we have, at present, no direct evidence for the identity of these peaks. Tryptophan, possibly because of binding to plasma proteins and decomposition during chromatography, was not detected.

The ninhydrin reagent used was a modification of that employed by Moore & Stein (1954), KCN being used instead of hydrindantin (Hill & Mangan, 1964).

Milk analyses. Milk-protein nitrogen was determined by Kjeldahl estimations of milk total nitrogen and non-protein nitrogen.

The amino acid composition of whole-milk hydrolysates was determined by refluxing 0.5 ml. samples of milk with 15 ml. of conc. HCl-formic acid (1:1, v/v) for periods of 24, 48 and 96 hr. After removal of excess of acid by repeated concentration of the hydrolysate at reduced pressure, and passage through a Dowex 2 (Cl⁻ form) column, the amino acid composition of the milk was determined from the results of the serial analyses. This procedure allowed compensation for the destruction of certain amino acids during hydrolysis and for the slow release of others (Tristram & Smith, 1963). The concentrations of amino acids in milk were calculated by using the chromatographic data in conjunction with the Kjeldahl nitrogen analyses. In practice the protein-nitrogen figures obtained from the chromatographic records agreed to within 10% with the Kjeldahl estimations, which indicates that the use of the

'serial hydrolysis' method to compensate for amino acid losses is satisfactory. Methionine was probably estimated with greater accuracy than in the plasma samples since no peaks at the theoretical elution times of the methionine derivatives were observed on the hydrolysate chromatograms. This was probably due to the decomposition of formic acid during hydrolysis, forming a layer of reducing gas over the hydrolysate.

α -Amino nitrogen. The α -amino nitrogen content of some carotid and venous plasma samples was estimated by the manometric method of Hamilton & Van Slyke (1943).

RESULTS

Arterial amino acids. The mean arterial concentrations of the 20 amino acids studied are given in Table 1. As in the cow (Verbeke & Peeters, 1965), glycine was present in the greatest amount (mean 6.85 mg./100 ml. of plasma) and glutamine, valine, proline, arginine, lysine and leucine were also present in high concentrations (means greater than 2 mg./100 ml. of plasma).

The amino acid pattern obtained was essentially similar to that reported by Shimbayashi, Itabisashi & Yonemura (1962) for a dry goat, except for glutamic acid and valine which were respectively higher and lower than the corresponding values obtained by these workers.

The mean arterial concentration of α -amino nitrogen (obtained from the chromatogram results) was 4.49 ± 0.32 (S.E.M.) mg./100 ml. of plasma, which is in marked contrast to the figure of 2.93 ± 0.24 reported for the cow (Verbeke & Peeters, 1965), the difference being due largely to the higher concentrations of arginine, lysine and non-essential amino acids in goat plasma. Throughout this paper the classification of amino acids into 'essential' and 'non-essential', on the basis of rat-growth experiments, has been used.

In addition to the common amino acids listed, taurine, urea, ammonia and a number of compounds which have not been positively identified were also present. Of the latter group it seems probable from the elution times of these compounds that three were α -amino-*n*-butyric acid, 1-methyl-histidine and 3-methylhistidine.

Arteriovenous differences. The mean arteriovenous differences of the results of ten pairs of samples from six lactating goats are shown in Table 1.

An examination of the arteriovenous technique led Barry (1961) to conclude that, because of erratic variations of as great as $\pm 10\%$ of the arterial level, to be convincingly demonstrated an arteriovenous difference should be consistently greater than 20%

Table 1. *Arterial plasma free amino acid concentrations and arteriovenous differences across the lactating goat mammary gland*

Concentrations, expressed as mg./100 ml. of plasma, are given as the mean $\pm$ S.E.M. For most amino acids each figure is the mean of ten pairs of arterial and venous samples, which were taken from six goats. The arteriovenous difference was calculated as a percentage of the arterial concentration for each pair of samples and the mean figure is given in the last column ($\Delta\%$). Coefficients of variations are represented by C.V.

Amino acid	Arterial concn.			Arteriovenous difference			$\Delta\%$
	Mean	$\pm$ S.E.M.	C.V.	Mean	$\pm$ S.E.M.	C.V.	
Aspartic acid	0.28	0.03	30	0.11	0.02	70	34
Asparagine	0.89	0.07	25	0.39	0.06	45	43
Threonine	0.96	0.06	20	0.58	0.04	24	62
Serine	1.41	0.12	27	0.03	0.08	625	0.1
Glutamic acid	1.93	0.11	18	1.08	0.04	13	58
Glutamine	3.74	0.27	23	0.98	0.18	58	25
Citrulline	1.93	0.18	23	0.02	0.10	1300	1.4
Proline	2.59	0.19	17	0.96	0.18	42	40
Glycine	6.85	0.78	36	0.36	0.10	89	5
Alanine	1.66	0.10	18	0.41	0.05	39	24
Valine	2.79	0.11	12	1.07	0.09	25	38
Methionine	0.27	0.02	21	0.19	0.02	26	77
Isoleucine	1.79	0.08	15	0.89	0.09	31	50
Leucine	2.07	0.08	12	1.30	0.08	20	63
Tyrosine	0.95	0.07	24	0.51	0.08	47	45
Phenylalanine	0.70	0.05	24	0.46	0.04	31	67
Ornithine	1.11	0.14	32	0.41	0.08	49	38
Lysine	2.13	0.19	27	0.96	0.09	27	47
Histidine	1.04	0.12	35	0.34	0.06	54	35
Arginine	2.53	0.11	13	1.24	0.14	33	48

of the arterial level. Applying this criterion to the results for the dry goat no amino acid showed a significant arteriovenous difference, whereas in lactating goats it will be seen (Table 1) that the uptake of the following amino acids where the arteriovenous difference was in all cases greater than 20% of the arterial value, may be considered established; methionine, phenylalanine, leucine, threonine, glutamic acid, isoleucine, arginine, lysine, tyrosine, proline, valine and histidine.

The mean arteriovenous differences of aspartic acid, asparagine, glutamine, alanine and ornithine were all greater than 20% of the mean arterial concentrations, but some of the individual results were below this value. It seems likely that all these last-named amino acids were usually absorbed by the gland, but the very low arteriovenous differences in some animals may reflect a physiological variation in the requirements of the mammary gland (see next section). The mean arteriovenous differences of glycine, serine and citrulline were very low and at least for serine and citrulline, for which negative arteriovenous differences (venous concentration higher than arterial) were frequently obtained, absorption has not been established. Glycine was present in very high arterial concentrations so that the arteriovenous differences, though substantial, did not approach the 20% level of significance. Moreover, measurement of a small arteriovenous difference in such a large peak carried considerable experimental error. However, the fact that positive arteriovenous differences were obtained in all ten analyses suggests the absorption of glycine by the gland, a conclusion which is confirmed by the incorporation of [¹⁴C]glycine into milk protein by a lactating goat (Askonas, Campbell & Work, 1954).

Comparison of uptake with output. In four experiments measurement of the rate of blood flow through the gland immediately after sampling allowed calculation of the uptake of individual

amino acids per unit time. [Uptake (wt./unit time) equals the product of the arteriovenous difference (wt./unit volume of plasma) and the plasma flow (vol./unit time).]

To make an accurate assessment of the contribution of the observed uptakes to the mammary synthesis of protein it would be necessary to determine the concentration and amino acid composition of all the milk proteins synthesized in the gland; but since casein, β -lactoglobulin and α -lactalbumin, all of which are synthesized in the mammary gland (Larson & Gillespie, 1957), constitute over 95% of the total protein of goat milk (Macy, Kelly & Sloan, 1953) a whole-milk hydrolysate should give amino acid concentrations which do not differ greatly from the sum of the amino acids derived from the synthesized protein. On this assumption, the error involved in the comparison of the uptake and output of individual amino acids will be even less if, as in the cow (Hansen & Carlson, 1956), the amino acid composition of the milk proteins which are derived as such from the plasma proteins do not differ in any major way from the mammary synthesized milk proteins.

Consequently, the amino acid composition of whole milk, from three of the goats used in this work, has been determined and the output of individual amino acids per unit time in the four experiments was calculated by using the appropriate data for milk yield and milk-protein nitrogen, on the assumption that the amino acid composition of goat milk is relatively constant (see the Discussion section).

The α -amino and total amino acid nitrogen uptakes in the four experiments were determined by adding the amounts of nitrogen in the individual amino acids and the balances with milk-protein nitrogen are shown in Table 2. In two experiments, as an external check on the chromatographic method, α -amino nitrogen was estimated by the manometric procedure and these results are

Table 2. Nitrogen balances across the mammary gland

Results show the α -amino nitrogen and total amino acid nitrogen (TN) uptake and milk-protein nitrogen (PN) output of one mammary gland of four goats, analysed in detail in Figs. 1 and 2. The α -amino nitrogen figures were obtained by the manometric method of Hamilton & Van Slyke (1943) (man.) and by the chromatographic method (chr.); the total amino acid nitrogen uptake figures were obtained from the chromatographic results, and the milk-protein nitrogen figures from Kjeldahl estimations.

Expt. no.	Goat	State of lactation (days)	Amino acid N uptake (mg./hr.)			Output (mg./hr.) Milk-protein N	TN uptake PN output (%)
			α -Amino N (man.)	α -Amino N (chr.)	Total amino acid N		
1	Alice	46	—	290	410	420	98
2	Gladys	68	—	157	224	208	107
3	Hazel	25	210	217	315	288	109
4	Balham	132	167	153	244	214	114

included in the Table. It is apparent that in all cases the amino acid nitrogen uptake was adequate to account for the nitrogen of the milk protein, and thus the conclusion of workers who have used isotopic tracers has been verified.

The uptakes and outputs of the individual amino acids per unit time in the four experiments are shown in Figs. 1 and 2. It is apparent from the histograms that, allowing for minor variations, the uptakes of the following amino acids in all the experiments were quantitatively sufficient to account for all the corresponding amino acid residues in the milk protein; arginine, valine, lysine, leucine, isoleucine, threonine, histidine, tyrosine, phenylalanine and glutamic acid.

The balance between uptake and output of most of the non-essential amino acids was very variable. For example, in one experiment the uptake of glutamine was 2.6% of the output and in another it was 125% of the output. It seems unlikely that the low value here was an artifact due to deamidation before analysis because the uptake of glutamic acid in this experiment was not atypically large. The low uptake for asparagine would not seem to be a constant feature of the particular goat sampled because measurements in the same animal on

another occasion, for which blood-flow data are not available, showed an asparagine arteriovenous difference 20 times as large as that obtained in the result shown in Fig. 2. The lack of balance between the uptake and output of serine, which frequently showed negative arteriovenous differences, indicates the inadequacy of the uptake of this amino acid to account for the corresponding residues in the milk protein. The uptakes of glycine and methionine were also consistently less than the output figures, and while the measurements for glycine were subject to error, for reasons given above, the uptake values for methionine were probably under-estimates (see the Methods section), and since methionine is an essential amino acid it seems likely that it was, in fact, absorbed in amounts sufficient to provide all the methionine residues in the milk protein.

As citrulline does not occur in milk the lack of a significant uptake of this amino acid did not seem surprising. However, ornithine, also absent from milk, showed a consistent, and sometimes large, uptake. Further, the uptake of arginine referred to above as adequate was in fact consistently greatly in excess (2.7–4.4 times) of the requirement for arginine residues in the milk protein. Equally large arginine arteriovenous differences and positive

Fig. 1. Uptake from the plasma (■) and output into the milk protein (□) of essential amino acids and tyrosine in four lactating goats, the nitrogen balances for which are shown in Table 2. Uptake figures are expressed as mg. absorbed during the time in which the gland secreted 100 ml. of milk; output figures represent mg. of amino acid/100 ml. of milk. Details of the calculations are given in the text.

Fig. 2. Uptake from the plasma and output into the milk protein of non-essential amino acids in lactating goats. For details see the legend to Fig. 1. Outputs of glutamic acid, glutamine, aspartic acid and asparagine were calculated from the respective acid:amide ratios in goat casein reported by Barry (1956) and Sansom & Barry (1958).

ornithine arteriovenous differences were obtained in all ten pairs of samples analysed.

Verbeke & Peeters (1965) also reported arteriovenous differences (of up to 60%) in ornithine in lactating cows, but they did not observe the large excess uptake of arginine. In view of this, we considered it of interest to measure the arteriovenous difference of arginine across the mammary gland of a cow in the herd at this Institute to check whether or not the discrepancy was due to a species difference. Jugular venous (assumed to be approximately equivalent to arterial) and mammary venous blood samples were taken from a lactating Jersey cow while the external pudic vein was clamped and the plasma amino acids were estimated. In this experiment the nitrogen absorbed in the form of amino acids was 95% of the nitrogen of the milk proteins (a figure calculated from the known milk yield and the estimated udder weight of the cow, and a mean value for the blood flow/unit wt. of cow udder tissue; Rasmussen, 1965), and, in agreement with the finding of Verbeke & Peeters (1965), the arginine jugular-mammary venous difference was of about the magnitude required to account for the arginine residues in milk protein. To check whether the large arginine uptake was a peculiarity of the goats in our herd (due conceivably to the diet of concentrates and hay) jugular and mammary venous blood samples were taken from a lactating Toggenburg goat, which was predominantly on grass at another farm. On estimation of plasma amino acids the uptake of arginine was three times the estimated requirement for arginine residues in the milk protein. From these results it is apparent that cows and goats differ in the extent to which they absorb arginine from the blood.

DISCUSSION

Validity of results. As a result of the evidence of isotopic tracer experiments, the view that plasma free amino acids are the major precursors of the milk proteins which are formed in the mammary gland is now very widely held (though not universally so; see Livrea, Cambria & Campanella, 1963). However, an adequate uptake of amino acid nitrogen has not previously been convincingly demonstrated by arteriovenous-difference measurements. For example, in the most recent report (Verbeke & Peeters, 1965), although the authors accept the theory of the adequacy of amino acid uptake, calculation of α -amino nitrogen uptake from the reported mean arteriovenous difference of α -amino nitrogen and the assumptions about blood flow and milk protein yield, employed by the authors, shows that about 30% of the milk-protein nitrogen was unaccounted for.

Reynolds & Kronfeld (1964) in a preliminary communication report a value for amino acid nitrogen uptake by the udder of the lactating goat that is considerably lower than ours. However, M. Reynolds has stated (personal communication) that this result will be subject to revision and that later work is more in agreement with our findings.

One possible explanation of the low uptakes which have previously been obtained is the dilution of the mammary venous blood with blood of non-mammary origin. The manual clamping of the external pudic vein, which was adopted in our experiments, ensured both that only mammary venous blood was sampled, and that only the rate of blood flowing through the mammary gland was measured. It is considered that the degree of balance of amino acid nitrogen uptake and output observed in these experiments validates the comparison of the uptake and output of individual amino acids.

The output of individual amino acids has been calculated from the estimations of milk-protein nitrogen in each experiment and the mean amino acid composition of whole milk from three goats. The individual analyses differed only slightly (maximum $\pm 10\%$) and this finding, together with the evidence in the literature of the constancy of the amino acid composition of mature milk, even in animals with widely differing diets (e.g. Barnabas & Mawal, 1959; Virtanen, 1963; Featherstone, Frazeur, Hill, Noller & Parmelee, 1964), indicated the permissibility of calculating amino acid outputs from protein-nitrogen data.

The very close agreement between the uptake and output of certain amino acids (e.g. threonine and tyrosine, shown in Fig. 1) suggests that the method is capable of greater precision than has been allowed for by Barry (1961). Since most of the estimations of amino acid uptake were subject to the same degree of experimental error (the exceptions being glycine, to which reference has been made, and proline, aspartic acid and methionine, where the peaks were very small) the large variations in the balance between the uptake and output of most non-essential amino acids in the different experiments probably reflect variations in mammary absorption. For example, for glutamine, in two experiments (Fig. 2) the uptake was approximately equal to the output, results in agreement with the finding of Barry (1956), who, from experiments with isotopically labelled glutamine, concluded that at least 70% of the casein glutamine was derived from plasma free glutamine. However, in the other two experiments (Fig. 2) the outputs were only 45% and 2.6% of the respective output values. The balances between the uptake and output of asparagine, aspartic acid, proline, alanine and serine were similarly very variable.

Implications of the results. The uptakes of methionine, valine, lysine and tyrosine reported in this paper confirm the major importance of these amino acids to the lactating goat mammary gland, which was indicated by the use of ^{14}C -labelled compounds (Barry, 1952; Askonas *et al.* 1954). This work has demonstrated the similarly important uptakes of the other essential amino acids, which had not previously been investigated in the goat. The results obtained for glutamine, glutamic acid, asparagine and proline are also in conformity with the isotopic evidence of Barry (1956, 1958) and Sansom & Barry (1958), but our results suggest a variability in the requirements for asparagine, glutamine and most other non-essential amino acids in different animals and also possibly in the same animal at different times.

The consistently inadequate uptakes of serine by the gland, which were more marked than those reported in lactating cows (Sheldon-Peters & Barry, 1956; Verbeke & Peeters, 1965), and the low uptakes of other non-essential amino acids in some experiments, suggest that as plasma proteins do not contribute significantly to the mammary synthesis of milk protein (Askonas, Campbell, Godin & Work, 1955) synthesis of amino acids must have been taking place in the mammary gland to provide all the corresponding residues in the milk protein. The synthesis of amino acids in the mammary gland would clearly require an adequate source of nitrogen in a suitably metabolizable form; the results showing an uptake of arginine which was consistently greatly in excess of the requirement for arginine residues in the milk protein, and the consistent uptake of ornithine although ornithine is not present in milk, suggest the possibility that nitrogen from these amino acids was used for the synthesis.

The close structural similarity of arginine and ornithine may indicate a common metabolic pathway for these amino acids and the presence of arginase in goat mammary tissue (S. J. Folley & A. L. Greenbaum, unpublished work cited by Folley, 1949) supports this view. The arginase-catalysed conversion of arginine into ornithine would involve the production of urea, and negative urea arteriovenous differences across the lactating goat mammary gland, though since doubted (Barry, 1961), have been reported on several occasions (e.g. Graham, Houchin & Turner, 1937). We have found the estimation of urea by the chromatographic technique to be rather imprecise, replicate analyses showing considerable variations, and urea estimations by the Conway method have only shown small and variable arteriovenous differences, which were within the error of the method. The arterial concentration of urea in our goats is about 50mg./100ml. of plasma, so that

even if all the excess of arginine were converted into ornithine the resulting urea production would represent only about 1% of the arterial concentration. Present methods of measuring blood flow and blood urea are not sufficiently sensitive to decide, by using the arteriovenous difference method, whether the mammary gland produces urea, but the slight excess uptake of amino acid nitrogen over milk-protein nitrogen in three experiments (Table 2) is consistent with the formation of a non-protein nitrogenous compound from some fraction of the amino acid uptake.

Two pathways for the synthesis of serine in animal tissues have been suggested. In the first, for which there is considerable evidence, serine is derived from glycine by interaction with hydroxymethyltetrahydrofolic acid; in the second it may be formed by transamination between amino acids and hydroxypyruvic acid. That the former pathway operates in the lactating goat is shown by the results of an experiment in which, after intravenous injection of $[^{14}\text{C}]$ glycine, the glycine and serine residues of the casein and β -lactoglobulin secreted several hours later were both labelled (Askonas *et al.* 1954). However, the quantitative importance of this conversion in the mammary gland would seem to be ruled out by the fact that barely sufficient glycine was absorbed by the gland to provide all the glycine residues of the protein alone. The second pathway, involving the transamination of hydroxypyruvic acid, has been postulated by several workers (e.g. in lactating cows, by Black, Kleiber & Baxter, 1955), and although the validity of this scheme has been questioned, according to Greenberg (1961) it still represents the most likely route for serine biosynthesis.

Enzymes catalysing the transfer of the δ -amino group of ornithine to a number of keto acids have been described (Meister, 1953), so that taken with the 'alanine-hydroxypyruvate' transaminase catalysing serine formation (Sallach, 1956) the use of ornithine nitrogen for the mammary synthesis of all the non-essential amino acids becomes a possibility if enzyme systems which have been described in other tissues (see Greenberg, 1961) are also present in the lactating mammary gland.

Verbeke, Peeters, Cocquyt & Lauryssens (1965) have reported the incorporation of $[2-^{14}\text{C}]$ ornithine into the proline residues of casein by a perfused sheep mammary gland. This finding is consistent with the suggested metabolic role of ornithine.

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