

## Arginase in Lactating Bovine Mammary Glands: Implications in Proline Synthesis<sup>1</sup>

### ABSTRACT

The occurrence and subcellular distribution of arginase have been studied in mammary glands from lactating dairy cattle. The enzyme appears to be localized in the mitochondrial fraction, although a significant amount has been found to be associated with the cytosolic fraction. Both mitochondrial and cytosolic arginase are activated by heating with  $Mn^{2+}$ . The Michaelis constants for the two fractions, however, are different: 49.5 and 18.5 mM for the mitochondrial fraction and cytosolic fraction, respectively. Overall, the total enzyme concentration in the gland suggests that these enzymes contribute to the conversion of arginine to ornithine. Ornithine, in turn, may be converted by ornithine aminotransferase into an intermediate, L- $\Delta^1$ -pyrroline-5-carboxylate; concurrently,  $\alpha$ -keto-glutarate is transformed into glutamic acid. Finally, pyrroline-5-carboxylate reductase yields proline, an important amino acid that is needed for casein synthesis. Because pyrroline-5-carboxylate reductase requires NADPH, and because ornithine aminotransferase uses  $\alpha$ -ketoglutarate, this new pathway is linked to the Krebs cycle through the cytosolic isocitrate dehydrogenase, which is the source of both of these intermediates.

(**Key words:** milk secretion, enzymes, proline)

**Abbreviation key:**  $K_m$  = Michaelis constant, OAT = ornithine- $\delta$ -aminotransferase, P5C = L- $\Delta^1$ -pyrroline-5-carboxylate.

### INTRODUCTION

To maintain protein secretion at the onset of full lactation, the bovine mammary gland must assemble the complex casein micelle structure and efficiently secrete the resultant skim milk components (4, 11, 15). The uptake of amino acids and their metabolic

interconversions are important preludes to protein synthesis. Deficits in nonessential amino acids that are not taken up by the mammary gland in sufficient quantity to satisfy the requirements for protein synthesis can be overcome by synthesis from other precursors (15). Proline is one such amino acid. The metabolic conversion of arginine and ornithine to proline was postulated because amounts of the first two amino acids, which are in excess of the demands for casein synthesis, are removed from serum. In contrast, the amount of proline taken up is insufficient for casein synthesis (6, 18, 22). Enzymes related to the urea cycle, which can convert other metabolites into precursors of proline, were thought to occur in the bovine mammary gland, but the urea cycle has no known activity in the mammary gland per se (22). Activity of ornithine- $\delta$ -aminotransferase (OAT) developed in the mammary gland of the rat, increasing to a high level in parallel with arginase (22), which suggested another physiological role for arginase apart from its known function in the urea cycle. The conversion of arginine to ornithine leads to proline; Basch et al. (3) previously reported that a key enzyme in this process was OAT, which occurs primarily in the mitochondria of the lactating bovine mammary gland. This enzyme converts ornithine to L- $\Delta^1$ -pyrroline-5-carboxylate (P5C), which in turn can be reduced to proline by the action of P5C reductase. Recently, P5C reductase has been reported to occur in the lactating bovine mammary gland as well (2), but is rather evenly distributed among subcellular fractions. This study reports on the occurrence of arginase (L-arginine amidohydrolase; EC 3.5.3.1) in the lactating bovine mammary gland, as well as its subcellular distribution, and related the properties of the mammary enzyme to similar enzymes in other tissues (12, 22).

### MATERIALS AND METHODS

#### Materials

Arginine hydrochloride, 2,3-butanedione monoxime, manganese chloride, proline, putrescine, *p*-

hydroxymercuribenzoic acid, spermine, and spermidine were purchased from Sigma Chemical Company (St. Louis, MO). Mercuric chloride was obtained from Alfa Products (Ward Hill, MA). Glycine was purchased from Bio-Rad Laboratories (Richmond, CA). Ultrapure urea was obtained from Schwarz/Mann Biotech (Cleveland, OH), and guanidine hydrochloride was purchased from Eastman Kodak Company (Rochester, NY).

Whole mammary glands from three multiparous Holstein cows in midlactation that were of known good health and productivity (about 20 kg/d of milk) were obtained from the herd at the USDA (Beltsville, MD). The glands were collected at slaughter, trimmed to remove adipose tissue, and sectioned into 500-g pieces that were frozen on dry ice and stored at  $-80^{\circ}\text{C}$  until used. Tissue fractionation was carried out according to the method described by Basch et al. (1). First the mammary glands were minced and then suspended in a homogenizing buffer. This buffer was composed of 37.5 mM Tris-maleate (pH 7.0), 1 mM EDTA, 5 mM  $\text{MgCl}_2$ , 0.1 mM dithiothreitol, and 0.25 M sucrose. The glands were homogenized and passed through three layers of cheesecloth to remove connective tissue. Subcellular fractions (nuclei, mitochondria, microsomes, and cytosol) were isolated by differential centrifugation as previously described (1). All pellets were resuspended in homogenization buffer and recentrifuged at the appropriate speeds. Pellets were then redispersed in a volume equal to 10% of that used for homogenization and stored at  $-80^{\circ}\text{C}$ .

### Enzyme Assays

Arginase activity was measured by the spectrophotometric determination of urea according to the method of Jenkinson and Grigor (12). Each fraction of the bovine mammary gland was activated before assay by incubation at  $55^{\circ}\text{C}$  for 5 min in the presence of  $\text{Mn}^{2+}$  (10  $\mu\text{l}$  of 100 mM  $\text{MnCl}_2$  added to 90  $\mu\text{l}$  of the bovine mammary gland fraction). The reaction mixture, which contained 100  $\mu\text{l}$  of 250 mM arginine (dissolved in 50 mM glycine buffer; pH 9.7) and 100  $\mu\text{l}$  of the bovine mammary gland fraction containing the activated enzyme, was incubated at  $37^{\circ}\text{C}$  for 10 min. A freshly prepared solution of acidic monoxime was made by mixing 500  $\mu\text{l}$  of monoxime reagent (3% 2,3-butanedione monoxime in 95% ethanol) with 14.5 ml of acid solution (27% concentrated  $\text{H}_3\text{PO}_4$  and 9% concentrated  $\text{H}_2\text{SO}_4$ ). To stop the reaction, 1 ml of acidic monoxime solution was added to the samples that had been incubated at  $37^{\circ}\text{C}$ . Unincubated samples served as controls and contained all components;

the controls worked best when the acidic monoxime solution was added to arginine before the addition of the bovine mammary gland fraction. The tubes were vortexed, stoppered, and heated at  $100^{\circ}\text{C}$  for 30 min to complete the urea colorimetric response. The mixture was then centrifuged at  $10,800 \times g$  for 15 min at  $25^{\circ}\text{C}$  with a TOMY microcentrifuge (Peninsula Laboratories Inc., Belmont, CA). The upper pellicle was removed by suction, and the infranatant solution (ca. 0.6 ml) was carefully transferred into a 1-cm path semi-microcuvette. Optical density was recorded at 490 nm with a Beckman DU 650 spectrophotometer (Beckman Instruments, Fullerton, CA). The extinction coefficient of urea for the arginase assay was determined: 10 to 90  $\mu\text{l}$  of 10 mM urea in 50 mM glycine buffer (pH 9.7) were reacted with arginine and acidic monoxime solution at  $100^{\circ}\text{C}$  for 30 min. Neither  $\text{Mn}^{2+}$  activation nor incubation at  $37^{\circ}\text{C}$  was necessary, but the volume of urea samples needed to be adjusted with the 50 mM glycine buffer (pH 9.7).

Assays for the ratio of NADPH:cytochrome *c* reductase activities and succinate dehydrogenase activity were conducted as previously described (1). Citrate synthase activity was measured spectrophotometrically according to the procedure for the bovine mammary gland as described by Basch et al. (2, 3). Readings were recorded at 412 nm with a spectrophotometer (Beckman DU 650).

### Protein Assay

Protein was determined using the Pierce bicinchoninic acid protein assay reagent (Pierce Chemical Co., Rockford, IL) using the room temperature protocol. Bovine serum albumin was the standard.

### Enzyme Data Analysis

Nonlinear regression analyses of Michaelis curves and the estimates for parameter variance obtained as a function of concentration were carried out using the program Abacus, which is based on the Gauss-Newton iterative method (7). Choices between fits of models and statistical methods of analysis of the nonlinear fits were as described by Farrell et al. (10). For plots of velocity (*V*) against concentrations of substrate, [*S*], that deviated from standard patterns, data were fitted with equations that were originally derived from Wyman's theory of thermodynamic linkage and subsequently adapted for use in enzyme kinetics experiments (10). The simplest expression used in these experiments was an expansion of the general Michaelis constant ( $K_m$ ) expression:

$$V_{\text{obs}} = \frac{V_{\text{max}}[S]^n}{K_m^n + [S]^n} \quad [1]$$

where  $V_{\text{obs}}$  = observed V, and  $V_{\text{max}}$  = maximum V. For enzyme kinetics in general,  $n = 1$ ; for cooperative mechanisms,  $n > 1$ .

## RESULTS

### Reaction Conditions

Preliminary studies of lactating bovine mammary glands showed arginase activity in both mitochondrial and cytosolic fractions. The accuracy of the enzymatic assay was tested on both fractions. The arginase assay at 37°C was found to be linear at incubation times up to 60 min for both sample fractions. According to the method of Jenkinson and Grigor (12), an incubation time of 10 min is sufficient for the assay. Furthermore, undiluted bovine mammary gland fractions worked best with the assay. Centrifugation has a vital role in the assay. When the heated (100°C) samples and controls were not centrifuged, the absorbance was high, and the difference in absorbance between the sample and the control was small because the bovine mammary gland fractions contained lipid, which floats and can form an insoluble precipitate. When centrifugation was used, the interfering pellicles and precipitates were removed, the absorbance was lower, and accuracy was improved. With these modifications, the assay (12) appeared to work quite well with mammary fractions. The optimal pH for arginase enzymatic activity for both mitochondrial and cytosolic fractions was between 9 and 10; maximal activity was at pH 9.7 for both fractions, which is in agreement with arginase activity from other sources (8, 13, 16) and from the mammary gland of the rat (12).

### Heat Activation

The bovine mitochondrial and cytosolic fractions were activated at different temperatures for 5 min with  $\text{Mn}^{2+}$  in the standard assay (Figure 1). In general, both mitochondrial and cytosolic fractions showed significant activation with  $\text{Mn}^{2+}$ . On average, the degree of activation was 80% for the mitochondrial fraction and 130% for the cytosolic fraction. As shown in Figure 1, the two fractions, although differing in specific activity, were similar because their highest catalytic activity occurred after preincubation at 55°C. The results in Figure 1 are from one preparation; similar data were obtained with two other

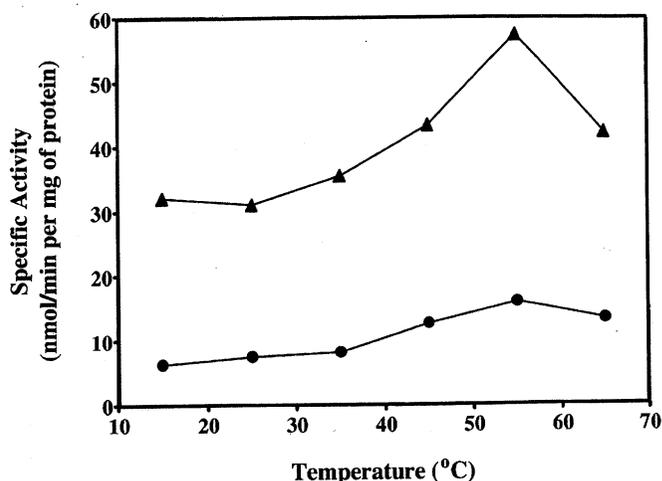


Figure 1. Temperature of activation curves for arginase of mitochondrial (▲) and cytosolic (●) fractions of the bovine mammary gland. Enzymes were activated at different temperatures for 5 min and then assayed under standard conditions at 37°C; results are means of triplicate determinations at each temperature for preparations from an individual cow; mean coefficient of variation is 5%.

preparations from two separate cows. These data are in agreement with the results of Jenkinson and Grigor (12) for rat mammary glands, which demonstrated that 55°C was the optimal temperature of activation for the cytosolic enzyme.

### Subcellular Distribution

The subcellular distribution of arginase in bovine mammary tissue is presented in Table 1. These subcellular preparations were made from frozen mammary tissues of three cows. Table 1 shows that the specific activity was the greatest in mitochondrial and cytosolic fractions; the ratio of the specific activity of the subcellular fraction to the specific activity of the homogenate was greatest for the same two fractions. Despite the fact that the specific activity and the ratio of the bovine fraction to homogenate were highest in the mitochondrial fraction, arginase yield in the cytosolic fraction was 4.5 times that in the mitochondrial fraction.

The 62% arginase yield in the cytosolic fraction (Table 1) could reflect mitochondrial breakage during freezing and thawing of the tissue or mitochondrial disruption during mincing and homogenization of the tissue. To test the effect of freezing and thawing or tissue damage, citrate synthase was assayed in three fractions. Table 2 shows the subcellular distribution of citrate synthase from the same preparations of bovine mammary tissue. The results for citrate

TABLE 1. Subcellular distribution of arginase in preparations from lactating mammary glands.

Bovine fraction <sup>1</sup>	Specific activity		Yield		Arginase: homogenate	Enzyme marker ratio <sup>2</sup>
	(nmol/min per mg of protein)		— (%) —			
	$\bar{X}$	SD	$\bar{X}$	SD		
Homogenate	19.1	6.2	...	...	...	1.0
Nuclear	18.3	3.5	2	1	1.0	...
Mitochondrial	36.6	3.5	14	7	1.9	5.7 <sup>3</sup>
Microsomal	9.4	5.6	3	1	0.5	7.0 <sup>4</sup>
Cytosolic	31.1	12.6	62	13	1.6	...

<sup>1</sup>Means and standard deviations from three preparations from three cows. Three assays were performed for each preparation.

<sup>2</sup>Mean values for the three preparations.

<sup>3</sup>Succinate dehydrogenase assay; ratio of specific activity in the mitochondrial fraction to specific activity in homogenate.

<sup>4</sup>Determined by NADPH:cytochrome *c* reductase assay; ratio of specific activity in the microsomal fraction to specific activity in the homogenate.

synthase were similar to those obtained for arginase (Table 1). After homogenization, bovine mammary glands showed 71 to 81% breakage of the mitochondrial fraction.

To determine whether arginase activity remained bound to the membrane in the purified mitochondrial preparations, the mitochondrial fraction was frozen, thawed, and centrifuged at 10,000 rpm prior to activation at 55°C. Without activation by Mn<sup>2+</sup>, greater than 80% of the activity remained in the pellets. However, when the samples were first treated with Mn<sup>2+</sup> at 55°C, nearly 95% of this activity became soluble and did not sediment with the pellets. In conclusion, arginase was still associated with mitochondrial membranes during the fractionation of the bovine mammary gland tissues but was released during Mn<sup>2+</sup> activation.

### K<sub>m</sub> Kinetics

Enzymes in the bovine mammary gland differ considerably in K<sub>m</sub> kinetics. In this study, the mammary arginase preparations yielded K<sub>m</sub> values of 49.5 ± 9.8 and 18.5 ± 2.4 for mM for the mitochondrial (Figure 2) and cytosolic (Figure 3) fractions, respectively. These values were computed from direct nonlinear fits to velocity versus substrate concentrations as previously described (10). The plots of velocity against substrate concentration for the mitochondrial and cytosolic fractions exhibited distinct differences in cooperativity at *n* = 1 and 2, respectively, as given in Equation [1]. For the cytosolic fraction, *n* = 2 occurred in all three preparations.

### Effector Studies

The influence of compounds containing nitrogen, amino acids, heavy metals, and polyamine second messengers on arginase activity was tested. Table 3 illustrates that none of the selected compounds were potent inhibitors, or activators, after heat activation at 55°C. The arginase activity was partially inhibited by a heavy metal, Hg<sup>2+</sup>, but not by *p*-hydroxymercuribenzoate. Arginase activity was partially inhibited at a very high concentration of proline (10 mM). An interesting note is that putrescine, spermine, and spermidine did not stimulate arginase activity much. These second messenger compounds contain diamine groups that generally affect enzyme activity for fractions related to membranes (19).

### DISCUSSION

Arginase is most commonly associated with liver function, but is also found in many extrahepatic tissues. The hepatic form of arginase is usually located

TABLE 2. Subcellular distribution of citrate synthase in preparations of lactating mammary glands.

Bovine fraction <sup>1</sup>	Specific activity		Yield		Ratio to homogenate
	(nmol/min per mg of protein)		— (%) —		
	$\bar{X}$	SD	$\bar{X}$	SD	
Homogenate	137	48	...	...	...
Mitochondrial	219	55	15	3	1.6
Cytosolic	128	30	51	10	0.9

<sup>1</sup>Means and standard deviations from three preparations from three cows. Three assays were performed for each preparation.

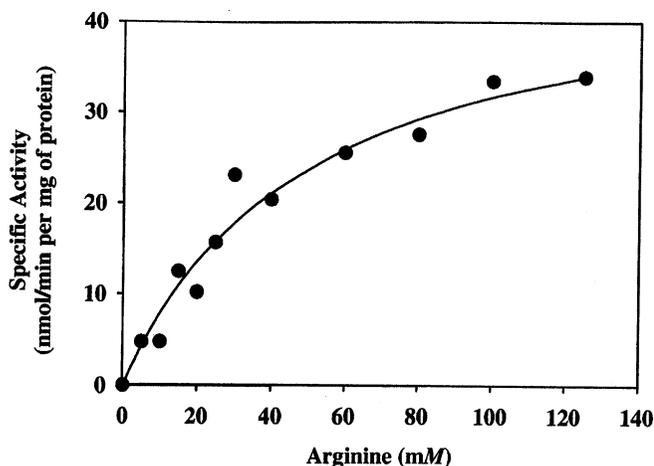


Figure 2. Change in the velocity of the arginase reaction in the mitochondrial fraction of the bovine mammary gland from one cow as concentrations of arginine substrate increased (duplicate analysis at each point). The overall fit was at the 95% confidence level. Similar results were obtained for two other cows, and the results are given in the text as the mean Michaelis constants.

in the mitochondria and is associated with urea cycle functioning in ureotelic (urea-excreting) species; the enzyme is often absent or occurs at low levels in uricotelic (uric acid-excreting) species (12, 13, 16).

Results from our study showed that arginase is present in the lactating bovine mammary gland. The specific activity in the crude homogenate is 19.1 nmol/min per mg of protein and is highest in the mitochon-

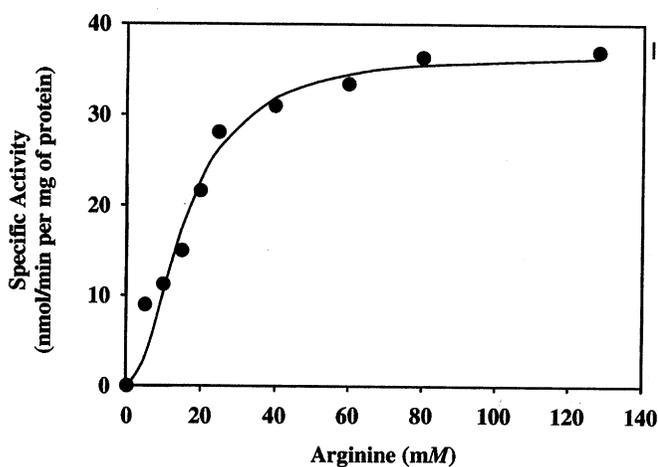


Figure 3. Change in the velocity of the arginase reaction in the cytosolic fraction of the bovine mammary gland as concentrations of arginine substrate increased. Note the cooperativity displayed here as  $n = 2$  in the equation  $V_{obs} = (V_{max}[S]^n)/K_m^n + [S]^n$ , where  $V_{obs}$  = observed velocity,  $V_{max}$  = maximum velocity,  $[S]$  = concentration of substrate, and  $K_m$  = Michaelis constant.

drial fraction, but the yield is greatest in the cytosolic fraction. Some arginase is located in the microsomal fraction (Table 1). Apparently, there is considerable breakage of the mitochondrial fractions of the bovine mammary gland, as was evidenced by parallel occurrence of citrate synthase and arginase in the cytosolic fractions. The data for subcellular distribution of citrate synthase in this report were very similar to those reported by Basch et al. (2, 3). Using citrate synthase as a marker, in this study, at pH 7.0, the frozen bovine mammary glands averaged 70% breakage of the mitochondrial fraction compared with a mean of 69 and 64% breakage in two previous studies (2, 3). At pH 7.4, with Tris buffer and fresh bovine tissue, Farrell et al. (10) reported 30% mitochondrial breakage. In contrast, at pH 6.7, breakage of mitochondria in fresh mammary tissue of the rat was 47% (2, 3). Apparently, subtle changes in homogenization buffer can alter the fragility of bovine mitochondria during the vigorous homogenization needed to disrupt mammary connective tissue. Some evidence suggests that there are two forms of arginase, a membrane-associated form and a soluble (cytosolic) form. Evidence for two forms of arginase in the bovine mammary gland arises from kinetic data because mitochondrial activity has a higher  $K_m$  and a different cooperativity. However, Jenkinson and Grigor (12) reported the occurrence of two isozymes of arginase in the mammary gland of the rat. The major form, AII, is characteristic of extrahepatic arginase, and AI appeared to be similar to hepatic arginase. The AII form increased 300-fold at midlac-

TABLE 3. Inhibitory and stimulatory effects of selected compounds on arginase activity.<sup>1</sup>

Compound	Concentration (mM)	Effect (%)	
		Cytosolic	Mitochondrial
Urea	1	+4	+4
Guanidine	1	-15	-4
Proline	5	-6	-1
Proline	10	-24 <sup>2</sup>	-10
HMB <sup>3</sup>	0.1	-10	+5
HgCl <sub>2</sub>	0.01	-21 <sup>2</sup>	-11
Putrescine	1	-2	-3
Putrescine	5	-11	+8
Spermine	1.25	-4	-4
Spermidine	1.25	+6	-1

<sup>1</sup>Mean values for three preparations (three assays each) are reported as percentages. + indicates activation; - indicates inhibition.

<sup>2</sup>Values that are two times the standard deviation for the mean of the three preparations.

<sup>3</sup>*p*-Hydroxymercuribenzoate.

tation over values found in the midpregnant gland. Aside from minor differences in isoelectric point and immunochemistry, the two mammary forms in the rat were kinetically indistinguishable from each other. The  $K_m$  values ranged from 12 to 14 mM. In this study with bovine mammary glands, examination of the kinetic behavior of the mitochondrial and cytosolic fractions showed that the apparent  $K_m$  for the cytosolic fraction,  $18.5 \pm 2.4$  mM, was in accord with previously reported values from the mammary gland, liver, and kidney of the rat. The value of 49.5 for the mitochondrial fraction contrasted greatly with the cytosolic fraction as well as with those from other tissues with the exception of some invertebrates (17) and a few plant species (8, 14, 21). The mitochondrial enzyme reported here was not studied directly in the mammary gland of the rat (12). In this study, we washed the mitochondrial pellets and retained the arginase with the membranes. In the study using the mammary glands of rats, the supernatant (cytosolic) fractions and pellet washes were used to purify the enzyme. It is possible, as reported by Jenkinson and Grigor (12), that upon liberation from mitochondrial membranes, the two isozymes are kinetically indistinguishable but that AI (mitochondrial form) exhibits differing kinetic properties when assayed in association with mitochondrial membranes. However, activation at 55°C with  $Mn^{2+}$  appeared to liberate the enzyme from the membranes, although they were not removed prior to assay.

Purified arginase from plant mitochondria is stimulated by polyamines (14). However, this was not the case in our study; polyamines were without effect (Table 3). With respect to mammary function, as reported by Jenkinson and Grigor (12), the cytosolic enzyme rises in response to lactation. The  $K_m$  for the curve for the cytosolic fraction from bovine mammary tissue displayed cooperativity as is shown in Figure 3 with  $n = 2$  in the equation. Such cooperativity could refer to activation induced by substrates,

which has been reported for uricotelic arginases (16). The cytosolic enzyme appears more likely to be related to mammary gland production of amino acids from arginine, which is present in higher concentrations (Table 1, is corrected in light of Table 2), and, if similar to the function of rat mammary gland, increases with stage of lactation. The mitochondrial enzyme may function only in response to greatly elevated arginine concentrations, hence, its greater  $K_m$ .

For arginase present in the lactating mammary gland, the specific activity in the crude homogenate was 19.1 nmol/min per mg of protein, which was similar to activities found in previous studies (2, 3) for OAT and P5C reductase when assayed under their optimum conditions in the homogenate (17.6 and 16.9 nmol/min per mg of protein, respectively) (Table 4). The specific activities and the ratios of the three enzymes to homogenate were highest in the mitochondrial fraction, but the yield was greatest in the cytosolic fraction. Studies on the uptake of proline by mammary cells (6, 15, 18) have indicated that insufficient proline is taken up to support casein production by fully lactating cells, which would indicate a need for proline biosynthesis within the mammary gland. The mechanism for conversion of arginine to proline may involve arginase, OAT, and P5C reductase (Figure 4). The first step in the conversion of arginine to proline is the action of arginase to yield ornithine and urea. The second step in this conversion is the action of OAT to produce  $\gamma$ -semialdehyde that cyclizes to yield P5C. This enzyme has previously been shown to occur in lactating tissue (3). The third step is the reduction of P5C to form proline, and P5C reductase was recently shown in lactating bovine mammary gland (2). The conversion of arginine to ornithine and the subsequent action of OAT couples the reaction product of isocitrate dehydrogenase,  $\alpha$ -ketoglutarate, to protein synthesis. In general, isocitrate dehydrogenase has been thought to be as-

TABLE 4. Comparison of subcellular distribution of three enzymes in preparations from lactating mammary glands.

Bovine fraction	Specific activity			Yield			Ratio to homogenate		
	Arginase	OAT <sup>1</sup>	P5C Red. <sup>2</sup>	Arginase	OAT	P5C Red.	Arginase	OAT	P5C Red.
	(nmol/min per mg of protein)			(%)					
Homogenate	19.1	17.6	16.9	...	...	...	...	...	...
Mitochondria	36.6	46.5	24.0	14	28	16	1.9	2.6	1.4
Microsomes	9.4	2.0	12.0	3	1	6	0.5	0.1	0.7
Cytosol	31.1	19.0	15.8	62	56	56	1.6	1.1	0.9

<sup>1</sup>Ornithine- $\delta$ -aminotransferase (3).

<sup>2</sup>L- $\Delta^1$ -Pyrroline-5-carboxylate reductase (2).

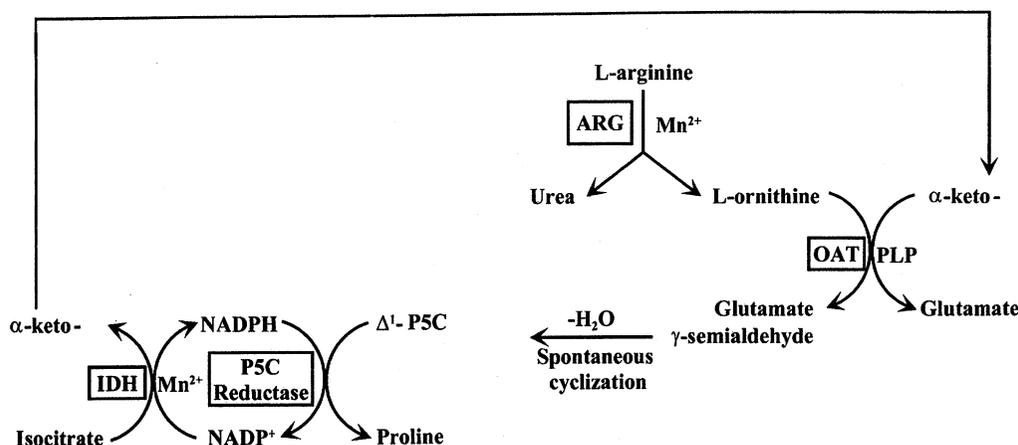


Figure 4. Proposed pathway for proline production in the lactating bovine mammary gland. IDH = Isocitrate dehydrogenase, P5C = pyrroline-5-carboxylate, OAT = ornithine- $\delta$ -aminotransferase,  $\alpha$ -keto- =  $\alpha$ -keto-glutarate, and ARG = arginase. The OAT uses the cofactor pyridoxal phosphate (PLP).

sociated only with fat synthesis, but, through this pathway, it provides a carbon skeleton for glutamic acid and glutamine synthesis. Furthermore, proline synthesis is completed by the action of P5C reductase. This latter enzyme, as discussed by Basch et al. (2), most likely uses NADPH (the second product of isocitrate dehydrogenase) as a reductant. Thus, the relationship of isocitrate dehydrogenase to protein synthesis is 2-fold in that it provides carbon skeletons and reducing equivalents for the three most abundant casein components, glutamate, glutamine, and proline.

For this pathway to be fully operative, some shuttling of carbon skeletons across the mitochondrial membranes must occur. As discussed previously, the soluble (cytosolic) form of arginase is important; OAT is definitely mitochondrial (3), but P5C reductase can be recruited to the mitochondrial surface by polyamines (2, 19). Conversely, isocitrate dehydrogenase, is almost completely cytosolic (9). Therefore,  $\alpha$ -ketoglutarate and ornithine must enter the mitochondria, and P5C and glutamic must emerge. Transferases for these metabolites are known in other tissues but have not been completely described in the mammary gland (M. Grigor, 1996, personal communication).

Using the starting assumptions of Waghorn and Baldwin (20), a moderately productive cow with a 21.7-kg udder could easily produce 15 kg/d of milk. Based on a mean casein content of 2.89% (5), the production is 433 g/d or 18.8 mmol/d of casein or roughly 3785 mmol/d of amino acids incorporated into casein protein. Because casein contains 12.3 mol of

proline/100 mol of amino acids (H. M. Farrell and H. J. Dower, 1994, unpublished data), 465 mmol/d or 323  $\mu$ mol/min of proline are incorporated into casein, and 15 nmol/min of proline per g of tissue are incorporated into casein. This value compares well with the mean proline incorporation (21 nmol/min per g of tissue) that was calculated from the overall data of Cant et al. (4). Basch et al. (3) reported that 960 nmol/min per g of tissue of P5C, the immediate precursor of proline, would be produced if OAT were to function near maximum velocity. Another study by Basch et al. (2) showed the potential to produce 610 nmol/min per g of tissue of proline if P5C reductase operated near maximum velocity. The comparable maximum velocity of isocitrate dehydrogenase is 43,000 nmol/min per g of tissue (9). The current study suggests that 1000 nmol/min per g of tissue of ornithine could be produced if arginase operated near maximum velocity. These values signify that, under theoretical conditions, arginase could convert arginine to ornithine, and P5C reductase could convert almost two-thirds of the P5C produced by OAT to proline; the pathway proposed by tracer studies appears to be present and potentially active.

## CONCLUSIONS

The goal of milk producers is to increase protein production and reduce fat production. To accomplish this goal, a better understanding of the mechanisms that control milk protein synthesis and secretion, coupled with an assessment of the rate-limiting steps in milk protein production, could offer new sites of fu-

ture intervention to alter the ratio of protein to fat. This study reported on the occurrence of arginase in lactating mammary glands of cow and compared the results of the crude enzyme preparations with OAT and P5C reductase in similar preparations. The indication was that very high  $K_m$  for arginase needs to be considered for a possible pathway to casein synthesis.

### ACKNOWLEDGMENT

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