the exception that arginine was omitted. The urea nitrogen in the blanks varied from 0.0 mg in the case of the dry glands to as much as 0.43 mg in the case of lactating glands. The amounts of urea nitrogen from 5 ml of glycerol extract as compared to 5 ml of glycerol extract and arginine hydrochloride are given in Table I.

From these data it is obvious that the active mammary gland contains appreciable arginase while the inactive gland contains less or none at all as revealed by the methods used.

It will be noted in the table that the amount of arginase in the mammary gland extract is proportional to the amount of preformed urea in the mammary gland extract, as shown by the blank determinations. This urea was probably formed in the gland or gland extract by arginase and urea precursors in the gland. Additional controls demonstrated that the arginine used did not contain free urea.

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Amino Acids and other Non-Protein Nitrogen Blood Substances in Relation to Milk Secretion.

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Since Cary[1] demonstrated that the amino-acid content of blood obtained from the mammary vein of lactating cows was significantly lower than that obtained from the jugular vein, it has been assumed that the amino-acids of the blood are the precursors of the milk proteins. Recently Graham,[2] and Shaw and Petersen[3] have presented evidence that the blood amino-acids are not the sole precursors of milk proteins. Graham, *et al.*,[4] have also demonstrated that the blood proteins may be involved in the protein metabolism of the mammary gland.

* The data in this paper are from a thesis presented by J. C. Shaw in partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of Minnesota, Minnesota Agricultural Experiment Station.

1 Cary, C. A., *J. Biol. Chem.*, 1920, **43**, 477.

2 Graham, W. R., Jr., *J. Biol. Chem.*, 1937, **122**, 1.

3 Shaw, J. C., and Petersen, W. E., *Proc. Am. Physiol. Soc.*, March 30, 31, April 1, 2, 1938, 50th Annual Meeting, Baltimore, Maryland, p. 183.

4 Graham, W. R., Jr., Peterson, V. R., Houchin, O. B., and Turner, C. W., *J. Biol. Chem.*, 1938, **122**, 275.

Data are presented in this paper which show that the amino-acids of the blood cannot account for more than 30 to 40% of the milk proteins.

Arterial and mammary venous bloods were obtained and handled as reported by Shaw, Boyd and Petersen.[5]

Amino-acids were determined in tungstomolybdic acid filtrates by the colorimetric method of Folin.[6] The apparent creatine and creatinine in blood were determined on the same filtrate by the method of Folin and Wu.[7] Uric acid was determined on blood plasma by Benedict's direct colorimetric method.[8] The Clark-Collip[9] modification of the Kramer-Tisdall method was employed for the determination of calcium. Urea was determined on blood plasma samples by the urease method of Van Slyke and Cullen.[10, 11]

Cary[1] made no attempt to determine the quantity of amino-acids which would have to be removed by the mammary gland to account for all of the proteins of the milk. In order to obtain a more complete picture of the nitrogen metabolism of the gland several non-protein fractions were determined on simultaneously drawn arterial and mammary venous bloods. Of these, herein are reported amino-acids, uric acid, urea, creatine and creatinine. Data are presented in Table I comparing the amino-acid and calcium arterio-venous difference in the passage of the blood through the mammary gland.

Amino-acids were removed from the blood by the gland in fairly constant amounts with an extreme variation of from 0.15 mg % to 0.97 mg %. The average arterio-venous difference was 0.46 mg %. On the basis of the protein in the milk this indicates that approximately 1200 volumes of blood per unit volume of milk would be necessary to supply enough amino-acids to account for the milk proteins if the amino-acids were the sole precursors of milk proteins. The average calcium loss of 0.32 mg % indicates that the volume flow of blood per unit volume of milk is approximately 390.

This is in good agreement with similar ratios calculated from fat and carbohydrate A-V differences and the quantity of fat and carbohydrate in the milk, Shaw and Petersen.[3] It is evident then that the amino-acid absorption by the mammary gland is much too small to

[5] Shaw, J. C., Boyd, W. L., and Petersen, W. E., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 579.

[6] Folin, O., *J. Biol. Chem.*, 1922, **51**, 377.

[7] Folin, O., and Wu, H., *J. Biol. Chem.*, 1919, **38**, 81.

[8] Benedict, S. R., *J. Biol. Chem.*, 1922, **51**, 187.

[9] Clark, E. P., and Collip, J. B., *J. Biol. Chem.*, 1925, **63**, 461.

[10] Van Slyke, D. D., and Cullen, G. E., *J. Biol. Chem.*, 1914, **19**, 211.

[11] Van Slyke, D. D., and Cullen, G. E., *J. Biol. Chem.*, 1916, **24**, 117.

TABLE I.
Amino Nitrogen in Simultaneously Drawn Samples of Arterial and Mammary Venous Bloods of Cows as Related to Calcium A-V Differences.

Amino nitrogen level		Plasma calcium		
Arterial, mg %	Venous, mg %	A-V difference, mg %	A-V difference, mg %	Remarks
3.92	3.25	—0.66	—0.31	2 hr. after milking
4.38	3.86	—0.52	—0.24	
3.72	3.26	—0.46	—0.54	
3.85	3.36	—0.49	—0.35	
3.08	2.93	—0.15	—0.19	3 " " "
4.23	3.72	—0.51	—0.30	
3.33	3.04	—0.29	—0.20	
3.76	3.23	—0.53	—0.32	4 " " "
3.30	3.05	—0.25	—0.46	
3.90	3.25	—0.65	—	
4.49	4.08	—0.31	—0.51	9 " " "
4.40	4.09	—0.31	—	
4.13	3.97	—0.16	—0.14	
4.28	3.31	—0.97	—0.29	
3.53	3.51	—0.02	—0.07	dry cow
Avg of lactating cows				
3.90	3.44	—0.46	—0.32	

account for all of the milk proteins. If all of the amino-acids absorbed by the mammary gland from the blood were converted into milk proteins there would still be about 65% of the milk proteins to be accounted for by some other nitrogen substances in the blood. Graham[2] conducted a carbohydrate balance experiment on the mam-

TABLE II.
Uric Acid in Simultaneously Drawn Samples of Arterial and Mammary Venous Bloods.

Cow No.	Uric acid level		Arterial Venous Difference, mg %
	Arterial, mg %	Venous, mg %	
241	1.50	1.32	—.18
445	1.72	1.30	—.42
235	1.39	1.34	—.05
432	1.50	1.65	+.15
447	1.64	1.55	—.09
419	1.79	1.72	—.07
433	1.80	1.73	—.07
23	1.43	1.36	—.07
25	1.41	1.41	0
A-7	1.76	1.82	+.06
586	1.75	1.79	+.04
Avg	1.60	1.55	—.05

mary gland and reported 2 observations on amino-acids in which the amino-acid loss when compared to the glucose and lactic acid loss appeared to be too small to account for the milk proteins. In a later paper Graham, *et al.*,[4] presented experimental data indicating that the blood proteins were involved in the protein metabolism of the mammary gland.

The fact that the amino-acids cannot account for the major portion of the milk proteins lends support to the hypothesis that the blood proteins are involved in the elaboration of milk proteins.

The data presented in Table II show that only very small amounts of blood uric acid are removed by the lactating mammary gland. In 11 analyses there was an average uric acid loss of –0.05 mg %.

Data are given in Table III on the arterio-venous creatinine and creatine differences. While there was a loss of creatinine and creatine in the majority of cases, the amounts absorbed by the mammary gland were extremely small. The average loss of each was 0.06 mg %.

TABLE III.
"Apparent" Creatine and Creatinine in Simultaneously Drawn Samples of Arterial and Mammary Venous Bloods.

Cow No.	Creatinine level		Arterial Venous Difference, mg %	Creatine level		Arterial Venous Difference, mg %
	Arterial, mg %	Venous, mg %		Arterial, mg %	Venous, mg %	
443	1.29	1.20	—.09	2.23	2.16	—.07
445	1.56	1.35	—.21	1.63	1.75	+.12
235	1.31	1.28	—.03	1.73	1.73	0
432	1.49	1.45	—.04	2.12	2.06	—.06
447	1.37	1.35	—.02	2.23	2.04	—.19
577	1.07	1.07	0	2.07	1.88	—.19
434	1.25	1.21	—.04	2.03	2.04	+.01
241	1.20	1.12	—.08	—	—	—
419	1.27	1.25	—.02	—	—	—
440	1.22	1.16	—.06	—	—	—
Avg	1.30	1.24	—.06	2.01	1.95	—.06

Conclusions. 1. The amino-acids absorbed from the blood by the mammary cannot account for more than 35% of the milk proteins. 2. While there is a slight loss of blood uric acid, creatine and creatinine to the lactating mammary gland, it is evident that these substances are not important in the nitrogen metabolism of the lactating gland.