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M. F. MILLER, *Director*

Studies on the Blood Precursors of Milk Protein

E. P. REINEKE, V. E. PETERSON, O. B. HOUCHIN, AND C. W. TURNER

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SUMMARY

The globulin differences obtained on arterial and venous samples of blood drawn simultaneously from lactating goats show that the mammary gland causes considerable shifting of increments of globulin from one fraction to another, but the uptake of globulin is not confined to any specific fraction.

During early lactation there is a consistent uptake of fibrinogen by the lactating mammary gland. From the 70th day of lactation onward, the mammary gland rather consistently returns fibrinogen in excess to the venous blood.

Data are presented showing that the mammary gland may absorb fibrinogen, globulin or albumin from the blood stream, with a return to the venous blood of an excess of one or more of these substances. It is suggested that the above proteins may undergo a reversible series of transformations in the mammary gland from albumin to globulin to fibrin. Portions of these proteins are apparently utilized in milk protein synthesis and the remainder returned to the blood stream.

The uptake of relatively large amounts of non-protein nitrogen suggests that unidentified portions of this fraction are concerned in milk synthesis. The absorption of amino acid nitrogen in normally lactating goats averages about 0.7 milligrams per 100 cc. of plasma. In goats fasted for 14 to 18 hours, but still lactating, the mammary gland is frequently in negative balance for amino acid nitrogen.

Arterio-venous differences of total nitrogen of the blood and plasma frequently show the mammary gland to be in negative balance for total nitrogen.

Analyses of samples drawn at consecutive intervals show that the variations in total nitrogen content of both arterial blood or plasma, from moment to moment, are great enough to mask in some cases the amount of nitrogen that would actually be involved in milk secretion.

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The mammary gland is the final assembly plant of the dairy animal, taking from the blood stream the products of the digestion of grains and roughages and converting them into milk, a uniform and highly refined food.

Studies of changes in the constituents of the blood in its passage through the mammary gland of lactating animals should give us information as to the exact nature and amount of each of the substances absorbed by the gland and utilized in the synthesis of milk, thus leading eventually to a better understanding of the whole mechanism of milk secretion. Such information holds great possibilities for practical results. A knowledge of the exact nutritional requirements of the mammary gland will be of fundamental value in determining more efficient methods of feeding for the production of milk and butterfat. Secondly, identification of the major precursors of milk will open the way to quantitative studies on the specific effect of the lactation stimulating hormones. This should eventually result in the revealing of information of prime importance for the breeding of more profitable dairy cattle.

REVIEW OF LITERATURE

Recent studies indicate that there is a close interrelationship between the chemical transformations taking place in the lactating mammary gland whereby substances absorbed from the blood are synthesized into milk sugar, protein and fat. There is evidence that significant amounts of amino acids are deaminized, thus leaving breakdown products that can be utilized in the formation of lactose. Other evidence suggests that there may be utilization of blood glucose in the synthesis of milk fat. Opposed to this is the theory that the fatty acids of the neutral fats of the blood are partially oxidized in the mammary gland in order to form the shorter chained fatty acids of milk and supply additional energy for the maintenance of the mammary gland. Thus there is the possibility of serious errors in the calculation and interpretation of data obtained by measuring the uptake of substances from the blood in its passage through the lactating mammary gland unless these interrelationships are given due consideration.

The recent investigations of Graham (1937) show that in addition to the uptake by the mammary gland of blood sugar, reported in earlier studies by Kauffman and Magne (1906), Herman and Turner (1932), Blackwood and Stirling (1932), Lintzel (1934), and Graham, Jones and Kay (1936), significant amounts of lactic acid are also taken up by the mammary gland from the blood circulating through it. Graham found it possible to obtain a carbohydrate balance for the lactating mammary gland only if both the glucose and lactic acid taken up were considered as precursors of milk sugar, and the absorbed amino acids were considered as being deaminized, leaving their residues as additional precursors for the formation of lactose. This calculation seemed justified by the fact that the urea produced in the mammary gland often exceeded the amount of amino acids that were found to be taken up at the same time. Calculations of the absolute amounts of milk precursors utilized were based on blood flow measurements by the thermostromuhr method.

The question of the carbohydrate exchanges of the lactating mammary gland is complicated further by the finding of Graham, Houchin, Peterson and Turner (1938) that the respiratory quotient of this gland is usually considerably above unity, indicating the formation of fat from carbohydrate, according to the classical method of interpretation.

Petersen and Shaw (1937) reported the synthesis of lactose *in vitro* from glucose and lactic acid in the presence of macerated mammary gland tissue, lending support to the concept of Graham (1937) that lactic acid is an important precursor of milk sugar.

In a later report Shaw and Petersen (1938) published results confirming Graham's observation that lactic acid is consistently taken up by the lactating mammary gland. Using the ratio of calcium absorbed to the amount of calcium in milk as a measure of the blood flow through the mammary gland, they showed that the arterial minus venous difference of glucose and lactic acid would be sufficient to account for all of the lactose produced. Their data on the uptake of fat indicated that more than enough fat is absorbed from the blood stream to account for all of the fat in milk. They postulated that the excess fat could be partially oxidized, thus accounting for the shorter chained fatty acids in milk. More extensive data on the uptake of blood glucose and lactic acid, with essentially the same findings as stated above, were published by Shaw, Boyd and Petersen (1938).

The theory of Shaw and Petersen that the fat is partially oxidized by the mammary gland is hard to reconcile with the results reported by Graham, Houchin, Peterson and Turner (1938) in which it was

shown that the respiratory quotient of the lactating mammary gland is above unity, indicating the formation of fat from carbohydrate. Both conditions would hardly be compatible in the same gland. However, in view of the later report of Shaw and Petersen (1938b) that the uptake of fat varies considerably, increasing with the length of time after milking, it would seem questionable if the amount of fat taken up by the mammary gland at a given time is a strictly quantitative measure of the amount actually used in the synthesis of milk fat.

The fact that amino acids are removed from the blood in its passage through the lactating mammary gland was established by the experiments of Cary (1920), Blackwood (1932), and Lintzel (1934). Because the inactive mammary gland was found not to absorb amino acids from the blood stream, this was accepted as proof that the amino acids were the sole precursors of milk protein.

Graham, Peterson, Houchin and Turner (1938) demonstrated that the amino acids absorbed by the mammary gland were not sufficient in amount to account for all of the milk protein. In fact it was reported that the urea nitrogen returned to the venous blood often exceeded the amount of amino acid nitrogen absorbed from the arterial blood. The first observation was confirmed by Shaw and Petersen (1938), who reported that the amino acids taken up could not account for more than 40 per cent of the milk protein, even when it is assumed that they are used entirely for protein synthesis. Graham et al. reported further that in addition to amino acids, the actively lactating mammary gland of goats apparently utilizes globulin and undetermined fractions of the non-protein nitrogen of the blood for the synthesis of milk proteins. Their published data show a decrease in the globulin and an increase in the albumin fraction of the blood during its passage through the actively lactating mammary gland. They interpret this to indicate that the milk protein is probably not built up piece by piece from amino acids, but that part of the molecule of globulin is used for the synthesis together with fractions of the non-protein nitrogen, including amino acids, and that the unused portions are then returned to the blood stream.

Jackson and Gortner (1938) reported that the only significant difference in the nitrogen partition of mammary gland tissue from lactating as compared to non-lactating cows was in the albumin-globulin ratio. Inactive glands yielded more albumin than globulin, but this ratio was markedly reversed for the active glands. Nitrogen distributions of the protein fraction showed no significant differences that could be correlated with glandular activity. It was stated that

the tissues from lactating and non-lactating glands seemed to be characterized by physical differences such as peptization response rather than by differences in their amino acid content.

The experiments reported by Graham et al. (1938) indicated that the globulin fraction of the plasma protein is in some way concerned in the synthesis of milk proteins.

Therefore, it was believed that further partitioning of the plasma proteins might provide some enlightening data in regard to the identity of the nitrogen fractions involved in milk secretion. The report of such experiments is included in this paper, together with additional experiments that were devised in an attempt to determine a plan of procedure that would show quantitatively the amounts of nitrogen that are utilized in the process.

EXPERIMENTAL

The experimental animals used were actively lactating goats which had been prepared for arterial sampling by exteriorization of the carotid artery according to the method of Graham, Turner and Gomez (1937). Blood samples were drawn simultaneously from the mammary vein and carotid artery. In most cases the goats were tied in their stalls and allowed to stand quietly for several hours before bleeding. The samples were then drawn, as rapidly and with as little disturbance to the animal as possible, care being taken not to produce stasis in the mammary vein. All samples except those used for serum determinations were drawn into vessels coated with potassium oxalate. The collected blood was chilled immediately by immersing in ice water. All precipitations were carried out at once, and in no case were the blood or plasma samples allowed to stand over night.

Globulins, albumin and fibrin were determined by a modification of Howe's method (Reineke, Peterson and Turner, 1939). Other methods used were as follows: non-protein nitrogen, Folin and Wu (1919); amino acids, Danielson (1933); amide nitrogen, Bliss (1929); urea, Van Slyke (1927); cell volume, hematocrit; oxygen capacity, Sendroy (1931); hemoglobin, Newcomer (1919). The actual determination of nitrogen in the micro methods for fibrin, NPN and amide nitrogen were carried out by the manometric method of Van Slyke (1927).

Partition of the Globulins of Arterial and Venous Plasmas of Lactating Goats

The results of seven experiments in which the 3 globulin fractions of arterial and venous samples from goats in early lactation were determined in addition to a number of other constituents of the blood and plasma are given in Table 1. Insofar as the uptake of total globulins is concerned the results are inconclusive. Three experiments show an apparent uptake of globulins by the mammary gland while four indicate the return of this constituent to the blood stream. The total plasma nitrogen shows a negative balance for the mammary gland in 5 out of 7 cases. With one exception these values showed a shift in the same direction as the globulin nitrogen. From a study of the data it is apparent that the interchange of globulins between the mammary gland and the blood stream is not confined to any one of the three fractions—euglobulin, pseudoglobulin I or pseudoglobulin II. There appears to be a constant, but unpredictable shifting of small increments of globulin from one fraction to another. The excellent agreement obtained between duplicate precipitations in es-

TABLE 1.—NITROGEN PARTITION OF ARTERIAL AND MAMMARY VENOUS BLOOD AND PLASMA OF LACTATING GOATS

Goat No.		Blood							Plasma						
		Total Nitrogen	NPN	Urea Nitrogen	Amide Nitrogen	Amino acid Nitrogen	Total Nitrogen	Euglobulin Nitrogen	Pseudo-globulin I Nitrogen	Pseudo-globulin II Nitrogen	Total globulin Nitrogen	Albumin Nitrogen	Fibrin Nitrogen	NPN	Amino acid Nitrogen
		Mg.%	Mg.%	Mg.%	Mg.%	Mg.%	Mg.%	Mg.%	Mg.%	Mg.%	Mg.%	Mg.%	Mg.%	Mg.%	Mg.%
403	A	2563	....	....	54.9	..	1294	127	286	148	561	619	87.5	23.7	7.1
	V	2555	....	....	55.6	..	1300	110	294	170	574	619	82.3	23.7	7.0
	A-V	+8	....	....	—0.7	..	—6	+17	—8	—22	—13	0	+5.2	0	+0.1
258	A	2674	....	....	...	..	1740	492	393	166	1051	550	87.1	51.4	..
	V	2618	....	....	...	..	1759	502	415	128	1044	593	74.2	47.5	..
	A-V	+56	....	....	...	..	—19	—10	—22	+38	+7	—43	+12.9	+3.9	..
258	A	2627	43.0	....	105.0	7.8	1683	480	377	90	947	595	103.1	37.6	6.9
	V	2639	40.4	....	101.7	7.8	1714	502	378	106	986	594	95.9	38.0	5.7
	A-V	—12	+2.6	....	+3.3	0	—31	—22	—1	—16	—39	+1	+7.2	—0.4	+1.2
254	A	2771	45.5	....	93.0	7.8	1482	166	329	225	719	610	110.5	41.8	5.7
	V	2751	46.6	....	85.7	7.3	1434	190	301	192	683	609	98.5	42.8	5.3
	A-V	+20	—1.1	....	+7.3	+0.5	+48	—24	+28	+33	+36	+1	+12.0	—1.0	+0.4
254	A	2283	57.4	24.5	78.2	7.9	1245	98	336	90	524	607	88.2	25.3	8.4
	V	2326	53.6	25.5	81.1	7.7	1251	131	321	121	573	582	75.9	20.3	7.3
	A-V	—43	+3.8	—1.0	—2.9	+0.2	—6	—33	+15	—31	—49	+25	+12.3	+5.0	+1.1
123	A	2344	36.3	31.2	103.6	6.9	1177	47	335	128	509	584	83.3	37.9	4.5
	V	2344	39.0	31.7	102.5	6.1	1201	79	332	121	531	602	67.7	40.0	3.6
	A-V	0	—2.7	—0.5	+1.1	+0.8	—24	—32	+3	+7	—22	—18	+15.6	—2.1	+0.9
254	A	2356	45.0	42.4	92.7	8.5	1300	196	295	136	627	572	55.6	45.8	8.7
	V	2381	50.1	46.0	95.4	7.8	1283	125	342	143	610	572	62.5	37.7	7.3
	A-V	—25	—5.1	—3.6	—2.7	+0.7	+17	+71	—47	—7	+17	—0	—6.9	+8.1	+1.4
Mean A-V Diff.		+0.6	—0.5	—1.7	+1.2	+0.5	—3.0	—4.7	—4.5	+0.3	—9.0	—4.8	+8.3	+1.9	+0.87

tablishing this method is strongly against the chance of these differences being entirely due to experimental error. Therefore, it is probable that this shifting of values is due to slight changes in the protein molecules brought about by the secretory processes taking place in the mammary gland.

All except one of the comparisons made show a significant uptake of fibrinogen by the mammary gland. Six experiments showed an uptake of fibrin nitrogen ranging from 5.2 mgs. to 15.66 mgs. per 100 cc. of plasma. One experiment showed a negative balance for fibrin nitrogen of 6.9 mgs., resulting in an average uptake for the 7 experiments of 8.3 mgs. Thus this series of experiments shows an apparent uptake by the mammary gland of approximately 10 per cent of the fibrinogen passing through it.

It is important to note here that all of the goats used were in early stages of lactation. Subsequent experiments showed a predominantly negative balance for fibrin nitrogen with advancing lactation.

The uptake of fibrin during early stages of lactation is compensated in part by the return of globulin or albumin or both to the mammary venous blood. This might suggest a cycle of reactions in the mammary gland involving the transformation of fibrinogen to globulin to albumin, with parts of the molecules being retained by the mammary gland to supply the nitrogen requirements of milk synthesis.

There was a small but persistent removal of amino acids by the lactating mammary gland, averaging 0.87 mg. per. 100 cc. of plasma in 6 comparisons. Three comparisons of the urea output showed that the urea produced approximately equalled the absorption of amino acids. It is realized that the number of analyses of urea nitrogen are too small to be of much significance in themselves, but they bear out the earlier observations of Graham et al. (1937) and Shaw and Petersen (1938). The non-protein nitrogen comparisons suggest that undetermined portions of this fraction other than the amino acids or urea are entering into the nitrogen metabolism of the mammary gland.

Nitrogen Exchanges of the Mammary Gland with Increasing Intervals After Milking

The foregoing experiments indicated that the apparent interchanges of the globulins between the blood stream and the mammary gland are not confined to any specific fraction of this protein, at least insofar as could be detected by the present methods of analysis. Therefore, 8 additional experiments were carried out, with the globulin fractionation omitted, in order to obtain additional data

on the uptake of protein and non-protein nitrogen constituents by the mammary gland with increasing intervals after milking.

The results are given in Table 2. In order to conserve space only the arterio-venous difference for each constituent is given. A plus sign before a figure means a decrease in the substance in the blood due to its passage through the mammary gland; a minus sign indicates an excess of the substance in the venous blood. Part of the data given in Table 2 are also contained in Table 1.

TABLE 2.—ARTERIO-VENOUS DIFFERENCES OF VARIOUS NITROGEN FRACTIONS WITH INCREASING INTERVALS AFTER MILKING

			Blood			Plasma						
Goat No.	Time After Milking Hrs.	Min.	Total Nitrogen	NPN	Amide Nitrogen	Total Nitrogen	Total globulin Nitrogen	Albumin Nitrogen	Fibrin Nitrogen	NPN	Amino acid Nitrogen	Remarks
			Mg.%	Mg.%	Mg.%	Mg.%	Mg.%	Mg.%	Mg.%	Mg.%	Mg.%	
403	0	10	+ 8	....	—0.7	— 6	—13	0	+ 7.2	— 0	+0.2	Fed and milked at regular time.
258	0	30	+56	....	....	—19	+ 6	—42	+12.9	+3.9	....	
258	2	00	—10	+2.6	+3.3	—32	—39	0	+ 7.2	—0.4	+1.2	
258	2	00	+64	+3.8	....	+10	+44	—32	— 4.4	+2.3	+0.7	Feed withheld in A.M. until sampling.
135	2	00	—19	+4.1	....	+10	+13	+ 6	— 8.6	—1.1	+0.1	
254	2	00	—38	....	....	....	—38	+ 7	— 6.1	—0.7	+0.4	
403	2	00	—13	....	....	....	—61	+23	+ 5.2	+1.2	+0.4	Fed and milked at regular time.
403	3	00	+15	—5.3	....	....	—33	+22	+ 9.2	—0.5	....	
254	3	00	+20	—1.0	+7.2	+48	+36	+ 1	+12.0	—1.0	+0.4	
254	4	00	—43	+3.7	—2.9	— 6	—49	+25	+12.2	+5.0	+1.2	
123	6	40	0	—2.7	+1.5	—24	+22	—16	+15.6	—2.0	+0.9	
254	8	30	—24	—5.0	—2.7	+17	+16	— 1	— 6.9	+8.2	+1.4	
442	7	30	—78	—7.8	....	—71	—54	— 3	—10.4	—3.3	+0.4	
254	9	00	—30	+0.5	....	—24	—16	+ 1	— 8.1	—0.8	+1.7	
403	9	00	—92	—0.6	....	—37	+23	—60	+ 7.7	+ .3	+0.2	
Mean			—12.3	—0.7	+1.0	—13.7	—9.5	—4.6	+ 3.0	+0.7	+0.7	

None of the data have been corrected for possible water losses from the venous blood to the mammary gland. However, calculations made on the basis of cell volume, hemoglobulin, or oxygen capacity determinations in the case of 11 of these experiments indicated that the negative values frequently obtained could not be wholly due to water losses.

Contrary to expectations, no consistent relationship was found between the length of time after milking and the uptake of total nitrogen of the blood or plasma by the mammary gland. Out of 15 pairs of blood samples, however, 9 were drawn within the first 3 hours, after milking, when one would expect to find a negative balance, in view of the work reported by Graham, et al. (1938). Of the samples ranging from 4 hours to 9 hours after milking, none showed a positive nitrogen balance for whole blood and only one showed an uptake of nitrogen for the plasma. Thus these data fail to confirm the report of

Graham, et al. that a positive nitrogen balance is obtained a few hours after milking. However, the results are not conclusive because of the limited number of experiments.

The uptake of amino acids by the mammary gland is fairly consistent, averaging 0.7 mgs. of nitrogen for 13 experiments. This is in good agreement with the earlier findings of Cary (1920), Blackwood and Stirling (1932), Lintzel (1934), Graham, et al. (1937), and Shaw and Petersen (1938).

Fibrin Exchange of the Mammary Gland with Advancing Lactation

The data presented in Table 1 showed that during early lactation there is a significant uptake of fibrin by the lactating mammary gland. In blood samples taken during later stages of lactation it was found that the balance for fibrin became consistently negative. Table 3 shows the partition of the plasma proteins of the goat during late pregnancy and advancing lactation. The data show that during late pregnancy, and up to the seventieth day of lactation the arterial blood is uniformly and significantly higher in fibrin than the venous blood indicating the absorption of fibrin by the mammary gland. From the seventieth to the 125th day of lactation there is considerable fluctuation of fibrin values from positive to negative. From about the 125th day of lactation, the lactating mammary gland shows a rather uniformly negative balance for fibrin nitrogen.

The data for the plasma proteins as a whole are made difficult of interpretation because of the fact that the mammary gland is in negative balance for total nitrogen in 23 out of the 36 experiments listed. However, the shifting of increments of nitrogen from one protein fraction to another might give a qualitative indication of some of the reactions taking place in the mammary gland even though they do not show quantitatively the amount of nitrogen actually involved in milk secretion.

The only significant change that can be correlated with advancing lactation is the change of fibrin from a positive to a negative balance. The arterio-venous differences for globulin and albumin show great variability. In 4 out of the 12 cases in which a positive balance was obtained for total nitrogen, there was an uptake of globulin, a relatively smaller uptake of albumin, and a return of fibrin to the blood stream. Other experiments show an absorption of fibrin and albumin, and a return of globulin to the blood. Still others show the uptake of only albumin, and the return to the blood stream of both fibrin and globulin.

TABLE 3.—ARTERIO-VENOUS DIFFERENCES OF THE PLASMA PROTEINS WITH ADVANCING LACTATION

Goat No.	Time in days	Total Nitrogen	Globulin Nitrogen	Albumin Nitrogen	Fibrin Nitrogen
	Ante Partum	Mg.%	Mg.%	Mg.%	Mg.%
258	7	+10	+39	−28	+ 2.8
536	Late pregnancy	....	....	....	+ 6.3
536	" "	−49	−109	+54	+11.5
	Post Partum	..	....	....	....
841	10	−30	−86	+44	+ 6.2
403	37	− 6	−13	0	+ 7.2
258	38	−32	−39	0	+ 7.2
258	45	−19	+ 6	−42	+12.9
254	58	+48	+36	+ 1	+12.1
123	65	−24	−22	−16	+15.6
258	70	− 3	....	....	− 0.5
254	70	− 6	−49	+25	+12.2
258	76	− 4	....	....	− 5.5
403	73	−12	....	....	− 7.2
254	79	+17	+16	− 0	− 6.9
254	81	− 7	....	....	+ 1.4
403	85	+ 7	− 0	+16	− 5.1
254	87	+19	....	....	− 4.5
123	91	−16	....	....	+ 8.7
836	95	+15	+15	+ 7	− 7.1
403	98	− 2	−33	+22	+ 9.2
254	99	+ 4	−18	+13	+ 3.2
403	102	+ 5	+29	−20	− 9.0
123	104	−15	− 8	− 9	+ 2.3
836	111	+11	+17	+ 9	−15.1
403	115	−32	−61	+23	+ 5.2
836	117	−12	−40	+27	− 5.5
403	124	−37	+23	−61	+ 7.7
258	136	+10	+44	−33	− 4.4
254	139	−38	−38	+ 7	− 6.1
254	148	−24	−16	+ 1	− 8.1
442	Advanced Lactation	−71	−54	− 3	−10.4
135	"	−36	− 4	−41	− 5.8
135	"	−35	−12	−17	− 5.2
135	"	+10	+13	+ 6	− 8.6
443	"	+19	+12	+10	− 1.2
443	"	− 2	−38	+50	−12.2

The Effect of Hourly Milking on the Arterio-Venous Difference for Total Nitrogen

Graham, et al. (1938) reported that the mammary gland is in negative balance for nitrogen during the first few hours after milking, and that the balance becomes positive in a few hours. In the experiments reported thus far, the venous blood often exceeded the arterial blood in its total nitrogen content, although no uniform relationship was observed between the nitrogen balance and the time after milking. The experiments of Turner and Garrison (1936) showed that in dairy cows milk secretion is a continuous process, proceeding at a uniform rate from milking to milking. Further unpublished data from experiments on the dairy goat show that goats may be milked at intervals of as little as one-half hour for a period of 24 hours with a surprisingly constant output of milk at each milking. Such a constant process as this hardly seems compatible with the concept of a periodic storage and release of nitrogen in the mammary

gland. Even though corrections for water losses failed to show a concentration of the blood in its passage through the mammary gland, it was believed that the negative balances for total nitrogen might be caused by the considerable drop in hydrostatic pressure of the udder incidental to the removal of milk, and the increase of pressure that takes place when the mammary gland becomes distended with milk.

In order to reduce these factors to a minimum, 14 experiments were conducted in which the goats were milked out at 3 successive 1-hour intervals immediately before drawing arterial and venous blood samples. The morning feed was also withheld until after sampling, resulting in a fast of 14 to 18 hours.

TABLE 4.—THE EFFECT OF MILKING AT HOURLY INTERVALS ON THE NITROGEN PARTITION OF ARTERIAL AND MAMMARY VENOUS BLOOD AND PLASMA**

Goat No.	Time After Milking (Minutes)	Blood		Plasma					
		Total Nitrogen	NPN	Total Nitrogen	Total Globulin Nitrogen	Albumin Nitrogen	Fibrin Nitrogen	NPN	Amino Acid Nitrogen
		Mg.%	Mg.%	Mg.%	Mg.%	Mg.%	Mg.%	Mg.%	Mg.%
135	24	—84	—0.2	—86	— 4	—41	— 5.7	+13.6	—0.5
443	27	—15	—1.5	+19	+12	+10	— 1.2	— 1.2	....
123	28	—49	+4.0	—12	—19	+ 3	— 0.2	+ 4.0	+0.1
836	33	+81	—2.4	+11	+17	+ 9	—15.1	— 0.4	+0.7
135	40	—83	+1.0	—35	—12	—17	— 5.2	— 0.7	—0.7
403	45	+13	+3.9	+ 5	+29	—20	— 9.0	+ 4.8	+0.4
254*	45	....	....	—15	+52	—68	.....	+ 1.2	+0.2
443*	55	....	....	—10	+11	—21	.....	+11.7	....
443	55	— 9	....	— 2	—38	+49	—12.2	— 1.7	....
123	45	+ 9	—3.9	—15	— 8	— 9	+ 2.3	+ 1.5	....
836	53	+34	—0.2	—12	—37	+27	— 5.5	+ 2.8	—0.4
836	60	+66	+3.9	+15	+15	+ 7	— 7.1	— 0.3	....
403	60	—68	+4.8	+ 7	0	+16	— 5.1	— 3.1	....
254	60	+19	+1.0	+ 4	—18	+13	+ 3.2	+ 5.4	....
Mean		—7.2	+0.2	—5.4	0	—3.0	— 5.1	+ 2.7	—0.03

*Determination made on serum.
**Feed withheld 14 to 18 hours and goats milked at 3 one hour intervals before drawing blood sample.

The arterio-venous differences of nitrogen following this plan of treatment are given in Table 4. The differences for total nitrogen and the plasma proteins were also included in Table 3, showing the effect of advancing lactation on nitrogen exchanges of the mammary gland. Contrary to expectations the balances for total nitrogen continued to fluctuate from positive to negative. However, the A-V differences were confined to a much narrower range than was obtained in the preceding experiments.

Unfortunately the amino acid differences were obtained in only 7 out of the 14 experiments. It was surprising to find that in animals handled as outlined above, the amino acid content of the mammary

venous blood is occasionally higher than that of the arterial blood. In 4 cases there was a small positive balance of amino acids, and in 3 cases a slight excess of amino acids seemed actually to be returned to the venous circulation. This is further evidence that the amino acids do not play a major role in the synthesis of milk proteins. In further explanation of these results it should be stated that the amino acid level of the blood was considerably lower in these partially fasted goats than it is in goats that have been fed normally before sampling.

The non-protein nitrogen data show a relatively large uptake by the mammary gland in 8 cases. Although some negative values were obtained for this fraction as a whole, the average for the 14 experiments was +2.7 mg. per 100 cc. of plasma. The recent work of Shaw and Petersen (1938) has shown that the uptake by the lactating mammary gland of uric acid, creatine and creatinine is negligible. The experiments of Graham, et al. (1938) as well as the present report, show that positive differences of this magnitude cannot be accounted for by absorption of amino acid or urea nitrogen. Therefore, it is apparent that an unidentified fraction of the non-protein nitrogen of the blood is taking part in the nitrogen metabolism of the mammary gland.

Variations in Total Nitrogen and Fibrin Concentration of the Blood

In order to determine whether or not some of the arterio-venous differences observed in the case of fibrin, and total nitrogen of the blood and plasma could be attributed to disturbances of the animal or length of time taken in sampling, 6 experiments were run in which 3 consecutive arterial and venous samples of blood were drawn from the same goat at intervals about 1 minute apart.

Ten cc. samples of blood were drawn into separate containers coated with potassium oxalate, and the fibrin and total nitrogen were determined by the methods stated previously.

The results are given in Table 5. It will be noted that for fibrin the values for both arterial and venous blood remained quite constant over the period of approximately 1 minute to 4½ minutes that were taken to draw the three consecutive samples.

Any disagreement between the arterio-venous differences in each series of consecutive samplings should indicate the amount of experimental error involved in the method used, due either to actual changes in concentration of a given substance in the blood or to analytical errors. The range of differences between the consecutive samplings run from 0.43 to 1.52 mg. for the fibrin analyses. The arterio-venous differences obtained for this constituent are considerably higher than the above variations.

TABLE 5.—FIBRIN AND TOTAL NITROGEN LEVELS OF ARTERIAL AND VENOUS BLOOD AND PLASMA DRAWN AT SUCCESSIVE ONE MINUTE INTERVALS

Goat No.	Sample No.	Fibrin Nitrogen			Total Nitrogen-Blood			Total Nitrogen-Plasma		
		Arterial	Venous	Arterial Minus Venous	Arterial	Venous	Arterial Minus Venous	Arterial	Venous	Arterial Minus Venous
		Mg.%	Mg.%	Mg.%	Mg.%	Mg.%	Mg.%	Mg.%	Mg.%	Mg.%
254	1	73.2	71.2	+2.0	2402	2466	—64	1251	1258	— 7
	2	73.3	71.0	+2.3	2407	2423	—16	1251	1263	—12
	3	73.7	72.5	+1.2	2466	2393	+73	1251	1251	0
258	1	64.5	64.8	—0.3	2378	2390	—12	1317	1309	+ 8
	2	64.2	64.1	+0.1	2425	2466	—41	1300	1312	—12
	3	65.0	66.5	—1.5	2387	2429	—42	1302	1300	+ 2
403	1	78.2	86.0	—7.8	2352	2381	—29	1246	1258	—12
	2	81.3	87.7	—6.4	2393	2378	+15	1251	1261	—10
	3	79.6	87.0	—7.4	2369	2356	+13	1249	1262	—13
254	1	77.1	81.5	—4.4	2351	2303	+48	1241	1215	+26
	2	76.9	81.6	—4.7	2390	2347	+43	1239	1239	0
	3	76.5	81.0	—4.5	2318	2313	+ 5	1251	1222	+29
123	1	75.6	67.8	+7.8	2364	2337	+27	1236	1256	—20
	2	76.5	67.4	+9.1	2332	2381	—49	1215	1222	— 7
	3	75.5	68.1	+7.4	2332	2296	+36	1198	1220	—22
258	1	64.4	69.9	—5.5	2259	2281	—22	1270	1251	+19
	2	64.6	70.9	—6.3	2301	2293	+ 8	1234	1263	—29
	3	63.9	69.7	—5.8	2306	2301	+ 5	1249	1258	— 9

TABLE 6.—PER CENT VARIATION IN FIBRIN AND TOTAL NITROGEN OF ARTERIAL AND MAMMARY VENOUS BLOOD AND PLASMA SAMPLES DRAWN AT SUCCESSIVE INTERVALS.

Goat No.	Total Nitrogen-Blood				Total Nitrogen-Plasma				Fibrin Nitrogen			
	A_1/A_2	A_1/A_3	V_1/V_2	V_1/V_3	A_1/A_2	A_1/A_3	V_1/V_2	V_1/V_3	A_1/A_2	A_1/A_3	V_1/V_2	V_1/V_3
254	99.79	97.43	101.75	103.04	100.00	100.00	99.63	100.59	99.91	99.32	100.32	98.28
258	98.09	99.90	96.93	98.39	101.30	101.10	99.80	100.74	100.48	99.15	100.93	97.35
403	98.28	99.29	100.10	101.03	99.60	99.80	99.81	99.71	96.15	98.29	98.11	98.95
254	98.37	101.46	98.12	99.57	100.19	99.22	98.03	99.40	100.41	100.81	99.87	100.77
123	101.35	101.35	98.15	101.77	101.79	103.24	102.69	102.89	98.64	100.00	100.47	99.47
258	98.18	97.98	99.46	99.14	102.95	101.75	99.02	99.41	99.81	100.78	98.64	100.21
Mean	99.01	99.56	99.08	100.49	100.97	100.85	99.82	100.45	99.23	99.72	99.72	99.17

The total nitrogen values for whole blood showed considerably more variation, the A-V differences within a series differing by amounts ranging from 137 mgs. to 30 mgs. per 100 cc. The values for the total plasma nitrogen showed somewhat better agreement, fluctuating within a series, by amounts of from 3 mgs. to 48 mgs. per 100 cc. of plasma.

In Table 6 the amount of nitrogen found in the first sample of each series is expressed as the percentage of the second and third samples. This gives the percentage of variation between samplings and should also provide some indication as to the amount of error that is due to actual variations of concentration of nitrogenous constituents in the blood and plasma during the period of sampling. A check on the method used in this laboratory for the determination of total nitrogen in blood shows that the experimental error of the method is not in excess of 0.5 per cent. The error of the plasma nitrogen analysis is usually within 0.1 per cent and never more than 0.5 per cent.

The differences obtained on whole blood from consecutive samplings range from —2.5 per cent to +1.46 per cent in the arterial blood and from —3.07 per cent to +3.04 per cent for the venous blood. The fact that the values for arterial blood fluctuate as well as those for the venous blood, would indicate that these differences are probably not due to cessation of milk secretion because of the disturbance incidental to drawing the sample, nor would it appear that they could be due to stasis caused by pressure unintentionally exerted on the blood vessel sampled.

Thus it would appear that the total nitrogen content of whole blood is not entirely constant, but that it varies within narrow limits, from moment to moment. These variations, though small, are larger than would be required to account for the total amount of nitrogen that must be removed from the blood for the synthesis of milk.

The data for plasma show slightly less variation than those for whole blood, ranging from —1.78 per cent to +3.24 per cent in arterial plasma and from —1.97 to +2.69 per cent for venous plasma. These fluctuations in nitrogen values are sufficient in amount to mask in some cases the arterio-venous differences that are actually due to milk secretion.

It should be stated here that the time taken for drawing the three consecutive samples was longer than that allowed for sampling as a regular procedure. When single samples of arterial and venous blood were drawn for our regular experiments they were taken as rapidly as possible and were discarded if the total bleeding time exceeded 2½

minutes. However, the data in Tables 5 and 6 do not show a consistent relationship between the nitrogen values obtained and the length of time taken to draw the samples. It appears that the fluctuations in total nitrogen values obtained with consecutive sampling is due to actual fluctuations in the level of nitrogen in the blood. It is not possible to state at this time whether these variations are due to changes in the water balance, whether they represent the periodic release of nitrogen compounds, from storage depots in the body, or whether some other mechanism is involved.

DISCUSSION

The data reported in this paper confirm the earlier reports of Graham, et al. (1938) and Shaw and Petersen (1938) that the amino acids absorbed by the mammary gland are insufficient to account for the nitrogen contained in milk.

The amino acid level of mammary venous blood is lower than that of arterial blood by approximately 0.7 mg. per 100 cc. of blood. According to data that have accumulated in this laboratory (Peterson and Turner, 1939), the average nitrogen content of normal goat milk is 555 mg. per cent. If the amino acids were to supply this nitrogen requirement, a volume of blood flow through the mammary gland of nearly 800 cc. per cc. of milk would be required. The actual rate of blood flow through the mammary gland is approximately 150 to 250 cc. per cc. of milk (Graham, Houchin, Peterson and Turner, 1938). Furthermore, the amount of urea nitrogen produced in the mammary gland often equals or exceeds the amount of amino acid nitrogen taken up. Thus it is apparent that the mammary gland makes demands upon the blood stream for nitrogenous substances other than amino acids for the synthesis of milk proteins.

The partition of the plasma proteins of arterial and mammary venous blood into fibrin, albumin and the three globulin fractions show considerable shifting of values between these fractions, due to the passage of the blood through the lactating mammary gland. However, the data show considerable variability. In a number of cases there is an uptake of globulin, a relatively smaller uptake of albumin and a return of fibrin to the blood stream. It is not unusual to find the apparent absorption of fibrin and albumin by the mammary gland and a return of globulin to the blood. Other experiments show an uptake of only albumin and a return to the blood stream of both fibrin and globulin. This shifting of nitrogen values is large enough in most cases that it appears unlikely that the variations could be attributed entirely to experimental error. However, a point that cannot be dis-

regarded is the fact that venous blood is about 6.5 volumes per cent higher in its carbon dioxide content than arterial blood. The effect that this difference in carbon dioxide concentration of the two bloods would have upon the partition of the proteins cannot be stated at this time.

If we disregard the possible effect of experimental error upon the results outlined above, the explanation is suggested that the plasma proteins are capable of undergoing a series of reversible transformations in the mammary gland involving changes from fibrin to globulin to albumin. This process might involve as suggested by Graham, et. al. (1937), the partial breakdown of the protein molecule, with portions being used for synthesis of milk proteins, and the residues being returned to the bloodstream. Another explanation would be that the blood proteins are not broken down to any appreciable extent, but that the molecules themselves are used as building blocks in the synthesis of the casein molecule. If, as is generally assumed, the synthesis of milk protein is activated by an enzyme system, it may be that these enzymes have the capacity to convert the albumin of the blood stream into the larger molecules of globulin or fibrin, or to break these molecules down to albumin, according to the equilibrium of the moment. If a particular protein is absorbed in excess, it might be converted to either a larger or smaller molecule, this process continuing until a form is reached that is stable to the enzyme system present. A hypothesis such as this is highly theoretical, and has little support in the way of decisive proof as to reactions which the blood proteins will undergo in a complex physico-chemical system such as the mammary gland. However, it would explain the variability observed in the uptake and discharge by the mammary gland of blood globulins, albumin and fibrin.

The most uniform and consistent data obtained upon the exchange of plasma proteins between the blood stream and the mammary gland are those reported for fibrin. Following a consistent uptake of fibrin during the first 70 days of lactation, the fibrin balance of the mammary gland becomes consistently negative subsequent to this period.

Data presented in Table 5 show that the amounts of the non-protein nitrogen taken up by the mammary gland in a majority of cases are too large to be accounted for by the amino acids or urea. Petersen and Shaw (1938) have published experiments ruling out uric acid, creatine and creatinine as precursors of milk nitrogen. Thus, it appears that an unidentified fraction or fractions of the non-protein nitrogen of the blood is participating in the nitrogen metabolism of the mammary gland. Additional investigation is needed on this phase of the problem.

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