The Uptake of Plasma Constituents by the Mammary Gland of the Sow

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1. Measurements were made of arterial and coccygeal concentrations of plasma constituents and of arteriovenous differences across the mammary gland in two anaesthetized lactating sows, and of coccygeal-mammary-venous differences in three conscious sows when lactating and again later when 'dry'. 2. With the possible exception of acetate concentration, the compositions of arterial and coccygeal plasma were similar, and arteriovenous differences in the anaesthetized lactating sow corresponded closely to coccygeal-venous differences in the conscious animal. 3. In the 'dry' sow coccygeal-venous differences were in all instances small. 4. In the lactating sow there were large arteriovenous (or coccygeal-venous) differences (mean value as a percentage of arterial or coccygeal concentration) in glucose (31%), acetate (46%), arginine (27%), glutamate (42%), histidine (26%), isoleucine (36%), lysine (25%), leucine (39%), methionine (38%), phenylalanine (32%), proline (31%), threonine (22%), tyrosine (32%) and valine (27%), and in palmitate (19%), oleate (23%), linoleate (21%) and stearate (16%) of the plasma triglycerides. The values for the following constituents were in all instances small: β -hydroxybutyrate, acetone + acetoacetate, citrate, lactate, alanine, glycine, aspartate, palmitoleate of the plasma triglycerides, phospholipids, cholesterol, cholesteryl esters and free fatty acids. 5. Of the total recorded uptake of plasma constituents by the lactating gland, 59% was accounted for by glucose, 28% by amino acids, 11% by plasma triglycerides and 2% by acetate. The relative uptakes of glucose and amino acids were higher in the sow than values reported previously for the goat, and the relative uptakes of acetate and triglycerides much less.

Studies of the uptake of blood plasma constituents by the mammary gland and of their quantitative contribution to the synthesis of individual milk constituents have been undertaken almost exclusively in ruminant animals. Because of the major differences between ruminant and non-ruminant animals in the products of digestion, information for the ruminant may not apply to the non-ruminant, and studies comparable with those undertaken in the cow and goat are therefore being carried out with the pig.

The method most commonly employed for the identification of the precursors of milk constituents in the blood plasma has been the arteriovenous-difference technique. Its limitations have been discussed by Folley (1949), Barry (1961) and more recently Linzell (1966). For an accurate assessment of the quantitative contribution of individual plasma constituents, not only has a measurement to be made of blood flow across the udder, but special precautions have to be taken to ensure that the samples of arterial and venous blood are wholly representative of the mammary-arterial supply and of the venous drainage respectively. There is little

published information on the circulatory system of pig mammary units, and in this work the arteriovenous-difference technique was used in a limited qualitative way to identify the major precursors.

Although it proved comparatively easy to maintain functional catheters in the mammary vein, catheters inserted into the mammary artery under total anaesthesia did not function adequately when the animal had recovered consciousness, and comparisons of mammary-venous and mammary-arterial blood could not be made in the conscious animal. However, mammary-arterial and coccygeal blood were found to differ only slightly in composition in the anaesthetized animal. In the conscious animal, coccygeal blood has therefore been taken as equivalent to mammary-arterial blood.

MATERIALS AND METHODS

Animals and their management. Three sows from the University herd of Large White pigs were used. They were housed in open pens and piglets were kept with the sows when they were lactating. The feeding routine was normal.

Experimental procedure. Mammary-arterial, mammary-venous and coccygeal blood samples were taken simultaneously under Nembutal anaesthesia from two of the sows in their fifth or sixth week of lactation. Mammary-venous and coccygeal samples were taken (under conditions of minimal disturbance) from all three animals when conscious in the fifth or sixth week of lactation and again 10–14 days later when 'dry'. Mammary-arterial and mammary-venous samples were withdrawn through a catheter into a heparinized syringe. The catheters were inserted previously under Nembutal anaesthesia. The major supply of arterial blood to the mammary gland in the sow is by a vessel, derived from the internal pudic artery, that commences in the inguinal region and runs between the mammary tissue and the body wall. The mammary vein is placed more laterally and less deeply than the artery. They were located most readily in the depressions between mammary units. Coccygeal samples were obtained from an incision of the tail and taken into a tube containing heparin. Blood plasma was prepared by centrifuging immediately at 0° and the plasma removed and used directly or stored at -20° until analysed.

Amino acids. To allow for possible losses arising from deproteinization or other steps in the procedure, norleucine was added to the original plasma (5 μ moles/10 ml. of plasma). Plasma proteins were precipitated by the addition of 5 vol. of 1% (w/v) picric acid (Hamilton & Van Slyke, 1943). Samples were centrifuged and excess of picric acid was removed from the supernatant by a Dowex 2 (Cl⁻ form) column (Stein & Moore, 1954). Free amino acids were analysed by an automatic amino acid analyser of the type described by Spackman, Stein & Moore (1958) and manufactured by Evans Electroscelenium Ltd. (Halstead, Essex), with Amberlite IR-120 resin.

Extractions of blood plasma lipids. Plasma was extracted by the method of Folch, Lees & Sloane-Stanley (1957). The antioxidant 2,6-di-*tert*.-butyl-*p*-cresol (butylated hydroxytoluene) was included in all solvents at the concentration recommended by Wren & Szezepanowska (1964). Heptadecanoic acid, glyceryl triheptadecanoate and cholesteryl heptadecanoate were added as internal markers to the methanol-chloroform (1:2, v/v) extract in amounts equivalent to 0.05, 0.1 and 0.5 mg./ml. of plasma respectively. Heptadecanoic acid was obtained from Koch-Light Laboratories Ltd. (Colnbrook, Bucks.), and the glyceryl triheptadecanoate and cholesteryl triheptadecanoate were each synthesized via the appropriate acyl chloride and purified by thin-layer chromatography. The extract was stored at -20°.

Thin-layer chromatography. The plates and solvent system used were the same as those of West & Rowbotham (1967). After separation of the lipid fractions the plates were sprayed with Ultraphor (Badische Anilin und Soda Fabrik A.-G., Ludwigshafen-am-Rhein, Germany) and dried at 50°. The lipid bands were individually scraped off the plate and the lipids eluted with diethyl ether-light petroleum (b.p. 40–60°)-formic acid (50:50:1, by vol.).

Analysis of lipid fractions. Triglycerides and cholesteryl esters were transesterified by using sodium methoxide (Storry, Rook & Hall, 1967). The free fatty acids were esterified by using methanol-boron trifluoride in H₂SO₄ (Metcalf & Schmitz, 1961). The methyl esters were separated at 170° on a column of diethylene glycol succinate (20%, w/w) on Chromosorb W (hexamethyldisilazane-

treated; 60–80 mesh size) in a Pye model 410 instrument. Cholesterol was determined by a modification of the Liebermann-Burchard method (Huang, Chen, Wefler & Raftery, 1961) against a standard run in a side line on the same thin-layer plate. Phospholipids were determined as phosphorus (Chen, Toribara & Warner, 1956) on a sample of the methanol-chloroform extract.

Methods of analysis for other plasma constituents. The total steam-volatile fatty acids of plasma were determined by steam-distillation (Scarlsbrick, 1952) and the acids in the distillate separated by gas-liquid chromatography on a column of 5% Carbowax 20M-terephthalic acid on AW Chromosorb G (dichlorodimethylsilane-treated; 80–100 mesh). Details of other methods of analysis are as follows: glucose (Huggett & Nixon, 1957), ketone bodies (Reid, 1960), citrate (McArdle, 1955) and lactate (Scholz, Schmitz, Bücher & Lampen, 1959).

RESULTS

Comparison of concentrations of plasma constituents in mammary-arterial and coccygeal blood. The plasma concentrations of the various blood constituents in mammary-arterial and coccygeal blood from two anaesthetized lactating sows are given in Tables 1–3. For most constituents values agreed to within 5% and for all constituents, except acetate, to within 8%. The concentration of acetate in mammary-arterial blood was 12% higher in one sow and 13% in the other than the corresponding values for coccygeal blood. It appears that, with the possible exception of acetate, coccygeal blood may be taken as equivalent to mammary-arterial blood for the measurement of arteriovenous differences across the mammary gland.

Arteriovenous and coccygeal-venous differences. The arteriovenous differences for the various plasma constituents determined in each of the two sows under anaesthesia, and the mean coccygeal-venous differences in the conscious animals for a total of seven measurements in three sows when lactating and for five measurements in the same sows when 'dry', are given in Tables 1–3. For all constituents examined, including acetate, the arteriovenous differences in the anaesthetized animals were of the same order as the coccygeal-venous differences in the conscious animals. The values are therefore taken as equivalent and are referred to below jointly as 'arteriovenous differences'.

Barry (1961) concluded that, because of erratic variations in the arteriovenous difference of as much as $\pm 10\%$ of the arterial concentration, to be convincingly demonstrated an arteriovenous difference should be consistently greater than 20% of the arterial concentration. By this criterion, no constituent showed a significant uptake in the 'dry' sow. The highest percentage difference observed was a value of 12% for methionine, which

Table 1. *Plasma concentrations of glucose, acetate, β -hydroxybutyrate, acetone + acetoacetate, citrate and lactate in coccygeal blood, comparison of mammary-arterial and coccygeal concentrations and arteriovenous (or coccygeal-venous) differences across the mammary gland in lactating and 'dry' sows*

Results for anaesthetized animals are individual values for two sows. Results are given for the arterial concentration minus the coccygeal concentration calculated as percentages of the arterial concentration, and for the arteriovenous difference calculated as percentages of the arterial concentration. Results for conscious animals are the means $\pm$ S.E.M. for a total of seven samplings from three sows when lactating and for a total of five samplings from the same sows when 'dry'. Results are given for the coccygeal concentration expressed as mg./100 ml. of plasma, and for the coccygeal-venous difference expressed as mg./100 ml. of plasma (Δ) or calculated as a percentage of the coccygeal concentration ($\Delta\%$).

Constituent	Status of sow	Anaesthetized animals				Conscious animals		
		Arterial-coccygeal difference (%)		Arteriovenous difference (%)		Coccygeal concentration	Coccygeal-venous difference	
		(i)	(ii)	(i)	(ii)		(Δ)	(Δ%)
Glucose	Lactating	5	7	35	27	122.3 ± 4.0	37.5 ± 3.6	31 ± 2
	‘Dry’	—	—	—	—	92.0 ± 2.1	6.7 ± 10.1	7 ± 3
Acetate	Lactating	12	13	49	44	1.32 ± 0.08	0.61 ± 0.05	46 ± 2
	‘Dry’	—	—	—	—	1.04 ± 0.66	0.06 ± 0.02	6 ± 1
β-Hydroxybutyrate	Lactating	0	−5	3	10	1.93 ± 0.08	0.21 ± 0.05	11 ± 2
	‘Dry’	—	—	—	—	1.78 ± 0.01	0.06 ± 0.01	3 ± 0.5
Acetone + acetoacetate	Lactating	1	−6	7	5	0.58 ± 0.03	0.01 ± 0.08	2 ± 4
	‘Dry’	—	—	—	—	0.47 ± 0.01	0.01 ± 0.02	2 ± 2
Citrate	Lactating	−7	−5	2	1	1.65 ± 0.14	−0.01 ± 0.03	−1 ± 2
	‘Dry’	—	—	—	—	1.33 ± 0.18	0.04 ± 0.03	3 ± 3
Lactate	Lactating	−3	−5	11	5	12.0 ± 1.0*	1.4 ± 1.0*	12 ± 3
	‘Dry’	—	—	—	—	11.5 ± 1.2	0.5 ± 0.2	5 ± 2

* Expressed as mg./100 ml. of whole blood.

is present in plasma in low concentration. However, in the lactating sow many constituents showed an arteriovenous difference in excess of 20%.

Glucose, acetate, β -hydroxybutyrate, acetone + acetoacetate, citrate and lactate (Table 1). The largest percentage arteriovenous difference observed was for acetate (mean value 46%), but because of the low concentration of acetate in sow's blood the concentration difference was small (mean value 0.6 mg./100 ml.). For glucose the arteriovenous difference was in all instances greater than 20% and, in view of the high concentration in arterial blood, glucose must make a major contribution to mammary-gland metabolism. Arteriovenous differences in acetone + acetoacetate and citrate were small, but statistically significant ($P < 0.05$) mean differences of 10% and 11% respectively were obtained for β -hydroxybutyrate and lactate. The biological significance of these values is uncertain, but in view of their low concentrations in arterial blood the contributions of β -hydroxybutyrate and of lactate to mammary-gland metabolism must be quantitatively unimportant.

Amino acids (Table 2). Arteriovenous differences for arginine, glutamate, histidine, isoleucine,

leucine, methionine, phenylalanine, proline, tyrosine and valine were invariably at least 20% and their uptake by the gland may be considered established. For lysine and threonine the mean values were greater than 20%, although individual values fell below this value, and it appears probable that these amino acids, too, are usually taken up by the gland. The other amino acids analysed, alanine, glycine and aspartate, showed mean differences considerably less than 20% and, with the possible exception of aspartate, there was no evidence of uptake.

Phospholipids, cholesterol, cholesteryl esters, triglycerides and free fatty acids (Table 3). Arteriovenous differences in phospholipid, cholesterol, cholesteryl esters and free fatty acids were in all instances small: the mean values for cholesteryl esters and free fatty acids were 2% and -6% respectively. However, fatty acids of the triglyceride fraction showed consistent positive arteriovenous differences and for palmitate, oleate and linoleate the mean values were close to 20%. In the cow and the goat uptake of fatty acids is exclusively from the chylomicra and very-low-density-lipoprotein triglycerides. If this applies also to the sow the observed differences for

Table 2. *Plasma concentrations of amino acids in coccygeal blood, comparison of mammary-arterial and coccygeal concentrations and arteriovenous (or coccygeal-venous) differences across the mammary gland in lactating and 'dry' sows*

Details are as given for Table 1.

Constituent	Status of sow	Anaesthetized animals				Conscious animals		
		Arterial-coccygeal difference (%)		Arteriovenous difference (%)		Coccygeal concentration	Coccygeal-venous difference	
		(i)	(ii)	(i)	(ii)		(Δ)	$(\Delta\%)$
Aspartate	Lactating	3	-2	15	17	0.58±0.15	0.06±0.01	10±3
	'Dry'	—	—	—	—	0.36±0.06	0.03±0.02	8±4
Threonine	Lactating	-4	1	17	23	3.13±0.15	0.73±0.04	23±2
	'Dry'	—	—	—	—	2.76±0.13	0.14±0.09	5±3
Glutamate	Lactating	-1	-1	50	42	6.87±0.36	2.86±0.18	41±3
	'Dry'	—	—	—	—	4.82±0.25	0.17±0.06	4±1
Proline	Lactating	-1	-4	26	31	4.51±0.24	1.43±0.22	32±3
	'Dry'	—	—	—	—	4.47±1.48	0.15±0.04	3±1
Glycine	Lactating	1	1	7	1	6.93±0.68	-0.01±0.13	-1±1
	'Dry'	—	—	—	—	5.96±1.16	0.29±0.14	5±1
Alanine	Lactating	1	1	10	9	4.46±0.42	0.42±0.05	9±2
	'Dry'	—	—	—	—	3.71±0.48	0.14±0.05	4±1
Valine	Lactating	1	1	21	20	5.34±0.39	1.56±0.19	29±2
	'Dry'	—	—	—	—	6.04±0.62	0.21±0.12	4±2
Methionine	Lactating	-2	-6	37	39	0.93±0.10	0.35±0.03	38±2
	'Dry'	—	—	—	—	0.67±0.11	0.08±0.02	12±3
Isoleucine	Lactating	-1	-1	38	35	3.26±0.11	1.18±0.11	36±3
	'Dry'	—	—	—	—	3.68±0.65	0.36±0.11	10±3
Leucine	Lactating	-2	-5	29	24	4.90±0.26	1.91±0.25	39±4
	'Dry'	—	—	—	—	5.35±0.89	0.35±0.13	7±3
Tyrosine	Lactating	-5	4	35	26	2.84±0.13	0.95±0.15	33±4
	'Dry'	—	—	—	—	2.32±0.42	0.18±0.10	8±3
Phenylalanine	Lactating	-5	1	31	25	2.33±0.13	0.76±0.11	33±1
	'Dry'	—	—	—	—	2.17±0.31	0.10±0.05	5±2
Lysine	Lactating	6	-2	17	24	4.47±0.29	1.08±0.05	26±1
	'Dry'	—	—	—	—	4.29±1.12	0.23±0.15	5±2
Histidine	Lactating	1	0	23	21	2.47±0.02	0.61±0.02	25±1
	'Dry'	—	—	—	—	2.04±0.17	0.14±0.05	7±2
Arginine	Lactating	-1	-1	26	22	4.10±0.26	1.16±0.29	28±2
	'Dry'	—	—	—	—	3.71±0.29	0.21±0.13	6±3

palmitate, oleate and linoleate, and possibly also for stearate (mean difference 16%), are strong evidence for uptake by the gland. An uptake of palmitoleate, the other major fatty acid of the plasma triglycerides, was not, however, established.

DISCUSSION

The observed arteriovenous differences indicate that major precursors of milk constituents in the sow are glucose, amino acids (arginine, glutamate, lysine, histidine, isoleucine, leucine, methionine, phenylalanine, proline, threonine, tyrosine and valine) and palmitate, oleate and linoleate of the

plasma triglycerides; the quantitative contribution of acetate is small. Linzell, Mephram, Annison & West (1967) reported an average arteriovenous difference in certain plasma amino acids of 16.5 mg./100 ml., which is close to the mean value of 17.6 mg./100 ml. for the same amino acids in the present investigation. Other values reported by Linzell *et al.* (1967) were for whole blood. Assuming a plasma volume of 75%, the calculated mean plasma arteriovenous differences were for glucose, 28.0 mg./100 ml., for triglyceride fatty acids, 2.1 mg./100 ml., and for acetate, 1.3 mg./100 ml. These are to be compared with the mean values determined in the present investigation of 37.5, 7.2 and 0.6 mg./100 ml.

Table 3. *Plasma concentrations of lipids in coccygeal blood, comparison of mammary-arterial and coccygeal concentrations and arteriovenous (or coccygeal-venous) differences across the mammary gland in lactating and 'dry' sows*

Details are as given for Table 1.

Lipid constituent	Status of sow	Anaesthetized animals				Conscious animals		
		Arterial-coccygeal difference (%)		Arteriovenous difference (%)		Coccygeal concentration	Coccygeal-venous difference	
		(i)	(ii)	(i)	(ii)		(Δ)	($\Delta\%$)
Phospholipid	Lactating	3	8	1	1	101.8 $\pm$ 1.8	4.1 $\pm$ 1.1	4 $\pm$ 1
	'Dry'	—	—	—	—	103.1 $\pm$ 0.5	4.9 $\pm$ 1.8	5 $\pm$ 2
Cholesterol	Lactating	1	1	1	-5	30.1 $\pm$ 1.5	0.1 $\pm$ 0.4	1 $\pm$ 2
	'Dry'	—	—	—	—	26.2 $\pm$ 0.9	-0.1 $\pm$ 0.3	-1 $\pm$ 1
Triglyceride palmitate	Lactating	3	-1	19	22	10.9 $\pm$ 0.5*	2.1 $\pm$ 0.1*	19 $\pm$ 2
	'Dry'	—	—	—	—	13.0 $\pm$ 0.9	0.4 $\pm$ 0.1	3 $\pm$ 1
Triglyceride palmitoleate	Lactating	0	0	5	5	1.9 $\pm$ 0.1*	0.2 $\pm$ 0*	11 $\pm$ 3
	'Dry'	—	—	—	—	1.8 $\pm$ 0.1	-0.1 $\pm$ 0.1	-6 $\pm$ 6
Triglyceride stearate	Lactating	4	5	10	20	3.2 $\pm$ 0.3*	0.5 $\pm$ 0.1*	16 $\pm$ 1
	'Dry'	—	—	—	—	3.5 $\pm$ 0.5	0.2 $\pm$ 0	6 $\pm$ 1
Triglyceride oleate	Lactating	1	2	21	22	14.1 $\pm$ 1.0*	3.2 $\pm$ 0.1*	23 $\pm$ 1
	'Dry'	—	—	—	—	16.4 $\pm$ 0.4	0.1 $\pm$ 0.1	6 $\pm$ 1
Triglyceride linoleate	Lactating	6	-2	26	21	5.9 $\pm$ 0.4*	1.2 $\pm$ 0.1*	20 $\pm$ 1
	'Dry'	—	—	—	—	8.9 $\pm$ 0.3	0.4 $\pm$ 0.1	5 $\pm$ 1

* Concentration expressed as mg. of fatty acid/100 ml. of plasma.

Table 4. *Relative uptake of plasma constituents by the mammary gland in the sow and in the goat*

Results are expressed as percentages of the total uptake of constituents for which a significant arteriovenous difference has been demonstrated. The values for the pig are calculated from (i) the present results and (ii) those of Linzell, Mephram, Annison & West (1967) and for the goat from observations summarized by Barry (1964).

Constituent	Relative uptake by the mammary gland		
	Sow		Goat
	(i)	(ii)	
Acetate	2	3	13
Amino acids	28	34	18
β -Hydroxybutyrate	—	—	7
Glucose	59	58	37
Triglycerides	11	5	25

respectively. When arteriovenous differences in individual constituents are calculated as percentages of the total uptake, the two sets of results are in good agreement (Table 4).

There are, however, marked differences between values for the sow and those reported elsewhere for the goat (Table 4). Acetate, β -hydroxybutyrate and triglyceride fatty acids are relatively less

important in the sow. Since on average the fat content of sow's milk is considerably higher than that in the goat, direct incorporation of fatty acids of the plasma triglycerides into milk fat must be quantitatively less important in the sow than in the ruminant, and new synthesis within the gland must be correspondingly increased, and from glucose as opposed to acetate and β -hydroxybutyrate in the goat. The relative contribution of amino acids also is higher in the sow, which is consistent with the higher protein content of sow's milk than goat milk.

Although measurements of blood flow through the udder of lactating sows are not available, Linzell (1960) has shown that in the goat at peak lactation about 375 volumes of plasma flow through the udder for each volume of milk secreted. Application of this value to the arteriovenous differences observed with the lactating sow gives an estimated uptake of 14.1 g. of glucose, 6.2 g. of amino acids, 2.7 g. of triglyceride fatty acids, 0.21 g. of acetate and 0.7 g. of β -hydroxybutyrate/100 ml. of milk secreted, which on average (Rook & Witter, 1967) would contain approx. 5 g. of lactose, 6 g. of protein and 8 g. of fat.

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