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Mammary metabolism in lactating sows: arteriovenous differences of milk precursors and the mammary metabolism of [^{14}C]glucose and [^{14}C]acetate

By J. L. LINZELL and T. B. MEPHAM
Institute of Animal Physiology, Babraham, Cambridge
and E. F. ANNISON and C. E. WEST
Unilever Research Laboratory, Sharnbrook, Bedford

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1. The following techniques, which have been applied successfully to goats, were used to study mammary metabolism in lactating sows: (1) measurements of mammary arteriovenous (A-V) differences in milk precursors in the conscious undisturbed animal (five sows); (2) continuous intravenous infusion of [U-^{14}C]glucose with concomitant arterial and mammary venous blood sampling for measurement of mammary blood flow and specific radioactivity of glucose and CO_2 (one sow); (3) perfusion of the isolated gland in vitro (eight glands from four sows), with the inclusion of [U-^{14}C]glucose (two glands) and [U-^{14}C]acetate (two glands) in the substrate mixture.

2. Sow mammary tissue was similar to that of goats in its milk yield, blood flow, O_2 and glucose uptake per unit weight of tissue. As in goats, mammary uptake of glucose was many times that of the rest of the body and the total mammary tissue was utilizing about half of the total glucose entering the circulation. Glucose was a major source of milk lactose and glycerol and of mammary CO_2.

3. Of the plasma lipid components, only the triglyceride fraction was consistently and significantly removed by the gland. In contrast to the results obtained for the goat, both [U-^{14}C]acetate and [U-^{14}C]glucose carbon were used for milk fatty acid synthesis, and although the pattern of labelling of fatty acid from each precursor was similar, the formation of fatty acids from glucose was at least five times greater than that from acetate. Quantitative evaluation of the contribution of these precursors was not possible, but the RQ (1·09–1·63) suggest that in some instances it may have been considerable.

4. The substantial A-V differences of most plasma essential amino acids suggest that these are the sole precursors of the corresponding residues in the mammary synthesized protein. The low A-V differences for several non-essential amino acids suggest that these are synthesized in the gland; this suggestion is supported by the incorporation of glucose carbon into non-essential amino acid residues of casein observed in one experiment. However, in contrast to results with the goat, mammary absorption of serine was consistently large.

Although efficient lactation is of great economic importance in farm animals, little is known about milk secretion in pigs. The reasons for this are clear. Much of our knowledge of mammary physiology has been obtained from cows and goats of the docile dairy breeds that are easily milked and handled, and from which it is relatively easy to obtain arterial and mammary venous blood in order to study the mammary uptake of milk precursors. In contrast, most lactating sows are unsuitable experimental animals, being strong and intractable and unused to handling, and their superficial arteries and mammary veins are covered by a thick layer of fat. A further difficulty is that anaesthesia can reduce mammary metabolism (Linzell, 1960) and that conscious cows and goats must be handled with the minimum of disturbance in order to avoid alterations in mammary metabolism due to stress (Graham, Kay & McIntosh, 1936; Shaw & Petersen, 1939).

In the present work we have obtained blood samples from conscious undisturbed animals in their own pens with their piglets by catheterizing an artery and a mammary vein under anaesthesia 1–4 days earlier, as reported briefly by Linzell, Mepham, Annison & West, (1967). Apart from the intrinsic value of obtaining information about a previously neglected species, the pig is a convenient animal to use in studying the interesting differences which exist between ruminants and omnivores in the mammary metabolism of glucose. The rat, from which most of the earlier findings for omnivores have been obtained, is too small for many quantitative studies. Accordingly we have also studied the mammary metabolism of [^{14}C]glucose in one of the sows during the arteriovenous measurements and, in addition, have compared the metabolism of [^{14}C]glucose and of [^{14}C]acetate by isolated perfused sow mammary glands.

METHODS

Animals. Six sows (five Large White and one Landrace) were used; five in the conscious state were used in the studies of arteriovenous differences (Table 1) and four (nos. 1, 2, 5 and 6) were later used to provide mammary glands for perfusion in isolation (Table 2). The animals were kept under good commercial conditions and fed twice daily (at the rate of 1·5 kg plus 0·25 kg per piglet) a standard pelleted cereal mixture, containing about 2% fat and 15% crude protein.

Table 1. *Details of experimental sows*

Sow no.	Breed	Body-weight (kg)	Age (years)	Lactation	Days lactating	No. of piglets being suckled	Milk composition (g/100 ml)		
							Lactose	Fat	Protein
1	Landrace	206	4	5th	61	7	4·75	5·3	6·65
2	Large White	191	2	3rd	37	11	4·5	4·74	5·46
3	Large White	165	5	11th	43	10	3·3	6·0	4·5
3*	Large White	153	6	12th	43	6	4·65	6·5	4·4
4	Large White	190	7	12th	51	5	3·4	4·51	7·72
5	Large White	189	5	10th	12	11	3·0	4·25	4·25
6	Large White	174	3	7th	28	8	—	—	—

Mammary arteriovenous differences. In these experiments samples of arterial and mammary venous blood were taken from five conscious animals, which were under normal conditions in their own pens with their piglets. From 1 to 4 days before the experiment polyvinyl chloride catheters were inserted under general anaesthesia into an artery (carotid, internal saphenous or external pudic) and a mammary vein draining a functioning gland and brought to the surface of the skin in the flank, so that samples could subsequently be taken from the conscious animal without restraining it in any way. They were in fact taken whilst the animals were feeding or wandering around

the pen and once during suckling. Before all experiments the experimenter accustomed the sows to his presence by visiting them for several days to scratch their backs and bellies whilst giving them tit-bits; they were never handled roughly, i.e. pulled by the ear or tail or roped by the snout.

Table 2. *Sow mammary gland perfusions. Two glands of each animal were perfused separately, using autologous blood*

Sow no.	Perfusion no.	Duration (h)	Maximum rate of blood flow (ml/100 g min)	Mean glucose uptake (mg/100 g min)	Mean O_2 uptake (ml/100 g min)	Mean RQ	Total milk yield (ml)
1	1	8	20	0	—	—	35
	2	8	28·5	1·3	—	—	38
2	3	4	0·1	—	—	—	Dry
	4	5·25	34	2·2	—	—	—
5	5*	5·25	23	6·2	0·68	1·62	35
	6†	5·25	17	6·1	0·36	1·4	24
6	7*	9	26	4·1	—	—	24·5
	8†	7	38	2·6	0·3	2·0	29·5

* [U-^{14}C]acetate included in the substrates.
† [U-^{14}C]glucose included in the substrates.

The operation for catheterization was begun by tranquillizing the animal in its pen. Injections of tranquillizers (Chlorpromazine (Largactil; May & Baker), 250 mg, or Spiperone (Janssen Pharmaceutica) 0·05–0·1 mg/kg body-weight) were given to the unrestrained animals subcutaneously, with fine sharp needles, just behind the ear or over a hind mammary gland whilst the animal was feeding. The tranquillized animal was enticed with food into a trolley and taken to the operating theatre, where anaesthesia was induced with pentobarbitone sodium, 20–35 ml of 10% solution or methohexitone, 20–40 ml 2·5% solution, injected into an ear vein again without restraint, or if sufficiently tranquil with halothane and O_2 by face mask. After the induction of anaesthesia, the trachea was intubated and anaesthesia was maintained with halothane and O_2 on a closed circuit with a Goldman vapourizer in the circuit (in the circle). After the operation the animal was returned to its pen to recover consciousness.

In two experiments catheterization and sampling were carried out under local anaesthesia after the sow had been tranquillized with Spiperone (0·05 and 0·1 mg/kg body-weight). The mammary vein and an artery (ear or saphenous) were catheterized by Seldinger's (1952) technique.

The catheters, which protruded through the skin of the flank or neck, were well tolerated by the sows. In fact the sows appeared to be so unaware of their presence that they allowed the piglets to nuzzle the catheters and any plaster used to cover them; several experiments were terminated by damage believed to be caused in this way (Pl. 1). Fig. 1 and Pl. 1 show two methods used for closing the catheters and fixing

them to the skin; these methods afforded protection and yet allowed samples to be taken and catheters to be flushed through with heparin.

Glucose metabolism and mammary substrate uptake. These were studied in one of the tranquillized sows (no. 3) by the techniques used with goats by Annison & Linzell (1964). [U-^{14}C]glucose (essentially carrier-free) was infused intravenously at constant rate (1·96 μc/min) for 4 h after a priming dose of 100 μc, and during the last hour four pairs of simultaneous samples of arterial and mammary venous blood were taken for the measurement of glucose and CO_2 specific radioactivity (SRA). Blood flow through the mammary gland sampled was measured by the Kety & Schmidt method, using

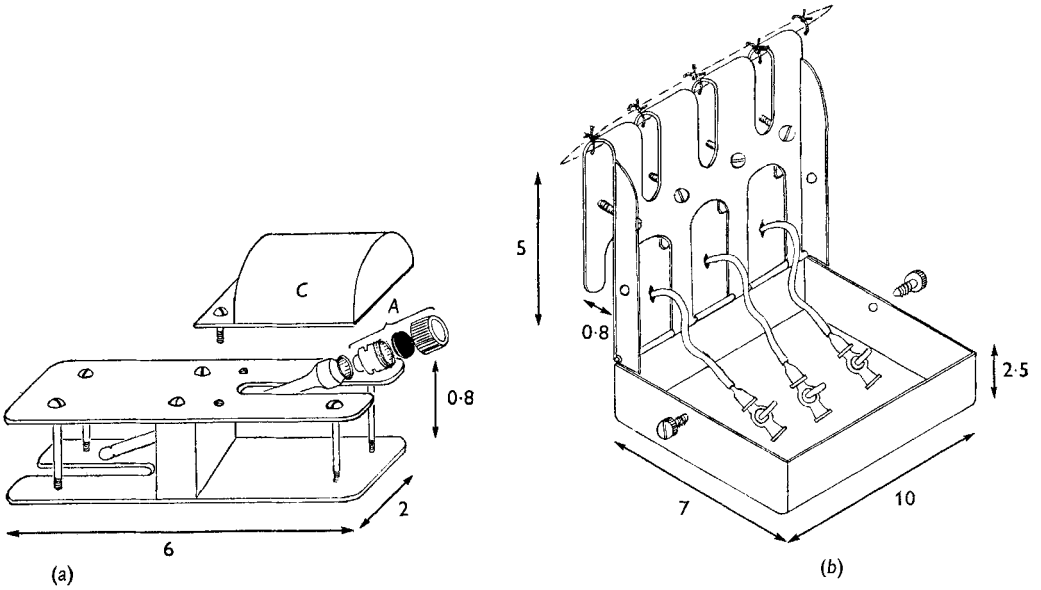

Fig. 1. Illustration of two methods used of bringing catheters through the skin of sows, which allowed easy sampling and prevented damage when not in use. (*a*) Luer-lok hypodermic needle fixed to a block, which is sandwiched between two plates fixed to the skin, one inside and the other outside. The catheter can be flushed through by injecting through the rubber diaphragm of the male adaptor (*A*), which is removed for sampling. A cover (*C*) protects the assembly from investigation by the piglets. (*b*) Metal box fixed to the skin, which can be closed over catheter holes and also contains the light plastic tap closing the catheters. Both devices were made of stainless steel, dimensions in cm; the only critical dimension is 0·8 cm, the thickness of the skin. Not drawn to scale.

^3H_2O instead of N_2O, as described by Setchell & Waites (1964) for the testis. The milk yield of the gland sampled was measured by milking out the gland at the start of infusion and at 3, 4 and 5 h after the start of infusion, using oxytocin intravenously (ten doses of 0·5 i.u. at each milking) to aid complete emptying of the gland. At the end of the experiment the animal was killed and all the functional mammary glands were weighed (4·62 kg) so that total mammary blood flow and milk yield could be calculated, assuming, as is true for goats (Linzell, 1960, 1966*a*), that these values are constant for each g of tissue.

Mammary gland perfusion. Eight isolated glands from four sows were perfused for

5–9 h by the technique developed by Hardwick & Linzell (1960) for goat glands, except that there was no artificial kidney (dialyser) in the circuit (Table 2). The glands and the autologous heparinized blood used as the perfusate were removed under general anaesthesia. The glands were milked out at hourly intervals using repetitive doses of oxytocin (each 20 mille units intra-arterially) until they were empty; generally five doses were required. A substrate mixture was infused into the blood continuously at about 50 ml/h; it had the following composition (g/l.): cystine hydrochloride 0·3, aspartic acid 0·1, glutamic acid 3, threonine 0·5, proline 1·5, glycine 0·1, alanine 0·4, valine 1·1, methionine 0·4, isoleucine 1·0, leucine 1·7, tyrosine 0·5, phenylalanine 0·7, lysine hydrochloride 1·6, histidine hydrochloride H_2O 0·7, arginine 1·2, hydroxyproline 0·25, serine 1·0, glutamine 2·0, asparagine 1·0, tryptophan 0·5, glucose 50 and emulsified cottonseed oil (Infonutrol Astra; Sodertalje, Sweden) 30.

In two perfusions [U-^{14}C]glucose and in two others [U-^{14}C]acetate were included in the substrates.

Analytical methods. These were the same as those used in similar experiments on goats (Annison & Linzell, 1964; Mepham & Linzell, 1966; Annison, Linzell, Fazakerley & Nichols, 1967; Annison, Linzell & West, 1968), plus the enzymic methods of measuring lactate (using the kit provided by Boehringer, Mannheim, Germany) and β-hydroxybutyrate (Williamson, Mellanby, & Krebs, 1962). The errors of analytical methods used were determined from replicates or duplicates and the standard deviations expressed as a percentage of the means (no. of observations) were as follows: O_2 2% (63), CO_2 1·5% (63), glucose 6% (63), volatile fatty acids 6% (54), lactate 7% (7), cholesterol 11% (8), cholesterol esters 12% (8), phospholipids 6% (6). The accuracy of measuring methionine and proline was about 10% and for the other amino acids 4%. The fatty acids in the free fatty acid and triglyceride fractions of plasma were estimated by the recovery of C_{17} fatty acid added as a marker (West & Rowbotham, 1967); the error for major peaks was 0·8% (5), and for minor ones 1–3% (5).

RESULTS

Validity of results

In cows and goats it is important to avoid stress in order to obtain results valid for normal mammary metabolism; this appears to apply to pigs also. In two sows mammary arteriovenous (A-V) differences were measured under general anaesthesia and in another animal 2 h after the operation to catheterize blood vessels. In two sows there were negligible uptakes of glucose, in one greatly reduced uptakes of amino acids, and one sow under anaesthesia had a very high level of free fatty acids (1·38 m-equiv./l. plasma); in all instances the same animals studied when conscious showed consistent A-V differences for glucose and low plasma concentrations of free fatty acids. In contrast, when one sow was studied on 2 successive days, normal or tranquillized with Spiperone (0·05 mg/kg), no significant changes in the A-V differences of six plasma components were noted between the 2 days.

The sow mammary glands did not perfuse as well as those of goats under similar conditions; they invariably became oedematous and showed petechial haemorrhages

at the end of perfusion. The milk yield was only 5–8 ml/h, compared with about 50 ml/h per gland in conscious animals (Turner, 1952); in goats the yield during perfusion is $57 \pm 2{\cdot}3$ (SE) % of that before perfusion (J. L. Linzell, unpublished). In perfused mammary glands of sows the blood flow frequently fell to low levels (less than 10 ml/min per 100 g tissue), which may be associated with the intense spasm that pig blood vessels develop on handling. Nevertheless, milk secretion, mammary uptake of glucose and of O_2 continued throughout perfusion (all less than in conscious animals) and isotopically labelled precursors were incorporated into milk components.

General mammary metabolism

Mammary blood flow, glucose and O_2 uptake and CO_2 output were measured in perfusion experiments and in one conscious sow tranquillized with Spiperone. In the conscious animal the milk yield of one gland, measured for 5 h during and after an intravenous infusion of radioactive glucose, was 43 ml/h, which is equivalent to 134 ml/day per 100 g tissue. The blood flow through this gland was 41 ml/min per 100 g, the O_2 uptake 2·06 ml/min per 100 g, and the glucose uptake 8·6 mg/min per 100 g. All these values are very similar to those measured for the same milk yield per 100 g udder tissue in goats (Linzell, 1960, 1966*b*). In perfused glands the maximum rate of blood flow was 20–38 ml/min per 100 g, of O_2 uptake 0·2–0·5 ml/min per 100 g and of glucose uptake 1·3–6·2 mg/min per 100 g (Table 2).

Table 3. *Mammary respiration and carbohydrate uptake in lactating sows*

(Analyses of blood taken simultaneously from an artery (A) and a mammary vein (V) of conscious undisturbed animals)

Sow no.		Haematocrit (%)	Blood glucose (mg/100 ml)	Blood volatile fatty acids (m-equiv./l.)	Plasma lactate (mg/100 ml)	Blood O_2 (ml/100 ml)	RQ
1	A	33·5	45·5	0·25	18·4	13·6	1·3
	V	34·5	36	0·12	12·9	10·1	
2	A	33·5	55	0·37	—	13·7	1·63
	V	33·2	34	0·20	—	6·6	
3	A	33	51	—	10·3	—	—
	V	33	44	—	—	—	—
3*	A	32	48·5	0·46	20·5	12·3	1·09
	V	33	28	0·31	17·6	7·3	
4	A	25	65	0·23	37·8	10·75	1·45
	V	27	54	0·24	20	7·8	
5	A	32	50	—	17·5	14·0	1·21
	V	31	38	—	14·3	11·1	

Mammary CO_2 production was determined eight times in conscious sows and six times in perfused glands. In all conscious sows it was always greater than the O_2 uptake. This is to be expected when lipid is being synthesized from carbohydrate and is characteristic of lactating mammary tissue. The RQ varied from 1·09 to 1·63 (mean 1·39, SE 0·13) (Table 3). During perfusion the RQ was 0·67–3·35 (mean 1·59, SE 0·39) (Table 2). The oxidation of glucose appeared to be an important source of energy.

When [^{14}C]glucose was intravenously infused into the conscious sow comparison of the SRA of mammary CO_2 (determined from the rise in the CO_2 content and SRAs during passage through the gland) with that of the arterial plasma glucose showed that 54% of mammary CO_2 originated from the oxidation of glucose. In the two perfusion experiments the values were 13 and 40% for glucose but only 2% for [U-^{14}C]acetate.

Carbohydrate metabolism

Measurements of mammary A-V differences showed an uptake of glucose and lactic acid in all six sows (Table 3). The extraction (A-V difference expressed as a percentage of the arterial concentration) of glucose varied from 14 to 40% and that of lactic acid varied from 14 to 47%. In the experiments where radioactive glucose was included the main products labelled with ^{14}C were CO_2, lactose and glycerol. In the conscious sow at least 40% of glycerol, 54% of CO_2 and 59% of lactose were derived from plasma glucose; 34% of the glucose taken up by the mammary glands was oxidized, whereas lactose output accounted for 80% of the remainder. The animal itself was utilizing glucose at 4·8 mg/min per kg but was deriving only 14% of its CO_2 from the oxidation of glucose, and the six active mammary glands were utilizing 54% of the total glucose entering the circulation.

Fat metabolism

A-V differences of possible milk fat precursors are shown in Table 4. The only component to show a consistent uptake was triglyceride (extraction, 13–53%); A-V differences of phospholipids, sterols and free fatty acids were small or variable. There were A-V differences of volatile fatty acids and β-hydroxybutyrate, but the arterial concentrations of these substances were so low that their mammary uptake was of minor importance. Chromatographic resolution of the individual fatty acids of the free fatty acids and triglyceride fractions revealed no consistent A-V differences in fatty acids, and triglyceride fractions revealed no consistent A-V differences in fatty acid composition, suggesting that all fatty acids are taken up equally (Table 4).

In the experiment (3*), where [U-^{14}C]glucose was infused and mammary A-V samples were taken, mammary blood flow and milk yield were also measured. Thus it was possible to make a balance of mammary uptake of fatty acids versus output in the milk. This was only approximate, since the concentration of fat in the milk rose during the experiment from 6·5 to 12·3 g/100 ml. However, the SRA of the milk fat was maximal 1 h after the end of [U-^{14}C]glucose infusion, so that the output of milk fat at this time was compared with the A-V difference 1 h before, and this balance showed that the output of total fatty acids and of some particular acids (palmitic, stearic and oleic) was equal to, or somewhat greater than, the uptake from the blood, suggesting there were other precursors of milk fat. One of these was glucose because in this experiment milk fat was labelled from radioactive glucose. Although the SRA was low, hydrolysis of the milk fat showed that both the fatty acid and glycerol moieties were labelled. A more detailed analysis of the contribution of glucose to milk fatty acid synthesis was therefore made using four isolated perfused glands, in which a much higher SRA of precursor can be achieved with a similar quantity of isotope;

Table 4. *Precursors of milk fat in lactating sows*

(Analyses of plasma from blood taken simultaneously from an artery (A) and a mammary vein (V) of conscious undisturbed animals)

Sow no. ...	1		2		3		3*		4		5	
	A	V	A	V	A	V	A	V	A	V	A	V
Cholesterol (mg/100 ml)	9·7	13·9	—	—	—	—	12·82	10·22	—	—	14·55	14·36
	—	—	—	—	—	—	—	—	—	—	—	—
Cholesteryl esters	22·5	22·4	—	—	—	—	29·01	21·65	—	—	21·65	17·03
(mg/100 ml)	—	—	—	—	—	—	—	—	—	—	—	—
Phospholipid P	0·86	0·80	—	—	—	—	1·81	1·50	—	—	2·38	1·94
(mg/100 ml)	—	—	—	—	—	—	—	—	—	—	—	—
β-hydroxybutyrate	—	—	0·5	0·4	0·8	0·6	0·9	0·8	0·8	0·6	—	—
(mg/100 ml)	—	—	—	—	—	—	—	—	—	—	—	—
Free fatty acids	0·285	0·277	0·35	0·38	0·19	0·18	0·87	0·59	0·55	0·78	0·255	0·255
(m-equiv./l.)	—	—	—	—	—	—	—	—	—	—	—	—
Composition of free fatty acids (moles %)†												
12:0			1·2	1·3	9·5	6·9	0·1	0·6	0·8	0·6		
14:0			3·6	3·1	6·6	5·1	1·5	2·0	2·5	2·3		
15:0			1·0	2·1	4·4	3·5	0·4	0·6	0·5	0·5		
16:0			34·8	30·1	37·5	35·1	23·3	24·6	28·0	27·5		
16:1			4·5	5·4	5·1	5·1	3·2	3·6	4·9	5·2		
18:0			12·0	11·5	16·5	15·8	16·3	14·9	6·7	6·0		
18: branched			1·4	1·2	tr	tr	0·8	0·4	1·0	0·7		
18:1			27·6	28·1	21·2	24·8	39·7	34·4	30·4	30·2		
18:2			11·9	14·7	1·2	1·8	9·9	11·0	22·0	23·6		
18:3			2·0	2·5	tr	tr	3·5	6·8	1·9	2·3		
20:0			tr	tr	tr	tr	1·1	1·0	1·2	1·0		
Triglycerides			12·5	tr	12·5	tr	32·2	tr	41·8	tr		
(mg/100 ml)			tr	10·9	tr	8·3	tr	15·1	tr	34·5		
Composition of triglycerides (moles %)†												
12:0			0·6	0·6	1·0	0·9	0·4	0·5	1·0	0·1		
14:0			2·6	2·6	3·3	3·8	2·3	3·0	3·0	1·2		
15:0			1·3	1·3	4·0	2·8	1·2	2·7	0·7	0·6		
16:0			33·9	29·3	29·6	31·6	24·0	23·7	27·3	27·7		
16:1			5·3	6·1	6·0	5·5	5·1	5·5	3·5	4·0		
18:0			11·1	10·0	10·0	12·9	6·1	7·2	9·8	8·1		
18:branched			0·5	tr	tr	tr	1·2	1·3	0·7	1·0		
18:1			35·9	38·7	37·4	28·9	36·8	34·9	31·6	32·0		
18:2			8·8	11·4	8·4	5·3	20·7	18·3	19·6	22·7		
18:3			tr	tr	tr	tr	2·0	1·3	1·3	1·4		
20:0			tr	tr	tr	tr	tr	1·7	0·9	1·0		

tr, trace.

† Moles fatty acid/100 moles total fatty acid.

Table 5. *Labelling of fat from glucose and acetate by two isolated perfused mammary glands of a sow. Perfusions* 5 *and* 6 (*see Table* 2)

(Both glands were perfused for 5·25 h with autologous blood and received 250 ml of a substrate mixture of glucose, 53 g/l., and amino acids 20 g/l., plus [U-^{14}C]glucose, 0·5 mc, or [U-^{14}C]-acetate, 0·5 mc. Milk was removed hourly: it contained 7·5–13·2 g lipid/100 ml of milk the fatty acid composition of which did not vary significantly. Lipid extracted from secretory tissue was obtained at the end of perfusion. Analyses are shown for the 4th and 5th h of perfusion)

Glucose

Specific radioactivity of substrate in blood μc/g carbon (SRA)	48 (acetate 33)
Tissue CO_2 SRA	26·52
Glycerol SRA	7·89

Fatty acid	Milk (4th h)		Milk (5th h)		Tissue	
	Moles (%)*	SRA	Moles %*	SRA	Moles %*	SRA
6:0	tr	ND	0·8	ND	tr	ND
10:0	0·3	ND	0·4	ND	0·4	ND
12:0	0·5	ND	0·6	ND	0·5	ND
14:0	4·4	0·93	4·4	1·17	4·0	6·38
14:1	0·6	tr	0·6	tr	0·4	tr
16:0	32·4	0·61	32·2	0·62	32·7	3·39
16:1	10·7	0·45	10·9	0·49	9·9	3·52
18:0	4·4	< 0·02	4·7	< 0·02	8·6	1·05
18:1	34·7	< 0·02	34·0	< 0·02	33·4	0·66
18:2	10·3	tr	10·3	tr	8·7	tr
18:3	1·1	tr	1·1	tr	0·7	tr
18:branched	0·3	tr	tr	tr	tr	tr
20:0	0·3	tr	tr	tr	0·7	tr

Acetate

Specific radioactivity of substrate in blood μc/g carbon (SRA)	788 (glucose 0·67)
Tissue CO_2 SRA	16·7
Glycerol SRA	—

Fatty acid	Milk (4th h)		Milk (5th h)		Tissue	
	Moles %*	SRA	Moles %*	SRA	Moles %*	SRA
6:0	tr	ND	tr	ND	tr	ND
10:0	0·4	ND	0·5	48·3	0·3	ND
12:0	0·7	14·25	0·6	21·1	0·4	56·5
14:0	4·3	14·95	4·5	21·5	4·1	52·8
14:1	0·3	tr	0·4	tr	0·5	tr
16:0	34·5	5·23	33·3	8·78	34·0	24·3
16:1	10·5	3·38	10·5	6·48	8·8	27·6
18:0	4·4	1·70	5·1	3·29	8·5	5·00
18:1	34·1	0·79	34·6	1·71	33·5	2·89
18:2	9·6	tr	9·5	tr	8·7	tr
18:3	1·2	tr	1·0	tr	0·6	tr
18:branched	tr	tr	tr	tr	tr	tr
20:0	tr	tr	tr	tr	0·6	tr

ND, not determined; tr, trace.
* Moles fatty acid 100 moles total fatty acid.

two glands were perfused with [U-^{14}C]glucose in the substrates and two with [U-^{14}C]-acetate. Similar results were obtained with each labelled substrate and the differences between the milk fatty acid labelling from glucose and acetate for two glands of one sow are shown in Table 5.

In all four perfusions the fatty acid composition of the milk fat secreted was similar to that produced in vivo, and, as in the goat, tissue lipid extracted at the end of perfusion, after repeated doses of oxytocin to remove retained milk, also had a similar fatty acid composition. This probably means that, as in goats (Hardwick, Linzell & Mepham, 1963), there is, in mammary tissue, a large pool of lipid with a slow turnover rate. This raises the possibility that none of the milk fat secreted had in fact been synthesized during perfusion. However, in both acetate and glucose experiments radioactivity was transferred to milk fat and to a much greater extent to mammary tissue lipids. This confirms that, as in goat udder perfusions (Linzell, Annison, Fazakerley & Leng, 1967), in view of the slow turnover time of mammary tissue lipid pool, perfusions must be continued for a considerable time before constant SRAs are obtained; this is not always technically feasible. Thus in the present experiments the contributions of glucose and acetate to milk fat were not maximal. However, when two glands of one sow were perfused for the same time the results with acetate and glucose may be compared. They show (Table 5), in marked contrast to results for the goat, substantial labelling of all fatty acids from both precursors, and in particular synthesis of stearate and oleate, which is negligible in goats. A further difference is that, in sows, glucose is quantitatively a much more important lipid precursor than acetate; e.g. the SRA of tissue myristate was 13% of the glucose SRA and only 7% of the acetate, and yet the concentration of blood glucose was much higher than that of acetate. The pattern of labelling of individual fatty acids was similar for each precursor, the SRAs being in the order: myristate > palmitate > palmitoleate > laurate ≫ stearate > oleate.

Protein metabolism

A-V difference measurement on five pairs of blood plasma samples from four lactating sows indicated that considerable quantities of free amino acids were absorbed by the glands (Table 6). Since the arterial concentrations of amino acids in the sow were in most instances much higher than in the goat (Mepham & Linzell, 1966), the mean extraction was, with the exception of serine, less than in the goat, despite the higher protein content of sow's milk. The highest mean extraction for the sow was 37% (for glutamic acid), whereas in the goat the values for three amino acids were greater than 60%.

The high extraction of aspartic acid, threonine, serine, glutamic acid, valine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine and arginine would appear to provide good evidence for mammary absorption, but the mean quotients of the other amino acids studied, except methionine (see p. 323), were all less than 10% and as such cannot be regarded as good evidence for their consistent absorption. The absorption of most non-essential amino acids was, however, very variable and it is clear that some of this group were appreciably absorbed in some animals; e.g. in the experiment on sow 3* the extraction of ornithine was 41%, while in sow 5 it was zero.

Methionine and proline presented analytical problems and the values given for the former are not reliable. The lability of methionine was indicated by the fact that the mild hydrolytic treatment used in estimating the amides (Mepham & Linzell, 1966) caused complete destruction of this amino acid. In most instances it was not possible to obtain sufficiently good chromatographic separation of citrulline and proline to allow accurate estimation.

Table 6. *Precursors of milk protein in lactating sows*

(Analyses of (mg/100 ml plasma) of blood taken simultaneously from an artery (A) and a mammary vein (V) of conscious undisturbed animals)

Sow no. ...	2		3		3*		5		5†	
	A	V	A	V	A	V	A	V	A	V
Aspartate	0·54	0·48	0·41	0·16	0·28	0·19	0·35	—	0·55	0·37
Asparagine	—	—	0·42	0·52	0·65	0·62	0·79	0·67	0·64	0·58
Threonine	2·54	2·02	1·48	1·03	1·11	0·80	2·18	1·92	1·72	1·38
Serine	—	—	2·02	1·69	1·65	1·19	1·53	1·22	1·45	1·08
Glutamate	7·08	4·06	3·50	1·92	5·00	3·41	4·54	3·34	5·18	3·28
Glutamine	—	—	4·56	4·37	4·45	3·54	9·56	8·71	7·03	6·81
Proline	5·63	4·19	—	—	2·03	1·97	—	—	4·54	—
Glycine	6·02	6·10	4·27	4·12	4·36	3·70	4·10	4·06	5·17	5·00
Alanine	4·57	4·15	2·97	2·45	1·97	1·84	2·43	2·43	3·15	2·88
Valine	5·59	4·45	3·67	3·17	3·53	2·39	5·00	4·42	5·00	4·48
Methionine	0·99	0·61	—	—	0·19	0·12	0·37	0·25	—	—
Isoleucine	2·91	1·92	1·69	1·21	1·21	0·78	1·93	1·49	1·67	1·17
Leucine	4·70	3·04	2·75	2·00	2·23	1·40	2·86	2·30	2·50	1·73
Tyrosine	2·64	2·13	1·70	1·44	0·99	0·64	1·85	1·42	1·34	1·20
Phenylalanine	2·18	1·55	2·01	1·61	1·14	0·76	2·02	1·47	1·56	1·30
Ornithine	2·32	2·27	1·49	1·28	1·22	0·72	2·19	2·27	2·29	1·69
Lysine	5·10	3·85	3·44	3·01	1·20	0·69	4·08	3·33	2·52	1·52
Histidine	1·77	1·33	1·65	1·07	1·37	1·12	1·70	1·32	1·31	0·90
Arginine	4·75	3·62	3·77	2·81	1·46	0·84	6·00	5·20	3·77	3·20

* At the time of the experiment with radioactive glucose.
† Tranquillized.

As in the goat, the A-V difference of non-essential amino acids (as defined by Mepham & Linzell, 1966) was much more variable between animals than that for essential amino acids (coefficient of variation: non-essential 120; essential 33), although the variation in arterial concentration was similar for the two groups (coefficient of variation: non-essential 25, essential 29). In marked contrast to the results for the goat, however, serine was absorbed in appreciable amounts and with the lowest degree of variation of all amino acids (see Table 6). In the goat, when correlation coefficients were computed from the results of ten determinations of A-V difference, there was a significant correlation between arterial concentration and A-V difference for aspartate, alanine, threonine and histidine, whereas the admittedly more limited results obtained for the sow did not show a significant correlation between these two measurements for any amino acid.

In the case of sow 3*, mammary blood flow was measured at the time of one blood sampling, and an attempt has been made to define the degree of balance between the uptake of essential amino acids and the output of the corresponding residues in the

milk protein synthesized in the mammary tissue. To calculate the latter, the results for milk yield and milk protein nitrogen were used in conjunction with the findings of Wünsche, Herrmann & Bock (1967) on the essential amino acid composition of sow's milk protein, and those of Morgan & Lecce (1964) on the proportion of protein in sow's milk that is synthesized in the mammary tissue. Table 7 shows that the degree of balance between uptake and output was fair for most amino acids, but it appears that almost twice as much valine was absorbed as was secreted in milk protein in the same time. This result is somewhat puzzling, but it should be appreciated that the Fick principle, on which the calculations were based, assumes that all the factors concerned are in a completely steady state. It is possible that the excess valine absorption was a transient effect which was immediately followed after sampling by a much decreased absorption. Alternatively the excess uptake may have been genuine, thus indicating that valine is metabolized in the mammary tissue. There is evidence that, in the isolated perfused goat gland, valine is oxidized to an appreciable extent (T. B. Mepham and J. L. Linzell, unpublished).

Table 7. *Expt* 3* (*see p.* 329 *and Table* 6). *An estimate of the essential amino acid uptake from the plasma and output into mammary synthesized protein by a single gland of a sow*

Amino acid	Uptake (mg/h)	Output (mg/h)
Threonine	40	53
Isoleucine	55	62
Leucine	105	105
Tyrosine	44	46
Phenylalanine	48	49
Valine	145	61
Lysine	53	89
Histidine	30	32
Arginine	79	59

In the goat the absorption of arginine greatly exceeds its output in the protein (Mepham & Linzell, 1966), an observation which is consistent with the appreciable hydrolysis of this amino acid in the mammary tissue (Mepham & Linzell, 1967). It is doubtful whether the slight excess uptake of arginine found for the sow's gland (Table 7) was significant, but if it were so it would suggest that arginine is similarly metabolized in this species, although to a much smaller extent than in the goat.

The low A-V differences of most non-essential amino acids in some or all of the sows suggests that the gland is capable of their synthesis. To investigate the possible significance of glucose, which may be presumed to be a probable carbon precursor, to mammary synthesis of amino acids, a sample of casein obtained from the glandular tissue at the end of a perfusion in which [U-^{14}C]glucose was infused, was acid-hydrolysed and the separated amino acids were assayed for radioactivity. Aspartate, glutamate, proline and alanine were significantly labelled, but since the maximum SRA (that of alanine) was only 1% of that of the plasma glucose, it is apparent that any amino acid synthesis in this experiment was quantitatively insignificant. As would be predicted from the results for A-V difference, serine, which had the highest SRA after infusion of glucose into goats in vivo (Linzell & Mepham, 1968), was only negligibly labelled in the experiment with sows.

DISCUSSION

Although information is now accruing on the effect of diet on the composition of sow's milk (Salmon-Legagneur, 1967; Rook & Witter, 1968), there appears to have been only one study specifically of mammary metabolism in sows. Gutte, Kleiber, Raggi & Black (1961) made single intravenous injections of [^{14}C]acetate and [^{14}C]glucose and compared the labelling of CO_2 and milk components with that obtained in parallel experiments in cows. Great similarities were encountered, the main difference being the greater contribution of glucose to CO_2 and milk fat in pigs. The present paper explores the possibility of obtaining more detailed information of mammary metabolism itself from an animal that is somewhat unsuitable for physiological experiments.

It has proved possible to obtain arterial and mammary venous blood from conscious sows. The animals were undisturbed as judged by their general demeanour and behaviour (one suckled its piglets during sampling), and from the fact that blood glucose and free fatty acid levels were usually low. More ambitious experiments (continuous infusion, mammary blood flow measurement, etc.) are greatly facilitated by the use of tranquilizers. Spiperone was better than chlorpromazine in immobilizing the animal but the free fatty acid levels were raised at higher dosages, although no effects on mammary metabolism were detected.

Quantitative information on mammary metabolism has been obtained most comprehensively for the goat, and, since all the techniques we have used with sows had been used previously with goats, fed a similar concentrate diet plus hay *ad lib.*, it seems profitable to summarize the similarities and differences between the results obtained for the two species in our laboratories.

Points of similarity are: Mammary blood flow, O_2 uptake and CO_2 production are of a similar magnitude at comparable milk yields. The RQ in both species is greater than unity. Glucose is the major source of energy for the mammary glands and during lactation the mammary glands are the main users of glucose. As in the goat, most of the glucose appears to be used to synthesize lactose and milk fat glycerol and most of the remainder is oxidized. All of the essential and much of the non-essential amino acid residues of milk protein are derived from the corresponding free amino acids of the plasma. Plasma long-chain fatty acids are the main precursors of milk fatty acids, which, in the sow, consist mainly of those of chain-length C_{14} and longer. As with the goat, but not the rabbit, mammary tissue of the sow can dehydrogenate stearate to form oleate (Bickerstaffe & Annison, 1968).

Points of difference between the pig and the goat are: Associated with the ruminant form of digestion, plasma levels of volatile fatty acids (mainly acetate) and β-hydroxybutyrate are high in goats, and these acids are exclusively used to form milk fatty acids of chain length up to and including C_{16} which account for about 40% of the weight of milk fatty acids in ruminants. By contrast, plasma levels of acetate and β-hydroxybutyrate are low in pigs, so mammary uptake is slight and the short-chain fatty acid content of sow's milk fat is low. An equally striking related difference is that milk fatty acids are extensively derived from glucose in the sow, but not at all in goats.

In the sow, mammary tissue can synthesize fatty acids up to chain length C_{18}, both from glucose and from acetate, whereas in the goat only acetate can be used and then only up to C_{16}. Thus in the sow there do not appear to be two pools of acetyl co-enzyme A in mammary tissue as there appear to be in the mammary tissue of goats (Hardwick *et al.* 1963; Hardwick, 1965). Although sow's mammary glands are able to utilize acetate for fat synthesis, this is of limited quantitative significance, since mammary uptakes are so low.

In the goat, mammary uptake of long-chain fatty acids from the free fatty acid of the plasma is masked by the release of fatty acids from plasma triglycerides during the passage of blood through the glands. Further work with labelled long-chain fatty acids is required to see if this applies to the sow also and to decide the exact proportion of milk long-chain fatty acids (C_{18} and longer) that are synthesized from glucose and derived from plasma fatty acids.

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EXPLANATION OF PLATE

Pl. 1. Sow with two catheters in mammary veins and one in the external pudic artery, brought to the surface of the flank by means of the protector illustrated in Fig. 1*a*. The left-hand device has the male adaptor in place for flushing through, in the right-hand one it is removed for sampling, and the centre one has the protecting cover in place. Note the keen interest of one of the piglets, whose exploration of the catheters demanded the use of these devices.

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