THE EFFECT OF STARVATION ON THE UPTAKE OF THE PRECURSORS OF MILK FAT BY THE BOVINE MAMMARY GLAND

By P. E. HARTMANN* and A. K. LASCELLES*

[*Manuscript received February* 26, 1965]

Summary

The relative changes imposed by a 4-day starvation on the arteriovenous difference across the mammary gland, for triglyceride, free fatty acids, glucose, β-hydroxybutyrate, and acetate have been studied in three lactating cows.

The arterial concentration of free fatty acids increased up to tenfold during starvation, but this was not associated with a corresponding increase in its arteriovenous difference across the mammary gland. There was a marked decrease in both the arterial concentration and the arteriovenous difference for acetate. These results indicated that the uptake of free fatty acids by the mammary gland of the cow is not a function of its arterial concentration while that of acetate is. There was little change in the uptake of triglyceride and β-hydroxybutyrate during starvation. The arterial concentration of glucose was maintained at close to non-fasting levels during the 4-day fast and glucose was not readily utilized by the mammary gland.

I. INTRODUCTION

The fatty acids of the milk fat of cows are synthesized within the mammary gland from acetate and β-hydroxybutyrate or derived directly from the plasma triglycerides (Barry 1964) and possibly from the plasma free fatty acids (Hartmann and Lascelles 1964). In addition, the glycerol moiety of the triglycerides of milk fat appears to come partly from the plasma triglycerides (Barry 1964) and is partly synthesized from blood glucose (Luick and Kleiber 1961).

Starvation of the dairy cow causes marked changes in the yield and composition of milk fat. Since a marked reduction in blood flow through the mammary gland occurs during starvation (McClymont, personal communication), it is clear that this alone could be responsible, to a large extent, for the decrease in milk fat production. However, the production of fat is suppressed to rather less than that of the other milk components (Overman and Wright 1927) and its fatty acid content is changed markedly (Smith and Dastur 1938). It is therefore of interest to determine the relative changes imposed by starvation on the arteriovenous differences of the important precursors of milk fat.

II. METHODS

(*a*) *Animals*

Three cows were starved for a period of 4 days. During this period they were completely deprived of food but allowed free access to water. Cow 1 (a 6-yr-old Australian Illawarra Shorthorn Cross) was in the ninth month of her third lactation, cow 2 (a $3\frac{1}{2}$-yr-old Guernsey) was in the fourth month of her second lactation, and cow 3 (a $7\frac{1}{2}$-yr-old Guernsey) was in the third month of her fifth lactation.

* Dairy Husbandry Research Foundation, Department of Animal Husbandry, University of Sydney, University Farms, Camden, N.S.W.

(*b*) *Management*

The cows grazed a mixed pasture containing subterranean clover, white clover, and rye grass and were supplemented with strip grazing (3 hr/day) on good quality green winter oats. Apart from the starvation period the cows were managed according to normal dairy farm practice.

(*c*) *Collection of Samples*

The surgical techniques and the methods used for sampling arterial and venous blood have been described previously (Hartmann and Lascelles 1965). Samples of blood were taken 20 days before, at the commencement, after 2 days, and at the end of the 4-day period of starvation. Samples were again taken 3, 7, 19, 29, and 39 days after starvation. Samples from cows 1 and 3 were collected at 11 a.m. while those from cow 2 were collected at 2.30 p.m.

(*d*) *Analytical Technique*

The following analyses were carried out: glucose by the glucose oxidase method of Huggett and Nixon (1957) in which Boehringer blood sugar kits were used; free fatty acids by the method of Dole (1956); β-hydroxybutyrate by the method of Peden (1964); acetate, propionate, and butyrate were separated by liquid–liquid partition chromatography on a silicic acid column by the method of Brown as described by Mackenzie (1964); total protein by the method of Gornall, Bardawill, and David (1949); phospholipids by the method of Zilversmit and Davis (1950). The extraction of plasma lipids with chloroform–methanol (2 : 1 v/v) and analysis for cholesterol, triglycerides, and cholesterol esters by thin-layer chromatography was carried out as previously described by Hartmann and Lascelles (1965). Milk fat was determined by the Babcock method as described by Davis and MacDonald (1953). Haematocrits were determined on all blood samples using the Hawksley microhaematocrit method.

The volatile fatty acids and β-hydroxybutyrate analyses were carried out on whole blood while the other analyses were carried out on blood plasma.

Free fatty acids were determined in triplicate while all other determinations were carried out in duplicate.

III. Results

(*a*) *Milk and Milk Fat Production*

The production of milk and milk fat for each of the cows on the days when blood samples were collected is presented in Table 1. It can be seen that, during starvation, the decrease in the production of milk was much more marked than that of milk fat. Milk production in cow 1 did not return to more than a third of her pre-fast level. This may have been related to the fact that at the time of starvation this cow was in the ninth month of lactation and was $6\frac{1}{2}$ months pregnant.

(*b*) *β-Hydroxybutyrate and Acetate*

The arterial concentrations and the arteriovenous differences of β-hydroxybutyrate and acetate are presented in Table 2. The average concentration of β-hydroxybutyrate in the arterial blood of grazing cows was $10{\cdot}4 \pm 2{\cdot}5$ (S.D.) mg/

100 ml. Although an increase in the concentration of β-hydroxybutyrate was observed in one of the cows (cow 3) on the fourth day of starvation, clinical symptoms of ketosis were not apparent. The mean arteriovenous difference for β-hydroxybutyrate across the mammary gland was $2{\cdot}8 \pm 0{\cdot}6$ (S.D.) mg/100 ml which was approximately 1 mg/100 ml higher than observed by Shaw (1941) but similar to the finding of McClymont (1949). It can be seen from Table 2 that there was no appreciable increase in the uptake of β-hydroxybutyrate by the mammary gland during starvation.

The large uptake of acetate by the lactating mammary gland of grazing cows (Table 2) was similar to that found previously (Hartmann and Lascelles 1964) for volatile fatty acids. In all cows the arterial concentration of acetate fell to approximately 50% of its pre-fast value during starvation and then returned to the pre-fast

TABLE 1

PRODUCTION OF MILK AND MILK FAT FOR EACH OF THE COWS ON THE DAYS WHEN BLOOD SAMPLES WERE COLLECTED

	Cow	Before Starvation		During Starvation		After Starvation		
		20 Days	0 Days	2 Days	4 Days	3 Days	7 Days	19–39 Days*
Milk production (kg/24 hr)	1	9·0	9·0	2·5	1·3	1·5	2·3	2·7
	2	12·0	12·3	6·4	3·6	7·5	8·3	9·7
	3	12·0	11·9	5·0	4·4	10·8	12·9	13·9
Milk fat production (g/24 hr)	1	423	457	173	164	99	105	132
	2	509	523	425	339	291	328	426
	3	520	447	303	306	496	552	641

* Average of the 19-, 29-, and 39-day samples.

concentration during the following 7 days. After 2 days of starvation, the arteriovenous differences for acetate had decreased to less than 15% of its previous level and remained at this low level until the end of starvation. These findings are similar to those described by McClymont (1951).

The concentration of propionate and butyrate in the arterial blood was $0{\cdot}9 \pm 0{\cdot}3$ (S.D.) and $1{\cdot}0 \pm 0{\cdot}4$ (S.D.) mg/100 ml respectively. Although separation of these compounds at this low concentration was not very precise, both appeared to have a lower concentration in the venous blood. The arterial concentration of both propionate and butyrate in cows 2 and 3 was lowered by at least 50% during starvation.

(c) *Triglycerides, Free Fatty Acids, and Glucose*

The arterial concentrations and arteriovenous differences for triglycerides, free fatty acids and glucose (F.F.A.), are shown in Table 3.

The arterial concentration of triglyceride increased two- to threefold on the fourth day of starvation in cow 1, but was not noticeably altered in the other two cows. In all cows the concentration was lowest 3 days after starvation and returned to pre-fasting levels in 7–19 days. Arteriovenous differences for triglyceride were not consistently altered by starvation.

TABLE 2

CONCENTRATIONS OF β-HYDROXYBUTYRATE AND ACETATE IN BLOOD SAMPLES COLLECTED FROM THE CAROTID ARTERY OF EACH OF THE COWS BEFORE, DURING, AND AFTER STARVATION, TOGETHER WITH THEIR ARTERIOVENOUS DIFFERENCES

Results are expressed as mg/100 ml blood. The negative sign indicates that the concentration in the venous blood was higher than that in the arterial blood

Cow	Sample	Before Starvation		During Starvation		After Starvation		
		20 Days	0 Days	2 Days	4 Days	3 Days	7 Days	19–39 Days*
				β-Hydroxybutyrate†				
1	Carotid artery	—	8·9	5·2	7·8	4·5	8·8	11·3
	Arteriovenous difference	—	3·8	1·8	1·2	1·9	3·4	2·3
2	Carotid artery	9·5	6·4	6·6	10·2	8·0	7·3	10·3
	Arteriovenous difference	2·8	2·3	1·7	1·8	2·2	2·3	2·8
3	Carotid artery	11·9	14·1	8·8	24·1	9·4	11·0	12·1
	Arteriovenous difference	2·5	3·8	2·6	3·3	3·3	2·7	4·1
				Acetate‡				
1	Carotid artery	11·1	14·6	7·1	7·7	13·9	18·5	—
	Arteriovenous difference	4·1	8·5	−0·1	0·8	7·2	7·9	—
2	Carotid artery	16·3	12·8	6·4	4·6	10·1	12·5	18·9
	Arteriovenous difference	8·6	7·0	1·1	<0·1	4·1	6·6	10·4
3	Carotid artery	18·8	21·5	6·0	7·0	12·7	16·4	19·9
	Arteriovenous difference	10·4	11·3	2·8	3·2	5·4	9·5	9·3

* Average of 19-, 29-, and 39-day samples.

†, ‡ Standard deviation of duplicates 0·4 and 0·5, respectively.

During starvation the concentration of F.F.A. in plasma increased four to tenfold but returned to normal by 3 days after feeding; their uptake by the mammary gland was slightly greater than in the fed state (Table 3). Some of the arteriovenous differences for F.F.A. were small or slightly negative. The mammary glands of high-producing cows appear to take up F.F.A. more consistently than lower-producing cows (Hartmann and Lascelles 1964) and since the cows in this experiment were not in high production, the variable uptakes of F.F.A. were understandable.

Although the arterial concentration was only slightly lowered, the uptake of glucose by the mammary gland was substantially decreased by the fourth day of starvation (Table 3). The glucose uptake had recovered in cows 2 and 3 by the third day after starvation.

(*d*) *Cholesterol Esters, Cholesterol, Phospholipids, and Haematocrit*

The concentration of these lipids did not alter to any marked extent during starvation but declined rapidly thereafter in two of the cows, reaching a minimum of approximately 50% of the pre-fast level 7 days later (Table 4). It is interesting to compare these results with those of Riis (1961) who found a consistent decrease in the concentration of these lipids in the plasma of cows during a more prolonged fast.

The haematocrit increased by 6–10% during starvation although the animals were allowed free access to water. This was associated with the rise in plasma protein concentration.

(*e*) *Diurnal Variation in Arteriovenous Differences*

Blood samples were collected from cow 3, 30 min after the morning milking and 30 min before the afternoon milking. The arterial concentration and arteriovenous differences for triglycerides, β-hydroxybutyrate, glucose, acetate, and F.F.A. from these collections were determined. The respective arteriovenous differences for triglyceride, β-hydroxybutyrate, and glucose did not indicate that there was any change in their uptake between morning and afternoon collections. These uptakes were of a similar order to those found for this cow, 4–5 hr after milking (Tables 2 and 3). There was a greater uptake of acetate in the afternoon.

IV. Discussion

The limitations of the arteriovenous difference technique for studies on the net uptake of various substances by the mammary gland of the cow has been discussed in an earlier publication (Hartmann and Lascelles 1964). Folley (1949) considered that diurnal variation in the uptake of the various precursors may result in misleading conclusions. This was supported by the finding of Shaw and Petersen (1940) that the arteriovenous difference for blood fat increased from almost zero just after milking to a maximum some hours later. This finding was not borne out in the present experiment in which the arteriovenous difference for triglyceride was 6·6 mg/100 ml for a cow sampled just after milking compared with 5·3 mg/100 ml some hours later. The results for this cow indicated that, with the exception of acetate, the uptake of which appears to be governed to a large extent by its concentration in arterial blood (and possibly F.F.A.), diurnal variation does not appear to be important. This

TABLE 3

CONCENTRATION OF TRIGLYCERIDE, FREE FATTY ACIDS, AND GLUCOSE IN BLOOD PLASMA SAMPLES COLLECTED FROM THE CAROTID ARTERY OF EACH OF THE COWS BEFORE, DURING, AND AFTER STARVATION, TOGETHER WITH THEIR ARTERIOVENOUS DIFFERENCES

Results are expressed as mg/100 ml blood plasma. The negative sign indicates that the concentration in the venous plasma was higher than that in the arterial plasma

Cow	Sample	Before Starvation		During Starvation		After Starvation		
		20 Days	0 Days	2 Days	4 Days	3 Days	7 Days	19–39 Days*
		Triglyceride†						
1	Carotid artery	17·0	10·9	14·0	33·0	8·1	9·9	13·5
	Arteriovenous difference	7·7	5·9	4·7	2·6	2·5	4·8	5·5
2	Carotid artery	10·6	14·6	6·7	11·7	5·1	5·0	10·4
	Arteriovenous difference	4·6	8·9	4·0	4·2	1·8	1·5	4·4
3	Carotid artery	8·9	9·9	10·2	9·0	7·2	9·3	11·2
	Arteriovenous difference	3·1	2·5	3·5	2·8	2·5	3·0	4·1
		Free Fatty Acids‡						
1	Carotid artery	12·8	8·9	61·2	67·9	12·3	8·2	8·9
	Arteriovenous difference	2·5	0·9	1·1	2·4	1·3	1·1	−0·4
2	Carotid artery	7·6	6·3	32·8	67·2	8·2	9·2	6·8
	Arteriovenous difference	1·0	−0·2	6·4	1·6	2·2	1·9	1·5
3	Carotid artery	11·3	12·5	46·0	48·8	10·9	10·5	9·2
	Arteriovenous difference	1·9	2·2	3·6	0·3	2·1	0·5	1·6
		Glucose§						
1	Carotid artery	61·9	68·2	63·4	65·0	74·4	67·4	67·1
	Arteriovenous difference	15·4	15·3	4·2	7·7	6·5	10·5	9·4
2	Carotid artery	69·8	72·4	59·0	56·2	75·2	72·9	65·7
	Arteriovenous difference	14·0	15·1	11·8	5·0	14·3	16·8	18·6
3	Carotid artery	67·9	58·1	54·7	42·2	72·3	66·7	67·6
	Arteriovenous difference	18·2	14·5	13·0	8·3	15·0	13·7	16·3

* Average of 19-, 29-, and 39-day samples. †, ‡, § Standard deviation of duplicates 0·3, 0·5, and 1·2, respectively.

conclusion is supported by the consistency of the arteriovenous differences obtained from a large number of determinations on a number of dairy cows from each of which samples were collected at various times throughout the day.

The finding that the arterial concentration of acetate fell to a low level during fasting agrees with the results of McClymont (1951) and Riis (1961). This fall was closely related to a marked decrease in its arteriovenous difference across the mammary gland (Table 2). It has been found that there is an increased uptake of acetate by the mammary gland of the starved cow when the arterial concentration is increased by intravenous infusion of sodium acetate (McClymont 1951). Thus, it would appear that the uptake of acetate by the mammary gland is related to its concentration in arterial blood.

Shaw (1941) has shown that there is an increase in the uptake of β-hydroxybutyrate by the mammary glands of ketotic cows. However, in the cows used in the present experiment, fasting did not produce clinical symptoms of ketosis and the arteriovenous difference for β-hydroxybutyrate was of the same order in the fasted and fed states (Table 2).

It has been suggested by Lauryssens, Verbeke, and Peeters (1961) and Barry *et al.* (1963) that F.F.A. may assume greater importance in the synthetic activities of the mammary gland of the ruminant during fasting. The utilization of F.F.A. by muscle and liver tissue has been found to be a function of their concentration in the medium (Steinberg 1964). However, this does not appear to apply to the mammary gland during starvation, since our results show that a large increase in the arterial concentration of F.F.A. was associated with only a doubtful increase in its uptake (Table 3).

The uptake of plasma triglyceride throughout starvation was similar to that in the fed state and on the average would appear to be quantitatively, more than twice as important as plasma F.F.A. in the contribution of long-chain fatty acids to the mammary gland (Table 3). However, the recent demonstration that there is a marked decrease in specific radioactivity of F.F.A. across the mammary gland of the lactating goat following the continuous intravenous infusion of ^{14}C-labelled long-chain fatty acids indicate that unlabelled fatty acids are continuously being added to the blood during its passage through the mammary gland. This unlabelled fatty acid may have been derived from plasma triglyceride taken up by the mammary gland. Thus, the net uptake of triglyceride fatty acid and F.F.A. reported in our experiments should more properly be considered together rather than individually. It is interesting to note that a two- to threefold increase in the arterial concentration of triglyceride in one of the cows was not associated with an increased uptake (cow 1, Table 3).

The finding that the concentration of plasma glucose decreased only slightly during the 4-day fast (Table 3) is in conformity with the finding of Riis (1961) for the dairy cow but is in marked contrast to that observed in the monogastric animal (Yensen 1964). It has been shown that a lactating goat utilizes 60–85% of its daily turnover of glucose for milk production (Annison and Linzell 1964). The substantial decrease in milk production which occurs during starvation would be expected to significantly diminish total body glucose utilization. This, together with the more efficient mechanisms for the control of glucose production in the ruminant than in

TABLE 4

CONCENTRATION OF TOTAL FAT, CHOLESTEROL ESTERS, CHOLESTEROL, PHOSPHOLIPIDS, AND TOTAL PROTEIN IN BLOOD PLASMA SAMPLES COLLECTED FROM THE CAROTID ARTERY OF EACH OF THE COWS BEFORE, DURING, AND AFTER STARVATION, TOGETHER WITH THE HAEMATOCRIT FOR THE BLOOD SAMPLES

Results are expressed as mg/100 ml blood plasma. S.D., standard deviation of duplicates

Compound	Cow	Before Starvation		During Starvation		After Starvation					S.D.
		20 Days	0 Days	2 Days	4 Days	3 Days	7 Days	19 Days	29 Days	39 Days	
Total fat	1	547	507	579	555	364	274	359	453	417	8·0
	2	672	724	779	766	420	260	438	487	489	
	3	304	267	359	334	319	302	387	462	446	
Cholesterol esters	1	254	217	233	222	156	112	154	184	179	5·0
	2	315	348	357	334	202	109	188	223	220	
	3	112	97	132	128	130	128	162	198	194	
Cholesterol	1	31·8	32·0	34·2	30·5	23·0	17·9	21·6	27·1	26·9	0·4
	2	41·0	45·8	50·8	47·3	26·5	20·3	26·1	31·9	30·2	
	3	18·4	13·9	19·3	14·6	16·9	17·1	21·7	25·6	26·2	
Phospholipids	1	192	236	223	217	157	126	168	184	182	2·0
	2	302	291	291	255	169	127	179	200	194	
	3	156	130	154	128	130	134	175	197	219	
Total protein	1	7·45	7·49	8·54	8·74	7·35	6·96	6·96	7·41	7·16	0·06
	2	7·28	7·86	8·02	8·27	6·89	6·39	7·03	7·03	7·28	
	3	7·84	6·94	7·72	7·97	7·19	7·09	7·21	8·24	8·09	
Haematocrit	1	—	35·5	39·0	45·5	34·0	38·0	32·0	35·0	34·5	0·5
	2	36·5	39·0	42·0	47·0	40·0	40·5	36·0	38·0	38·0	
	3	32·5	29·0	34·5	35·5	30·5	29·0	28·5	31·5	33·0	

the monogastric animal, as suggested by Annison and White (1961), are undoubtedly important factors responsible for the maintenance of plasma glucose concentration during starvation at essentially pre-fasting levels.

Annison and White have also measured glucose utilization in fed and fasted sheep by isotope dilution and their results indicate that the tissues of intact non-lactating ewes have the ability to utilize glucose at low plasma concentrations. They suggest that under these conditions mechanisms to "spare" glucose utilization are absent in the sheep. However, this does not appear to apply to the mammary gland of the lactating cow, since there was a progressive decrease in the uptake of glucose by the mammary gland during the fasting period (Table 3). As this occurred in the absence of a marked decrease in the arterial concentration, it would appear that glucose is not utilized readily by the mammary tissue of fasting cows. Some support for this idea is provided by the finding that there is a marked fall in the concentration of lactose in the milk of cows during starvation (Smith, Howat, and Ray 1938).

It has been suggested (Evans 1964) that the function of plasma phospholipids, cholesterol esters, and cholesterol (components of the lipoprotein complexes) is to stabilize triglyceride for plasma transport. However, the large reductions in the arterial concentration of these lipids observed in the present experiment (Table 4) did not appear to be associated with changes in the concentration of triglyceride or its utilization by the mammary gland (Table 3).

V. Acknowledgments

The authors would like to thank Miss R. L. Pell, and other members of the Experimental Dairy Unit, Camden, N.S.W., for valuable technical assistance.

VI. References

Annison, E. F., Fazerkerly, S., Linzell, J. L., and Nichols, B. W. (1965).—*Biochem. J.* **94**: 21P.

Annison, E. F., and Linzell, J. L. (1964).—*J. Physiol.* **175**: 372.

Annison, E. F., and White, R. R. (1961).—*Biochem. J.* **80**: 162.

Barry, J. M. (1964).—*Biol. Rev.* **39**: 194.

Barry, J. M., Bartley, W., Linzell, J. L., and Robinson, D. S. (1963).—*Biochem. J.* **89**: 6.

Davis, J. G., and MacDonald, F. J. (1953).—Richmond's "Dairy Chemistry". 5th Ed. p. 358. (Charles Griffin & Co. Ltd.: London.)

Dole, V. P. (1956).—*J. Clin. Invest.* **35**: 150.

Evans, J. R. (1964).—*Can. J. Biochem.* **42**: 955.

Folley, S. J. (1949).—*Biol. Rev.* **24**: 316.

Gornall, A. G., Bardawill, G. J., and David, M. M. (1949).—*J. Biol. Chem.* **177**: 751.

Hartmann, P. E., and Lascelles, A. K. (1964).—*Aust. J. Biol. Sci.* **17**: 935.

Hartmann, P. E., and Lascelles, A. K. (1965).—*Aust. J. Biol. Sci.* **18**: 114.

Huggett, A. St. G., and Nixon, D. H. (1957).—*Biochem. J.* **66**: 12P.

Lauryssens, M., Verbeke, R., and Peeters, G. (1961).—*J. Lipid Res.* **2**: 383.

Luick, J. R., and Kleiber, M. (1961).—*Am. J. Physiol.* **200**: 1327.

Mackenzie, D. D. S. (1964).—M. Rur. Sc. Thesis, New England University.

McClymont, G. L. (1949).—*Biochem. J.* **45**: i.

McClymont, G. L. (1951).—*Aust. J. Agric. Res.* **2**: 158.

Overman, O. R., and Wright, K. E. (1927).—*J. Agric. Res.* **35**: 637.

Peden, V. H. (1964).—*J. Lab. Clin. Med.* **63**: 332.

Riis, P. M. (1961).—Ann. Rep. R. Vet. Agric. Coll., Copenhagen. p. 159.

SHAW, J. C. (1941).—*J. Dairy Sci.* **24**: 500.
SHAW, J. C., and PETERSEN, W. E. (1940).—*J. Dairy Sci.* **23**: 1045.
SMITH, J. A. B., and DASTUR, M. N. (1938).—*Biochem. J.* **32**: 1868.
SMITH, J. A. B., HOWAT, G. R., and RAY, S. C. (1938).—*J. Dairy Res.* **9**: 310.
STEINBERG, D. (1964).—*Metabolism* **13**: 1264.
YENSEN, R. (1964).—*Nature, Lond.* **203**: 327.
ZILVERSMIT, D. B., and DAVIS, A. K. (1950).—*J. Lab. Clin. Med.* **35**: 155.