Lactose and fatty acid synthesis in lactating-rat mammary gland

Effects of starvation, re-feeding, and administration of insulin, adrenaline, streptozotocin and 2-bromo- α -ergocryptine

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1. Lactose synthesis and fatty acid synthesis in intact lactating-rat mammary gland were measured simultaneously by incorporation of [U- 14 C]glucose and of both [U- 14 C]glucose and $^3\text{H}_2\text{O}$ respectively. 2. Both processes were almost abolished by overnight starvation. Self-re-feeding caused recovery of lipogenesis to 100% of normal by 2 h and to 170% by 5 h. Lactose synthesis recovered to 80% of normal by 5 h. 3. Food intubated to starved rats caused partial recovery in 3 h, standard diet favouring lactose synthesis and sugars favouring lipogenesis. Casein and starch were ineffective. 4. Olive oil intubated to fed rats suppressed lipogenesis greatly and lactose synthesis slightly. Paraffin oil or water partly mimicked these effects. 5. Adrenaline (subcutaneous) decreased lipogenesis from glucose, whereas insulin (subcutaneous) caused hypoglycaemia associated with loss of lactose synthesis but unchanged fatty acid synthesis. Streptozotocin and 2-bromo- α -ergocryptine (CB-154) impaired lipogenesis but not lactose synthesis. 6. The results are interpreted in terms of competition for intracellular glucose by biosynthetic pathways for lactose and fat, and the possible implications for variations in milk composition are discussed.

The rate of lactose synthesis in the intact lactating rat, measured by a radioisotopic technique, varies markedly with the time of day, the availability of food and the stage of lactation (Carrick & Kuhn, 1978; Wilde & Kuhn, 1979). Measurement *in vivo* is important because such changes in metabolic rate are not reflected by measurements *in vitro* (Wilde & Kuhn, 1979). Similarly, mammary lipogenesis measured in intact rats is greatly inhibited by starvation, recovers fully by 2 h of re-feeding (Robinson *et al.*, 1978), and shows pronounced diurnal variation (Munday & Williamson, 1983).

In the present work lactose synthesis, lipogenesis from glucose and lipogenesis from all precursors were measured simultaneously in lactating-rat mammary gland *in vivo*, in order to document in greater detail the effects of overnight starvation, of re-feeding *ad lib* or by intubation, and of administration of various hormones and drugs. The results are discussed in terms of the competi-

tion of fatty acid- and lactose-biosynthetic pathways for intracellular glucose as a consequence of the rate-limiting entry of glucose across the plasma membrane (Threadgold *et al.*, 1982; Threadgold & Kuhn, 1984).

Methods and materials

Animals

Primiparous rats of a Wistar-derived strain bred in the Department, and with litters of at least six pups, were used on days 14–16 of lactation. They were exposed to artificial lighting between 06:00 and 20:00 h each day, with food and water supplied *ad lib* unless otherwise stated. Normal food was a Modified Rat and Mouse Breeding Diet (Pilsbury Ltd., Birmingham B5 7UG, U.K.) containing 2.5% (w/w) digestible crude oil, 17.6% (w/w) digestible crude protein and 46.8% (w/w) digestible crude carbohydrate. All animal measurements were carried out between 08:00 and 12:00 h, except where re-feeding extended into early afternoon. Stomach intubation was performed on animals that had been lightly anaesthetized with diethyl ether.

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Radioisotopic procedures

A solution of 0.9% NaCl (0.3 ml) containing [U- ^{14}C]glucose (approx. 10 nmol, 3 μCi) and $^3\text{H}_2\text{O}$ (1 mCi) was injected via the femoral vein into each rat maintained under anaesthesia with approx. 2.5% (v/v) halothane in O_2 . Blood samples (0.2 ml) were taken from the tail at 1, 3, 6, 12, 20 and 30 min into chilled heparinized tubes and were centrifuged to obtain plasma. One portion of plasma (50 μl) was taken for determination of ^3H , and another portion (50 μl) was washed with 2 ml of water through a small (0.5 ml) column of Dowex-1 resin (formate form; 200 mesh; X8). One portion of the weighed eluate was assayed enzymically for glucose, and another portion (1 ml) was evaporated to dryness at about 40°C to remove the large excess of $^3\text{H}_2\text{O}$ before determination of ^{14}C , which comprised essentially only glucose. Mammary tissue was removed from each rat 30 min after injection of the radioisotopes, and was ground to a powder under liquid N_2 . Duplicate weighed portions were taken for extraction of lactose (about 0.7 g) and for saponification and extraction of fatty acids (about 1 g).

Rates of lactose biosynthesis and of fatty acid biosynthesis were calculated from the amount of radioactive lactose or fatty acid per g of tissue and the specific radioactivity of the appropriate plasma precursor. For lactose synthesis the falling specific radioactivity of plasma glucose with time was plotted for each rat and, after arbitrary division into fifteen 2-min periods, was integrated graphically (Carrick & Kuhn, 1978) to yield the formula:

$$\text{Lactose synthesized } (\mu\text{mol/h per g of tissue}) = \frac{15 \times [^{14}\text{C}]\text{lactose (d.p.m.)/g of tissue}}{\text{sa}_1 + \text{sa}_2 + \dots + \text{sa}_{15}}$$

where sa_1 , sa_2 etc. are the mean specific radioactivities (d.p.m./ μmol) of plasma glucose in successive 2-min intervals. (Note that this formula was incorrectly given in the paper quoted, although the correct version was in fact used for calculation.) Results were multiplied by 12 to give μg -atoms of glucose carbon incorporated into lactose (see the Tables). The same procedure served to calculate the rate of incorporation of glucose carbon into fatty acids, the appropriate formula being:

$$\text{Glucose C incorporated } (\mu\text{g-atoms/h per g of tissue}) = \frac{180 \times [^{14}\text{C}]\text{fatty acids (d.p.m.)/g of tissue}}{\text{sa}_1 + \text{sa}_2 + \dots + \text{sa}_{15}}$$

where sa_1 etc. are the same as in the formula above.

The rate of fatty acid synthesis from all carbon precursors, known or unknown, was calculated from the incorporation of ^3H from body water (Windmueller & Spaeth, 1966). Tissue fatty acids were saponified and extracted by the method of

Stansbie *et al.* (1976) and counted for ^3H and ^{14}C radioactivities. The specific radioactivity of plasma water, which was not always constant during the 30 min experiment, was taken as the mean value from the six blood samples obtained. Rates of lipogenesis were first calculated as μg -atoms of H incorporated/h per g of tissue. Assuming an average incorporation of 1 H atom for each C atom arising from malonyl-CoA (Jungas, 1968) and an average chain length of 12 C atoms for the newly synthesized fatty acids (Smith & Abraham, 1975), rates of H-atom incorporation were multiplied by 6/5 to obtain rates of lipogenesis from all carbon precursors as μg -atoms of C incorporated/h per g of tissue.

Glucose was determined by use of a glucose oxidase kit (Boehringer). All radioactive samples were made up to 1 ml with water, except for lipid samples, and dissolved with 10 ml of scintillation fluid, consisting of 1 vol. of Triton X-100 plus 2 vol. of xylene containing 2,5-diphenyloxazole (6 g/l) and 1,4-bis-(5-phenoxazol-2-yl)benzene (0.12 g/l). All vials were counted for radioactivity by a liquid-scintillation technique by using a programme suitable for $^3\text{H}/^{14}\text{C}$ dual label.

Insulin ('Lente') was from Wellcome Foundation, Hither Green, London SE13 6TL, U.K. 2-Bromo- α -ergocryptine (CB-154) was generously given by Professor E. Flückiger and Dr. H. Friedli, Pharmaceutical Division, Sandoz Ltd., Basle, Switzerland.

Expression of results

Results are given in the text, Tables and Figures

as means $\pm$ S.E.M. Error bars denote S.E.M. in Figures.

Results

Effects of starvation and re-feeding

The mean rate of lactose synthesis in fed rats was $102 \pm 9 \mu\text{g}$ -atoms of C/h per g of tissue, equivalent to $8.54 \pm 0.77 \mu\text{mol}$ of lactose/h per g of tissue (10 rats). This compares with mean values of 8–

$10 \mu\text{mol}$ of lactose/h per g tissue reported previously (Carrick & Kuhn, 1978; Wilde & Kuhn, 1979). The mean rate of fatty acid synthesis from glucose in the same rats was $174 \pm 28 \mu\text{g}$ -atoms of C/h per g of tissues, and from all precursors was $278 \pm 41 \mu\text{g}$ -atoms of C/h per g. The latter value

Fig. 1. Recovery of lactose synthesis and fatty acid synthesis on re-feeding of rats starved overnight

(a) Lactose synthesis ($\blacktriangle$), and fatty acid synthesis from glucose ($\bullet$) and from all precursors ($\circ$). Results are shown as percentages of mean values for fed rats (see the text), and each point is the mean $\pm$ S.E.M. for eight rats. Also shown (....) for comparison is the recovery of monosaccharide transport from plasma (Threadgold & Kuhn, 1984). (b) Percentage contribution of glucose carbon to newly synthesized fatty acid carbon ($\bullet$), and ratio of glucose carbon used for fatty acid synthesis to glucose carbon used for lactose synthesis ($\circ$), calculated from the mean values in (a).

compares with a mean recalculated value of $290 \pm 136 \mu\text{g-atoms of C/h per g}$ reported by Robinson *et al.* (1978).

Fig. 1. shows the rates of lactose and fatty acid synthesis, as percentages of rates in the fed state, in rats that had been starved for 15–18 h overnight or subsequently allowed to re-feed for up to 5 h. Lactose synthesis was decreased to 3% by starvation, thence rising to 20% after 30 min of re-feeding and reaching 80% by 5 h of re-feeding. Fatty acid synthesis from all precursors was decreased to a similar extent by starvation, whereas that from glucose was virtually abolished ($<0.1\%$). During re-feeding both values rose with roughly similar time courses to reach the value typical of fed rats by 2 h

and 170% of this value by 5 h. For comparison, Fig. 1 also shows the apparent recovery of monosaccharide transport in starved/re-fed rats measured at about the same time in our laboratory (Threadgold & Kuhn, 1984). It is evident that lactose synthesis, fatty acid synthesis and monosaccharide transport all followed separate time courses of recovery.

The percentage contribution that glucose carbon made to the total carbon atoms incorporated into fatty acids at different times is shown in Fig. 1(b). A contribution of 63% in fed rats, virtually abolished on starvation, recovered to a maximum of 75% after re-feeding for 3 h. The ratio of glucose carbon flow into fatty acid to that into lactose, also shown, fell drastically on starvation and rapidly recovered to severalfold greater values on re-feeding.

These data reveal that lactose synthesis and lipogenesis both decrease greatly on starvation and recover rapidly on re-feeding. Yet within this similarity there were marked shifts in the distribution of available glucose carbon between the two pathways and substantial changes in the contribution of glucose carbon to overall rates of lipogenesis.

Effects of re-feeding starved rats by intubation

Table 1 shows rates of lactose synthesis and fatty acid synthesis in rats that were first starved overnight and then given various nutrients, by stomach tube, 3 h before measurement. Rates are compared with those from starved rats and from starved rats subsequently allowed to re-feed *ad lib.* for 3 h (data from Fig. 1). Intubation of powdered diet restored lactose synthesis to 68% of the value seen in self-fed rats, but restored total lipogenesis to only 15% and lipogenesis from glucose to even less. Intubated glucose ($P < 0.001$) and maltose ($P < 0.01$) were significantly more effective than the diet in restoring lipogenesis. This appeared to be achieved at the expense of lactose synthesis, as judged by the significant ($P < 0.01$) redirection of glucose carbon from lactose to fatty acid. Sucrose was inferior to glucose ($P < 0.05$), and possibly to maltose ($P < 0.1$), in stimulating total lipogenesis, whereas intubated starch and casein were almost devoid of effect. Although it was not possible to intubate an amount of starch equal to the weight of glucose or maltose given, the resultant turgidity of the filled intestine made it seem unlikely that the rate of absorption of carbohydrate was thereby inhibited. Thus the recovery of mammary lipogenesis seems to require a rapidly absorbable sugar that is not generated by starch or starch-containing diet administered by intubation. As in Fig. 1, there were marked changes in the relative flow of glucose carbon to fatty acids and to lactose, and in the percentage contribution of glucose to total fatty acid synthesis.

Table 1. *Lactose and fatty acid synthesis in rats starved overnight and re-fed with different diets*

Also shown are concentrations of plasma glucose, the calculated percentage of total new fatty acid carbon arising from glucose carbon, and the calculated ratio glucose carbon converted into fatty acid/glucose carbon converted into lactose. Each value shown is the mean ($\pm$ S.E.M.) for eight rats, except for starch re-feeding, where only three rats were used. Amounts of substances intubated were: powdered diet, 4g; maltose, sucrose, glucose, 4g each; casein, 2.5g; starch, 1.5g. Times shown refer to the duration of each treatment before the start of measurements. Synthesis rates are expressed as μ g-atoms of C incorporated/h per g.

Treatment	Plasma glucose (mM)	Lactose synthesis	Total fatty acid synthesis	Fatty acid synthesis from glucose	Mean glucose contribution to total fatty acid synthesis (%)	Relative fate of glucose carbon (ratio fatty acid to lactose synthesis)
Starved	5.57 $\pm$ 0.27	3.2 $\pm$ 2.2	7.0 $\pm$ 2.2	0.1 $\pm$ 0	1	0.03
Starved + self-re-feeding (3h)	6.42 $\pm$ 0.21	71.6 $\pm$ 7.9	330 $\pm$ 21	247 $\pm$ 34	75	3.4
Starved + intubated diet (3h)	6.76 $\pm$ 0.17	48.7 $\pm$ 15.6	48.0 $\pm$ 14.5	13.6 $\pm$ 7.3	28	0.3
Starved + intubated glucose (3h)	8.19 $\pm$ 0.74	34.6 $\pm$ 6.0	200 $\pm$ 37	102 $\pm$ 25	51	2.9
Starved + intubated maltose (3h)	7.17 $\pm$ 0.67	33.2 $\pm$ 5.8	180 $\pm$ 42	92.2 $\pm$ 25.1	51	2.8
Starved + intubated sucrose (3h)	9.94 $\pm$ 0.29	27.0 $\pm$ 7.2	102 $\pm$ 16.3	25.0 $\pm$ 6.7	25	0.9
Starved + intubated casein (3h)	6.45 $\pm$ 0.01	12.8 $\pm$ 6.0	11.5 $\pm$ 2.9	0.4 $\pm$ 0.1	3	0.03
Starved + intubated starch (3h)	6.27	1.5	8.4	0.2	2	0.1

Effects of intubated oils and water in fed rats

The intubation or feeding of triacylglycerol to fed rats has been shown to suppress the rate of fatty acid synthesis in the rat mammary gland *in vivo* (Agius & Williamson, 1980; Grigor & Warren, 1980). In confirmation of this, Table 2(a) shows that intubated olive oil greatly inhibited total lipogenesis when this was compared with lipogenesis in untreated rats. Lipogenesis from glucose was even more impaired, so that the contribution of glucose carbon to fatty acid synthesis fell to only 23%. However, intubated water alone significantly inhibited total lipogenesis ($P < 0.002$) and lipogenesis from glucose ($P < 0.02$). Paraffin oil, which is presumably not digested to fatty acids, did not affect total lipogenesis, but apparently halved the incorporation of glucose. The rate of lactose synthesis was depressed significantly by olive oil ($P < 0.05$) and by water ($P < 0.05$), and not significantly by paraffin oil.

These experiments suggest that some of the inhibitory effects of olive oil may not arise from the products of its digestion, but from procedures associated with its administration. Brindley *et al.* (1981) have observed an increase in plasma corticosterone to follow the intubation of saline into rats. Whatever its mechanism, however, it served to show how differently the mammary pathways of lactose and fatty acid biosynthesis can be affected.

Effects of administered adrenaline, insulin, streptozotocin and CB-154

Adrenaline administered 30 min previously significantly ($P < 0.05$) inhibited glucose carbon incorporation into fatty acid, despite the distinct hyperglycaemia that developed, but had no significant effect on total lipogenesis or lactose synthesis (Table 2b). Thus there was a relative redistribution of glucose carbon away from fatty acid synthesis towards lactose synthesis. Because hyperglycaemia was not a feature of the intubation experiments described above, any effects that may have arisen from stress cannot reasonably be ascribed to the release of adrenaline.

Lactose synthesis was markedly inhibited ($P < 0.001$) by insulin administered 5 min previously, and profoundly inhibited by insulin administered 30 min previously (Table 2b). These effects correlated with the extent of hypoglycaemia and, confirming observations in other species (Kronfeld *et al.*, 1963; Reynolds & Rook, 1977; Hove, 1978a), underline the sensitivity of lactose synthesis to the immediate supply of glucose. Despite the hypoglycaemia, the rate of lipogenesis and the contribution of glucose to this process were unaffected. Naturally there was a great increase in the proportion of glucose directed towards fatty

Table 2. Lactose and fatty acid synthesis in rats intubated with (a) oil or water, and in rats given (b) adrenaline or insulin or (c) streptozotocin or CB-154 by injection. Also shown are concentrations of plasma glucose, the calculated percentage of total new fatty acid carbon arising from glucose carbon, and the calculated ratio glucose carbon converted into fatty acid/glucose carbon converted into lactose. Each value shown is the mean ($\pm$ s.e.m.) for at least eight rats. Times shown refer to the duration of each treatment before the start of measurements. Abbreviation: s.c., subcutaneous. Synthesis rates are expressed as μ g-atoms of C incorporated/h per g.

Treatment	Plasma glucose concn. (mM)	Lactose synthesis	Total fatty acid synthesis	Fatty acid synthesis from glucose	Mean glucose contribution to total fatty acid synthesis (%)	Relative fate of glucose carbon (ratio fatty acid to lactose synthesis)
(a) Fed, untreated	7.61 $\pm$ 0.25	110 $\pm$ 17	198 $\pm$ 27	145 $\pm$ 34	73	1.3
Fed, intubated olive oil (2h)	6.89 $\pm$ 0.24	63.4 $\pm$ 8.2	27.0 $\pm$ 6.0	6.2 $\pm$ 1.8	23	0.10
Fed, intubated water (2h)	7.39 $\pm$ 0.17	54.1 $\pm$ 16.2	83.9 $\pm$ 14.3	36.0 $\pm$ 8.2	43	0.67
Fed, intubated paraffin oil (2h)	7.27 $\pm$ 0.12	74.8 $\pm$ 9.7	142 $\pm$ 25	71.1 $\pm$ 15.8	50	0.95
(b) Fed, + NaCl s.c. (30min)	7.05 $\pm$ 0.29	112 $\pm$ 6	206 $\pm$ 34	113 $\pm$ 23	55	1.0
Fed, + adrenaline bitartrate s.c. (20 μ g/100g body wt.; 30min)	9.01 $\pm$ 0.37	89.8 $\pm$ 11.0	160 $\pm$ 19	55.4 $\pm$ 7.8	35	0.62
Fed, + adrenaline bitartrate s.c. (40 μ g/100g body wt.; 30min)	10.35 $\pm$ 0.46	123 $\pm$ 13	172 $\pm$ 13	64.0 $\pm$ 4.9	37	0.52
Fed, + insulin s.c. (0.2 unit/100g body wt.; 5min)	6.24 $\pm$ 0.27	63.5 $\pm$ 8.0	211 $\pm$ 22	110 $\pm$ 14	52	1.7
Fed, + insulin s.c. (0.2 unit/100g body wt.; 30min)	3.76 $\pm$ 0.29	10.3 $\pm$ 2.3	161 $\pm$ 22	92.1 $\pm$ 13.6	57	8.9
(c) Fed, + streptozotocin (5mg/100g body wt.; 3h)	14.4 $\pm$ 0.89	72.1 $\pm$ 22.8	31.6 $\pm$ 15.2	8.8 $\pm$ 7.3	28	0.12
Fed, + streptozotocin (5mg/100g body wt.) + insulin (0.2–0.4 unit/100g body wt.)	4.57 $\pm$ 1.98	11.4 $\pm$ 0.6	151 $\pm$ 33	89 $\pm$ 26	59	7.8
Fed, + glucose (0.13–0.4g/100g body wt.; 3h)	8.08 $\pm$ 0.24	69.6 $\pm$ 25	132 $\pm$ 25	50.7 $\pm$ 11.8	38	0.73
Fed, + CB-154 (0.17mg/100g body wt.; s.c. 12 and 0.4h)						

acids as opposed to lactose, in which respect therefore insulin and adrenaline acted in opposite directions.

The constancy of the rate of lipogenesis after insulin administration implies that the hypoglycaemia was not due to increased mammary withdrawal of plasma glucose, and suggests that activation of critical glycolytic and lipogenic enzymes within the mammary gland was compensating for the decreased availability of glucose as a precursor.

In accord with this interpretation, administered streptozotocin, an inhibitor of insulin release that causes marked hyperglycaemia, greatly inhibited lipogenesis, but did not significantly affect lactose synthesis (Table 2c). This effect on lipogenesis was largely reversed by administration of insulin and glucose, but lactose synthesis was impaired, possibly because of the accompanying hypoglycaemia.

Administration of 2-bromo- α -ergocryptine (CB-154) significantly ($P < 0.01$) decreased both total lipogenesis and that from glucose, but the apparent impairment of lactose synthesis did not reach statistical significance.

Discussion

Measured rates of milk-solid synthesis *in vivo* vary markedly between apparently comparable animals. Thus lactose synthesis varied 2.6-fold and lipogenesis 5-fold within a group of ten fed rats examined within a few weeks of each other. It is therefore important to collect enough data and to intersperse the different treatments under study throughout the period of data collection, which can be several weeks. Especially valuable is the simultaneous measurement of two or more processes within each animal, the activity ratios of which generally yield mean values with appreciably smaller standard errors. The effect of a given treatment on the activity of one pathway relative to another is consequently more readily discerned than that on its absolute activity.

Effect of nutritional change

The profound inhibition of lactose and fatty acid synthesis caused by starvation overnight confirms the nutritional sensitivity of these processes (Carrick & Kuhn, 1978; Robinson *et al.*, 1978; Wilde & Kuhn, 1979) and is to be expected of an organ that consumes so much of the available body nutrients. The speed with which lipogenesis recovers during re-feeding is indicative of enzyme activation rather than induction, and one can speculate that in such empty glands the considerable overshoot that is reached by 5h may reflect an absence of negative feedback by any accumulated milk products. It is suggested that the rapid flow of glucose carbon into fatty acid may be achieved at the expense of lactose

synthesis, the recovery of which was distinctly slower and never exceeded 80% of that in fed rats.

Glucose contributed about 63% of the carbon required for lipogenesis in the fed state; however, this may be a minimum value, owing to experimental stress during 30 min anaesthesia causing the release of adrenaline, which we have shown does direct glucose utilization away from lipogenesis (Table 2). In any case the lower percentage contribution of glucose in the starved or partially re-fed rat indicates an inhibition of glycolysis separate from, and greater than, that of lipogenesis. The contributions *in vivo* of other likely precursors, such as alanine, lactate and glutamine, remain to be quantified (Katz *et al.*, 1974; Bartley & Abraham, 1976; Vina & Williamson, 1981).

Intubation experiments, intended to pinpoint the dietary constituent most essential for restoration of lactose and fatty acid synthesis, showed how difficult it is to mimic the natural food intake. That the most effective substances, glucose and maltose, fell short of natural re-feeding in the biosynthetic rates that they produced may indicate inhibitory effects of stressful handling. Consistent with this interpretation was the inhibition caused by intubation of water to fed animals. The relative inability of intubated diet, and especially of starch, to restore lipogenesis is particularly striking and may indicate the importance of salivary amylase in initiating starch hydrolysis.

Proposed competition between biosynthetic pathways for lactose and fatty acid

The results of starvation and self-re-feeding of lactating rats emphasize the overall similarity of lactose and fatty acid biosynthetic responses to nutritional change, while nevertheless revealing quantitative differences in these responses. By contrast, the remaining, more experimentally contrived, situations emphasize rather the differences between the two responses. These apparently opposing responses may arise from a difference in the relationship that each process bears to insulin, the hormone most likely to mediate nutritional change. Lipogenesis is readily enhanced by insulin, which probably activates the transport, phosphorylation and glycolysis of plasma glucose as well as the two rate-limiting lipogenic enzymes pyruvate dehydrogenase (EC 1.2.4.1) and acetyl-CoA carboxylase (EC 6.4.1.2). Control probably rests more with the state of activation of the enzymes than with the concentrations of precursors such as glucose, lactate etc.

On the other hand, the influence of insulin on lactose synthesis is likely to be mixed, insofar as it both replenishes intracellular glucose by stimulating transport and depletes it by hastening its conversion into fat. The concentration of intracellular

glucose seems likely to dominate the control of lactose synthesis in established lactation, where α -lactalbumin is in excess. Cytosolic glucose almost certainly equilibrates into the Golgi lumen, where its concentration (about 0.1–0.5 mM) remains below the K_m for glucose of lactose synthetase (1.5 mM) (Kuhn *et al.*, 1980; Wilde & Kuhn, 1981; Faulkner *et al.*, 1981). Other things being equal, the rate of lactose synthesis can be expected to be depressed by a high rate of lipogenesis and enhanced by a low rate of lipogenesis. This situation arises from the rate-limiting nature of glucose transport across the plasma membrane (Threadgold *et al.*, 1982). The consequently unpredictable net influence of insulin on lactose synthesis probably accounts for the lack of correlation that has been observed between the two in the rat and goat (Wilde & Kuhn, 1979; Hove, 1978a,b).

The inhibition of total lipogenesis by CB-154 confirms a previous report (Agius *et al.*, 1979) and extends it to lipogenesis specifically from glucose. The effect can be explained by the fall in concentration of plasma prolactin (Agius *et al.*, 1979) and the known sensitivities of pyruvate dehydrogenase, acetyl-CoA carboxylase and fatty acid synthetase activities to this hormone (Field & Coore, 1976; McNeillie & Zammit, 1982; Speake *et al.*, 1976), even though Robinson & Williamson (1977) did not observe any decreased mammary glucose uptake in CB-154-treated rats. The decreased lipogenesis of CB-154-treated rats may have had a glucose-sparing effect that compensated lactose synthesis for the impairment otherwise to be expected from the known prolactin-dependence of α -lactalbumin (Turkington & Hill, 1969).

In the rat glucose provides acetyl-CoA, ATP, reducing power and glycerol 3-phosphate for milk triacylglycerol synthesis. In different species, where certain of these essentials may arise from other sources (see Kuhn, 1978), the requirement for glycerol 3-phosphate and NADPH may nevertheless constitute a drain on intracellular glucose. Therefore a reciprocal relationship between milk fat and lactose contents might be sought among other species generally. Fig. 2 reveals a pronounced inverse relationship between the fat and lactose contents reported for the milks of 85 different species (data from Jenness, 1974). Primate milk, for example, is characterized by much lactose but little fat, whereas the reverse obtains for the milks of many aquatic mammals. Palmiter (1969) has also drawn attention to this, albeit on the basis of fewer data, but was unable to provide an experimentally supported explanation. An inverse relationship is also strikingly exhibited by the changing composition of rabbit milk during the course of lactation (Cowie, 1969), whereas a decreased fat content accompanies the rise in lactose content of

Fig. 2. Reciprocal relationship between milk lactose and fat content

Each point shows a mean milk composition from several individuals of a single species (data from Jenness, 1974). The curve was fitted by eye.

the milk of bears brought from the wild into captivity (Jenness *et al.*, 1972).

As a recently evolved process, lactose synthesis may not have acquired the stringent hormonal control characteristic of lipogenesis, and may therefore be dominated by the latter. If the present viewpoint is correct, then the study of the regulation of lactose biosynthesis cannot ignore simultaneous changes in milk fat synthesis. Moreover the rate of milk fat synthesis may influence the final fat content of secreted milk both by direct contribution and by affecting the synthesis of an important osmotic constituent of the aqueous phase. The decreased milk yield of starved or of nutritionally impaired ruminants is accompanied by an elevated fat content (Rook & Line, 1961; Van Soest, 1963; Linzell, 1967; Dawson & Rook, 1972). The present study suggests that this reflects the superior ability of fat synthesis to compete with lactose synthesis for low concentrations of intracellular glucose. This explanation would accord with the correlation of milk fat content with acetate/propionate ratios in ruminant plasma (Van Soest, 1963), since acetate is a lipogenic precursor, whereas propionate is an insulin secretagogue and a crucial precursor of plasma glucose. By contrast the yield and composition of human milk appears to be much less sensitive to maternal malnutrition (Walker *et al.*, 1952; Gopalan & Belavady, 1961; unnamed author, 1975). This may be connected with the unusually high glucose concentration in human milk, possibly reflecting a high intracellular concentration (Faulkner *et al.*, 1981).

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