The Topography of Lactose Synthesis

By NICHOLAS J. KUHN and ADRIAN WHITE
*Department of Biochemistry, University of Birmingham,
P.O. Box 363, Birmingham B15 2TT, U.K.*

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1. At short incubation times, and under suitable osmotic conditions, the lactose synthesized by Golgi-derived vesicles of rat mammary gland is 85-90% particulate. Evidence is presented for its occlusion within the lumen of the vesicles. 2. Ovalbumin is used as a bulky active-site inhibitor to show that the active site of lactose synthase lies on the inner face of the Golgi membrane. 3. Phlorrhizin and phloretin inhibit lactose synthesis by such vesicles, indicating the presence of a glucose-transport system. 4. The relationship of this topography to the synthesis of *N*-acetylneuraminyllactose and to the secretion of milk sugars is discussed.

Recent studies on the enzyme lactose synthase (EC 2.4.1.22) have not only clarified the enzymic features of lactose synthesis but have also thrown light on the mechanism of secretion of lactose from the mammary epithelial cell. Of the two component proteins, galactosyltransferase is bound firmly to the membrane of what appears to be the Golgi apparatus, whereas α -lactalbumin is present in soluble form within the Golgi lumen. According to Brew's (1969) hypothesis it is the flow of α -lactalbumin from the ribosomes, past the stationary galactosyltransferase and into the alveolar lumen that determines the rate of lactose synthesis and accounts for the preponderance of α -lactalbumin over galactosyltransferase in the milk.

Although this scheme accords with current views on the role of the Golgi apparatus in synthesis-secretion processes, very little direct evidence has been adduced in its support. In particular, no studies have yet dealt with the topographical locations of the substrates and products of lactose synthase, although it may be assumed that UDP-galactose occurs in the cytosol because this is the location of UDP-glucose 4-epimerase (EC 5.1.3.2). Similarly, glucose is presumably also present in the cytosol after its facilitated transport across the plasma membrane; consistent with this is the predominantly cytosol location of mammary hexokinase (EC 2.7.1.1) (Gumaa *et al.*, 1971).

The present paper describes experiments on a particulate fraction from homogenates of lactating rat mammary gland which presumably contains pinched-off vesicles derived from the Golgi apparatus. Evidence is given for the production of lactose on the luminal side of the membrane and for the transport of glucose to that site by facilitated diffusion. The bearing of this topography on the subsequent synthesis of *N*-acetylneuraminyllactose is discussed.

Experimental

Materials

Rats were a Wistar-derived stock bred in the Department, and were generally used during their first lactation. UDP-galactose was from Sigma (London) Chemical Co., London S.W.6, U.K. Radiochemicals were from The Radiochemical Centre, Amersham, Bucks., U.K.

Methods

Mammary particles. Mammary tissue (4g wet wt.) from 15-day lactating rats was trimmed, chopped and homogenized with 36ml of 0.9% (w/v) NaCl or 0.25M-lactose (or mannitol where indicated) in a glass tube fitted with a Teflon pestle of 0.45mm total clearance. About six strokes of the pestle were used, but this was not standardized. The homogenate was centrifuged for 10min at 10000*g*_{av.}, and the supernatant further centrifuged for 30min at 105000*g*_{av.}. The high-speed sediment was resuspended by brief homogenization, in the apparatus described above, with 3.5ml of 50mM-glycylglycine-NaOH buffer, pH 7.5, in either 0.9% (w/v) NaCl, 0.25M-lactose or 0.25M-mannitol as indicated. Portions of this suspension, which contained about 40mg of protein/ml, were diluted 10-fold into the same medium and used in reaction mixtures as 'intact' particles. Other portions, diluted fivefold, were mixed with an equal volume of 2% (w/v) digitonin in 25mM-Tris-HCl, pH 7.5, and used as 'lysed' particles. All procedures were carried out at 0-4°C, and intact particles were used within 2h of preparation.

Sonication of particles (Table 2) was performed with a probe of 1cm diameter operating at 60W, 20000 cycles/s.

Determination of [^{14}C]lactose. Two methods were used to determine the [^{14}C]lactose formed during incubations from radioactive substrates.

(a) With UDP-[^{14}C]galactose as the substrate, small columns of Dowex-1 (formate form) resin were used to isolate the [^{14}C]lactose as described previously (Kuhn, 1968). In this case control incubations from which glucose was omitted were necessary to correct for the formation of [^{14}C]galactose by hydrolysis of the substrate.

(b) With either UDP-[^{14}C]galactose or [U- ^{14}C]glucose as the substrate, the [^{14}C]lactose formed could be isolated by repeated precipitation in the presence of carrier lactose.

Reaction mixtures or tissue extracts were denatured with 6% (w/v) HClO_4 . After centrifugation, 1 ml of the clear acid extract was added to 200 mg of solid lactose and warmed at 70°C to effect solution. After cooling, the lactose was precipitated out by the addition of 8 ml of ethanol-diethyl ether (3:1, v/v). After 30 min at about 20°C the sediment was collected by centrifugation, redissolved with 1 ml of water and reprecipitated in the same way. Two further precipitations followed, with 0.8 ml of water to dissolve the sediment and 6 ml of ethanol-ether to precipitate it. The final sediment was dissolved with 1 ml of water, giving a final volume of about 1.3 ml. Of this, 1 ml was taken for determination of radioactivity in the presence of 0.5 ml of water and 10 ml of scintillation fluid, and 0.1 ml was diluted to 100 ml with water and duplicate 1 ml portions were assayed for lactose by the phenol- H_2SO_4 method (Ashwell, 1966). In this way the final amount of radioactive lactose could be corrected for the measured recovery (about 60%) that occurred during precipitation. In control experiments where radioactive glucose, glucose 1-phosphate, UDP-galactose or mixed metabolites prepared by extracting liver slices that had been incubated with [^{14}C]glucose were added, less than 1% of the initial radioactivity was recovered in the final lactose precipitate.

In suitable cases this technique is an alternative to the isolation of radioactive lactose by chromatography on paper or on borate ion-exchange columns, and has the merit of extreme simplicity. It offers a new way of assaying low activities of lactose synthase in the presence of more active UDP-galactose hydrolysis, and supersedes the charcoal-resin method (Kuhn, 1969), which we found difficult to reproduce reliably.

Determination of [^{14}C]galactosyl-ovalbumin. Ovalbumin, which was used as a galactosyl acceptor, was precipitated out of solution, after the addition of 2 mg of bovine serum albumin carrier, with 10 mM-silicotungstic acid-50 mM- H_2SO_4 (final concn.). The precipitate was collected and washed three times with the precipitant, dissolved in 1 ml of 1 M-NaOH

and a portion mixed with scintillation fluid for radioactivity counting.

Scintillation fluid was prepared by mixing 1 vol. of Triton X-100 with 2 vol. of xylene containing 2,5-diphenyloxazole (6 g/l) and 1,4-bis-(5-phenoxazol-2-yl)benzene (0.12 g/l).

Results

Retention of newly synthesized lactose by particles

When mammary-gland particles are incubated briefly with glucose and UDP-galactose in the presence of Mn^{2+} ions and then centrifuged in a cold, iso-osmotic medium for 30 min at $105000g_{\text{av}}$, much of the newly synthesized lactose sediments with the particles. Table 1 shows that, in a NaCl medium, about 50 and 40% of the total lactose synthesized was retained by the particles after incubation at 37°C for 1 and 2 min respectively. The same result was obtained whether glucose or UDP-galactose was radioactively labelled. In control incubations to which [^{14}C]lactose was added initially, less than 2% was retained, indicating that the above results did not represent mere adsorption of lactose to the particles.

Since the newly made lactose that was found in the suspending medium might have already leaked out of the particles, conditions were explored to find its maximum retention. The time-course studies in Fig. 1 revealed the greatest retention of

Table 1. *Retention of newly synthesized, or added, [^{14}C]lactose by mammary particles*

Particles were prepared in 0.9% (w/v) NaCl and incubated for 1 or 2 min at 37°C with UDP-galactose+glucose, one of these being labelled with ^{14}C . The final reaction mixtures contained particles (equivalent to 0.14 g wet wt. of tissue), 123 mM-NaCl, 40 mM-glycylglycine-NaOH buffer, pH 7.5, 18 mM- MnCl_2 , 0.5 mM-UDP-galactose (0.5 $\mu\text{Ci}/\mu\text{mol}$ where labelled) and 50 mM-glucose or 21 mM-[^{14}C]glucose (0.2 $\mu\text{Ci}/\mu\text{mol}$) in a final volume of 0.2 ml. The total amount of [^{14}C]lactose formed was about 20 and 40 nmol at 1 and 2 min respectively. In Expt. 4 the [^{14}C]lactose was 0.5 mM. Expt. 1* was carried out with particles aged at 0°C for 24 h. Each value shown is the result of a single reaction mixture.

Expt. no.	Incubation time Substrates	Percentage of lactose retained	
		1 min	2 min
1	UDP-[^{14}C]galactose+glucose	50	39
1*	UDP-[^{14}C]galactose+glucose	19	—
2	UDP-[^{14}C]galactose+glucose	55	42
3	UDP-galactose+[^{14}C]glucose	54	41
4	[^{14}C]Lactose	<2	<2

Fig. 1. Retention of newly synthesized lactose by particles incubated for different times in different media

The incubation media contained 35mM-glycylglycine-NaOH buffer, pH 7.5, 0.05mM-UDP- ^{14}C galactose (50nCi), 50mM-glucose, 2mM-MnCl₂, particles (equivalent to 0.16g wet wt. of tissue) and either 0.2M-lactose at 37°C (Δ), 0.72% (w/v) NaCl at 37° ($\circ$) or 25° ($\bullet$), or 0.2M-mannitol at 37°C ($\blacksquare$) in a final volume of 0.2ml. Particles were correspondingly prepared in 0.25M-lactose, 0.25M-mannitol or 0.9% (w/v) NaCl. After incubation each reaction mixture was diluted into 1.8 ml of ice-cold 0.25M-lactose, 0.25M-mannitol or 0.9% (w/v) NaCl and sedimented. Each point shows the result of a single reaction mixture.

lactose at very brief incubation times. Further, the retention in a NaCl medium was increased by lowering the temperature of the incubation to 25°C. The best retention was seen in 0.25M-lactose medium at 37°C, when 85–90% of the newly made lactose sedimented with the particles after incubation for 0.5–2min. Mannitol was a poor substitute. It is probable that some loss of lactose is unavoidable during the 30min period of centrifugation to recover the particles. The inability of the lactose medium to displace newly synthesized lactose from the particles appears to rule out the presence of specific adsorption sites. Surprisingly, the percentage retention of lactose under given conditions was very reproducible, as may be seen by comparing the appropriate results in Tables 1 and 2 and in Fig. 1.

Release of bound lactose from particles

Since these experiments implied the trapping of newly generated lactose within the particles, it was expected that treatments which rupture membranes should cause its release. Particles were prepared and incubated with substrates in 0.25M-lactose to generate retained lactose. Then, before

Table 2. Effects of disruptive treatments on the retention of ^{14}C lactose by mammary particles

Particles that had been prepared in 0.25M-lactose were loaded with ^{14}C lactose by incubation for 2min with UDP- ^{14}C galactose+glucose under the conditions given in Fig. 1. They were then diluted into 1.8ml of the appropriate medium at 0°C and, after 5min, were collected by centrifugation for 30min at 105000g_{av}. Freezing and thawing of diluted samples (Expt. 3) was performed within the 5min period by alternate exposure to an acetone-solid CO₂ freezing mixture and to a water bath at 30°C. The results of individual reaction mixtures are shown and expressed as percentage retention of the total lactose synthesized. Abbreviation: EGTA, ethanedioxybis(ethylamine)tetra-acetic acid.

Expt. no.	Conditions of dilution medium	Percentage of lactose retained
1	0.25M-Lactose	89
	Water	13
2	0.25M-Lactose	90
	10mM-EGTA in 0.25M-lactose	91
	0.01% Deoxycholate in 0.25M-lactose	87
	0.03% Deoxycholate in 0.25M-lactose	81
	0.1% Deoxycholate in 0.25M-lactose	14
	Water	10
3	0.25M-Lactose	84
	Sonication for 30s in 0.25M-lactose	27
	Sonication for 60s in 0.25M-lactose	15
	Freezing and thawing $\times$ 2	81
	Freezing and thawing $\times$ 4	81

sedimentation, they were subjected to osmotic shock by dilution into water, or, in the presence of 0.25M-lactose, to sonication, deoxycholate or repeated freezing and thawing. Table 2 shows that the percentage of sedimentable lactose was drastically decreased by all treatments except for low concentrations of deoxycholate and for freezing and thawing. It is notable that freezing and thawing have not found a use in the rupture of microsomal vesicles of liver (Dallner & Ernster, 1968). Moreover, mammary homogenates may be stored frozen without loss of lactose synthase activity, and so presumably without loss of retained α -lactalbumin (Brodbeck & Ebner, 1966; Jones, 1972).

Particulate lactose in intact mammary tissue

Experiments were carried out to check that the synthesis of particulate lactose was also a feature of intact cell preparations.

Slices of lactating mammary tissue were incubated for 2min at 37°C with $[\text{U-}^{14}\text{C}]$ glucose. They were then rapidly rinsed in 0.9% (w/v) NaCl at 0°C, homogenized in ice-cold 0.25M-lactose and centrifuged to sediment particulate matter. Soluble

Table 3. *Particulate nature of [^{14}C]lactose synthesized by mammary slices*

Tissue slices (0.5g wet wt.) were incubated in duplicate flasks (1 and 2) for 2min at 37°C in 5ml of Krebs-Ringer bicarbonate buffer, pH7.4, containing 2mM- ^{14}C glucose (approx. 0.5 μCi). They were then blotted, chilled and homogenized in 3ml of 0.25M-lactose at 4°C. Homogenates were centrifuged for 30min at 105000 g_{av} directly to give high-speed sediments (S_2), in Expt. 1, or after an initial centrifugation for 5min at approx. 500 g_{av} to give a low-speed sediment (S_1), in Expt. 2. Sediments and supernatants were analysed for [^{14}C]lactose and for total ^{14}C . The results are expressed as the percentage of the total tissue lactose or ^{14}C that was sedimented. In Expt. 2 values for the low-speed (S_1) and the high-speed (S_2) sediments are given.

	% radioactivity in particles	
	[^{14}C]Lactose	Total ^{14}C
Expt. 1		
Flask 1	77	—
Flask 2	71	—
Expt. 2		
Flask 1	66 ($S_1 = 6$; $S_2 = 60$)	18
Flask 2	70 ($S_1 = 22$; $S_2 = 48$)	14

and particulate fractions were assayed for [^{14}C]lactose and, in one experiment, for total radioactivity. The results of two experiments are given in Table 3. Of the total [^{14}C]lactose in the tissue 66–77% was particulate. In Expt. 2, where the particulate matter was separated into ‘low-speed’ and ‘high-speed’ sedimenting material, the bulk of the [^{14}C]lactose was in the latter. By contrast, only 14–18% of the total radioactivity was particulate.

These findings suggested that even in normal lactating tissue a measurable proportion of the endogenous lactose might be found to be particulate at any one time. To confirm this one mammary gland was removed from each of two rats under anaesthesia on day 15 of lactation. The tissue was chilled in 0.9% (w/v) NaCl at 0°C, chopped, and homogenized in the same solution or in 0.25M-sucrose. After being strained through muslin, the homogenate was centrifuged for 30min at 105000 g_{av} and the supernatant and sediment were separately analysed for lactose by a specific enzymic assay (Reithel, 1963). In the two rats the total lactose content was respectively 21.3 and 9.0 $\mu\text{mol/g}$ wet wt. and respectively 21 and 23% was particulate.

Location of the active site of vesicular lactose synthase

The presence of newly made lactose within the vesicles, after the substrates have been presented to the outside of the vesicles, implies

Fig. 2. *Ovalbumin inhibition of lactose synthesis*

The incubation media contained 10mM-glycylglycine-NaOH buffer, pH7.5, 7.5mM-MnCl₂, 0.2M-lactose, 0.05mM-UDP-galactose, 1mM- ^{14}C glucose (50nCi), 0–1mM-ovalbumin, α -lactalbumin (0.4mg/ml, added only with lysed particles), and intact (○) or digitonin-lysed (●) particles (equivalent to 1mg wet wt. of tissue) in a final volume of 0.05ml. The incubation was for 20min at 37°C. Each point shows the mean result of duplicate determinations.

that either the lactose has been synthesized first and then transported across the membrane, or vice versa. To distinguish between these possibilities experiments were designed to locate the position on the membrane of the enzyme active site. The mechanistic studies of Morrison & Ebner (1971*a,b,c*) show that there is probably a single type of galactosyl acceptor site, which may be occupied by glucose or by *N*-acetylglucosamine. *N*-Acetylglucosamine is a suitable substrate both when it is free and when attached in β -(1 $\rightarrow$ 4) linkage to a larger molecule. Therefore we used ovalbumin, which has a terminal *N*-acetylglucosamine residue (Montgomery *et al.*, 1965), as a competitive inhibitor of lactose synthesis. It was reasoned that an active site on the inside of a membrane vesicle should not be accessible to a molecule as large as ovalbumin, which has a molecular weight of 45000. Fig. 2 shows that lactose synthesis by intact particles was indeed poorly inhibited by ovalbumin. By contrast, much more effective inhibition occurred with particles that had been previously lysed with digitonin. Fig. 3 shows that *N*-acetylglucosamine, which might be expected to penetrate membranes more readily, inhibited both preparations to a similar extent; again, however, there were indications of slightly greater effectiveness in lysed preparations.

In the lysed-particle preparations it was necessary to add α -lactalbumin to replace that which had been diluted out during lysis. It could be argued that the

Fig. 3. *N*-Acetylglucosamine inhibition of lactose synthesis. Incubations were carried out with intact (■, ●) and lysed (□, ○) particles as described in the legend to Fig. 2, except that ovalbumin was replaced by 0–5 mM-*N*-acetylglucosamine. The temperature was 25° (■, □) or 37° (●, ○). Each point shows the mean result of duplicate determinations.

concentration of added α -lactalbumin differed from that within the particles, which is unknown, in such a way as to favour greater inhibition by ovalbumin. Brew *et al.* (1968) reported that α -lactalbumin inhibited the reaction of *N*-acetylglucosamine with galactosyltransferase, although Schanbacher & Ebner (1970) reported that this was not the case with ovalbumin. In control experiments, however, we found that the ability of *N*-acetylglucosamine to inhibit lactose synthase activity in lysed-particle preparations was unaffected by concentrations of α -lactalbumin up to 1 mg/ml.

A more direct test of ovalbumin accessibility to the active site is to measure its own activity as a galactosyl acceptor. In a single experiment it was found that it accepted 1 nmol of galactose from intact particles, and 2.7 nmol from the same amount of lysed particles. Its significant activity with the intact particles probably reflects the presence of ruptured membranes which, having lost their α -lactalbumin, can react with ovalbumin or *N*-acetylglucosamine though not with glucose.

Evidence for a glucose transport system

Since the active site of lactose synthase appeared to be on the inside of the vesicle membrane, the question arose as to how it was reached by substrate added on the outside. In other cells where glucose passes through a membrane there is evidence for a specific transport system. We therefore used phlorrhizin and phloretin, classical inhibitors of such systems, to try to block glucose

Fig. 4. *Phlorrhizin* inhibition of lactose synthesis

Incubations with intact (●) or lysed (○) particles were carried out as described in the legend to Fig. 2, except that ovalbumin was replaced by 0–10 mM-phlorrhizin and the substrates were 0.05 mM-UDP-[14 C]galactose+1 mM-glucose. Each point shows the mean result of duplicate determinations.

Fig. 5. *Phloretin* inhibition of lactose synthesis

Incubations with intact (●) and lysed (○) particles were carried out as described in the legend to Fig. 2, except that ovalbumin was replaced by 0–0.5 mM-phloretin and the substrates were 0.05 mM-UDP-[14 C]galactose+1 mM-glucose. The results shown are the means of five separate experiments.

entry into intact mammary particles and thereby to inhibit lactose synthesis.

Fig. 4. shows that phlorrhizin strongly inhibited the synthesis of lactose by intact particles, whereas it had little effect on synthesis by lysed particles. Phloretin showed the same difference, though at lower concentrations (Fig. 5). However, since it was rather more inhibitory to the solubilized lactose synthase than was phlorrhizin, the experiment was repeated several times to establish clearly the difference between intact and lysed particles. Fig. 5 shows the mean values of five such experiments. Higher concentrations of glucose were not able to reverse the inhibitions by phlorrhizin or phloretin.

A number of sugars or derivatives were tested as competitive inhibitors either of glucose transport or of glucose as a galactosyl acceptor in the presence of lysed particles, with the hope that they might serve as tools to investigate the topographical locations of these steps. The compounds included lactose, cellobiose, D-galactose, L-arabinose, D-xylose, 2-deoxy-D-glucose, and 3-O-methyl-D-glucose. None was effective at the concentrations used (10–100 mM).

Retention of glucose by particles

Since the results with phlorrhizin and phloretin provide only indirect evidence for a glucose-transport process, direct evidence was sought for the entry of glucose into these particles and its inhibition by one of these agents. It was found that particles incubated with [^{14}C]glucose (21000 d.p.m.) retained some radioactivity (4614 d.p.m.) when subsequently diluted into cold 0.25 M-lactose containing 1 mM-phloretin and sedimented. Less radioactivity was retained if either the phloretin (2446 d.p.m.) or the lactose (2397 d.p.m.) was omitted, or if the [^{14}C]glucose was added directly to the lactose-phloretin medium (1617 d.p.m.). These experiments were repeated several times with essentially the same results.

These data indicate the entry of glucose into vesicles by a phloretin-sensitive step. They do not, however, show that these vesicles are the same as those that make lactose. Indeed, the presence of considerable 5'-nucleotidase activity in these preparations (results not shown) suggested appreciable contamination by the plasma membrane, with its associated glucose-transport system. In an attempt to remove plasma membrane, the lactose-synthesizing vesicles were purified by centrifugation in discontinuous and in continuous gradients of sucrose, in a manner similar to that described by Coffey & Reithel (1968). However, the particles so obtained had a markedly poorer ability to retain either glucose or lactose, so they were not suitable for experiments of this nature.

Discussion

When a tissue is homogenized the endoplasmic reticulum is broken up into small pieces, many of which pinch off into spherical vesicles (see, e.g., Redman *et al.*, 1966; Ehrenreich *et al.*, 1973). In the preparation of mammary homogenates with the Potter-Elvehjem homogenizer such pinching off is sufficiently rapid that the α -lactalbumin originally enclosed in the Golgi apparatus is retained, or partly so, by the final vesicles. Thus it resists dilution but may be released by disruptive treatments. Such vesicles may be detected in mammary homogenates by their ability to synthesize lactose in the absence of added α -lactalbumin. These vesicles form the subject of the present study, and the assumption is made that their properties directly reflect those of the original Golgi apparatus. The evidence that they are derived from the Golgi region of the endoplasmic reticulum rests on the electron-microscopic identification of particles enriched in galactosyltransferase activity (Keenan *et al.*, 1972*a,b*). Scheme 1, which bears on the following discussion, summarizes our current view of the topography of lactose and *N*-acetylneuraminyl-lactose synthesis. It supersedes the scheme proposed earlier (Kuhn, 1971).

The demonstration that vesicles retain the newly synthesized lactose, in a manner not explicable by adsorption, forms the most direct evidence to date that mammary lactose is concentrated within the Golgi tubules. This is supported by our finding that about 22% of the total lactose is particulate in intact tissue; the rest is probably extracellular. From data on the volume of mammary cell occupied by Golgi apparatus (7.4% of lipid-free cell volume; Hollman, 1968) and the amount of intracellular water (about 0.2 g/g fresh wt. of tissue; Murphy *et al.*, 1973), and by assuming 15 μmol of lactose/g fresh wt., it is possible to estimate a lactose concentration of about 330 mM within the Golgi lumen. This is consistent with the high concentration of lactose that is characteristic of milks generally, and illustrates the ability of the mammary cell to concentrate lactose. Our experiments with ovalbumin as a bulky active-site inhibitor show that lactose synthase is probably embedded in the inner face of the Golgi membrane, with the active site exposed to the substances in the lumen. It is therefore of obvious importance that even high concentrations of lactose are unable to inhibit the activity of the enzyme (Palmiter, 1969).

The presence of lactose synthase inside the Golgi lumen is consistent with the belief that it is by origin a part of the machinery for glycosylating glycoproteins, since one can envisage that the (glyco)-protein substrates flow down the Golgi lumen in the manner proposed for α -lactalbumin (Brew, 1969). In this light it is reasonable that the adaptation of the

Scheme 1. *Proposed topography of lactose and N-acetylneuraminyl-lactose synthesis in the Golgi apparatus*

The scheme shows the penetration of reactants through the membrane by facilitated transport (in the case of glucose) or by unknown mechanisms (indicated by ?). A, Galactosyltransferase; B, α -lactalbumin; NLS, N-acetylneuraminyl-lactose synthase; AcNeu, N-acetylneuraminic acid.

galactosyltransferase for the synthesis of lactose should have been accompanied by the appearance of a transport system to allow entry of glucose from the cytosol. The ability of two classical inhibitors of glucose-transport systems in cell membranes to inhibit lactose synthesis constitutes the first evidence for a similar system in the Golgi membrane. The rather high concentrations of these inhibitors that are needed may indicate that the glucose-transport

system is in great excess over the lactose synthase, and that it only becomes limiting when a large degree of inhibition has been attained. This could also explain why lactose synthesis was not inhibited by sugars that might be expected to inhibit glucose transport competitively.

The transport mechanism of UDP-galactose is still unknown. We have so far failed to get evidence for any galactosyl-lipid intermediate similar to the

glycosyl phosphodolichols that appear to participate in the synthesis of some mammalian glycoproteins. If such a mechanism exists, however, the UDP-galactose would have to be regenerated on the inside of the membrane, since studies on the purified galactosyltransferase have shown conclusively that it is the true substrate. An alternative mechanism would be the specific transport of UDP-galactose across the membrane, perhaps coupled with the outward transport of UDP.

The above model for lactose synthesis also throws light on the studies *in vitro* that have been made of *N*-acetylneuraminyl-lactose synthesis. This is an important sugar during part of lactation in the rat and mouse (Kuhn, 1972) and is synthesized by isolated microsomal fractions in the presence of lactose and CMP-*N*-acetylneuraminic acid (Barra *et al.*, 1969; Carlson *et al.*, 1973). It is suggested that the unusual kinetics, which both groups of workers have observed, and the presence of endogenous *N*-acetylneuraminyl acceptor that Carlson *et al.* (1973) found so difficult to wash out, are both explained by the fact that the lactose is normally generated from within the particle. Indeed, Barra *et al.* (1969) showed that endogenously generated lactose was used in preference to added lactose. Their results appear to be more simply explained in terms of Scheme 1 than by their suggestion of enzyme-bound lactose and the possibility of two distinct *N*-acetylneuraminyl-lactose synthases.

In rat milk the ratio lactose/*N*-acetylneuraminyl-lactose varies greatly during lactation (Kuhn, 1972). Scheme 1 implies that the ratio may reflect on the one hand the ratio lactose synthase/*N*-acetylneuraminyl-lactose synthase and their associated substrates, and on the other hand the rate of milk secretion. At high rates of milk secretion it might be expected that newly synthesized lactose will be rapidly secreted before there is much chance of it being converted into *N*-acetylneuraminyl-lactose. These ideas remain to be tested.

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References

- Ashwell, G. (1966) *Methods Enzymol.* **8**, 85–95
 Barra, H. S., Cumar, F. A. & Caputto, R. (1969) *J. Biol. Chem.* **244**, 6233–6240
 Brew, K. (1969) *Nature (London)* **223**, 671–672
 Brew, K., Vanaman, T. C. & Hill, R. L. (1968) *Proc. Nat. Acad. Sci. U.S.* **59**, 491–497
 Brodbeck, U. & Ebner, K. E. (1966) *J. Biol. Chem.* **241**, 5526–5532
 Carlson, D. M., Jourdian, G. W. & Roseman, S. (1973) *J. Biol. Chem.* **248**, 5742–5750
 Coffey, R. G. & Reithel, F. J. (1968) *Biochem. J.* **109**, 177–183
 Dallner, G. & Ernster, L. (1968) *J. Histochem. Cytochem.* **16**, 611–632
 Ehrenreich, J. H., Bergeron, J. J. M., Siekevitz, P. & Palade, G. E. (1973) *J. Cell Biol.* **59**, 45–72
 Gumaa, K. A., Greenbaum, A. L. & McLean, P. (1971) in *Lactation* (Falconer, I. R., ed.), pp. 197–238, Butterworths, London
 Hollman, K. H. (1968) *Z. Zellforsch. Mikrosk. Anat.* **87**, 266–277
 Jones, E. A. (1972) *Biochem. J.* **126**, 67–78
 Keenan, T. W., Huang, C. M. & Morré, D. J. (1972a) *J. Dairy Sci.* **55**, 51–57
 Keenan, T. W., Huang, C. M. & Morré, D. J. (1972b) *J. Dairy Sci.* **55**, 1577–1585
 Kuhn, N. J. (1968) *Biochem. J.* **106**, 743–748
 Kuhn, N. J. (1969) *J. Endocrinol.* **44**, 39–54
 Kuhn, N. J. (1971) in *Lactation* (Falconer, I. R., ed.), pp. 161–176, Butterworths, London
 Kuhn, N. J. (1972) *Biochem. J.* **130**, 177–180
 Montgomery, R., Lee, Y. C. & Wu, Y. C. (1965) *Biochemistry* **4**, 566–577
 Morrison, J. F. & Ebner, K. E. (1971a) *J. Biol. Chem.* **246**, 3977–3984
 Morrison, J. F. & Ebner, K. E. (1971b) *J. Biol. Chem.* **246**, 3985–3991
 Morrison, J. F. & Ebner, K. E. (1971c) *J. Biol. Chem.* **246**, 3992–3998
 Murphy, G., Ariyanayagam, A. D. & Kuhn, N. J. (1973) *Biochem. J.* **136**, 1105–1116
 Palmiter, R. D. (1969) *Nature (London)* **221**, 912–914
 Redman, C. M., Siekevitz, P. & Palade, G. E. (1966) *J. Biol. Chem.* **241**, 1150–1158
 Reithel, F. J. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), p. 103, Academic Press, New York and London
 Schanbacher, F. L. & Ebner, K. E. (1970) *J. Biol. Chem.* **245**, 5057–5061