Biosynthesis of Lactose by Mammary Gland Slices from the Lactating Rat*

J. C. Bartley,† S. Abraham, and I. L. Chaikoff‡

From the Department of Physiology, University of California, Berkeley, California 94720

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SUMMARY

The incorporation of the isotope from D-glucose-1-14C, D-glucose-1-3H, D-glucose-2-14C, D-glucose-3-14C, D-glucose-6-14C, D-glucose-6-3H, uniformly labeled D-glucose-14C, uniformly labeled D-galactose-14C, and D-mannose-1-14C into lactose by slices of mammary glands of lactating rats (14 to 20 days post partum) was studied. The lactose formed was hydrolyzed either with acid or with β-galactosidase, and the component hexoses were isolated and assayed for isotopic content.

Three findings indicate that D-glucose, and not α-D-glucose 1-phosphate, acts as the galactosyl acceptor during lactose biosynthesis. (a) The extent of conversion of the label of the variously labeled glucoses to lactose was the same regardless of the site of the label and regardless of whether it was 14C or 3H. (b) The addition of unlabeled glucose to the incubation medium was required for incorporation of 14C from D-mannose-1-14C into lactose. (c) Lactose formed in the experiments with D-mannose-1-14C and unlabeled glucose as substrates was labeled almost exclusively in the galactose portion.

The results of periodate degradation of the hexose moieties of lactose formed in experiments in which D-glucose-6-14C served as substrate revealed that little carbon from the triose stage enters the phosphohexose pool via reversal of the Embden-Meyerhof pathway.

In the presence of unlabeled D-glucose (1 × 10−3 M), the incorporation of the label of uniformly labeled D-galactose-14C into lactose was at least 10 times that into CO2, indicating that galactose probably enters the metabolic scheme in mammary gland as D-galactose 1-phosphate.

It has been established that the two hexose moieties of lactose in the mammary gland are derived from blood glucose (1, 2), but the mechanism of their incorporation into the lactose molecule is currently in doubt (Fig. 1). From experiments with cell-free extracts of lactating bovine mammary glands, Gander, Petersen, and Boyer (3, 4) have concluded that α-D-glucose 1-phosphate is the galactosyl acceptor and lactose 1-phosphate is the initial product. Subsequent hydrolysis by phosphatases in the gland would convert the phosphorylated disaccharide to lactose (Scheme A). On the other hand, the evidence that Watkins and Hassid (5, 6) obtained with particulate preparations of bovine and guinea pig mammary glands and that obtained by Babad and Hassid (7) with an enzyme isolated from bovine milk has shown that free α-glucose is the galactosyl acceptor and that lactose is the initial product (Scheme B). It is generally accepted that uridine diphosphate-α-galactose, derived from glucose 1-phosphate via uridine diphosphate-glucose (8–10), is the galactosyl donor (4, 6).

These findings with homogenate preparations raise the question as to which of the proposed mechanisms operates in the intact cell. Studies with lactating rat mammary gland slices (11), perfused udders (12), and intact lactating cows (13–15) support the view that glucose rather than glucose 1-phosphate is the galactosyl acceptor in the formation of lactose. However, as pointed out by Wood et al. (14) and Hansen et al. (15), the interpretation of the results of these experiments is equivocal because of alternative explanations based on unequal rates of equilibration of precursor pools or of the existence of more than one D-glucose 6-phosphate pool in mammary gland.

The studies reported here were designed to elucidate whether glucose or glucose 1-phosphate is the galactosyl acceptor in the formation of lactose by slices of lactating rat mammary glands.

EXPERIMENTAL PROCEDURE

Treatment of Rats Lactating rats of the Long-Evans strain, which were raised and maintained on an adequate stock diet (Wayne Lab-Blox) and had each suckled at least six pups, were killed 14 to 20 days post partum by a blow on the neck. All of the visible mammary glands were then quickly excised, trimmed of connective tissue, and placed in an ice-cold, oxygenated Krebs-Henseleit bicarbonate buffer (16) of pH 7.3 to 7.4.

Incubation Procedure—Slices of the mammary glands (0.4 mm thick) were prepared with the apparatus described by McLain and Buddle (17). The slices were washed repeatedly with the ice-cold bicarbonate buffer until milk was no longer observed in the wash and then gently blotted dry with coarse filter paper. Then 250 mg of slices were transferred to the main compartment of a flask, provided with a center well (18), which contained the labeled and unlabeled substrates indicated in the tables. The flask was gassed with a mixture consisting of 95% O2 and 5% CO2, closed with a self-sealing rubber serum cap, and the mixture...
was incubated for 3 hours, with shaking, in a water bath main-
tained at 37°. At the end of that time, the tissue was inactivated
by immersing the flask in boiling water for 1 min. The medium
was then acidified and the CO₂ was collected as described previ-
ously (19), except that 0.3 ml of a 1:1 (v/v) mixture of ethanol-
amine and 2-ethoxyethanol was used to trap the CO₂ (20).

Isolation and Isotopic Assay of Lactose—The contents of the
incubation flask were filtered through glass wool to separate
the slices from the acidified medium. A measured aliquot of
the filtrate was deionized by passing it through a column (10 mm
× 20 cm) containing Amberlite IR 112 (H⁺ form) in the upper
one-third and Dowex 3 (OH⁻ form) in the lower two-thirds.
At least 100 ml of distilled water were then added to the column,
and the neutral eluate was concentrated in a vacuum at 50°
and adjusted to a known volume.

The material in the neutral eluate was separated by paper
chromatography with the aid of the 1-butanol-acetic acid-water
(by volume, 100:22:50) solvent system. Unlabeled sugars,
applied to the paper to serve as standards, were located with the
benzidine-trichloracetic acid spray reagent (21). Radioautog-
raphy with Kodak medical x-ray film or liquid scintillation assay
of the paper chromatogram revealed two areas of radioactivity.
One corresponded to lactose and the other corresponded to the
labeled substrate that was added to the medium, either glucose,
galactose, or mannose. All of the radioactivity in the neutral
eluate was associated with these two areas.

In the experiments in which either 1 × 10⁻⁴ M or 2 × 10⁻⁴ M
sugar served as the labeled substrate, the specific activity of
the glucose isolated on the paper chromatogram, calculated from
the glucose concentration and the isotopic activity, was the
same as that of the glucose added at the start of the incubation.
The isotopic activity of glucose in the neutral eluate could there-
fore be calculated from the final glucose concentration. The
isotopic activity of lactose in the neutral eluate was taken as the
difference between the total isotopic activity and isotopic activity
of glucose. The reliability of this calculation was substantiated
by separating the compounds in the neutral eluate of the experi-
ments with ²³C-labeled glucose by paper chromatography with
the solvent system composed of 1-butanol, acetic acid, and water.
The isotopic activity of the lactose isolated in this manner was
the same as that calculated from the difference between the total
isotopic activity in the neutral eluate and the activity in the
neutral eluate attributable to lactose.

In the experiments in which the substrate was labeled with
³⁵S, the sugars in the neutral eluate were separated chroma-
tographically on paper, eluted from the paper with water, and
assayed in the liquid scintillation spectrometer.

In the experiments in which ¹⁴C-labeled substrates were used,
the ¹⁴C content of the chromatographically separated sugars was
determined (a) as in the experiments with ³⁵S, (b) by direct
scintillation assay of the samples on paper (22), or (c) by oxida-
tion of the sugars on paper with persulfate followed by assay of
the CO₂ in the liquid scintillation spectrometer (23). The
reliability of isotopic assay of sugars on paper by these methods
has been established (23).

Degradation of Lactose—For determination of ¹⁴C or ³⁵S activity
in its galactose and glucose moieties, lactose was isolated chroma-
tographically on paper, eluted from the paper, and hydrolyzed
with either 0.5 N H₂SO₄ at 95° for 3 hours or purified β-galac-
tosidase at 37° for 2 hours (9). The acidic hydrolysate was
neutralized by addition of solid BaCO₃ and the BaSO₄ precipitate
was removed by centrifugation.

The glucose and galactose so obtained were separated by paper
chromatography in the solvent system composed of ethyl acetate,
pyridine, acetic acid, and water (by volume, 5:5:2:3).¹ The
multiple descending technique was used to insure adequate
separation of the hexoses. Glucose and galactose were eluted
with water. The eluates were concentrated in a vacuum at 50°
to a known volume, and an aliquot of the concentrated solution
was taken for assay in the liquid scintillation spectrometer. The
isotopic activity of the glucose or galactose moieties is presented
as percentage of the total isotopic activity of the original
lactose.

The ¹³C activities of carbon 6 of the chromatographically
separated glucose and galactose moieties of lactose, in the experi-
ments with glucose-6-¹³C, were determined by oxidizing each
hexose with periodate, which yields formaldehyde from the
primary alcoholic carbon atom (25). The ¹⁴C in the formalde-
hyde was determined as described by Abraham and Hassid (26).
The radioactivity in the glucose and galactose was measured
after persulfate oxidation to CO₂ (27). The ¹³C in C-6 as well
as in the whole molecule was determined as the ¹⁴C activity per
mg of BaCO₃.

Verification of Formation of Lactose—The following evidence
shows that the product isolated in the experiments with lactating
rats was truly lactose. (a) The neutral material eluted from
the union and cation exchange column contained a compound
that could not be separated chromatographically (filter paper).
From authentic lactose in the butanol-acetic acid-water as well as
in the ethyl acetate-pyridine-acetic acid-water solvent system.
(b) When the compound moving with authentic lactose on paper
chromatograms was hydrolyzed with acid or, more important,
with β-galactosidase (an enzyme specific for the glycosidic
linkage of lactose), paper chromatography of either hydrolysate
in the ethyl acetate-pyridine-acetic acid-water solvent system
revealed that all of the radioactivity resided in the areas corre-
sponding to glucose and galactose.

Determination of ³⁵S and ¹⁴C Activities—The BaCO₃ was assayed
for ¹³C activity on planchets with a Nuclear-Chicago scaler,
model 161A. A liquid scintillation spectrometer (Packard
Tri-Carb, model 314) was used for all of the other analyses of
radioactivity. The liquid scintillation medium for counting
the samples on paper has been described (22, 23). The medium
for all of the other liquid scintillation assays was 10 ml of a 2:1
(v/v) mixture of toluene and 2-ethoxyethanol containing 50 mg
of 2,5-diphenyloxazole. For assay of ³⁵S-labeled compounds, 10
ml of the scintillation mixture contained 100 mg of 2,5-
diphenyloxazole and 2 mg of 1,4-bis-2'-(5'-phenyloxazolyl)-
benzene.

Determination of Glucose—Glucose was determined by a
glucose oxidase method (Glucostat, Worthington Biochemical
Corporation) in which 0.1 M phosphate buffer, pH 7.2, was
substituted for water in the reaction mixture.

Materials—Glucose-U-¹³C (28) and galactose-U-¹³C were
prepared photosynthetically from Conna indica (29) and Irides
laminaroides (30), respectively. D-Glucose-1-¹³C, 1-¹⁴C, 1-¹⁴C,
and 6-¹³H and acetate-2-¹⁴C were purchased from the New
¹ M. Torri and B. E. Volcani, personal communication cited
by Carsten and Pierce (24).
² Glucose-U-¹³C and galactose-U-¹⁴C refer to the uniformly
labeled d isomers of glucose and galactose.
England Nuclear Corporation. d-Glucose-2-14C and d-mannose-1-14C were obtained from Dr. H. S. Isbell, National Bureau of Standards, Washington, D. C. D-Glucose-3,4-14C, prepared as described by Kogstad, Kemp, and Katz (51), was generously provided by Dr. J. Katz, Cedars of Lebanon Hospital, Los Angeles, California. d-Glucose-3,4,5-14C was prepared and isolated as described previously (32). dL-Lactate-2-14C was kindly furnished by Dr. R. M. Lemmon of the Donner Laboratory, University of California. β-Galactosidase, purified from Escherichia coli, was a gift from Dr. W. Z. Hassid of the University of California.

RESULTS

Experiments with 14C- and 3H-Labeled Glucoses

Effect of Glucose Concentration on Conversion of 14C of Glucose-U-14C to Lactose—The incorporation of the 14C of glucose-U-14C into lactose by 250 mg of lactating rat mammary gland slices increased as the concentration of glucose in the medium was varied from 2 × 10^{-3} to 2 × 10^{-2} M (Table I). At the lowest glucose concentration, namely, 2 × 10^{-3} M, 0.12 μmole of glucose carbon was incorporated into lactose. When the concentration of glucose in the medium was raised to 4 × 10^{-2} M, the incorporation of glucose carbon into lactose was 0.44 μmole. A 7-fold increase in the incorporation of the 14C into lactose was observed when slices were incubated with 1 × 10^{-2} M glucose. Increasing the glucose concentration 2-fold increased incorporation of the label into lactose 1.5-fold. This indicates that a glucose concentration of 2 × 10^{-2} M was approaching that for maximal synthetic capacity under our incubation conditions.

Extent of Lactose Synthesis from Variously Labeled Glucoses—The incorporation of the isotopes from glucose-1-14C, glucose-2-14C, glucose-3,4-14C, glucose-6-14C, glucose-1-3H, glucose-3-3H, and glucose-6-3H into lactose by lactating mammary gland slices is shown in Table II. The site of the label, regardless of whether it was carbon or tritium, had no appreciable effect on the extent of incorporation of isotope into lactose (Table I). The average incorporation of label into lactose was about 19% of the glucose utilized during the 3-hour incubation period.

Incorporation of 14C from Glucose-6-14C into C-6 of Glucose and Galactose Moieties of Lactose—The lactose formed in experiments with glucose-6-14C was hydrolyzed, and the individual hexoses were isolated. When the glucose was oxidized with periodate, the specific activity of the formaldehyde, which represents carbon 6 of the hexose, was 6 times that of the entire hexose molecule. This finding shows that all of the 14C in the glucose moiety of the lactose formed in the experiments with glucose-6-14C still resided in carbon 6. When, in the same experiments, the distribution of 14C in the galactose moiety was examined, about 84% of it was found in the sixth carbon.

Experiments with Galactose-U-14C and Mannose-1-14C

Conversion of 14C of Galactose-U-14C and Mannose-1-14C to Lactose and CO2—The extent of incorporation of the 14C of galactose-U-14C and mannose-1-14C into lactose was increased markedly by the addition of glucose to the medium (Table III). The addition of 25 μmoles of unlabeled glucose to the medium enhanced about 10-fold the incorporation of the 14C of galactose-U-14C into lactose when the concentration of the labeled galactose in the medium was less than 1 × 10^{-4} M, and about 3-fold when the galactose concentration was higher. The addition of 25 μmoles of unlabeled glucose to the medium increased the conversion of mannose-1-14C into lactose about 7-fold.

When glucose was added to an incubation medium containing 4 × 10^{-3} M of labeled galactose, the incorporation of 14C into lactose was about the same as that observed in experiments with 1 × 10^{-3} M glucose-U-14C in the medium (Table III). This level of incorporation appeared to be maximum, since increasing the concentration of galactose-U-14C 2.5-fold produced no further increase in the amount of galactose carbon incorporated into lactose.

In the experiments in which the medium contained 1 × 10^{-4} M labeled galactose and 1 × 10^{-3} M unlabeled glucose, the incorporation of 14C into lactose exceeded that into CO2 (Table III). In contrast, at the same concentration, the label of glucose-U-14C was converted to an equal extent to CO2 and lactose.

### Table I

Incorporation of 14C of glucose-U-14C into lactose by slices of mammary glands of lactating rats

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>Glucose</th>
<th>Glucose utilized</th>
<th>% utilized</th>
<th>μmoles</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>2 × 10^{-4}</td>
<td>95.8 ± 1.4</td>
<td>2.6 ± 0.6</td>
<td>0.12</td>
</tr>
<tr>
<td>6</td>
<td>4 × 10^{-4}</td>
<td>95.1 ± 1.4</td>
<td>4.6 ± 0.9</td>
<td>0.44</td>
</tr>
<tr>
<td>4</td>
<td>1 × 10^{-3}</td>
<td>71.2 ± 3.1</td>
<td>17.0 ± 2.6</td>
<td>3.0</td>
</tr>
<tr>
<td>1</td>
<td>2 × 10^{-3}</td>
<td>27.4</td>
<td>33.1</td>
<td>4.5</td>
</tr>
</tbody>
</table>

### Table II

Incorporation of isotope of glucose variously labeled with 14C and 3H into lactose by mammary gland slices prepared from lactating rats

See Table I for details of incubation procedures. The substrate concentration was 1 × 10^{-4} M in all cases. Each value is the mean, followed by its standard error, of determinations from duplicate incubations of tissue from each rat.

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>Substrate</th>
<th>Isotope of glucose incorporation into lactose</th>
<th>% utilized glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Glucose-1-14C</td>
<td>20.1 ± 1.5</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Glucose-2-14C</td>
<td>17.6</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Glucose-3,4-14C</td>
<td>18.5 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Glucose-6-14C</td>
<td>20.8 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Glucose-U-14C</td>
<td>17.0 ± 2.6</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Glucose-1-3H</td>
<td>17.7 ± 2.4</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Glucose-3-3H</td>
<td>19.0 ± 4.3</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Glucose-6-3H</td>
<td>20.2 ± 1.6</td>
<td></td>
</tr>
</tbody>
</table>
TABLE III
Incorporation of isotope of variously labeled glucoses, galactose-U-14C, and mannose-1-14C into lactose by slices of mammary glands of lactating rats and distribution of isotope among hexose moieties of lactose

See Table I for details of incubation procedures. The substrates and concentrations are given in the table. The values are the means of duplicate determinations from at least two incubations.

<table>
<thead>
<tr>
<th>Labeled substrate</th>
<th>Unlabeled substrate</th>
<th>Added isotope recovered in</th>
<th>Label in lactose as</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CO2</td>
<td>Lactose</td>
</tr>
<tr>
<td>Glucose-1-14C, 1 x 10^{-3} M</td>
<td>None</td>
<td>23.1</td>
<td>8.1</td>
</tr>
<tr>
<td>Glucose-3,4-14C, 1 x 10^{-3} M</td>
<td>None</td>
<td>37.8</td>
<td>11.2</td>
</tr>
<tr>
<td>Glucose-6-14C, 1 x 10^{-3} M</td>
<td>None</td>
<td>2.3</td>
<td>9.7</td>
</tr>
<tr>
<td>Glucose-6-1, 1 x 10^{-3} M</td>
<td>None</td>
<td>10.5</td>
<td>58</td>
</tr>
<tr>
<td>Glucose-U-14C, 2 x 10^{-3} M</td>
<td>None</td>
<td>1.2</td>
<td>3.2</td>
</tr>
<tr>
<td>Glucose-U-1, 1 x 10^{-3} M</td>
<td>None</td>
<td>22.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Mannose-U-1, 1 x 10^{-3} M</td>
<td>Glucose*</td>
<td>11.9</td>
<td>1.4</td>
</tr>
<tr>
<td>Galactose-U-1, 4 x 10^{-3} M</td>
<td>None</td>
<td>8.6</td>
<td>11.2</td>
</tr>
<tr>
<td>Galactose-U-1, 4 x 10^{-3} M</td>
<td>None</td>
<td>1.2</td>
<td>3.2</td>
</tr>
<tr>
<td>Galactose-U-1, 4 x 10^{-3} M</td>
<td>None</td>
<td>0.6</td>
<td>24.5</td>
</tr>
<tr>
<td>Galactose-U-1, 4 x 10^{-3} M</td>
<td>None</td>
<td>0.5</td>
<td>2.7</td>
</tr>
<tr>
<td>Galactose-U-1, 4 x 10^{-3} M</td>
<td>None</td>
<td>0.4</td>
<td>8.1</td>
</tr>
</tbody>
</table>

* The concentration of glucose used was 1 x 10^{-3} M.

The 14C of mannose-1-13C was oxidized to CO2 as readily as was that of glucose-1-14C. This result confirms previous observations (33). The extent of incorporation of 14C of mannose-1-13C into lactose in the presence as well as in the absence of unlabeled glucose in the medium was much lower than that observed with glucose-1-14C.

Incorporation of Label from Galactose-U-14C and Mannose-1-14C into Glucose and Galactose of Lactose—Lactose that was synthesized in experiments with galactose-U-14C and with mannose-1-14C was labeled almost exclusively in the galactose moiety (Table III).

Experiments with Acetate-2-14C and Lactate-2-14C

When slices of rat mammary glands were incubated with 1 x 10^{-3} M acetate-2-14C or with 1 x 10^{-3} M lactate-2-14C, incorporation of 14C into lactose could not be detected, regardless of whether unlabeled glucose (1 x 10^{-3} M) had been added to the medium or not. Both of these substrates are readily converted to CO2 and fatty acid by lactating rat mammary gland (34, 35).

DISCUSSION

Two mechanisms have been proposed for the biosynthesis of lactose from glucose (Fig. 1). Cander, Peterson, and Boyer (3, 4) isolated lactose-1-P in experiments in which glucose-1-P served as substrate for soluble enzymes obtained from homogenates of lactating bovine mammary gland, and from this they (3, 4) concluded that lactose is formed via Scheme A (Fig. 1). Watkins and Hassid and Babad and Hassid, working with particulate preparations from lactating guinea pig and bovine mammary glands (5, 6) and a soluble enzyme from bovine milk (7), concluded that lactose is synthesized from glucose and UDP-galactose according to Scheme B. They obtained no evidence for formation of lactose-1-P (5, 6).

Of these two mechanisms, the one involving glucose as the galactosyl acceptor (Scheme B) appears consistent with the earlier work of Wood et al. (12-15) and with the observation that incorporation of the isotope into glucose was about twice that into the galactose moiety of lactose formed in our experiments with labeled glucose. Wood et al. (12-15) showed that lactose synthesized from 14C-labeled acetate and propionate by perfused bovine udders (12) and from glycerol-1,3-14C by lactating cows in vivo (14, 15) was labeled to a greater extent in the galactose moiety than in the glucose moiety. Hansen et al. (15) have presented additional evidence for free glucose as the galactosyl acceptor in the intact cell. When the isolated bovine udder was perfused simultaneously with glucose-2-14C and glycerol-1,3-14C, the glucose moiety of lactose was labeled almost exclusively in C-2 and the galactose moiety was labeled as it was in the experiments in which only glycerol 1,3-14C was employed. If glucose-1-P were the galactosyl acceptor, both the glucose and galactose moieties of lactose of these experiments should have had the same labeling pattern. Because of alternative explanations of the results of these studies, based on the rate of equilibration between precursor pools or the existence of more than one glucose-6-P pool in mammary gland (14, 15), it is impossible to state categorically which scheme operates in the intact cell.

The recent finding (11) that lactose isolated from lactating mammary glands incubated with glucose-U-14C for 1 min was labeled exclusively in the glucose moiety was taken as evidence for the operation of Scheme B. However, a much more rapid rate of entry of the 14C of glucose into the glucose moiety than into the galactose moiety of lactose by either scheme, as well as unequal equilibration rates of precursor pools, could also account for such a labeling pattern.

If all of the glucose were phosphorylated before being incorporated into lactose (Scheme A), one would expect the incorporations of isotope into glucose to be about twice that into the galactose moiety.
corporation of isotope from glucose-1-14C, glucose-1-3H, glucose-2-14C, and glucose-3-3H to be lower than that from glucose-6-14C and glucose-6-3H because of loss of the label from the 1, 2, and 3 labeled sugars during their initial passage through the pentose phosphate cycle. This cycle is active in mammary gland tissue (35, 37). If the equilibration of the d glucose-6-P ketal isomerase reaction in lactating mammary gland is complete, the loss of label during passage through the pentose phosphate cycle would reduce the isotopic activity of the glucose-6-P and, thereby, of glucose-1-P and lactose. The similarity in the extent of conversion of labeled glucose to lactose in the present study, regardless of the site and whether the label was 14C or 3H, thus favors Scheme B rather than Scheme A.

In either of the proposed schemes, the isotopic activity of the galactose moiety of lactose derived from glucose-1-14C, glucose-1-3H, glucose-2-14C, and glucose-3-3H should be lower than that from glucose-6-14C and glucose-6-3H because of the cyclical activity of the pentose phosphate cycle. Since most of the isotopes of the labeled sugars reside in the glucose portion of lactose, it is conceivable that a reduction in incorporation of isotopes from glucose-1-14C, glucose-1-3H, glucose-2-14C, and glucose-3-3H into galactose could not be detected by the methods used here.

Evidence that glucose is incorporated intact into lactose, whereas the galactose moiety arises from the hexose phosphate pool (Scheme B), is provided by the experiments in which mannose-1-14C was incubated with lactating rat mammary gland slices in the presence and absence of glucose. In this tissue, mannose is phosphorylated to mannose-6-P, which is then converted to glucose-6-P via fructose-6-P (33) (Fig. 1). The finding that mammary gland does not produce glucose means that lactose can be formed solely from mannose only if the glucose moiety is derived from glucose-1-P. The fact that 14C from mannose-1-14C was incorporated into lactose to an appreciable extent only in the presence of exogenous glucose indicates that the major pathway of biosynthesis of lactose is a coupling of free glucose, presumably with UDP-galactose derived from the phosphohexose pool (Scheme B). That the lactose formed from mannose-1-14C, in the presence of unlabeled glucose, was labeled almost exclusively in the galactose moiety confirms the conclusion that glucose is the galactosyl acceptor. The results of the experiments with mannose-1-14C, therefore, strongly support the view of Wood et al. that glucose (12-15, 36) is incorporated into lactose without first being phosphorylated, and indicate that the enzyme system described by Watkins and Hassid (5, 6) operates in the intact cell. In addition, the lack of appreciable labeling in the glucose portion of lactose supports the conclusion that glucose is not produced by the lactating rat mammary gland.

Degradation of the galactose moiety of lactose formed in the experiments with glucose-6-14C shows the extent to which reversal of the Embden-Meyerhof pathway contributed carbon from the triose stage of glycolysis to lactose. If carbon from the triose phosphates was incorporated into lactose via a reversal of the Embden-Meyerhof pathway, some of the 14C of glucose-6-14C would have appeared in C-1 of the galactose of lactose because of equilibration between glyceraldehyde-3-P and dihydroxyacetone-P. If, on the other hand, carbon arising from the triose stage of glycolysis were incorporated by a transaldolase-transketolase reaction, as suggested by Hansen et al. (15), none of the label derived from glucose 6-14C would have been lost from C-6. Thus, our findings that most of the label remained in C-6 indicates that carbon from the phosphorylated trioses did not reenter the glucose-6-P pool via reversal of the Embden-Meyerhof pathway; however, the possibility that conversion of carbon from the phosphorylated trioses to hexose phosphates via the transaldolase-transketolase reactions is not excluded.

Slices prepared from mammary glands of lactating rats failed to incorporate the 14C of acetate-2-14C or of lactate-2-14C into lactose. This is probably the result of a nonreversal of glycolysis at a level below the triose phosphates (34, 35) rather than nonreversal between triose phosphates and hexose phosphates. Acetate and lactate become important sources of lactose carbon in the intact animal probably only after their conversion, in tissues other than the mammary gland, to compounds that can be utilized for lactose formation by the mammary gland (2).

The results of the experiments with labeled galactose throw light on the first steps of galactose utilization in the mammary gland. It has been shown that in other tissues galactose is first activated to galactose-1-P (39, 40) (Fig. 1). Conversion of galactose to galactose-1-P in the mammary gland is consistent with the findings presented here, i.e. that in contrast to glucose, galactose enters the metabolic pathway as a compound readily converted to lactose but oxidized to CO2 with difficulty. Galactose-1-P can be converted to UDP-galactose and thence to lactose by UDP-galactose pyrophosphorylase, an enzyme whose presence has been shown in the lactating mammary gland (41). Galactose-1-P uridylintransferase, the enzyme catalyzing the conversion of galactose-1-P to glucose-1-P, is absent (10) or is of negligible activity (41) in the mammary gland. Therefore, conversion of galactose-1-P to glucose-1-P and glucose to glucose-6-P and CO2 can occur only to a small extent.

The higher incorporation of the label of galactose than that of mannose into galactose of lactose is consistent with the view that mannose competes for the same phosphorylating system as does glucose, and enters the same phosphorylated intermediate pool (33), whereas galactose enters as galactose-1-P (39, 40). Glucose is required as the galactosyl acceptor for lactose synthesis from mannose only as far as from galactose; but in the case of mannose, glucose inhibits the uptake of mannose (42) and, by entering the same phosphohexose pool, dilutes the label from mannose. Galactose, on the other hand, enters the metabolic scheme at a point where it competes with less than 10% of the glucose utilized when glucose and galactose are both present in the incubation medium in a concentration of 1 × 10^-2 M.

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REFERENCES