The Synthesis of Lactose from Glucose in the Mammary Gland

By O. K. REISS and J. M. BARRY*
Department of Biochemistry, University of Chicago

(*Received* 30 *May* 1953)

The mammary gland of goats and cows has been shown to absorb glucose from the blood stream during lactation (Folley, 1949), and lactose is formed when slices or suspensions of the mammary glands of guinea pigs and rats are incubated with glucose (Malpress & Morrison, 1950; Reithel, Horowitz, Davidson & Kittinger, 1952; Caputto & Trucco, 1952; Hills & Stadie, 1952). This work strongly suggested that lactose is synthesized from glucose absorbed from the blood stream by the mammary gland, and a recent experiment with [1-^{14}C]glucose did in fact prove that in the goat the glucose and galactose residues of lactose are derived, at least partly, from blood glucose (Barry, 1952*a*). This experiment, however, did not show quantitatively the significance of this synthesis of lactose from glucose; the possibility remained that the bulk of the lactose in milk had been synthesized from some unlabelled compound taken from the blood.

In the present experiments, uniformly labelled glucose was injected intravenously into a lactating goat, and blood and milk samples were taken at intervals up to 12 hr. after injection. The specific activities of blood glucose, of lactose, and of the glucose and galactose residues of lactose, were measured. The results show that blood glucose was the principal source of both the glucose and galactose residues of lactose.

EXPERIMENTAL

Radioactive glucose. Glucose labelled uniformly with ^{14}C was obtained from the U.S. Atomic Energy Commission. In order to test its purity, carrier glucose was added and two derivatives, phenyl-D-glucosazone and phenyl-D-glucosotriazole, were prepared and purified by recrystallization. The specific activities of these derivatives, after correcting for their content of unlabelled carbon, were within 10% of that of the original glucose, indicating that at least 90% of its radioactivity was due to glucose. Paper chromatography of the original material with aqueous phenol as a solvent (Partridge, 1948) gave only one radioactive spot, in the position of glucose.

In order to determine the distribution of activity in the molecule, the glucose was partially degraded by yeast fermentation: carbon atoms 3 and 4 were obtained as CO_2, while the ethanol from carbon atoms 1, 2, 5 and 6, was oxidized and isolated as silver acetate. The specific activities of these two groups of carbon atoms were identical with that of the original glucose, and with those of its derivatives after correcting for their content of unlabelled carbon.

Procedure in experiments on goat. The labelled glucose was dissolved in a few ml. of water and injected into the left jugular vein of a lactating goat which was giving about 1 l. of milk a day. The methods of obtaining blood plasma and milk samples were the same as described previously (Barry, 1952*b*).

Measurement of specific activities

Plasma glucose. Blood plasma glucose was fermented with baker's yeast and the CO_2 formed was carried in a stream of N_2 into NaOH solution; the procedure was adapted from methods of determining blood glucose by fermentation (Holden, 1937; Conway, 1950), and gives carbon atoms 3 and 4 of glucose as CO_2 (Koshland & Westheimer, 1950). The CO_2 was precipitated as $BaCO_3$ which was centrifuged on to the base of a standard aluminium cup to give a layer of uniform thickness. The sample was counted for sufficiently long to reduce the probable error to less than 3% and, after correction to infinite thickness, the specific activities were calculated using a factor previously determined with standard samples.

Lactose. A very convenient procedure was devised for isolating lactose from milk, based on the industrial method of Leviton (1949). The milk was centrifuged, the fat-free milk poured off from the cake of fat, and then freeze-dried. The resulting powder was added slowly with shaking to 85% (v/v) methanol at 10–15°, using 10–15 ml./g. of powder. The mixture was shaken for 5 min., centrifuged to remove protein, and the supernatant filtered through a sintered glass funnel (fine porosity), the filtration being completed within 15 min. of adding the powder to the methanol. The filtrate was acidified to pH 3–4 with 5N-HCl, left at room temperature with occasional shaking for 48 hr., and then left at 0° for a further 48 hr.; crystals began to appear within 5–10 hr. at room temperature. The crystals were filtered off, washed with ice-cold 70% (v/v) ethanol, and recrystallized by dissolving in hot water and adding ethanol to make 70% (v/v) ethanol; after 48 hr. at 0° the crystals were filtered off, washed and dried. This procedure yielded 60–70% of the lactose in milk as colourless crystals with a nitrogen content of less than 0·1%. Colorimetric assay with the anthrone reagent (Morris, 1948) indicated that the material was pure. The lactose was burnt in a stream of O_2 and the CO_2 converted into $BaCO_3$ and counted as before.

Glucose residue of lactose. Lactose was hydrolysed to glucose and galactose by heating with 0·01N-HCl in a sealed test tube in an autoclave at 15 lb./in.^{2} for 2 hr. (Ramsdell & Webb, 1945). The hydrolysate was neutralized and the specific activity of carbon atoms 3 and 4 of the glucose determined by fermentation with yeast as described above. Under the conditions used, the yeast did not ferment galactose.

* Present address: Department of Agriculture, University of Oxford.

Galactose residue of lactose. The galactose residue of lactose was converted into mucic acid by oxidation of the lactose with HNO_3. The product was reprecipitated twice from hot water and gave colourless crystals (m.p. 206°); the specific activity was determined after combustion to CO_2. From one of the mucic acid samples the di-*p*-bromophenacyl ester of mucic acid was prepared and purified by recrystallization; its specific activity, after correcting for its content of unlabelled carbon, was identical with that of the original mucic acid.

RESULTS

In Fig. 1 are shown the specific activities of lactose and of its glucose and galactose residues in five milk samples taken at intervals after the intravenous injection of uniformly labelled glucose into a lactating goat. The results of a second similar experiment are given in Table 1. It can be seen that, within the limits of experimental error, the specific activities of the glucose and galactose residue of each lactose sample were the same.

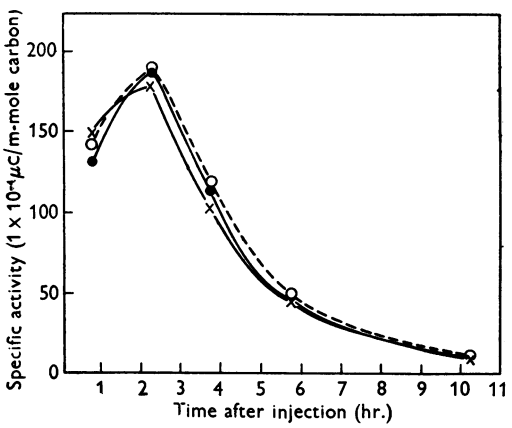

Fig. 1. Expt. 1. Specific activities of lactose and its glucose and galactose residues in milk samples taken at intervals after injecting 14 μC of uniformly labelled glucose intravenously into a lactating goat. The specific activities of each sample are plotted at the time half-way through the period in which the milk was secreted. O---O, lactose; ●—●, galactose; ×—×, glucose.

Blood plasma samples were also taken during these experiments, and the relation between the specific activities of blood glucose and milk lactose in Expt. 2 is shown in Fig. 2; the results of expt. 1 were similar. It has been assumed here that the blood glucose has remained uniformly labelled throughout the experiment, and that the specific activity of carbon atoms 3 and 4 which was actually measured was equal to the specific activity of the whole molecule; that this assumption is justified is suggested by the results in Table 1, which show that in the glucose residue of each lactose sample the specific activity of carbon atoms 3 and 4 was equal to the specific activity of all six carbon atoms. It can be seen that the specific activity of the lactose, after it had risen to a maximum at 1–2 hr. after the injection, was always equal to the specific activity of the blood glucose about 1–1·5 hr. earlier. During

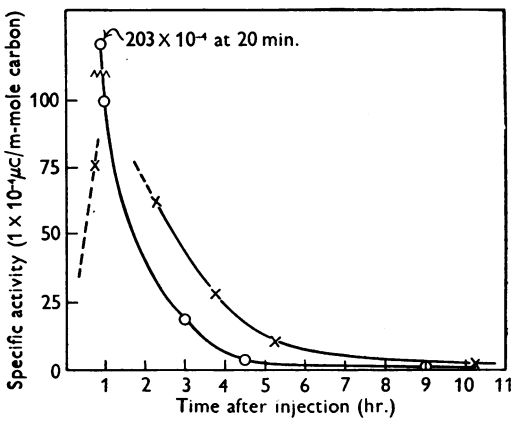

Fig. 2. Expt. 2. Uniformly labelled glucose (6 μC) injected intravenously into a lactating goat. The specific activity of each lactose sample is plotted at the time half-way through the period in which the milk was secreted; the curve drawn through these points gives approximately the specific activity, at any time, of lactose which has just reached a point in the mammary gland from which it can be drawn off by milking. O—O, blood glucose; ×—×, milk lactose.

Table 1. *Specific activities of lactose and its glucose and galactose residues after intravenously injecting 6 μC of uniformly labelled glucose into a lactating goat*

Experiment 2

Time of sampling (hr. after injection)	Specific activity (1×10^{-4} μC/m-mole carbon)			
	Lactose	Galactose*	C-3 and C-4 of glucose†	Glucose‡
1·5	79·3	76·3	76·0	82·3
3	63·3	63·2	60·5	63·4
4·5	29·4	27·1	27·7	31·7
6	10·5	10·8	10·7	10·2
12	2·05	2·28	1·79	1·82

* Measured as mucic acid. † Obtained by fermentation. ‡ Calculated from lactose and galactose values.

the last few hours of the experiment the specific activity of the blood glucose remained almost constant, and was approximately equal to the specific activity of the lactose. The curves are similar to those found previously for the specific activities of lysine, tyrosine, and inorganic phosphorus in blood plasma, and the lysine, tyrosine and phosphorus of casein in milk (Barry, 1952*b*).

From the results in Fig. 2 it may be concluded that glucose was the principal compound taken from the blood stream by the mammary gland for the synthesis of lactose and that glucose carbon took, on the average, about 1–1·5 hr. to pass from the blood stream to a point in the mammary gland from which it could be drawn off in the lactose of milk. While glucose may have been the only compound used in the synthesis, the possibility cannot be excluded that a small proportion of the lactose came from unlabelled blood constituents. The possibility that some or all of the radioactivity in the lactose arose, not from glucose absorbed from the blood, but from some compound in the blood formed by the degradation of the injected glucose has been virtually excluded by a previous experiment performed under similar conditions (Barry, 1952*a*). It was then found that when [1-^{14}C]glucose was infused intravenously into the same goat and milk collected at 1·5 and 3·5 hr. after the end of the infusion, none of the radioactivity in the lactose was contained in carbon atoms 4, 5 and 6 of the glucose and galactose residues. Had the radioactive glucose been first degraded to C_3 or C_2 compounds and these then taken from the blood stream for the synthesis of lactose, it is highly improbable that this result would have been obtained.

The fact that the specific activities of the glucose and galactose residue of each lactose sample were identical shows that equal amounts of blood-glucose carbon were used by the mammary gland in the synthesis of both the glucose and galactose residue, and that there was no appreciable time lag between the appearance of blood-glucose carbon in the glucose and in the galactose residue. This agrees with the results of French, Popják & Malpress (1952), who also found equal specific activities in the glucose and galactose of lactose after feeding [^{14}C]starch by stomach tube to lactating rabbits. However, after infusing [1-^{14}C]glucose into a goat the specific activity of the galactose residue was about 30% lower than that of the glucose (Barry, 1952*a*); and recently Schambye, Wood & Popják (1953) have reported different distributions of activity in the glucose and galactose of lactose after administering various labelled compounds to lactating rabbits. Therefore, although our experiments show that blood glucose is the principal precursor of both the glucose and galactose residue of lactose in the goat, these results suggest that the synthesis of galactose has a more complex mechanism than a direct conversion of the glucose carbon chain into that of galactose.

SUMMARY

1. Glucose labelled uniformly with ^{14}C was injected intravenously into a lactating goat and the specific activities of blood glucose, of lactose, and of the glucose and galactose residues of lactose were measured in blood and milk samples taken up to 12 hr. after the injection.

2. The results show that blood glucose was the principal source of both the glucose and galactose residues of the milk lactose.

REFERENCES

Barry, J. M. (1952*a*). *Nature, Lond.*, **169**, 878.
Barry, J. M. (1952*b*). *J. biol. Chem.* **195**, 795.
Caputto, R. & Trucco, R. E. (1952). *Nature, Lond.*, **169**, 1061.
Conway, E. J. (1950). *Microdiffusion Analysis and Volumetric Error*, p. 220. 3rd ed. London: Crosby, Lockwood and Son.
Folley, S. J. (1949). *Biol. Rev.* **24**, 316.
French, T. H., Popják, G. & Malpress, F. H. (1952). *Nature, Lond.*, **169**, 71.
Hills, A. G. & Stadie, W. C. (1952). *J. biol. Chem.* **194**, 25.
Holden, R. F. (1937). *J. biol. Chem.* **119**, 347.
Koshland, D. E. & Westheimer, F. H. (1950). *J. Amer. chem. Soc.* **72**, 3383.
Leviton, A. (1949). *Industr. Engng Chem. (Anal.)* **41**, 1351.
Malpress, F. H. & Morrison, A. B. (1950). *Biochem. J.* **46**, 307.
Morris, D. L. (1948). *Science*, **107**, 254.
Partridge, S. M. (1948). *Biochem. J.* **42**, 238.
Ramsdell, G. A. & Webb, B. H. (1945). *J. Dairy Sci.* **28**, 677.
Reithel, F. J., Horowitz, M. G., Davidson, H. M. & Kittinger, G. W. (1952). *J. biol. Chem.* **194**, 839.
Schambye, P., Wood, H. G. & Popják, G. (1953). *Fed. Proc.* **12**, 264.