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Galactose Metabolism in Suckling and Adult Isolated Rat Hepatocytes

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Summary

The metabolism of galactose during the postnatal developmental period has been examined in isolated parenchymal cells of livers from fasted rats aged 7, 14, 21, 28, and 42 days, by measuring the disappearance of 1 and 4 mM 1-[^{14}C]galactose from the incubation media as well as [^{14}C]galactose conversion to [^{14}C]glucose and [^{14}C]lactate and oxidation to [$^{14}CO_2$]. Cells from 7- and 14-day-old suckling animals consistently utilized galactose more rapidly (4 times greater) than those of adult. Hepatocytes of suckling rats also converted greater amounts of galactose to glucose and oxidized galactose 2–3 times faster than the adult. The conversion to glucose by suckling cells occurred with minor recycling of labeled C-1 galactose to C-6 of glucose. A comparison with [1-^{14}C]glucose as substrate showed that oxidative rates by the young cells were 3 times faster for galactose than for glucose. This was not due to the presence of a direct galactose oxidative pathway as assessed by the [$^{14}CO_2$] yield from C-1 and C-2 labeled galactose. Incubation of hepatocytes with galactose appeared to augment the production of glucose from endogenous precursors, 33 nmoles/mg cells in 30 min. The enhanced glucose output from endogenous sources in isolated suckling hepatocytes incubated with galactose contrasts with the sugar's suppression of glucose output observed in isolated perfused liver even though galactose metabolism in both preparations are similarly greater in the suckling than in the adult.

Abbreviations

KRB, Krebs-Ringer bicarbonate
KRB-A, Krebs-Ringer bicarbonate containing 3% albumin
LDH, L-lactic dehydrogenase

In the young mammal, galactose is an important dietary carbohydrate. Because the liver is the major organ involved in the metabolism of galactose (34), our laboratory has focused on the mechanism whereby this hepatic process occurs. Past developmental analyses of rat liver galactokinase (10), galactose-1-phosphate uridyltransferase (5), and uridine diphosphogalactose-4-epimerase (9) (the major enzymes of galactose disposition), indicated their specific activity to be maximal during the suckling period. In order to more precisely learn the details of galactose uptake and conversion to glucose by suckling rat livers, we have recently utilized the isolated liver perfusion technique (3, 22). A comparison of isolated perfused livers of suckling and adult rats revealed that the liver of the suckling rat not only took up more galactose from the perfusate than the adult, but put out proportionately larger amounts of glucose (2). Indeed, over 80% of perfused galactose was converted to glucose by livers of suckling animals (3).

Whereas the isolated perfused liver has been extensively used as a physiologic model system to study metabolic processes (13, 15, 39), isolated hepatocytes have become a tool used to promote evaluation at the cellular level and to augment results obtained with the intact organ system (1, 19, 38). The use of the hepatocyte preparation eliminates the metabolic contribution of other hepatic cellular elements, facilitates easy manipulation of experimental conditions and provides ready determination of oxidative metabolic rates, which could not be done in our own perfusion experiments inasmuch as the perfusion apparatus did not permit CO_2 collection. For these reasons, the differences in galactose uptake and glucose output observed in suckling and adult galactose perfused livers (2, 3) have been more extensively studied in isolated hepatocytes to determine other metabolic fates, especially galactose oxidation by cells from young and adult livers. Our results form the basis of this report.

EXPERIMENTAL

Animals. Adult, 42-day-old, Sprague-Dawley male rats weighing 135–150 g and 7-, 14-, and 28-day-old, non-litter mates were the animals used. At the age of 5 days, litters were randomly mixed and culled to nine pups each. Pups were weaned and separated according to sex at 21 days. Animals were fasted for 18 h with free access to water before surgery. Young animals were removed from the mother and placed in a warm, dark area for fasting. The anesthesia was 50 mg/kg of sodium pentobarbital injected intraperitoneally.

Chemicals. Collagenase was obtained from Worthington Biochemical Corporation, Freehold, NJ. Glucose-free galactose was a product of Sigma, St. Louis, MO. D-[1-^{14}C]galactose, D-[1-^{14}C]glucose and D-[2-^{14}C]glucose (50 mCi/mM) were purchased from Amersham Corp., Arlington Heights, IL. Radiolabeled glactose did not contain glucose as a contaminant and was used without additional purification. D-[2-^{14}C]galactose (0.55 mCi/mM) was supplied by Calbiochem, Los Angeles, CA. Bovine serum albumin, Fraction V came from Miles Laboratories, Elkhart, IN, hydroxide of hyamine-10x from Packard, Downers Grover, IL and a glucose analysis kit was supplied by Biodynamics, Brookfield, CT. Whatman 3 mm paper and all other chemicals of the highest quality available were obtained through Fisher Scientific, King of Prussia, PA.

Isolated hepatocyte preparation. Liver cells were isolated by the procedure of Berry and Friend (4) with some modifications by Seglen (28) such as the addition of EGTA to Ca^{2+}-free buffer for the non-recirculating perfusion phase and the omission of Mg^{2+} from the media for the recirculating phase. Warmed, oxygenated Krebs-Ringer bicardonate buffer (KRB), pH 7.4 was the basic perfusion and incubation media used for all experiments.

Hepatocytes were isolated from livers of fasted rats aged 7, 14, 21, 28, and 42 days. To minimize biologic variations due to individual differences, each batch of hepatocytes was prepared from tissue of more than one animal. Livers of two to three adult or three to four young rats were simultaneously perfused on a Mortimore apparatus (20) utilizing the surgical techniques described previously (2). Because livers of younger animals are smaller and more fragile than the adult, volumes and perfusion rates varied according to the size and lability of livers at various

developmental stages. Adult rat livers were perfused with 100 ml of Ca^{2+}-free KRB containing 0.5 mM EGTA at 12.5 ml/min; livers of animals at 21- and 28-days were perfused with 50 ml at 5 ml/min, 14-day at 4 ml/min and 7-day at 2 ml/min. At the end of the non-recirculating period when the media flasks were nearly empty, Mg^{2+}-free KRB containing 0.05% collagenase was added. The perfusion was continued in a recirculating mode 20–25 min for adult livers or until the livers had swelled, softened and started to 'leak.' Fragile, immature livers leak sooner than adult livers, usually after less than 15 min of recirculating collagenase media. Livers were combined, minced in collagenase media and digested an additional 15 min. The suspension was filtered through a bilayer of nylon to remove debris. Preparations of adult cells were filtered through 170 and 67 μm pore size, whereas young cells were filtered through 67 and 30 μm. All preparations were centrifuged at 50 g for 2 min and washed twice with KRB containing 3% albumin (KRB-A). The final pellet was weighed and diluted to 10 mg (wet wt)/ml KRB-A for incubation with hexose.

This isolation procedure yielded about 300 mg cells/g liver, a somewhat lower yield was obtained from young than from adult liver. Light microscopic examination of the final pellet revealed round, refractile cells that were free of cell debris and excluded 95% trypan blue. Cell viability was also assessed by LDH leakage into the media and ability to convert lactate to glucose as recommended by Krebs (19).

Incubation conditions. Incubation flasks containing 2 ml of the cell suspension were gassed with O_2/CO_2 95:5%, sealed and prewarmed for 3 min in a 37°C shaking water bath. One-hundred microliters of either 200 or 800 mM hexose containing 0.08 μCi of radiolabeled substrate were added to each flask to give a final concentration of 1 or 4 mM sugar. Incubation intervals began with the introduction of substrate. Cell viability was maintained throughout the incubation time period.

Metabolite analysis. Changes in the concentration of radiolabeled substrates and products were measured in 500 μl samples withdrawn from the incubation flasks at 30, 60, and 90 min and centrifuged through a silicone layer to remove cells from the media. Media was deproteinized with sodium hydroxide and zinc sulfate (31) and the metabolites, [^{14}C]galactose, [^{14}C]glucose and [^{14}C]lactate were separated by paper chromatography using ethylacetate/pyridine/water (12:5:4 by vol) as the solvent phase (29). Areas corresponding to galactose, glucose and lactate were identified by chromatographing appropriate standards. Segments corresponding to the individual sugars were cut out for radioactive counting in scintillation fluid.

In order to test whether the galactose was converted to glucose directly or whether the glucose was synthesized after glycolysis to triose phosphate, the % of [6-^{14}C]glucose was measured. After chromatographic separation of the labeled metabolites in 40 μl of media from hepatocytes incubated with 4 mM galactose, the [^{14}C] glucose was eluted and the labeling of C-6 was determined by isotope dilution technique. One hundred mg of unlabeled glucose was added as a carrier and the sample dried. The glucose was isolated as potassium gluconate (6) which was degraded by periodate oxidation (12). The resulting formaldehyde from C-6 was measured as the dimedon derivative (7).

The amount of oxidation was determined by collecting the CO_2 released after 0.3 ml of 6 N sulfuric acid was injected into the incubation flask. CO_2 evolved was absorbed by 0.4 ml of hyamine placed in centerwells. The radioactive content of the hyamine was assessed by placing the entire centerwell into scintillation fluid and counting it in a liquid scintillation spectrometer.

Glucose was chemically measured with a kit (33) supplied by BioDynamics, Brookfield, CT. Uptake and conversion are expressed as nmoles per mg cells, wet weight. Values are mean ± S.E.M. for n preparations from two to four livers. Rates are expressed as nmole·mg^{-1}·min^{-1}.

RESULTS

Galactose uptake. The uptake of galactose, as measured by the disappearance of the sugar from the incubation medium, is shown in Figure 1. At 1 mM, hepatocytes from both young and adult animals removed galactose at a linear rate. The suckling hepatocytes, however, utilized the sugar more rapidly, at a rate about five times faster than the adult cells, 1.08 *versus* 0.20 nmole·mg^{-1}·min^{-1}. At 4 mM, the pattern of uptake was somewhat different from that at 1 mM in that there was a faster uptake before 30 min by both groups of cells. The utilization by suckling cells at each time point studied was again about 4-fold higher than the adult cells. These curves for 4 mM galactose uptake differed from those found previously with perfused rat liver (2). In the perfused suckling liver, there was a 30 min lag before the onset of a linear rate, whereas in the perfused adult liver the curve was linear through the origin (2).

The 30 min uptake was also determined at 0.1 and 0.25 mM galactose. At the lower concentration, the suckling cells extracted 8.6 ± 0.29 (n = 5) (mean ± S.E.M.) *versus* 4.47 ± 0.17 (n = 8) nmoles/mg adult cells. At the higher concentration, the suckling value was 20.0 ± 1.4 (n = 5) *versus* 5.31 ± 0.76 (n = 11) for adult hepatocytes; thus, at all galactose concentrations studied, the suckling cells utilized galactose faster than adult cells.

Glucose output. Hepatocytes from both suckling and adult liver released glucose into the medium with more glucose appearing after incubation with galactose than in medium initially free of hexose (Fig. 2). The amount found after 30 min incubation of suckling hepatocytes in galactose-free medium was 76 nmoles/mg cells and was essentially the same at 60 and 90 min (Fig. 2A). Adult hepatocytes in buffer alone released only 20% of the amount formed by suckling cells by 30 min. This increased during the subsequent 60 min of incubation at a rate of 0.48 nmoles·mg^{-1}·min^{-1} (Fig. 2B). In response to 1 and 4 mM galactose, suckling cells released glucose at a linear rate of 2.35 nmoles·mg^{-1}·min^{-1} between 30 and 90 min (Fig. 2A). There was no increase in glucose output when the galactose concentration was raised from 1 to 4 mM, an observation also made during suckling liver perfusion (2). At 90 min, the suckling cells had added 190 nmoles glucose/mg cells above the basal level (Fig. 2A).

When adult cells were incubated with galactose, there was much less glucose added to the medium than was released by suckling cells. More glucose was found in the medium of adult cells when galactose was increased from 1 to 4 mM (Fig. 2 B and C). After 30 min incubation in 1 mM galactose, adult cells released 16 nmoles glucose/mg cells above the basal level in galactose-free medium, compared to 55 nmoles/mg cells for suckling hepatocytes. Glucose output by adult cells in 1 mM galactose increased linearly after 30 min incubation, at a rate of 0.90 nmoles·mg^{-1}·min^{-1} with a net rate above the basal of 0.52. At 90 min, adult hepatocytes added 41 nmoles glucose/mg cells above the basal level compared to 190 for suckling cells. After correction for the basal output, adult cells incubated in 4 mM galactose released twice as much glucose as cells incubated in 1 mM galactose. This, however, was not sustained after 60 min of incubation, the slope describing the increase in medium glucose appears lower (Fig. 2C). At 90 min, the medium of adult hepatocytes incubated in 4 mM galactose contained only 52 nmoles glucose/mg cells above the basal level.

Galactose conversion to glucose. To more exactly determine the relationship of galactose removed from the medium and glucose added to the medium by the cells, the conversion of [1-^{14}C]-galactose to [^{14}C]glucose was studied. The results are shown in Figure 1. When suckling cells were incubated in 1 mM [^{14}C] galactose, radioactive glucose appeared in the medium at a rate of 0.89 nmoles·mg^{-1}·min^{-1}, which is 82% of the rate of galactose uptake, 1.08 nmoles·mg^{-1}·min^{-1}. This value for the % conversion of 1 mM galactose to glucose by suckling cells is the same as that observed in the perfused suckling liver (3). Adult liver cells incubated with 1 mM [^{14}C]galactose converted the sugar to [^{14}C] glucose at a rate of 0.12 nmoles·mg^{-1}·min^{-1}, which is 60% of the rate of galactose uptake, 0.20 nmoles·mg^{-1}·min^{-1}.

Figure 1B shows the appearance of [^{14}C]glucose when cells are incubated in 4 mM [1-^{14}C]galactose. Labeled glucose was released into the medium at a linear rate of 0.98 nmoles·mg^{-1}·min^{-1} for suckling and 0.14 for adult hepatocytes. These rates are essentially

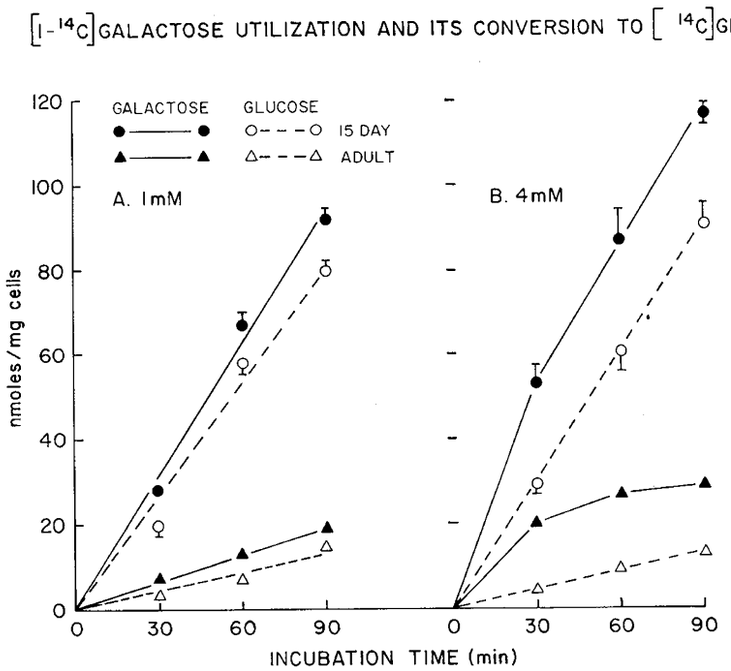

Fig. 1. [1-^{14}C]Galactose utilization and its conversion to [^{14}C]glucose by suckling and adult rat hepatocytes. Twenty mg (wet wt.) of hepatocytes isolated from fasted 15-day-old (○, ●) and adult (△, ▲) rats were incubated with 0.08 μCi of [1-^{14}C]galactose in 2 ml of media containing 1 mM (*A*) or 4 mM (*B*) galactose. *Solid symbols with solid lines* indicate galactose utilization, whereas, *open symbols with dashed lines* indicate conversion to glucose. Aliquots of cellular suspension withdrawn at the time indicated were analyzed for metabolite levels. Experimental conditions and analyses are described in the text. Each point is the mean + S.E.M. of at least five preparations of livers pooled from two to four animals. Some S.E.s are not visible because there are less than the size of the geometric figure.

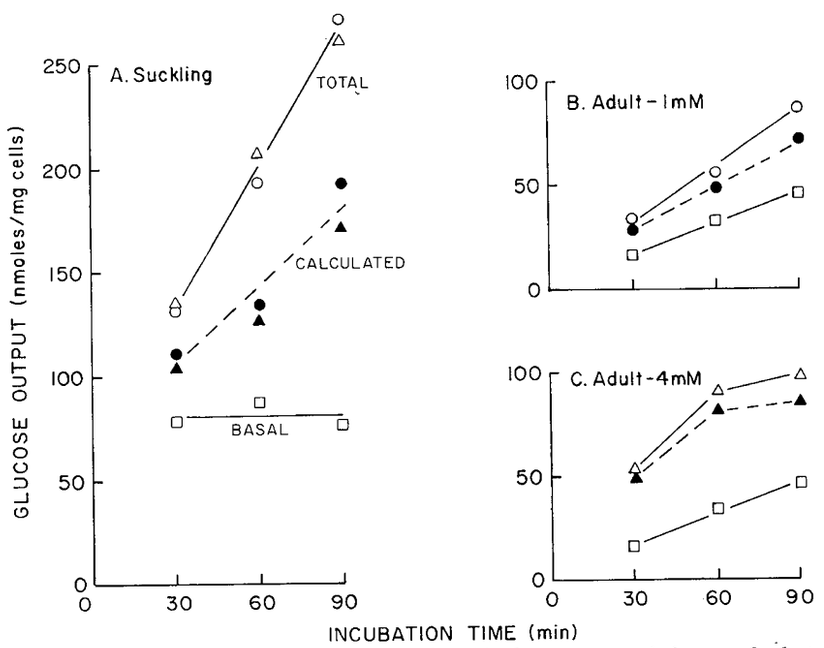

Fig. 2. Glucose output by suckling (*A*) and adult (*B, C*) hepatocytes incubated in the presence and absence of galactose. Liver cells were incubated without hexose to determine glucose output in the basal (□) state. Total chemical glucose was also measured in media of cells incubated with 1 (○, *A* and *B*) and 4 (△, *A* and *C*) mM galactose. The calculated *dashed lines* (●, ▲) represent the difference between the total glucose output and the amount of [^{14}C]glucose output obtained from galactose conversion. Experimental conditions are given in Figure 1.

the same as those observed in Figure 1A for conversion of 1 mM galactose. The rates of labeled glucose released are similar to the rates at which galactose is taken up after 30 min.

Figure 3 is a plot of the correlation of [^{14}C]galactose taken up and labeled glucose put out into the medium. These data indicate a linear relationship in both suckling and adult cells. The fact that at both galactose concentrations the adult and suckling values fit the same line suggests the same regulatory processes are operative in both young and older cells.

The recycling of C-1 of galactose in its conversion to glucose was assessed as suggested by Van Schaftingen *et al.* (35) and Rognstad and Katz (23) by assaying the % of the radioactivity in glucose which resides in the C-6 position. The results of such a study are shown in Table 1. Only 1.5–2% of the [^{14}C] in glucose was found in C-6, indicating that most of the medium glucose was derived from hexose phosphate generated from galactose and not from triose phosphates. These results are similar to those observed with cells derived from adult liver (35).

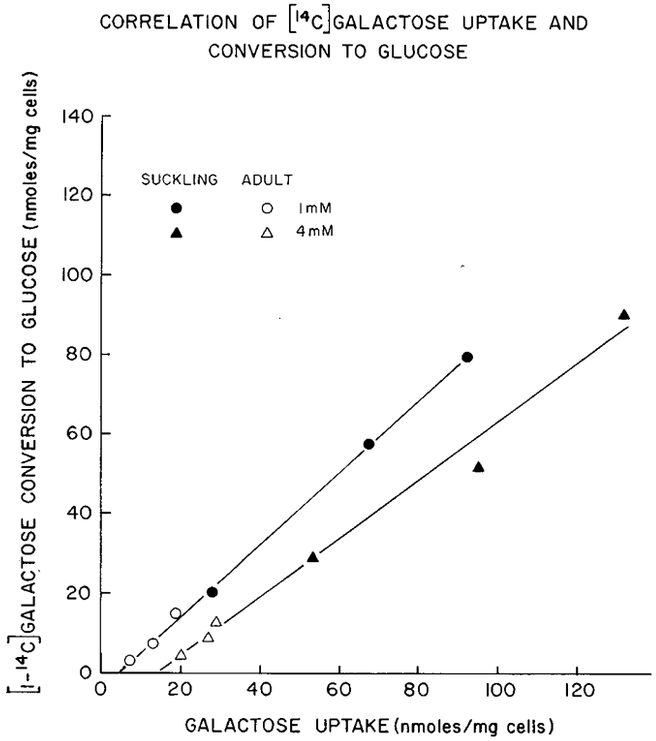

Fig. 3. Correlation of [^{14}C]galactose uptake and conversion to glucose. Data from Figure 1 is used to depict the relationship between galactose uptake and conversion to glucose by suckling (*closed symbols*) and adult (*open*) cells. The line representing 1 mM galactose (○, ●) is described by the equation y = 0.914x − 4.18, with a 0.999 correlation and 4 mM galactose (△, ▲) by y = 0.732x − 10.44, 0.992 correlation.

The increment in total chemically determined glucose as shown in Figure 2 appears to be greater than the amount of glucose in the medium converted from [^{14}C]galactose that is shown in Figure 1. If the amount of glucose in the medium accounted for by direct conversion from galactose is subtracted from the total chemically determined glucose, the dashed curves of Figure 2 are obtained. The difference between the basal glucose output values and the dashed line curves is the glucose appearing in the medium from carbon sources other than the added galactose. For the suckling cells (Fig. 2A) incubated in 1 and 4 mM galactose for 90 min, this is about 100 nmole/mg cells, a value slightly greater than that observed for direct conversion (Fig. 1). For adult cells (Fig. 2B and C) the amount of glucose appearing in excess of the direct galactose-glucose conversion is smaller than for suckling cells, is greater at 4 mM galactose than at 1 mM galactose and is actually much greater than that converted from galactose. At 30 min of incubation with 4 mM galactose, for example, 4 nmoles glucose/mg cells is converted from galactose (Fig. 1B) and 28 nmole/mg cells is derived from endogenous sources (Fig. 2B).

Galactose conversion to lactate. [^{14}C]Galactose is converted to [^{14}C]lactate when cells of either age group are incubated with galactose; media contained 3.4 ± 0.3 nmole/mg cells and 1.6 ± 0.3/mg cells for seven suckling and adult preparations, respectively, at 30 min with 1 mM galactose. By 60 min, the amount of [^{14}C]lactate had doubled (6.0 ± 0.2, n = 6) in media of suckling cells whereas in the adult, levels remained constant. At 4 mM galactose the 30 min lactate values were 7.8 ± 1.2 nmoles/mg cells (n = 7) for suckling, and 4.9 ± 0.5 (n = 8), for adult cells. The lactate levels for suckling cells had increased to 12.1 ± 0.7 nmole/mg cells (n = 6), by 60 min whereas levels were constant in media of adult cells. At both 1 and 4 mM galactose, the amount converted to lactate by suckling cells was two to four times the amount converted by adult cells and this corresponded to the greater amounts of galactose taken up by suckling cells. Although the labeled lactate increased during the first 60 min of incubation of suckling cells, that from adult cells was at a steady-state level at the first time point, 30 min, and did not increase after that time. The adult cells at most time points studied had converted a greater fraction of the galactose extracted from the medium to lactate. This was most pronounced at 30 min. Results obtained with 1 mM galactose reveal that the suckling cell converted 12% of the galactose taken up compared to 21% for adult cells. At 4 mM, values were 15 and 24% for suckling and adult cells respectively.

Galactose oxidation to [$^{14}CO_2$]. The amount of [1-^{14}C]galactose metabolized to [$^{14}CO_2$] is shown in Figure 4. Suckling hepatocytes metabolized more galactose to [$^{14}CO_2$] than adult cells. The rate of oxidation of both 1 and 4 mM galactose was the same in the adult cells but the younger cells increased their oxidation when the substrate was increased from 1 to 4 mM. For 1 mM galactose, the oxidation rate, 0.10 nmole·mg^{-1}·min^{-1}, was 9% of the galactose uptake rate in suckling cells whereas the rate in adult cells, 0.04 nmole·mg^{-1}·min^{-1}, was 25% of the sugar's rate of uptake.

[1-^{14}C]Galactose oxidation to [$^{14}CO_2$] was also compared to [1-^{14}C]glucose oxidation by suckling and adult hepatocytes. Figure 4 shows that the oxidation of 1 mM galactose is about three times more rapid than that of glucose in both suckling and adult hepatocytes. In the suckling cells, 4 mM galactose is also more rapidly oxidized than glucose; but in adult cells, this is not the case.

Because the differences in galactose conversion to metabolites by hepatocytes of different ages appeared to reflect the differences in removal of galactose from the medium, we examined glucose removal to ascertain if the difference in galactose and glucose oxidation could be explained in this way. Such appeared to be the case. Suckling hepatocytes incubated for 30 min in 1 mM glucose had a net removal of 9.5 ± 2.0 nmole/mg cells (n = 5), compared to 27.7 ± 2.8 (n = 5), for galactose. Additionally, no further removal of glucose from the medium occurred over an ensuing 60 min incubation interval while galactose was continuously extracted. The same differences in removal of the hexoses were observed at 4 mM where the younger cells at 30 min removed 30.7 ± 5.6 nmoles glucose/mg cells (n = 5) and 52.7 ± 3.9 nmoles galactose/mg cells for n = 7 preparation. Although galactose removal was sustained after 30 min, net glucose removal was not.

Comparison of [1-^{14}C]galactose and [2-^{14}C]galactose oxidation. Because the metabolism of galactose to galactonate has been shown in rat liver (21) and the subsequent decarboxylation of the hexonic acid to pentose has been proposed (11), evidence was sought for such a sequence by comparing the oxidation of C-1 and C-2 labeled sugar. The rationale for this was that preferential loss of the first carbon as [$^{14}CO_2$] might be observed. Figure 5 shows the oxidation of 4 mM galactose by suckling and adult liver cells where [$^{14}CO_2$] was derived from [1-^{14}C]galactose at a rate 3 times that from [2-^{14}C]galactose. Because the major pathway of galactose metabolism to [$^{14}CO_2$] is via prior conversion to glucose-6-phosphate, the oxidation of glucose labeled in the same carbon positions was also examined. A similar ratio is obtained for [$^{14}CO_2$]

Table 1. *Radioactivity in C-6 of glucose present in media from hepatocytes incubated with 4 mM [1-^{14}C]galactose*[1]

Incubation time (min)	10^{-3} × [^{14}C] in glucose (cpm/ml)	[^{14}C] in C-6 of glucose (% of total [^{14}C] in glucose)
30	6.3	1.6
60	11.0	1.5
90	19.3	2.1

[1] Hepatocytes, isolated from fasted 14-day-old suckling rat livers, were incubated with 4 mM [1-^{14}C]galactose. Aliquots, withdrawn at timed intervals, were centrifuged through silicone oil to remove cellular matter and the media were analyzed for [6-^{14}C]glucose content. The analytic method is described in the text. Data are the average of duplicate assays.

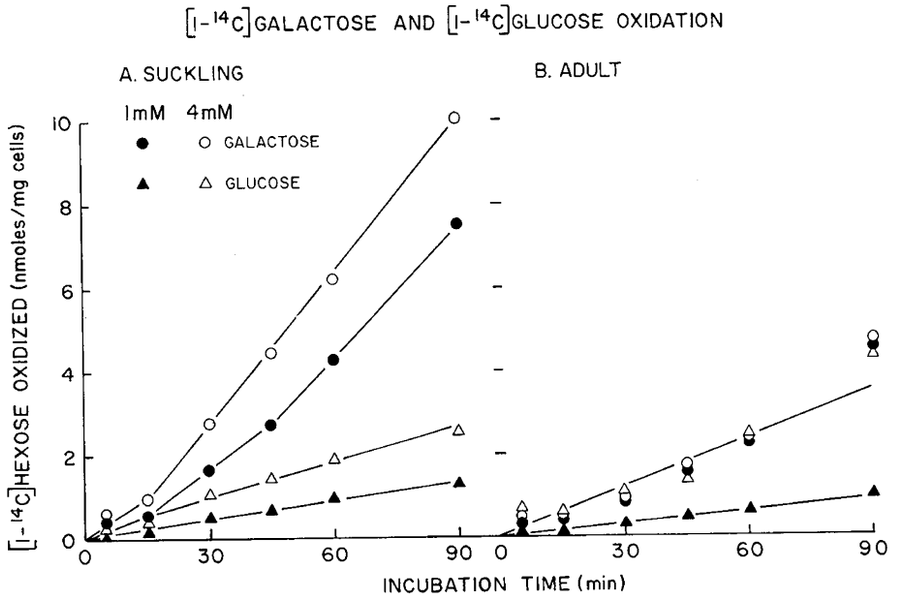

Fig. 4. [1-^{14}C]Galactose and [1-^{14}C]glucose oxidation by suckling (*A*) and adult (*B*) hepatocytes. [$^{14}CO_2$] production during incubation with galactose (○, ●) and glucose (△, ▲) at concentrations of 1 mM (*solid symbols*) and 4 mM (*open*) are graphed with respect to time interval. Each point is the mean of [$^{14}CO_2$] collected for n varied between 5 and 25. The S.E. was less than 10% of the mean value. Experimental conditions are given in Figure 1 and the procedures are detailed in the text.

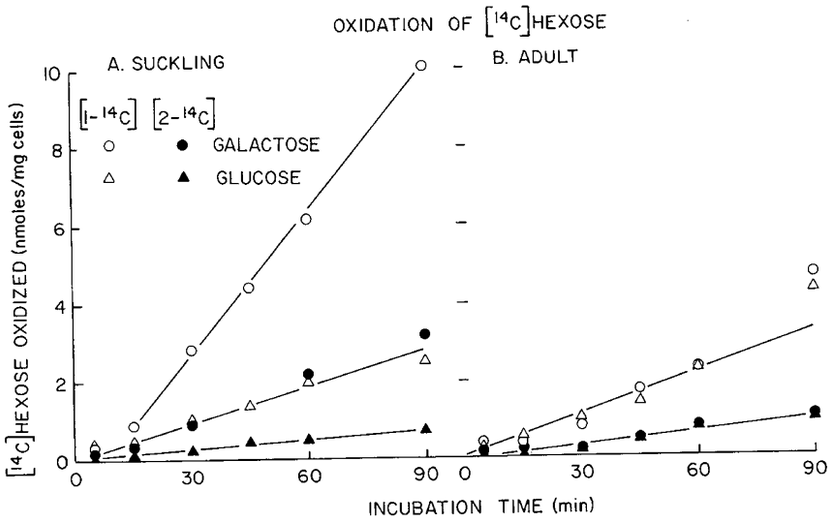

Fig. 5. Oxidation of [^{14}C] hexose by suckling (*a*) and adult (*b*) heptocytes. Galactose (*circles*) or glucose (*triangles*) labeled in the first position (*open symbols*) and second position (*closed*) was incubated with rat hepatocytes. The concentration of either hexose was 4 mM. Each point is the mean of [$^{14}CO_2$] collected from 3—25 The S.E. was less than 10% of the mean value. Experimental conditions are given in Figure 1.

yield from the C-1 and C-2 labeled glucose; thus, at 4 mM galactose there was no indication for operation of a direct oxidative pathway of galactose metabolism with preferential loss of carbon one.

Galactose metabolism with development. Results for galactose uptake and conversion to glucose, lactate and CO_2 by hepatocytes of various age animals incubated for 30 min in 4 mm galactose are shown in Figure 6. Galactose removal and subsequent metabolism is the same at 7 days of age as it is for the 14-day-old suckling cells. After 14 days there is an almost linear decrease in galactose removal with a parallel decrease in galactose conversion to glucose. The decrease in conversion of galactose to lactate and CO_2, observed between 14 days of age and the adult is not as marked as that of removal and conversion to glucose.

[^{14}C] Balance. Table 2 shows the isotopic balance in the medium of hepatocytes incubated with [1-^{14}C]galactose. At 1 mM, up to 92% of the labeled sugar was removed by the suckling cells in the 90 min incubation period but only 19% by adult hepatocytes. The conversion to glucose, lactate and CO_2 accounted for most of the [^{14}C]galactose removed from the medium by the suckling cells. After 90 min incubation in 4 mM galactose, 33% of added galactose was removed by suckling cells but only 7% by adult cells. At this higher concentration, there was a large unaccounted difference in total isotopic recovery which in the suckling cells was about 25% and in adult about 50%. This can also be seen in Figure 6 where the discrepancy between galactose removed and the sum of metabolites assayed increases with age. In similar studies using adult hepatocytes with gluconeogenic precursors such as lactate, Katz *et al.* (17) found large amounts of label located in the amino acid pools, products not assayed in the present studies.

DISCUSSION

There appear to be marked differences in the disposition of galactose by hepatocytes from 14-day-old suckling rats and cells from adult livers. Young cells remove galactose from the medium at a rate several-fold faster than older cells. This is accompanied by a parallel increase in galactose conversion to glucose, is reflected in metabolism to lactate, and oxidation to CO_2 and a decreased conversion to other undetermined metabolites. The present data amplify our previous findings (27) employing a liver brei from young animals and the observations of Fukushima *et al.* (14), that primary hepatocyte cultures, when first initiated from young rat liver, utilize greater amounts of galactose over a 24 h period than cells from adult liver.

Our present findings, in some ways, are similar to those we reported in liver perfusion studies and, in other ways, different. As with hepatocyte incubations, 15-day-old suckling livers perfused with 4 mM galactose took up more substrate and put out glucose at a faster rate than the adult (2). A detailed comparison of the results of hepatocyte incubation and whole liver perfusion can be made from our more extensive results reported for suckling liver perfusion (3). In both types of experiments, about 80% of the [^{14}C]galactose taken up by suckling tissue was converted to [^{14}C]glucose. A difference between the results obtained with the two techniques can be found in the patterns of galactose uptake. When 4 mM galactose was perfused, there was an initial 35 min lag in uptake before a linear rate was established (2), whereas with hepatocytes, there was an initial surge of uptake before the linear rate was observed.

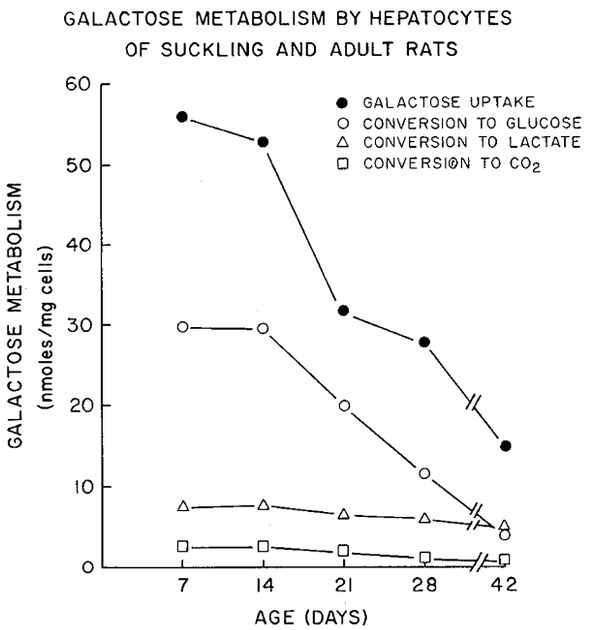

Fig. 6. Comparison of [^{14}C]galactose metabolism during postnatal development. The galactose uptake (●) and its conversion to glucose (○) lactate (△) and oxidation to CO_2 (□) are given for the ages indicated. Each point represents the mean of duplicate assays of hepatocyte preparations from two to four pooled livers. Experimental conditions are the same as those given in Figure 1.

Another disparity observed in the results from suckling hepatocyte preparations and liver perfusion relates to the effect of galactose on total glucose output. We reported (3) that the total chemical glucose put out during galactose perfusion was less than the sum of basal output when only buffer was circulated and the [^{14}C]glucose derived from labeled galactose, concluding that galactose suppressed endogenous glucose production of other precursors. Just the opposite was found, however, with isolated cells (Fig. 2), which produce much more total glucose than the sum of that converted from galactose and the basal glucose production, leading to the presumption that galactose stimulates gluconeogenesis from endogenous precursors. Similar observations have been reported by Kleigman *et al.* (18) who found greater endogenous glucose production after enteric galactose administration to newborn puppies than for those fed glucose. These observations deserve further study with radioactive glucose precursors such as lactate.

The higher galactose utilization rate observed for young liver reflects higher levels of specific activities of the galactose metabolizing enzymes—galactokinase, galactose-1-phosphate uridyltransferase, and UDPgalactose-4-epimerase—in the suckling period and parallels the fall to lower enzyme levels over the same time period in which the utilization rate falls (Fig. 6). This is also the period when the rat pups are decreasing milk intake and beginning to eat lab chow, thus diminishing the amount of galactose in the diet. Our animals are fully weaned at 21 days. Attempts to prevent the enzyme specific activities from falling by weaning onto high galactose diets have not succeeded (10) and high galactose in the medium could not prevent the fall in galactose utilization by suckling hepatocytes cultured for several days (14) suggesting that dietary galactose levels are not responsible for the changes. We are in the process of examining the role of diet on total hepatocyte galactose utilization.

Whether there are other factors besides hyperactivity of the Leloir pathway enzymes, which are responsible for the high galactose utilization by young cells, deserves comment especially in view of the increased potential for gluconeogenesis by perfused

Table 2. *Media isotope balance (% of galactose added)*[1]

Substrate concentration (mM)		Incubation time (min)	Galactose uptake	Conversion to [^{14}C]glucose	Conversion to [^{14}C]lactate	Oxidation to [$^{14}CO_2$]	(Unaccounted difference from 100%)
1	S	30	27	20	3.4	1.7	1.9
		60	67	58	6.0	4.3	−1.3
		90	92	80	5.7	7.5	−1.9
	A	30	7	3	1.6	0.9	1.5
		60	13	7	1.5	2.2	2.3
		90	19	15	1.3	4.4	−1.7
4	S	30	13	7	2.0	0.7	3.3
		60	24	13	3.1	1.6	6.3
		90	33	23	3.5	2.5	4.0
	A	30	5	1	1.2	0.2	2.6
		60	7	2	1.2	0.6	2.2
		90	7	1	1.2	1.2	3.6

[1] Isolated hepatocytes from fasted suckling (S) and adult (A) rats were incubated with 0.8 μCi [1-^{14}C]galactose. Media withdrawn at timed intervals were analyzed for the radiolabeled metabolites listed. Experimental conditions are detailed in the text. Percentages were calculated from mean values with S.E. less than 10% of the mean for n varied between 4 and 14 preparations.

liver and hepatocytes of suckling rats (1). Indeed, the basal level of glucose output from suckling cells in our own studies was much higher than from adult cells (Fig. 2) and the rate of galactose conversion to glucose several-fold higher in suckling tissue (Fig. 1). Perhaps the hormonal and other factors responsible for increased gluconeogenesis from lactate are also operative with regard to galactose, thus contributing to the high rate of galactose extracted from the incubation medium. Simkins *et al.* (30) have shown that glucocorticoids enhance the formation of glucose from galactose by fetal rat liver explants in organ culture.

Van Schaftinger and his colleagues (35), however, have shown that there is little or no recycling of label in C-1 of galactose to C-6 in fasted adult hepatocytes and we have shown this to be the case in suckling cells. Katz *et al.* (16) have pointed out that tritiated galactose in the C-2 position loses 80% of its label when converted to glucose by adult hepatocytes. Both observations are consistent with galactose metabolism to fructose-6-phosphate followed by conversion back to glucose and little recycling from breakdown to triose phosphate. The presentation of increased amounts of glucose-6-phosphate via the Leloir pathway plus the increase in glucose-6-phosphatase activity in the suckling period (8) would explain the increased conversion to medium glucose. Vernon and Walker (36) have reported that there is little or no recycling of glucose via the Cori cycle in the intact suckling rat.

The use of isolated hepatocytes permitted an examination of the oxidation of [1-^{14}C]galactose to [$^{14}CO_2$], which could not be done in our liver perfusion apparatus. The results indicate that metabolism of galactose to CO_2 is not nearly as extensive as conversion to glucose nor, indeed, the major metabolic fate of galactose in short term studies. *In vivo* studies in normal man have shown that within half an hour after injection of radioactive galactose, more than 50% of the label is present in the circulating glucose pool with much less conversion to CO_2 (24). It appears that under normal circumstances the labeled CO_2 from galactose arises from the oxidation of glucose derived from galactose (25). When, however, the Leloir pathway is defective in humans (26) or when rats are exposed to high galactose diets (21), the oxidation product, galactonate, is formed which Cuatrecasas and Segal (11) indicated could be decarboxylated to CO_2 and pentose. A preferential loss of C-1 of radioactive galactose compared to C-2 has been demonstrated in humans lacking galactose-1-phosphate uridyltransferase (26); therefore, preferential oxidation was examined in the present studies to determine if the higher oxidation rate of suckling cells compared to adult cells could be explained by this mechanism. This was found not to be the case when 4 mM galactose was used as the substrate. There was a difference in the rate of oxidation of C-1 and C-2 labeled galactose but the relative difference was similar in suckling and adult cells. Our data also indicated that the difference in [$^{14}CO_2$] yield from the C-1 and C-2 labeled galactose was paralleled by the same difference in the yield from C-1 and C-2 labeled glucose, suggesting that a direct oxidative pathway for galactose metabolism was not present in the hepatocyte. This is not surprising in view of the very high Km for the system responsible for galactose conversion to galactonate (21).

The rate of labeled galactose conversion to [$^{14}CO_2$] was found to be much higher than that of labeled glucose. It should be pointed out, however, that at the substrate levels used we found very little net uptake of glucose compared to that of galactose. Katz *et al.* (16) have indicated that there is extensive recycling of glucose and glucose-6-phosphate at the low glucose concentrations we used and that in view of the low net of uptake one cannot really calculate the amount of glucose oxidized nor truly compare the relative rates of galactose and glucose oxidation.

From accumulating data, it seems clear that the ability of liver to metabolize galactose is optimal in the suckling period when galactose is a prime nutrient and there is a greater need to efficiently utilize the sugar. There are indications, however, that galactose may have other important aspects than providing usable calories. Sparks and Glinsman (32) have shown that galactose perfusion of liver augments glycogen synthetase activity and other reports have indicated that galactose may be a factor in regulation of glucokinase (37). Our present finding that galactose augments gluconeogenesis adds another important function to this growing list.

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