Screening for Errors in Galactose Metabolism with the Erythrocyte

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We propose determination of the ratio of the rate of galactose metabolism to glucose metabolism by erythrocytes as a screening test for abnormalities in glucose and galactose metabolism. Packed erythrocytes (20 μl) are incubated for 1 h at 37 °C in 0.42 ml of a solution comprising phosphate buffer (pH 7.4), 0.4 mg of glucose, 60 mg of methylene blue, and 50 nCi of either [1-14C]glucose or [1-14C]galactose. Metabolism is then stopped by injecting dilute H2SO4 through a rubber septum sealing the flasks. On incubating the acidified solution for 1 h, the evolved CO2 is trapped in a well containing ethanolamine, which is suspended from the septum. The radioactivity of the well and its contents is measured in a scintillator, and from these data CO2 is calculated. (The scintillation medium is preheated with ethanolamine to eliminate chemiluminescence.) For normal adults mean values for CO2 are 0.468 μmol/liter of erythrocytes per minute for galactose and 37.8 μmol/liter of erythrocytes per minute for glucose. Homozygous galactosemics exhibit no galactose metabolism but the rate for glucose metabolism is normal. Results for parents of homozygotes are described. We review various causes for galactosemia and point out that transfer of galactose through the cell wall and into the erythrocyte is markedly reduced in certain cats and, although unreported, may possibly be a cause for galactosemia in humans.

Additional Keyphrases: genetics • inherited disorders • normal values • feline metabolism • interspecies comparison • pediatric chemistry • erythrocytic metabolism in adults and newborns • galactosemia • galactose transfer into cells

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The problem of screening for genetic defects is complicated by the many different enzyme activities one would be required to determine in order to assay for the numerous reported deficiencies (1). In addition, the returns are small for the amount of time invested, because of the relatively low prevalence of a particular defect. For this reason, many institutions limit their programs to one particular enzyme or metabolite. An example of this is the phenylketonuria screening program.

A more efficient approach would be to exploit the interdependence of enzymatic pathways. Thus, a single screening procedure could be used to test the integrity of an entire metabolic system, which would eliminate further study of the vast majority of specimens and so would focus on a few aberrations that require more specific identification.

For these reasons, we decided to explore the overall metabolism of galactose and glucose by the erythrocyte (2, 3), the strategy being that if glucose was metabolized normally while galactose was not, then one was necessarily dealing with a case of galactosemia. This finding could be caused by a deficiency of any one of three enzymes: galactokinase (EC 2.7.1.6), galactose-1-phosphate uridylyltransferase (EC 2.7.7.10) or UDPglucose 4-epimerase (EC 5.1.3.2) (4). In this case, the defect could be pinpointed by measuring the activities of these enzymes. Relatively few clinical assays would then need to be done on a given case.

If glucose were not being metabolized normally, then one would be prompted to look elsewhere. By judicious use of the short common screening procedures, such as for glucose-6-phosphate dehydrogenase (EC 1.1.1.49) or pyruvate kinase (EC 2.7.1.40), one could then identify most of the problems. The others would then be the subject of further study. In all of these cases it would
be ascertained first that a defect did in fact exist, making the detailed studies fruitful.

A screening test based on this concept consists of incubating a sample of the patient's erythrocytes with [1-14C]galactose and with [1-14C]glucose in separate containers. The 14CO2 produced is trapped in ethanolamine and measured by measuring its radioactivity in a liquid scintillation counter. The amount of glucose-6-phosphate channeled through the monophosphate shunt to yield CO2 may be increased 14-fold by adding NADPH-oxidizing agents such as methylene blue (5, 6). The reason for this can be seen in Figure 1, which summarizes the reactions taking place when the erythrocyte acts upon glucose or galactose.

Despite the theoretical attractiveness of this method, there is a severe experimental drawback. Addition of a strong base such as ethanolamine to common scintillation media produces an objectionable chemiluminescence (7). Our initial attempts to perform this test as reported in the literature (8, 9), resulted in a procedure that required at least 24 h for the spuriously elevated count rate to disappear, a delay that is particularly undesirable in the screening of neonates. The current trend toward earlier discharge of newborns from the nursery makes this imperative that results be available promptly, so that abnormal results may be rechecked while the newborn is still at hand in the hospital.

To solve this problem, we tried substituting 15 different bases for ethanolamine, including KOH, NaOH, benzethonium hydroxide ("Hyamine 1622"), triethanolamine, 1,2-diaminoethane, and various tetra-alkyl ammonium hydroxides—all without success. Acid could not be added to the scintillation solutions to decrease the pH because the bound 14CO2 would be lost.

We eventually obviated the difficulty by warming the scintillation medium with ethanolamine. When more ethanolamine is then added, there is no transient in-

crease in count rate. Presumably, an impurity in the medium itself undergoes total irreversible reaction with ethanolamine on such pretreatment and is rendered innocuous. The impurity cannot be the scintillation solutes, solvent, or emulsifier, because the desirable property of the medium—efficient counting—is unchanged.

Besides incubating patient’s bloods with [1-14C]galactose, we also did parallel experiments involving incubation with [1-14C]glucose, believing that the ratio of the metabolic activities of these two sugars would be more diagnostic than values for either activity alone. We have applied this procedure to healthy newborns and adults, to establish a reference for comparison when screening for this defect.

During this study, we encountered two children who are homozygous for galactosemia, and we also report results when these subjects and their parents were so tested.

In routine screening of certain members of the cat family, we noted that markedly less galactose was transferred across the erythrocytic membrane of certain species of cats as compared to the human. We explored this phenomenon and describe preliminary results.

**Materials and Methods**

**Equipment**

- *Unilux III Liquid Scintillator* (Nuclear Chicago, Elk Grove Village, III. 60007).
- *Diffusion flasks*, 25-ml Erlenmeyer flasks, No. K-882310, with rubber stopper, penetrated by a wire that holds a plastic center well, No. K-882320, above the surface of the reaction mixture (all from Kontes Glass Co., Vineland, N. J. 07052).

**Reagents**

- Except as noted, the following solutions are stable for several months when kept refrigerated.
- *Phosphate buffer, 0.1 mol/liter, pH 7.4*: Dissolve 245 mg of KH2PO4 and 1.244 g of K2HPO4 in 100 ml of water.
- *Methylene blue stock solution, 26.7 mmol/liter*: Dissolve 50 mg of methylene blue (Merck, Rahway, N. J. 07052) in 5 ml of the phosphate buffer.
- *Methylene blue working solution, 534 µmol/liter*: Dilute 1 ml of the methylene blue stock solution to 50 ml with the phosphate buffer.
- *[1-14C]Galactose, 0.5 mCi/liter*: Dissolve 50 µCi of crystalline [1-14C]galactose (New England Nuclear Co., Boston, Mass. 02118) in 10 ml of water. This is the stock solution. Dissolve 40 mg of α-D-glucose (Grade III; Sigma Chem. Co., St. Louis, Mo. 63178) in 1 ml of the stock solution and dilute to 10 ml with NaCl solution (9 g/liter). This is the working solution, 0.5 mCi/liter.
- *[1-14C]Glucose, 0.5 mCi/liter*: Dissolve 50 µCi of crystalline [1-14C]glucose (New England Nuclear) in 10 ml of water. This is the stock solution. Dissolve 40 mg of α-D-glucose in 1 ml of the stock solution and dilute
to 10 ml with NaCl solution (9 g/liter). This is the working solution.

Radioactive standard: A [14C]toluene standard was obtained from New England Nuclear Corp. The radioactivity of the sample was stated to be 4.94 × 10^6 dpm/ml.

Pretreated scintillator media: Dissolve 2 ml of ethanolamine in 200 ml of “3a70B scintillator media” (Research Products International Corp., Elk Grove Village, Ill. 60007). Heat this mixture in a volumetric flask, with the glass stopper secured tightly with a rubber band, for 1 h at 90-93 °C. The mixture is stable at room temperature, when stored in the dark.

Galactose, 1 mol/liter: Dissolve 4.5 g of α-D-galactose (Sigma, No. G0750) and dilute to 25 ml with water.

Galactokinase assay reagent: Following the method of Beutler and Matsumoto (10), mix the following: 250 μl of 100 mmol/liter NaF, 500 μl of 100 mmol/liter MgCl2, 1000 μl of a 1 mol/liter tris(hydroxymethyl)- aminomethane HCl buffer (pH 7.5), 250 μl of 7.6 mmol/liter galactose, 500 μl of 2 mCi/liter [1-14C]galactose, 250 μl of 0.12 mol/liter ATP, 1000 μl of a 10 g/liter solution of saponin, and 250 μl of water. The solution is stable for one month when stored frozen.

Assay Procedures

Human blood samples: The adults selected were apparently healthy individuals whose blood was sampled by venipuncture, with use of heparinized evacuated blood-collection tubes. These samples were drawn for routine, periodic screening purposes. Blood from newborns was sampled by heel prick into heparinized Natelson tubes, and were obtained for the primary purpose of screening for phenylketonuria.

Blood samples from galactosemics were also obtained by use of heparinized evacuated blood-collection tubes.

Feline blood samples: The blood from the cats became available because the animals were either being moved to another zoo or to another cage within the Lincoln Park Zoo. In these and other cases, blood was being drawn in order to monitor the well-being of the animals by various laboratory tests. All samples were obtained from animals that had been anesthetized with ketamine (“Vetalar”, Parke Davis & Co., Detroit, Mich. 48232; or “Ketaset”, Bristol Myers Products, Syracuse, N. Y. 13201). Blood drawn from either the saphenous or radial veins was collected with heparinized evacuated tubes.

Sampling of erythrocytes: Centrifuge heparinized blood in Natelson capillary tubes, sealed with Crito- cap-K (Scientific Products, McGaw Park, Ill. 60085) for 20 min at 900 × g. Score the tube with a file under the leukocyte layer, break the tube at this point, and discard leukocytes and plasma. Remove the Crito cap and aspirate 20 μl of packed erythrocytes into a disposable pipet. Using another disposable pipet filled with isotonic saline, introduce a bubbled saline column into the pipet containing the erythrocytes, by periodically joining and separating the ends of both pipets, while allowing the erythrocytes to flow downward. The volume vacated by the erythrocytes is thereby filled with slugs of saline interspersed with air bubbles. By blowing on the top of the bubbled stream, wash the erythrocytes and saline into a 25-ml Erlenmeyer flask containing 0.3 ml of the methylene blue working solution and 0.1 ml of either the [1-14C]galactose or [1-14C]glucose solutions (0.5 mCi/liter).

Measurement of 14CO2 evolution: seal the diffusion flask containing the reaction mixture with a rubber septum cap, from which is suspended the above-mentioned plastic well, containing 0.1 ml of ethanolamine. Incubate the flask at 37 °C for 1 h in a metabolic shaker, oscillating at 100 vibrations per minute. Inject 0.5 ml of 9 mol/liter H2SO4 through the cap with a syringe and needle, to stop the reaction. Incubate for an additional hour at 37 °C. Remove the stopper, cut the plastic well from its stem, and deposit it in a plastic scintillator vial (Research Products International Corp.) containing 10 ml of pretreated scintillator media. Count for 1 min in the liquid scintillator.

Activity of glucose working solution: Add 0.1 ml of the 0.5 mCi/liter [1-14C]glucose solution to 10 ml of the pretreated scintillation media and count for 1 min for five successive times. The average of these counts is the activity of the glucose working solution, subsequently abbreviated as Gα.

Counting efficiency: Dilute 0.3 ml of the radiolabeled standard (typical activity, 4.94 × 10^6 dpm/ml) to 100 ml with the pretreated scintillation medium. To 10 ml of this solution add 0.1 ml of ethanolamine and determine the cpm by counting for 1-min intervals five times and averaging the values obtained. The cpm obtained divided by the calculated dpm (e.g., 14820 dpm) multiplied by 100, gives the counting efficiency. Typically, we found values of 87%.

Calculation: For glucose, divide the counting rate (cpm) observed for the CO2 generated at the end of 1 h of incubation by 60; this is the count rate per minute of incubation and a measure of the rate at which glucose is being metabolized via the hexose monophosphate shunt, on a 1:1 molar basis, because the glucose is labeled only in the 1-position. Because 2.22 μmol of glucose was placed in the flask, the activity of the radioactive glucose (Gα) divided by 2.22 yields the cpm per micromole of glucose metabolized. The cpm per minute of incubation obtained for CO2, divided by the cpm per micromole of glucose metabolized gives the micromoles metabolized per minute by the 20 μl of packed cells used. Multiplying by 50 000 yields the micromoles per liter of erythrocytes per minute. This is summarized as follows:

\[ \text{cpm (CO}_2\text{) × (1850/G}_\alpha\text{) = μmol/liter of erythrocytes per minute} \]

For a typical Gα of 78 000 cpm, the factor 1850/Gα becomes 0.0237. If the value 1700 cpm were obtained for a particular specimen, the corresponding value would be 1700 × 0.0237, or 40.3 μmol/liter of erythrocytes per minute.
For galactose, no nonradioactive galactose was added. One Ci yields $2.22 \times 10^{12}$ decompositions per minute (dpm).

The specific activity stated on the vial of radiolabeled galactose we used was $6.75 \times 10^{-6}$ Ci per $\mu$mol of galactose.

Multiplying the specific activity of $6.75 \times 10^{-6}$ Ci/$\mu$mol by the factor $2.22 \times 10^{12}$ gives the dpm per micromole of galactose. This figure, when multiplied by 0.87 counting efficiency, yields the cpm per micromole of galactose ($1.304 \times 10^{7}$).

The cpm observed for the $CO_{2}$ generated after 1 h of incubation is divided by 60 to give the cpm per minute of incubation. This number divided by $1.304 \times 10^{7}$ gives the micromoles of $CO_{2}$ obtained per minute of incubation. Because only 20 $\mu$L of packed erythrocytes was in the flask, this figure is multiplied by 50 000 to obtain the results in $\mu$mol/liter of erythrocytes per minute.

In summary: cpm ($CO_{2}$) $\times 6.392 \times 10^{-5} = \mu$ mol/liter erythrocytes per minute.

For example, a sample galactose test resulting in 7320 cpm implies a metabolic rate of $7320 \times 6.392 \times 10^{-5} = 0.468 \mu$mol/liter erythrocytes per minute.

**Galactokinase assay:** We followed the method of Beutler and Matsumoto (10), with several modifications. Mix packed erythrocytes with an equal volume of isotonic saline. Add 50 $\mu$L of this mixture to 200 $\mu$L of the galactokinase assay reagent. Transfer two 50-$\mu$L aliquots of this mixture to separate tubes, each containing 20 $\mu$L of 1 mol/liter galactose solution, and mix. Spot 50-$\mu$L aliquots from each tube onto 18-mm diameter Whatman DE-81 paper circles. Permit the “100% sample” to dry completely. Place the other circle, the “zero time sample,” in a small beaker of water. Incubate the remainder of the reaction mixture for 1 h at 37 °C. Quench a 50-$\mu$L aliquot by adding 20 $\mu$L of 1 mol/liter galactose, and spot 50-$\mu$L of the resulting mixture on a paper circle. Place this 60-min sample in a cup of water. Place the “zero time” and 60-min samples on fritted glass disks, and wash with 800 ml of water. Dry thoroughly and count all disks in 10 ml of 3a70B media.

**Galactose-1-phosphate uridylyltransferase assay:** We followed the method of Beutler and Mitchell (11).

**Results**

**Normal subjects:** Table 1 compares the metabolic rate observed for neonates with that for galactose for erythrocytes obtained from 25 apparently healthy adults and neonates. A mean value of 0.468 $\mu$mol/liter of erythrocytes per minute ±0.146 (SD) was obtained for the galactose metabolism test performed for adults; for glucose metabolism the corresponding mean value was 37.8 ± 9.81 (SD) $\mu$mol/liter. Thus, the ratio of the rate at which glucose is metabolized relative to that for galactose is 81 for normal adults.

Neonates showed substantially higher rates of metabolism: the corresponding means were 1.507 ± .325 (SD) and 56.0 ± 11.4 (SD), respectively. Erythrocytes from neonates therefore metabolize glucose 37 times as rapidly as galactose. The coefficients of variation of 21.6% for galactose metabolism and 20.4% for glucose metabolism in the neonate approximate the values of 31.1% and 25.9% we found for the corresponding tests performed upon erythrocytes from adults.

The coefficients of variation for the populations in Table 1 are understandably much larger than the precision of the test. Table 2 shows the within-run precision of the metabolic activities computed on the basis of duplicate determinations on each subject. All CV's lie in the range of 5.4% to 7.9%. Stated another way, interindividual variation is substantially greater than intra-individual variation (including analytical variation).

**Galactosemia.** Results for two galactosemics and their parents are presented in Table 3, which shows activities for the metabolism of galactose and glucose, each compared with a normal control assayed concurrently. As determined by independent assays for galactose-1-phosphate uridylyltransferase, both parents

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<th>Table 1. Comparison of Erythrocyte Activity of Galactose and Glucose Metabolism for 25 Healthy Adults and Neonates</th>
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Mean: 0.4677 ± 37.83 ± 1.507 ± 56.00

CV, %: 31.1 ± 25.9 ± 21.6 ± 20.4

* The coefficient of variation is the variation from individual to individual.
of both families are heterozygous for deficiency of this enzyme.

Proband No. 1 had received a transfusion before her galactose transferase activity (14% of normal) was present one day after she had received a 30-ml transfusion of blood. Since the estimated blood volume of the infant was 170 ml after transfusion, the infant should theoretically have 16% transferase activity, as compared to 14% observed. In Proband No. 2, a transfusion was given shortly after birth. Thirteen days after blood transfusion the child still exhibited 53.9% of the normal transferase activity. Thus, cells from a donor can metabolize, and so decrease, circulating galactose.

Activities for glucose metabolism, measured for all family members, ranged from 73 to 108% of normal.

Table 3 also shows the ratio of the rates at which galactose and glucose are being metabolized. One homozygote showed a ratio of zero, as expected. The other, who had been transfused, showed a ratio of 13, about that expected as calculated from the amount of blood transfused. Heterozygotes show a ratio that extends from 56 to 83%.

Results for cats: Table 4 lists data obtained for erythrocytes from various cats. Those tigers with cataracts generated CO₂ from galactose at a rate at least threefold lower than was the case for tigers without cataracts. Erythrocytes from all cats metabolize galactose at a substantially lower rate than humans. For certain cats, such as the housecat, the puma, and the lion, erythrocyte galactokinase activity is much greater than in humans. In the tigers, the galactokinase activity is much lower. Galactose transferase activity is about the same as observed in humans, except for the lion, where it is lower but still substantially active. Overall glucose metabolism is in a similar range for all cats, somewhat less than half the rate observed in humans.

Discussion

Three types of galactosemia have been reported (12), corresponding to deficiencies in one of the three enzymes: galactokinase (ATP-D-galactose 1-phosphotransferase, EC 2.7.1.6) (13), hexose-1-phosphate uridylyltransferase (UDP glucose:α-D-galactose-1-phosphate uridylyltransferase, EC 2.7.7.12) (14), or UDPglucose 4-epimerase (EC 5.1.3.2) (15). Figure 1 shows the interdependence of these enzymes.

The screening test for the galactosemias proposed in this paper examines the integrity of the system from galactose to 6-phosphogluconate formation and decarboxylation, by measuring the end product of metabolism, carbon dioxide.

In vivo, only a fraction of the galactose metabolized appears as CO₂; most follows the Embden–Meyerhof pathway to lactate (3, 16). To increase the sensitivity of the method, the activity of the hexose monophosphate shunt was accelerated by adding methylene blue. As shown in Figure 1, this dye couples the oxidation of NADPH to the reduction of molecular oxygen, and thus drives the shunt pathway.

A low value for ^14CO₂ trapped, when [1-14C]galactose is used as substrate, indicates that any of several enzymes—galactokinase, -transferase, or -epimerase, or glucose-6-phosphate dehydrogenase—may be deficient. If, on incubating the same blood sample with [1-14C]-glucose, it is found that a normal amount of ^14CO₂ is trapped, then the metabolic block is located before the participation of glucose-6-phosphate dehydrogenase (1). The deficiency must then lie with decreased activity of galactokinase, hexose-1-phosphate uridylyltransferase, or UDPglucose 4-epimerase. If, on incubation with either [1-14C]glucose or [1-14C]galactose, diminished activity is observed, then a defect must exist beyond the conversion of galactose to glucose. In this case, a deficiency in glucose-6-phosphate dehydrogenase is
probably present, and this may be confirmed by measuring the glucose-6-phosphate dehydrogenase activity of the erythrocytes. It is also conceivable that a galactosemia co-existing with a deficiency of either hexokinase or glucose-6-phosphate dehydrogenase would result in diminished activities of both galactose and glucose metabolism. In any case, assay for specific enzymes will clarify this matter.

The ratio of the metabolic rate of galactose to glucose was found to be twice as great in newborns as for adults (Table 1). This reflects the greater reticulocyte count in newborns and also the presence of a greater percentage of younger erythrocytes in the blood of the newborn. In younger cells, the activities of both galactokinase and hexokinase are increased. These two enzymes limit the rate of metabolism of the two sugars, respectively, in the erythrocyte (17). Because galactokinase activity is increased more, the increase in the rate of galactose metabolism is more marked (2, 18).

Table 1 shows that there is a large variation between individuals. That this variation is indeed characteristic of the population is supported by the degree of reproducibility when erythrocytes of a single individual are repeatedly examined (Table 2). One reason for the large coefficient of variation observed for the population on using the $^{14}$CO$_2$ evolution technique is undoubtedly the cumulative variations for each individual enzyme involved in the overall conversion of galactose to CO$_2$.

A second reason for the large variation between individuals shown in Table 1 is the existence of significant numbers of individuals with variants for transferase and kinase deficiency. The Duarte variant shows 50% of the normal erythrocyte transferase activity, the heterozygote 75%. These patients exhibit no symptoms. It is estimated that 8 to 13% of the population are heterozygous for the Duarte gene (24). A milder form of transferase deficiency also occurs in the Negro population (25). Thus several of the individuals of Table 1 might be heterozygotic for some variant form of galactosemia.

If one measures the rate of glucose metabolism in the blood of an adult who has the same ratio of reticulocytes to erythrocytes observed in the newborn, then the blood of the newborn will metabolize the glucose less rapidly (19). It has been suggested that this serves a useful purpose during the neonatal period by presenting less competition with more vital organs for the limited substrate, glucose. At the same time, the needs of the neonatal erythrocyte are met by metabolism of galactose, which is converted to glucose. In infancy, galactose constitutes a substantial proportion of all carbohydrates consumed, because of the milk regimen. Interestingly,
an isoenzyme of hexokinase is present in the erythrocyte of the neonate, with a $K_m$ for glucose that is sixfold higher than that for the isoenzyme found in adult erythrocytes (19). This isoenzyme is not found in the adult.

In the two families with galactosemia, the disease state accrued from a deficiency of hexose-1-phosphate uridylyltransferase. Table 3 also shows the ratios of the galactose to glucose activities, a parameter that is not more diagnostic of the heterozygote than the value for galactose activity, because all of the values for glucose activity fall within a relatively narrow range in this table. The homozygotes were unambiguously identified by the inability of their erythrocytes to metabolize galactose to yield CO$_2$. The heterozygotes exhibited somewhat more than half the activity observed in an individual without the galactosemia trait. For this reason, positive identification of a carrier is more difficult.

From our data, the values for galactose metabolism are not proven to be diagnostic of the carrier state by the $^{14}$CO$_2$ evolution technique. This is true because of the large coefficient of variation exhibited by the healthy adult population (Table 1). The efficiency in detecting the heterozygote for galactosemia by this procedure could only be determined with a substantial number of additional cases. However, all heterozygotes (Table 3) had decreased activity, about 60% of the control in three of four cases.

Note the positive correlation between the values for galactose metabolic activity, as determined by $^{14}$CO$_2$ evolution, and the figures for transferase activity. The relative values obtained for the two tests for each heterozygous parent of Proband No. 2 are almost identical.

Analysis of the data obtained from samples of blood from the cats shows very low values of galactose metabolism when whole erythrocytes were used to generate CO$_2$. In the case of the lions, this metabolism is much lower than would be expected from the appreciable activity of galactokinase and hexose-1-phosphate uridylyltransferase found from tests performed on hemolysates. It is important to note that the assay for transferase activity depends on adequate enzyme activity of UDPglucose 4-epimerase, phosphoglucomutase, glucose-6-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase. The fact that appreciable transferase activity was found shows that, in addition, a metabolic block did not exist in those enzymes in the erythrocytes of the lions.

One must conclude, therefore, that the ability of galactose to permeate the membrane of the erythrocyte of the lion is blocked. Corroborative evidence of such a theory is forthcoming from assays for galactokinase activity in whole cells rather than hemolysates. In the assay, radioactive galactose is incubated with erythrocytes, which are subsequently washed, lysed, and chromatographed. Radioactivity is quantitated from three peaks corresponding to galactose, galactose-1-phosphate, and UDPgalactose (20). The test, when performed on galactokinase-deficient erythrocytes, exhibits a peak only for galactose, because on entering the cells it is not further metabolized under the conditions of the assay.

When we subjected the erythrocytes from tigers with cataracts (Table 1) to this test, we found no radioactive peaks at all. Thus, the galactose did not penetrate the cell. These assays were done before we realized that a defect existed in the passage of galactose across the cell wall. The tigers were shipped to other zoos before we could obtain blood for assay of kinase activity on hemolysates.

Some radioactivity, corresponding to a peak for galactose, was detected when erythrocytes from the Indian lion were so tested by using whole cells. Thus, while tigers and lions have galactokinase and transferase activities comparable to that in the human when erythrocyte hemolysates are used, they are much less able to transport galactose into the erythrocyte. Possibly such a defect may exist and has as yet not been diagnosed in human subjects. It has been shown that hexoses pass the membrane of the erythrocyte by facilitated transport (21). In humans, this is the rate-limiting step in glucose and galactose metabolism (22).

It cannot be inferred from the limited galactose metabolic activity of the erythrocyte that a relatively decreased galactose metabolism is characteristic of all organs of the cat. Each organ is under separate genetic control. Appreciable galactose metabolism occurs in the liver and kidney cortex of the house cat (23).

From the above, it can be seen that our procedure is practicable for testing the integrity of the enzyme systems responsible for metabolism of galactose by the erythrocyte. The test is theoretically attractive because any defect preventing conversion of [1-14CO$_2$]galactose to CO$_2$ will cause less 14CO$_2$ to be generated. Using this procedure, we found that the ability of the erythrocytes of certain cats to transport galactose across the erythrocyte membrane is markedly reduced relative to that observed in the human. Utilization of current screening methods to detect enzyme activity of hemolysate preparations would have failed to detect this metabolic defect. We suggest that when it is found that galactose is present in the urine or blood in excessive amounts then consideration be given to the fact that this may be a defect in the mechanism for transfer across the erythrocyte membrane.

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