Measurement of "True" Glucose Production Rates in Infancy and Childhood with 6,6-Dideuteroglucose

Dennis M. Bier, M.D., Rosemary D. Leake, M.D., Morey W. Haymond, M.D., Kenneth J. Arnold, M.D., Larry D. Gruenke, Ph.D., Mark A. Sperling, M.D., and David M. Kipnis, M.D., St. Louis, Los Angeles, and San Francisco

SUMMARY

"New" glucose production has been measured in 54 infants and children for the first time by continuous three-to-four-hour infusion of the safe, nonradioactive tracer 6,6-dideuteroglucose. The use of combined gas chromatography–mass spectrometry with monitoring of selected ions allowed deuterium enrichment in blood glucose to be measured on microliter samples with an error of less than 2 per cent. In the young child, glucose production increased in a slightly curvilinear manner from 1 kg. to 25 kg. body weight, when it reached 140 mg. per minute, almost the adult value of 173 mg. per minute (2.28 ± 0.23 mg./kg.·min., mean ± S.E.). Normalized for weight, glucose production in premature infants was 5.46 ± 0.31 mg./kg.·min., in term neonates averaged 6.07 ± 0.46 mg./kg.·min., in children below the age of six years was 7.1 ± 0.27 mg./kg.·min., and in late childhood averaged 5.4 ± 0.28 mg./kg.·min. Relative to estimated brain weight, however, glucose production was essentially linear from the 1-kg. premature infant to the 80-kg. adult. These data, the first measurements of "new" glucose production in childhood, suggest that brain size may be a principal determinant of those factors that regulate hepatic glucose output throughout life. DIABETES *26*:1016-23, November, 1977.

Despite the fact that hypoglycemia is relatively common in children, little is known about the quantitative dynamic aspects of glucose production and utilization in them because of the justifiable reluctance to use radioactive tracers or invasive techniques for obtaining such information. In an accompanying paper[1] we have described and validated precise microtechniques that allow carbohydrate metabolism to be studied in vivo with stable isotopically labeled glucose. Furthermore, rapid sample preparation and analysis time permit the undertaking of routine clinical studies that generate large numbers of samples. This report describes the application of these microtechniques to a systematic determination of glucose turnover throughout childhood. Portions of this work were presented previously.[2,3]

From the Departments of Medicine and Pediatrics, Washington University School of Medicine; Department of Pediatrics, University of California, Los Angeles (Harbor General Hospital Campus); and Departments of Pediatrics and Pharmaceutical Chemistry, University of California, San Francisco.

Address reprint requests to Dennis M. Bier, M.D., Washington University School of Medicine, Metabolism Division, 660 South Euclid, St. Louis, Missouri 63110.

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MATERIALS

6,6-Dideuteroglucose (glucose-6,6-d_2) was purchased from Merck, Sharp and Dohme, Ltd., Pointe Duval, Quebec, Canada, and rigorously tested for chemical purity as previously described.[1] Mass spectrometric analysis confirmed isotopic purity of 98 per cent d_2. It was pyrogen-free.[4] Organic solvents and reagents were purchased from various chemical suppliers.[1]

Preparation of materials for human use. Appropriate quantities of glucose-d_2 were dissolved in sterile pyrogen-free 0.5 N saline with an aseptic technique and sterile glassware. The solution was then passed through a sterile 0.22-μ Millipore filter into sterile vials that were subsequently sealed with rubber stoppers and crimped metal caps. Aliquots were removed aseptically as needed.

METHODS

Heparinized venous blood samples of 250-500 μl. were drawn from older children and capillary samples

of 100-200 μl. obtained from newborns at appropriate intervals. After immediate centrifugation at 4° C., the plasma proteins were precipitated with an equal volume of 3 M perchloric acid. The resultant supernatant was neutralized with 20 per cent KOH and aliquots used for microfluorometric measurement of glucose content by the coupled hexokinase-glucose-6-phosphate dehydrogenase method[5] and for preparation of the glucose-acetate-boronate derivative[1,6] after its sequential anion- and cation-exchange chromatography.[1] Whole-blood deuterated-glucose isotopic enrichment was measured in the glucose-acetate-boronate derivative by combined gas chromatography–mass spectrometry (GC-MS) with selected ion monitoring on one of two systems,[7,8] each capable of measuring the isotopic enrichments found in the described studies with an error of less than 2 per cent. Ions of nominal mass 297 and 299 representing $[M-C_4H_9]^+$ and the corresponding fragment enriched with two deuterium atoms, respectively, were those monitored for the present experiments. Analysis of a sample lasted approximately four to six minutes.

All glucose production measurements were made by the technique of a continuous intravenous infusion of tracer.[9] In this case, glucose-6,6-d_2 was infused at a rate estimated to be about 1-2 per cent of the glucose production rate. In the case of the newborn studies, the volume of saline administered was adjusted to also satisfy the infant's fluid requirements for the period of observation. Samples were drawn at the onset of dideuteroglucose infusion and half-hourly from 90 to 180 minutes in the newborn or 90 to 240 minutes in the remainder of the patients. Glucose flux rates for the latter group were calculated from steady-state isotopic enrichments according to standard formulas.[10] Since blood glucose values and/or isotopic enrichments were not completely constant during the study in approximately half of the newborns, both glucose production and utilization rates were calculated by the non-steady-state approximations of Steele.[11] For these estimations, the extracellular space was calculated by nomogram[12] and 75 per cent of this space was considered the mixing pool, as recommended by Steele.[13] If an infant lost weight after birth and before the study, this loss was subtracted from the extracellular volume prior to calculation of mixing-pool size. In practice, these corrections had little influence on the results since deviations from the steady state (where pool size does not enter into production rate calculations) were slight. For example, linear regression analysis of newborn glucose production versus utilization revealed a slope of 0.96 and an r^2 value of 0.91, indicating that the group as a whole did not deviate significantly from steady state.

6,6-Dideuteroglucose was chosen for the tracer since the label is nonrecycling. That is, once initially utilized, glucose labeled in position 6 will not reënter the blood stream as glucose-6,6-d_2 because virtually all the deuterium is removed during the gluconeogenic process by the keto-enol tautomerism of pyruvate, by the action of pyruvate carboxylase,[14] and by randomization of dicarboxylate acids in the citric acid cycle.[15] Furthermore, during gluconeogenesis, deuterium is lost from labeled alanine formed from 6,6-dideuteroglucose through the additional action of alanine aminotransferase.[16,17] Therefore, glucose production rates obtained with 6,6-dideuteroglucose represent "true" glucose output rates rather than the somewhat slower rates obtained with carbon labels that are recycled.

PATIENTS

Glucose turnover measurements were made in 19 newborn infants and 35 children whose clinical characteristics are presented in tables 1 and 2, respectively. All studies were performed under the guidelines of the Committees on Human Research at Washington University, St. Louis, the University of California, San Francisco, and the University of California, Los Angeles (Harbor General Hospital Campus).

Of the newborns, 11 were prematurely born between the 27th and 36th gestational weeks as estimated from menstrual history and physical characteristics according to the system of Dubowitz et al.[18] All were of normal size for gestational age. Four had mild respiratory embarrassment at the time of study, and two had biochemical abnormalities of calcium and bilirubin metabolism. Since no infant was hypoglycemic at any time during its nursery stay, we considered these infants to be representative of "normal" prematures, at least as far as glucose homeostasis is concerned. All eight term infants were well at the time of study, although the oldest infant (14 days) had recently recovered from a febrile episode of unknown etiology.

Ten of the prematures were receiving intravenous glucose-containing solutions prior to study, and half were also being fed orally. All of the term infants were receiving formula or breast feeding. Glucose-6,6-d_2 infusions were begun either at termination of intravenous alimentation or three hours after the last

TABLE 1

Clinical characteristics of the newborn infants studied

Patient	Weight (kg.)	Gestational age (wk.)	Age at study (days)	Conditions
1	1.25	29	1.5	Premature rupture of membranes; status—post-transient-tachypnea
2	1.00	27	7	Well
3	1.22	30	5	Placenta previa. Twin. Status—post-mild-RDS
4	1.30	30	4	Resolving moderate RDS
5	1.33	32	4	Hypocalcemia
6	1.10	29	7	Well
7	2.16	36	6	Status—post-transient-tachypnea
8	1.40	32	3	Well
9	2.02	35	4	Well
10	1.40	34	1	Well
11	1.58	31	3	Hyperbilirubinemia
12	3.58	40	14	Well; status—post-FUO
13	3.20	40	1	Well
14	3.20	40	1	Well
15	2.81	40	2	Well
16	3.43	40	3	Well
17	2.66	38	1	Well
18	2.73	38	2	Well
19	3.00	40	1	Well

TABLE 2

Clinical characteristics of the children studied

Classification	Number	Age range (yr.)	Weight range (kg.)
Normal	9	3 8/12 - 12 4/12	15.8 - 42
Ketotic hypoglycemia	7	2 2/12 - 6 6/12	9.6 - 18.8
Congenital heart disease	6	1 - 5 6/12	7.5 - 13.6
Muscle-wasting diseases	9	4 10/12 - 14 2/12	13.0 - 37.8
Partial lipodystrophy	1	7 10/12	23.1
Galactosemia	1	7/12	7.2
Maple-syrup urine disease	1	4/12	4.8
Hyperlipoproteinemia 2B	1	5 8/12	18.8

milk feeding. Subsequent glucose flux rates, calculated from values obtained 90-180 minutes into the course of the investigation, did not correlate with antecedent oral or intravenous carbohydrate intake.

The remaining 35 subjects were older children representing different clinical groups. There were nine normal children, seven with ketotic hypoglycemia, six suffering from cyanotic congenital heart disease, nine with various myopathies, and one each with lipodystrophy, galactosemia, maple-syrup urine disease, and hyperlipoproteinemia type IIB. Their ages ranged from four months to 14 years, and each was studied in the postabsorptive state after an eight-to-nine-hour fast. Although the older children represent diverse populations, including nine with a history of hypoglycemia, we consider the glucose production rates obtained representative of normal postabsorptive values for the following reasons: Each of the children had been well fed prior to the study (having eaten a late-night carbohydrate-rich snack the evening before), and each remained normoglycemic during the period of observation. Furthermore, of those children with a history of hypoglycemia, low-blood-sugar episodes were infrequent as well as intermittent and none had had a recent attack related to the time of study. For example, the galactosemic child had been normoglycemic since the neonatal period, had normal hepatic enzyme values, and had a normal liver shown by histology of a biopsy specimen obtained the day after measurement of glucose turnover. Likewise, to our knowledge, none of the nine children who were hypoglycemic previously has had a low-blood-sugar episode subsequent to the glucose flux studies.

RESULTS

Blood glucose values for newborn infants and older children during the course of dideuteroglucose infusion are shown in figure 1. After the newborn period, blood glucose was constant throughout. Because newborn infants were receiving intravenous glucose–containing solutions and/or oral feedings until shortly before the measurement of deuterated glucose turnover, blood glucose fell from a mean of 78 mg./dl. at the start of isotope infusion to an average value close to 60 mg./dl. from 90-180 minutes later. Neonatal glucose production rates, calculated from values obtained during the final 90 minutes of study, did not correlate ($p > 0.05$) with initial blood sugar level.

Figure 2 demonstrates the relationship between neonatal glucose production and body weight. A highly significant correlation between the two variables fit equally well ($r = 0.91$) to either a linear or a quadratic function. In term newborns, glucose production was 6.07 ± 0.46 mg./kg.·min. (mean ± S.E.), while in premature infants it averaged 5.46 ±

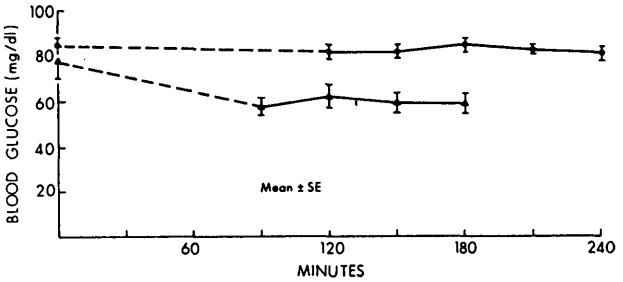

FIG. 1. Blood glucose levels during the course of dideuteroglucose infusion in 19 newborn infants (▲-▲-▲) and 35 children (●-●-●).

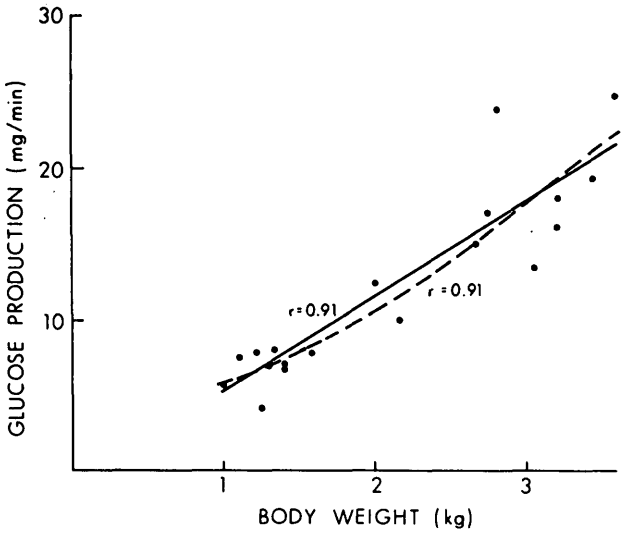

FIG. 2. Relationship between glucose production and body weight in newborn infants. The solid and dashed lines represent the linear and quadratic functions $Y = 6.31X - 1.14$ and $Y = 0.261X^2 + 5.15X - 0.034$, respectively.

0.31 mg./kg. · min., a value not statistically different from that of the term infants.

In the adult, the central nervous system is the leading consumer of daily glucose production.[19] If a similar relationship holds true for the neonate, one might expect a close correlation between hepatic glucose output and brain size. Figure 3 shows newborn glucose production related to brain weight estimated from the perinatal organ weight data of Gruenwald.[20] As for body weight, there was a highly significant direct correlation ($r = 0.91$). Although the data points appear to be slightly curvilinear, a quadratic regression ($r = 0.92$) fit no better statistically than did a simple linear relationship. Since brain weights were estimated, a more critical analysis of this relationship is speculative.

The relationships between glucose production and body weight are extended when the newborn data are combined with information obtained from the older children studied as well as with glucose production rates determined in five postabsorptive normal men by four-hour infusion of glucose-6,6-d_2. Figure 4 shows that total glucose production increased in a slightly curvilinear manner from 1 kg. body weight to 25 kg. (about the age of six), when it reached 140 mg. per minute, a value only slightly less than the adult average of 173 mg. per minute. In fact, 12 of the 13 children heavier than 20 kg. body weight had glucose production rates in the normal adult range.

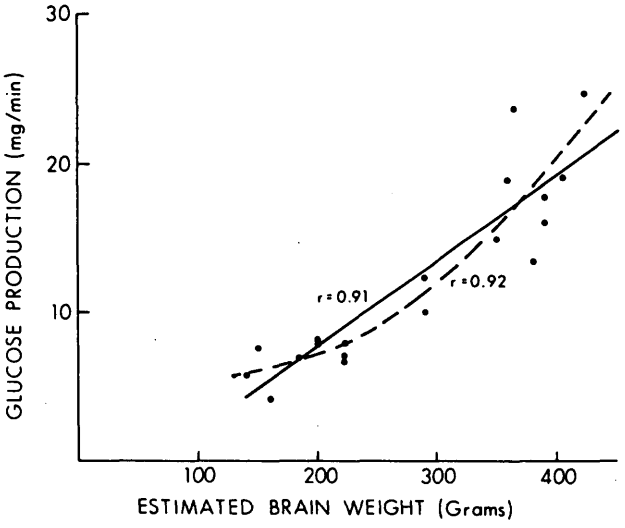

FIG. 3. Relationship between glucose production and estimated brain weight in neonates. The linear function $Y = 0.058X - 4.107$ and the quadratic expression $Y = 0.00016X^2 - 0.03X + 6.85$ are depicted by solid and dashed lines, respectively.

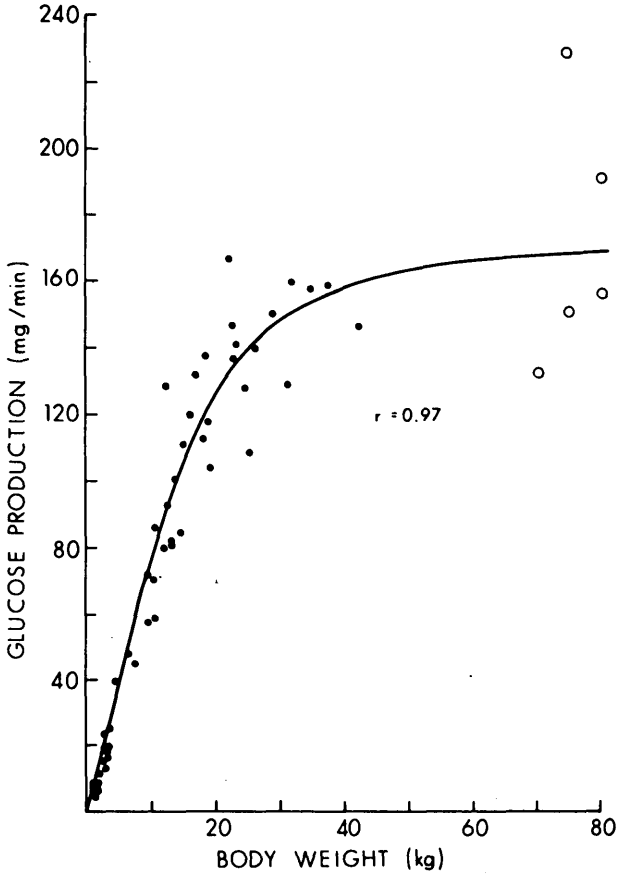

FIG. 4. Glucose production as a function of body weight from infancy to adulthood. The solid line depicts the cubic expression $Y = 0.0014X^3 - 0.214X^2 + 10.411X - 9.084$. Children are represented by closed and adults by open circles, respectively.

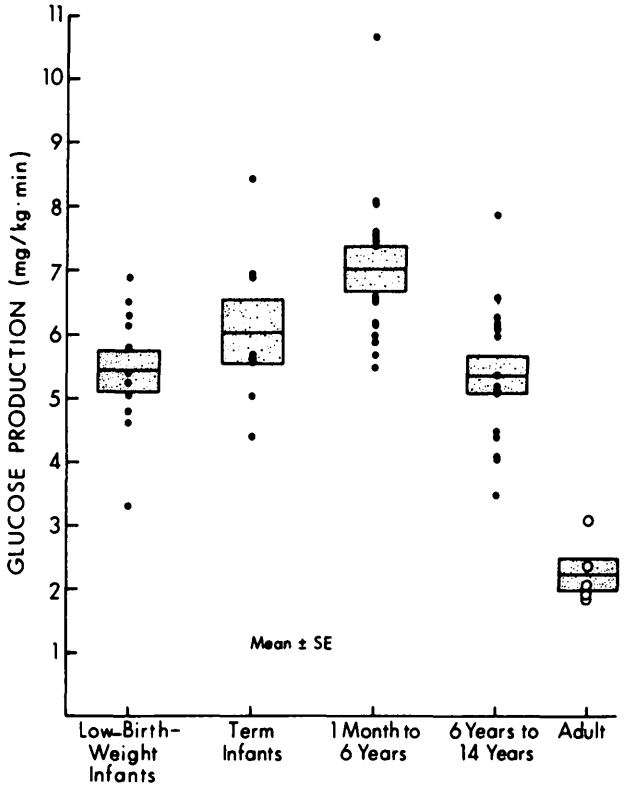

FIG. 5. Glucose production normalized for body weight in the 54 children (solid circles) and five adults (open circles) studied with dideuteroglucose infusion.

Figure 5 shows glucose production rates corrected for body weight throughout childhood. The bulk of children, from premature infants to age six years, produced glucose at the rate of 5-8 mg./kg.·min. After that age, glucose production as a function of body weight began to fall, approaching the adult value of 2.28 ± 0.23 mg./kg.·min. In the present series, six of the eight children older than eight years of age had glucose production rates less than 5.1 mg./kg.·min.

Potential explanation for the curvilinear relationship between childhood glucose metabolism and body weight became available when we examined glucose production relative to brain weight, estimated from age and head circumference.[21-24] Figure 6 shows a highly significant linear relationship ($r = 0.94$) between glucose production and estimated brain weight throughout the pediatric age group. Once again, a quadratic function fit the data no better than a linear correlation. If the child's brain is responsible for the bulk of daily glocose utilization, as is the situation in the adult,[19] it is not surprising that adult glucose production rates are reached in midchildhood, since average brain weight at age five to six years is 1,250 gm., about 90 per cent that of the mature brain.

DISCUSSION

It has been estimated that the newborn infant and young child must produce glucose at a rate in excess of that in the adult on a body-weight basis.[25-27] The present studies agree with previous indirect estimates of childrens' glucose production obtained by stepwise infusion of unlabeled glucose[28,29] and confirm that pediatric glucose production is two-to-four-fold greater than the adult rate, at least until midchildhood. In this regard, the young human being is similar to the immature rhesus monkey,[30] dog,[31] and sheep,[32] which produce glucose at rates of 4-8 mg./kg.·min., values also two to four times those of the respective adult animal.

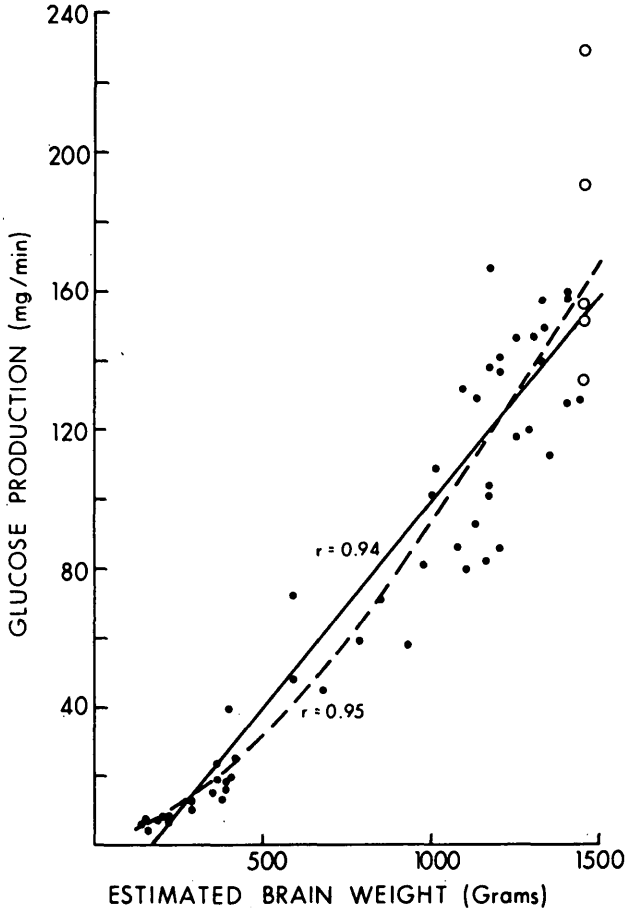

FIG. 6. Relationship between glucose production and estimated brain weight during childhood (solid circles) and adult life (open circles). The solid line represents the linear regression $Y = 0.122X - 22.75$, while the dashed line depicts the quadratic function $Y = 0.000041X^2 + 0.05X - 5.64$.

There are virtually no other direct measurements of glucose production in children. Following pulse injection of glucose-^{14}C(U), Somersalo[33] determined glucose turnover rates of 2.8 to 3.9 mg./kg.·min. in three eight-year-old children (two of whom had malignancies) and a flux rate of 3.4 mg./kg.·min. in an 11-month-old child with M. Cushing [sic] disease. Using a continuous infusion of glucose-1-^{13}C, Kalhan et al.[34] measured glucose production rates of 4-5 mg./kg.·min. in four normal infants at two hours of age and rates of 3.86 and 3.83 mg./kg.·min. in two additional infants at one day of age. Several important differences exist between the present studies and those of Kalhan and Somersalo. Both of the latter investigators used isotopic-carbon-tagged glucose, which can be metabolically recycled through pyruvate, lactate, and amino acids.[14] Therefore, glucose production rates thus obtained will be lower than the "true" glucose production rates found in the present studies employing the "irreversible" tracer 6,6-dideuteroglucose. The use of glucose-1-^{13}C in Kalhan's study would be expected to produce less discrepancy since some of the labeled carbon is lost as CO_2 in the hexose monophosphate shunt and other carbon-13, redistributed by recycling to positions 2, 5, and 6 of glucose, is not measured by Kalhan's technique. Furthermore, our infants were studied without rigid control of the prepartum maternal glucose administration, postpartum infant glucose infusion, or strict thermal neutrality in the infant's environment. Kalhan et al.,[34] on the other hand, prohibited maternal glucose infusion during labor and delivery and studied their infants in a temperature- and humidity-controlled room. In addition, none of our infants were studied as early as two hours of age. Lastly, the orally fed infants were studied three hours after the last feeding, at which time the neonate is primarily utilizing carbohydrate fuels, as demonstrated by Gentz et al.,[35] whereas Kalhan's infants were not studied until six hours after formula ingestion.

Thus, our results are in reasonable agreement with those of Kalhan et al.[34] when one accounts for the somewhat higher glucose flux rates expected in the present investigations due to use of a nonrecycling label, the presence of a carbohydrate-based fuel economy,[35] the shorter period of fasting,[36] and the increased energy demands for maintaining body temperature.[37,38] Furthermore, the glucose production rates of 5-8 mg./kg.·min. found in the present studies are not excessive when one considers central nervous system energy requirements in the infant and young child. Recent in-vivo studies of cerebral metabolism in children under a variety of circumstances[39-42] confirm that the immature brain can utilize glucose at rates in excess of 25 μmoles per 100 gm. brain weight·minute. Thus, a term 3.5-kg. newborn with a 420-gm. brain would require hepatic glucose production rates of 19 mg. or more per minute (5.5 mg./kg. body weight·min.) to satisfy cerebral glucose needs alone. Likewise, a six-year-old, 25-kg. child with a 1,250-gm. brain would need a hepatic glucose production of at least 3 mg./kg. body weight·minute to maintain normal cerebral glucose consumption. If central nervous system glucose consumption represents 60-80 per cent of daily hepatic glucose output in the child, as it does in the adult,[19] total glucose production rates of 5-8 mg./kg. body weight·minute, as found in the current studies, are realistic values.

In support of these estimates, Villee[43] showed more than 20 years ago that human fetal liver slices could produce glucose at the rate of 0.45 to 0.5 μmoles/gm.·min., and recent studies by Adam et al.[44,45] demonstrated that isolated perfused human fetal liver produced glucose at 0.4 to 1.2 μmoles/gm.·min. Thus, a 3.5-kg. term unfant with a 150-gm. liver is capable of producing glucose at the rate of 10-30 mg./min., or about 3-9 mg./kg. body weight·minute, within the range of glucose production values found in the present investigation.

Perhaps the most exciting finding in the present studies was the linear relationship found between glucose production and brain weight from premature infant to adult. Although not unexpected from the considerations described above, this observation confirms the pivotal role of the central nervous system in regulating carbohydrate balance throughout life. The mechanisms by which CNS glucose utilization might regulate hepatic glucose output are unknown. The effect may be mediated by a simple substrate feedback loop. Subtle blood sugar decrements (due to an enlarging brain with increasing glucose requirements) might exert negative feedback control on pancreatic insulin secretion, with consequent increased hepatic glucose output. Alternatively, glucose production might be reduced directly through the effect of lowered blood sugar on a hepatic glucokinase-glucose-6-phosphatase substrate cycle.[46] However, it is also well established that the brain exercises carbohydrate homeostatic control by neural and hormonal pathways that transmit glucoregulatory information from cortical or hypothalamic centers.[47-56] Presumably the

energy requirements of the brain play an important role in regulating such effector pathways, and glucose concentration in blood bathing the hypothalamus[57] as well as glucose utilization within hypothalamic centers[58] are felt to be afferent signals for such feedback regulation. Thus, brain size, by virtue of its energy drain on the carbohydrate fuel supply, may be the principal component of those factors responsible for glucose production throughout life.

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