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Glucagon
glucose
neonate

Glucose Production in the Newborn Dog. I. Effects of Glucagon *in Vivo*

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Extract

Systemic glucose production rates were evaluated 4 hr after feeding in 14 newborn beagle dogs at ages between 1 and 5 days. After a prime injection of radioisotopic tracers, glucose production was determined during infusion of intermixed tracer [$2\text{-}^3\text{H}$]glucose and [$1\text{-}^{14}\text{C}$]glucose at a constant rate. Seven of the newborn dogs served as controls throughout the 3-hr period of infusion, while seven of their littermates, infused simultaneously, received glucagon at a constant rate of $3.3\text{ }\mu\text{g/min}$ between 90 and 180 min of study. In control dogs, mean glucose production, determined by dilution of [$2\text{-}^3\text{H}$]glucose, was $55 \pm 3\text{ }\mu\text{mol/min}\cdot\text{kg}$ body weight. During the control period, their littermates produced glucose at a similar rate; however, glucagon infusion raised glucose production to $81 \pm 4\text{ }\mu\text{mol/min}\cdot\text{kg}$.

The average glucose production rate estimated with [$1\text{-}^{14}\text{C}$]glucose was 88% of that with the ^3H tracer during the initial control period and 77% during the glucagon infusion. In order to confirm that this discrepancy reflected the recycling of ^{14}C and the early development of gluconeogenesis, an additional 14 newborn dogs were infused with potential substrates for [^{14}C]glucose. In separate studies, [$U\text{-}^{14}\text{C}$]lactate, [$3\text{-}^{14}\text{C}$]lactate, [$U\text{-}^{14}\text{C}$]alanine, and [$6\text{-}^{14}\text{C}$]glucose were incorporated into glucose and [$1\text{-}^{14}\text{C}$]glucose. Quantification of gluconeogenesis by simultaneous infusion of [$6\text{-}^3\text{H}$]glucose and [$3\text{-}^{14}\text{C}$]lactate in a 5-day-old dog demonstrated that 25% of the glucose produced originated from lactate, whereas 10% was incorporated into carbon 1. Thus, systemic glucose production was established rapidly in newborn dogs and responded to stimulation with glucagon. A significant proportion of the glucose originated from recycling via the gluconeogenic pathway.

Speculation

Even in well nourished newborn mammals, recycling of glucose is important in maintaining homeostasis during the postabsorptive period. Based on comparisons between dogs infused with [$3\text{-}^{14}\text{C}$]lactate or [$6\text{-}^{14}\text{C}$]glucose, 25% or more of hepatic glucose production may originate from glucose recycled through lactate and pyruvate. Presumably most of the rest is derived from hepatic glycogen stored *in utero*.

Shortly after separation from the maternal glucose supply, a newborn animal must establish hepatic and perhaps renal glucose production in order to maintain the blood glucose at physiological levels (1). In the fasting newborn infant or mammal, plasma insulin remains at relatively low levels, whereas plasma glucagon levels rise (8, 13). Since the normal newborn infant or mammal is able to stabilize the blood glucose concentration shortly after birth, normoglycemia is attributed to stimulation of hepatic glucose production by a preponderance of glucagon over insulin effect.

Hepatic glucose production has been quantified previously in newborn dogs utilizing uniformly labeled [^{14}C]glucose (11) or [$2\text{-}^3\text{H}$]glucose as a tracer (15). In studies of adult dogs, however, uniformly labeled glucose has underestimated systemic glucose production rates (10), particularly in the presence of hormonal stimulation with methylprednisolone. The presumed reason for this reduced estimate is recycling of ^{14}C in glucose.

Such recycling in the newborn would depend on development of enzymatic activities associated with gluconeogenesis. In recent studies hepatic fructose diphosphatase, phosphoenolpyruvate carboxykinase, and mitochondrial pyruvate carboxylase activities rise to physiological levels within the first day after birth (4, 5). Thus, the potential for recycling exists, could cause underestimation of neonatal glucose production, and may contribute substantially to glucose homeostasis.

The potential for hormonal control of hepatic glucose production by insulin, pancreatic glucagon, and splanchnic neural norepinephrine has been evaluated *in vivo* and *in vitro* in newborn dogs. From 4.5 hr to 55 days of age, infusion of glucose alone or with physiological quantities of insulin suppresses glucose production in young dogs ineffectively compared with adults, indicating "resistance" to insulin action (15). Pharmacological quantities of glucagon *in vivo* raise systemic glucose production 36% during [$U\text{-}^{14}\text{C}$]glucose infusion (11), whereas norepinephrine, 10^{-6} M , *in vitro*, raises glucose production by the isolated perfused liver 71% above control levels (4, 5). The response to glucagon 10^{-6} M *in vitro* is of a similar magnitude (4).

The present study was done to quantify hepatic glucose production, gluconeogenesis, and recycling of glucose in the newborn dog. In addition the effects of pharmacological doses of glucagon were

re-examined in the context of a methodology which estimates the minimum values for recycling of carbon.

METHODS AND MATERIALS

MATERIALS

Isotopic tracers used in the studies were purchased from New England Nuclear (16); enzymes used in the assays were purchased from Sigma (17); glucagon and insulin were purchased from Eli Lilly (18).

SURGICAL PROCEDURES

Pregnant beagles were maintained in kennels and fed a standard diet for 1 week before delivery. Newborn puppies were fed *ad libitum* by their mothers until 4 hr before the study. Two hours before infusion with radioisotopes the newborn dogs were restrained without general anesthesia; then indwelling polyethylene catheters were inserted under local anesthesia in one femoral vein for infusion, and in the right external jugular vein to the level of the right heart for sampling. These catheters were kept patent with a 100 μ U/ml heparin-saline solution. The body temperature of newborn dogs was monitored rectally and maintained at 36–38° with a radiant lamp. During each study water was offered and ingested from soft-tipped syringes. Under these conditions the puppies remained at rest and appeared to be healthy.

STUDY PROTOCOLS

Estimation of Systemic Glucose Production with [2-³H]Glucose (G²³H) and [1-¹⁴C]Glucose (G¹¹⁴C). Fourteen newborn dogs, ages 1, 3, and 5 days of age were studied by infusion of intermixed G²³H and G¹¹⁴C. A solution of tracers in isotonic saline containing 11.54 μ Ci G²³H and 5.77 μ Ci G¹¹⁴C was injected rapidly and followed with a constant rate infusion of G²³H (0.13 μ Ci/min) and G¹¹⁴C (0.064 μ Ci/min) for 3 hr. Seven of the newborn dogs served as controls throughout the 3-hr period of study, whereas seven of their littermates, infused simultaneously, received glucagon at a constant rate of 3.3 μ g/min between 90 and 180 min of the study period. Half-milliliter blood samples were obtained during fasting and at 30, 60, 75, 90, 120, 150, 165, and 180 min of the study. The continuous infusion of tracers in isotonic saline solution replaced the blood removed for study. Samples were cooled to 0° on ice, plasma separated from red blood cells by centrifugation, and plasma stored at –20° until assay. The plasma samples were assayed for glucose, G²³H specific activity, and G¹¹⁴C specific activity as indicated in the analyses.

Incorporation of Substrates into Glucose and [1-¹⁴C]Glucose. An additional 14 newborn dogs were studied between 4 hr and 8 days of age with constant rate infusions of potential substrates for [1-¹⁴C]glucose. The dose of substrates was standardized so that all puppies received an 11.54 μ Ci prime and 0.13 μ Ci/min infusions of ¹⁴C-labeled tracer substrates for a period of 180 min. Four of these newborn dogs, ages 4 hr–6 days, received [U-¹⁴C]lactate (LU¹⁴C); three, ages 1–5 days, received [3-¹⁴C]lactate (L3¹⁴C); and one, 8 days old, received [U-¹⁴C]alanine (AU¹⁴C). Six of the dogs, ages 4 hr–8 days, received [6-¹⁴C]glucose (G6¹⁴C) in order to demonstrate directly the recycling of C-6 into the C₁ position of glucose.

Five of the dogs served as controls throughout the study period; four received a single dose of insulin (0.1 U/kg iv) at 90 min of the study; and five received glucagon at a lower dose (0.17 μ g/min after 90 min) than the previous newborn dogs. Plasma samples were obtained from these dogs for the assay of [1-¹⁴C]glucose at 15, 30, 60, 90, 105, 120, 150, and 180 min. Insulin or glucagon at the doses used did not affect incorporation of label, so that the results have been pooled and normalized for body weight.

Quantification of Gluconeogenesis. One 5-day-old dog infused with [3-¹⁴C]lactate was infused with [6-³H]glucose at the same dosage level. In this dog, plasma [¹⁴C]glucose was separated from [¹⁴C]lactate chromatographically, and the specific activities of [¹⁴C]lactate, [¹⁴C]glucose, [1-¹⁴C]glucose, and [6-³H]glucose were

determined to evaluate the contribution of gluconeogenesis to the glucose pool.

ANALYSES

Plasma samples were precipitated with 10% perchloric acid and neutralized with K₂CO₃. Glucose was determined spectrophotometrically by coupling hexokinase and glucose 6-phosphate dehydrogenase activities (2); [1-¹⁴C]glucose radioactivity was assayed in an aliquot using 6-phosphogluconate dehydrogenase to cleave CO₂ from C₁ of glucose as reported previously (2). Before assessment of [³H]glucose by column chromatography and liquid scintillation, supernatants from precipitated plasma specimens were evaporated to dryness and reconstituted with fresh unlabeled water. The evaporation served to remove tritiated water from plasma filtrates so that label remained only in glucose and its metabolic derivatives. As reported previously, negligible quantities of tritium from [2-³H]glucose are incorporated into glycerol or lactate *in vivo* during studies of newborn, young, and adult dogs (10, 15). In contrast, the quantity of ³H in plasma organic acid during the infusion of [6-³H]glucose was approximately 50% of that in glucose at a steady state. Under these conditions, radioactivity in glucose was separated from that in lactate by anion exchange as reported previously (2); plasma lactate was assayed with lactate dehydrogenase (2).

Radioactivity in ¹⁴C and ³H were determined by liquid scintillation with an external standard. All counts were corrected for background, the appropriate blank, and quenching. Intermixed isotopes, recovered in the dried samples and from the anion exchange columns, were determined by subtraction after the appropriate corrections documented above. Radioactivity in all samples was expressed as the disintegration rate (disintegrations per min).

CALCULATIONS

Glucose Production Rate. Glucose production rate (Fig. 1, Table 1) was calculated assuming a steady state. As indicated in the results (Fig. 1), a virtual steady state was established after 30 min in the control studies. During infusions with glucagon, a steady state was not established until 60 min after initiation of hormonal treatment, so that comparisons during hormonal infusions were

Fig. 1. Glucose metabolism in 14 newborn dogs, ages 1–5 days, during simultaneous infusions of [2-³H]glucose (0.13 μ Ci/min) and [1-¹⁴C]glucose (0.064 μ Ci/min) after tracer priming. Results are expressed as mean $\pm$ SEM.

Table 1. Glucose metabolism in newborn dogs

	Fasting	30-90 min	120-180 min	<i>t</i> ²	<i>P</i>
A. Controls (7) ¹					
Glucose (μmol/ml)	8.3 ± 0.7	9.4 ± 0.4	8.8 ± 0.4	0.99	>0.3
Production ³ H (μmol/min·kg) ³		54.7 ± 3.4	48.7 ± 2.3	1.47	>0.05
Production ¹⁴ C (μmol/min·kg) ⁴		48.6 ± 2.4	40.4 ± 1.6	2.84	<0.05
<i>t</i> ⁵		2.49	5.69		
<i>P</i>		<0.01	<0.0001		
Clearance ³ H (ml/min·kg) ³		5.8 ± 0.2	5.3 ± 0.2	1.60	>0.05
Clearance ¹⁴ C (ml/min·kg) ⁴		5.3 ± 0.2	4.7 ± 0.2	2.04	<0.025
<i>t</i> ⁵		1.89	4.00		
<i>P</i>		<0.05	<0.0005		
B. Glucagon ⁶ (7)					
Glucose (μmol/ml)	9.4 ± 0.9	9.1 ± 0.4	12.1 ± 0.4	4.97	<0.0001
Production ³ H (μmol/min·kg) ³		52.7 ± 2.7	80.8 ± 4.2	5.84	<0.0001
Production ¹⁴ C (μmol/min·kg) ⁴		45.6 ± 1.9	62.4 ± 4.1	4.03	<0.0005
<i>t</i> ⁵		4.05	7.55		
<i>P</i>		<0.0005	<0.0001		
Clearance ³ H (ml/min·kg) ³		5.9 ± 0.2	6.7 ± 0.3	2.14	<0.025
Clearance ¹⁴ C (ml/min·kg) ⁴		5.1 ± 0.2	5.1 ± 0.2	0.05	>0.4
<i>t</i> ⁵		3.99	7.31		
<i>P</i>		<0.0005	<0.0001		

¹ Number of pups studied in each group.

² Student's *t*-test comparing pooled data from the control period between 30 and 90 min with the later study period assuming equal variance in the samples.

³ Metabolic parameters estimated with [2-³H]glucose tracer.

⁴ Metabolic parameters estimated with [1-¹⁴C]glucose tracer.

⁵ Student's *t*-test of the mean difference between paired samples with [2-³H]glucose tracer and [1-¹⁴C]glucose tracer.

⁶ Glucagon (3.3 μg/min) infused continuously between 90 and 180 min.

restricted to samples obtained between 150 and 180 min of the tracer infusions. $P = I^*/W(G^*/G)$, where P = systemic glucose production rate (micromoles per min·kg); I^* = rate of infusion of isotopic tracer (disintegrations per min per min); W = weight of newborn dog (kilograms); G^*/G = specific activity of plasma glucose with respect to the tracer isotope infused (disintegrations per min per μmol).

The quantity of glucose tracer infused was negligible compared with glucose production rates in all studies; therefore, there was no need to correct for the infused tracer by subtraction as would be required with chemical quantities of infused glucose.

Fractional C₁ Recycling (Fig. 1, Table 1). The amount of recycling of C₁ was inferred and quantified from the difference between glucose production rates determined with G2³H and G1¹⁴C. $R = P^3H - P^{14}C/P^3H$, where R = the fraction of carbon recycled into C-1, P^3H = glucose production rate estimated from dilution of G2³H (micromoles per min·kg), and $P^{14}C$ = glucose production rate estimated with G1¹⁴C (micromoles per min·kg). Recycling of glucose also was demonstrated directly by the incorporation of [6-¹⁴C]glucose into [1-¹⁴C]glucose as reported in Figure 2.

Fraction of Glucose Pool Derived from Lactate (Table 2). This was determined by the ratio of (total [¹⁴C]glucose specific activity) to 2([¹⁴C]lactate specific activity). This result was confirmed by comparing G¹⁴C specific activity with that of G6³H as follows: $R = G^{14}C \text{ specific activity}/G6^3H \text{ specific activity}$, where R = the fraction of the glucose pool derived from lactate. The specific activities were corrected appropriately for the assayed ratio of L3¹⁴C and G6³H in the infusate, and the calculation reported only after a steady state specific activity ratio of glucose to lactate was established (Table 2).

Metabolic Clearance Rate. Metabolic clearance rate (C) calculated in a steady state when glucose production rate (P) equaled glucose uptake (U). $C = U/G$ (milliliters per min·kg), where U = glucose uptake (micromoles per min·kg), G = plasma glucose concentration (micromoles per ml).

Fig. 2. Incorporation of ¹⁴C-substrates into [1-¹⁴C]glucose (mean ± SEM). Number of pups in each group is indicated by the number in parentheses after the age range.

Differences between Production Rates Estimated with G2³H and G1¹⁴C tracers. These were compared with the paired Student's *t* test. Pooled data in control and treated periods were compared in order to assess the change of glucose production rates with time and during glucagon infusion. All results are expressed as mean ± SEM. Calculations were done with a Hewlett-Packard 9830 calculator system employing standard programs in BASIC.

RESULTS

HEPATIC GLUCOSE PRODUCTION (FIG. 1, TABLE 1)

In 14 newborn dogs the mean plasma glucose stabilized during control periods in the range of 8–10 μmol/ml. Infusion of glucagon

Table 2. Fractional incorporation of [$3\text{-}^{14}\text{C}$] lactate into [$1\text{-}^{14}\text{C}$]glucose in one dog 5 days old

Tracer ¹	[$3\text{-}^{14}\text{C}$]Lactate
(n) ²	(8)
Glucose ($\mu\text{mol/ml}$)	4.3 ± 0.3
Lactate ($\mu\text{mol/ml}$)	1.11 ± 0.11
$\text{G}^{14}\text{C}/2\text{L}^{14}\text{C}^3$	0.254 ± 0.030
$\text{G}^{14}\text{C}/\text{G}^3\text{H}^4$	0.252 ± 0.026
$\text{G}^{14}\text{C}/\text{G}^3\text{H}^4$	0.102 ± 0.012

¹ ^{14}C -Labeled substrate infused at a rate of $0.13 \mu\text{Ci/min}$.

² Number of samples after steady state [$1\text{-}^{14}\text{C}$]glucose specific activity (Fig. 2).

³ Ratios determined from the relative specific activities. G^{14}C , [^{14}C]glucose; L^{14}C , [^{14}C]lactate; G^3H , [^3H]glucose separated chromatographically. [$1\text{-}^{14}\text{C}$]glucose (G^{14}C) was determined enzymatically.

⁴ G^3H derived from [$6\text{-}^3\text{H}$]glucose infused simultaneously with L^{14}C .

at $3.3 \mu\text{g/min}$ raised the plasma glucose concentration from $9.1 \pm 0.4 \mu\text{mol/ml}$ to a mean value of $12.1 \pm 0.4 \mu\text{mol/ml}$ between 150 and 180 min of the study.

In seven control dogs, both G^{23}H and G^{14}C specific activities were relatively stable from 30 min of study onward, but there was a gradual rise that was more marked with [$1\text{-}^{14}\text{C}$]glucose. Glucagon treatment reduced the specific activity of both G^{23}H and G^{14}C , an effect that was measurable from 120 min onwards. The specific activity of labeled glucose was relatively constant for both isotopes between 150 and 180 min of study.

Mean glucose production rates measured with tracer G^{23}H stabilized at $53\text{--}55 \mu\text{mol/min}\cdot\text{kg}$ in control periods and declined slightly to $49 \mu\text{mol/min}\cdot\text{kg}$ between 120 and 180 min of study in the control dogs. Glucagon infusion raised systemic glucose production rates from $52.7 \pm 2.7 \mu\text{mol/kg}\cdot\text{min}$ in the control period to 60.8 ± 5.7 at 120 min and $80.8 \pm 4.2 \mu\text{mol/kg}\cdot\text{min}$ at 150–180 min.

During the control periods, mean glucose production rates assessed with G^{14}C were 89% of those with G^{23}H tracer in control dogs, and 87% in those treated later with glucagon. This result implies that 11% to 13% of the C-1 in glucose was recycled to the same position. Although there was a slight decline in the proportion of glucose production estimated with G^{14}C during the final 60 min of infusion in controls, glucagon infusion at pharmacological rates reduced the fractional estimate further to 77%. This resulted in a reduction of the estimated hormonal effect on glucose production from a 53% increase with G^{23}H to 37% with G^{14}C tracer. Nevertheless, there was a significant increase of systemic glucose production rate from 45.6 ± 1.9 to $62.4 \pm 4.1 \mu\text{mol/min}\cdot\text{kg}$ when estimated with G^{14}C .

CONVERSION OF SUBSTRATE TO [$1\text{-}^{14}\text{C}$]GLUCOSE (FIG. 2)

Although glucagon infusion at the lower rate ($0.17 \mu\text{g/min}$) raised blood glucose on the third and fifth day of life, it had no effect on the incorporation of substrates into [$1\text{-}^{14}\text{C}$]glucose. Similarly, insulin (0.1 U/kg) lowered blood glucose transiently but did not modify the plasma level of [$1\text{-}^{14}\text{C}$]glucose compared with that in control animals. Thus, results from the incorporation studies have been grouped according to the ^{14}C -labeled substrate infused.

In Figure 2, the conversion of ^{14}C -labeled substrates to [$1\text{-}^{14}\text{C}$]glucose is displayed. Since there was a threefold variation in puppy size whereas each tracer was infused at a standardized rate, the results are normalized as indicated for puppy weight. This permitted gross comparisons of incorporation into [^{14}C]glucose. All the substrates studied were incorporated into G^{14}C . When adjusted by weight, the plasma concentrations of [$1\text{-}^{14}\text{C}$]glucose varied little and were indistinguishable for each substrate between 1 and 8 days of age. Only those obtained at 4 hr were clearly different, and have been displayed separately.

In dogs between 1 and 8 days of age [$U\text{-}^{14}\text{C}$]lactate, [$3\text{-}^{14}\text{C}$]lactate, and [$U\text{-}^{14}\text{C}$]alanine- $\text{U}\text{-}^{14}\text{C}$ infusions resulted in a marked rise of [$1\text{-}^{14}\text{C}$]glucose within 15 min of the prime infusion, with a tendency to stabilize thereafter during the constant rate infusion of substrate. In contrast, [$6\text{-}^{14}\text{C}$]glucose infused to five dogs between 1 and 8 days of age resulted in a continuous rise of plasma [$1\text{-}^{14}\text{C}$]glucose during the first 90 min. Between 90 and 180 min of infusion, [$1\text{-}^{14}\text{C}$]glucose tended to stabilize at a relatively constant level.

As indicated, two newborn dogs at 4 hr of age incorporated lactate into glucose or recycled glucose at much lower rates than at age 1–8 days (Fig. 2). During these infusions of LU^{14}C at 4 hr of age plasma G^{14}C rose continuously at a very low rate throughout the infusions, and did not stabilize. Even at the end of the studies, plasma G^{14}C level was only 30% during LU^{14}C infusion and 22% with G^{6}C of the mean levels obtained at later ages.

QUANTIFICATION OF GLUCONEOGENESIS (TABLE 2)

As quantified in Table 2, total plasma [^{14}C]glucose specific activity was one-half of plasma [^{14}C]lactate specific activity after a steady state was established, indicating that 25% of the glucose pool originated from L^{14}C . The proportion of the glucose pool originating from ^{14}C -labeled substrate also equaled 25% when [^{14}C]glucose specific activity was compared with that of [$6\text{-}^3\text{H}$]glucose in plasma (Table 2).

METABOLIC CLEARANCE RATES (TABLE 1)

Metabolic clearance of glucose estimated with G^{23}H tracer in control periods varied between 5 and $6 \text{ ml/min}\cdot\text{kg}$ body weight. The metabolic clearance of G^{14}C was always significantly less. Treatment with pharmacological quantities of glucagon ($3.3 \mu\text{g/min}$) raised G^{23}H clearance slightly by 17%, while having no effect on the clearance of G^{14}C .

DISCUSSION

REGULATION OF GLUCOSE

As indicated in the present study and others, systemic glucose production is established early in the newborn dog at rates which exceed those in the adult (11, 15). Presumably, most of the glucose is released by neonatal liver in response to events associated with birth such as hypoglycemia or hypoxia (1, 6, 8, 9). Based on measurements of plasma pancreatic hormones, glucose, and glucogenic substrate, various investigators have inferred that hepatic glucose production is initiated after birth by the rise of plasma glucagon occurring while plasma insulin stabilizes in the fasting range (9, 13). Furthermore, Varma *et al.* (15) have demonstrated that young dogs between 4.5 hr and 55 days of age are less sensitive to insulin action in the physiological range than are adult dogs. During glucose infusions which raised plasma insulin to levels resembling those in the adult, these young dogs failed to suppress systemic glucose production completely or to increase the metabolic clearance of glucose effectively. In the present study the newborn dogs had low metabolic clearance rates for glucose, less than those observed previously in the young and adult dogs. These data imply that blood glucose was stabilized in the postabsorptive state by an underdeveloped or unstimulated insulin effect upon glucose uptake.

Stimulation with continuous glucagon infusion ($3.3 \mu\text{g/min}$) raised blood glucose approximately $3 \mu\text{mol/ml}$, in a manner resembling the response to a single glucagon pulse of $300 \mu\text{g/kg}$ between 24 and 96 hr of age (3). Similarly, blood glucose stabilized between 60 and 120 min after the pulse or infusion, a fact which led previous investigators to suggest that hepatic glucose production was increased, although the uptake of glucose was not (3, 11).

In the present study, basal systemic glucose production in the postabsorptive state was the same as that observed in the isolated perfused canine liver (4, 5). In addition, the rise of glucose production stimulated by a pharmacological rate of glucagon

infusion was the same as that observed in the isolated perfused canine liver stimulated with either glucagon or norepinephrine 10^{-6} M (4, 5). Thus the canine liver, at least potentially, could account for all the glucose produced *in vivo*. Furthermore, the metabolic clearance of glucose rose only 16% in the newborn dogs. Based on present evidence in the newborn mammal and infant, therefore, direct stimulation of the newborn liver by pancreatic glucagon or sympathetic neural norepinephrine (5, 7) raises hepatic production. Plasma insulin tends to remain in the fasting range unless combined stimulation with amino acids as well as glucose occurs (12). Finally, insulin is relatively ineffective in suppressing hepatic glucose production or elevating the metabolic clearance of glucose (15). Thus, the postnatal stabilization of blood glucose may reflect the unopposed action of pancreatic glucagon and sympathetic neural norepinephrine.

SOURCES OF GLUCOSE

As in other species, the canine fetus accumulates glycogen in the liver as term gestation approaches; the newborn dog has hepatic glycogen levels of approximately 500 μ mol/g, 3 times those in the postabsorptive adult (3). After birth, hepatic glycogen declines even in the fed newborn dog, stabilizing at low levels of 65–75 μ mol/g between 2 and 4 days of age (3, 4). Presumably this decline reflects the mobilization of hepatic glycogen to sustain blood glucose either while the newborn is fasting or as adequate caloric intake is being established.

Pharmacological doses of glucagon administered *in vivo* cause a decline of hepatic glycogen which correlates with the rise of blood glucose at ages earlier than 96 hr (3); glucagon or norepinephrine *in vitro* cause a decline of hepatic glycogen which accounts for 80% of the glucose produced after 1 day (4, 5). Thus, it appears that hepatic glycogen accumulated *in utero* serves as a major source of systemic glucose production in the postabsorptive or hormonally stimulated state.

Nevertheless, the enzymatic activities required for gluconeogenesis from C-3 intermediates are established within 3 hr of birth (4) and measurable gluconeogenesis occurs from this time onwards. As would be expected, this has led to a discrepancy between methods for measuring hepatic glucose production. Estimates of systemic glucose production rate with uniformly labeled glucose are lower than those utilizing tritiated glucose as tracer (10). In the present study, $[1-^{14}\text{C}]\text{glucose}$ and $[2-^3\text{H}]\text{glucose}$ tracers were utilized simultaneously to permit the evaluation of recycling. The assay of $[1-^{14}\text{C}]\text{glucose}$ quantified only C-1 and estimated the ^{14}C -label independently of tritiated glucose. Since C-1 randomized away from its position during recycling and distributed in the carbon skeleton of glucose, a greater agreement was expected between estimates of glucose production utilizing $\text{G}1^{14}\text{C}$ and $\text{G}2^3\text{H}$ than would occur when uniformly labeled glucose was utilized. As indicated $\text{G}1^{14}\text{C}$ tracer infused at a constant rate gave an estimate of systemic glucose production in the newborn dog which was 11–13% below that with $\text{G}2^3\text{H}$ tracer. There was a moderate increase of recycling between 90 and 180 min of study, and a marked increase with the high doses of glucagon (3.3 $\mu\text{g}/\text{min}$).

Direct measurement of gluconeogenesis and recycling in this study demonstrated that $[^{14}\text{C}]\text{lactate}$ was incorporated into glucose, and that $[6-^{14}\text{C}]\text{glucose}$ recycled as early as 4 hr of age. Within the first day, functional gluconeogenesis and recycling of glucose *in vivo* had increased to a relatively constant level similar to that in the isolated perfused canine liver at 3–5 days of age.

Quantification of gluconeogenesis in a postabsorptive 5-day-old dog demonstrated that 25% of the glucose formed originated from lactate. The exactness of this estimate is affected by exchange of labeled carbon causing its loss from intermediate pools, plus the dilution of intermediate pools with other sources of unlabeled carbon. For example, a marked influx of unlabeled carbon from fatty acid metabolism during fasting would serve to dilute the specific activity and cycling would redistribute the labeled carbon of the intrahepatic oxaloacetate pool. Thus, the quantified estimate of gluconeogenesis represents a minimum value.

Nevertheless, these results resemble those in the isolated perfused liver with only ^{14}C -labeled lactate as substrate in which lactate incorporation into glucose was established after birth, was 20% of functional CO_2 fixation by intact mitochondria, and correlated directly with mitochondrial pyruvate carboxylase activity.

Finally, a comparison of plasma $[1-^{14}\text{C}]\text{glucose}$ in newborn dogs infused with $[3-^{14}\text{C}]\text{lactate}$ or $[6-^{14}\text{C}]\text{glucose}$ suggests that most of the gluconeogenesis in the first 5 days represents recycled glucose. In this context, recycling of glucose in the postabsorptive state may account for 25% or more of the hepatic glucose production. Thus, recycling apparently represents an important aspect of fuel economy and glucose homeostasis in the newborn dog.

SUMMARY

In the postabsorptive newborn dog hepatic glucose production is established shortly after birth; the potential for gluconeogenesis can be demonstrated within 4 hr after spontaneous delivery. Most of the glucose produced originates from glycogen or unlabeled sources; but approximately 25% of it may originate from gluconeogenesis or recycling of glucose. The newborn canine liver is a target organ which responds to stimulation by pancreatic glucagon or sympathetic neural norepinephrine; however, hormonal stimulation of hepatic glucose production by the newborn dog may be relatively unopposed by pancreatic insulin.

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