Spontaneous and Amino Acid-Stimulated Glucagon Secretion in the Immediate Postnatal Period

RELATION TO GLUCOSE AND INSULIN

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ABSTRACT The extent and significance of spontaneous glucagon secretion in the immediate postnatal period were investigated in groups of normal infants studied cross-sectionally and longitudinally. Arginine- and alanine-stimulated glucagon secretion was also studied. Plasma glucagon concentrations were correlated with prevailing glucose and insulin concentrations.

The characteristic fall in blood glucose, reaching a nadir within hours of birth, was associated with a significant increase in glucagon concentration. Despite persistence of relative glucopenia, glucagon did not change appreciably between 2 and 24 h of life. A further significant elevation in glucagon concentration occurred from day 1 to day 3 of life associated with a return of glucose to euglycemic levels. In contrast to the sluggishness of pancreatic glucagon release, glucagon-like immunoreactivity rose markedly to mean levels of approximately 2,000 pg/ml after introduction of formula feeding. No significant changes in insulin levels were observed in these studies.

Arginine infusion via an umbilical vein catheter into six infants within 6 h of birth elicited a brisk, almost threefold increment in glucagon concentration (from 339±85 to 940±254 pg/ml) in blood obtained from, or close to, the portal circulation. Bolus injection of alanine (1 mmol/kg) into a peripheral vein to six infants resulted in significant increments in glucagon (mean maximal, 128 pg/ml) as well as glucose and insulin.

The observations suggest that spontaneous glucagon secretion may be an important factor in neonatal glucose homeostasis. Secretion seems more brisk in response to amino acid stimulation than to a falling glucose concentration.

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INTRODUCTION

Glucagon has been known to be a potent stimulant of glycogenolysis (1) and gluconeogenesis (2), but only recently has the physiology of endogenous glucagon been defined (3). By employing radioimmunoassay (RIA)[1] procedures with a high degree of specificity for pancreatic glucagon, it has been established that glucagon secretion is suppressed by high blood glucose concentrations and stimulated by hypoglycemia (4, 5), amino acids such as arginine (6) and alanine (7, 8), and by exercise (9), infection (10), and anxiety (11). The concept that glucagon secretion plays a significant role in glucose homeostasis is now generally accepted.

Only a few data are available concerning glucagon secretion in the neonatal period, a time of acute energy need. The transition from the intrauterine to an extrauterine environment is characterized by a transient fall in plasma glucose levels, which is not due to lack of several gluco-homeostatic hormones. Moreover, plasma insulin concentrations are low and insulin secretion after stimulation is obtunded (12). There is evidence that gluconeogenesis is deficient in the newborn (2, 12, 13), and it is possible that this deficiency may be related to inadequate glucagon secretion. Although this

[1] *Abbreviations used in this paper:* GLI, glucagon-like immunoreactivity; RIA, radioimmunoassay.

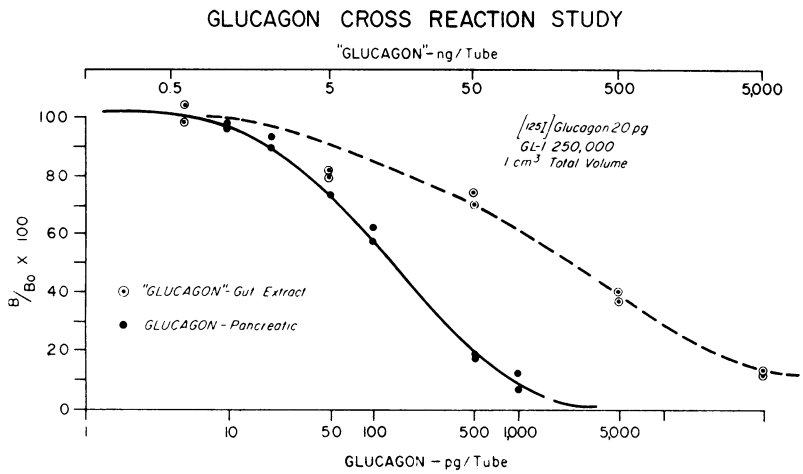

FIGURE 1 Standard curve with GL-1 antiserum. This antiserum detects 20 pg of crystalline glucagon but cross reacts significantly with a pork gut extract whose potency is reputedly 2,000 ng equivalents/mg of powder. Thus, the extent of cross reaction approximates 5 : 1, although exact comparisons are not valid since the displacement curves are not parallel.

hormone has been identified in the feal pancreas (14) and in the plasma of the human fetus and newborn (14–17), the significance of glucagon secretion in the immediate neonatal period is not clear. The present investigations in normal newborn infants were thus undertaken to define the spontaneous changes in plasma glucagon concentration in the newborn period, to clarify the interrelationships of plasma glucagon, glucose, and insulin during the first hours and days of life, and to study the capacity of the newborn pancreas to secrete glucagon in response to arginine or alanine infusion.

METHODS

The study population consisted of newborn infants delivered vaginally at term; all had a clinically uneventful neonatal course. Several separate groups of infants were studied.

Cross-sectional studies. Blood samples were obtained by venipuncture or by heelstick from infants at several of the following time periods after birth: ½, 1, 1½, 4, 12, 60, 72, and 96 h. All infants were fasted for the initial 12 h and given water at 12 h. Formula was offered beginning at 24 h.

Longitudinal studies. An initial cord blood sample was obtained from six infants, after which each had peripheral blood sampled at 15, 30, 60, 90, and 120 min after birth. An additional ten infants were each sampled sequentially at 2, 4, 6, 12, and 24 h after birth. Finally a third group of eight infants were sampled sequentially at approximately 24, 48, and 72 h of life, just before a scheduled feeding, so that 3–4 h had elapsed since the last feeding.

Stimulation tests. Arginine monohydrochloride (Cutter Laboratories, Berkeley, Calif.) was infused at a dose of 0.5 g/kg over 30 min into five newborn infants within the first 6 h of life. The infusion was performed with a Harvard constant infusion pump (Harvard Apparatus Co., Inc., Millis, Mass.) via an umbilical vein catheter which was inserted under aseptic technique. Although each catheter was inserted for an equal distance, the location was not radiographically controlled, and a site close to or in the portal circulation is assumed. Blood was withdrawn via the same catheters before (0 time), immediately after (30 min), and a half-hour after ceasing the infusion (60 min).

Sterile, pyrogen-free, 5% alanine in water was infused into a separate group of six infants, also within 6 h of birth. The alanine was infused into a peripheral vein at a dose of 1 mmol per kg (89.1 mg/kg) over 2 to 3 min. Peripheral blood was obtained before and 15, 30, 45, and 60 min after the bolus injection.

Each blood sampling consisted of no more than 2 ml of blood so that in no infant was more than 10 ml of blood withdrawn. Each sample was placed into chilled tubes containing 10.5 mg EDTA and 1,000 kallikrein inhibitor units Trasylol (FBA Pharmaceuticals, Inc., New York), and promptly spun in a refrigerated centrifuge. The plasma was separated and stored frozen at −20°C until assayed. All study procedures were reviewed and approved by the Human Use Committee at our institution. In conformity with the Declaration of Helsinki, informed written parental consent was obtained before experimental procedures. No deleterious effects were noted from any of the infusions.

Assays. Plasma glucose was determined by a glucose oxidase technique using 10-μl aliquots (18). Insulin was determined by a modification of the double antibody RIA of Morgan and Lazarow (19), employing 100-μl aliquots in duplicate. Plasma immunoreactive glucagon was determined by a double antibody RIA employing 200-μl samples in duplicate. The glucagon RIA systems were developed in our laboratories and recently described.[2] Two antisera were employed. Antiserum GL-1 (Fig. 1) detects 20 pg of crystalline pork glucagon, but cross reacts significantly with an extract of pork gut (kindly provided by Dr. Lise Heding, Novo Institute, Denmark), said to have a potency

[2] Sperling, M. A., P. V. DeLamater, M. Kazenelson, R. H. Fiser, and D. A. Fisher. 1974. The development and application of radioimmunoassay for plasma glucagon. *Clin. Chem.* In press.

of 2,000 ng equivalents of glucagon/mg of gut extract powder. Since in our system the crystalline glucagon standard and gut extract do not give parallel displacement curves, exact potency comparisons are not possible, but the extent of cross reaction approximates 5:1. Thus, this antiserum measures total glucagon-like immunoreactivity (GLI).

The RIA system using antiserum GL-5 permits detection of 10 pg crystalline pork glucagon and cross reacts minimally with the gut extract. In addition, (Fig. 2) this system give displacement curves which are essentially identical in both sensitivity and specificity to Dr. Unger's 30K antiserum, widely used and considered specific for glucagon (3, 8, 10).

Plasma from a volunteer undergoing arginine stimulation gave a reading of 583±29 pg/ml with GL-1 antiserum in seven separate assays; the value with GL-5 antiserum in six separate assays was 450±39 pg/ml ($P < 0.01$). Thus, assays with GL-1 antiserum overestimate pancreatic glucagon concentration. GL-1 antiserum was used in the cross-sectional and arginine stimulation studies; GL-5 antiserum was used in all other glucagon determinations.

In the assay, 200-μl plasma samples are reacted with 100 μl antibody in a 1:300,000 final dilution, 50 μl Trasylol, (500 U), 100 μl 0.1 M EDTA, and 450 μl phosphate saline buffer (0.01 M, pH 7.4 with 0.25% normal rabbit serum added). Throughout incubation the protein concentration in the standard curve is controlled with pancreatectomized dog plasma which gave near zero readings in the RIA. Addition of [^{125}I]glucagon, 20 pg in 100 μl, is delayed 48 h after which the reaction is incubated for an additional 72 h at 4°C. Free and bound hormones were separated by using a second antibody (goat anti-rabbit antiserum). The intra-assay coefficient of variation does not exceed 8% and approximated 4% in the mid portion of the standard curve. The interassay coefficient of variation is 13% for antiserum GL-1 and 23% for antiserum GL-5.

Student's t test and paired t analysis were used to determine levels of significance between groups at various time intervals, or for changes in any given individual over time. Simple linear regression analysis was used to assess correlations between parameters.

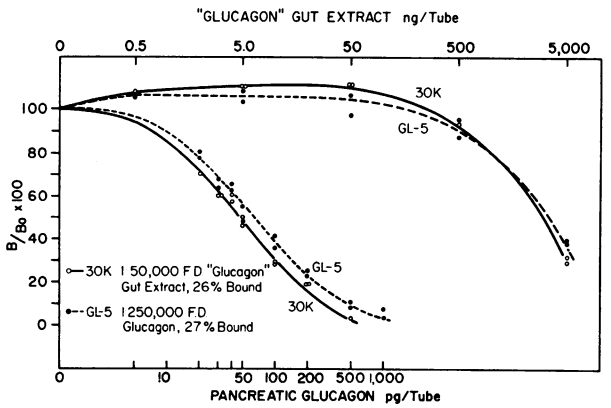

FIGURE 2 Standard curve with GL-5 antiserum. This antiserum detects 10 pg of crystalline glucagon and reacts only minimally with the pork gut extract. Sensitivity and degree of cross reaction with "gut" glucagons are almost identical to Dr. Unger's 30K antiserum, considered specific for pancreatic glucagon. F.D., final dilution.

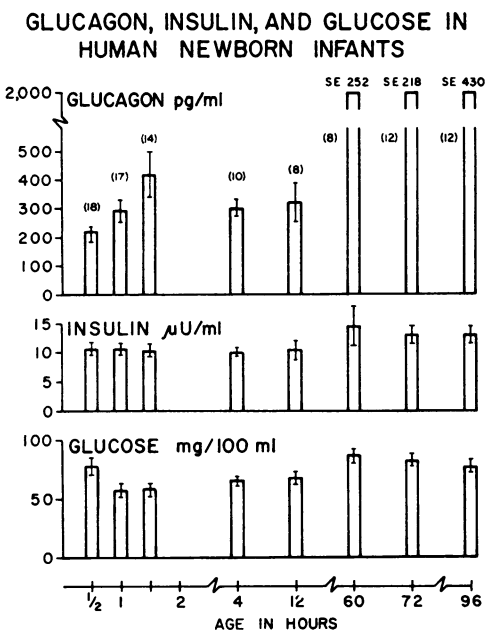

FIGURE 3 Cross-sectional studies showing the spontaneous changes in immunoreactive glucagon, insulin, and glucose in the postnatal period. The vertical lines indicate 1 SEM. The numbers of patients are in parentheses.

RESULTS

Cross-sectional studies (*Fig. 3*). Glucose declined from a mean (±SEM) of 77±6 mg/100 ml at ½ h after birth to 57±5 mg/100 ml at 1½ h ($P < 0.02$), and by 60 h had risen to 87±4 mg/100 ml ($P < 0.01$ in comparison to 1½ h). Mean plasma glucose remained at this level thereafter. Plasma insulin values did not change significantly at any time during the study. Immunoreactive plasma glucagon concentration increased significantly from 225±26 pg/ml at ½ h to 355±52 pg/ml at 1½ h ($P < 0.05$), and the mean glucagon concentration was still elevated at 4 h (307±32 pg/ml), although this value was not statistically different from the ½ h value.

At 1½ h the plasma glucose and glucagon concentrations were negatively correlated, (coefficient of correlation [r] = − 0.73, $P < 0.01$). A significant correlation between glucose and glucagon also was present at 4 h ($r = -0.65$; $P < 0.05$), but not at 12 h.

After the introduction of formula feeding at 24 h immunoreactive plasma glucagon concentrations increased markedly to plateau values of approximately 2,000 pg/ml at 60 h. These high levels largely represent immunoreactive glucagon of nonpancreatic and presumably gut origin, as shown by comparing RIA results in 10 samples at varying time intervals after birth assayed by using both the GL-1 and GL-5 antisera (Table I). The mean value for total GLI was 2,983±449 pg/ml, whereas the mean pancreatic (GL-5) glucagon concen-

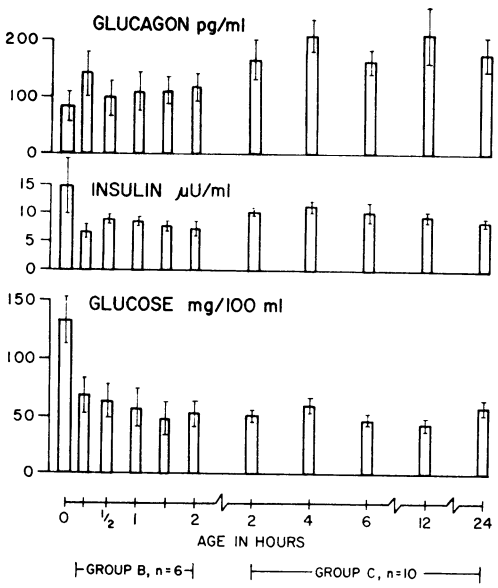

FIGURE 4 Longitudinal studies showing the sequential changes in plasma glucagon, insulin, and glucose in two groups of infants. Group B, birth to 2 h; group C, 2 to 24 h after birth. The vertical lines indicate 1 SEM.

tration was 374±59 pg/ml. Moreover, there was no significant correlation between paired values ($r = 0.27$; $P > 0.1$).

Longitudinal studies: The sequential changes in plasma glucose, insulin, and glucagon in the longitudinal studies are shown in Figs. 4 and 5.

As in the cross-sectional studies, glucose fell rapidly from cord values of 133±21 mg/100 ml to a nadir of 49±14 mg/100 ml in peripheral blood at 90 min ($P < 0.01$). Plasma insulin concentration fell from 15±5 μU/ml in cord blood to levels of 6.5±0.7 at 15 min, and 8.7±0.8 at 30 min, but this fall was not significant. The plasma glucagon concentration increased from a mean value of 83±27 pg/ml in cord blood to 139±41 pg/ml at 15 min; mean values subsequently remained in the range of 100–110 pg/ml throughout the first 2 h of life. Paired t analysis for each individual revealed a significant elevation in plasma glucagon concentration ($P < 0.05$) above cord blood levels. The fall in glucose and increase in glucagon concentrations during the first 15 min were negatively and significantly correlated ($r = -1.00$, $P < 0.01$). Beyond 15 min no significant correlation existed between these parameters.

No significant changes in plasma glucose, glucagon, or insulin occurred between 2 and 24 h of life, and the values at 2 h in this study were not significantly different from the values at 2 h in the previous (cross-sectional) series. Thus, in cross-sectional and longitudinal studies during the first day of life, the major changes in glucose and glucagon occurred within the first 2 h after birth.

The plasma glucose, insulin, and glucagon concentrations on days 1, 2, and 3 are shown in Fig. 5. Plasma glucose concentration increased from 44±5 mg/100 ml on day 1 to 61±5 mg/100 ml on day 2 ($P < 0.05$) and 61±6 mg/100 ml on day 3. In each individual infant, the blood glucose concentration was higher on days 2 or 3 of life than on day 1. The plasma insulin level was similar on days 1, 2, and 3. In contrast, the plasma glucagon level was appreciably higher on days 2 and 3 than on day 1 ($P < 0.01$), and paired t analysis showed that both the 1–2 day change and the 1–3 day change

TABLE I

*Comparison of Total Immunoreactive Glucagon and Pancreatic Glucagon Concentrations in Plasma of Ten Normal Human Infants**

Infant	Hours after birth	Cross-reacting antibody (GL-1)	Specific antibody (GL-5)
		pg/ml	*pg/ml*
1	1	260	280
2	15	715	435
3	60	2,325	400
4	60	3,400	100
5	72	3,350	87
6	72	2,700	450
7	96	2,200	480
8	96	5,000	600
9	96	5,000	600
10	120	5,000	305

$r = 0.27$, $P > 0.1$.

* Infants delivered normally without complications. Neonatal course uneventful.

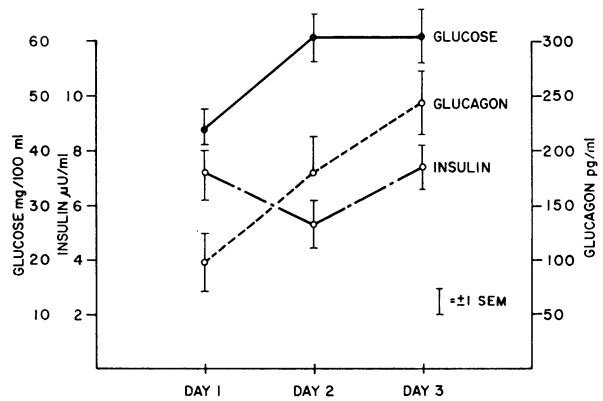

FIGURE 5 Sequential changes in glucose, glucagon, and insulin in eight infants each of whom was sampled at approximately 24, 48, and 72 h after birth. The increments in glucose and glucagon are significantly correlated ($r = 1.0$; $P < 0.01$).

were significant ($P < 0.05$, $P < 0.005$, respectively). The changes in mean glucose and glucagon from day 1 to day 2 were highly correlated ($r = 1.0$; $P < 0.01$). Individual glucose and glucagon concentrations were correlated only on day 1 ($r = -0.91$; $P < 0.01$).

Stimulation studies: Arginine infusion into the umbilical vein (Table II) did not change blood glucose appreciably, but the mean plasma insulin level increased in an erratic fashion in some, but not all, of the infants studied. In contrast, the glucagon response was dramatic; mean plasma glucagon levels were significantly increased at 30 and 60 min (in comparison to control values $P < 0.05$); by paired t analysis the differences at 30 and 60 min were even more significant ($P < 0.005$).

The results of the alanine infusion tests are shown in Table III. Glucose concentrations at 30 min were significantly greater than the mean value before infusion. Plasma insulin also tended to increase; the mean concentration was significantly greater than the baseline value at 60 min ($P < 0.05$) and by paired t analysis the maximal increment above basal also was significant ($P < 0.05$).

The mean glucagon level, too, was significantly elevated at 60 min ($P < 0.05$) and the mean maximal increment above the basal concentration was 128 pg/ml, highly significant by paired t analysis ($P < 0.005$). Thus, alanine evoked significant increments in blood glucose, insulin, and glucagon concentrations. However, the increments in glucose and insulin were small in comparison to the glucagon response.

TABLE II
*Response of Newborn Infants to Intravenous Arginine (0.5 g/kg)**

		Time in minutes		
		0	30	60
Infant				
Ca	Glucose (*mg/100 ml*)	72	67	67
	Glucagon (*pg/ml*)	103	310	235
	Insulin (*μU/ml*)	31	42	30
Cd	Glucose	61	—	—
	Glucagon	260	375	850
	Insulin	16	18	13
Hc	Glucose	54	63	75
	Glucagon	575	1,525	1,100
	Insulin	42	86	60
Ch	Glucose	69	68	70
	Glucagon	268	1,088	950
	Insulin	—	—	—
St	Glucose	97	84	84
	Glucagon	490	1,400	1,250
	Insulin	37	—	116
Means±SE				
	Glucose	71±7	71±5	74±4
	Glucagon	339±85	940±254‡	877±174‡
	Insulin	32±6	49±20	55±23

* Infused and sampled via an umbilical vein catheter.
‡ $P < 0.05$ in comparison to 0 time.

DISCUSSION

The initial fall in blood glucose with subsequent return to euglycemic levels by days 2–3, as well as the sluggishness of insulin secretion in fetal and newborn humans, primates, and other animals models is well documented in the literature (20–28). Plasma glucagon concentrations in the present studies were observed to increase during the first 2 h of life in infants studied both cross-sectionally and longitudinally. Both the absolute concentrations and the magnitude of the early increase were similar to those recently reported by Bloom and Johnston in a series of infants in whom glucagon concentrations were determined in cord blood and once again in peripheral plasma 2 h after birth (17). Moreover, the baseline glucagon values in the plasma from close to or in the portal circulation are in the range recently reported by Luyckx, Massi-Benedetti, Falorni, and Lefebvre in newborn portal plasma (15). The present results suggest that the pancreatic islet alpha cell in the newborn is capable of responding to the fall in plasma glucose during the first 2 h of life. Although this increment in blood glucagon is statistically significant and correlates well with the fall in blood glucose, it is, in absolute terms, quite small, especially when compared to the dramatic rise in glucagon found in the early postnatal period in the rat (29). It is conceivable that this initial rise in glucagon prevents the further fall of glucose. But it is apparently inadequate to evoke appropriate glycogenolytic and gluconeogenic responses in order to raise glucose, since glucagon in pharmacologic doses is capable of increasing glucose concentrations in human newborns (30) and has been shown to activate key gluconeogenic liver enzymes in rats (31–33). However, the plasma concentrations achieved with pharmacologic doses of glucagon may exceed the secretory capacity of the neonatal pancreas.

A progressive and more significant increment in basal plasma glucagon concentration occurs between day 1 and day 3 of life; mean plasma glucagon level on the third day of life is 2–3 times the values seen on day 1 of life. This secondary rise is accompanied by, and correlated with, a significant rise in blood glucose concentrations, without alteration in the mean circulating plasma insulin level. It is thus possible that on day 1 the secreted glucagon may be incapable of evoking an appropriate glycemic response by virtue of immaturity of liver glycogenolytic and gluconeogenic enzymes. Reisner et al. (13) have recently reported that in newborn infants pharmacologic doses of glucagon are more effective in activating spanchnic gluconeogenic mechanisms on day 3 than on day 1 of life. These authors suggest that the

TABLE III

Response of Newborn Infants to Intravenous Alanine (1 mmol/kg)

		Time in Minutes				
		0	15	30	45	60
Infant						
V	Glucose (*mg/100 ml*)	50	52	—	44	47
	Glucagon (*pg/ml*)	148	340	—	224	242
	Insulin ($\mu U/ml$)	7.2	14	—	9.4	10.3
H	Glucose	41	39	—	47	49
	Glucagon	215	307	—	287	263
	Insulin	—	6.0	—	—	9.3
Y	Glucose	59	63	68	71	69
	Glucagon	129	115	247	126	—
	Insulin	5.0	5.5	6.0	7.0	6.4
P	Glucose	38	54	66	63	59
	Glucagon	319	543	410	426	347
	Insulin	7.9	6.8	9.4	9.4	12.5
M	Glucose	52	54	51	47	54
	Glucagon	227	293	273	213	240
	Insulin	—	—	10.5	—	10.3
J	Glucose	47	53	52	46	55
	Glucagon	207	158	116	140	280
	Insulin	4.8	8.6	—	7.6	7.0
Mean±SE						
	Glucose	47.8±3.1	52.5±3.1	59.3±4.5*	53±4.6	56±3.2
	Glucagon	207±27	293±62	262±60	236±45	274±20*
	Insulin	6.5±0.7	8.2±1.5	8.6±1.4	8.5±0.5	9.6±1.0*

* $P < 0.05$ in comparison to 0 time.

inability of neonatal liver to extract the amino acids for gluconeogenesis after glucagon stimulation on day 1 may account for the neonatal susceptibility to hypoglycemia. Our studies support this concept since there was no increment in glucose on day 1 despite spontaneous increments in plasma glucagon, and in the absence of significant increment in blood insulin, while on day 3 spontaneous increments in glucagon were correlated with a return of glucose to normal concentrations.

In contrast to pancreatic glucagon, the response of GLI after oral feeding is striking; plasma GLI concentrations of 5,000–6,000 pg/ml are achieved within 48 h after birth. The precise physiological role of GLI has not yet been determined, although recent studies indicate that GLI is capable of stimulating the release of immunoreactive insulin as well as of free fatty acid in vitro (34). No consistent rise in plasma insulin was noted in our studies, and the physiologic significance, if any, of the high plasma GLI concentrations after feeding in infants is not clear.

The dramatic rise in immunoreactive glucagon concentration after arginine infusion suggests that the islet alpha cell in the newborn responds more briskly to this amino acid stimulus than to spontaneous hypoglycemia. In the absence of prior feeding and from the manner of infusion, it is more likely that the increase reflects pancreatic glucagon rather than GLI secretion. And the lack of concommitant rise in glucose may be due to depletion of hepatic glycogen stores and/or the "prehepatic" sampling site.

Our findings that an increase in both glucose and glucagon occurs in response to the infusion of 1 mmol/kg alanine into a peripheral vein suggests that lack of substrate, or inability to mobilize substrate, may also play a role in the genesis of neonatal hypoglycemia. Alanine was chosen because of its significance as the principal gluconeogenic precursor in what has been called the glucose-alanine cycle (35), and because it is a known stimulus to glucagon secretion in adults (8). The glucagon response to both arginine and alanine suggests that the pancreatic islet alpha cell, like the beta cell, is more able at birth to respond to an amino acid signal than a glucose signal (36).

The present investigations suggest the possibility that

both relative inadequacy of glucagon secretion and hepatic unresponsiveness to the glucagon signal contribute to the tendency to glucopenia during the first day of life. In part, the initial sluggishness of the glucagon response to a falling blood glucose may be due to neonatal extension of the observed sluggishness of glucagon secretion in utero (28), possibly as a result of maternal glucose constantly transported across the placenta. Significantly lower glucagon concentrations have been reported in infants born to diabetic hyperglycemic mothers (11). The positive correlation between glucose and glucagon concentrations suggests that endogenous glucagon secretion plays a significant role in neonatal glucose homeostasis, particularly on days 2 and 3. However, the negative correlation between glucose and glucagon on day 1, and the glycemic response to alanine imply some hepatic unresponsiveness, relative substrate deficiency, and possibly other factors as being involved in immediate postnatal glucose homeostasis.

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