Fuels, Hormones, and Liver Metabolism at Term and during the Early Postnatal Period in the Rat

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Abstract The metabolic response to the first fast experienced by all mammals has been studied in the newborn rat. Levels of fuels and hormones have been compared in the fetal and maternal circulations at term. Then, after cesarean section just before the normal time of birth, sequential changes in the same parameters were quantified during the first 16 h of the neonatal period. No caloric intake was permitted, and the newborns were maintained at 37°C. Activities of three key hepatic enzymes involved in glucose production were estimated.

Marked differences in maternal and fetal hormones and fuels were observed. Lower levels of glucose, free fatty acids, and glycerol but higher levels of lactate, α-amino nitrogen, alanine, and glutamine were present in the fetus. Pyruvate, glutamate, and ketone bodies were not significantly different. The combination of a strikingly higher fetal immunoreactive insulin and a slightly lower immunoreactive glucagon (pancreatic) resulted in a profound elevation in the insulin-to-glucagon ratio, a finding consistent with an organism in an anabolic state.

The rat at birth presents a body composition with respect to fuels available for mobilization and conversion which is dominated by carbohydrate and protein, since little fat is present. However, at birth a transient period of hypoglycemia occurred, associated with a rapid fall in insulin and rise in glucagon, causing reversal of the insulin-to-glucagon relationship toward ratios such as were observed in the mother. After a lag period, hepatic activities of phosphorylase, glucose-6-phosphatase, and phosphoenolpyruvate carboxykinase increased. Concurrent with these enzyme changes, the blood glucose returned to levels at or above those of the fetus. Interestingly, the fall observed in levels of the gluconeogenic precursors, lactate and amino acids, preceded the rise in enzyme activities and restoration of blood glucose. After 4 h, however, hypoglycemia recurred, during a period of decreasing hepatic glycogen content and blood lactate, pyruvate, and glycerol levels but of stable or increasing amino acid concentrations. Hepatic gluconeogenesis in this phase of depleted glycogen stores was insufficient to maintain euglycemia.

Substrates derived from fat showed early changes of smaller magnitude. The rise in free fatty acids which occurred was less than twofold the value at birth, though this rise persisted up to 6 h. Whereas glycerol rose transiently, acetoacetate did not change and β-hydroxybutyrate concentration fell. Both ketone bodies showed a marked rise at 16 h, at a time of diminished free fatty acid levels. Plasma growth hormone, though higher in the fetal than the maternal circulation, showed no consistent change during the period of observation.

The changes in levels of the endocrine pancreatic hormones at birth were appropriate in time, magnitude, and direction to be implicated as prime regulators of the metabolic response during the neonatal period in the rat.

Received for publication 3 May 1973 and in revised form 20 July 1973.

INTRODUCTION

The interruption of the continuous transplacental fuel supply to the fetus at birth imposes a completely altered metabolic environment upon the newborn. It must rapidly convert from a long-term "fed" to an absolute "fasted" situation, not only to survive the neonatal period, but to establish lifelong mechanisms for the adaptation to the alternating feeding and fasting of extrauterine life. Acutely, however, it must make oxidative fuel available from endogenous reserves until commenc-

ing alimentation in the form of milk from the mother. In the rat, this period of 4–6 h is invariably associated with profound hypoglycemia, spontaneously returning toward normoglycemia by 4 h (1–4). This occurs whether birth is spontaneous at term or by cesarean section near the end of gestation (2, 3). Glucose, the principal intrauterine oxidative fuel, must therefore be produced from liver glycogen stores or via *de novo* synthesis from lactate, pyruvate, glycerol, and amino acids.

It is well-established that in the rat the fetal liver accumulates large glycogen stores, which are rapidly mobilized after birth (1, 3–5). By contrast, the capacity for gluconeogenesis is acquired only postpartum (5, 6). The mechanisms of postnatal activation of metabolic pathways for glycogenolysis and gluconeogenesis have not been fully elucidated.

A role for glucagon in these processes has been proposed (1, 2). Indeed, it has recently been demonstrated that plasma glucagon levels rise during the early postnatal period in the rat (4, 7). However, glucose homeostasis in vivo (8) and hepatic metabolism in vitro (9–11) in mature animals appear to be determined principally by the interaction of insulin and glucagon levels. For example, the disposition of gluconeogenic amino acids into hepatic glucose production versus muscle protein synthesis is probably determined by such an interaction (12, 13). It has been suggested that adipose tissue fuel balance may be determined likewise (14).

In light of such observations, the present study was performed to correlate secretion of insulin and glucagon with the changes observed in metabolites during the early postnatal fasting period. Since growth hormone has effects which would render it an appropriate fasting hormone, its levels have also been determined. Further, the activities of certain key hepatic enzymes have been assayed and correlated with the hormone-substrate alterations. Hence the data presented represent corroboration of the findings of a number of previous investigators, but in a context which allowed for simultaneous correlations among levels of circulating fuels, hormones, and hepatic enzyme activities.

METHODS

Experimental procedures. Albino rats of the Sherman strain, bred in the laboratory, and fed ad lib. on laboratory chow (U.A.R. B.03 carbohydrate 47%, protein 20%, fat 8%) were employed. Since in the females it has been established that ovulation usually occurs around 1 a.m., caging them with a male from 5 p.m. to 9 a.m. on one occasion allowed for estimation of the gestational age to within 8 h. Normal gestation in this strain is 22 days, and parturition lasts up to 2 h. Since precise timing of birth and sampling was desired, delivery was by cesarean section at 21.5 days gestation. The newborns (in all instances 10–12 in number) were immediately transferred to a "humidicrib" (Jouan, S. A., Paris) in which temperature was maintained at 37°C and relative humidity at 70%. They remained unfed for the whole of the present study.

Blood was sampled via an incision across the axillary vessels after careful cleaning of the axillary region. For the data designated as "fetal," the pregnant rat was anesthetized by 30 mg/kg pentobarbital intraperitoneally, and the fetuses were successively exteriorized from the uterus, leaving placenta and umbilical cord *in situ.* Maternal samples were drawn from the bifurcation of the aorta. All samples were obtained between 5 and 15 min after administration of pentobarbital. Since the transplacental transfer of pentobarbital precludes extrauterine survival, those samples designated as "newborn" were drawn from animals delivered in the same fashion, but within 2 min after cervical dislocation of the pregnant rats. Hence, zero time in this portion of the study refers to samples obtained immediately after separation from the uterus. All pregnant rats were allowed free access to food before cesarean section, which was performed at 9 a.m.

Adequate blood collection in fetuses and newborns necessitated exsanguination; hence, each time period is represented by different animals. Individuals from a given litter were employed for different time periods, such that several litters were studied longitudinally, for any given parameter. Though all measurements could not be made on a single animal, at least two determinations of hormones or substrates were made to overlap with other groups, to assure that for a given time point, all individuals represented a homogenous metabolic state.

Blood was collected in heparinized polyethylene microtubes cooled in ice water, and containing the antiprotease Iniprol,[1] in samples intended for hormone assays. This antiprotease is a polypeptide similar to the commonly used Trasylol, and was added to inhibit enzymatic breakdown, particularly of glycagon. An aliquot of whole blood was immediately deproteinized in cold 30% (wt/vol) perchloric acid or in barium hydroxide-zinc sulfate. The remainder was centrifuged at 4°C and aliquots of plasma deproteinized in cold 7.5% (wt/vol) perchloric acid or 5% (wt/vol) trichloroacetic acid (TCA). Plasma and unneutralized filtrates were refrigerated at −20° until analysis, which for glutamine, pyruvate, and acetoacetate was within 3 days. Under the conditions employed, no significant decrease in the concentration of these substances could be demonstrated for this time interval, provided that freezing was immediate and that the sample was thawed only once, just before assay. The perchloric acid filtrates were used for assay of metabolic intermediates except glucose, α-amino nitrogen, and free fatty acids.

Upon completion of blood collection (30 s), the liver was rapidly removed, weighed, and either frozen at −20°C for subsequent glycogen extraction, or homogenized at 0°C in a Potter-Elvehjem tissue homogenizer for assay of liver enzyme activities.

Biochemical methods. Assay of most metabolic intermediates was by enzymic fluorimetric methods modified from published techniques to enable determination on as little as 10 μl of filtrate. The modifications consisted of decreasing the amount of nucleotide cofactors to levels appropriate to the quantity of substrate in the sample, and of the utilization of an Aminco Fluoromicrophotometer (American Instrument Co., Travenol Laboratories, Inc., Silver Spring, Md.) equipped with appropriate filters. Lactate

[1] CHOAY Laboratory, France; 200,000 Peptidase Inhibitor U/ml.

Table I

*Circulating Substrates and Hormones in 21.5-Day Pregnant Rats and Their Fetuses**

Substrate or hormone		Maternal	Fetal	$P<$‡
Blood glucose	mmol/liter	5.0±0.4 (10)	3.7±0.2 (15)	0.01
Blood pyruvate	mmol/liter	0.16±0.02 (10)	0.10±0.01 (15)	0.01
Blood lactate	mmol/liter	2.0±0.1 (10)	7.8±0.3 (15)	0.01
Lactate/pyruvate ratio	—	12.5	78	
Plasma α-amino nitrogen	mmol/liter	3.0±0.2 (10)	9.2±0.6 (10)	0.01
Plasma alanine	μmol/liter	210±16 (10)	560±40 (10)	0.01
Plasma glutamine	μmol/liter	305±25 (10)	610±50 (10)	0.01
Plasma glutamate	μmol/liter	100±20 (10)	120±20 (10)	NS
Plasma FFA	μmol/liter	230±20 (10)	160±20 (10)	0.05
Blood glycerol	μmol/liter	90±10 (10)	38±5 (10)	0.01
Blood β-hydroxybutyrate	μmol/liter	70±8 (10)	81±7 (10)	NS
Blood acetoacetate	μmol/liter	19±2 (10)	18±2 (10)	NS
β-Hydroxybutyrate/acetoacetate ratio	—	3.69	4.50	
Plasma immunoreactive insulin	ng/ml	0.92±0.11 (9)	8.0±0.8 (12)	0.01
Plasma immunoreactive glucagon	pg/ml	560±40 (10)	270±29 (18)	0.01
Plasma growth hormone	ng/ml	103±19 (12)	147±8 (29)	0.05
Insulin/glucagon molar ratio		0.92	16.4	

* Mean ±SEM of the number of observations shown in parentheses.

‡ P value represents the significance of the difference between maternal and fetal values, by the Student unpaired t test.

(15), pyruvate (16), β-hydroxybutyrate (17), acetoacetate (18), and glycerol (19) were determined in blood filtrates; alanine (20), glutamine (21), and glutamate (22) were estimated in plasma filtrates. Blood barium hydroxide-zinc sulfate filtrates were used for glucose determination with a glucose oxidase method (23). This precipitation method removes reduced glutathione, which is present in high concentration in newborn erythrocytes, and has been shown to interfere with color development in the glucose oxidase system. The TCA filtrates were used for colorimetric determination of α-amino nitrogen (24) using serine as standard. Plasma free fatty acids (FFA) were estimated by the radiochemical microtechnique of Ho (25).

Liver glycogen was estimated by the glucose oxidase method, applied to samples after dissolution of liver in hot KOH, subsequent precipitation with cold ethanol, and acid hydrolysis of the purified glycogen.

10% (wt/vol) liver homogenates were employed for assay of enzyme activities. Soluble phosphoenolpyruvate carboxykinase (PEPCK, EC 4.1.1.32) [2] was estimated by the method of Ballard and Hanson (26) in the 100,000 g supernate of liver homogenized in a buffer containing 0.2 M sucrose, 0.02 M triethanolamine, 1 mM reduced glutathione, at pH 7.5. Activity is reported as nanomoles of $NaH^{14}CO_3$ fixed per hour, per milligram protein, at 37°C. Glucose-6-phosphatase (EC 3.1.3.9) activity in crude homogenates (in 0.1 M maleate buffer, pH 6.5) was measured by the method of Swanson (27). The inorganic phosphate released was assayed by the procedure of Fiske and Subbarow (28). Phosphorylase (EC 2.4.1.1) was similarly assayed in crude homogenates with a modification of the procedure of Cake and Oliver (29). The medium used was 0.1 M maleate, 0.05 M NaF, 0.005 M EDTA, 0.04 M mercaptoethanol, at pH 6.5. Protein was determined by the biuret method (30).

[2] *Abbreviations used in this paper:* IRG, immunoreactive glucagon; IRI, immunoreactive insulin; PEPCK, phosphoenolpyruvate carboxykinase.

Statistical analyses were performed using the Student unpaired t test, to establish the significance of changes with time in the parameters studied.

Hormone assays. Plasma insulin concentrations (IRI) were estimated using a radioimmunoassay system separating bound and free hormone with uncoated charcoal (31), an anti-insulin serum reacting poorly with proinsulin, and purified rat insulin as standard (lot R169, 24 U/mg, supplied by Novo Research Institute, Copenhagen). Plasma immunoreactive glucagon (IRG) was determined with a pancreatic-glucagon-specific antibody K47, kindly supplied by L. G. Heding (Novo Research Institute) in an assay system described previously (4). The assay for glucagon is considered to provide values representing immunoreactivity originating primarily on the pancreas. This is based upon data demonstrating the poor cross-reactivity of the antiserum employed, with gut extracts (32). Although the antiserum K47 has been shown to react to some extent with pancreatic glucagon fragments, it gives absolute values for human plasma consistent with those of other pancreatic-glucagon-specific antisera, and these values decline after oral glucose load (32).

Growth hormone levels in plasma were also measured by radioimmunoassay using an uncoated charcoal separation technique (33), with rat growth hormone standard and antiserum provided by the National Institute of Arthritis and Metabolic Disease, Bethesda, Md. (Dr. A. F. Parlow).

RESULTS

At birth, all the animals weighed between 5.0 and 5.7 g. They had no visible white adipose tissue, and the triglyceride reserves of brown adipose tissue (2.5 mg/animal, representing 5% of the tissue wet weight), and

Table II

Circulating Substrates and Hormones in the Newborn Rat

	0	Newborn 0.5	Hours after birth 1	2	4	6	16
Blood glucose, *mM/liter*	3.0±0.1 (45)	2.1±0.2 (28) ‡	1.0±0.2 (39) ‖	1.9±0.3 (36) §	4.0±0.3 (43) ‖	3.5±0.2 (42) NS	1.0±0.1 (31) ‖
Blood lactate, *mM/liter*	8.07±0.47 (12)	—	6.07±0.70 (12) §	3.23±0.20 (12) ‖	3.82±0.25 (12) ‖	3.66±0.18 (12) ‖	1.30±0.20 (12) ‖
Blood pyruvate, *mM/liter*	0.116±0.009 (12)	—	0.160±0.012 (12) ‖	0.135±0.009 (12) §	0.149±0.017 (12) §	0.160±0.013 (12) ‖	0.055±0.010 (14) ‖
Lactate/pyruvate ratio	70	—	38	24	26	23	24
Plasma α-amino nitrogen, *mM/liter*	9.1±0.3 (15)	7.7±0.4 (6) ‡	5.15±0.15 (12) ‖	4.7±0.2 (10) ‖	4.1±0.2 (12) ‖	4.6±0.3 (12) ‖	5.8±0.2 (21) ‖
Plasma alanine, *μM/liter*	425±53 (12)	—	113±22 (12) ‖	104±16 (12) ‖	118±26 (12) ‖	142±26 (12) ‖	407±20 (19) NS
Plasma glutamine, *μM/liter*	514±40 (12)	—	202±40 (12) ‖	200±22 (12) ‖	237±20 (12) ‖	335±28 (12) ‖	367±40 (15) ‡
Plasma glutamate, *μM/liter*	104±19 (12)	—	50±8 (12) ‖	43±4 (12) ‖	58±7 (12) ‖	68±6 (12) ‖	139±7 (15) ‡
Plasma FFA, *μM/liter*	127±10 (11)	—	180±9 (12) §	200±10 (12) ‖	226±16 (12) ‖	201±15 (12) ‖	133±30 (4) NS
Blood glycerol, *μM/liter*	23±5 (12)	—	124±15 (12) ‖	85±5 (12) ‖	36±8 (12) NS	28±7 (12) NS	15±2 (12) NS
Blood β-hydroxybutyrate, *μM/liter*	78±10 (12)	—	33±3 (12) ‖	28±3 (12) ‖	33±7 (12) ‖	14±6 (12) ‖	175±14 (14) ‖
Blood acetoacetate, *μM/liter*	16±9 (12)	—	8±6 (12) NS	6±4 (10) ‡	8±4 (10) ‡	5±3 (12) ‡	90±8 (10) ‖
β-Hydroxybutyrate/acetoacetate ratio	4.87	—	4.14	4.68	4.14	2.80	1.94
Plasma insulin, *ng/ml*	6.2±0.5 (16)	3.1±0.3 (9) §	1.7±0.2 (13) ‖	1.1±0.1 (14) ‖	1.3±0.1 (14) ‖	1.0±0.1 (17) ‖	0.5±0.1 (8) ‖
Plasma glucagon, *pg/ml*	330±30 (15)	1038±110 (16) ‖	950±70 (16) ‖	620±40 (14) §	560±50 (14) §	510±70 (16) §	1050±50 (8) ‖
Insulin/glucagon, molar ratio	10.5	1.68	1.00	1.00	1.29	1.10	0.27
Plasma growth hormone, *ng/ml*	267±27 (15)	329±12 (9) NS	205±18 (13) NS	263±18 (13) NS	247±17 (11) NS	316±30 (13) NS	362±35 (6) NS

* Mean ±SEM of the number of observations shown in parentheses.
‡ $P < 0.05$ when compared with 0 time value, Student unpaired t test.
§ $P < 0.02$.
‖ $P < 0.01$.
NS, The difference observed was not significant.

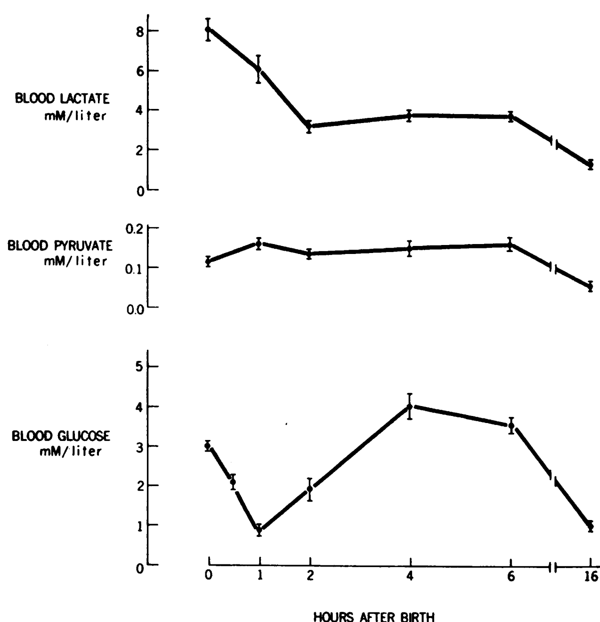

FIGURE 1 Blood glucose, lactate, and pyruvate in newborn rats during a 16 h fast starting at birth by cesarean section at term. Animals were maintained at 37°C and 70% relative humidity throughout. Each time period is represented by different groups of animals, since sampling required exsanguination. Values are mean ±SEM. Absolute values, *n*, and statistical analyses appear in Table II.

liver (0.7 mg/animal, or 2% of liver wet weight) were minimal.[3] By contrast, hepatic glycogen stores were plentiful at birth, 99 mg/g wet weight of liver, or 32 mg/animal. Hence, potential fuel stores were predominantly carbohydrate and protein.

The composition of circulating substrates in the fetus similarly demonstrated a predominance of carbohydrate and amino acids. These are shown in Table I, where maternal arterial levels are shown for comparison. Of note are the markedly higher fetal lactate, α-amino nitrogen, glutamine, alanine, and IRI, and slightly higher growth hormone levels. By contrast, concentrations of glucose, FFA, glycerol, and IRG were much lower in the fetus. The ketone bodies (acetoacetate and β-hydroxybutyrate) and glutamate levels were similar in fetal and maternal circulations. With reference to the metabolic state of the pregnant rats, it is of interest to note that the observed levels of IRG and growth hormone exceeded those of fed virgin rats of equivalent age, though insulin levels were not different. Values for virgin rats with equivalent blood glucose concentrations were: IRI 0.85±0.06 ng/ml ($n = 6$), IRG 200±20 pg/ml ($n = 6$), insulin/glucagon molar ratio 2.4, growth hormone 28±4 ng/ml ($n = 8$).

[3] Ricquier, D., and P. Hémon. Personal communication.

The responses of circulating energy substrates and hormones from delivery to 16 h of extrauterine life are presented in Fig. 1–4 and Table II. Significant differences between fetal (Table I) and 0 time values were present only for blood glucose and growth hormone ($P < 0.01$). After delivery, blood glucose fell precipitously to a nadir at 1 h (Fig. 1). It then rose slowly to a level at 4 h above the value at delivery. Thereafter, it fell progressively to hypoglycemic levels by 16 h (as previously demonstrated by a number of workers [1, 3, 5]). Blood glucose values for 8, 10, and 12 h (not shown in Table II) were 2.2±0.1 ($n = 15$), 1.6 ±0.2 ($n = 8$), and 1.1±0.1 ($n = 6$) mM/liter respectively. Blood lactate was markedly elevated at birth, decreased to a plateau by 2 h, then again between 6 and 16 h. By contrast, blood pyruvate concentration rose slightly over the 1st h, remained unchanged until 6 h, then fell. The net effect on the lactate to pyruvate ratio was that of progressive fall from high initial values over the first 2 h, then of no change thereafter.

Plasma α-amino nitrogen, extremely elevated *in utero* and at birth, also showed a profound fall over 2 h to less than half the initial values, followed by a plateau (Fig. 2), only slightly higher than the maternal concentration, then rose again at 16 h. Individual amino acids measured showed a similar pattern: alanine and glutamine showed an initial acute fall followed by an upward trend in concentration from 4 h onward.

Plasma FFA were low at birth and showed a rise by 1 h, which was maximal at 4 h then declined (Fig. 3). Blood glycerol, similarly low at birth, rose only

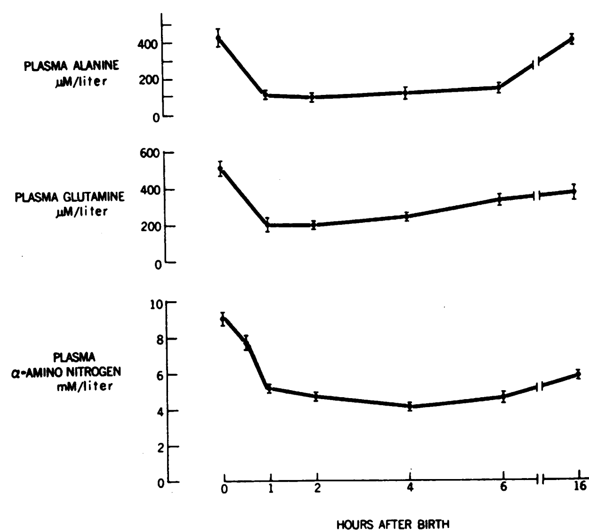

FIGURE 2 Plasma α-amino nitrogen, alanine, and glutamine in newborn rats during a 16 h fast starting at birth. Values are mean ±SEM. Plasma glutamate is shown in Table II.

during the 1st h, but thereafter declined progressively to 0 time levels by 6 h. Concentrations of ketone bodies were exceedingly low at birth and a significant change during the first 6 h was apparent only for β-hydroxybutyrate, which declined progressively. At 16 h, both demonstrated a marked elevation. The ratio of β-hydroxybutyrate to acetoacetate remained constant until 4 h, then decreased.

IRI, elevated *in utero,* fell dramatically with detachment from the uterus, then continued a progressive fall until 2 h, remained constant in the succeeding 4 h interval (at concentrations similar to maternal), then fell again at 16 h. By contrast, IRG values increased threefold to a peak between 30 and 60 min, then decreased after 2 h but persisted at values significantly greater than zero time. A second marked rise was observed at 16 h. The insulin/glucagon molar ratio was high in the fetus, fell precipitously during the 1st h, then remained low (Fig. 4). Growth hormone levels, high in the fetus, rose even higher to 0 time and at 30 min postpartum, then showed a significant ($P < 0.02$) transient decline at 1 h. No clear trend in either direction occurred after 1 h (Table II).

During the period of falling blood glucose, IRI, and gluconeogenic substrate levels, and of the rise in IRG, striking changes in liver glycogen (Table III) and enzyme activities (Table IV) were observed. By the nadir of blood glucose, and 30 min after IRG reached its peak, (i.e., at 1 h) no change in glycogen content had occurred, and only small rises in phosphorylase

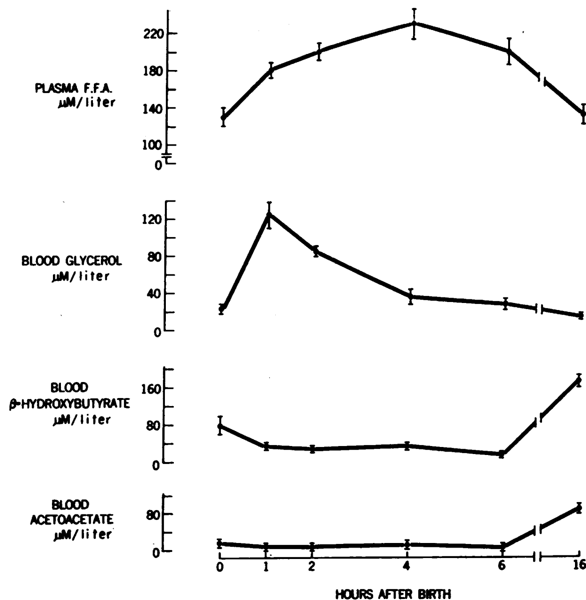

FIGURE 3 Plasma FFA, and blood glycerol, β-hydroxybutyrate, and acetoacetate in newborn rats during a 16 h fast starting at birth. Values are mean ±SEM.

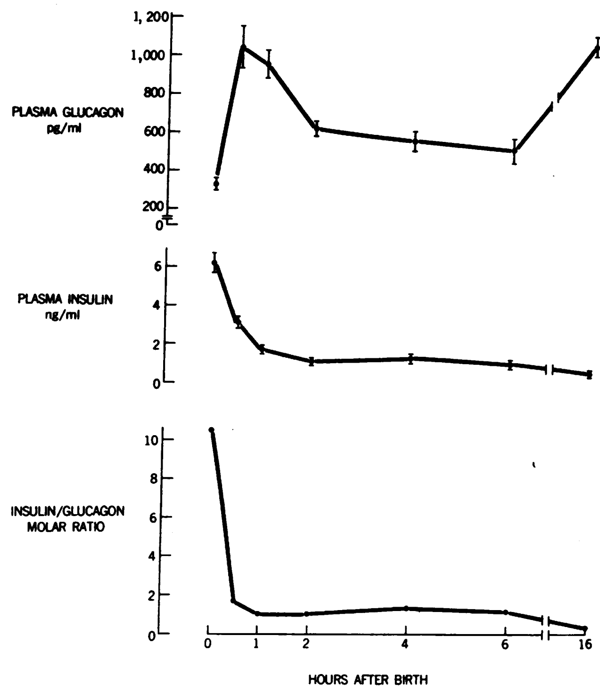

FIGURE 4 Plasma immunoreactive insulin, glucagon, and insulin/glucagon molar ratio in newborn rats during a 16 h fast starting at birth. Values are mean ±SEM.

and glucose-6-phosphatase were detected. PEPCK activity doubled. With the subsequent rise in blood glucose, progressive and marked decrease in liver glycogen, and increase in all three enzyme activities were observed.

DISCUSSION

During gestation, the unremitting maternal supply of substrates and cofactors is directed toward synthetic processes, for which the energy supply is principally from glucose oxidation. Before term, some glucose is diverted to hepatic lipogenesis and part is stored as glycogen in muscle, heart, and liver (34). Though fetal plasma amino acid levels are extremely elevated (they are transported across the placenta against a concentration gradient [35]), the nonruminant mammalian fetus appears unable either to oxidize them or to convert them to glucose (36). In the rat, the absence of white adipose tissue, the low triglyceride content of brown adipose tissue, and the relative impermeability of the placenta to FFA (35) probably account for the low fetal FFA and glycerol levels. Furthermore, the fetal liver has a low capacity for ketogenesis (37–39). Hence, most sites of fatty acid utilization are underdeveloped (39, 40). Unlike the fatty acids, ketone bodies are water soluble and are present in fetal blood

TABLE III
Liver Glycogen in the Newborn Rat

Hours	0	0.5	1	2	4	6	8	10	12	16
mg/g wet wt	93±4	96±7	95±5	80±4	58±3	44±2	18±1	6±1	4±1	3±1
mg/animal	28±2	30±3	29±2	25±3	18±3	13±1	7±1	3.4±0.4	2.6±0.2	2.0±0.1
n	45	14	37	36	48	42	15	8	6	20
P<*	—	NS	NS	0.05	0.01	0.01	0.01	0.01	0.01	0.01
mg glycogen mobilized/h‡	—	—	—	4.0	3.5	2.5	3.0	1.8	0.4	0.15
µmol glucose available from glycogen/h‡	—	—	—	22.3	19.5	13.9	16.7	10.0	2.2	0.8

* *P* value represents the significance of the difference from the 0 time value (Student unpaired *t* test).
‡ Expressed for the time interval preceding the column in which the value appears.

at concentrations approximating maternal blood, suggesting free transplacental transfer (35). Since fetal tissues can derive energy from ketone bodies (41, 42), these are alternate fuels, which may be utilized during times of maternal glucopenia.

Hormone levels in the term fetus would appear appropriate to the maximally anabolic state. The extremely high IRI (43) and low levels of glucagon (7, 44) create an insulin-glucagon relationship which in the adult is considered to favor anabolism (8). (It is of note that the observed maternal insulin/glucagon molar ratio would favor catabolism, which in the gravid organism would be appropriate for provision of substrate to the fetus [45].) The significance of the high fetal growth hormone levels is unclear, since normal growth of the rat fetus occurs in the absence of this hormone (34).

The postnatal activation of hepatic enzymes critical for glucose production has been demonstrated previously (1, 2, 5, 29). Though it was postulated that glucagon (and catecholamine) secretion might be responsible, this was not previously correlated temporally with enzyme changes. Substrate levels and Michaelis constants (*Km*) of the enzymes studied are such that increase in activity as assayed in the previous and present studies would be expected to produce increased glucose output (5). The period between birth and recovery from hypoglycemia corresponds to the time course of the increases in enzyme activity. Such findings in rats have been reported previously for phosphorylase (4, 29), glucose-6-phosphatase (1), and PEPCK (5, 6). Equivalent changes have been shown in other species (42).

Several lines of evidence support a role for glucagon in the induction of the observed changes in hepatic metabolism. First, the rise in endogenous IRG levels preceded the hepatic changes. Second, exogenous glucagon administered to fetal rats *in utero* has been shown to be capable of provoking premature glycogen degradation (46–48), increasing activities of glucose-6-phosphatase (49, 50), PEPCK (2, 51), and phosphorylase (3, 50). The third suggestive line of evidence is the hypoaminoacidemic effect of exogenous glucagon in the fetus[4] as well as the adult (10, 12). Fourth, the glucagon induction of autophagocytosis due to increased lysosomal activity has been shown in the adult rat liver (52), and such changes occur spontaneously after birth (53). Fifth, a marked increase in liver 3',5'-cyclic AMP has been demonstrated after birth, which would be expected with increased endogenous glucagon, particularly if associated with decrease in the insulin/glucagon ratio (53, 54). It is probable that any one or a combination of such glucagon-mediated effects could be inhibited by insulin (10). Thus the concurrent fall in IRI levels and in insulin/glucagon ratio probably contributed significantly to the changes observed.

Increased activity of the autonomic nervous system at birth, acting via epinephrine secretion or local norepinephrine release could equally influence hepatic glucose release, and stimulate lipolysis in adipose tissue. Evidence for its role in mobilization of hepatic glucogen has been presented in the newborn calf (55). The recent demonstration of a considerably more rapid effect of epinephrine than glucagon upon glycogenolysis (56) argues as well for an important role for catecholamines in the neonatal period.

The early phase of hypoglycemia after birth thus results from the inability of the newborn liver to produce adequate glucose in the face of continuing glucose catabolism (57). The restoration of blood glucose levels by 4–6 h corresponds in time with the increment in enzyme activity. Other potential mechanisms include

[4] Girard, J. R., and E. B. Marliss. Unpublished results.

Table IV
Liver Enzyme Activities in the Newborn Rat

Enzyme	Hours after birth					
	0	1	2	4	6	16
Phosphorylase						
μM P_i/h/mg Prot.	5.30±0.19	5.87±0.21	6.87±0.29	7.11±0.34	6.54±0.32	6.44±0.19
n	13	9	13	10	9	13
$P<$*	—	0.05	0.01	0.01	0.02	0.02
Glucose-6-phosphatase						
μM P_i/h/mg Prot.	0.75±0.04	1.00±0.03	1.21±0.06	1.43±0.04	1.61±0.04	2.75±0.24
n	13	11	12	15	12	13
$P<$	—	0.01	0.01	0.01	0.01	0.01
PEPCK						
nM CO_2/h/mg Prot.	24±3	48±4	240±28	1140±90	1440±120	1800±120
n	12	12	15	13	13	12
$P<$		0.02	0.01	0.02	0.01	0.01

* P value represents the significance of the difference from the 0 time value (Student unpaired t test).

a contribution of glucose by the kidney (though renal gluconeogenic capacity is reportedly low at this stage) (58) and a decrease in glucose utilization (57).

The distribution of the increased hepatic glucose output between glycogenolysis and gluconeogenesis has not been quantified. According to Snell and Walker (57) and to Ballard (5) the rate of glucose utilization in such newborn rats is 10–15 μmol/h. At maximal rates, gluconeogenesis (using lactate as precursor) could account for only 15% of the glucose required in the 4 h old newborn rat (5). The sharp decrease in glucogenic precursors observed even before the rise in glycemia would appear to be at variance with such a calculation. For lactate, the initial fall in levels may be attributed to rates of utilization and conversion to glucose in excess of production rates (57). Lactate was the substrate most readily converted to glucose in liver slices from newborn rats (58). The initial hypoaminoacidemia could be due to increase in hepatic uptake, perhaps with continuing peripheral uptake. The fall in alanine, in particular, might be related to hepatic uptake (36, 58, 59) and diminished muscle output owing to decreased glycolysis. In addition, there may be a more rapid hepatic uptake than conversion to glucose, with net intracellular substrate accumulation (60). The suggestion that such accumulation might be related to the activity of transaminases is raised by the data of Reisner et al. (61).

Thus, both recovery from the first, and the development of the second period of hypoglycemia probably relate to the mobilization of liver glycogen. According to the reported rates of glucose utilization, glycogenolysis would be sufficient to maintain euglycemia only up to 10 h. The rise in plasma levels of amino acids at 16 h though hypoglycemia persists, implies that gluconeogenesis is not sufficient at this stage to maintain blood glucose levels, as previously suggested by Ballard (5), despite a further rise in glucagon levels.

It is noteworthy that similar trends in levels of glucose and precursors have been reported in newborn rats, though with some differences in absolute values likely attributable to variations in protocol (7, 36, 57, 62). Such variations include the use of differing strains of rats; altered maternal diet; the use of newborns after spontaneous parturition; study of newborns at temperatures other than 37°C; and use of whole blood rather than plasma for certain of the substrate assays.

The slight rise in FFA and the transient rise in glycerol might be attributable to lipolysis in brown adipose tissue. However, the primary stimulus to lipolysis in this tissue is local norepinephrine release, particularly in response to cold exposure. Hence, lipid mobilization might have been inhibited by maintaining the newborns at thermal neutrality. The decrease observed in blood glycerol starting 2 h after birth might be attributable in part to its conversion to glucose (63). The fall in blood β-hydroxybutyrate with no change in acetoacetate cannot be explained by the data available. The late rise in ketone body levels at a time of falling FFA levels suggest more active conversion of fatty acids to ketones, which might serve as alternate nervous system energy substrates in the presence of hypoglycemia (64).

The control of the insulin and glucagon secretory changes observed has not been defined by the present study. Though the early cataglycemia would be expected to induce the early decrease in IRI and increase in IRG, it is likely that it is not the primary mediator because newborn β- and α-cells are remarkably unresponsive to changes in glycemia (44, 65, 66). If the high amino acid levels are to be implicated in islet cell function, it is difficult to account for the low fetal IRG

with high amino acid levels, and rise in IRG while plasma amino acids fall in concentration. The relation between changes in plasma amino acids and IRI secretion would be more appropriate, since the newborn β-cell is responsive to these substances (67).

A further mechanism to account for changes in levels of both hormones might be the activity of the autonomic nervous system. The stress of removal of the fetus from the intrauterine environment undoubtedly augments sympathetic activity, which has been shown in the adult organism to stimulate IRG (68) and inhibit IRI (69) release. Furthermore, the administration of norepinephrine to the term fetus raises IRG and lowers IRI levels (44).[5] The second rise in IRG at 16 h is also not explained, but in this instance hypoglycemia developing over the preceding 10 h might be responsible. Neural stimulation may be responsible as well for the acute rise in growth hormone levels between fetal and immediate newborn periods, though an effect of the maternal anesthesia used in obtaining fetal samples is not excluded.

Observations upon the metabolic state of the rat at birth may not be directly extrapolated to other mammals, because of a number of peculiar features of this species, including absent white adipose tissue, primary energy store in the form of glycogen, and very high basal metabolic rate. However, both a decrease in glycemia and an increase in IRG have been reported in the human newborn (70, 71). The secretory behavior of the human α-cell at birth may thus be relevant to normal extrauterine adaptation, and altered secretion may contribute to abnormal states of glucoregulation demonstrated in the infants of diabetic mothers. The reported failure of glucagon release (71) might be associated with delay in neonatal maturation of hepatic mechanisms for glucose production. In addition, the "small-for-dates" newborn is characterized by lack of adipose tissue and is subject to hypoglycemia for which the pathophysiology is as yet incompletely defined. The present model could provide potential insights into the mechanism of disturbed fuel homeostasis in this state.

ACKNOWLEDGMENTS

The authors express their gratitude to Misses J. Boillot, A. Delage, M. Deloron, B. Meier, and to Mr. D. Caquet for conscientious technical assistance, to Mrs. S. Dunlop for secretarial assistance, and to Professor A. E. Renold for discussion and review of the manuscript. The guidance and advice of Professor A. Jost is gratefully acknowledged.

These studies have been supported in part by the Fonds National Suisse pour la Recherche Scientifique (Grant 3.384.70 SR) and by the Délégation Générale de la Recherche Scientifique et Technique Française (Contract 71. 7.3250.01).

[5] Girard, J. R., A. Kervran, E. Soufflet, and R. Assan. Factors affecting the secretion of insulin and glucagon by the rat fetus *in utero*. Submitted for publication.

REFERENCES

1. Dawkins, M. J. R. 1963. Glycogen synthesis and breakdown in fetal and newborn rat liver. *Ann. N. Y. Acad. Sci.* **111**: 203.
2. Yeung, D., and I. T. Oliver. 1968. Factors affecting the premature induction of phosphopyruvate carboxylase in neonatal rat liver. *Biochem. J.* **108**: 325.
3. Cake, M. H., D. Yeung, and I. T. Oliver. 1971. The control of postnatal hypoglycemia. *Biol. Neonate.* **18**: 183.
4. Girard, J., D. Bal, and R. Assan. 1972. Glucagon secretion during the early postnatal period in the rat. *Horm. Metab. Res.* **4**: 168.
5. Ballard, F. J. 1971. Gluconeogenesis and the regulation of blood glucose in the neonate. *In* Diabetes. R. R. Rodriguez and J. Vallance-Owen, editors. Excerpta Medica Foundation, Publishers, Amsterdam. 592.
6. Yeung, D., and I. T. Oliver. 1967. Development of gluconeogenesis in neonatal rat liver. Effect of premature delivery. *Biochem. J.* **105**: 1229.
7. Blazquez, E., T. Sugase, M. Blazquez, and P. P. Foa. 1972. The ontogeny of metabolic regulation in the rat, with special reference to the development of insular function. *Acta Diabetol. Lat.* **9** (Suppl. 1): 13.
8. Unger, R. H. 1971. Glucagon and the insulin: glucagon ratio in diabetes and other catabolic illnesses. *Diabetes.* **20**: 834.
9. Mackrell, D. J., and J. E. Sokal. 1969. Antagonism between the effects of insulin and glucagon upon the isolated liver. *Diabetes.* **18**: 724.
10. Exton, J. H., and C. R. Park. 1972. Interaction of insulin and glucagon in the control of liver metabolism. *Handb. Physiol.* **1** (Sect. 7): 437.
11. Parrilla, R., C. J. Toews, and M. N. Goodman. 1972. The influence of the glucagon: insulin ratio on hepatic metabolism. *Diabetes.* **21**: 341.
12. Marliss, E. B., T. T. Aoki, and G. F. Cahill, Jr. 1972. Glucagon and amino acid metabolism. *In* Glucagon. P. J. Lefebvre and R. H. Unger, editors. Pergamon Press Ltd., Oxford. 123.
13. Cahill, G. F., Jr., T. T. Aoki, and E. B. Marliss. 1972. Insulin and muscle protein. *In* Handbook of Physiology: The Endocrine Pancreas. American Physiological Society, Washington. 563.
14. Lefebvre, P., 1972. Glucagon and lipid metabolism. *In* Glucagon. P. J. Lefebvre and R. H. Unger, editors. Pergamon Press Ltd., Oxford. 109.
15. Passonneau, J. V. 1970. Lactate. *In* Methoden der Enzymatischen Analyse. H. U. Bergmeyer, editor. Verlag-Chemie, Weinheim. 1430.
16. Passonneau, J. V., and O. H. Lowry. 1970. Pyruvate. *In* Methoden der Enzymatischen Analyse. H. U. Bergmeyer, editor. Verlag-Chemie, Weinheim. 1412.
17. Mellanby, J., and D. H. Williamson. 1970. β-Hydroxybutyrate. *In* Methoden der Enzymatischen Analyse. H. U. Bergmeyer, editor. Verlag-Chemie, Weinheim. 1772.
18. Mellanby, J., and D. H. Williamson. 1970. Acetoacetate. *In* Methoden der Enzymatischen Analyse. H. U. Bergmeyer, editor. Verlag-Chemie, Weinheim. 1776.
19. Wieland, O. 1970. Glycerol. *In* Methoden der Enzymatischen Analyse. H. U. Bergmeyer, editor. Verlag-Chemie, Weinheim. 1367.

20. Williamson, D. H. 1970. Alanine. *In* Methoden der Enzymatischen Analyse. H. U. Bergmeyer, editor. Verlag-Chemie, Weinheim. 1634.
21. Lund, P. 1970. Glutamine. *In* Methoden der Enzymatischen Analyse. H. U. Bergmeyer, editor. Verlag-Chemie, Weinheim. 1670.
22. Berut, E., and H. U. Bergmeyer. 1970. Glutamate. *In* Methoden der Enzymatischen Analyse. H. U. Bergmeyer, editor. Verlag-Chemie, Weinheim. 1659.
23. Huggett, A. S. G., and D. A. Nixon. 1957. Use of glucose oxidase, peroxidase and O-dianisidine in determination of blood and urinary glucose. *Lancet.* **2**: 368.
24. Malangeau, P., R. Bourdon, A. M. Nicaise, and B. Masson. 1963. Dosage des acides aminés dans les liquides de l'organisme. *Ann. Biol. Clin.* **21**: 3.
25. Ho, R. J. 1970. Radiochemical assay of long-chain fatty acids using ^{63}Ni as tracer. *Anal. Biochem.* **36**: 105.
26. Ballard, F. J., and R. W. Hanson. 1967. Phosphoenolpyruvate carboxykinase and pyruvate carboxylase in developing rat liver. *Biochem. J.* **104**: 866.
27. Swanson, M. 1955. Glucose-6-phosphatase from liver. *Methods Enzymol.* **2**: 541.
28. Fiske, C. H., and Y. Subbarow. 1925. The colorimetric determination of phosphorus. *J. Biol. Chem.* **66**: 375.
29. Cake, M. H., and I. T. Oliver. 1969. The activation of phosphorylase in neonatal rat liver. *Eur. J. Biochem.* **11**: 576.
30. Gornall, A. G., C. J. Bardawill, and M. M. David. 1949. Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.* **177**: 751.
31. Albano, J. M. D., R. P. Ekins, G. Maritz, and R. C. Turner. 1972. A sensitive precise radioimmunoassay of serum insulin relying on charcoal separation of bound and free hormone moieties. *Acta Endocrinol.* **70**: 487.
32. Heding, L. G. 1971. Radioimmunological determination of pancreatic and gut glucagon in plasma. *Diabetologia.* **7**: 10.
33. Rieutort, M. 1972. Dosage radio-immunologique de l'hormone somatotrope de Rat à l'aide d'une nouvelle technique de séparation. *C. R. H. Acad. Sci. Ser. D.* **274**(Pt. 6): 3589.
34. Jost, A., and L. Picon. 1970. Hormonal control of fetal development and metabolism. *Adv. Metab. Disord.* **4**: 123.
35. Szabo, A. J., and R. D. Grimaldi. 1970. The metabolism of the placenta. *Adv. Metab. Disord.* **4**: 185.
36. Yeung, D., and I. T. Oliver. 1967. Gluconeogenesis from amino acids in neonatal rat liver. *Biochem. J.* **103**: 744.
37. Drahota, Z., P. Hahn, A. Kleinzeller, and A. Kostolánsleá. 1964. Acetoacetate formation by liver slices from adult and infant rats. *Biochem. J.* **93**: 61.
38. Lee, L. P. K., and I. B. Fritz. 1971. Hepatic ketogenesis during development. *Can. J. Biochem.* **49**: 599.
39. Lockwood, E. A., and E. Bailey. 1971. The course of ketosis and the activity of key enzymes of ketogenesis and ketone-body utilization during development of the postnatal rat. *Biochem. J.* **124**: 249.
40. Wittels, B., and R. Bressler. 1965. Lipid metabolism in the newborn heart. *J. Clin. Invest.* **44**: 1639.
41. Dierks-Ventling, C., and A. L. Cone. 1971. Acetoacetyl-CoA thiolase in brain, liver and kidney during maturation of the rat. *Science (Wash. D. C.).* **172**: 380.
42. Dierks-Ventling, S. 1971. Prenatal induction of ketone-body enzymes in the rat. *Biol. Neonate.* **19**: 426.
43. Felix, J. M., R. Jacquot, and B. C. J. Sutter. 1969. Insulinémies maternelles et foetales chez le rat. *Horm. Metab. Res.* **1**: 41.
44. Girard, J., R. Assan, and A. Jost. 1973. Glucagon in the Rat Fetus. *In* Foetal and Neonatal Physiology. Proceedings of the Sir. J. Barcroft Centenary Symposium. Cambridge University Press, London. 456.
45. Freinkel, N., B. E. Metzger, M. Nitzan, J. W. Hare, G. E. Shambaugh, III, R. T. Marshall, B. Z. Surmaczynska, and T. C. Nagel. 1972. "Accelerated starvation" and mechanisms for the conservation of maternal nitrogen during pregnancy. *Isr. J. Med. Sci.* **8**: 426.
46. Hunter, D. J. S. 1969. Changes in blood glucose and liver carbohydrate after intrauterine injection of glucagon into foetal rats. *J. Endocrinol.* **45**: 367.
47. Girard, J., and D. Bal. 1970. Effets du glucagon-zinc sur la glycémie et le teneur en glycogène du foie foetal du rat en fin de gestation. *C. R. H. Acad. Ser. D.* **271** (Pt. 1): 777.
48. Greengard, O., and H. K. Dewey. 1970. The premature deposition or lysis of glycogen in livers of fetal rats injected with hydrocortisone or glucagon. *Dev. Biol.* **21**: 452.
49. Greengard, O., and H. K. Dewey. 1967. Initiation by glucagon of the premature development of tyrosine aminotransferase, serine dehydratase and glucose-6-phosphatase in fetal rat liver. *J. Biol. Chem.* **242**: 2986.
50. Girard, J. R., D. Caquet, D. Bal, and I. Guillet. 1973. Control of rat liver phosphorylase, glucose-6-phosphatase and phosphoenolpyruvate carboxykinase by insulin and glucagon during the perinatal period. *Enzyme (Basel).* In press.
51. Phillippidis, H., and F. J. Ballard. 1970. The development of gluconeogenesis in rat liver. Effects of glucagon and ether. *Biochem. J.* **120**: 385.
52. Rosa, F. 1971. Ultrastructural changes produced by glucagon, cyclic 3'5'-AMP and epinephrine on perfused rat livers. *J. Ultrastruct. Res.* **34**: 205.
53. Novák, E., G. I. Drummond, J. Skála, and P. Hahn. 1972. Developmental changes in cyclic AMP, protein kinase, phosphorylase kinase, and phosphorylase in liver, heart and skeletal muscle of the rat. *Arch. Biochem. Biophys.* **150**: 511.
54. Butcher, F. R., and V. R. Potter. 1972. Control of the adenosine 3',5'-monophosphate-adenyl cyclase system in the livers of developing rats. *Cancer Res.* **32**: 2141.
55. Edwards, A. V., and M. Silver. 1970. The glycogenolytic response to stimulation of the splanchnic nerves in adrenalectomized calves. *J. Physiol. (Lond.).* **211**: 109.
56. Clark, C. M., Jr., B. Beatty, and D. O. Allen. 1973. Evidence for delayed development of the glucagon receptor of adenylate cyclase in the fetal and neonatal rat heart. *J. Clin. Invest.* **52**: 1018.
57. Snell, K., and D. G. Walker. 1973. Glucose metabolism in the newborn rat. Temporal studies *in vivo*. *Biochem. J.* **132**: 739.
58. Zorzoli, A., I. J. Turkenkopf, and V. L. Mueller. 1969. Gluconeogenesis in developing rat kidney cortex. *Biochem. J.* **111**: 181.
59. Vernon, R. G., S. W. Eaton, and D. G. Walker. 1968. Carbohydrate formation from various precursors in neonatal rat liver. *Biochem. J.* **110**: 725.

60. Christensen, H. N., and J. B. Clifford. 1963. Early postnatal intensification of hepatic accumulation of amino acids. *J. Biol. Chem.* **238**: 1743.
61. Reisner, S. H., J. V. Aranda, E. Colle, A. Papageorgiou, D. Schiff, C. R. Scriver, and L. Stern. 1973. The effect of i.v. glucagon on plasma amino acids in the newborn. *Pediatr. Res.* **7**: 184.
62. Haymond, M., I. Karl, A. Pagliara, and D. Kipnis. 1972. Glucose homeostasis in the newborn rat: role of gluconeogenic substrates and ketones. *J. Clin. Invest.* **51**: 43*a*. (Abstr.)
63. Bossi, E., and R. E. Greenberg. 1972. Sources of blood glucose in the rat fetus. *Pediatr. Res.* **6**: 765.
64. Krebs, H. A., D. H. Williamson, M. W. Bates, M. A. Page, and R. A. Hawkins. 1971. The role of ketone bodies in caloric homeostasis. *Adv. Enzyme Regul.* **9**: 387.
65. Edwards, J. C., K. Asplund, and G. Lundquist. 1972. Glucagon release from the pancreas of the newborn rat. *J. Endocrinol.* **54**: 493.
66. Lernmark, Å., and B. I. Wenngren. 1972. Insulin and glucagon release from the isolated pancreas of foetal and newborn mice. *J. Embryol. Exp. Morphol.* **28**: 607.
67. Grasso, S., N. Saporito, A. Messina, and G. Reitano. 1968. Serum-insulin response to glucose and aminoacids in the premature infant. *Lancet.* **11**: 755.
68. Marliss, E. B., L. Girardier, J. Seydoux, Y. Kanazawa, L. Orci, A. E. Renold, C. B. Wollheim, and D. Porte, Jr. 1973. Glucagon release induced by pancreatic nerve stimulation in the dog. *J. Clin. Invest.* **52**: 1246.
69. Porte, D., Jr., L. Girardier, J. Seydoux, Y. Kanazawa, and J. Posternak. 1973. Neural regulation of insulin secretion in the dog. *J. Clin. Invest.* **52**: 210.
70. Luyckx, A. S., F. Massi-Benedetti, A. Falorni, and P. J. Lefebvre. 1972. Presence of pancreatic glucagon in the portal plasma of human neonates. Differences in the insulin and glucagon responses to glucose between normal infants and infants from diabetic mothers. *Diabetologia.* **8**: 296.
71. Bloom, S. R., and D. I. Johnston. 1972. Failure of glucagon release in infants of diabetic mothers. *Br. Med. J.* **4**: 453.