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J Clin Invest. 1976;**58**(3):571-578. <https://doi.org/10.1172/JCI108503>.

Research Article

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Development of Glucagon Sensitivity in Neonatal Rat Liver

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ABSTRACT The ontogenesis of the hepatic glucagon-sensitive adenylate cyclase system has been studied in the rat. With a partially purified liver membrane preparation, fetal adenylate cyclase was less responsive to glucagon than the enzyme from neonatal or adult livers. Similar results were obtained in gently prepared liver homogenates, suggesting that destruction of essential components of the fetal liver membrane did not account for the relative unresponsiveness of the adenylate cyclase enzyme to glucagon.

Investigation of other factors that might account for diminished fetal hepatic responsiveness to glucagon indicate (a) minimal glucagon degradation by fetal membranes relative to 8-day or adult tissue; and (b) available adenylate cyclase enzyme, as suggested by a 13-fold increase over basal cyclic AMP formation with NaF in fetal liver membranes. These results indicate that neither enhanced glucagon degradation nor adenylate cyclase enzyme deficiency accounts for the relative insensitivity of the fetal hepatic adenylate cyclase system to glucagon.

In early neonatal life, hepatic adenylate cyclase responsiveness to glucagon rapidly developed and was maximal 6 days after birth. These changes were closely paralleled by a fivefold increase in glucagon binding and the kinetically determined V_{max} for cyclic AMP formation.

These observations suggest that (a) fetal hepatic unresponsiveness to glucagon may be explained by a limited number of glucagon receptor sites; (b) during the neonatal period, the development of glucagon binding is expressed primarily as an increase in adenylate cyclase

V_{max} ; (c) the ontogenesis of hepatic responsiveness to glucagon may be important in the resolution of neonatal hypoglycemia.

INTRODUCTION

In the adult, glucagon plays a central role in the regulation of hepatic glycogenolysis and gluconeogenesis by activating the membrane-bound enzyme, adenylate cyclase, and increasing cyclic AMP concentrations (1, 2). During the perinatal period, however, the exact role of the hepatic adenylate cyclase system in the control of glucose homeostasis has not been clearly defined.

In the last third of gestation, fetal animals store glycogen in a variety of organs (3, 4). At birth in the rat, there is an immediate increase in plasma glucagon and a decrease in plasma insulin concentrations (5, 6), as well as a shift in hepatic carbohydrate metabolism from glycogen synthesis to glycogen breakdown and gluconeogenesis (3, 7).

While alterations in the hormonal environment appear essential in the regulation of carbohydrate metabolism, the capacity of the liver to respond to these hormonal changes may be equally necessary in the maintenance of euglycemia during the neonatal period. Other investigators have demonstrated that before birth, fetal hepatic glycogenolytic and gluconeogenic enzyme systems are relatively insensitive to glucagon, but not to dibutyryl cAMP (8, 9). In contrast, the neonatal hepatic adenylate cyclase system in the rat is very responsive to glucagon (10-12), suggesting that the development of hepatic sensitivity to glucagon may be important in the establishment of neonatal glucose homeostasis.

In the present report, we have investigated the development of hepatic adenylate cyclase sensitivity to glucagon in partially purified membranes from fetal, neonatal, and adult rats.

This study was presented in part at the 35th Annual Meeting of the American Diabetes Association, June 1975.

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Received for publication 16 August 1975 and in revised form 11 May 1976.

TABLE I
Increases in Membrane Marker Enzyme

	5'-Nucleotidase	
	Fetal	Adult
	<i>nmol/μg/20 min</i>	
Homogenate	0.22±0.04	0.60±0.1
Partially purified membrane	2.00±0.23	5.90±0.4
Increase in specific activity	9.1	9.8

Mean±SEM of two fetal or three adult experiments, each with a different tissue preparation.

METHODS

[α - 32 P]ATP (10–16 Ci/mmol) was purchased from New England Nuclear (Boston, Mass.); [3 H]cAMP (15–20 Ci/mmol) was obtained from ICN Pharmaceuticals Inc. (Life Sciences Group, Cleveland, Ohio); glucagon (crystalline) was a gift from Eli Lilly and Company (Indianapolis, Ind.) (Dr. William Bromer); carrier-free [125 I]iodide was obtained from Union Carbide Corp. (New York); all unlabeled chemicals were analytical grade.

Tissue preparation

Whole homogenates. Pregnant rats (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) of known gestational ages were stunned, the fetuses removed and livers rapidly excised, and placed in an ice-cold solution of 0.25 M sucrose, 10 mM Tris-HCl, pH 7.6. Adult females (>60 days of age; 200–250 g) and neonatal livers were obtained similarly. Minced liver tissue was homogenized at 4°C with one plunge of a loose-fitting Teflon-glass homogenizer, filtered through cheesecloth, and centrifuged for 5 min at 600 *g*. Pellets were resuspended in the sucrose-Tris buffer to a protein concentration of 3–5 mg/ml.

Hepatic membranes. Livers from rats of known age were removed and placed in ice-cold 1 mM NaHCO₃. At 4°C, partially purified plasma liver membranes were prepared by the method of Neville et al. (13) as modified by Pohl et al. (14). The preparation sequence was rigidly followed for membranes of all ages, and increases in the specific activity of the membrane marker enzyme, 5'-nucleotidase, were similar (15) (Table I). Membranes were stored at –60°C for up to 3 mo without decrease in adenylate cyclase activity.

Adenylate cyclase assay

Adenylate cyclase activity was measured by a modification of the method of Pohl et al. (14) as previously described (16). Unless specified otherwise, the assay medium (50 μ l final volume) contained the following reactants: 0.4 mM [32 P]ATP (10–20 dpm/mmol); 4.5 mM MgCl₂; 1 mM EDTA; 8 mM theophylline; 20 mM Tris-HCl, pH 7.6; an ATP-regenerating system (20 mM phosphoenolpyruvate and 4 μ U/ml of pyruvate kinase); human serum albumin or glucagon diluted in human serum albumin; and 20–40 μ g of protein (partially purified membrane or homogenate) diluted in 0.25 M sucrose, 10 mM Tris-HCl, pH 7.6. Reactions were initiated by the addition of 10 μ l of membrane and continued for 5 min at 30°C. The adenylate cyclase reaction was terminated by the addition of 50 μ l of recovery mix (40 mM ATP, 12.5 mM [3 H]cAMP [30,000–50,000

cpm] and boiling for 3.5 min. [32 P]cAMP was separated by the method of Salomon et al. (17) and [3 H] and [32 P] were measured by liquid scintillation counting. Final values for cAMP formed were calculated from the efficiency of counting, recovery of [3 H]cAMP, subtraction of "blanks" (cAMP formed in absence of membrane), and specific activity of [32 P]ATP. Protein was determined fluorometrically (18) and adenylate cyclase activity was expressed as picomoles of cAMP formed per milligram protein per 5-min incubation.

Iodination of glucagon

Glucagon was iodinated with [125 I]iodide by the chloramine-T method of Hunter and Greenwood (19). To a 12 $\times$ 75-mm test tube containing 10 μ l (16 μ g) of glucagon in 0.05 N HCl, the following were added in rapid succession: 75 μ l of 0.5 M NaPO₄, pH 7.4, 5 μ l (1–2 mCi) of [125 I]iodide in 0.01 N NaOH, 20 μ l of freshly prepared chloramine-T (16 mg in 4 ml of 0.5 M NaPO₄, pH 7.4) with slow mixing for 30 s, and 100 μ l of sodium metabisulfite (12 mg in 5 ml of 0.5 M NaPO₄, pH 7.4). 1 ml of 1 mM NaPO₄, pH 7.4, was added and separation of 125 I-glucagon was carried out by modification of the method of Rosselin et al. (20). The entire reaction volume was added to 30 mg of microfine silica (QUSO-G-32, Philadelphia Quartz Co., Philadelphia, Pa.), mixed, and centrifuged for 10 min at 1,000 *g*. The supernate was withdrawn and the pellet thoroughly resuspended in 2 ml of 1 mM NaPO₄, pH 7.6, and centrifuged for 10 min at 1,000 *g*, and the supernatant was discarded. The pellet was again resuspended in 1.5 ml of 10 mM NaPO₄, 1% albumin, pH 10, and centrifuged for 10 min at 1,000 *g*. The supernate, which contained 125 I-glucagon, was saved. The iodinated hormone (sp act 100–200 μ Ci/ μ g) was chemically characterized by trichloroacetic acid precipitability (>90% precipitation) and paper counterelectrophoresis (>95% remained at the origin). Molar concentrations of 125 I-glucagon were determined by activation of adenylate cyclase in adult, partially purified liver membranes, with unlabeled glucagon as standards. Yields of labeled glucagon were 30–50%. Aliquots of the iodinated hormone were frozen and used within 2 wk.

Glucagon binding assay

Binding of 125 I-glucagon to liver membranes was assayed by a modification of the method of Rodbell et al. (21). Liver membranes were incubated with 125 I-glucagon under conditions identical to the adenylate cyclase assay (see Methods). At the appropriate time, 50- μ l aliquots were removed and layered on top of 300 μ l ice-cold 2.5% albumin in 20 mM Tris-HCl, pH 7.6, in plastic microcentrifuge tubes. These tubes were centrifuged for 1 min at 10,000 *g* in a Beckman microcentrifuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.), the supernate was aspirated, the pellet was carefully washed with 300 μ l of ice-cold 10% sucrose, and the supernatant fluid was again aspirated. The tips of the centrifuge tubes were carefully cut off just above the pellet and the 125 I-radioactivity remaining in the tips was determined. With each experiment, two controls were run: (a) incubation as above with 125 I-glucagon in the absence of liver membrane; the amount of radioactivity in the tip was always <1% of 125 I counts added; (b) incubation as above, except that in addition to 125 I-glucagon, a 1,000-fold excess of cold glucagon was added; the difference between 125 I counts bound without and with excess cold glucagon represents nonspecific binding and was usually

TABLE II
Adenylate Cyclase Activity in Liver Homogenates

	18-day fetal	1-day neonatal	6-day neonatal	Adult
	<i>pmol cAMP formed/mg protein/5 min</i>			
Basal	24.9±2.8*	43.4±0.5	62.2±2.6	43.1±3.1
Glucagon (0.4 μM)	58.9±3.6†§	146.9±4.5	233.9±7.2	173.6±2.5
Percent activity over basal	240	340	375	400

* Mean±SEM of two separate experiments, performed in triplicate, each with different membrane preparations for each age.

† Compared to basal, $P < 0.01$ by Student *t* test.

§ Compared to 1- and 6-day neonatal and adult, $P < 0.001$ by Student *t* test.

|| Compared to basal, $P < 0.001$ by Student *t* test.

< 5% of the ^{125}I counts bound. Nonspecific binding was subtracted from total binding in calculating picomoles of glucagon bound.

Since the biological activity of chloramine-T-prepared monoiodinated glucagon is similar to the unlabeled hormone (22, 23), binding and adenylate cyclase activity were determined in the same test tube. As an example, under adenylate cyclase assay conditions as previously described, ^{125}I -glucagon was incubated with liver membranes in 200 μl final volume. At the appropriate time, two 50-μl aliquots were rapidly removed for determination of ^{125}I -glucagon binding and the remaining reaction mix was immediately assayed for adenylate cyclase activity. This procedure allowed examination of the relationship between glucagon binding and activation of adenylate cyclase under identical conditions.

Glucagon degradation

Reaction A: degradation of ^{125}I -glucagon by liver membranes. ^{125}I -Glucagon was incubated with 150 μg of liver membrane under adenylate cyclase assay conditions (see Methods) (24). At 5 min, the entire reaction volume (300 μl) was rapidly transferred to a microfuge tube and centrifuged for 1 min at 10,000 *g*. 20-μl aliquots of the supernate (supernate A) were counted for ^{125}I and identical aliquots were assayed for adenylate cyclase activation in reaction B.

Reaction B: adenylate cyclase activation by [^{125}I]-glucagon. In fresh adult liver membranes, activation of adenylate cyclase by varying concentrations of ^{125}I -glucagon was determined and the results plotted as counts of ^{125}I versus picomoles of cAMP formed. 20-μl aliquots of supernate A were also assayed for adenylate cyclase activity in the same experiment. If the 20-μl aliquots produced less adenylate cyclase activation than an identical number of counts of ^{125}I -glucagon, it was assumed that glucagon had been degraded in reaction A. The percent of glucagon degraded was calculated by dividing the number of ^{125}I counts no longer associated with biologically active ^{125}I -glucagon by the total number of ^{125}I counts added to reaction A.

Adenylate cyclase kinetics

In partially purified liver membranes from animals of different ages, cyclic AMP formation was determined at varying concentrations of ATP in basal and glucagon-stimulated conditions. To insure linear cAMP formation at ATP concentrations less than 0.4 mM (data not shown), the reaction was allowed to proceed for only 4 min. K_m and V_{max} were determined by the Wilkinson formula (25), and

results were confirmed by least-squares analysis (26) and double-reciprocal plots (data not shown).

RESULTS

Effects of glucagon concentration on adenylate cyclase activity. In experiments summarized in Fig. 1, adenylate cyclase activity in partially purified hepatic membranes from 20-day fetal, 8-day neonatal, and adult animals was determined in the presence of varying glucagon concentrations. Whereas the glucagon concentration giving half-maximal stimulation of adenylate cy-

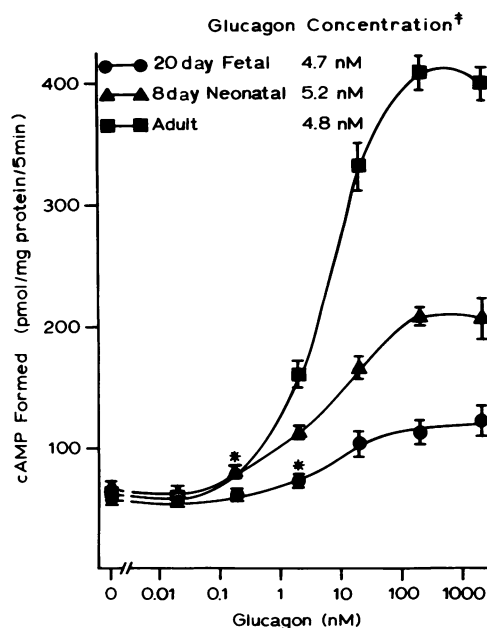


FIGURE 1 Effects of glucagon concentration on adenylate cyclase activity. Partially purified liver membranes (20–40 μg protein/tube) were incubated for 5 min at 30°C for determination of cAMP formation. Points are means±SEM of triplicate determinations in three experiments on three different membranes for each age. * Concentration of glucagon giving initial significant stimulation of hepatic adenylate cyclase over basal activity. ‡ Concentration of glucagon giving half-maximal stimulation of hepatic adenylate cyclase.

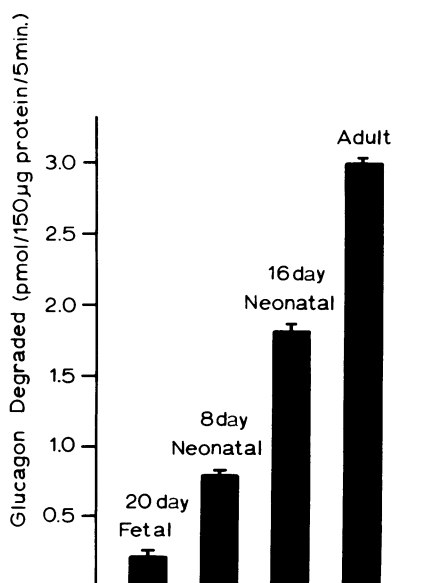


FIGURE 2 Effects of age on degradation of [125 I]glucagon. In the adenylate cyclase assay, partially purified hepatic membranes (150 μ g of protein) were added to incubation mix containing [125 I]glucagon (30 nM). After 5 min at 30°C, the amount of glucagon degraded was determined (see Methods). Bars represent means $\pm$ SEM of two experiments performed in triplicate with different membrane preparations for each age.

class is remarkably similar for the three ages, around 5 nM, the fetal membrane was less responsive than the 8-day neonatal or adult hepatic membrane at any glu-

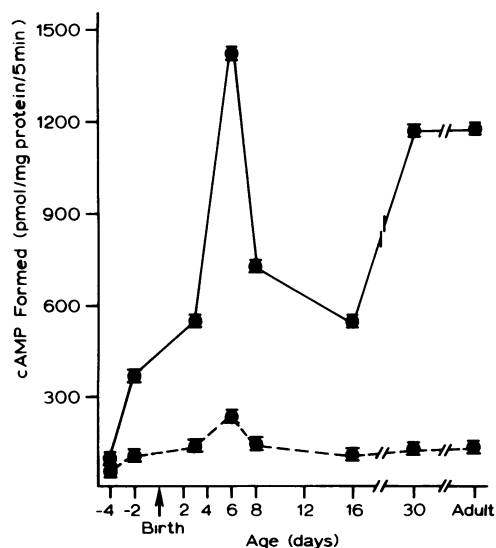


FIGURE 3 Effects of age on adenylate cyclase activity. Partially purified hepatic membranes (20–40 μ g) were incubated for 5 min at 30°C in the absence (---) or presence (—) of [125 I]glucagon (77 nM) and cAMP formation was determined. Points are means $\pm$ SEM of three experiments, each performed in triplicate with three different membrane preparations for each age.

TABLE III
Effects of Glucagon and NaF on Adenylate Cyclase Activity

	20-day fetal	8-day neonatal	Adult
<i>pmol cAMP formed/mg protein/5 min</i>			
Basal (HSA)	53 $\pm$ 4*	66 $\pm$ 11	64 $\pm$ 7
Glucagon (2 μ M)	147 $\pm$ 15‡	201 $\pm$ 12	478 $\pm$ 17
Fluoride (15 mM)	699 $\pm$ 21§	478 $\pm$ 22	493 $\pm$ 29
Glucagon (2 μ M) +fluoride (15 mM)	690 $\pm$ 13	490 $\pm$ 28	529 $\pm$ 41

* Mean $\pm$ SEM of two experiments, each performed in quadruplicate with different membrane preparations at each age.

‡ Compared to neonatal and adult, $P < 0.001$ by Student's t test.

§ Compared to neonatal and adult, $P < 0.01$ by Student's t test.

cagon concentration greater than 10 pM. This difference was greater at the higher glucagon concentrations. Furthermore, while fetal hepatic adenylate cyclase activity was significantly activated at a glucagon concentration of 1 nM, activation of adenylate cyclase in the 8-day

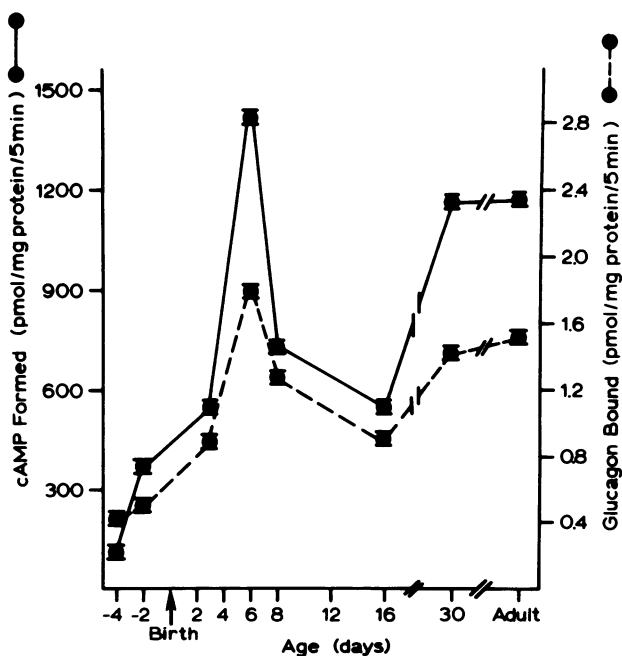


FIGURE 4 Effects of age on adenylate cyclase activity and glucagon binding. Under adenylate cyclase assay conditions, partially purified hepatic membranes (20–40 μ g) were incubated for 5 min at 30°C in the presence of [125 I]glucagon (77 nM), and cAMP formation (—) and glucagon binding (---) were simultaneously determined (see Methods). Points are means $\pm$ SEM of three experiments, each performed in triplicate with three different membrane preparations for each age.

and adult hepatic membrane occurred at 0.1 nM glucagon, close to the physiologic concentration of this hormone (1).

Effects of tissue preparation on adenylate cyclase activity. To investigate the possibility that some essential component of the fetal adenylate cyclase system was destroyed in the preparation of partially purified hepatic membranes, adenylate cyclase activity was determined in gently prepared liver homogenates from animals of varying age (Table II). While glucagon significantly increased cAMP formation over basal at all ages studied, the 18-day fetal tissue was significantly less responsive than at other ages.

Effects of age on degradation of ^{125}I -glucagon. To examine the possibility that enhanced glucagon degradation by the fetal hepatic membrane accounts for the apparent insensitivity of this tissue to this hormone, glucagon inactivation was studied (Fig. 2). There appeared to be an age-dependent increase in the capacity of the liver membrane to inactivate glucagon: in contrast to the fetal tissue, which inactivated only 0.22 ± 0.01 pmol/150 μg protein per 5 min, or about 2% of glucagon added, the 8-day, 16-day, and adult hepatic membrane degraded 0.78 ± 0.02 (6.5%), 1.8 ± 0.05 (15.1%), and 2.99 ± 0.05 (25%) pmol of glucagon/150 μg protein per 5 min under these conditions.

Effects of glucagon and NaF on adenylate cyclase activity. To investigate whether an insufficient quantity of the adenylate cyclase enzyme itself may account for the relative insensitivity of the fetal hepatic membrane to glucagon, adenylate cyclase activity was determined in

response to maximal stimulating concentrations of glucagon, NaF, or both (Table III). While the 20-day fetal membrane responded minimally to glucagon compared to the 8-day neonatal and adult tissue, the presence of 15 mM NaF increased cAMP formation 13 times basal in fetal tissue, greater than in either the 8-day (7.5-fold) or the adult (7.7-fold) animals. Previous studies by Birnbaumer et al. indicate that in the adult liver, glucagon and fluoride act on the same adenylate cyclase enzyme (27). Similar results were obtained in these studies, in which maximal concentrations of fluoride and glucagon failed to stimulate this enzyme more than fluoride alone.

Effects of age on adenylate cyclase activity and ^{125}I -glucagon binding. In Fig. 3, studies of hepatic adenylate cyclase activity in the presence and absence of glucagon are summarized. Whereas glucagon increased adenylate cyclase activity 2.5-fold over basal in the 18-day fetal membrane, the youngest tissue examined (11 ± 0.5 to 29 ± 2 pmol/mg protein per 5 min, $P < 0.01$), by the 6th day of life, glucagon stimulated cAMP formation 6 times basal to $1,472 \pm 37$ pmol/mg protein per 5 min. For unexplained reasons, hepatic responsiveness to glucagon then decreased in later neonatal life to 730 ± 6 (8 days) and 544 ± 3 (16 days) pmol/mg protein per 5 min.

Experiments in which specific ^{125}I -glucagon binding was simultaneously compared to glucagon-stimulated adenylate cyclase activity are summarized in Fig. 4. A close age-dependent relationship existed between these two parameters: as hepatic adenylate cyclase

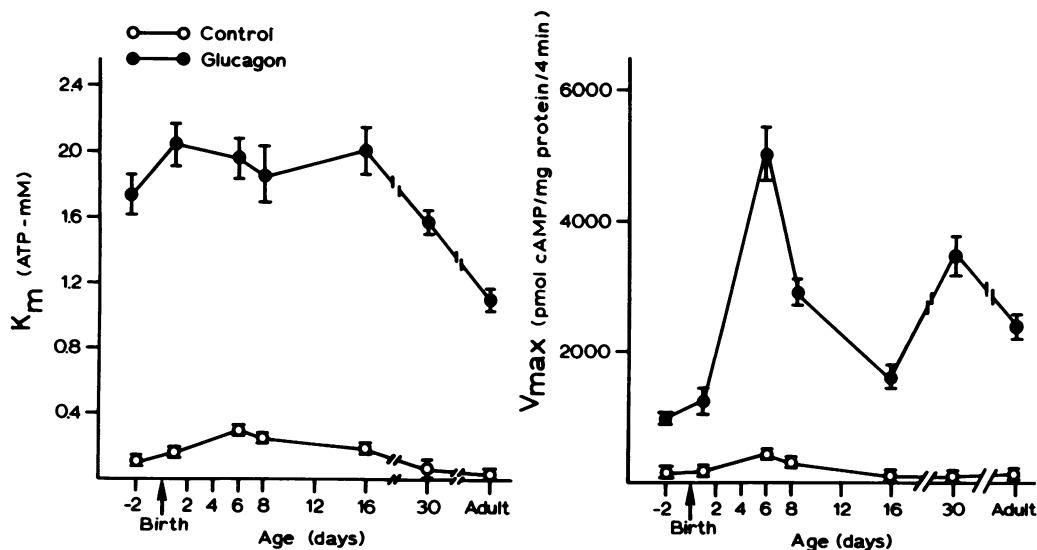


FIGURE 5 Adenylate cyclase kinetics. cAMP formation was determined at 30°C for 4 min in the absence (○—○) and presence (●—●) of glucagon (0.18 μM) and varying concentrations of ATP. K_m and V_{max} were determined by the formula of Wilkinson (25). Points are means $\pm$ SEM of two separate experiments performed in triplicate.

responsiveness to glucagon developed in early neonatal life, there was a 4.5-fold increase in glucagon binding from 0.43 ± 0.06 (18-day fetal) to 1.82 ± 0.08 (6-day neonatal) pmol/mg protein per 5 min. Similarly, as cAMP formation decreased in later neonatal life at 8 and 16 days, glucagon binding decreased to 1.28 ± 0.06 and 0.92 ± 0.11 pmol/mg protein per 5 min. In adult hepatic membranes, ^{125}I -glucagon binding again increased to 1.5 ± 0.05 pmol/mg protein per 5 min.

Adenylate cyclase kinetics. Under our assay conditions, there were no statistical differences among the K_m 's for ATP in fetal and neonatal partially purified membranes (Fig. 5). In contrast, development of glucagon binding was expressed primarily as an increase in maximum velocity of cAMP formation: 986 ± 85 (20-day fetal) vs. $5,028 \pm 403$ (6-day neonatal) pmol cAMP formed/mg protein per 4 min.

DISCUSSION

Before birth, fetal gluconeogenic and glycogenolytic capacities are severely limited (9, 28); fetal plasma glucose concentrations are maintained by transfer of glucose from mother to fetus (3). To maintain euglycemia at birth, the animal must (a) store a potential glucose source, e.g. glycogen, before birth for use during the initial neonatal period; (b) alter the hormonal environment so that endogenous glucose formation is maximal; and (c) develop the hepatic capacity for glycogenolysis and gluconeogenesis in response to hormonal stimulation. Previous investigations have documented prenatal glycogen storage (3, 4) and a postnatal catabolic hormonal milieu (5, 6, 29). The development of the capacity of the neonatal liver to respond to one of these catabolic hormones, glucagon, is the subject of the present report.

The results of our studies indicate that whereas the 18-day fetal hepatic adenylate cyclase system can respond to glucagon at a concentration greater than 1 nM, this response is quite limited when compared to that of neonatal and adult animals, both in quantity of cyclic AMP formed and sensitivity to glucagon. Relative fetal hepatic adenylate cyclase insensitivity to glucagon has been observed by others in liver homogenates (11, 30), particles (10), and slices (10), and in vivo (10, 12). This lack of response to glucagon, together with a high insulin-glucagon ratio (31), would insure glycogen storage during the last third of gestation.

Several reasons could explain the relative unresponsiveness of the fetal hepatic adenylate cyclase to glucagon. Friedman et al. have indicated that purification of fetal lamb myocardial tissues damages the glucagon-responsive adenylate cyclase system (32). Destruction of a component of the fetal adenylate cyclase system during preparation of partially purified membranes seems an unlikely explanation from our results, however, be-

cause similar observations of fetal hepatic unresponsiveness to glucagon have been made in gently prepared liver homogenates (Table II) (11), in intact livers (10), and in liver slices (10), conditions in which tissue damage should be negligible.

Differences in the cellular composition of the fetal liver may explain the relative unresponsiveness of this tissue to glucagon compared to neonatal liver. Very few hematopoietic and reticulo-endothelial cellular elements are present in the liver in the late gestational and early newborn period. However, and at most, there is a two- to fourfold increase in hepatocytes after birth (33, 34). In contrast, changes in adenylate cyclase responsiveness to glucagon in the perinatal period are 50-fold (Fig. 3), suggesting real changes in the capacity of the liver membrane to respond to the hormone.

Pohl et al. have documented the existence of a potent hepatic glucagon-degradation system (24). Furthermore, evidence exists that the hepatic adenylate cyclase and glucagon degradation systems are independent processes (35, 36). Studies of glucagon degradation (Fig. 2), however, indicate that enhanced hormone degradation by the fetal liver does not occur and thus cannot explain the relative unresponsiveness of this membrane to glucagon.

An insufficient quantity of adenylate cyclase enzyme itself might account for the insensitivity of the fetal hepatic membrane to glucagon. As noted by others (10, 11), however, the fetal adenylate cyclase system is very responsive to NaF and, in our system, more sensitive than in neonatal or adult tissue. Since fluoride and glucagon appear to act on the same adenylate cyclase enzyme in the liver (27, Table III), we conclude that in the fetal liver, this enzyme system is potentially very active and the relative inability of glucagon to stimulate this enzyme must reflect delayed development of a component other than the enzyme itself.

When comparisons were made between glucagon-stimulated adenylate cyclase activity and glucagon binding, there was a close age-dependent relationship between these two properties. Furthermore, alterations in hormone binding appeared to account in large part for the ontogenesis of hepatic adenylate cyclase responsiveness to glucagon in the early neonatal period. The 18-day fetal membrane bound very little glucagon and the adenylate cyclase system was relatively insensitive to the hormone. As noted by others in different tissue preparations (10-12), hepatic adenylate cyclase stimulation by glucagon then peaked during the 1st wk of neonatal life and in our experiments was closely paralleled by an increase in specific glucagon binding to neonatal hepatic membranes (Fig. 4). Furthermore, these changes in glucagon-binding were accompanied primarily by increases in the maximum velocity of cAMP formation

(Fig. 5), perhaps due to activation of more adenylate cyclase enzyme molecules.

Recent investigations that indicate (a) a dissociation between rates of glucagon-stimulated hepatic adenylate cyclase activation and ^{125}I -glucagon binding (37); and (b) binding of hormones to nonreceptor materials (38) suggest that one must be exceedingly careful in the interpretation of hormone binding studies. Similarly, the application of our results to other species or disease states must be done with caution. Nevertheless, possible consequences of delayed hepatic responsiveness to glucagon deserve comment. Offspring of diabetic mothers are prone to develop severe neonatal hypoglycemia (39), presumably due to hyperinsulinemia (40), and an inadequate glucagon response to this hypoglycemia (41). Experimental evidence suggests that hormone induction of gluconeogenic and glycogenolytic enzymes can be blocked by hyperglycemia (9, 42). If glucagon's effect on these enzymes is mediated via cAMP, hyperglycemia may delay the normal development of hepatic adenylate cyclase responsiveness to glucagon and thus contribute to the profound hypoglycemia observed in offspring of diabetic mothers.

"Small-for-gestation" infants are also at risk for the development of hypoglycemia (43, 44). This "hypoglycemic potential" appears not to be due to defects in hormone secretion, including glucagon (45, 46) or lack of potential glucose substrates (45). Rather, hepatic enzyme induction by glucagon may be limited in these small-for-gestation infants (45). In normal-weight newborns, gluconeogenic (47), hyperglycemic, and urinary cAMP responses (48) to pharmacological doses of glucagon do not reach maturity until 3–4 days of life. This same immaturity of hepatic responsiveness may be exaggerated in small-for-gestational infants and reflect delayed development of glucagon-stimulated hepatic adenylate cyclase system. Clearly, further work is necessary to validate this hypothesis.

Our findings suggest that the development of hepatic adenylate cyclase responsiveness to glucagon may be important in the normal developmental pattern of carbohydrate homeostasis: in the fetal period, a relative lack of adenylate cyclase responsiveness to glucagon would favor glycogen storage; in the neonatal period, development of glucagon binding is correlated with heightened sensitivity of adenylate cyclase to glucagon, a situation optimal for endogenous glucose formation via glycogenolysis and gluconeogenesis.

The cellular factors involved in the ontogenesis of hepatic responsiveness to glucagon, the signal for the development of functional hepatic glucagon receptors, the influence of environmental factors (e.g. hyperglycemia) on neonatal adenylate cyclase sensitivity to glu-

cagon, and the relationship of these events to human physiology and disease will require further investigation.

ACKNOWLEDGMENTS

We thank Mrs. Roberta Bergman for secretarial assistance and Drs. Norman Bell, Sherry Queener, and T. K. Li for helpful criticism of this paper.

This work was supported in part by grants from the American Heart Association and from the American Heart Association, Indiana Affiliate.

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