# Advances in the Biosciences 13

# Hormones and Embryonic Development

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# Introduction

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Although it is impossible to cover all aspects of the topic – hormones and embryonic development – during one symposium, it would be desirable to find the answers to a number of open questions, including:

a) Which maternal hormones are essential for normal mammalian embryonic development?
b) What is the time course of the occurrence of endocrine systems during mammalian fetal development?
c) What indication is there that maternal or fetal hormones act directly on embryonic or fetal cells and that they play a key role in the induction and differentiation processes during embryonic or fetal development?
d) Which adverse effects on embryonic or fetal development could be visualized to occur after an administration of hormones or hormonelike substances during pregnancy?

It is hoped that the papers selected will help to answer some of these questions or at least give some indication of what the present status of our knowledge is.

A symposium such as this one should provide a survey for scientists who are not actively engaged in this field and, furthermore, it may be expected to stimulate discussion among researchers. It is also hoped that some investigators may be inspired to do research in this field.

Hormone actions in general may be classified into:

a) "physiological" effects of hormones – these are effects occurring with doses close to physiological concentrations,

and

b) "pharmacological" effects – these are effects seen with doses of the hormone far exceeding "physiological" concentrations or some effects produced by artificial compounds with hormonelike actions.

The papers presented during this symposium will give examples of both types of effects.

Experimental studies during embryonic development are complicated by a number of factors. On one hand, the amounts of tissue available are small when compared with many organs of an adult organism. Furthermore, one is faced (Fig. 1) with a two-compartment system – mother/embryo – and the fact that embryonic development can certainly be impaired by indirect means affecting processes such as implantation, etc., and affecting tissues such as decidua or placenta.

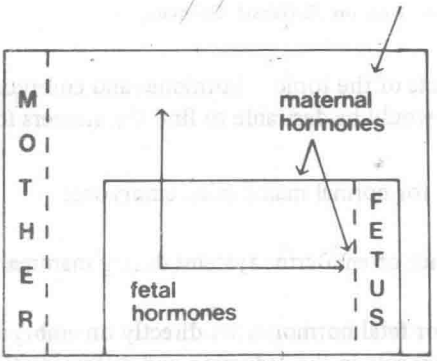


Fig. 1

For many hormones, very little information is available as to what concentrations they attain in the embryo and whether the embryo possesses any target cells for such hormones at all. Such information may be of great value for the determination of factors which govern the regulation of normal and abnormal embryonic development.

In recent years, information from various sources has accumulated that may justify a symposium as this one on a field where our knowledge on basic factors certainly is still quite limited. In this introduction, only two examples are given which represent two extreme situations in this field and which show that information is needed at all levels:

The first example deals with basic research in developmental biology. Outstanding work in this field has certainly been performed by Dr. *William Rutter* and his group, first in Seattle and later in San Francisco. The rates at which different proteins from the exocrinic and the endocrinic pancreas develop in vivo and in organ culture have been measured, and these studies were combined with electron microscopic investigations [3]. This work has very much increased our knowledge on the differentiating processes occurring during organogenesis of an endocrinic tissue. The

rates of synthesis of the two hormones glucagon and insulin by the fetal pancreas show a typical pattern (cf. Fig. 2) during differentiation as measured by sensitive biochemical methods.

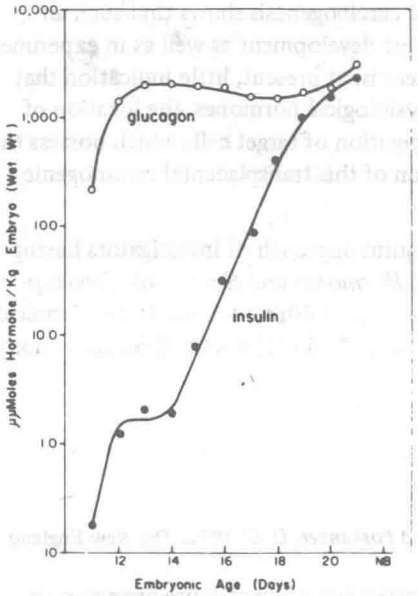


**Fig. 2**
*Concentration of glucagon and insulin during embryonic development of the rat* [3]

It is interesting that during the stage of organogenesis there is obviously little need for several hormones known to be important for cell metabolism in an adult organism. Studies in our laboratory [2] showed that, for example, insulin has no effect on glucose metabolism of 12-day-old rat embryos (Fig. 3). In these rodent embryos in the late stage of organogenesis, the glucose uptake already proceeds in the absence of insulin at a high rate which is reached in many tissues of an adult organism, such as muscle, only in the presence of insulin.

| Additions to medium | n | pmoles X μg DNA$^{-1}$ X min$^{-1}$ Glucose utilization | Lactate formation | Lactate from glucose |
|---|---|---|---|---|
| no | 10 | 39.8 ± 3.8 | 65.6 ± 7.5 | 82 % |
| 10 mU/ml insulin | 5 | 41.7 ± 2.4 | 63.2 ± 13.2 | 76 % |

**Fig. 3.** *Effect of insulin on glucose utilization and lactate formation of 12-day-old rat embryos in vitro (1.25 mM glucose, 37 °C, $O_2$)*

The second example to be mentioned here concerns a clinical observation. It is the alarming finding made in the USA that daughters of mothers treated during pregnancy with the hormonelike substance stilbestrol developed a high incidence of vaginal carcinomas at the age of about 17 years. The original observation by *Herbst* et al. [1] was subsequently confirmed by several other investigators. This tragic and clear-cut example of a drug-induced prenatal carcinogenesis shows that such an event can be induced during human embryonic development as well as in experimental studies on animals (cf. [4]). Although there is, at present, little indication that such a toxic situation can be induced by physiological hormones, the location of the carcinomas certainly points to the participation of target cells which possess the hormone receptors. The exact mode of action of this transplacental carcinogenic event is still rather obscure.

Today in many fields of medical research, a joint approach of investigators having a different scientific background is required. *Hormones and Embryonic Development* presents such a "puzzle" providing a variety of different aspects: biochemical, endocrinological, embryological, toxicological, and clinical ones and possibly more.

## References

[1] *Herbst, A. L.; Kurman, R. J.; Scully, R. E.;* and *Poskanzer, D. C.* 1972. The New England J. of Med. **287**, 25: pp. 1259–64.

[2] *Neubert, D.* 1970. *In* Metabolic pathways in mammalian embryos during organogenesis and its modification by drugs, eds. Bass et al., pp. 225–48. Berlin: Free University Press.

[3] *Pictet, R.,* and *Rutter, W. J.* 1972. *In* Handbook of physiology, Endocrinology I, chapter 2, pp. 25–66.

[4] *Tomatis, L.,* and *Mohr, V.,* eds. 1973. Transplacental Carcinogenesis, IARC Scientific Publ. No. 4, WHO, Lyon.

# Glucagon and Perinatal Metabolism in the Rat

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**Abstract:** In the rat, plasma glucagon levels rise and plasma insulin falls immediately after birth. The changes in the levels of pancreatic hormones precede the development of liver glycogenolysis and gluconeogenesis. Several lines of evidence support the role of glucagon in these hepatic adaptations: (a) The rise of glucagon precedes the hepatic changes concomitant with an increase in liver 3′5′ cyclic AMP. (b) Exogenous glucagon injected into a fetal rat is capable of provoking premature liver glycogenolysis and gluconeogenesis in vivo and in vitro. (c) The intracellular mediator of glucagon in the liver, 3′5′ cyclic AMP, can reproduce the metabolic effects of the hormone in fetal rat. (d) A dramatic fall of plasma gluconeogenic substrates (lactate, amino acids) occurs at birth, and exogenous glucagon increases lactate and amino acid utilization in perinatal rats. Evidence is also presented that glucagon increases hepatic amino acid uptake and liver transaminase activities. All these metabolic effects of glucagon can be antagonized by insulin, and it is proposed that the fall of insulin at birth could also contribute to the metabolic adaptation of the newborn rat. The physiological role of glucagon during fetal and neonatal life is discussed.

## Problem

During gestation, the rat fetus receives a continuous, unremitting, maternal fuel supply across the placenta, and this may be considered to be the only continuously fed state known in a physiologic context. Oxidative fuels are supplied by maternal circulation in the form of glucose and ketone bodies, and amino acids are used for protein synthesis since the rat fetus appears to be unable to oxidize or to convert them to glucose. In the rat, the absence of white adipose tissue, the low triglyceride content of brown adipose tissue, the relative impermeability of the placenta to free

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fatty acid, and the underdeveloped sites of fatty acid utilization suggest that fat plays a quantitatively minor role as fuel during the perinatal period. Glucose, which is the main oxidative fuel, is also diverted to hepatic lipogenesis, and a part is stored as glycogen in many tissues (see reviews [1, 18, 27, 47]). Since glucose is the principal intrauterine oxidative fuel, the newborn must make glucose available from the liver glycogen stores built during gestation or by gluconeogenesis from lactate, pyruvate, glycerol, and amino acids. It is well established that in the rat, liver glycogen is rapidly mobilized after birth [3, 11, 20, 31, 72] and that liver gluconeogenesis also appears within the hours following detachment [3, 33, 72, 81]. An analysis of enzyme activities in the liver of newborn rats shows that phosphorylase [10, 31] and all key gluconeogenic enzymes [2, 37, 78] increase; while the increase in glucose-6-phosphatase, fructose diphosphatase, and pyruvate carboxylase were 3-fold, the changes in gluconeogenic flux and in phosphoenol pyruvate carboxykinase (PEPCK) activity were 30- to 50-fold [4]. A linear correlation has been found between gluconeogenic flux from pyruvate and the PEPCK activity in newborn rat livers [81].

Using an immunological measurement of rat liver PEPCK, it has been demonstrated that the increase in this enzyme activity at birth was due to an increase in enzyme synthesis [63]. These results suggest that liver PEPCK is a key regulatory enzyme in liver gluconeogenesis [23, 41, 42] in the rat. Since the neonatal hypoglycemia is a frequent occurrence in many species [16, 69], the understanding of the mechanisms which regulate the sequential appearance of hepatic glucose production is of considerable interest. In the adult mammals, glucose homeostasis [75], hepatic metabolism in vitro [24, 53], and disposition of gluconeogenic amino acids into hepatic glucose production or muscle protein synthesis [9, 51, 56–58] are principally determined by the interactions of insulin and glucagon. It is the purpose of this review to examine the metabolic effects of insulin and glucagon in fetal and newborn rats and to discuss their physiologic significance during the perinatal period in rat and other species.

## Material and Methods

Albino rats of the Sherman strain, bred in the laboratory and fed *ad libitum* on laboratory food, were employed. Since ovulation occurs, around 1:00 a. m., caging them with a male over night on one occasion allowed for estimation of the gestational age to within eight hours. Normal gestation is 22 days, and parturition lasts up to two hours. Since precise timing of birth was desired, delivery was by Caesarian section at day 21.5 of gestation. The newborns were immediately transferred to an incubator in which the temperature was 37 °C and the relative humidity 70 %. They remained unfed for the whole of the study. Experimental and biochemical procedures have been described in detail previously [32–35].

## Results and Discussion

### Glucagon and liver carbohydrate metabolism in perinatal rats

Several lines of evidence support a role of glucagon in the appearance of glycogenolysis and gluconeogenesis in the liver of newborn rats. First, an unequivocal increase in plasma glucagon has been observed immediately after birth by Caesarian section [30, 31, 33] (Fig. 1) or by vaginal delivery [7] in the rat. The analysis of sequential appearance of liver glycogenolysis and gluconeogenesis and of the increase in key regulatory enzyme activities (phosphorylase, glucose-6-phosphatase, and PEPCK) show that the rise in plasma glucagon precedes the hepatic changes ([31, 33] and Figs. 2 and 3). The delay in development of liver glycogenolysis and gluconeogenesis is also compatible with the time course of induction of PEPCK in fetal rat liver by glucagon [40] and with the kinetics of glucagon-induced glycogen degradation in the liver of newborn rats [13]. Second, a marked rise in liver 3'5' cyclic AMP occurs at birth [8, 60] which would be expected if associated with increased plasma glucagon. Third, a dramatic fall in blood and plasma lactate level ([33, 72] and Fig. 4) in parallel with a decline in hepatic lactate concentration [3] occurs at birth concomitant with the rise in plasma glucagon (Fig. 1), and it has been recently reported that lactate utilization and conversion to glucose was increased by intraperitoneal administration of glucagon to newborn rats [73].

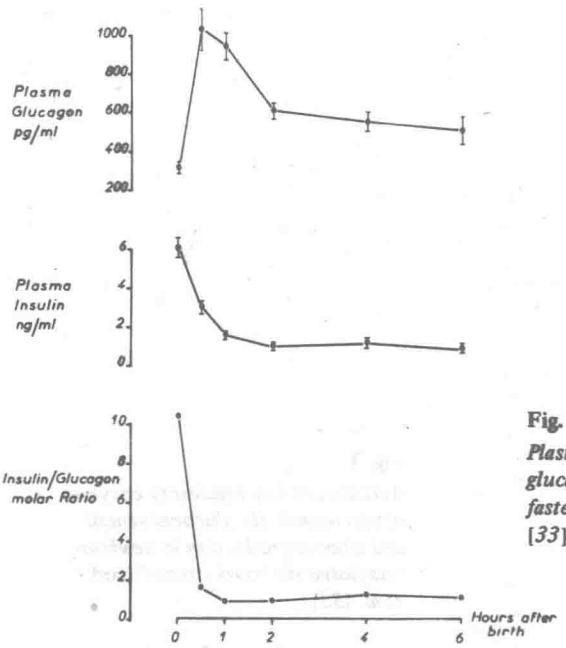


**Fig. 1**
*Plasma insulin, glucagon and insulin/glucagon molar ratio in newborn rats fasted six hours (from* Girard *et al. [33])*

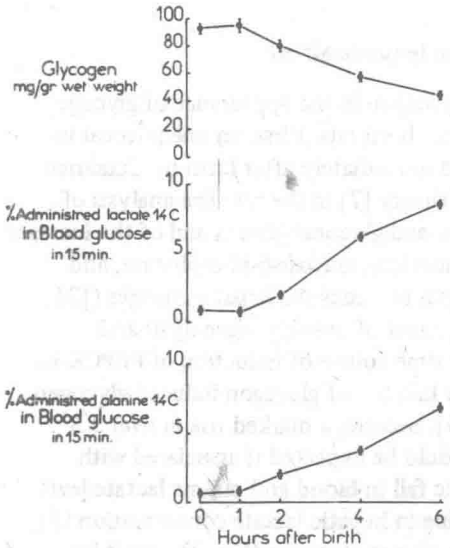


**Fig. 2**

***Liver glycogenolysis and gluconeogenesis in newborn rats fasted six hours***

Gluconeogenesis from lactate or alanine was measured 15 minutes after intraperitoneal administration of 0.1 μCi of labeled substrate/gr of body weight of newborn rat and expressed as % of administered radioactivity converted to labeled glucose on a basis of a glucose space of 85 % of body weight according to [72] (from *Girard* and *Marliss* [35])

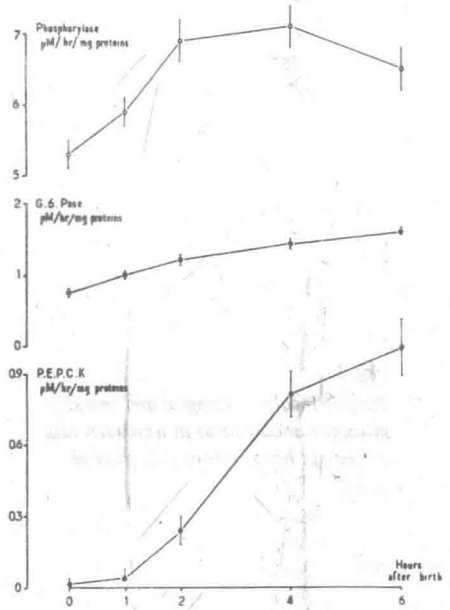


**Fig. 3**

***Activities of key regulatory enzymes of glycogenolysis, gluconeogenesis and glucose production in newborn rats fasted six hours (from*** Girard ***et al. [33])***

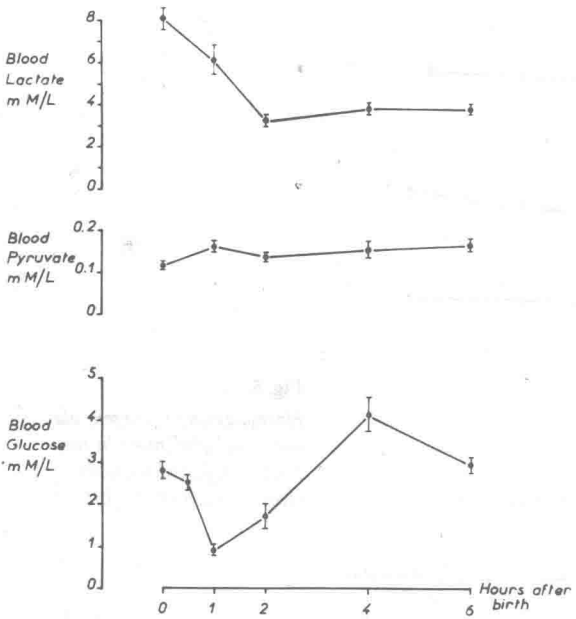


**Fig. 4**
*Blood glucose, lactate, and pyruvate in newborn rats fasted six hours (from* Girard *et al. [33])*

Fourth, exogenous-administered glucagon to the fetal rat in utero has been shown to be capable of provoking premature liver glycogen degradation [29, 39, 46], of increasing the activities of glucose-6-phosphatase [32, 38], PEPCK [5, 32, 40, 62, 82], and phosphorylase [32, 67] in the liver of the fetal rat. Addition of glucagon to fetal rat liver explant in vitro induces glycogenolysis [65, 68], phosphorylase activation [19, 70], and PEPCK synthesis [49, 79]. It is interesting to point out that glucagon-induced liver phosphorylase activation appears only after 20.5 days of gestation [32] when increase of liver glucose-6-phosphatase and PEPCK can be observed in younger fetuses [32, 40]. A permissive effect of glucocorticoids in glucagon-induced liver glycogenolysis in fetal and newborn rats has recently been suggested [32, 73]. All the hepatic effects of glucagon are probably mediated by 3′5′ cyclic AMP since fetal rat liver adenyl cyclase has been shown to be responsive to glucagon four days before term [6, 8, 12, 45, 48], and this cyclic nucleotide produces liver glycogenolysis [39], and increases the activities of glucose-6-phosphatase [36], PEPCK [40, 83], and phosphorylase [70] in perinatal rat liver.

## Glucagon and liver amino acid metabolism in perinatal rats

A sudden fall in plasma amino acids including alanine and glutamine – the two main gluconeogenic amino acids in the adult mammals [25, 56, 57] – occurs at birth in the rat ([17, 33, 43] and Fig. 5). An intensification of hepatic uptake of

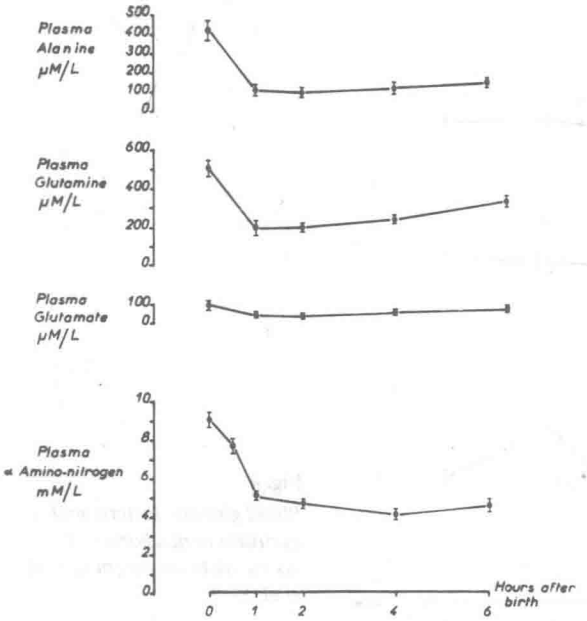


**Fig. 5**
*Plasma amino nitrogen, alanine, and glutamine in newborn rats fasted six hours (from* Girard *et al. [33])*

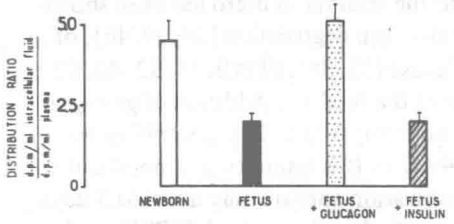


**Fig. 6**
***Cycloleucine uptake by fetal and newborn rat liver in vivo***
Distribution ratio was measured four hours after subcutaneous injection of 0.5 µCi of cycloleucine 14 C to fetal or newborn rats. Some rat fetus received either 10 µg of glucagon or 400 mU of insulin (from *Girard* and *Marliss* [35])

nonmetabolizable amino acid (cycloleucine) has also been observed ([35] and Fig. 6) in newborn rats. The injection of exogenous glucagon to fetal rat in utero decreases plasma amino acids levels and increases the hepatic uptake of cycloleucine ([35] and Fig. 6). This hepatic effect is independent of insulin released by the fetal pancreas in response to glucagon [76] since exogenous insulin injected in the same experimental conditions to fetal rats remains without effect on hepatic uptake of cycloleucine ([35] and Fig. 6). Available data on conversion of exogenous substrates to glucose by neonatal rat liver slices show that amino acids are poor gluconeogenic substrates when compared to pyruvate, lactate, or oxalo acetate [77, 80]. Similar experiments performed in vivo with tracer doses of alanine $^{14}C$ and lactate $^{14}C$ show that lactate is a better gluconeogenic substrate ([35] and Fig. 2) than alanine.

This suggests that transamination of amino acids may be rate limiting. In adult rat liver, the activity of transamination is not limiting for gluconeogenesis in physiologic conditions [54]. The Km of hepatic transaminases is very high, and a rise of amino acid concentration in physiological ranges in plasma and tissues automatically causes an increased rate of amino acid degradation [50]. But nutritional and hormonal conditions can also cause an adaptative adjustment of transaminase activities by variation in the rate of enzyme synthesis or degradation. Such a situation occurs at birth in the rat since it has been found that the activity of several hepatic transaminases are very low or absent in the fetus and dramatically increase after birth [37, 66, 71]. The injection of glucagon to the fetal rat increase the activity of several hepatic transaminases: tyrosine amino transferase [38, 44, 52], serine dehydratase [38, 59, 84], threonine dehydratase [85], and alanine glyoxylate amino transferase [71] which also suggests a role for glucagon at this level of the gluconeogenic pathway.

## Antagonistic role of insulin on liver metabolic effects of glucagon during the perinatal period

At the end of the gestation, the plasma insulin levels are very high in the rat fetus [14, 15, 26, 34], and the fetal liver becomes sensitive to insulin. Administration of insulin to fetal rat in utero [55] or addition of insulin to fetal liver explants in vitro [21, 22, 68] stimulate glycogen synthesis and convert glycogen synthetase to its active form. Administration of exogenous insulin to newborn rats (in order to prevent the fall of plasma insulin which normally occurs, Fig. 1) decreases liver gluconeogenesis from alanine $^{14}$C (Fig. 7) and inhibits the development of liver glucose-6-phosphatase [20, 32], PEPCK [32, 83], serine dehydratase [59], and tyrosine amino transferase [44]. Injection of antiinsulin serum to newborn rats also suppresses the delay in onset of liver glycogenolysis [74]. These results clearly show that insulin probably plays a significant role in liver metabolism of perinatal rats.

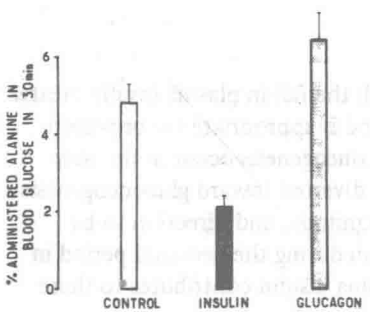


Fig. 7

*Effect of pancreatic hormones on gluconeogenesis from alanine 14 C in 4-hours-old newborn rats*

Gluconeogenesis from labeled alanine was measured after intraperitoneal administration of 0.1 μCi of 14. C. alanine/gr of body weight of newborn rats and expressed as in Fig. 2. Some newborn rats received at birth a subcutaneous injection of either 10 μg of glucagon or 40 mU of insulin (from *Girard* and *Marliss* [35])

## Physiological significance of glucagon in perinatal metabolism

Injection of pharmacological doses of glucagon in the fetal rat have been reported to have several metabolic effects on fetal liver which can be antagonized by exogenous insulin. But during late fetal development, the metabolic effects of the low plasma glucagon levels are probably prevented by the high plasma insulin levels (Fig. 8). The very elevated insulin-glucagon molar ratio in fetal plasma is highly appropriate to a metabolic set for which no endogenous glucose is required (active liver glycogen synthesis, no glycogenolysis or gluconeogenesis) and in which available amino acids are channeled into protein synthesis and thus relegate glucagon to a role of minor importance from a metabolic point of view. But it is not excluded that glucagon can play a role in differentiation of fetal tissues. The detailed ontogenic study of the pancreas in the rat [64] has shown that the A cells are the first differentiated endocrine cells in the rat embryo and suggests that glucagon may play a role in the early development of fetal pancreas. The closely paralleled evolution of insulin and glucagon in the plasma of the late fetal rat (Fig. 8) might result from the well-established insulin-tropic effect of glucagon on the fetal rat pancreas [76]. Whether the presence of glucagon is required to assure the appearance of the appropriate enzyme for glycogenolysis and gluconeogenesis, though their activities are low, remains unanswered pending a model of intrauterine A-cell deficiency.

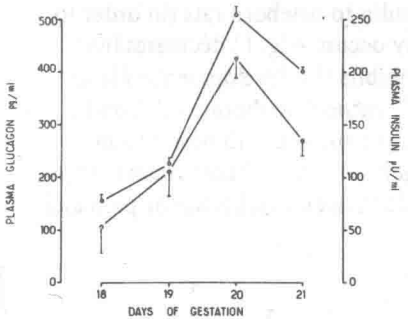


**Fig. 8**
*Insulin and glucagon in fetal rat plasma (from* Girard *et al.* [34]*)*

At birth, the rise of plasma glucagon associated with the fall in plasma insulin creates an insulin-glucagon ratio which favors catabolism and is appropriate for provision of glucose to the newborn. Glycogenolysis and gluconeogenesis occur in the newborn rat liver, and a part of plasma amino acids are diverted toward gluconeogenesis. The effects of glucagon are appropriate in time, magnitude, and direction to be implicated as prime regulators of hepatic metabolism during the neonatal period in the rat, but undoubtedly the concurrent fall in plasma insulin contributes to these changes.