Recently, another species of RNA containing a poly A tail has been isolated from the posterior silk gland. This mRNA exhibits a high template activity for the synthesis of fibroin small subunit in a wheat germ cell-free system. This finding of the small size mRNA strongly supports the opinion that 2 polypeptide chains of large and small subunits in a fibroin molecule are synthesized individually in the posterior silk gland. The site at which the 2 polypeptides are jointed to a whole fibroin molecule presumably by disulfide bond(s) remains to be determined.

The program of the synthesis of silk proteins in the silk glands and the spinning of the proteins synthesized is apparently under direct or indirect control of 2 major hormones, ecdysteroids and juvenile hormones. Throughout most of the larval instar period, the ecdysone level is very low or not detectable in the hemolymphs of *B. mori* and *Galleria mellonella.* However, before ecdysis - at the 4th day of the 4th molt and day 8 of the 5th molt - the ecdysone level sharply rises, the peak at the last larval instar being twice as high as that at the 4th instar. This peak is preceded by secondary peaks: spinning starts during that period.

On the other hand, juvenile hormone levels in *G. mellonella* hemolymph show 2 peaks during the 4th instar and decrease during the 4th ecdysis. They rise immediately after re-feeding at the last instar and then decrease throughout the period of the last instar. The ratio of juvenile hormones to ecdysteroids in the hemolymph of the silkworm at the last instar is an important factor for larval developmental processes. Treatment of the larvae with juvenile hormones during the early stage of the last instar induces a prolongation of the instar (that is, a delay in cocoon spinning), and a 30–50% increase in silk secretion. Thus, it is most likely that the silk gland is a target tissue for juvenile hormones which act as a potent inhibitor of protein synthesis, presumably, at the transcriptional level.

Although many steroid compounds play important roles in insect metabolism, insects lack the capacity for de novo sterol synthesis, and therefore, they require a dietary or exogenous source of sterol for their normal growth, development and reproduction. This sterol requirement of insects is, in most cases, satisfied by plant sterols such as sitosterol. The conversion of sitosterol to cholesterol has been shown to occur in a number of species of insects including the silkworm, *B. mori.* Recent biochemical works by Ikekawa demonstrated that fucosterol epoxide is a key intermediate in the conversion of sitosterol to cholesterol and that the site of ecdysone synthesis is the prothoracic gland, which is under the control of brain hormones. Further detailed studies in this area provide a great deal of valuable information not only on insect but also general sterol metabolisms.

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Biochemistry of liver development in the perinatal period

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Summary. Just before birth, changes occur in the metabolic capacities of rat liver so that the animal can adapt to changes in the substrate supply. In utero, glucose is the main energy-generating fuel and the liver metabolism is directed towards glucose degradation. The activities of the rate-limiting enzymes of glycolysis, hexokinase and phosphofructokinase, are high. In preparation for post-natal life, when the continuous glucose supply from the mother is interrupted, very large amounts of glycogen are stored in the late fetal liver. With the intake of the fat-rich and carbohydrate-poor milk diet, the animal develops the ability to synthesize glucose de novo from non-carbohydrate precursors. During suckling, metabolic energy is derived mainly from the β-oxidation of fatty acids, which in turn is an essential prerequisite for the high rate of gluconeogenesis, by yielding acetyl-CoA for the activation of pyruvate carboxylase and by generating a high NADH/NAD ratio for the shift of the glyceraldehyde 3-phosphate dehydrogenase reaction in the direction of glucose formation. - The developmental adaptation of metabolism and the process of enzymatic differentiation are closely connected with the maturation of the endocrine system and the changes in the concentration of circulating hormones. The neonatal regulation of phosphoenolpyruvate carboxykinase and of tyrosine aminotransferase by variations in the hormonal milieu around birth, and also the interaction of hormonal and nutritional factors in the induction of serine dehydratase and glucokinase at the end of the suckling period, will be discussed in detail.

Introduction

In the course of perinatal development significant metabolic changes in the growing organism are taking place. The environment of the fetus is determined by the maternal organism, which provides the fetus continuously with substrates, oxygen and other essential compounds and removes the metabolic products. Development is characterized by growth as well as by structural and functional differentiation. In the developing tissues and organs specific enzyme patterns are expressed enabling the growing organisms to cope with the demands of fetal life and life after separation from the mother. Hence, profound alterations in the enzymatic equipment of a growing and differentiating organ may be expected to occur.

The timing of the successive developmental processes is one of the most fundamental aspects of ontogenesis. According to present views the early phases of development depend primarily on the genetic program; in later stages, however, the sequential gene expression is apparently under the control of metabolic influences as the dominant, though perhaps not exclusive, mechanism. The metabolic influences may be divided into internal, i.e. hormonal, and external, primarily nutritional, factors.

The intention of this contribution is to discuss the changes in the enzymatic equipment of the developing liver in the late prenatal and the early postnatal stages of development. In order to investigate the mechanisms of timing, the changes in the enzyme pattern are correlated with variations in the hormonal environment and in nutrition. The significance of the 2 types of signals, the mutual interaction and the underlying mechanisms will be discussed.

The substrates of the fetus

The fetus requires substrates from the mother both for energy production and for growth. Only those substances which are capable of penetrating the placental barrier can be utilized by the fetal organism.

Glucose

Glucose, passing the placenta by 'facilitated diffusion'[1], is a main substrate for the fetus. In large mammals, like sheep, cow, and man, glucose is completely degraded by the fetus to carbon dioxide and water. Burd et al.[2] and Char and Creasy[3] found a significant positive venoarterial difference in blood lactate in the umbilical vessels of the fetal lamb. This points to an uptake of lactate by the fetus. It was calculated that approximately 25% of the fetal oxygen consumption can be attributed to lactate oxidation. Although at present a partly anaerobic metabolism of some fetal tissues cannot be excluded, their 'oxygen debt' is apparently paid by other organs, which means that the fetus of a large mammal is a lactate consumer and not a lactate producer.

In contrast, fetuses of small laboratory animals (e.g. the rat) apparently convert substantial amounts of glucose to lactate during the last 3 days of gestation. A major part of this metabolite flows back to the mother, as is demonstrated by the negative venoarterial difference in the umbilical circulation[4-6].

The species-dependent differences in lactate metabolism appear to be closely related to differences in the rate of oxygen consumption per unit of body weight. Whereas in extrauterine life, the weight-specific oxygen consumption is inversely proportional to the body weight[7], the weight-specific fetal oxygen consumption is similar in animals differing widely in their fetal masses[8]. Apparently, the fetal weight-specific oxygen consumption is less than the maternal one in small mammals and greater than the maternal one in large mammals.

The comparatively small oxygen uptake by the fetal tissues of small mammals can possibly be attributed to the immaturity of the mitochondria in this state of development rather than to a limitation of the oxygen supply. The neonatal maturation of the mitochondria[9], triggered by the metabolic changes taking place in the course of labor, results in a dramatic increase in the rate of oxidative metabolism. In the newborn rat the oxidation of glucose accounts for the total oxygen consumption[10]. In contrast, the fetuses of large mammals are a site of high oxygen consumption in comparison with the maternal organism. A drastic increase in the oxygen uptake after birth is observed.

In addition to glucose, fructose might be a potential substrate for the fetus, since in some species (e.g., sheep, cow, pig) its concentration in the fetal blood is 3–4 times higher than the concentration of glucose. However, neither a significant umbilical uptake nor a conversion of fructose to glucose or to lactate could be detected in fetal sheep[11]. In rats in the last days of gestation the incorporation of fructose into fetal liver glycogen is low; it increases in the newborn animal[12]. Maternal starvation leads to a decrease of the fructose concentration in ovine fetal blood[13]. Thus, in some mammalian species fructose may represent a fetal carbohydrate reserve which becomes utilizable under specific circumstances, for instance during starvation of the mother. In contrast to fructose, in several mammalian species galactose incorporation into fetal liver glycogen could be demonstrated; however, the galactose concentration in the fetal blood is very low.

Lipids

The nutritional role of maternal blood lipids for the fetus is poorly understood, since the views about the permeation of fatty acids through the placental barrier are rather controversial. Koren and Shafir[14], from their experiments with ^{14}C-labeled fatty acids, con-

cluded that in the rat no appreciable transport of fatty acids occurs between the 17th and 19th days of gestation. In the fetal lamb[15], no umbilical uptake of fatty acids could be found either. On the other hand, Hummel et al.[16] reported for the rat that maternal plasma triglycerides as a source of fetal lipids cannot be neglected. They estimated a net flux of fatty acids from the mother to the fetuses of about 0.12 µmoles per min and litter. Two-thirds of this amount is free fatty acid, and one-third is maternal VLDL. The latter must be degraded before crossing the placenta. The triglycerides of the chylomicrons cannot be used for meeting the fetal requirement for fatty acids, although the placenta contains a lipoprotein lipase.

Despite the possibility of receiving lipids from the mother, the newborn rat possesses only small fat stores, which account for about 1–2% of the body weight[17]. The ovine fetus, which does not receive lipids via the umbilical circulation, also has a very small fat content at term[18]. In comparison, fat constitutes about 16% of the birth weight in man and 10% in the guinea-pig[17]. The placentas of the latter 2 species allow the transfer of fatty acids from the mother to the fetus. All in all, the significant differences in the fat stores of different species apparently originate from dissimilarities in the fetal lipid metabolism rather than from the lipid supply through the placenta.

In the fetal rat plasma on the 19th day of gestation, only LDL could be detected[19]. Whereas VLDL could be found 1 day before birth, HDL were absent during the whole period of gestation. The appearance of LDL before VLDL is in contrast to the well established pathway of metabolism of these lipoproteins in the adult animal, where VLDL is synthesized in the liver and is than converted to LDL in peripheral tissues by partial loss of triglycerides[20]. Although lipoproteins from fetal plasma may have different separation properties in comparison to those of adults, the fetal hepatic lipoprotein lipase[21], which prevents the secretion of VLDL, must also be taken into consideration. The catabolism of VLDL in the liver, giving rise to the deposition of triglycerides in the organ itself, in addition to the absence of HDL, known to contain activators of the extrahepatic lipoprotein lipase[22], might be the reasons for the small fat deposition in rat fetuses.

Ketone bodies

In respect of the uptake by the fetus, ketone bodies behave similarly to fatty acids. In those species in which the fetuses are capable of extracting them from the maternal circulation, ketone bodies are probably important substrates for extrahepatic tissues. Their rapid appearance in the fetal blood and incorporation into the lipids of the brown adipose tissue, the pancreas and to a smaller extent also of the liver and lung has been shown by Seccombe et al.[23] after i.v. application of ^{14}C-labeled β-hydroxybutyrate to the mother. Their incorporation into the fetal lipids was many times higher than into the maternal lipids.

Whether fatty acids and ketone bodies delivered to the fetus are really important fuels for it is not completely clear at present. Although fetal tissues are capable of degrading these substances [24,25], evidence exists that the fetal fat is not utilized as an energy source before birth[26].

Amino acids

In the fetal plasma, the concentration of amino acids is higher than in the maternal plasma[27,28]; this suggests active placental transfer of these substrates. Not all amino acids incorporated into fetal proteins are extracted from the maternal circulation. Lemons et al.[29] found that neutral and basic amino acids permeate the ovine placenta, whereas the acidic amino acids do not. Glutamic acid was found to be produced by the fetal lamb itself. The fetus of man[30] is also capable of forming glutamic acid. Through the rat placenta[31,32], little or no transfer of glutamate and asparate takes place, in contrast to a rapid permeation of glutamine.

The absorbed amino acids are utilized by the fetus mainly for the synthesis of proteins, i.e., for the formation of new tissue. Little is known concerning the significance of amino acids as oxidizable fetal substrates. The urea concentration in the fetal plasma of sheep[33] and man[34] is higher than in the maternal plasma, which indicates amino acid catabolism. In fetal rats, the plasma urea concentration is not significantly different from that of the maternal plasma[6].

The metabolism of the liver in the perinatal period

Carbohydrate metabolism

In 1925 Negelein[35], in Warburg's laboratory, demonstrated that the fetal rat is capable of carrying out the conversion of glucose to lactate. Later, it was possible to establish the great glycolytic capacity of the fetal rat liver, and it was shown that the rate-limiting enzymes of the glycolytic pathway, hepatic hexokinase and phosphofructokinase, have high activities during this developmental stage[36]. Their activities decrease in the course of further gestation and during the first days of extrauterine life. Owing to the immaturity of the mitochondria, in the second decade of gestation the fetal rat liver shows a high rate of aerobic glycolysis. As shown in figure 1, in the fetal liver very high concentrations of lactate and pyruvate are found. After birth the rate of glycolysis and the lactate level decrease rapidly as the mitochondria mature and oxygen consumption increases. The activities of other glycolytic enzymes are relatively low in

the fetal liver and pass through a maximum after birth (fig. 2).

During perinatal development there is not only an alteration in the amounts of enzymes, but variations in the isoenzyme composition also occur. Changes in the hepatic isoenzyme pattern are of importance, since liver-type isoenzymes in the adult organ are often equipped with distinct regulatory properties; for example, pyruvate kinase and phosphofructokinase are targets for the action of effectors regulating the coordination of glycolysis and gluconeogenesis.

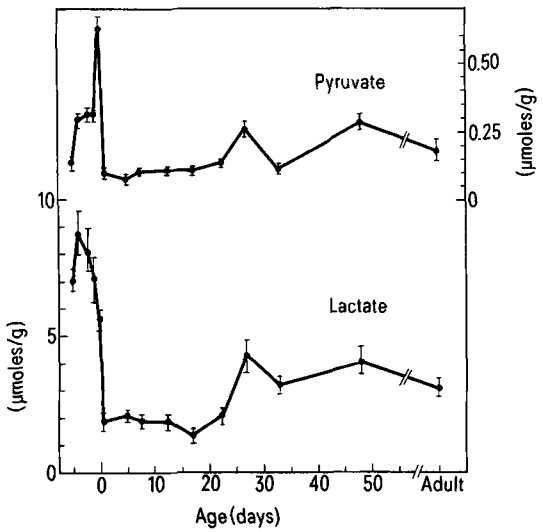

Figure 1. Changes in rat liver lactate and pyruvate during perinatal development. The values are given as means ±SEM.

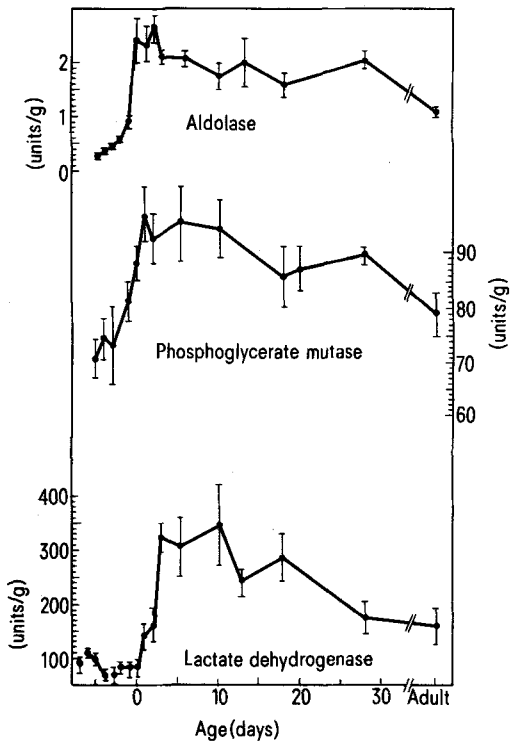

Figure 2. Developmental changes in selected glycolytic enzymes. Activities are given as means ±SEM.

As in other mammals, in rat tissue 3 types of phosphofructokinase subunits exist; the muscle (M)-, the liver (L)- and the spleen (S)-type. All 3 types can be identified in adult and fetal tissues, but characteristic differences between the isoenzymic pattern of fetal and adult organs are discernible. Fetal rat liver at the 15th day of gestation contains M- and L-type subunits. The proportion of the muscle isoenzyme decreases during the late gestational period (from day 17 of gestation until birth) whereas the proportion of the liver isoenzyme increases (fig. 3).

The presence of S-type subunits in the liver at early stages of development has been shown by electrophoretic analyis. Fetal brain contains the muscle and the liver isoenzymes. During perinatal development the latter is replaced by the spleen form.

The liver-specific aldolase B, which is capable of cleaving fructose 1,6-bisphosphate and fructose 1-phosphate, is absent from the fetal rat liver which contains only aldolase A (muscle-type) and aldolase C (brain-type). Both isoenzymes decrease progressively whereas aldolase B increases[37]. In the case of pyruvate kinase the 'fetal' isoenzyme (kidney-type) is replaced in the third week of extrauterine life by the adult form (liver-type)[38].

The above-mentioned temporary increase in the activity of most glycolytic enzymes during neonatal life is probably closely related to their involvement both in glycolysis and in gluconeogenesis. In contrast to the fetuses of ruminants[39], rat fetuses are unable to utilize lactate and alanine as gluconeogenic precursors apparently because of a prenatal lack of cytoplasmic phosphoenolpyruvate carboxykinase[40–42]. Small amounts of phosphoenolpyruvate carboxykinase can be found in the mitochondria of fetal liver; however, in the rat this enzymic activity is apparently not involved in gluconeogenesis[40]. More recently, Mac

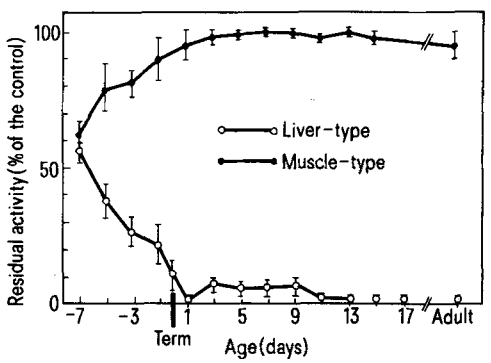

Figure 3. Variation of the isoenzyme pattern of phosphofructokinase during the perinatal development of rat liver. Residual activities after incubation of liver extracts with antibodies against the muscle- or the liver-type phosphofructokinase are plotted.

Donald et al.[43] demonstrated the presence of a significant amount of phosphoenolpyruvate carboxykinase in fetal rat liver (about 15% of the adult value); however, these authors did not differentiate between the 2 types of the enzyme. In the rat, although there is little mitochondrial phosphoenolpyruvate carboxykinase activity, and that in the cytoplasm is very low, fetal gluconeogenesis can be induced by maternal starvation[44].

The other gluconeogenic enzymes, pyruvate carboxylase[40], fructose 1,6-bisphosphatase[36,45], and glucose 6-phosphatase[36,45,46] are present in only small amounts in the livers of rat fetuses. After birth the levels of the 4 gluconeogenic enzymes and the rate of glucose synthesis increase considerably. The formation of glucose from substrates like glycerol which do not require phosphoenolpyruvate carboxykinase does indeed occur in rat fetuses. Gilbert[47] observed a more rapid incorporation of ^{14}C-labeled glycerol into fetal than into maternal liver glycogen.

The intensive hepatic glycogen deposition in the late gestational phase is very important for the survival of the animal after separation from the continuous maternal glucose supply. Glycogen synthetase[48] and phosphorylase[36] appear in fetal rat liver after the 16th day of gestation. The developmental activity changes of these and other enzymes involved in glycogen metabolism give rise to glycogen deposition (fig. 4). Since the ratio of active phosphorylase to active glycogen synthetase never approaches zero during intrauterine life, it was concluded that degradation

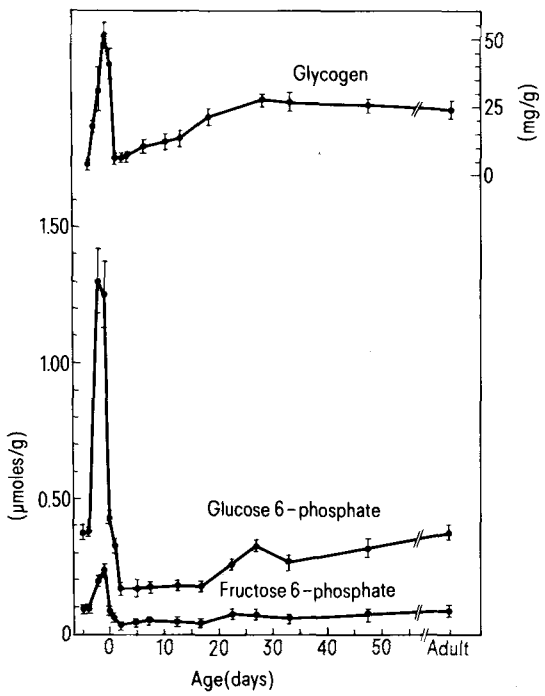

Figure 4. Concentration of glycogen and of hexosemonophosphates in the developing rat liver. The values are given as means ±SEM.

and synthesis of glycogen take place simultaneously in the fetal liver[48]. On the other hand, Devos and Hers[49] calculated that in the fetal liver during the last 5 days of gestation the rate of glycogen synthesis does not allow glycogenolysis to occur to a significant extent. However, it was reported recently that in fetal liver the active glycogen synthetase has a much higher K_M for UDPG than the enzyme of the adult organ[50]. Since in the calculations of Devos and Hers[49] the lower affinity of fetal glycogen synthetase for UDPG was not taken into account, the maximal activity of the enzyme was underestimated.

A prenatal glycogen degradation also becomes evident when the developmental changes in the concentrations of the hexosemonophosphates are followed (fig. 4). Both glucose 6-phosphate and fructose 6-phosphate are high in the fetal liver and increase further to a remarkable extent during the last 2 days of gestation, apparently because of an increasing glycogen breakdown. Most probably the low activity of glucose 6-phosphatase in fetal liver[36,45,46] does not permit a significant hepatic glucose production from the glucose 6-phosphate formed by glycogenolysis. Hence, glucose 6-phosphate accumulates in the late fetal liver. Not until shortly after birth can a steep decrease in the hexosemonophosphate level be observed.

The correctness of the assumption that in the fetal liver glucose 6-phosphatase limits the rate of hepatic glucose production from glycogen was substantiated by the precocious induction of the enzyme by glucagon. In the first hour after intrauterine application of the hormone, the glucose 6-phosphate level increased in consequence of the cAMP-mediated activation of glycogen phosphorylase. Subsequently, in the next 3 h the glucose 6-phosphate level decreases to about 50% of that in the saline-treated fetuses used as controls (fig. 5). The decrease in the concentration of glucose 6-phosphate correlates well with the simultaneous increase in the glucose 6-phosphatase activity. Actinomycin D, which prevents the precocious induction of the enzyme by glucagon, is also capable of preventing the decline of glucose 6-phosphate (fig. 5). In fact, after combined application of glucagon and actinomycin D, the glucose 6-phosphate concentration is higher and the glucose-6-phosphatase activity lower than in the saline-treated controls. The latter effect is apparently due to an inhibition of the endogenous induction of the enzyme.

Beyond the 20th day of gestation the active glycogen synthetase is inhibited, probably because of the high glycogen level[50,51]. Since glucose 6-phosphate is known to act as an activator of the inactive form of glycogen synthetase[52], the high concentration of glucose 6-phosphate in the late fetal liver is thought to be of great physiological significance for the deposition of the large amounts of glycogen found which are higher than in the adult liver.

Fatty acid metabolism

All mammalian fetuses studied so far are capable of synthesizing lipids[53-56]. In rats the rate of lipid synthesis exhibits a triphasic behavior. In the fetal liver, lipid synthesis from glucose is very active (twice the adult rate). Near term the rate falls; it becomes almost zero after birth, and remains at a very low level until weaning. After weaning the rate of lipid synthesis increases again. These changes are reflected by the intake of glucose, which is high in the fetus and after weaning. Conversely, the changes in lipid synthesis are reciprocal to the fat intake, which is low during fetal life and high during suckling.

The perinatal decrease in the rate of liver lipogenesis is temporally correlated with an accelerated lipogenesis in the fetal lung[57], as a prerequisite for the synthesis of surfactant and the maturation of the lung before parturition[58].

The cytoplasmic synthesis of fatty acids needs acetyl-CoA and NADPH. In the adult liver the 2 metabolites are provided by the co-ordinated action of the ATP-citrate lyase and the extra-mitochondrial malate dehydrogenase couple as shown in figure 6. ATP-citrate lyase splits citrate into acetyl-CoA and oxaloacetate. The latter is then converted by the NAD- and NADP-dependent malate dehydrogenases into pyruvate; this process is accompanied by an increase in the cytoplasmic NADPH at the expense of NADH. In fetal rat liver, the NADP-dependent malate dehydrogenase is virtually absent. This suggests that the lipogenic system receives the NADPH from other sources, mainly from the direct oxidation of glucose 6-phosphate. The activity patterns of other lipogenic enzymes, like ATP-citrate lyase[53], pyruvate dehydrogenase[59], and fatty acid synthetase[60], in addition to pyruvate kinase[36] which produces the precursor substrate for fatty acid synthesis, resemble the triphasic course of hepatic lipid synthesis.

After birth, in the suckling period, the high fat and low carbohydrate content of milk makes it necessary for the animal to degrade lipids in order to cover its energy requirements. The onset of β-oxidation in the newborn is apparently essential for the maintenance of a normal blood glucose level. It has been shown

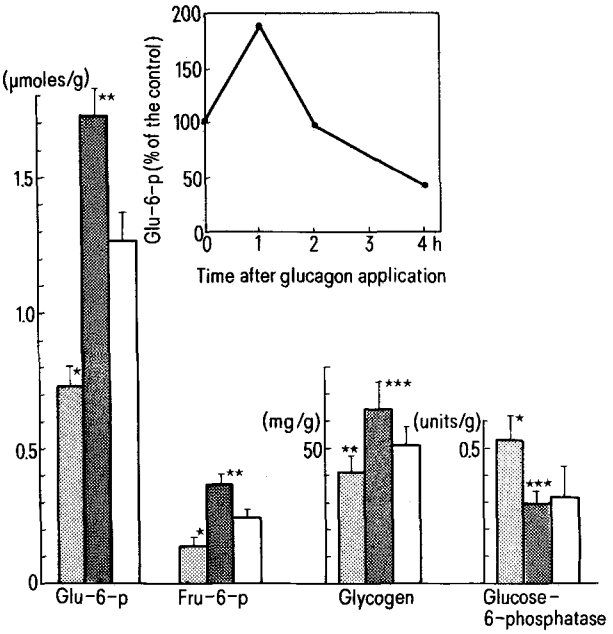

Figure 5. Effect of glucagon on the concentration of hexosemonophosphates and of glycogen as well as on the activity of glucose 6-phosphatase in fetal rat liver (21th day of gestation). The values correspond to means ±SEM of 4-9 litters. Asterisks indicate the statistical comparison between each group of hormone-treated animals and the control group. * $p < 0.005$, ** $p < 0.05$, *** not significant. ▨, Glucagon alone; ▩, glucagon + actinomycin; □, controls (saline). Inset: Time dependence of the glucagon effect on the glucose 6-phosphate concentration.

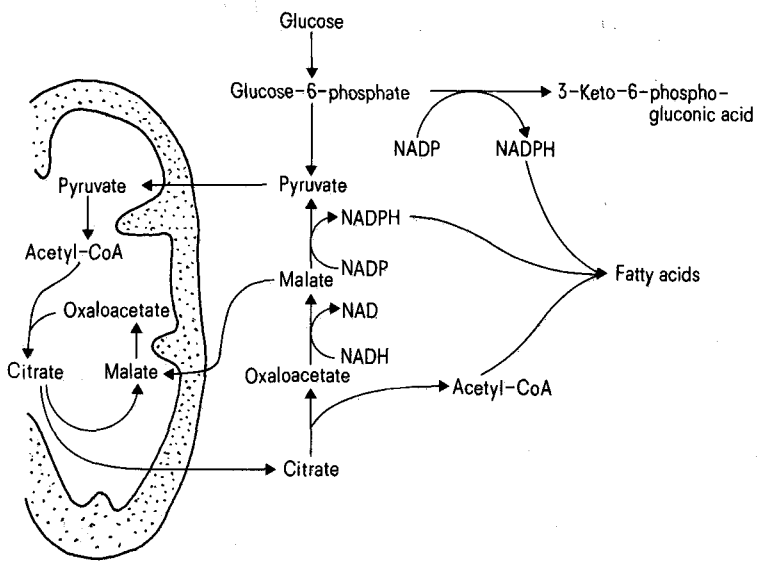

Figure 6. Lipogenesis in rat liver.

that the administration of pent-4-enoate, an inhibitor of fatty acid oxidation, gives rise to hypoglycemia owing to a decreased rate of gluconeogenesis[61]. Starved newborn rats also become rapidly hypoglycemic[62]. In contrast to the adult, the newborn rat is not capable of responding to starvation by mobilization of fat reserves because of a lack of white adipose tissue and of other fat stores[63]. Ferre et al.[64] concluded from their results that in neonatal rats hepatic fatty acid oxidation promotes gluconeogenesis by providing acetyl CoA as allosteric activator of the pyruvate carboxylase, as well as by supplying the NADH, which is essential for shifting the glyceraldehyde 3-phosphate dehydrogenase equilibrium in the direction of gluconeogenesis.

The extent of ketone body utilization in the neonatal period shows species-dependent differences. Ketosis can be observed in newborn infants[65], rabbits[66] and rats[67], whereas in sheep[68] and pigs[69] the blood ketone body concentration remains neonatally very low. Ketone bodies are apparently important neonatal fuels in those species having high blood concentrations. In newborn rats the blood ketone body concentration increases slowly during the first 12 h of extrauterine life. In the following 4 h it rises sharply[70]. No correlation could be found between the plasma concentration of non-esterified fatty acids and the ketone body concentration. The mechanisms by which neonatal ketogenesis is regulated are not well understood at present. The low activities of mitochondrial carnitine palmitoyl transferase, changes in the plasma insulin and glucagon concentrations and also the increase in the rate of gluconeogenesis seem to be involved (for a review see Girard et al.[71].

Amino acid metabolism

The perinatal period is characterized by rapid growth. The amino acids supplied to the fetus from the maternal blood by placental transport, and to the newborn by the milk diet, are consumed mainly for synthetic processes; protein biosynthesis, and the synthesis of nucleotides, nucleic acids, and other nitrogenous products. Urea synthesis and excretion are low during fetal life and suckling[72], indicating a low rate of amino acid degradation and of gluconeogenesis from amino acids in these periods. The de novo synthesis of glucose is very low in rat fetuses, but high in the suckling animal[73]. During suckling about 85% of the glucose metabolized is provided by gluconeogenesis. Inhibition of this metabolic pathway by 3-mercaptopicolinate leads to pronounced hypoglycemia and to a rise of the plasma lactate level. In contrast to the adult animal the plasma concentration of alanine remains unchanged[74], which suggests that alanine is not directly used for gluconeogenesis in the suckling animal. Correspondingly, the activity of alanine aminotransferase in rat liver, although present, is low in the period of suckling and does not show a significant increase before the 20th postnatal day[75]. Apparently, lactate is the prevailing gluconeogenic substrate in this period of life.

Snell[74] proposed that the liver of suckling rats is an alanine producer rather than a consumer. The high rate of fatty acid oxidation leads to a high NADH/NAD quotient within the mitochondria which shifts the equilibrium of the glutamate dehydrogenase reaction in the direction of glutamate formation, and consequently the mitochondrial alanine aminotransferase reaction towards pyruvate consumption. The apparent incorporation of alanine into glucose in suckling rats is probably due to a preceding extrahepatic conversion of alanine to lactate or pyruvate, respectively. The CORI-cycle operates with high activity[73] in suckling rats.

The low rate of urea synthesis in suckling rats is apparently not caused by a limited supply of ammonia and aspartate. The activities of glutamate dehydrogenase and of aspartate aminotransferase are high in the neonatal rat liver[75]. The enzymes of the urea cycle emerge during liver development in a biphasic manner. A first rapid increase in their activities is observed perinatally, allowing the production of urea after birth, when gluconeogenesis from amino acids begins. The 2nd rise occurs during weaning. The small urea production in connection with the high activities of the enzymes of the ornithine cycle and the high rate of ammoniagenesis, which amounts to about 50% of ureagenesis[74] (in the adult only 10%), gives rise to the assumption that in the neonatal rat liver the operation of the urea cycle is slowed down. Very likely the diminished operation of the ornithine cycle originates from the high concentration of non-esterified fatty acids[76]. It may be considered to be of regulatory significance, since more carbamoyl phosphate is made available for the synthesis of the pyrimidines which are required for the formation of nucleotides and nucleic acids.

The role of hormones in enzymatic differentiation

The development of an organ-specific enzyme pattern proceeds in the form of clusters and not proportionately. Greengard[77] was able to identify 4 such clusters. The enzymes of cluster A were found to decrease during the late fetal and/or neonatal development, those belonging to the other 3 clusters to increase. They differ between each other according to whether they rise in the late fetal period (cluster B) or soon after birth (cluster C) or just before weaning (cluster D), respectively (table 1). The sequence of emergence and its timing reflects the necessity of the corresponding metabolic pathway for the developing organism. For example, hepatic glycogen deposition is clearly not essential for life in utero; however, in anticipation of the vital requirements after birth,

Table 1. Enzyme clusters in developing rat liver

Cluster A Enzyme activity decreases during development	Cluster B Late fetal cluster	Cluster C Neonatal cluster	Cluster D Late suckling cluster
Hexokinase[36,78]	Carbamoyl phosphate synthetase[81]	Phosphoenolpyruvate carboxykinase[40-42]	ATP-citrate lyase[53]
Phosphofructokinase[36]	Ornithine transcarbamylase[81]	Tyrosine aminotransferase[83-85]	Pyruvate dehydrogenase[59]
Thymidine kinase[79]	Arginase[81,82]	Serine dehydratase[86]	Malate dehydrogenase (NADP)[53]
Phosphoserine phosphatase[80]	Phosphorylase[36]	Fructose 1,6-bisphosphatase[36,45]	Fatty acid synthetase[60]
Pyruvate dehydrogenase[59]	Glycogen synthetase[48]	Glucose 6-phosphatase[36,45,46]	Glucokinase[78]
Fatty acid synthetase[60]	Fructose 1,6-bisphosphatase[36,45]	Alcohol dehydrogenase[87]	Pyruvate kinase[36]
Pyruvate kinase[36]	Glucose 6-phosphatase[36,45,46]	Aspartate aminotransferase[75]	Alanine aminotransferase[75,88]
ATP-citrate lyase[53]	Pyruvate carboxylase[40]		Ornithine aminotransferase[89]
			Serine dehydratase[86]

glycogen deposition and the appearance of the enzymes necessary for hepatic glucose production occur shortly before bith, which enables the newborn to maintain an adequate blood glucose level. The de novo synthesis of glucose from non-carbohydrates is demanded when the hepatic glycogen store is depleted; hence, during the 1st day post partum the set of gluconeogenic enzymes is completed.

The stepwise emergence of enzymes in the developing liver is closely related to changes in the hormonal environment and alterations in the fuel supply.

The appearance of enzymes during late fetal life (cluster B) is apparently triggered by the sudden increase in the secretion of glucocorticoids and thyroxine on the 17th day of gestation[90,91]. An intrauterine application of hydrocortisone was found to cause a precocious deposition of glycogen in the liver of fetal rats[92], and the treatment of rat fetuses with thyroxine gives rise to an increase of NADPH dehydrogenase and glucose 6-phosphatase[93].

The next profound change in the hormonal milieu of the fetus which is of significance for the enzymatic differentiation of the liver takes place around birth. In the perinatal period changes in the plasma concentrations of glucagon and of insulin and consequently in the insulin/glucagon ratio have been described[91,94,95]. The increase of glucagon and the decrease of insulin commencing between the 21st and 22nd day of gestation cause a diminution of the insulin/glucagon ratio from 15.6 to 5.9. Immediately after delivery a further reduction of the insulin/glucagon ratio takes place; it reaches a plateau within 30 min. Although enzymes of the neonatal cluster (cluster C), e.g. tyrosine aminotransferase[83-85] and phosphoenolpyruvate carboxykinase[96,97], can be induced precociously by intrauterine application of glucagon, evidence exists that the decrease of the insulin/glucagon ratio rather than a mere increase of glucagon alone is responsible for the appearance of these enzymes[98]. In response to these variations in the hormonal milieu, the cAMP level in the liver increases[98]. Apparently, a critical level of cAMP must be exceeded in order to induce the synthesis of tyrosine aminotransferase and of phosphoenolpyruvate carboxykinase. A positive correlation between the cAMP concentration and the activity of the 2 enzymes has been established[99]. In cultures of fetal hepatocytes from the rat, Bulanyi et al.[100] studied the role of hormones in respect of the initiation of phosphoenolpyruvate carboxykinase synthesis. The enzyme was found to be inducible by epinephrine, glucagon, dibutyryl-cAMP as well as by the phosphodiesterase inhibitors isobutylmethylxanthine and theophylline. They proposed epinephrine as the initial signal for the induction of hepatic phosphoenolpyruvate carboxykinase at birth, whereas the subsequent decrease of the insulin/glucagon ratio acts as a prolonging signal. In contrast to adult liver cells in which the stimulation of gluconeogenesis is mainly mediated by α-adrenergic receptors, in the perinatal hepatocytes the induction of phosphoenolpyruvate carboxykinase is apparently dependent on the β-adrenergic system. The induction of phosphoenolpyruvate carboxykinase by cAMP is due to an effect on the transcriptional level[101]. Fetal rat liver contains little, if any, functional mRNA for this enzyme. The administration of dibutyryl-cAMP was found to result in a marked increase of the level of translatable mRNA for phosphoenolpyruvate carboxykinase[102].

In addition to cAMP-dependent processes, cAMP-independent mechanisms seem to be involved in the perinatal regulation of phosphoenolpyruvate carboxykinase. This was demonstrated by Mencher et al.[103] by investigating this enzyme in diabetic rat fetuses.

The administration of glucagon to fetal rats results in the precocious formation of several enzymes, including phosphoenolpyruvate carboxykinase, tyrosine aminotransferase, and serine dehydratase, the synthe-

sis of which in the adult is under the control of glucocorticoids and not of glucagon. Interestingly, within the 1st day after birth, these enzymes become inducible by glucocorticoids, which are without any effect on their induction in the fetus. This is demonstrated in table 2 for tyrosine aminotransferase.

The data of Cake et al.[104] suggest that the inability of fetal liver cells to respond to glucocorticoids before day 18 of gestation is due to a lack of the glucocorticoid receptor. The glucocorticoid receptor activity which has been found in the whole fetal liver in still earlier stages of development, is evidently associated with hemopoietic cells being there in large amounts at this stage of development[105]. After the 18th day of gestation the glucocorticoid receptor in hepatocytes becomes detectable. Then the cells respond to exogeneous glucocorticoid administration with an increase of their glycogen content.

The fact that phosphoenolpyruvate carboxykinase and tyrosine aminotransferase are not inducible by exogenous glucocorticoids in utero but in isolated hepatocytes led Cake et al.[104] to the conclusion that the high fetal insulin concentration accounts for the inability of glucocorticoids to induce tyrosine aminotransferase precociously.

Table 2. Influence of glucagon and of dexamethasone on the activity of tyrosine aminotransferase in perinatal rat liver

Age (days)	Hormone	Enzyme activity [units/g]
−2	Control	0.03 ± 0.02 (5)
	Glucagon	0.29 ± 0.05 (5)*
−1	Control	0.33 ± 0.04 (6)
	Glucagon	1.82 ± 0.37 (8)*
	Dexamethasone	0.43 ± 0.05 (6)***
5	Control	5.49 ± 1.62 (5)
	Glucagon	6.08 ± 1.09 (8)***
	Dexamethasone	19.53 ± 4.15 (3)**
14	Control	2.23 ± 0.17 (3)
	Dexamethasone	9.51 ± 0.77 (4)**

The results are expressed as means ± SEM. The significance of the effect of hormones in each experiment is indicated by: *$p < 0.0025$, **$p < 0.005$, ***not significant.

When all observations about the regulation of phosphoenolpyruvate carboxykinase and tyrosine aminotransferase synthesis in the perinatal liver are summarized, the following can be inferred:

1. In the perinatal liver, a cAMP-dependent process initiates the formation of the system responsible for the synthesis of phosphoenolpyruvate carboxykinase and tyrosine aminotransferase. After that, when this system is formed the synthesis of both enzymes is independent on cAMP but can be affected by glucocorticoids.

2. An increase of the hepatic cAMP level above a critical value brought about either by hormonal stimulation of adenylate cyclase or by inhibition of phosphodiesterase leads to a stimulation of the synthesis of the 2 enzymes.

3. The high insulin level in the fetal blood restrains the appearance of the 2 enzymes. A decrease of insulin brought about either physiologically around term or artificially by inducing diabetes in the fetus makes the liver cells sensitive towards glucocorticoids when the specific hormone receptor is present.

4. In the absence of insulin either in diabetic animals or in isolated cultured hepatocytes the endogenous cAMP level is high enough to initiate the formation of the enzyme-synthesizing system.

In the rat liver the final step of enzymatic differentiation occurs just before weaning. The late suckling cluster (cluster D) is composed of enzymes necessary for adaptation to solid food. Members of this cluster are glucokinase, pyruvate kinase (the liver isoenzyme) and several enzymes of amino acid degrading pathways. In the regulation of their synthesis both hormonal and nutritional factors are involved. In many cases corticosteroids have been found to affect an actinomycin D-sensitive step before dietary components are capable of causing the emergence of an enzyme by the action of an actinomycin D-insensitive mechanism[106].

Snell and Walker[86] observed that hydrocortisone can increase the activity of serine dehydratase as a member of the neonatal and of the late suckling cluster. The 2nd increase of the enzyme at the 18th day of extrauterine life ensues from an actinomycin D-sensitive mechanism. The actinomycin D-insensitive stimulation of serine dehydratase by tryptophan is due to a stabilization of the enzyme against degradation. As in the adult animal, glucose is capable of inhibiting completely the effect of hydrocortisone when both are simultaneously administered. It has been suggested[86] that glucose acts at the translational level.

Glucokinase is also an enzyme of the late suckling cluster normally appearing in rat liver around the 16th day after birth. Wakelam et al.[107] were able to demonstrate that treatment of younger animals with triiodothyronine and glucose gives rise to a precocious formation of the enzyme, whereas glucose alone has only a weak inductive effect. Because glucose remains effective in animals with marked hypothyroidism, and triiodothyronine administration did not enhance the glucose effect in these animals, a connection between the ability of glucose to induce glucokinase and the thyroid status does not seem to exist. By working with isolated hepatocytes Wakelam and Walker[108] demonstrated that the induction of glucokinase by the combined action of glucose and triiodothyronine requires a definite basal insulin level. Whereas in the presence of 5.5 mM glucose in the culture medium no glucokinase could be detected, increasing amounts of the enzyme were found when a higher glucose concentration was applied; such concentrations may

occur in portal blood after oral ingestion of glucose. When investigating in more detail the joint action of glucose and of insulin on glucokinase synthesis in hepatocytes from suckling rats Wakelam and Walker[109] interestingly found that the insulin effect depends strongly on the preceding presence of glucose but does not require the presence of glucose itself. Whereas the action of glucose can be suppressed by actinomycin D but not by cycloheximide, the insulin effect is sensitive towards cycloheximide and not towards actinomycin D. This points to a stimulation of the formation of glucokinase mRNA by glucose and a promotion by insulin of its subsequent translation to the enzyme protein. However, since normally glucokinase appears during weaning when the rats are accustomed to a carbohydrate-free diet[110], but also when weaning is prevented[111], the existence of additional inducing factors have to be assumed operating around the 16th day after birth. Perhaps glucose, insulin, triiodothyronine and other presently unknown factors cooperate in the initiation of glucokinase synthesis.

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Full Papers

A new method for the large scale preparation of antitoxic antibodies exhibiting high specific protective activities[1]

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Summary. A method using polyethylene-glycol and immobilized pepsin for purifying heterologous antitoxic antibodies is described. Using horse antitetanus plasma or sera, $F(ab')_2$ fragments exhibiting specific activities in the range of 150 IU per mg protein were repeatedly isolated with a yield around 80%. The procedure was scaled up from 200 ml up to 20 l.

Introduction

Heterologous antitoxic sera have long been used for the treatment of toxi-infections: diphtheria and tetanus, as well as snake and scorpion envenoming. With the purpose of eliminating the risk of adverse reactions, whole sera have been subjected to pepsin digestion which results in the destruction of the Fc fragment responsible for the reactogenicity of the