Symposium on Drugs in the Newborn Child, N.Y., March 15-16, 1973.

Effects of hormones on development of fetal enzymes

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This investigation was supported by U. S. Public Health Service Grant CA 08676 from the National Cancer Institute and by U. S. Atomic Energy Commission Contract AT(11-1)-3085 with the New England Deaconess Hospital (COO-3085-23).

DISCLAIMER

Abstract

The adaptation of newborn mammals to extrauterine life is conditioned, to a large extent, by the maturity that their biochemical functions have attained during prenatal tissue differentiation. We concentrated on studying the development of enzyme systems in rat liver during late fetal and neonatal life; this was combined with the morphometry of cell types and of subcellular organelles. A cluster of enzymes which emerge during late fetal life and attain their adult concentrations at term has been distinguished from another cluster which makes its appearance immediately after birth. Different hormones (e.g., thyroxine, glucagon and glucocorticoids), the secretion of which is the natural stimulus for the expression of specific groups of genes, have been identified. With the administration of these hormones to fetuses it is possible to evoke the synthesis of chosen enzymes before the scheduled time and to produce newborns (delivered at term or prematurely) with precociously mature enzyme profiles. The potential medical application of the numerous experimental means by which we can now manipulate the course of enzymic differentiation remains to be evaluated.

A significant portion of serious perinatal medical problems may be due to a short delay in the onset of essential physiological functions.[1] Understanding of the mechanisms which regulate the sequential appearance of enzymes in developing organs is thus of considerable importance. This paper will illustrate, with a few examples, the ways in which we can experimentally enhance or delay the normal course of biochemical differentiation in the most extensively studied mammalian organ, rat liver. These experiments helped to elucidate the mechanisms underlying the normal process of enzymic differentiation and point to possible methods for correcting gestational abnormalities or retarded maturation.

Normal and experimentally altered biochemical differentiation in rat liver

Biochemical differentiation of mammalian liver around the time of birth can be divided into two distinct phases, the late fetal and the neonatal, each associated with the simultaneous emergence of a cluster of 10 to 12 enzymes.[4] It appears that the potentiality for some hepatic functions, such as urea synthesis, must be ready at birth while others, such as the gluconeogenic capacity, can develop during the ensuing day. Fig. 1 illustrates the rate of formation of typical enzymes of the late fetal and neonatal clusters. As shown by the solid lines, in whole liver and therefore in preparations used for determining enzyme concentrations, the mass attributable to hepatocytes increases during development about 1.4-fold (from 60% to the adult value of 87%). The much more precipitous rise in enzyme concentrations can obviously not be attributed to enrichment with hepatic cells at the expense of hematopoietic elements but

must represent intracellular differentiation of the hepatocytes themselves.[8] However, as will be seen later, the elimination of hematopoietic cells may be relevant to another aspect of biochemical differentiation, the loss of enzymes associated with growth per se rather than with hepato-specific functions.

Since thyroid and pituitary-adrenocortical activity begins in fetal rats around the 18th day of gestation,[12] it is very likely that the corresponding hormones trigger the synthesis of enzymes of the late fetal cluster. If this is so, then the administration of these hormones prior to the time at which their natural secretion is high should initiate enzyme synthesis. Indeed, thyroxine can cause the upsurge of several enzymes to occur prematurely.[3,10,6] One of these is illustrated in Fig. 2. In some experiments the fetuses were not sacrificed after thyroxine administration but were allowed to be delivered at the normal time. These were born with higher than normal levels of NADPH-cytochrome c reductase (EC 1.6.99.2) and glucose-6-phosphatase (EC 3.1.3.9)[3] as high as those attained by their untreated littermates 24 hours later (see Fig. 2). Since NADPH-cytochrome c reductase is a component of the microsomal drug metabolism system it is conceivable that these newborns, with a precociously mature enzymic equipment, have an enhanced detoxifying potential.

Fig. 2

Thyroxine also enhances the prenatal accumulation of both isozymes of hepatic aspartate aminotransferase (EC 2.6.1.1) (GOT) (Fig. 3), though it does not increase their levels in adult liver. This is one of the many examples showing that the stimulus which triggers the developmental formation of an enzyme does not necessarily regulate the synthesis of

Fig. 3

the same enzyme in the adult organ.[4] The soluble (but not the particulate) variant of aspartate aminotransferase exhibits another frequently obscured phenomenon: in the course of maturation enzymes become responsive to new regulators,[4,3] in this case to cortisol.[10] The normal accumulation of the particulate form of aspartate aminotransferase in fetal liver can be inhibited by an injection of estradiol (Fig. 3). Estradiol did not have such an effect on other enzymes of the late fetal cluster, including the soluble isozyme of aspartate aminotransferase (see Fig. 3).

Glucocorticoids are responsible for initiating the formation of those enzymes of the neonatal cluster (e.g., glycogen synthetase, see Fig. 1) which are required for glycogen deposition.[15] As would be expected, cortisol administration to fetal rats enhances the accumulation of glycogen in fetal liver.[12,7] Of the hormones that have been used in fetal enzyme induction experiments (thyroxine, glucagon, epinephrine, cortisol, estrogen) only cortisol caused microscopically visible cytological changes: it enhanced the elimination of hematopoietic cells. Since these cells are much smaller than are the hepatocytes, their loss had little effect on the number of hepatocytes per gram. The decrease in total cell nuclei per gram mainly reflects the elimination of the hematopoietic cells. As seen in Fig. 4, such a change became apparent 48 hours after the cortisol injection. In the same experiments we also determined the level of one of those nontissue-specific enzymes which decrease in amount prior to term, i.e., during the same time when the late fetal cluster of enzymes emerges (cf. Fig. 1 and Fig. 4). The enzyme, thymidine kinase (EC 2.7.1.21), is necessary for DNA synthesis and must be present in both cell types. Its concentration may be higher in hematopoietic

than in parenchymal tissue. As seen in Fig. 4, cortisol decreased the thymidine kinase level (units per gram liver); however, this decrease was maximal at 24 hours, i.e., before there was any sign of hematopoietic involution. The two processes may thus be either independent or the decrease in the enzyme may be one of the causes of the subsequent disappearance of the hematopoietic cells. The latter explanation is plausible since the cortisol-induced involution of thymus and neonatal spleen is also associated with the loss of thymidine kinase.[9]

Glucagon is a hormone which plays an important role in the adaptation of the neonate to extrauterine conditions.[3,5] This hormone, secreted in response to hypoglycemia (seen shortly after cutting the cord) has two distinct roles: 1, it activates liver phosphorylase and permits immediate glycogenolysis and 2, it stimulates the synthesis of enzymes of the neonatal cluster. As seen in Table I, the administration of glucagon in utero can cause both of these events to occur prenatally. Phosphopyruvate carboxylase, a key enzyme in gluconeogenesis, and serine dehydratase (EC 4.2.1.13) are other enzymes of the neonatal cluster which, like the tyrosine aminotransferase (EC 2.6.1.5) shown in Table I, can be prematurely evoked by glucagon.[17,3] The activation of phosphorylase (role 1 of glucagon) does not require de novo protein synthesis, whereas its evocation of new enzymes (role 2) depends on both protein and RNA synthesis. Thus, as shown in Table I, actinomycin D does not interfere with the glycogenolytic action of the hormone but prevents the glucagon induction of tyrosine aminotransferase. The latter effect of actinomycin D is, of course, nonspecific; this inhibitor of RNA synthesis prevents the normal or induced developmental formation of several enzymes.

Conclusions

The detailed description of the process of enzymic differentiation in normal rat liver, which now provides a basis for studies of mechanisms and for the detection of pathological deviations, is a result of work by many laboratories. The cumulative value of these individual studies depended on the use of adult rat liver as the standard tissue[13]- only by reference to the "adult concentration" is it possible to judge the developmental age at which an enzyme has or has not yet attained a level of possible physiological significance. - There is an urgent need for more information about the process of biochemical differentiation in human liver. But reports of "presence" or "absence" of various enzymes at a particular embryonic age may give entirely erroneous impressions about the fetal age at which these functions do in fact develop. The detection of one enzyme as opposed to another may depend entirely on the different sensitivity of assay methods that happen to be available. Even carefully quantified enzyme activities are of little meaning without reference to those in the fully differentiated tissue. The few reports which do include mature values[2,14,16] indicate that in human and monkey livers enzymic differentiation during late fetal and neonatal life is not unlike that in rat liver.

Rats are often used for testing the teratogenic effects of drugs during early embryonic development. In late fetal life when morphological differentiation is essentially accomplished, the harmful effect of drugs can best be detected by their interference with the normal evolution of enzyme patterns. Rats would be suitable experimental animals for studying such "biochemical malformations." Some four dozen rat liver enzymes

have now been classified into four major clusters, one present before the last trimester and three others which emerge during late fetal, neonatal and late suckling life, respectively.[5] There is thus a wide scope for diagnosing deviations from normal differentiation. The rapid synthesis of a newly emerging enzyme in late fetal liver may be sensitive to antimetabolites and antibiotics in concentrations lower than those which cause malformation in the early embryo. The resulting unbalanced enzyme pattern, not reflected in histological tests, may interfere with neonatal viability.

As illustrated here with a few examples, the process of enzymic differentiation is not an unresolvable succession of predestined events. In rat liver, individual enzymes or small groups of enzymes can be caused to be formed "out of step": a few hours after the injection of an appropriate stimulus they can attain levels normally associated with a more mature morphology and overall enzymic profile. There are also agents which can cause selective delays in enzyme synthesis. Treatments of the fetal rat a few days before term with hormones or other substances usually did not interfere with gestation and postnatal viability. This is due in part to the efficiency of the maternal detoxifying mechanisms and also to the fact that the hormones given were often the same ones that the normal fetus was scheduled to secrete shortly.

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Fig. 1. Enzymic and cytological differentiation in rat liver. Broken lines depict the accumulation of typical enzymes of the late fetal cluster, glycogen synthetase (EC 2.4.1.11)[11] and of the neonatal cluster, phosphopyruvate carboxylase (EC 4.1.1.38).[18] The solid lines refer to changes in the fraction of liver volume occupied by parenchymal (o) and hematopoietic cells (•) determined morphometrically.[8] All values are expressed as percent of that in adult liver.

Fig. 2. The effect of thyroxine on the developmental formation of NADPH-cytochrome c dehydrogenase. Enzyme activity is expressed in μmoles of NADP reduced per hour per gram liver. The solid and broken line refers to control and treated animals, respectively. Thyroxine (0.03 μg.) was administered to fetal rats in utero, once, on the 19th day of gestation (they were killed 24 hours later, see circle on day 20) or on the 21st day of gestation (these were born naturally and their enzyme levels were determined at the indicated postnatal hour).

Fig. 3. Hormones and the developmental formation of aspartate aminotransferase (EC 2.6.1.1) in rat liver. Soluble or particulate aspartate aminotransferase (AAT) activities per gram liver are shown as percent of that in adult rats where the absolute values are 47 and 85 μmoles/min./g., respectively. The solid lines with crosses refer to normal fetuses. Fetuses were injected with saline (—x—), 1 μg. thyroxine (Δ), or 25 μg. estradiol benzoate (o) 24 hours before assay.

Fig. 4. The effect of cortisol on cellularity and thymidine kinase (TK) in fetal liver. On the 18th day of gestation, fetal rats received an intraperitoneal injection of 0.12 mg. of cortisol (hydrocortisone acetate); some dams were killed 24 and others 48 hours later to determine the thymidine kinase activity (--Δ--) and cellularity (--●--) per gram fetal liver. The solid lines with open symbols refer to control fetuses.

Table I. The effect of glucagon on enzyme synthesis and glycogenolysis.

Glucagon (i.p.)	Glycogen (mg.) (per gram fetal liver)	Tyrosine aminotransferase (units)
None	81 ± 4 (6)	< 2 (10)
35 mg., to mother	82 ± 7 (3)	< 2 (3)
0.5 µg., to fetus	40 ± 6 (4)	25 ± 3 (5)
0.5 µg., to fetus + actinomycin D	36 (2)	< 2 (2)

Rats were killed 5 hours after injection (20 to 21st day of gestation); glycogen and tyrosine aminotransferase activity were determined in pools of fetal livers as previously described.[7] The values are means (± SD) with the number of litters used in parentheses. When indicated, actinomycin D (10 µg.) was injected together with glucagon.

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Fig. 1

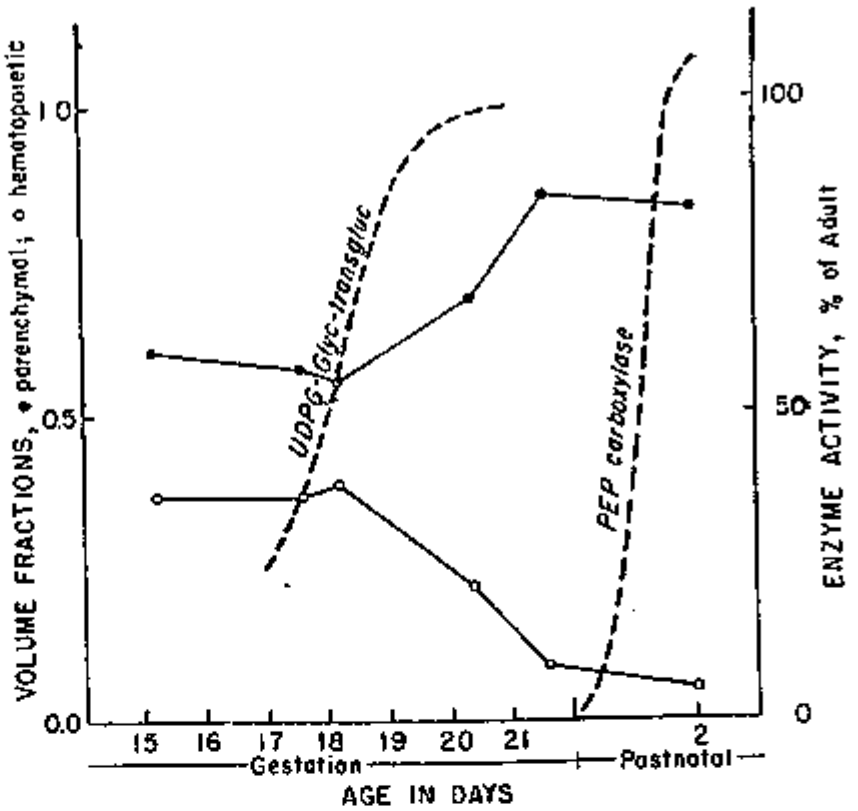
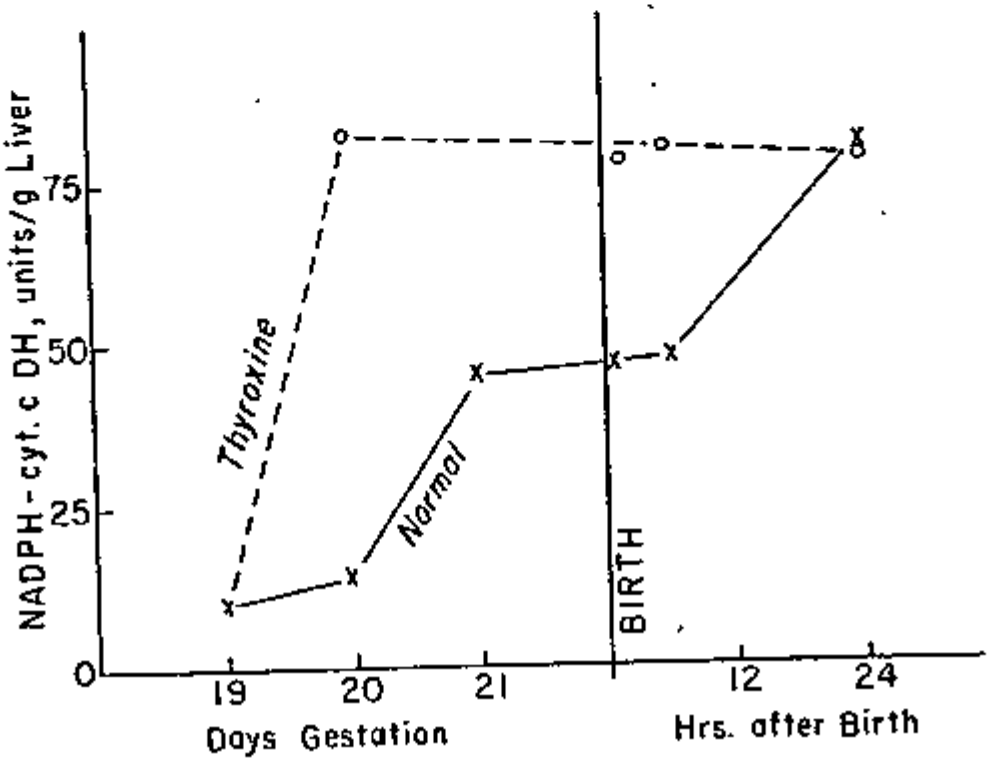


Fig. 2



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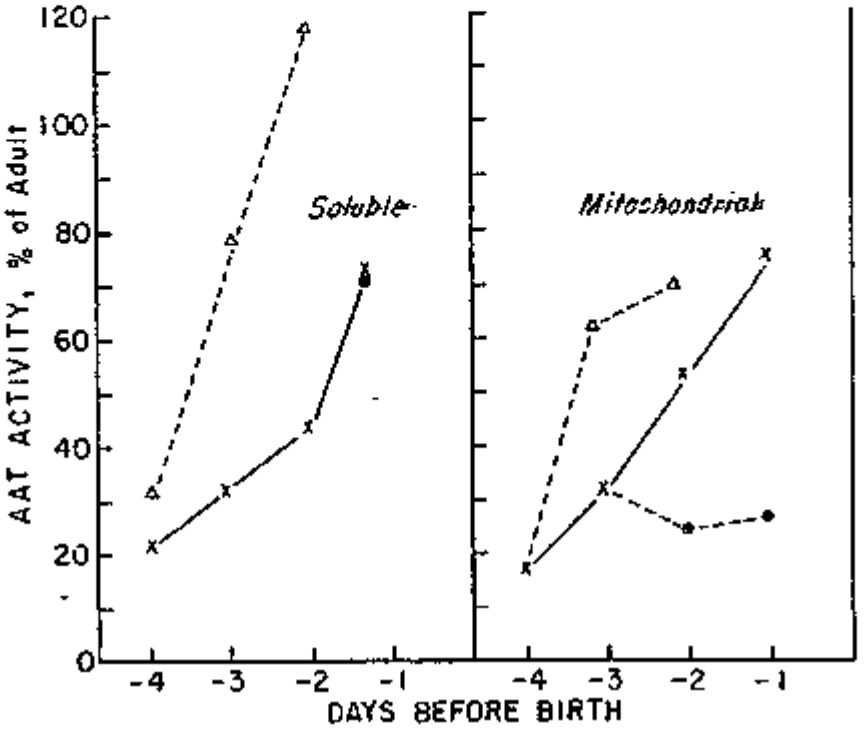

Fig. 3

Fig. 4