Enzymic and Morphological Differentiation
of Rat Liver

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Running title: Hepatic Differentiation

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INTRODUCTION

The metabolic functions and associated enzymic patterns which distinguish mature tissues from each other continue to evolve long after the organs acquire their morphological distinctness. In fact, most of our present knowledge about the mechanism of enzyme differentiation comes from investigations of mammalian organs at rather advanced stages of development; namely, during late gestation and early postnatal life. Work in this area received considerable impetus in the sixties from Dawkins (1966), who suggested that delays in the adaptation of the enzymic equipment to extrauterine conditions may be a frequent cause of perinatal mortality and of permanent defects in surviving babies, particularly in premature ones. Soon after that we learned that chemical differentiation of tissues occurs in a series of molecular steps under adaptive controls. The knowledge which has been accumulated since then, especially from studies of developing rat liver, provides guidelines for detecting subtle but physiologically crucial deviations from the normal process of enzymic differentiation. In the last few years we have also learnt to enhance, with the injection of various regulatory substances, the developmental formation of some 20 enzymes in rat liver. The principles which emerged from this type of work are beginning to be evaluated as possible bases of therapeutic applications.

EMERGING ENZYME CLUSTERS AND CYTOMORPHOMETRIC CHANGES IN DEVELOPING LIVER

With few exceptions, the many dozen enzymes that have been studied in rat liver conform to one of four developmental patterns. The enzymes of one group (Cluster I, not discussed in detail here) appear to be mandatory for growth, are present in all early fetal tissues and decrease in amount towards the end of gestation. Most enzymes concerned with specific hepatic functions are distributed among three other clusters. As reviewed previously (Greengard, 1971), the late fetal cluster (II), emerging between the 17th and 21st days of gestation, includes the enzymes of the urea cycle and of glycogen synthesis. The neonatal cluster (III) brings forth much of the equipment for gluconeogenesis and detoxification processes; while the upsurge, in the third postnatal week, of the late suckling cluster (IV) represents enrichment with those enzymes involved in fatty acid synthesis and in the subtle regulation of amino acid and glucose levels in the blood. Typical examples are shown in Fig. 1.

It is reasonable to ask whether the massive synthesis of new enzymes is associated with detectable morphological changes. In what way are changes in the distribution of cell types in liver or subcellular compartments in the hepatocyte related to the emergence of enzyme clusters during the late fetal, neonatal or late suckling periods? Until

recently, the analysis of such possible correlations was precluded by the qualitative, impressionistic nature of the available morphological data. The first quantitative information about the cytocomposition of adult liver became available a few years ago as a result of extensive work by Weibel, Kistler, and Scherle (1966) and Loud (1968). We applied the same stereological method described by these authors to the study of liver throughout late fetal and early postnatal life (Greengard, Pederman, and Knox, 1972; Herzfeld, Pederman, and Greengard, 1973).

As seen in Fig. 1, in whole liver, up to the 18th day of gestation, and thus in preparations used for determining enzyme concentrations, hematopoietic cells (though present in large number) occupy only 35% of the total volume. Before term the mass attributable to hepatocytes increases from about 60 to 85%. Clearly, several-fold increases during this time in the concentration of enzymes per gram liver cannot be accounted for by this 1.4-fold enrichment with hepatic tissue proper. Thus, upsurge of the late fetal cluster of enzymes and, of course, that of the two postnatal clusters, represents intracellular differentiation, that is, changes in the composition of the hepatocytes themselves.

Between fetal and adult life the total number of cells per gram liver decreases from about 11×10^8 to 2×10^8 . Hematopoietic involution accounts for part of this decrease.

The rest is the 4-fold enlargement of the mean volume of the hepatocytes themselves. It occurs in two major steps, at term and between the 12th and 28th postnatal days (Greengard, Federman, and Knox, 1972). Because of these changes the expression of enzyme amounts per cell or per milligram DNA can give a distorted picture of the process of biochemical differentiation. For example, enzymes in the hepatocyte cytoplasm which during fetal life increase 3-fold per milligram DNA do not in fact contribute to hepatic, biochemical differentiation because their amount per unit hepatocytic mass remains constant. Similarly, when hepatocytes double in volume after the 12th postnatal day, 2-fold increases in enzyme level per cell do not reflect alterations in the biochemical composition of the hepatocytes' cytoplasm. In general, then, as long as activities are measured in aliquots of whole liver rather than in physically separated, homogeneous cell populations, expression per gram tissue (or per milligram protein) is the safest way of avoiding misinterpretations.

Some constituents may be concentrated in Kupffer's cells. It should be noted that, because of hematopoietic involution and the enlargement of hepatocytes, the relative number of Kupffer's cells in liver rises between fetal and adult life from about 4 to 35% of the total. But they never occupy more than 3% of the liver mass and their absolute number

per cm^3 liver does not change significantly with age (Greengard, Federman, and Knox, 1972).

Differences in the subcellular morphology of immature and adult liver have been frequently observed. Such qualitative impressions could not be easily correlated with the precisely timed and quantified changes in enzyme composition. Using the method of electronmicroscopic stereology, we followed changes in mitochondria and endoplasmic reticulum from the 15th day of gestation to adult life, and determined some biochemical parameters in subfractions of homogenates of the same livers (Herzfeld, Federman, and Greengard, 1973). The results show that the volume occupied by mitochondria doubles during the first 2 postnatal days. The increase in the amount of several mitochondrial enzymes per gram liver is much greater than doubling and occurs at various stages of development. Fig. 2 illustrates three mitochondrial enzymes belonging to the late fetal, the neonatal and the late suckling clusters, respectively. It is also apparent from Fig. 2 that elevation of these enzymes does not parallel the increase in the amount of mitochondrial protein. "Marker enzymes" thus characterize the quality but not the quantity of the evolving mitochondrial compartment. Fig. 2

Similar conclusions can be drawn about the endoplasmic reticulum. Enzymes located in this compartment (e.g.,

cytochrome c reductase and glucose-6-phosphatase) rise precipitously during late fetal or neonatal life (Greengard and Dewey, 1968). In contrast, the amount of "smooth ER" rises essentially only in the 2nd to 12th postnatal days and the amount of "rough ER" remains constant throughout the developmental period studied (Fig. 3).

The conclusion from both cellular and subcellular stereological observations is that the sequential emergence of known enzyme clusters is not geared to obvious morphological signs of differentiation. On the 15th prenatal day, the morphological features of the liver are well developed: the parenchymal volume fraction, the mitochondrial compartment and the area of smooth ER have attained 65, 67 and 30% of their adult levels. In contrast, at least three dozen enzymes characteristic of adult liver are still essentially absent. Important as these enzymes are to hepatic functions, they represent a very small fraction of the total protein. Consequently, their acquisition is not reflected by a major change in the size or shape of the respective subcellular compartments.

EXPERIMENTAL ENHANCEMENT OF SELECTIVE GENE EXPRESSION

The timing of the accumulation of different enzymes in developing liver of the healthy rat shows remarkably little biological variation. There is essentially no disagreement

between different laboratories, using different strains of rats, as to whether an enzyme approaches its adult level 1 day after or 1 day before birth. This precise scheduling is easily understandable for enzymes of the neonatal cluster whose synthesis is triggered by the sudden physiological changes associated with the events of birth. For the late fetal and late suckling clusters, the pituitary and its target glands appear to set the pace (Greengard, 1971).

Our approach to the mechanism of enzymic differentiation has been to attempt to identify specific, physiological substances responsible for triggering the synthesis of individual enzymes or particular groups of enzymes. It seems that the competence of the liver to respond develops considerably before the normal time of action of these natural triggers. Indeed, prematurely delivered animals could not survive if the liver was not "ready", sometime before term, to react to stimuli associated with the event of birth. It is due to this latitude in "readiness" that we can evoke, with various experimental stimuli, the precocious formation of some 20 enzymes. Injection of thyroxine and the glucocorticoid, cortisol, enhanced the development of enzymes of the late fetal cluster. The same hormones are likely to be the physiological stimuli for the "spontaneous" accumulation of these enzymes at the scheduled time. Indeed, thyroid and

pituitary adrenocortical activities start in rats around the 16th day of gestation (Jost, 1966).

Several enzymes, including the key catalyst of gluconeogenesis, phosphopyruvate carboxylase (see Fig. 1), which are essentially absent from normal rat liver up to the moment of birth, can be prematurely evoked by an injection of glucagon to fetuses (Greengard and Dewey, 1967; Yeung and Oliver, 1968). Fig. 4 illustrates this with the example of tyrosine aminotransferase: almost adult levels are attained in fetal liver 5 hrs after an injection of glucagon (solid triangle). The normal neonatal rise in tyrosine aminotransferase (open triangles), which coincides with the increase in plasma glucagon levels (crosses), was inhibited by glucose administration (open square). These observations suggest that glucagon, secreted in response to the normal, neonatal state of hypoglycemia, is the natural trigger for the synthesis of several enzymes of the neonatal cluster. (The same assumption is not valid for those enzymes of the neonatal cluster which cannot be evoked by glucagon in the fetus and which are not inhibited by glucose in the neonate).

Fig. 4

None of the enzymes of the neonatal cluster appear to respond to the administration in utero of thyroxine or cortisol. This ineffectiveness of cortisol is particularly striking in the case of tyrosine aminotransferase, whose

synthesis in postnatal liver is greatly enhanced by this hormone. Fig. 4 shows that an injection of glucagon not only evokes this enzyme (almost adult levels are attained in 5 hrs) but also renders the synthetic apparatus competent to respond to cortisol. It may be seen that 24 hrs after a single glucagon injection, the prematurely induced enzyme disappears again but cortisol (which has no effect without pretreatment with glucagon) stimulates its resynthesis. This alternate method of enzyme evocation can be of considerable practical importance. For example, as a result of prenatal glucagon treatment rats would be born with precociously high tyrosine aminotransferase levels; this might be desirable. But these rats would have the serious disadvantage of abnormally low hepatic glycogen levels (Greengard and Dewey, 1970). An injection to such rats of cortisol, which enhances hepatic glycogen deposition (Greengard and Dewey, 1970) as well as the synthesis of tyrosine aminotransferase would neutralize this disadvantage.

In the rat, pituitary-adrenocortical function occurs during late gestation, then is at an ebb from about the 3rd to the 10th postnatal day and increases thereafter (Devine and Mullins, 1966); there is also a considerable rise in thyroid secretion at this time (Hommes, Wilmink, and Richters, 1969). These endocrine changes, as well as the dietary

ones, occurring later with weaning, must regulate the synthesis of enzymes of the late suckling cluster. Some of these enzymes (exemplified by alanine aminotransferase, Fig. 5) can be prematurely evoked with cortisol, while others (e.g., ATP-citrate lyase) accumulate rapidly in response to the administration of glycerol or glucose (Greengard and Jamdar, 1971). Malic enzyme, which normally appears at the age of 18 days, can be evoked 2 weeks earlier with thyroxine treatment (Greengard and Jamdar, 1971; Lockwood, Bailey, and Taylor, 1970). On and after day 18, premature weaning to high carbohydrate diet can enhance the accumulation of this enzyme (Vernon and Walker, 1968). Apparently, the increase of thyroid secretion after the 10th postnatal day (Hommes, Wilmink, and Richters, 1969) not only initiates the synthesis of malic enzyme but confers on the synthetic apparatus the ability to produce more malic enzyme in response to diet.

As mentioned earlier, the successful evoking of enzymes prematurely is due to the fact that the competence of the liver to respond to the final trigger develops significantly before the time this trigger normally appears. A lack of success means either that we have not been able to devine the identity of the stimulus or that the liver was not yet ready to respond to it. The latter problem was reflected by two enzymes of the late suckling cluster, glucokinase and tryptophan oxygenase.

As soon as these enzymes become detectable, i.e., on the 13th to 15th postnatal day, their level can increase precipitously upon the administration of their respective substrates or cortisol. But before this time the synthetic apparatus is not competent to respond to the same regulators. To evoke these enzymes prematurely, treatment with substrate has to be preceded by treatment with cortisol (Greengard and Dewey, 1971; Jamdar and Greengard, 1970). This suggests that increased substrate availability might be the natural trigger for the synthesis of these enzymes but that a prior increase in pituitary-adrenocortical activity (normally on day 10 (Levine and Mullins, 1966)) is a prerequisite for its effectiveness.

The complexities described above are highly relevant to our views on the molecular mechanisms involved in new enzyme synthesis during differentiation. The frequently demonstrated need for more than one (hormonal or nonhormonal) stimulus for the appearance of an enzyme contradicts the assumption that overt gene expression depends on a single, controlling step. Studies of tryptophan oxygenase illustrated that an enzyme may be absent long after its messenger RNA is available, and that the eventual appearance of the enzyme under the action of a second stimulus need not require more, concomitant RNA synthesis (Greengard and Dewey, 1971). For

other enzymes, the emergence of which does require uninhibited RNA metabolism, mRNA synthesis may be the limiting step. The most reasonable assumption, for the present, is that the competence of a tissue to synthesize an enzyme develops in a stepwise manner, under the impact of a series of more or less specific stimuli. Each stimulus facilitates one of the many reactions (at the DNA, RNA or cofactor level) involved in the synthesis of a given protein. The actual emergence of the enzyme pinpoints the time at which all of these reactions have become operative.

PATHOLOGICAL ALTERATIONS IN ENZYMIC DIFFERENTIATION

The ease with which one can experimentally alter (accelerate or delay) the normal course of enzymic differentiation suggests that alterations may also occur "spontaneously" under the influence of moderate deviations from normal environmental conditions. There is essentially no information as yet relating to this important question. The pathological situations so far studied are rather extreme: we determined the levels of some liver enzymes in postnatal animals (18 to 30 days old) which had been X-irradiated or had received a tumor implant at the age of 10 days (Herzfeld and Greengard, 1972). Both conditions, probably because they are associated with stress, enhanced the accumulation of tryptophan

oxygenase, an enzyme which is exquisitely sensitive to glucocorticoid. The behavior of other enzymes varied in different directions in these debilitated rats. Tumor implants inhibited the emergence of enzymes of the late suckling cluster: ornithine aminotransferase, glucokinase, malic enzyme and alanine aminotransferase. Irradiation (300 R) also inhibited the expected rise in glucokinase but not in ornithine aminotransferase activity.

The mechanisms by which the above pathological states alter the evolution of the normal biochemical equipment of the liver is still obscure. It appears that the response of some enzymes to hormones (at least to the high experimental doses) remains normal in these animals. Tumor-bearing almost completely prevented the emergence of ornithine aminotransferase, but cortisol (which evokes this prematurely in normal rats) could reverse this (Herzfeld and Greengard, 1972). The premature induction of malic enzyme by thyroxine was also effective in tumor-bearing suckling rats. Thus, at least in principle, the correction of some enzymic abnormalities is highly feasible.

CONCLUSIONS

Our studies show that, at least during late fetal or postnatal life, periods of explosive enzyme synthesis are not necessarily reflected in changes in the cytochemistry

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of the liver or the fine structure of hepatocytes.

Experimental agents, such as glucagon and thyroxine which can enhance the synthesis of various enzymes, have no effect on these cytological parameters. Therefore, serious delays in functional maturation due, for example, to abnormal influences during late gestation, may not be detectable under the microscope or even the electron microscope.

Enzyme patterns would be the most sensitive and specific indicators of deviations from the normal state of differentiation. Because of our extensive knowledge about the behavior of some four dozen enzymes in the rat (in liver but also in other organs), this species provides a highly suitable model for studying the background and consequences of "biochemical malformations". The rat is also the species where we learnt to identify the natural stimuli of selective gene expression. With the administration of such agents, it is possible to evoke the synthesis of chosen enzymes before the scheduled time and to produce newborn animals (delivered at term or prematurely) with precociously mature enzyme profiles (Greengard, 1969). Phenobarbital prophylaxis for neonatal hyperbilirubinemia, now tested in man, is the outcome of studies in rats demonstrating the premature induction of detoxifying enzymes (Yeung et al., 1971) with this drug. Recent studies in rats and rabbits of the role of cortisol

in lung maturation (Blackburn, Travers, and Potter, 1972; Motoyama et al., 1971) may provide guidelines for ways of preventing the respiratory distress of hyaline membrane disease in newborn infants. The adaptation of newborns to extrauterine life is conditioned by the maturity their biochemical functions have attained during prenatal tissue differentiation. Exploration of the pattern and the experimental enhancement of enzymic differentiation provides direct insight into these crucial processes.

REFERENCES

Blackburn, W. R., Travers, H., and Potter, D. M. (1972):

The role of the pituitary-adrenal-thyroid axes in lung differentiation. I. Studies of the cytology and physical properties of anencephalic fetal rat lung.

Laboratory Investigation, 26:306-318.

Dawkins, M. J. R. (1966): The hazards of birth. Advances

in Reproductive Physiology, 1:217-264.

Girard, J., Bal, D., and Assan, R. (1972): Glucagon

secretion during the early postnatal period in the rat. Hormone Metabolic Research, 4:168-170.

Greengard, O. (1969): Enzymic differentiation in mammalian

liver. Science, 163:891-895.

Greengard, O. (1971): Enzymic differentiation in mammalian

tissues. Essays in Biochemistry, 7:159-205.

Greengard, O., and Dewey, H. K. (1967): Initiation by

glucagon of the premature development of tyrosine aminotransferase, serine dehydratase, and glucose-6-

phosphatase in fetal rat liver. Journal of Biological Chemistry, 242:2986-2991.

Greengard, O., and Dewey, H. K. (1968): The developmental

formation of liver glucose 6-phosphatase and reduced nicotinamide adenine dinucleotide phosphate dehydrogenase in fetal rats treated with thyroxine. Journal of

Biological Chemistry, 243:2745-2749.

- Greengard, O., and Dewey, H. K. (1970): The premature deposition or lysis of glycogen in livers of fetal rats injected with hydrocortisone or glucagon. Developmental Biology, 21:452-461.
- Greengard, O., and Dewey, H. K. (1971): The prematurely evoked synthesis of liver tryptophan oxygenase. Proceedings of the National Academy of Sciences of the United States of America, 68:1698-1701.
- Greengard, O., Federman, M., and Knox, W. E. (1972): Cytochrome of developing rat liver and its application to enzymic differentiation. Journal of Cell Biology, 52:261-272.
- Greengard, O., and Jamdar, S. C. (1971): The prematurely promoted formations of liver enzymes in suckling rats. Biochimica et Biophysica Acta, 237:476-483.
- Herzfeld, A., Federman, M., and Greengard, O. (1973): Subcellular morphometric and biochemical analyses of developing rat hepatocytes. Journal of Cell Biology, 57:475-483.
- Herzfeld, A. and Greengard, O. (1972): The dedifferentiated pattern of enzymes in livers of tumor-bearing rats. Cancer Research, 32:1826-1832.
- Kommes, F. A., Wilmink, C. W., and Richters, A. (1969): The development of thyroid function in the rat. Biologia Neonatorum, 14:69-73.

- Jacquot, R., and Kretchmer, N. (1964): Effect of fetal decapitation on enzymes of glycogen metabolism. Journal of Biological Chemistry, 239:1301-1304.
- Jamdar, S. C., and Greengard, O. (1970): Premature formation of glucokinase in developing rat liver. Journal of Biological Chemistry, 245:2779-2783.
- Jost, A. (1966): Problems of fetal endocrinology: the adrenal glands. Recent Progress in Hormone Research, 22:541-569.
- Levine, S., and Mullins, R. F. (1966): Hormonal influences on brain organization in infant rats. Science, 152:1585-1592.
- Lockwood, E. A., Bailey, E., and Taylor, C. B. (1970): Factors involved in changes in hepatic lipogenesis during development of the rat. Biochemical Journal, 118:155-162.
- Loud, A. V. (1968): A quantitative stereological description of the ultrastructure of normal rat liver parenchymal cells. Journal of Cell Biology, 37:27-46.
- Motoyama, E. K., Orzalesi, M. M., Kikkawa, Y., Kaibara, N., Wu, B., Zigas, C. J., and Cook, C. D. (1971): Effect of cortisol on the maturation of fetal rabbit lungs. Pediatrics, 48:547-555.

- Vernon, R. G., and Walker, D. G. (1968): Adaptive behaviour of some enzymes involved in glucose utilization and formation in rat liver during the weaning period. Biochemical Journal, 106:331-338.
- Weibel, E. R., Kistler, G. S., and Scherle, W. F. (1966): Practical stereological methods for morphometric cytology. Journal of Cell Biology, 30:23-38.
- Yeung, C. Y., Tam, L. S., Chan, A., and Lee, K. H. (1971): Phenobarbitone prophylaxis for neonatal hyperbilirubinemia. Pediatrics, 48:372-376.
- Yeung, D., and Oliver, I. T. (1968): Factors affecting the premature induction of phosphopyruvate carboxylase in neonatal rat liver. Biochemical Journal, 108:325-331.
- Yeung, D., Stanley, R. S., and Oliver, I. T. (1967): Development of gluconeogenesis in neonatal rat liver; effect of triamcinolone. Biochemical Journal, 105: 1219-1227.

FIG. 1. Enzymic and cytological differentiation in rat liver. Broken lines depict the accumulation (units per gram) of typical enzymes of the late fetal cluster, glycogen synthetase (UDPG-glyc-transgluc, EC 2.4.1.11) (Jacquot and Kretchmer, 1964); of the neonatal cluster, phosphopyruvate carboxylase (PEP carboxylase, EC 4.1.1.32) (Yeung, Stanley, and Oliver, 1967); and of the late suckling cluster, glucokinase (EC 2.7.1.2) (Jamdar and Greengard, 1970). The solid lines refer to changes in the fraction of liver volume occupied by parenchymal (●) and hematopoietic (○) cells determined morphometrically (Greengard, Federman, and Knox, 1972). All values are expressed as percent of that in adult liver.

FIG. 2. The development of mitochondrial enzymes in rat liver. The activities of cytochrome c oxidase (EC 1.9.3.1) (X—X), glutaminase (EC 3.5.1.2) (□—□), ornithine aminotransferase (EC 2.6.1.13) (●—●), mitochondrial volume (cm³ per cm³ hepatocyte cytoplasm (- - -) and mitochondrial protein (mg per gram liver) (·-·-·) are expressed as percent of the adult value. The data are from Herzfeld, Federman, and Greengard, 1973.

FIG. 3. The endoplasmic reticulum of liver during development. The surface areas (m^2 per cm^3 hepatocyte cytoplasm) of rough (RER) and smooth (SER) endoplasmic reticulum and the activity (units per gram liver) of glucose-6-phosphatase (G6P-ase, EC 3.1.3.9) (Herzfeld, Federman, and Greengard, 1973) are expressed as percent of that in adult liver.

FIG. 4. Tyrosine aminotransferase (TT, EC 2.6.1.5) and its regulators in fetal and neonatal liver. The two crosses denote plasma glucagon (ng per ml, right ordinate); from Girard, et al., 1972. All other symbols refer to the activity of TT (units per gram liver) as percent of that in adult liver (left ordinate) given as means (bar = 1 SD) of results with 4 to 10 rats. Fetal rats were injected in utero, with hormones (solid symbols) or vehicle (Δ), as previously described (Greengard and Dewey, 1967). Some litters were assayed 5, others 12 or 24 hrs after the administration of 0.25 μ g glucagon (G, $--\blacktriangle--$). Cortisol (Cort, 0.125 mg) was injected 5 hrs before assay to some of the same fetal rats which received glucagon 24 hrs earlier ($\bullet$). (Without such pretreatment, cortisol has no effect on TT in fetal liver (Greengard and Dewey, 1967).) The solid line with open triangles refers to normal rats; the neonatal administration of glucose (2 X 50 mg, $--\square--$) inhibited normal development of TT.

FIG. 5. The premature induction of alanine aminotransferase ("GPT", EC 2.6.1.2). The solid line refers to normal, untreated rats. Hydrocortisone acetate (HC, 0.2 mg, X) was given 24 and 48 hrs before assay.

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