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Diet- and hormone-induced reversal of the carbamoylphosphate synthetase mRNA gradient in the rat liver lobulus

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A hybridocytochemical analysis of adult liver from normal control and from hormonally and dietary-treated rats was carried out, using radioactively-labelled probes for the mRNAs of glutamine synthetase (GS), carbamoylphosphate synthetase (CPS) and phospho*enol*pyruvate carboxykinase (PEPCK). In line with previous findings, GS mRNA is exclusively expressed in a small pericentral compartment, CPS mRNA exclusively in a contiguous large periportal compartment and PEPCK mRNA across the entire porto-central distance. The density of labelling in CPS and PEPCK mRNA-positive hepatocytes decreases in a porto-central direction. Starvation resulted in a reversal of the gradient of CPS mRNA within its periportal compartment; glucose refeeding counteracted this effect. Livers of glucocorticosteroid-treated, starved or diabetic rats also revealed a reversal of the normal gradient of CPS mRNA, but now across the entire porto-central distance. The patterns of expression of GS and PEPCK mRNA remained essentially unchanged, notwithstanding substantial changes in the levels of expression. It is concluded that blood-borne factors constitute the major determinants for the expression patterns of CPS mRNA within the context of the architecture of the liver lobulus.

Carbamoylphosphate synthetase; Phospho*enol*pyruvate carboxykinase; Glutamine synthetase; Enzymic zonation; Hybridization, in situ; Rat liver

1. INTRODUCTION

The mammalian liver has a simple architecture that is uniform throughout the organ. The hepatocytes are arranged in microcirculatory units dubbed acini or lobules, that extend from the upstream portal to the downstream central venules [1,2]. Accordingly, domains of periportal and pericentral hepatocytes are distinguished, though their demarcation is not unequivocally defined in morphological or biochemical terms and has to be specified depending on the metabolic functions examined. Based on the various protein distribution patterns in the adult (rat) liver lobulus, two apparently different types of enzymic zonation can be distinguished [3].

Firstly, in a 'gradient type' of zonation, a protein or mRNA is expressed in all hepatocytes, the highest concentrations being observed in either the periportal or the pericentral hepatocytes. Classical paradigms of this type of distribution are the enzymes involved in glucose uptake and release [4], and albumin and α-fetoprotein [5]. Blood-borne modulatory factors like hormones, substrates and oxygen, are supposed to be the major determinants for the establishment and the dynamics of these gradients [4,6].

Secondly, if a gene can no longer be expressed in all hepatocytes, its expression is said to be confined to a compartment. This distribution is denoted as a 'compartment' type of zonation [3]. Well-documented examples of this type of zonation in rat are the ammonium-metabolizing enzymes GS, exclusively located in a small pericentral compartment [7,8] and CPS, exclusively located in a contiguous large periportal compartment [8,9]. In adult life the compartments are exceptionally stable [10] and, hence, a distinct differentiated state of the hepatocytes within the compartments has been suggested [11].

It only recently became clear that the zonal distribution of a variety of liver-characteristic proteins is primarily regulated at the pretranslational level [5,12-18]. The physiological cues that direct the spatial (and temporal) programs of gene expression in the liver are largely unknown. PEPCK, CPS and GS mRNA are representative examples of mRNAs with a zonation pattern of the 'gradient' type and of the periportal and pericentral 'compartment' type, respectively. It is well-known that the hormonal and dietary status is of major importance for the rate of expression of these mRNAs but not whether these conditions are important for the pattern of expression. Because of their shorter half-life, changes in expression pattern can be more readily established at the RNA rather than at the protein level. We have, therefore, carried out a hybridocytochemical analysis of the distribution patterns of these mRNAs under hormonal and dietary conditions that profoundly alter the rate of expression of the various mRNAs.

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2. MATERIALS AND METHODS

2.1. *Treatment of animals*

3–4-month-old Wistar rats (TNO animal farm; Zeist, The Netherlands) were used throughout the study. Diabetes was induced by intraperitoneal injection of streptozotocin (70 mg/kg body weight) after an overnight fast. One day later animals were checked for glucosuria and, if applicable, glucocorticosteroid hormone (dexamethasone, 2.5 mg/kg body weight/day) was administered 16 h before sacrifice. Starvation was for 3 days and, if applicable, dexamethasone (2.5 mg/kg body weight) was injected 16 h before sacrifice. Glucose (5 g/kg body weight) was administered by gastric gavage after an overnight fast and 2 h before sacrifice. Animals were killed 09.00 h.

2.2. *In situ hybridization*

Livers were fixed in 4% paraformaldehyde buffered in PBS, pH 7.4, for 4 h, quickly frozen in liquid Freon-22 that was cooled with liquid nitrogen, and stored at −70°C until use. 7 μm-thick cryostat sections were made and mounted on 3-aminopropyltriethoxysilane-coated microscope slides [19]. The sections were pretreated, hybridized and processed for autoradiography as described previously [13,18,20].

As probes for the detection of GS, CPS and PEPCK mRNA, the 663 bp DNA insert of the pGS3 cDNA subclone [21,22], several *Pst*I and *Eco*RI fragments of the 5.6 kb pCPS-KdG cDNA subclone [23] and the 1081 bp *Pst*I fragment of cDNA clone PCK-10 [24] were used, respectively. Probes were labelled overnight at 15°C with [α-^{35}S]dCTP to a specific activity of approx. 5–10 × 10^8 cpm/μg, according to the multiprime DNA labelling method [18,25]. The probe concentration was approx. 0.1 ng/μl hybridization solution.

3. RESULTS

The mRNA distribution patterns of GS, CPS and PEPCK are depicted in Fig. 1. As previously shown [13,15], GS mRNA is characteristically localized in a layer of 1–3 pericentral hepatocytes, CPS mRNA is present in the entire contiguous periportal compartment, with a shallow gradient in cellular concentration from portal to central, and PEPCK mRNA is present in all hepatocytes, with a steep fall in the gradient in the middle of the porto-central axis.

Starvation has been reported to result in a decrease in GS mRNA [10] and CPS mRNA [23], and an increase in PEPCK mRNA [15,26,27]. In support, hybridocytochemical analysis (Fig. 2) shows that after prolonged starvation (72 h), GS mRNA is no longer expressed in all hepatocytes of the pericentral compartment, CPS mRNA is expressed less abundantly in its periportal compartment and PEPCK mRNA is expressed to a higher extent, but in a similar pattern when compared to the expression in control liver. However, in these animals, the direction of the gradient of CPS mRNA expression has changed, the highest concentration now being observed at its pericentral boundary of expression. Despite a high expression of CPS mRNA in the hepatocytes close to the central vein it is not expressed in the pericentral hepatocytes that express GS mRNA. Administration of glucocorticosteroid hormone, 16 h before sacrifice of the fasted animals, not only induced CPS mRNA [23], but also extended CPS mRNA expression into the pericentral compartment, resulting in a definite gradient from the central to the portal venule. This treatment did not change the distribution pattern of PEPCK mRNA, but its concentration is reduced, probably due to the glucocorticosteroid hormone-induced increase of insulin [28]. Administration of glucose to starved animals abrogated the effect of starvation on the reversal of the CPS mRNA gradient, resulting in an almost normal distribution in the periportal compartment. GS mRNA remains present in the pericentral compartment, whereas the PEPCK mRNA concentration, as might be expected [26,27], has been considerably reduced.

Comparable, although less pronounced results, were observed in livers of diabetic animals. In these animals CPS mRNA was homogeneously distributed within the periportal compartment (data not shown), whereas administration of glucocorticosteroids resulted in a gra-

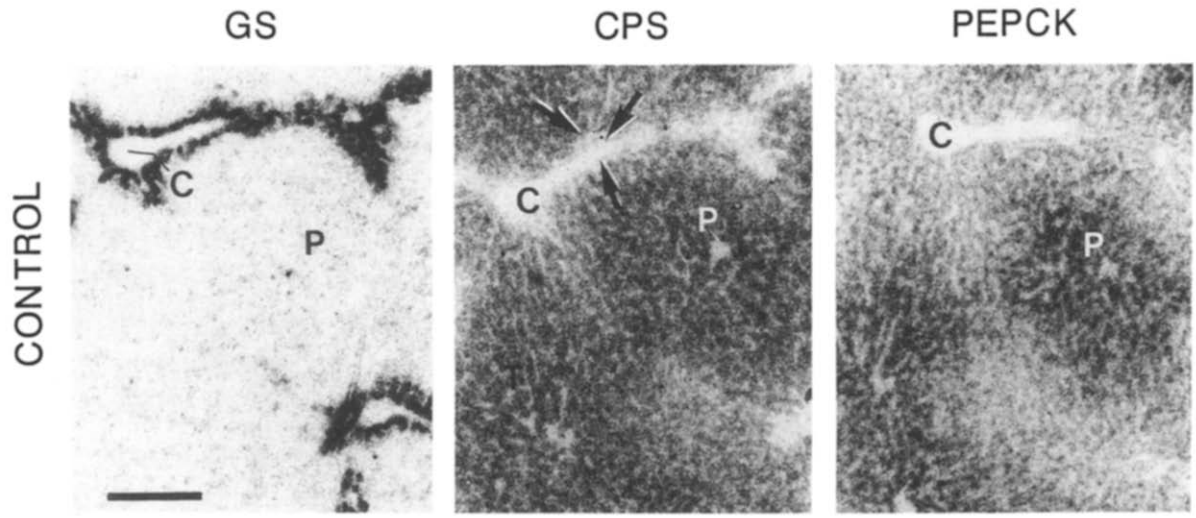

Fig. 1. Distribution of GS, CPS and PEPCK mRNA in the liver parenchyma of normal adult rat. p, portal tract; c, central venule; arrows point to the CPS mRNA-negative pericentral compartment (see for enlargements [13]). Bar = 0.2 mm.

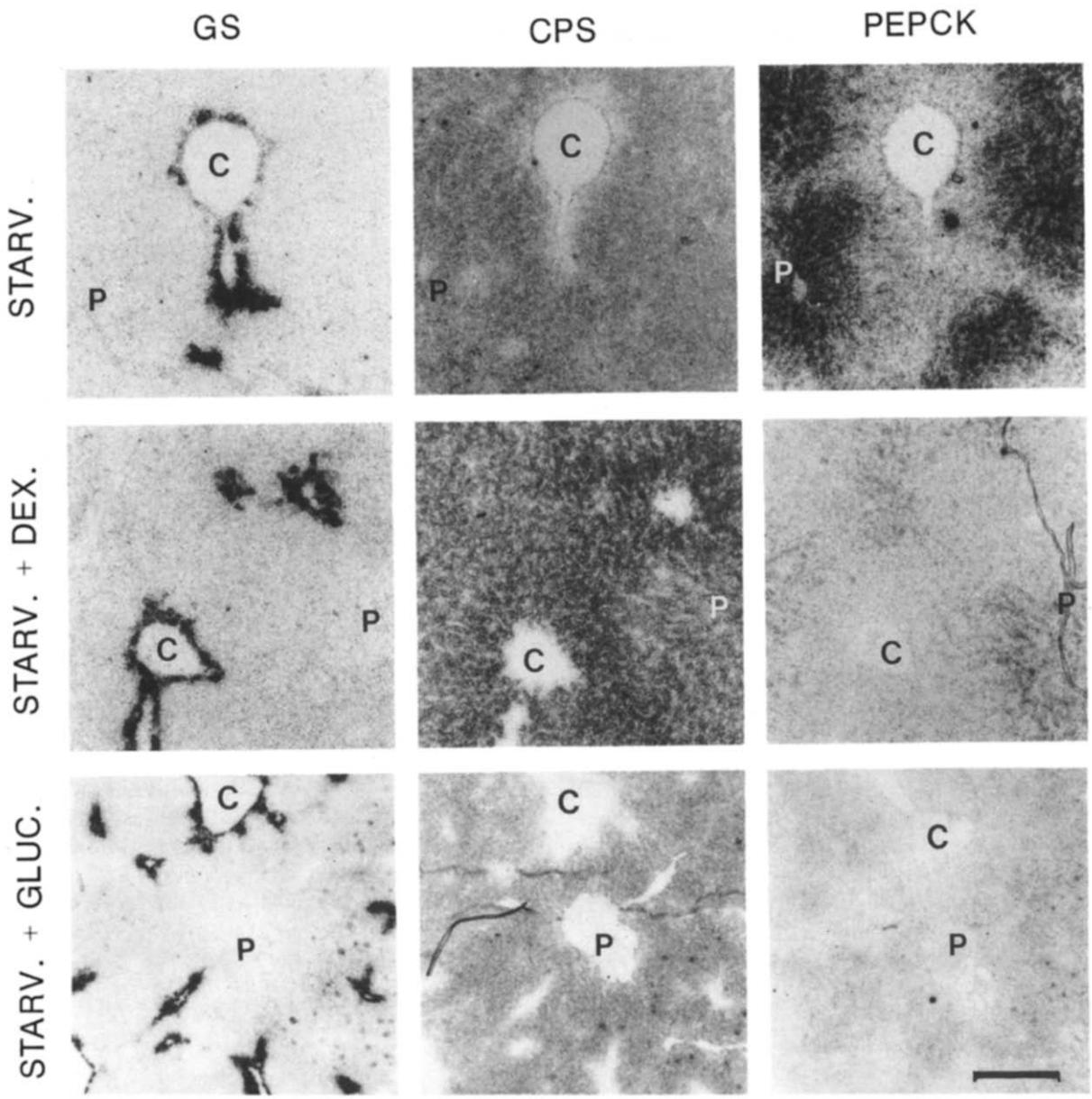

Fig. 2. Distribution of GS, CPS and PEPCK mRNA in the liver parenchyma of starved adult rat (control) and of starved rat treated with dexamethasone or refed with glucose. p, portal tract; c, central venule; Bar = 0.2 mm.

dient of expression in all hepatocytes receding in central-portal direction (Fig. 3). The patterns of GS and PEPCK mRNA were essentially unchanged. It should be noted that we previously showed that the latter condition leads to a homogeneous distribution of CPS protein [10]. Apparently, the decreases in the RNA levels are quenched at the protein level, probably due to the long half life of the protein [29].

4. DISCUSSION

The fundamental problem of how the spatial organization of gene expression is regulated in liver, has been shown to be rather elusive. The evidence presented in this study indicates that changes in the metabolic/hormonal conditions not only can reverse the direction of the gradient of CPS mRNA within its 'own' compartment, but can also expand the CPS mRNA expression over the entire porto-central distance. Under normal conditions, urea synthesis and gluconeogenesis are functionally linked [6], CPS and PEPCK both having a periportal expression pattern [9,30] and both being induced by glucocorticoids and by glucagon via cAMP [26,27,31,32]. Nevertheless, starvation and diabetes result in an opposite response of the spatial expression of the respective mRNAs, suggesting that an additional factor is involved in the expression of CPS mRNA. A change in the ratio of this

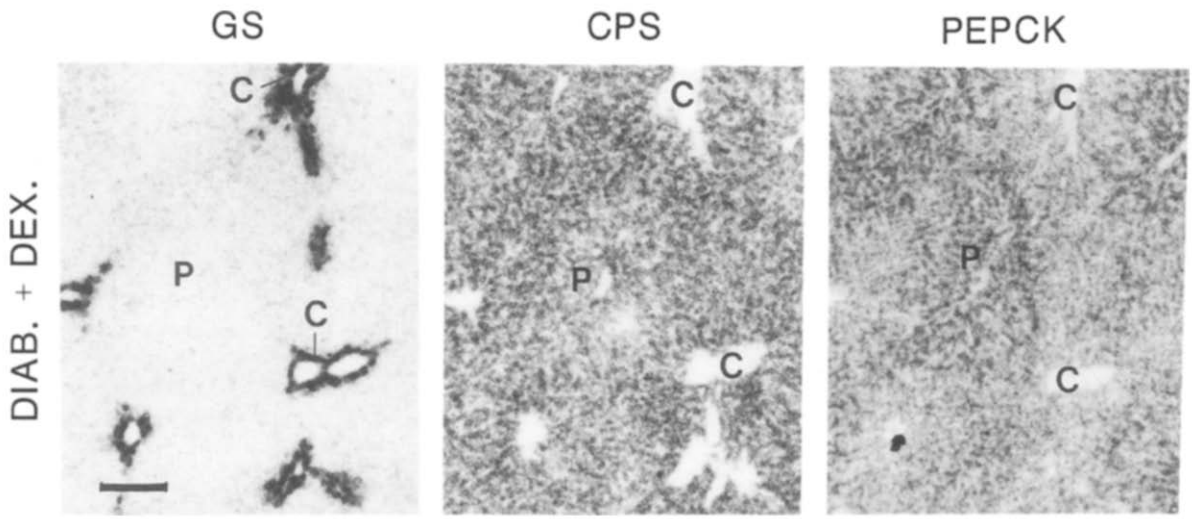

Fig. 3. Distribution of GS, CPS and PEPCK mRNA in the liver parenchyma of dexamethasone-treated diabetic rat. p, portal tract; c, central venule. Bar = 0.2 mm.

factor to cAMP, that induces both mRNAs, or to another factor, within the context of the liver architecture, might cause the opposite response.

CPS mRNA remains confined to the periportal compartment in the liver of starved and diabetic animals, whereas administration of glucocorticosteroids to such animals allows the expression of CPS mRNA in the pericentral compartment as well. The rapid induction of CPS mRNA expression in the pericentral compartment makes it unlikely to be the result of reprogramming of the genome during cell division [33]. These observations may, therefore, point to the presence of a threshold for glucocorticoid-dependent CPS mRNA expression in this compartment.

Finally, the fact that both the observed 'dynamic' zonation of CPS mRNA and the 'gradient' type of regulation are regulated by blood-borne factors, raises the question of whether the characteristic pattern of expression of CPS and GS represents a very stable variant of the 'gradient' type of zonation, or wether it represents the existence of essentially irreversible compartments of gene expression that, as such, may even have to be considered as two distinct differentiated populations of hepatocytes [11]. The available data tend to favor a model in which the expression of CPS and GS has an inherent reciprocal relationship, resulting in two relatively stable compartments of gene expression. Explantation of hepatocytes to fat pads [34] and spleen [35] has clearly demonstrated a reciprocal regulation of GS and CPS that, in liver, is directly related to the hepatic architecture. The reciprocal relationship of CPS and GS expression is demonstrated by the developmental expression of both enzymes. Embryonic rat hepatocytes do not express CPS mRNA [18] and protein in vivo [36], whereas GS mRNA, in turn, is initially highly expressed and becomes gradually confined to the pericentral compartment only after CPS mRNA starts to accumulate [18]. Conversely, in human embryonic liver CPS is expressed immediately after the formation of the liver from the embryonic foregut; GS cannot be detected as long as CPS remains expressed in all hepatocytes [37]. The fact that under appropriate hormonal and dietary conditions CPS mRNA and protein can occupy the entire porto-central distance at the expense of the expression of GS (this study and [10,23]) shows that, in adult liver, the expression pattern of CPS exhibits, in additions, the properties that are characteristic of the 'gradient' type of zonation.

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