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Early Bioenergetic Changes in Hepatocarcinogenesis: Preneoplastic Phenotypes Mimic Responses to Insulin and Thyroid Hormone

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Biochemical and molecular biological approaches *in situ* have provided compelling evidence for early bioenergetic changes in hepatocarcinogenesis. Hepatocellular neoplasms regularly develop from preneoplastic foci of altered hepatocytes, irrespective of whether they are caused by chemicals, radiation, viruses, or transgenic oncogenes. Two striking early metabolic aberrations were discovered: (1) a focal excessive storage of glycogen (glycogenosis) leading via various intermediate stages to neoplasms, the malignant phenotype of which is poor in glycogen but rich in ribosomes (basophilic), and (2) an accumulation of mitochondria in so-called oncocytes and amphophilic cells, giving rise to well-differentiated neoplasms. The metabolic pattern of human and experimentally induced focal hepatic glycogenosis mimics the phenotype of hepatocytes exposed to insulin. The conversion of the highly differentiated glycogenotic hepatocytes to the poorly differentiated cancer cells is usually associated with a reduction in gluconeogenesis, an activation of the pentose phosphate pathway and glycolysis, and an ever increasing cell proliferation. The metabolic pattern of preneoplastic amphophilic cell populations has only been studied to a limited extent. The few available data suggest that thyromimetic effects of peroxisomal proliferators and hepadnaviral infection may be responsible for the emergence of the amphophilic cell lineage of hepatocarcinogenesis. The actions of both insulin and thyroid hormone are mediated by intracellular signal transduction. It is, thus, conceivable that the early changes in energy metabolism during hepatocarcinogenesis are the consequence of alterations in the complex network of signal transduction pathways, which may be caused by genetic as well as epigenetic primary lesions, and elicit adaptive metabolic changes eventually resulting in the malignant neoplastic phenotype.

KEY WORDS: Hepatic preneoplasia; glycogenotic foci; amphophilic foci; mitochondria; peroxisomes; hepatocellular neoplasms.

INTRODUCTION

An aberrant energy metabolism is one of the most consistent features of the malignant neoplastic phenotype (Warburg, 1926; Weinhouse, 1972; Pedersen, 1978; Eigenbrodt *et al.*, 1994). Since the pioneering work of Warburg (1926) it has been well established that the majority of cancer cells is characterized by a high glycolytic glucose consumption even under aerobic conditions. However, the hypothesis propounded by Warburg (1926, 1966) that aerobic glycolysis results from a primary defect in mitochondrial respiration and eventually causes cancer has been refuted by a number of authors who regarded the aberrations in energy metabolism as secondary events appearing only in late stages of neoplastic development (Druckrey *et al.*, 1958; Aisenberg, 1961; Weinhouse, 1972; Weber, 1977). Although there is no reason to revive the original Warburg hypothesis, more recent results provide compelling evidence for a regular association of carci-

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nogenesis with early changes in energy metabolism which seem to elicit a gradual metabolic shift eventually resulting in the malignant neoplastic phenotype (Bannasch, 1984; Eigenbrodt *et al.*, 1994).

Thus, biochemical and molecular biological approaches *in situ* have revealed profound changes in energy metabolism in focal preneoplastic lesions emerging in the liver and kidney long before actual neoplasms, whether benign or malignant, become manifest (Bannasch *et al.*, 1984, 1986, 1997b; Eigenbrodt *et al.*, 1994; Mayer *et al.*, 1997). The present minireview focuses this point to hepatocarcinogenesis because this has been studied most extensively. The validity of this approach is supported by the striking similarities in preneoplastic hepatocellular phenotypes and their neoplastic descendents observed in various experimental models of hepatocarcinogenesis (Bannasch, 1996, Grisham, 1997), and also in man (Altmann, 1994; Bannasch *et al.*, 1997a; Grisham, 1997).

The two most conspicuous features of early focal lesions appearing in the liver parenchyma during hepatocarcinogenesis are an excessive storage of glycogen (glycogenosis), and an accumulation of mitochondria in so-called oncocytes and amphophilic cells. Knowledge how these altered hepatocellular phenotypes are integrated into distinct lineages leading to hepatocellular neoplasms is essential for an understanding of the metabolic changes which are closely linked with the conversion of highly differentiated hepatocytes into poorly differentiated cancer cells.

HEPATOCELLULAR LINEAGES IN HEPATOCARCINOGENESIS

The concept of a progressive dedifferentiation of mature hepatocytes during hepatocarcinogenesis has been substantiated by the discovery that hepatocellular adenomas and carcinomas (HCC) regularly arise from foci of altered hepatocytes (FAH) (Bannasch, 1968, 1996). The earliest types of FAH which emerge are comprised of differentiated populations, which show characteristic metabolic and molecular aberrations, and progress through various intermediate forms to the malignant phenotype.

Based on cytomorphological and simple cytochemical criteria there are at least eight types of preneoplastic FAH which can be induced in rodent liver, which obviously do not develop at random but are integrated within three cell lineages (Fig. 1) leading to hepatocellular neoplasms. The most prevalent sequence of cellular changes starts with glycogenotic clear and acidophilic cell foci and passes through mixed cell populations before formation of glycogen-poor, basophilic (ribosome-rich) neoplastic lesions. The glycogenotic-basophilic cell lineage was originally inferred from light and electron microscopic investigations of livers of *N*-nitrosomorpholine-treated rats (Bannasch, 1968, 1976; Bannasch *et al.*, 1980), and was subsequently confirmed by several stereological studies (Moore *et al.*, 1982; Enzmann and Bannasch, 1987; Weber and Bannasch, 1994a–c), as well as investigations on the proliferation kinetics of the different cellular phenotypes (Zerban *et al.*, 1989, 1994), and a number of cytochemical, microbiochemical, and molecular biological approaches as presented below. Further evidence for this sequence of cellular changes was furnished by results obtained with other models of hepatocarcinogenesis induced in rodents by chemicals (Hirota and Yokoyama, 1985; Steinberg *et al.*, 1991; Bannasch and Zerban, 1997), radiation (Ober *et al.*, 1994), hormones (Dombrowski *et al.*, 1996, 1997), hepadnaviridae (Toshkov *et al.*, 1990; Bannasch *et al.*, 1995), and oncogenic transgenes (Kim *et al.*, 1991; Toshkov *et al.*, 1994). Perusal of liver specimens from patients with HCC or at high risk of HCC because of chronic liver disease suggested that the same lineage is frequently involved in human hepatocarcinogenesis (Altmann, 1994; Bannasch *et al.*, 1997a). The second cell lineage leading to liver neoplasms is characterized by tigroid cells (containing abundant and highly ordered rough endoplasmic reticulum), foci of which apparently originate from enlarged hepatocytes (X-cells) and become transformed into homogeneously basophilic neoplastic cell populations (Bannasch *et al.*, 1985). This sequence of cellular changes probably represents a variant of the predominant glycogenotic-basophilic cell lineage, and is mainly caused by treatment with low doses of chemical hepatocarcinogens (Weber and Bannasch, 1994a).

The third cell lineage differs markedly from these two, and commences with amphophilic cells, characterized by a combination of densely packed granular acidophilic and homogeneously distributed or scattered basophilic cytoplasmic components at the light microscopic level (Weber *et al.*, 1988; Metzger *et al.*, 1995). Foci of such cells are very rare in rodents treated with most hepatocarcinogens but they constitute the main phenotype of FAH induced in rats by nongenotoxic agents eliciting peroxisomal proliferation (Bannasch *et al.*, 1989; Harada *et al.*, 1989). Studies on the sequential appearance and ultrastructure of amphophi-

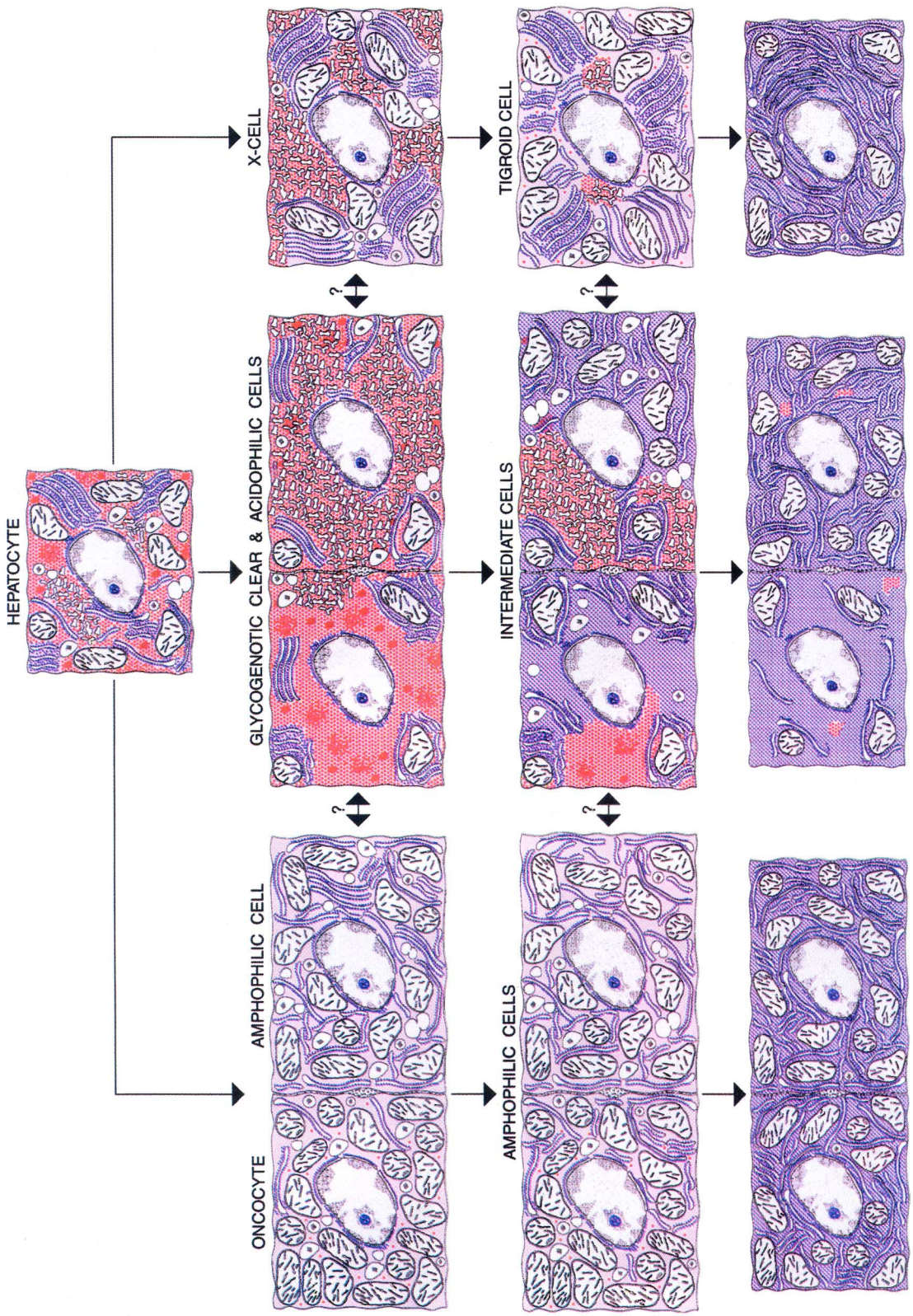

Fig. 1. Schematic diagram of hepatocellular lineages emerging in rodent liver during hepatocarcinogenesis. The predominant sequence of cellular changes (center) starts with glycogenotic clear and acidophilic (smooth endoplasmic reticulum-rich) hepatocytes and progresses through intermediate phenotypes in mixed cell populations to glycogen-poor, homogeneously basophilic (ribosome-rich) cellular phenotypes prevailing in undifferentiated hepatocellular carcinomas. The tigroid basophilic cell lineage (to the right), originating from enlarged hepatocytes (X-cells), is initially characterized by cells with abundant highly ordered stacks of the rough endoplasmic reticulum and apparently represents a variant of the glycogenotic-basophilic cell lineage, occurring especially after low-dose treatment with hepatocarcinogens. The amphophilic cell lineage (to the left), which has hitherto mainly been described in rats treated with nongenotoxic peroxisomal proliferators, and may include oncocytes in woodchucks chronically infected with the woodchuck hepatitis virus, consists of cells with a glycogen-poor cytoplasm containing both abundant granular-acidophilic (mitochondria and peroxisomes) and basophilic (ribosomes) components. Red color, glycogen particles; blue color, ribosomes (from Bannasch, 1997).

lic cell foci induced in rats by the adrenal hormone dehydroepiandrosterone, which is also a peroxisomal proliferator, have revealed that their amphophilic character is due to a marked proliferation of mitochondria wrapped in elements of rough endoplasmic reticulum, mixed to various degrees with increased numbers of peroxisomes (Metzger *et al.*, 1995). Recently, the amphophilic phenotype has also been identified in preneoplastic cell populations occurring during hepadnaviral hepatocarcinogenesis in woodchucks (Bannasch *et al.*, 1995). In the same model, oncocytes crowded with mitochondria have been found in focal preneoplastic lesions (Bannasch *et al.*, 1995) presenting essentially the same features as lesions associated with chronic human liver diseases linked to development of hepatocellular neoplasms (Altmann, 1994).

FOCAL HEPATIC GLYCOGENOSIS

Since the focal lesions occupy a maximum of 5–10% of the parenchyma this precludes biochemical analysis of glycogenotic foci using liver homogenates. However, microbiochemical investigations on individual glycogen storage foci dissected with the aid of a laser beam from freeze-dried tissue sections have revealed an average 100% increase in glycogen as compared to the parenchyma of untreated controls (Klimek *et al.*, 1984). In combination with biochemical studies *in situ* this approach has also demonstrated changes in levels of enzyme activity which constitute a distinct metabolic pattern (Fig. 2). Hepatocellular glycogenosis is generally associated with a disturbance in phosphorylytic glycogen breakdown (Hacker *et al.*, 1982), which is not due to the loss of phosphorylase protein (Seelmann-Eggebert *et al.*, 1987) but rather to a lack of phosphorylation which is apparently the consequence of alterations in superordinate regulatory mechanisms such as a dysfunction of signal transduction, as demonstrated by a reduction in the activity of adenylate cyclase (Ehemann *et al.*, 1986; Mayer *et al.*, 1998). In addition, a reduction in the glucose transporter protein GLUT 2, which is typically expressed in the liver parenchyma of adult rats to facilitate glucose transport at the plasma membrane in both directions, occurs (Grobholz *et al.*, 1993). Moreover, many glycogenotic foci show decreased activity of the microsomal glucose-6-phosphatase (Friedrich-Freksa *et al.*, 1969; Fischer *et al.*, 1987b) and the lysosomal α-glucosidase (Klimek and Bannasch, 1989). Thus, disturbances of phosphorylytic and hydrolytic glycogen breakdown, and of glucose transport at the plasma membrane, appear to act in concert, resulting in an accumulation of glycogen in preneoplastic hepatocytes. An increase in the concentration of glucose-6-phosphate, the central metabolite of carbohydrate metabolism, has been detected in homogenates of livers treated with nitrosomorpholine for 7 weeks (Enzmann *et al.*, 1988), but not in laser-dissected specimens of pronounced glycogenotic foci analyzed by microbiochemical methods.

Of particular interest are the increases in glycogen storage foci in the content or activities of key enzymes of the pentose phosphate and glycolytic pathways, i.e., glucose-6-phosphate dehydrogenase (Hacker *et al.*, 1982; Klimek *et al.*, 1984; Greaves *et al.*, 1986; Moore *et al.*, 1986) and pyruvate kinase (Klimek and Bannasch, 1990), respectively. These findings indicate the beginning of a metabolic shift in glycogenotic hepatocytes toward alternative metabolic pathways. The increased activity of glucose-6-phosphate dehydrogenase is associated with an enhanced level of the enzyme protein as detected by immunohistochemistry (Moore *et al.*, 1986), and with an abundance of the respective mRNA, demonstrated by *in situ* hybridization (Stumpf and Bannasch, 1994). This, and indeed the overall pattern of enzymatic changes in the glycogenotic foci, closely mimics the phenotype of hepatocytes exposed to insulin (Klimek and Bannasch, 1989, 1993; Bannasch, 1996). Alterations in enzymatic functions resembling the effects of insulin were also found in preneoplastic and neoplastic focal lesions induced in rat livers by transduction of retroviral vectors containing activated ras-genes (Pearline *et al.*, 1996). In accordance with these observations, an overexpression of the proto-oncogene c-raf was shown by *in situ* hybridization in glycogenotic and mixed cell foci (Bannasch, 1996), and in hepatocellular adenomas and carcinomas (Beer *et al.*, 1988). c-raf occupies a central position in the complex network of signal transduction pathways, including the insulin-stimulated mitogen-activated protein (MAP) kinase signaling cascade (Daum *et al.*, 1994).

When glycogen storage foci give rise to mixed or basophilic cell foci, adenomas and carcinomas, additional metabolic changes occur (Fig. 3). Glycogen, initially stored in excess, decreases (Bannasch, 1968; Klimek *et al.*, 1984), at the same time being often replaced by an accumulation of neutral lipids (Bannasch, 1976). While the expression of the glucose transporter protein GLUT 2 remains reduced, the fetal isoform GLUT 1 is frequently re-expressed (Grobholz

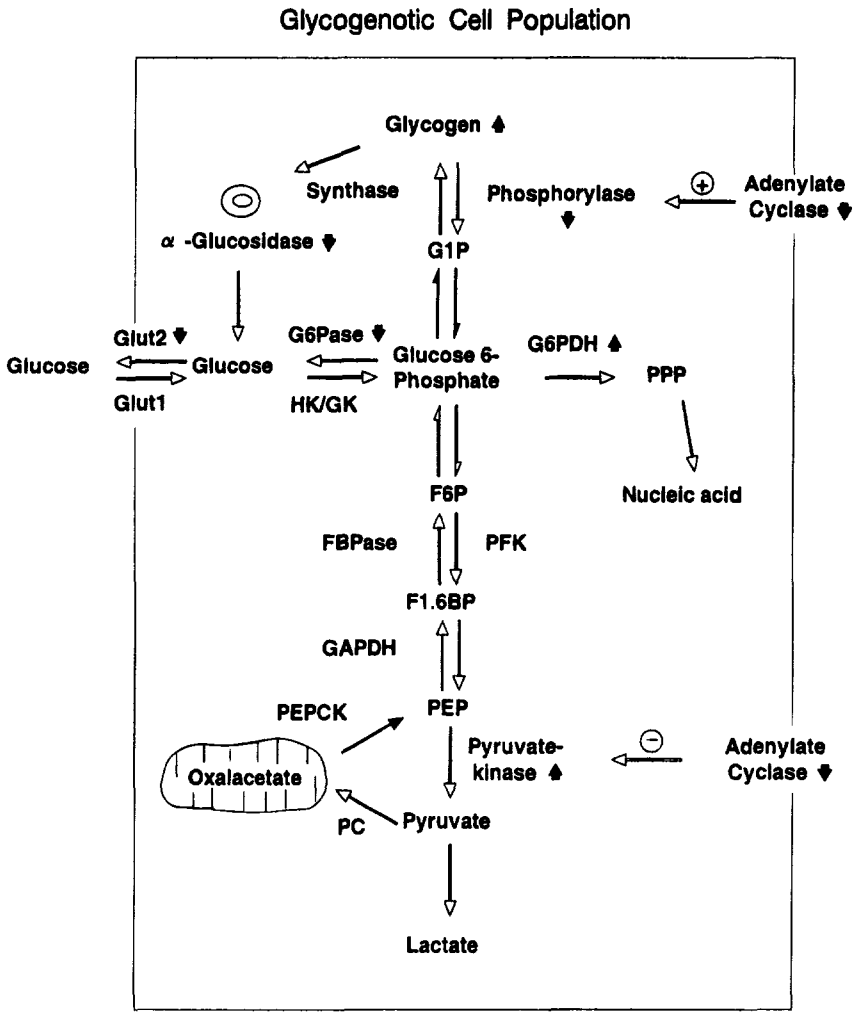

Fig. 2. Schematic diagram of changes in carbohydrate metabolism in glycogenotic preneoplastic cell populations of rat liver. Excessive storage of glycogen is associated with reduced activities of enzymes involved in phosphorylytic (adenylate cyclase, glycogen phosphorylase, G6Pase) and hydrolytic (lysosomal α-glucosidase) glycogen degradation, reduced expression of the bidirectional glucose transporter protein GLUT2 (which is typical for the adult liver), and increased activities of key enzymes of the pentose phosphate pathway (G6PDH) and glycolysis (pyruvate kinase). Adenylate cyclase leads to phosphorylation of glycogen phosphorylase and pyruvate kinase, resulting in activation ⊕ of phosphorylase but inactivation ⊖ of pyruvate kinase. Consequently, reduction in adenylate cyclase activity results in reduced phosphorylase but increased pyruvate kinase activities. FBPase, fructose bisphosphatase; F1.6BP, fructose 1,6-bisphosphate; F6P, fructose 6-phosphate; GK, glucokinase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; G1P, glucose 1-phosphate; G6Pase, glucose-6-phosphatase; G6PDH, glucose-6-phosphate dehydrogenase; GLUT1, glucose transporter protein 1; GLUT2, glucose transporter protein 2; HK, hexokinase; PC, pyruvate carboxylase; PEP, phosphoenolpyruvate; PEPCK, phosphoenolpyruvate carboxykinase; PFK, phosphofructokinase; PPP, pentose phosphate pathway. ↑ Increased content or activity. ↓ Decreased content or activity (from Bannasch, 1996).

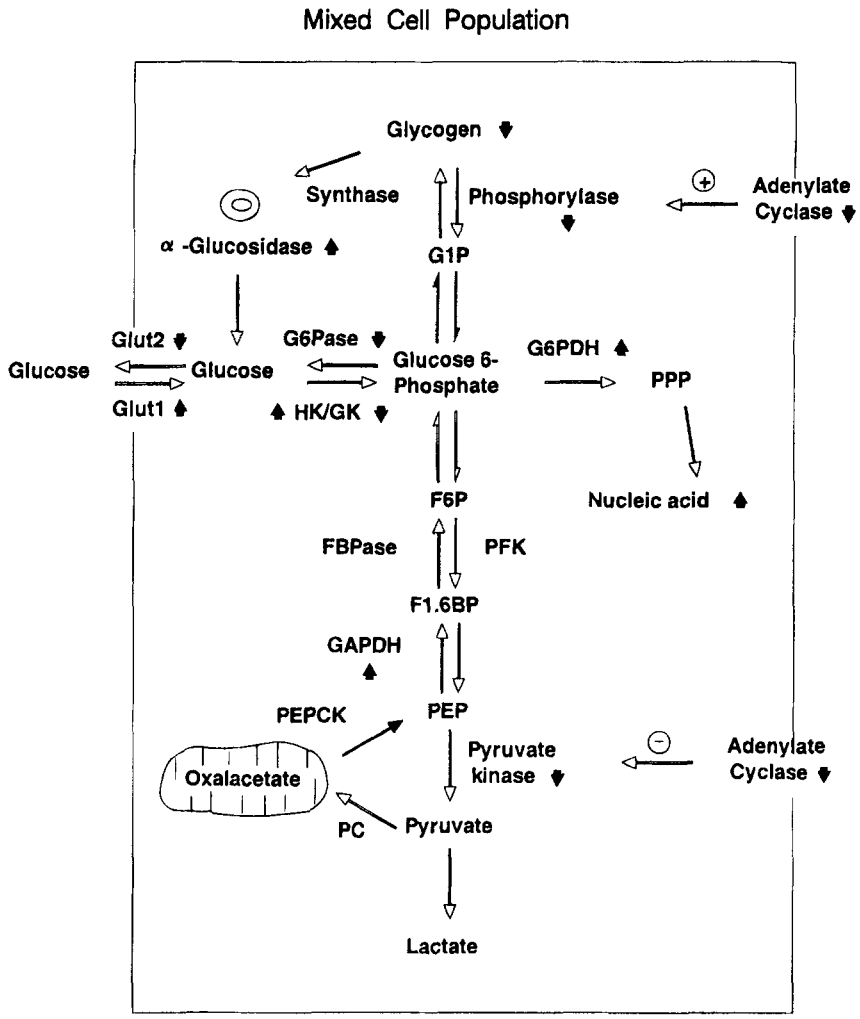

Fig. 3. Schematic diagram of changes in carbohydrate metabolism in preneoplastic or early neoplastic mixed cell populations of rat liver. The predominant metabolic pattern of this cell population is characterized by a reduced glycogen metabolism, a reexpression of the glucose transporter protein GLUT1 (which is strongly expressed in the fetal liver), an isoenzyme shift from glucokinase to hexokinase, and increased activities in enzymes involved in the pentose phosphate pathway and in glycolysis. Adenylate cyclase leads to phosphorylation of glycogen phosphorylase and pyruvate kinase, resulting in activation ⊕ of phosphorylase but inactivation ⊖ of pyruvate kinase. Consequently, reduction in adenylate cyclase activity results in reduced phosphorylase activity. In contrast to glycogenotic foci, this metabolic situation does not lead to an increased activity of pyruvate kinase compared to the normal liver parenchyma because the expression of this enzyme is strongly reduced in mixed cell foci. FBPase, fructose bisphosphatase; F1.6BP, fructose 1,6-bisphosphate; F6P, fructose 6-phosphate; GK, glucokinase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; G1P, glucose 1-phosphate; G6Pase, glucose-6-phosphatase; G6PDH, glucose-6-phosphate dehydrogenase; GLUT1, glucose transporter protein 1; GLUT2; glucose transporter protein 2; HK, hexokinase; PC, pyruvate carboxylase; PEP, phosphoenolpyruvate; PEPCK, phosphoenolpyruvate carboxykinase; PFK, phosphofructokinase; PPP, pentose phosphate pathway. ↑ Increased content or activity. ↓ Decreased content or activity (from Bannasch, 1996).

et al., 1993). The activities of glyceraldehyde-3-phosphate dehydrogenase (Hacker *et al.*, 1982) and α-glucosidase (Klimek and Bannasch, 1989) usually increase, while the content and activity of pyruvate kinase decrease (Fischer *et al.*, 1987a; Klimek *et al.*, 1988; Klimek and Bannasch, 1990). Microbiochemical studies have shown that these changes are accompanied by a drop in glucokinase and an increase in hexokinase activities (Fischer *et al.*, 1987b; Klimek and Bannasch, 1993; Mayer *et al.*, 1997), which thus are not early (Fischer *et al.*, 1987b) but rather late events (Klimek and Bannasch, 1993; Mayer *et al.*, 1997) in hepatocarcinogenesis. Hepatocellular carcinomas mainly express hexokinase II (Rempel *et al.*, 1994a,b; Mathupala *et al.*, 1995) generally bound to an elevated extent to the outer mitochondrial membranes (Rempel *et al.*, 1994b) where direct access to mitochondrial ATP facilitates maintenance of high rates of glycolysis typical of cancer cells (Arora and Pedersen, 1988; Mathupala *et al.*, 1995). Interestingly, the promoter regions of genes of all key glycolytic enzymes including hexokinase II contain response elements for insulin and cAMP (Granner and Pilkis, 1990; Mathupala *et al.*, 1995). The gene of hexokinase II has been shown to be stably amplified in the highly glycolytic AS-30D hepatoma cells (Rempel *et al.*, 1996). Valera *et al.* (1995) provided evidence in a transgenic mouse model for a regulation of hepatic glycolysis by the proto-oncogene c-myc, which is frequently overexpressed in preneoplastic and neoplastic lesions developing in chemical and viral hepatocarcinogenesis (Bannasch, 1996; Grisham, 1997).

Application of microbiochemical techniques has further demonstrated that the level of glucose-6-phosphate dehydrogenase activity progressively increases from glycogenotic foci through the mixed and basophilic cell populations which prevail in adenomas and carcinomas (Klimek *et al.*, 1984). Concomitantly, the growth rate as assessed by incorporation of ^{3}H-thymidine into DNA gradually increases from glycogenotic to mixed/basophilic cell foci (Zerban *et al.*, 1989, 1994). Ledda-Columbano *et al.* (1985) and Moore *et al.* (1986) have reported a positive correlation between the incorporation of ^{3}H-thymidine in the DNA of preneoplastic liver lesions and glucose-6-phosphate dehydrogenase activity, indicating that the expression of this enzyme may be proliferation-linked. This notion has been substantiated by Baba and colleagues (1989) with a combined enzyme histochemical and autoradiographic approach. It is unlikely, however, that the strong overexpression of glucose-6-phosphate dehydrogenase in preneoplastic and neoplastic lesions merely reflects increased cell proliferation, since only a slight increase in enzyme activity is found during the rapid cell replication which occurs after partial hepatectomy (Weber, 1982; Moore *et al.*, 1983). The production of reducing equivalents (NADPH), which are utilized in reductive biosynthetic processes, for example in cholesterogenesis, may be an important prerequisite for cell proliferation (Ledda-Columbano *et al.*, 1985; Moore *et al.*, 1986; Baba *et al.*, 1989). However, this metabolic state is obviously the consequence of a complex pattern of metabolic aberrations starting with the focal glycogenosis. In later stages of hepatocarcinogenesis, metabolites such as glucose 6-phosphate are apparently no longer used for glycogen synthesis but instead become channelled into the pentose phosphate pathway or used for glycolysis, providing energy and precursors for nucleic acid synthesis and cell proliferation (Bannasch *et al.*, 1980; Bannasch, 1996).

Recently, using cytochemical approaches to identify FAH in explanted or resected human livers we have been able to demonstrate focal glycogenotic lesions of the clear and mixed cell types, exhibiting changes in various enzymes, particularly a decrease in the activities of glycogen phosphorylase and glucose-6-phosphate, in all cases with HCC, and in 50% of the HCC-free cirrhotic livers, including those due to chronic HBV and HCV infection (Bannasch *et al.*, 1997a). The prevalence of glycogen-rich clear or ground glass cells in many hepatocellular adenomas, in focal nodular hyperplasia, in cases of so-called adenomatous hyperplasia, in hepatoblastomas, and in clear cell carcinomas is well documented (Altmann, 1994). The available information thus suggests that the predominant sequence of cellular changes leading to hepatocellular neoplasms is, in principle, identical in animals and man, beginning with hepatocellular glycogenosis as proposed years ago (Bannasch and Klinge, 1971).

A further very convincing line of evidence which supports this conclusion is provided by the fact that patients suffering from inborn hepatic glycogen storage disease type 1 due to a genetically fixed defect in glucose-6-phosphatase have an exceedingly high risk of developing multiple hepatocellular neoplasms (Bannasch *et al.*, 1984; Bianchi, 1993). Whereas the tumors are relatively rare in young children, the incidence rapidly increases when the patients pass through adolescence.

As mentioned above, the metabolic pattern and proliferative behavior characterizing the preneoplastic

focal glycogenosis (and lipidosis) resemble in many ways the response of hepatocytes to insulin (Klimek and Bannasch, 1989, 1993; Bannasch, 1996). This hormone stimulates signal transduction pathways involved in a vast array of metabolic processes including glycogen synthesis, lipogenesis, and the regulation of glycolysis, gluconeogenesis, and cell proliferation (Lawrence, 1992; Taub *et al.* 1994; Argaud *et al.*, 1996; O'Brien and Granner, 1996). Indeed, Dombrowski *et al.*, (1994, 1996, 1997) have shown that hepatic foci emerging in streptozotocin-induced diabetic rat livers after isologous pancreatic islet cell transplantation largely resemble the glycogenotic foci induced by oncogenic agents with regard to their biochemical phenotype, and give rise to hepatocellular adenomas and carcinomas after 15 to 22 months. These observations suggest that the high levels of insulin (and possibly other secretory products) derived from the transplanted islets are able to directly induce changes in energy metabolism in the surrounding hepatocytes which result in their eventual neoplastic conversion. In this context, it should be mentioned that a significantly increased incidence of HCC in patients with diabetes mellitus has been reported recently (Adami *et al.*, 1996).

FOCAL HEPATIC AMPHOPHILIA

No glycogenotic prestage has been demonstrated for amphophilic cell foci, even the smallest of which is usually poor in glycogen (Weber *et al.*, 1988; Bannasch *et al.*, 1989; Metzger *et al.*, 1995). They are the prevailing type of FAH after exposure of rats to peroxisome proliferators, but are also frequently observed as preneoplastic lesions in mice treated with various chemical hepatocarcinogens (Ruebner *et al.*, 1997), and in woodchucks chronically infected with the woodchuck hepatitis virus (Bannasch *et al.*, 1995). In rats treated with the peroxisomal proliferator dehydroepiandrosterone, enzyme histochemical (Weber *et al.*, 1988) and ultrastructural investigations (Metzger *et al.*, 1995) have revealed that amphophilic cell foci are characterized by a pronounced mitochondrial proliferation associated with variable changes in peroxisome numbers. This observation points to the mitochondrion as a new target for the analysis of hepatocarcinogenesis caused by peroxisomal proliferators, and is in line with a recent report on a thyromimetic action of several peroxisomal proliferators such as clofibrate and acetylsalicylic acid on rat liver, including changes in mRNA levels for certain genes involved in mitochondrial biogenesis (Cai *et al.*, 1996). A thyromimetic effect of peroxisomal proliferators on the activities of several enzymes such as glycerol-3-phosphate dehydrogenase, malic enzyme, and glucose-6-phosphate dehydrogenase was found in rat liver homogenates and cultured hepatocytes (Hertz *et al.*, 1991, 1993, 1996).

The biochemical phenotype of amphophilic cell foci in rats treated with dehydroepiandrosterone (Fig.4) differs in essential respects from that of the glycogenotic foci. Cytochemical approaches revealed that the glucose-6-phosphate dehydrogenase activity is unchanged in the early amphophilic cell foci, while glucose-6-phosphatase, acid phosphatase, cytochrome *c* oxidase, succinate dehydrogenase, and glycerol-3-phosphate dehydrogenase usually demonstrate increased activity. Hepatocellular adenomas with amphophilic phenotype and highly differentiated carcinomas arising from amphophilic lesions are characterized by elevated succinate dehydrogenase, cytochrome *c* oxidase, glycerol-3-phosphate dehydrogenase, glucose-6-phosphatase, and glucose-6-phosphate dehydrogenase. Similar enzymic patterns were observed in amphophilic cell populations appearing in hepadnaviral hepatocarcinogenesis in woodchucks. Mayer *et al.* (1988, 1996) have shown in the rat that dehydroepiandrosterone exerts a number of effects on carbohydrate metabolism in the liver resulting in a pattern that is in direct contrast to that observed in glycogenotic FAH. In spite of these obvious differences in the phenotype of glycogenotic and amphophilic cell foci, there is circumstantial evidence for a close relationship between the two. Glycogenotic clear cell foci induced by nitrosomorpholine or by dimethylaminoazobenzene in rats often undergo phenotypic modulation with a shift toward amphophilic phenotypes after additional administration of dehydroepiandrosterone (Moore *et al.*, 1988; Weber *et al.*, 1988). Change in the opposite direction has been described by Marsman and Popp (1994) who induced focal lesions with features of amphophilic cell foci (but called "homogeneous basophilic foci") by the peroxisomal proliferator Wy-14,632, which were replaced by clear cell populations after withdrawal of the chemical insult. Finally, a close spatial relationship between glycogenotic clear cell foci and amphophilic cell foci including a variety of intermediate cellular phenotypes frequently occurs in early stages of hepadnaviral hepatocarcinogenesis in woodchucks with and without oral administration of aflatoxin B_1 (Bannasch *et al.*, 1995). Given our very

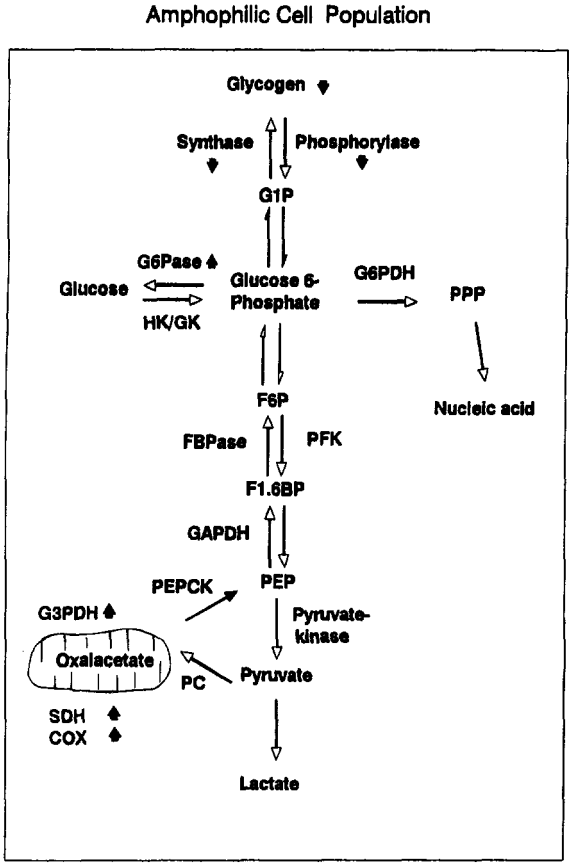

Fig. 4. Schematic diagram of changes in carbohydrate metabolism in preneoplastic amphophilic cell populations of rat liver. Glycogen content and the activity in the two glycogen-associated enzymes synthase and phosphorylase are reduced; the gluconeogenic enzyme glucose-6-phosphatase shows increased activity. The key glycolytic enzymes hexokinase, glucokinase, and pyruvate kinase, and the key enzyme of pentose phosphate pathway, glucose-6-phosphate dehydrogenase, are unchanged in the majority of the lesions. The mitochondrial enzymes glycerol-3-phosphate dehydrogenase, cytochrome *c* oxidase and succinate dehydrogenase show increased activity which is probably due to the strongly increased number of mitochondria in amphophilic cell foci. FBPase, fructose bisphosphatase; F1.6BP, fructose 1,6-bisphosphate; F6P, fructose 6-phosphate; GK, glucokinase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; G1P, glucose 1-phosphate; G6Pase, glucose-6-phosphatase; G6PDH, glucose-6-phosphate dehydrogenase; HK, hexokinase; PC, pyruvate carboxylase; PEP, phosphoenolpyruvate; PEPCK, phosphoenolpyruvate carboxykinase; PFK, phosphofructokinase; PPP, pentose phosphate pathway; G3PDH, glycerol-3-phosphate dehydrogenase; SDH, succinate dehydrogenase; COX, cytochrome *c* oxidase. ↑ Increased content or activity. ↓ Decreased content or activity.

incomplete knowledge of underlying molecular mechanisms, it is impossible to make any categorical statement regarding the interrelation between glycogenotic and amphophilic phenotypes in preneoplastic hepatic lesions, but it is tempting to speculate that crosstalk between different altered signal transduction pathways, such as the insulin-stimulated raf-MAP kinase signalling cascade and the cAMP-dependent PKA signalling pathway, may take place. This applies also to the PKC signaling pathway since PKCα was found to be elevated in nodular liver lesions produced by the Solt/Farber protocol (La Porta *et al.*, 1993). An activation of different isoforms of PKC including PKCα by insulin has been demonstrated in other cell types, namely fibroblasts and adipocytes, most recently (Bandyopadhyay *et al.*, 1997).

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