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Metabolic Regulation and Enzyme Alterations in the Morris Hepatomas[1]

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I would like to supplement the comprehensive talk by Dr. Pitot and the comments of Dr. Weber by pointing out several interesting enzymatic aspects of dedifferentiation associated with neoplasia in rat liver, resulting from studies of the Morris hepatomas. The biochemist who is grappling with the cancer problem has been plagued by his inability to establish, among the many observed metabolic and enzymatic alterations and deletions, which of these are intrinsically associated with the initial neoplastic transformation, which ones are reflections of differences in growth rate or blood supply, and which ones may be due to tumor progression, that is, to postneoplastic changes resulting from inherent instability of the cancer genome.

The work of Dr. Morris demonstrated that liver tumors could be induced in the rat by chemical carcinogens, tumors which were well differentiated by histologic criteria and which retained a number of enzymatic and metabolic characteristics of normal liver; yet they were malignant in the sense that they would kill their hosts, and even metastasize. For the first time the biochemist had available a spectrum of tumors which had definitely undergone the neoplastic transformation but had apparently not proceeded very far along the path of progression and dedifferentiation.

From studies carried out in our own and other laboratories, in collaboration with Dr. Morris (6, 14, 15), it became clear that many enzymes involved in liver function were retained in the slowly growing, well-differentiated hepatomas but were reduced or lost in the more rapidly growing, poorly differentiated liver tumors. These observations were interesting but not too surprising, examples of the molecular basis underlying the loss of function accompanying dedifferentiation. What is more surprising is that loss of differentiation may result not only in a loss in specific liver marker enzymes, but also in some instances may lead to the appearance of new enzymes, either absent or present in low activity in normal liver. I should like to describe three such instances under study in our laboratory. The first example concerns the enzymes which catalyze the initial step in glucose metabolism, namely, its phosphorylation to glucose-6-phosphate. In order to appreciate fully the complex situation with regard to glucose phosphorylation in the tissue of origin, namely, the normal liver, I have outlined some of the factors which regulate glucose phosphorylation in liver in Chart 1.

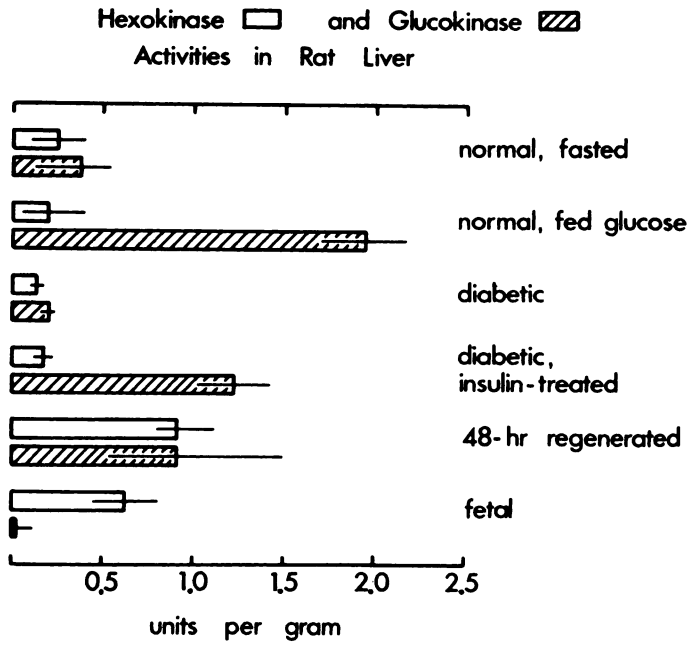

Chart 1. Bar graph showing levels of hexokinase and glucokinase in rat liver under various conditions. Values are in units (μmoles transformed per minute) per gram fresh tissue.

Normal rat liver has a low concentration of three isoenzymes which for convenience are grouped together and called hexokinase (3, 4). Their level remains relatively constant under a variety of nutritional and hormonal conditions. Their properties, in general, are similar to other animal hexokinases. Liver also has a high concentration of a specifically hepatic enzyme called glucokinase. This enzyme has a number of unusual properties among which are a low affinity for glucose, thus making its activity highly responsive to the ambient glucose levels, and a requirement for insulin, making it further responsive to the dietary and hormonal conditions under which the animal exists. Although the hexokinase level, shown in clear bars, does not change greatly under a variety of dietary and hormonal conditions, glucokinase drops markedly on fasting or in diabetes, and essentially recovers its normal level within 24 hours after insulin injection. In regenerating liver, hexokinase is somewhat elevated, but glucokinase is normal. In fetal liver, however,

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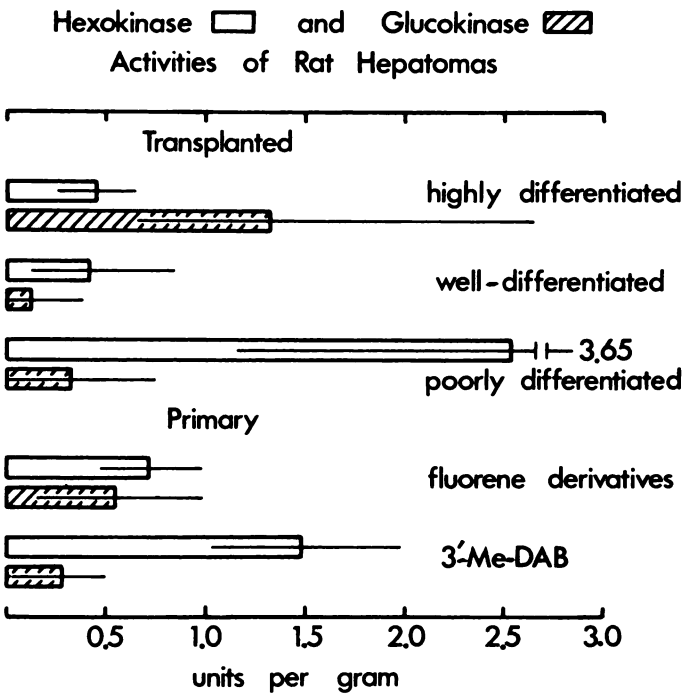

Chart 2. Hexokinase and glucokinase levels in poorly and well-differentiated transplanted and primary rat hepatomas. 3'-MeDAB, 3'-methyl-4-dimethylaminoazobenzene.

glucokinase is almost entirely absent and appears only after about 2 weeks following birth (13).

The levels of these enzymes in liver tumors are delineated in Chart 2. Only two tumors, either in the primary stage or in early generations, have the characteristic low hexokinase—high glucokinase pattern of normal liver. Histologically, these are the most highly differentiated tumors so far observed. In the next segment of this chart we see that all of the well-differentiated Morris tumors have about the same low hexokinase level as liver but have nearly or completely lost glucokinase. Representative primary tumors diagnosed histologically as well-differentiated hepatomas, as shown in the next segment, also have largely, though not entirely, lost glucokinase and have low hexokinase. The most striking change, however, as shown in the last segment, is the complete reversal of pattern in the poorly differentiated tumors. Not only is glucokinase lost, but hexokinase is increased, in some instances far beyond the original total phosphotransferase levels (15). These striking alterations in enzyme pattern have physiologic significance. It was noted previously that the well-differentiated Morris tumors differed sharply from other hepatic tumors studied hitherto in having a very low glycolytic capability. It is clear from the data presented and also has been confirmed (2) that the low glucose phosphotransferase activity is a major factor in this aberration of usual tumor behavior.

Thus, in liver neoplasia, there is a wide diversity of glucose phosphotransferase activity paralleling the diversity in glycolytic capability. What makes high glycolysis possible in the poorly differentiated tumors is that, with loss of differentiation, there is a replacement of a typically liver marker enzyme by a high activity of an enzyme that is normally very low in normal liver.

Chart 3 depicts another example of enzyme reversal occurring with loss of differentiation in hepatomas. Liver aldolase differs from the aldolases of other tissues in molecular structure (7) and is kinetically unique in having equal activity towards two substrates, fructose-1,6-diphosphate and fructose-1-phosphate, whereas other nonhepatic aldolases are about 50 times more active towards the diphosphate (10). The functional role of this enzyme in liver is to break down fructose-1-phosphate, a key step in fructose utilization by liver. The ratio of activities is still equal in regenerating liver, so that rapid growth *per se* is without influence. We see from the next segment of this chart that the aldolases of the well- and highly differentiated tumors have about the same activities towards both substrates; thus they consist at least preponderantly of the liver enzyme. In contrast, as shown in the bottom segment, the poorly differentiated tumor aldolase consists essentially entirely of the nonhepatic type. This is a particularly striking example of a virtually complete replacement of the liver type enzyme by a new molecular species which is not detectable in normal liver (1). The loss of the typical hepatic aldolase from hepatic tumors has also been under study by Schapira *et al.* (8).

A third enzyme is pyruvate kinase, one that may be of particular importance for its regulatory role in glycolysis. This enzyme exists in rat liver in two forms that can be separated by chromatography on DEAE-cellulose. These are designated as Isozymes 1 and 2 in Chart 4. Both forms are always present, but Isozyme 2 is predominant. Isozyme 1 has the same chromatographic behavior as the muscle enzyme and, according to Tanaka *et al.* (11), is nearly or entirely identical therewith. In a single highly differentiated hepatoma, 9618A, the pattern is essentially that of liver, whereas in the two well-differentiated tumors, the 7787 and the 5123, both isozymes are low in activity. In striking contrast, the Novikoff and the 3924A hepatomas, both poorly differentiated, have an overwhelming predominance of the nonhepatic form.

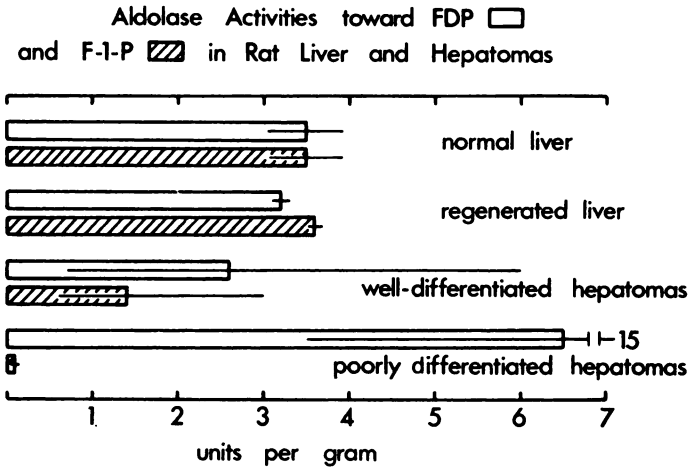

Chart 3. Aldolase activities towards fructose-1-phosphate (F-1-P) and fructose-1,6-diphosphate (FDP) in rat liver and hepatomas.

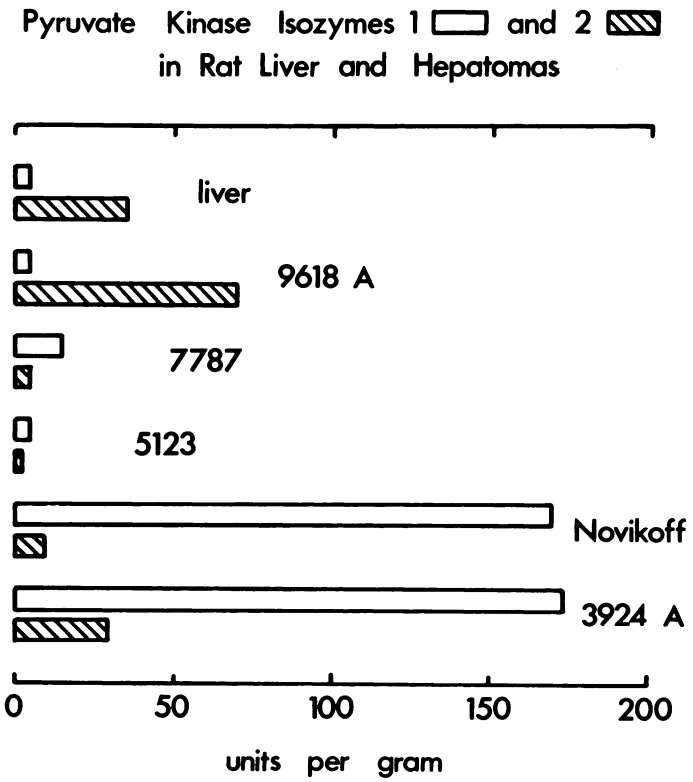

Chart 4. Pyruvate kinase isozymes in rat liver and in certain poorly and well-differentiated rat hepatomas.

Much remains to be learned about the kinetic properties of these forms of pyruvate kinase before we can understand the metabolic significance of these observed alterations. However, the strategic position of this enzyme in the sequence of glycolytic reactions suggests that its level in cells may be a determining factor in regulation of glycolysis. We have already indicated that the low glycolysis in the well-differentiated tumors is attributable to low glucose ATP phosphotransferase activity. There is now good evidence that the high glycolysis of the poorly differentiated tumors is due to high pyruvate kinase activity.

Most biochemists would seek rate-controlling steps at enzyme sites which are lowest in activity; but enzymes may also be rate controlling when highly active if they are in a competitive situation; and the flux of intermediates through the pyruvate kinase step depends on a supply of ADP, for which this enzyme has to compete with other ADP-acceptor enzymes, including the respiratory system. We now have good evidence that the high pyruvate kinase activity plays a major role in the high aerobic glycolysis of the poorly differentiated hepatic tumors (5), and it is plausible to assume that the explanation may apply more generally to all types of tumors which exhibit high glycolysis.

The foregoing data, in conjunction with an already vast accumulation from many laboratories (6) on the Morris tumors, demonstrate that tumors of a single, distinct cell type may have a wide diversity of phenotypes exhibiting a wide range of biologic and biochemical properties. The initiation and progression of cancer cells represent a progressive deterioration of those complex, integrative mechanisms which maintain normal cells under homeostasis. The underlying molecular foundation may be presumed to be a deletion or repression of those enzymes which are uniquely structured for hepatic function and, in certain instances, their replacement by new enzyme species not subject to such controls. At present no clue exists as to the nature of this phenomenon. However, if they do not advance our knowledge of the nature of neoplasia, these studies provide some criteria for distinguishing at least some of the initial stages of neoplasia from the subsequent stages. From this perspective the high aerobic glycolysis, once considered a *sine qua non* of cancer, may be looked upon as a late stage in the evolution of the neoplastic process.

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