Different Deviation Patterns of Carbohydrate-metabolizing Enzymes in Primary Rat Hepatomas Induced by Different Chemical Carcinogens

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ABSTRACT

The deviation patterns of nine enzymes of carbohydrate metabolism were examined simultaneously in the individual hyperplastic liver nodules and primary hepatomas induced by N-2-fluorenylacetamide, diethylnitrosamine, and 3'-methyl-4-dimethylaminoazobenzene, and the activities were considered according to the histological classification of the tissues. With decreased histological differentiation, the liver-specific marker enzymes of gluconeogenesis, glucose-6-phosphatase and fructose-1,6-bisphosphatase, as well as the hepatic isozymes of glucose-adenosine 5'-triphosphate phosphotransferase, pyruvate kinase, aldolase, and glycogen phosphorylase, decreased gradually and were replaced with the respective nonhepatic fetal or prototypic isozymes of undifferentiated tissues. In poorly differentiated hepatomas, glyco- gen synthase was also decreased and phosphofructokinase was increased. Glucose-6-phosphate dehydrogenase increased remarkably from nodular hyperplasia with no relation to differentiation. The degree of enzyme deviation from the adult male liver diameter among hepatomas induced by the different carcinogens, especially between those induced by N-2-fluorenylacetamide (or diethylnitro- samine) and 3'-methyl-4-dimethylaminoazobenzene, reflecting the difference in potential of the three carcinogens to induce the more dedifferentiated hepatoma. These differences were most evident in the isozyme deviation patterns of glucose-adenosine 5'-triphosphate phosphotransferase and pyruvate kinase. Some of the nodules and hepatomas retained exceptionally high activities of some hepatic isozymes, especially of the liver type of pyruvate kinase, near or above the normal level.

INTRODUCTION

It has been well demonstrated in the rat transplantable hepatomas, especially in the Morris hepatomas (16, 17), that liver marker isozymes decrease and nonhepatic fetal or prototypic isozymes of undifferentiated tissues increase as growth rate increases and dedifferentiation proceeds; many enzymes deviate from the normal adult (male) liver to a general common pattern similar to that in the fetal liver or in the whole early embryo (4, 30, 31, 48). This carcinofetal alteration is one of the most striking characteristics of phenotype alterations during carcinogenesis and has been observed also with many tumor-associated antigens, such as α-fetoprotein and carcinoembryonic antigen (3). Although the phenotypes, including isozyme pattern, in the transplantable tumors are known to be fairly stable through generations of transplantation (1, 17, 50), in certain instances some phenotypes in transplantable hepatomas alter gradually or suddenly along with increased growth rate during successive transplantations (1, 22, 25, 50). However, it is also strongly supposed that enzymes in transplantable hepatomas do not deviate simultaneously or in ordered progression as they do in embryonic development. Some enzymes, such as those in amino acid metabolism (10, 20), in the urea cycle, and in the synthesis of nucleic acid precursors (13, 23, 41), deviate apparently at random with no relation to growth rate or differentiation. Although key enzymes of glycolysis and gluconeogenesis show common ordered deviation patterns with increased growth rate and decreased differentiation (47), enzymes such as lactate dehydrogenase (26) and glucose-6-phosphate dehydrogenase (21, 46, 47) deviate apparently at random with no relation to growth rate or differentiation. We also reported that some Yoshida ascites hepatomas, although poorly differentiated, have exceptionally predominant activities of the liver-type isozyme of phosphorylase (28). Therefore, it is very important to extend the findings hitherto obtained with transplantable hepatomas to ascertain, in the precancerous states of the liver and primary hepatomas, to what degree these enzyme alterations are ordered, how specific they are to hepatomas, and at what stage they are fixed during hepatocarcinogenesis. Such studies on enzyme alterations in the precancerous states and primary hepatomas are necessary not only to understand mechanisms of hepatocarcinogenesis but also for clinical application, e.g., for enzymatic diagnosis on biopsy specimens. In this study we measured simultaneously the activities of 9 enzymes of carbohydrate metabolism, and in 4 of these the isozyme activities were measured kinetically and immunochemically in individual liver nodules and primary hepatomas induced by 2-FAA,3 DENA, or 3'-Me-DAB. In this paper we confirm in primary hepatic nodules and hepatomas the same general enzyme deviation patterns that are seen in transplantable hepatomas. We also observed differences among the 3 carcinogens in their potential to induce more dedifferentiated hepatomas with marked deviation. We also found that in some nodules and hepatomas the liver-specific isozyme of some enzymes was retained, whereas in most other nodules and hepatomas the enzyme pattern deviated from that of the liver.

1 This study was supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Education, Science, and Culture.
2 Present address: Second Department of Surgery, Hirosaki University School of Medicine.
3 The abbreviations used are: 2-FAA, N-2-fluorenylacetamide; DENA, diethylnitrosamine; 3'-Me-DAB, 3'-methyl-4-dimethylaminoazobenzene.

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Animals and Tumors. Sprague-Dawley rats were obtained from Oriental Yeast Co., Ltd., Tokyo, Japan, and were bred in a closed colony on our medical school farm. Only male rats were used in the experiments.

The Yoshida ascites hepatoma AH 130 (19) was maintained in Donryu or Sprague-Dawley rats by i.p. implantation in our laboratory and was used within 6 to 8 days after inoculation.

The administration of the following chemical carcinogens was started when the male rats weighed approximately 180 g (7 weeks of age). 2-FAA, DENA, and 3'-Me-DAB were used as carcinogens. For induction of mainly hepatomas by 2-FAA, the rats in the first group were fed a commercial stock diet (Oriental Yeast Co., Ltd., Tokyo, Japan) containing 0.025% 2-FAA continuously for 20 weeks and then fed the normal diet for 4 to 12 weeks, until the liver nodules and hepatomas were large enough for biochemical examination (above 0.5 g). DENA was administered with drinking water to the rats in the second group at a concentration of 0.01% for 3 weeks while rats were fed the Oriental MF diet. Liver nodules and hepatomas appeared after 26 to 37 weeks. 3'-Me-DAB was administered to the third group of rats at a concentration of 0.06% in the Oriental MF diet continuously for 25 to 29 weeks, and the rats were then killed for examination of hepatomas without further feeding of the normal diet. Animals were killed by decapitation and exsanguination. Individual tumors and nodules weighing over 0.5 g (minimum, 0.3 g) were immediately isolated on an ice-cold glass plate and chilled in ice-cold 0.9% NaCl solution. A small portion was cut off for histological examination and fixed immediately in 10% neutral formalin. A macroscopically normal portion of each liver bearing tumors or nodules was also resected as a nonnodular area and used for enzyme assay. The absence of nodules or abnormal cell foci was confirmed later microscopically.

Histological Examination. Each specimen fixed with formalin was sent to Nagoya after enzyme assay for histological examination, performed microscopically on routine sections stained with hematoxylin-eosin. The liver nodules, tumors, and the normal portions of the liver then were classified histologically into 5 groups mainly according to differentiation (or dedifferentiation) of the tissues as described by Reuber (25); Group 1, nonnodular area; Group 2, nodular hyperplasia; Group 3, highly differentiated hepatoma (hepatocellular carcinoma); Group 4, well-differentiated hepatoma; and Group 5, poorly differentiated hepatoma. Furthermore, cholangiofibrosis and cholangiocarcinoma were excluded. Livers of normal rats not treated with the carcinogens and of the same age as those treated were used as controls.

Tissue Preparation. After resection of a small portion for histological examination, a portion or all of the nonnodular area, nodule, or tumor weighing 0.5 to 1.0 g was homogenized with a Potter-Elvehjem homogenizer with a Teflon pestle to make a 20 or 10% homogenate in 40 mM Tris-HCl buffer (pH 7.4) containing 154 mM KCl, 4 mM EDTA, and 5 mM dithiothreitol. The supernatants obtained by centrifugation at 600 x g for 10 min were used for the assays of glucose-6-phosphatase (EC 3.1.3.2), glycogen synthase (EC 2.4.1.11), and phosphorylase (EC 2.4.1.1). The supernatants obtained by centrifugation at 105,000 x g for 45 min were used for the assays of other enzymes.

Enzyme Assay. Hexokinase (EC 2.7.1.1) and glucokinase (EC 2.7.1.2) (glucose-ATP phosphotransferases) were assayed kinetically according to the methods of Sato et al. (29). Phosphofructokinase (EC 2.7.1.11), fructose bisphosphate aldolase (EC 4.1.2.13), pyruvate kinase (EC 2.7.1.40), glucose-6-phosphate dehydrogenase (EC 1.1.1.49), glucose-6-phosphatase, and fructose-1,6-bisphosphatase (EC 3.1.3.11) were assayed according to the methods of Kemp (11), Blostein and Rutter (2), Tanaka et al. (39), Taketa and Watanabe (38), Kode and Oda (12), and Taketa and Pogell (35), respectively. Assays with NADP reduction of NADH oxidation at 340 nm were carried out with a recording Hitachi Model 100, type 10 spectrophotometer in an air-conditioned room at 23° to 25°. Glycogen synthase and phosphorylase were assayed as described previously (27, 28). One unit of all enzymes is defined as the amount of enzyme that catalyzes 1 µmol of substrate per min at the temperature described in each method. Enzyme activity was expressed routinely as units per g, wet weight, of tissue, but the degree of tissue dilution through homogenization was confirmed by determining protein concentration of homogenate. Protein concentration was determined according to the method of Lowry et al. (14). Glycogen was measured by the anthrone method (8).

Quantitative Determination of Isozyme Activities. For determination of the degree of isozyme alteration during hepatocarcinogenesis, the activities of the liver-specific isozymes and the nonhepatic prototypic isozymes of the following 4 enzymes were measured separately. The respective activities of glucokinase (liver-specific high-Km isozyme) and hexokinase (nonhepatic low-Km isozyme) of glucose-ATP phosphotransferases were measured kinetically as mentioned previously. The activity of aldolase A [muscle type (M)] was measured according to the method of Endo et al. (5) with the use of the specific antibody against aldolase A. The activity of aldolase B [liver type (L)] was measured by the difference between the total activity assayed with 2 mM fructose bisphosphate as substrate and the activity of aldolase A. Similarly, the activities of the liver (L) type and the nonhepatic M2 type (prototype) of pyruvate kinase were measured according to the method of Yanagi et al. (49) with the specific antibody against the muscle type (M2) of pyruvate kinase. As described by Yanagi et al. (49), an excess of this antibody inhibited about 97% of the activity of the M2 type. The activities of the liver type and fetal type (brain type) of phosphorylase were also measured with the use of the specific antibody against the fetal type or the liver type, as described previously (28). The isozyme compositions of these enzymes were confirmed by electrophoresis.

Preparation of Antibodies. The antibodies against aldolase A, pyruvate kinase M2, and liver- and brain-type phosphorlyases were prepared according to the methods of Ikehara et al. (9), Tanaka et al. (39), and Sato et al. (27, 28), respectively.

Chemicals. UDP-[U-14C]glucose and [U-14C]glucose-1-phosphate were purchased from New England Nuclear, Boston, Mass. Substrates, adenine nucleotides, NADP+,
NADH, and auxiliary enzymes were the products of Boehringer Mannheim, Mannheim, West Germany. 2-FAA was a product of Nakarai Chemicals, Ltd., Osaka, Japan, and 3'-Me-DAB was a product of Tokyo Kasei Industries, Ltd., Tokyo, Japan. Other chemicals were products of Wako Pure Chemical Industries, Ltd., Tokyo, Japan.

RESULTS

Histological Classification. The incidence and histological classification of hyperplastic liver nodules and hepatomas induced by 2-FAA, DENA, and 3'-Me-DAB are summarized in Table 1. Enzyme assays were carried out on all the samples indicated in Table 1. After administration of 2-FAA, nodular hyperplasia and highly and well-differentiated hepatomas were induced predominantly, and poorly differentiated hepatomas were induced very rarely. In contrast, poorly differentiated hepatomas together with cholangiocarcinoma were induced predominantly by administration of 3'-Me-DAB; neither nodular hyperplasia nor highly differentiated hepatomas were found by our schedule of 3'-Me-DAB administration at the stage when tumors occurred. The types of liver lesions induced by DENA administration ranged widely from nodular hyperplasia to poorly differentiated hepatoma.

Isozyme Alteration during Hepatocarcinogenesis. Charts 1 and 2 clearly show that the hepatic isozymes glucokinase, pyruvate kinase L, aldolase L (or B), and phosphorylase L were already somewhat reduced in the nonnodular areas of livers bearing tumors. They progressively decreased further as dedifferentiation proceeds from nodular hyperplasia to poorly differentiated hepatoma, while, on the other hand, the nonhepatic prototypic isozymes hexokinase, pyruvate kinase M2, aldolase M, and phosphorylase F increased very gradually with decreased differentiation. A similar pattern was already reported by Shatton et al. (33) for glucose-ATP phosphotransferase isozymes (glucokinase and hexokinase) of the Morris hepatomas. Comparison of Charts 1 and 2 shows there was no remarkable difference between isozyme deviation patterns induced by 2-FAA and DENA. In the exchange between the hepatic and nonhepatic isozymes, the latter did not completely replace the activities of the former; therefore the sums of the activities of hepatic and nonhepatic isozymes were lowered below the normal liver levels, even in the poorly differentiated hepatomas induced by 2-FAA and DENA. However, except for phosphorylase, the nonhepatic isozymes were increased markedly in the...
poorly differentiated hepatomas induced by 3'-Me-DAB, approaching the patterns observed in the Yoshida ascites hepatoma AH 130, one of the maximally deviated, poorly differentiated hepatomas. In these hepatomas the nonhepatic isozymes are the sole type, with extremely high activities of hexokinase, pyruvate kinase M₂, and aldolase M. Although the activities of the nonhepatic isozymes of these 3 enzymes were not as remarkably increased in our primary hepatomas as in transplantable hepatomas such as the Morris hepatomas (6, 48), they did increase significantly from the hyperplastic nodules with decreased differentiation. One exception was noted in Chart 2, that the activities of hexokinase and pyruvate kinase M₂ in the hyperplastic nodules were higher than those in the highly differentiated hepatomas, in carcinogenesis by DENA.

**Different Deviation Patterns of Isozymes among Carcinogens.** In Charts 3 to 5, the relationships between activities of the hepatic type and nonhepatic prototypic isozymes in the individual nodules and hepatomas are shown by individual symbols denoting the 3 carcinogens, but without regard for tissue differentiation. The symbols are distributed widely, ranging from high hepatic and low nonhepatic to low hepatic and high nonhepatic isozyme activities. However, in general, it seems that the activities of the hepatic isozymes are decreased whenever the activities of the nonhepatic isozymes are increased and vice versa. This relationship is most evident for glucokinase and hexokinase (Chart 3) and for pyruvate kinases L and M₂ (Chart 4). The glucokinase and pyruvate kinase L activities in hyperplastic nodules induced by 2-F FA and DENA ranged from those of normal livers to those of highly or poorly differentiated hepatomas but had low hexokinase and pyruvate kinase M₂ activities close to those of normal livers and highly and well-differentiated hepatomas. The same relationships hold, but are less marked, for aldolases and phosphorylases (Chart 5).

It is also noticeable in Charts 3B and 4B that, despite some exceptions, isozyme deviation patterns for the phosphotransferases and pyruvate kinases in the nodules and hepatomas seem to distribute within a limited range characteristic of each carcinogen. Table 2 shows clearly that differences in isozyme deviation patterns induced by the 3 carcinogens were statistically significant, irrespective of the degree of histological differentiation of the resultant tumors. The hepatomas induced by 2-F FA deviated least, those induced by 3'-Me-DAB deviated most, and those...
induced by DENA deviated intermediately between those induced by 2-FAA and 3'-Me-DAB.

Other Enzyme Deviation Patterns. The deviation patterns of other enzymes in hepatocarcinogenesis by 2-FAA are summarized in Chart 6. Similar patterns were observed in carcinogenesis by DENA (not shown). As dedifferentiation proceeds during hepatocarcinogenesis, the activities of phosphofructokinase do not change significantly before the stage of hepatoma but change clearly in the poorly differentiated hepatomas when a moderate increase occurs. This enzyme also increases markedly in transplantable hepatomas with increased growth rate and decreased differentiation (45, 47). Gluconeogenic enzymes, such as glucose-6-phosphatase and fructose-1,6-bisphosphatase, and glycogen synthase as well as glycogen content decrease markedly with dedifferentiation reaching a pattern similar to that seen in transplantable poorly differentiated hepatomas.

In contrast, glucose-6-phosphate dehydrogenase increases very markedly from nodular hyperplasia with no relation to differentiation, as pointed out by Weber et al. (46, 47) for transplantable hepatomas. Chart 6 also shows that, although the mean values deviate in accordance with the degree of differentiation, individual tumors vary over a wide range, especially in highly and well-differentiated hepatomas.

Nodules and Hepatomas Exhibiting Abnormal Isozyme Patterns. Despite an overall regularity in the deviation patterns, several exceptions are readily evident from the individual activities depicted in Charts 3 to 5.

The most remarkable examples are seen in Chart 4B, in which 4 nodules and 3 highly differentiated hepatomas exhibited pyruvate kinase L activities much higher than that of normal liver while the M2 activities were in the normal range. Several other nodules and hepatomas, even poorly differentiated, had high activities of the M2 type but still retained activities of the L type near the normal level. Several other nodules and hepatomas (Charts 3B and 5) exhibited the abnormal activities of other isozymes such as glucokinase, aldolase L, or phosphorylase L.

DISCUSSION

The results reported in this paper clearly show that the enzyme deviation patterns in the hyperplastic liver nodules and primary hepatomas are similar to those found for transplantable hepatomas. During hepatocarcinogenesis the liver-specific marker enzymes (or isozymes) decrease gradually and are replaced with the nonhepatic enzymes as dedifferentiation of hepatoma proceeds.

Those enzymes that display deviations in isozyme composition toward fetal patterns with decreased differentiation of transplanted hepatomas (4, 7, 30, 31, 48) also deviate progressively in the same direction during progressive stages in hepatocarcinogenesis. This is evident in Charts 1 and 2 for glucose-ATP phosphotransferases, aldolases, pyruvate kinases, and glycogen phosphorylases. Quantitatively, the deviations in the poorly differentiated primary hepatomas did not reach the extent exhibited by transplanted tumors. This could be due in part to a mixed population of cells in the former, consisting of some well- or poorly differentiated and perhaps some normal cells. This seems unlikely, however, since this would be evident easily by histological examination; moreover, to account for the degree of difference the highly differentiated cells would have to be preponderant. It is more likely that enzymatic dedifferentiation continues with successive transplantsations.

Also the sequential pattern of enzyme expression during hepatocarcinogenesis is the reverse of that seen during embryonic and perinatal development. Key enzymes of gluconeogenesis and glycogen metabolism increase remarkably just before birth (7), and the liver-type isozymes of hexokinase and pyruvate kinase increase rapidly with weaning (15, 43), while the nonhepatic types remain constant. The reverse occurs as the primary tumors progress from the preneoplastic nodules to well-differentiated hepatomas, with rapid decrease in the liver types and relatively constant or gradually increasing non-liver types. The same pattern was observed for aldolase and phosphorylase isozymes. Gluconeogenic enzymes and glycogen content also decreased rapidly as the stage in hepatocarcinogenesis progressed.

Several investigators have already reported on isozyme alterations in the preneoplastic liver and primary hepatomas.
Enzyme Deviation Patterns in Primary Hepatomas

Table 2
Activities of the hepatic and nonhepatic isozymes of glucose-ATP phosphotransferase, pyruvate kinase, aldolase, and phosphorylase in normal rat livers and hepatomas induced by 2-FAA, DENA, and 3'-Me-DAB

Assay procedures and the schedules of carcinogen administrations are given in the text. Activities are expressed in units/g tissue, 1 unit being defined as the amount of enzyme catalyzing 1 μmol of substrate per min.

<table>
<thead>
<tr>
<th>Enzyme Activity</th>
<th>Normal liver (17)</th>
<th>FAA (42)</th>
<th>DENA (17)</th>
<th>3'-Me-DAB (18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose-ATP phosphotransferase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucokinase</td>
<td>2.25 ± 0.52&lt;0.001</td>
<td>0.84 ± 0.64&lt;0.001</td>
<td>0.77 ± 0.57&lt;0.02</td>
<td>0.38 ± 0.15</td>
</tr>
<tr>
<td>Hexokinase</td>
<td>0.42 ± 0.19&lt;0.001</td>
<td>0.85 ± 0.45&lt;0.001</td>
<td>1.27 ± 0.71&lt;0.02</td>
<td>2.15 ± 1.13</td>
</tr>
<tr>
<td>Pyruvate kinase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>55.2 ± 16.5&lt;0.001</td>
<td>20.7 ± 11.8&lt;0.2</td>
<td>17.4 ± 8.9&lt;0.7</td>
<td>15.9 ± 11.1</td>
</tr>
<tr>
<td>M₁</td>
<td>7.1 ± 5.0&lt;0.001</td>
<td>16.6 ± 11.5&lt;0.001</td>
<td>19.9 ± 13.0&lt;0.001</td>
<td>92.5 ± 57.8</td>
</tr>
<tr>
<td>Aldolase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>7.96 ± 1.21&lt;0.001</td>
<td>3.90 ± 1.75&lt;0.001</td>
<td>4.44 ± 1.58&lt;0.001</td>
<td>1.91 ± 1.13</td>
</tr>
<tr>
<td>M</td>
<td>0.80 ± 0.34&lt;0.001</td>
<td>1.14 ± 0.53&lt;0.001</td>
<td>1.71 ± 0.93&lt;0.1</td>
<td>2.51 ± 1.32</td>
</tr>
<tr>
<td>Phosphorylase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>13.25 ± 2.31&lt;0.001</td>
<td>4.95 ± 2.44&lt;0.001</td>
<td>4.23 ± 2.36&lt;0.001</td>
<td>2.10 ± 1.09</td>
</tr>
<tr>
<td>M</td>
<td>0.35 ± 0.39&lt;0.001</td>
<td>2.01 ± 1.20&lt;0.001</td>
<td>4.14 ± 2.14&lt;0.5</td>
<td>3.44 ± 1.36</td>
</tr>
</tbody>
</table>

- Numbers in parentheses, number of samples.
- Statistical significance of the differences between activities of respective isozymes in normal liver and hepatoma induced by 2-FAA.
- Statistical significance of the differences between activities of respective isozymes in hepatomas induced by 3'-Me-DAB and 2-FAA or DENA.
- Mean ± S.D.

Chart 6. The deviation patterns of phosphofructokinase (PFK), glucose-6-phosphate dehydrogenase (G6PDH), glucose-6-phosphatase (G6Pase), fructose bisphosphatase (FDPase), glycogen synthase (GS), and glycogen content with decreased differentiation in the hepatocarcinogenesis by 2-FAA. Each point indicates the value of an individual. Shaded range, S.E.

induced by dimethylaminoazobenzene (or 3'-Me-DAB), 2-FAA, or other carcinogens. Sharma et al. (32) reported that during 3'-Me-DAB hepatocarcinogenesis glucokinase was gradually replaced by hexokinase in the preneoplastic liver and that primary hepatomas and cholangiocarcinomas induced by 3'-Me-DAB retained very low activity of glucokinase and high activity of hexokinase. We also confirmed that cholangiocarcinomas originating from nonparenchymal cells exhibit isozyme deviation patterns similar to those in the poorly differentiated hepatomas (not shown). This similarity is of practical interest because identification must be made by histological examination. Endo et al. (5) reported that, in the early stage of hepatocarcinogenesis induced by feeding of 3'-Me-DAB or 2-FAA (60 days), the activities of aldolase A (M) and pyruvate kinase M [M₂, characterized by Tanaka et al. (40)] were increased and that they persisted at increased levels for a long time after removal of the carcinogen from the diet.

Walker and Potter (44) emphasized the necessity of studies of nodular hyperplasia as a liver precancerous state. They observed the maximum increase of hexokinase, aldolase A, and pyruvate kinase type III (M₂ of Tanaka) between
the second and third months of 3'-Me-DAB feeding, a transitional phase when the oval cells occupy the major population before hyperplastic areas appear (24). Although they did not assay the activities in the individual hyperplastic liver, Yanagi et al. (49) pointed out the significant elevation of pyruvate kinase M2 activity in the individual nodules induced by 2-FAA. In our experiments, although hyperplastic nodules coexisted with hepatomas, significant differences between them were observed in pyruvate kinase isozyme patterns (Charts 1 and 2).

Glucose-6-phosphate dehydrogenase was increased strikingly in all neoplastic tissues, including nodular hyperplasia, apparently with no relation to differentiation, although this enzyme increases in hepatic transformation (46). As pointed out by Weber (46, 47), the increased activity of this enzyme may be specific to the neoplastic transformation, and indeed it represented a more potential marker of preneoplasia than did any other enzymes examined in this study. However, this enzyme is also induced by liver injury (36) and is not always increased in poorly differentiated transplanted hepatomas such as the Yoshida ascites hepatomas according to the report of Ohashi and Ono (21).

In general, it is believed that chemical carcinogenesis proceeds by similar processes irrespective of the carcinogen (18). However, it is also well known that individual hepatomas exhibit morphological characteristics reflecting the chemical carcinogen used for their induction. The mild carcinogen 2-FAA and its derivatives induce many transplanta-ble highly and well-differentiated hepatomas such as Morris hepatomas (16, 17), although they also include some poorly differentiated hepatomas; the strong carcinogen dimethylaminoazobenzene and its derivatives induce mainly poorly differentiated hepatomas such as the Yoshida ascites hepatomas (19, 50). Our results indicate that the degree of isozyme deviation may also differ among carcinogens. Furthermore, even among poorly differentiated hepatomas, the degree of isozyme deviation seems to differ among carcinogens (Charts 1 and 2). The patterns of glucose-ATP phosphotransferase (glucose hexokinase) and pyruvate kinase isozymes markedly differed between 2-FAA (or DENA) and 3'-Me-DAB. In our preliminary experiment dimethylnitrosamine induced isozyme deviation patterns similar to those induced by 3'-Me-DAB. These differences must be confirmed with larger numbers of primary hepatomas in each category and with those induced by different dose and time schedules of the same carcinogen, and they also should be extended to enzymes the activities of which are closely related to growth rate and differentiation.

Judging by mean values, our data show clearly that 9 enzymes deviate with decreased differentiation of the tissues during hepatocarcinogenesis. However, some nodules or hepatomas exhibited an abnormal activity of only some isozyme (Charts 3 to 5; e.g., some nodules and hepatomas in Chart 4B retained much higher pyruvate kinase L activities than that of normal liver while the M2-type activities were in the normal range. However, other enzymes or isozymes in these tumors deviated to a common pattern concomitantly with dedifferentiation of tissues (not shown). The retention of the pyruvate kinase L activity higher than that of normal liver was reported for the Morris 9618A by Farina et al. (6) and recently for human hepatoma by Taketa et al. (37). These characteristic phenotypes of each tumor are not fully understood at present but may be related to gene aberration (48), disdifferentiation (34), or unbalanced retrodifferentiation (42).

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