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REGULATION OF RAT LIVER PHOSPHOFRUCTOKINASE LEVELS IN MORRIS HEPATOMAS

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SUMMARY

The levels of the major liver phosphofructokinase isozyme (PFK-L_2) and the PFK regulatory factor were measured in adult and fetal liver as well as Morris hepatomas of different differentiation states. The results indicate that both the PFK-L_2 activity and the PFK regulatory factor levels in well and highly differentiated hepatomas are not statistically different from the amounts found in adult liver. In the poorly differentiated hepatomas and fetal liver, the levels of both PFK-L_2 and PFK regulatory factor are approximately 2 and 3 fold greater, respectively, than what was found in adult liver.

Previously we demonstrated an increased phosphofructokinase (PFK) concentration in hepatoma 3924-A (1). Subsequent work with this hepatoma indicated that the increased PFK activity was largely a consequence of a 2 fold increase in the level of the major liver enzyme (PFK-L_2) although a nonhepatic type PFK isozyme comprising about 20% of the total activity was found (2). Realizing that an imbalance in the turnover of PFK-L_2 had occurred in this hepatoma, PFK-L_2 synthesis and degradation were measured which suggested that its synthesis and degradation was increased approximately 2 fold (3). More recently it has been shown that nutritionally and hormonally induced changes in PFK-L_2 levels are mediated via a PFK regulatory factor (4,5).

In this paper we present data which indicates that the increased levels of PFK-L_2 and PFK regulatory factor are characteristic of only poorly differentiated hepatomas and fetal liver and that the increased PFK-L_2 activity correlates with increased levels of PFK regulatory factor.

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METHODS

Animals were maintained as previously described (6). Fetal liver was obtained from 19-day pregnant rats as described (2). Livers and tumors were excised rapidly, homogenized, and centrifuged as described (2). The assay of PFK activity and separation of PFK isozymes on DEAE-cellulose has been described (2). The total activity of PFK-L_2 was determined from the DEAE-cellulose chromatogram as described (2). The PFK regulatory factor activity was measured as previously described (5).

RESULTS AND DISCUSSION

Earlier work has demonstrated that PFK-L_2 was the major PFK isozyme in the poorly differentiated hepatoma 3924-A (1,2). Other investigations have indicated similar results with different Morris hepatomas (6,7). Herein, we present a systematic determination of the levels of PFK-L_2 in poorly differentiated hepatomas (3924-A, 9A, and 9618A_2), well differentiated hepatomas (44, 38B, and 8995), and highly differentiated hepatomas (66, 7794A, 16, and 7787) as well as fetal and adult liver. For reasons of condensation no DEAE cellulose chromatograms will be presented; however, typical examples for fetal and adult liver as well as hepatoma 3924-A can be found in reference 2. After separation of the PFK isozymes by DEAE cellulose chromatography the total PFK activity recovered was measured, and the amount of PFK-L_2 activity determined and expressed as area under the PFK-L_2 peak relative to total area under all PFK peaks. Typical results for total PFK activity recovered and % PFK-L_2 activity was 6.3 units/g and 73% for 3924-A, 6.8 units/g and 70% for 9A, 5.8 units/g and 69% for 9618A_2, 3.3 units/g and 82% for 44, 3.0 units/g and 83% for 38B, 3.2 units/g and 84% for 8995, 2.5 units/g and 88% for 66, 2.8 units/g and 86% for 7794A, 2.8 units/g and 88% for 16, 2.5 units/g and 84% for 7787, 2.5 units/g and 88% for adult liver, and 6.3 units/g and 75% for fetal liver. In Figure 1 PFK-L_2 activity in hepatomas of different differentiation state are shown. The PFK-L_2 activity in poorly differentiated hepatomas and fetal liver was approximately 2-fold greater than adult liver PFK-L_2 activity, and the PFK-L_2 activity in the well and highly differentiated hepatomas was not different from that found in adult liver.

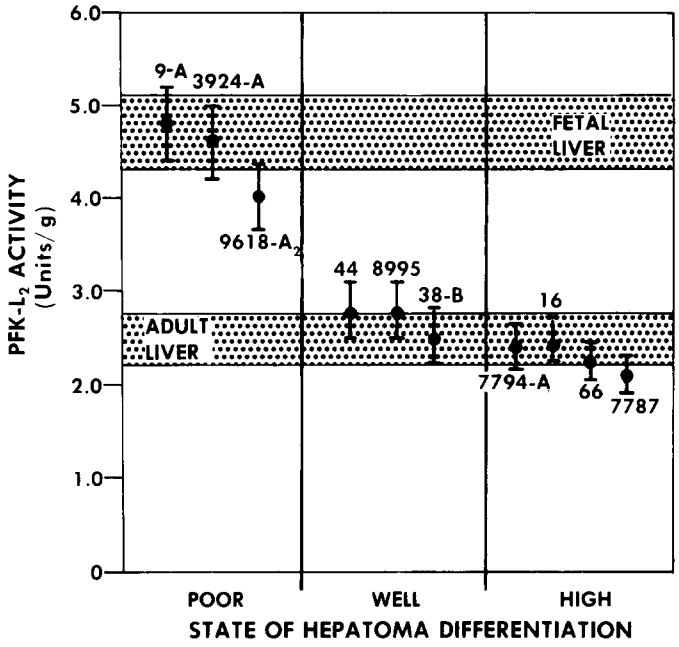

Figure 1: Amount of PFK-L_2 Activity in Morris Hepatomas. The amount of PFK-L_2 was estimated by its separation from other PFK isozymes by DEAE-cellulose chromatography. The amount of PFK-L_2 activity was determined by multiplication of the fractional area under the PFK-L_2 peak times the total eluted PFK activity. At least 90% of the added PFK activity was recovered.

Since the rate of PFK-L_2 synthesis was increased approximately 2-fold in the poorly differentiated hepatoma 3924-A, the increased level of PFK-L_2 could be a consequence of increased translation and/or transcription. However, the expression of repressed genes, which are usually genes for fetal proteins, is a common phenomenon in neoplastic liver (8,9). Since increased levels of PFK-L_2 are found in fetal liver and poorly differentiated hepatomas, this could be interpreted as a consequence of expression of additional PFK-L_2 genes which are normally functional in the fetal liver but expressed in poorly differentiated hepatomas.

The levels of PFK regulatory factor in well and highly differentiated hepatomas were found to be identical with adult liver levels of regulatory factor ($p<.01$), whereas, the poorly differentiated hepatomas and fetal liver exhibited approximately a 3 fold increase relative to adult liver (Figure 2).

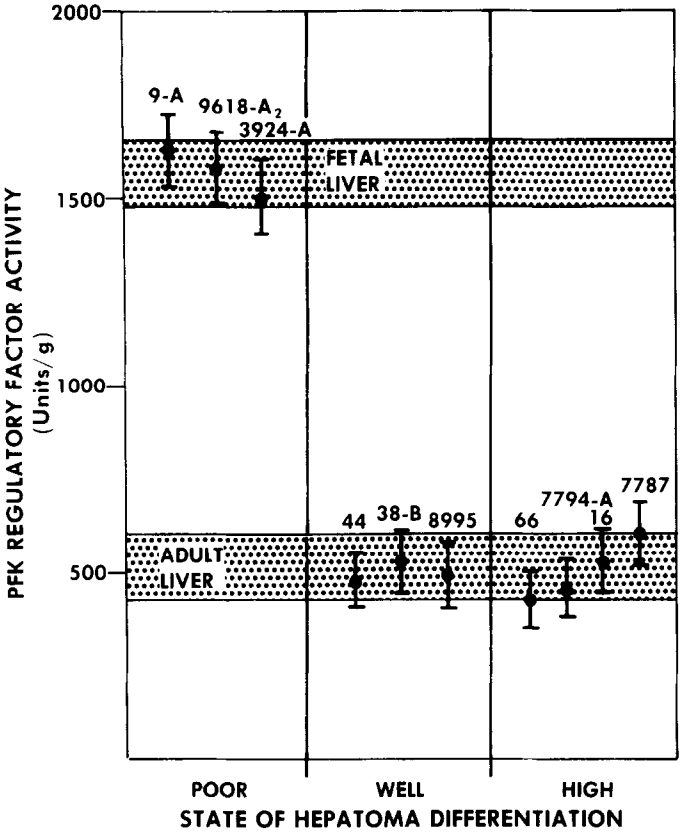

Figure 2: Amount of PFK Regulatory Factor in Morris Hepatomas. The amount of PFK regulatory factor was determined as previously described (4).

Comparison of Figures 1 and 2 indicates that the increased PFK-L_2 activity observed in fetal liver and poorly differentiated hepatoma was accompanied by increased levels of PFK regulatory factor activity. Further, the PFK-L_2 activity and PFK regulatory factor activity in well and highly differentiated hepatomas were the same as that observed for adult liver.

Other work has suggested that the PFK regulatory factor mediates the increased PFK-L_2 activity following refeeding of fasted animals or insulin treatment of diabetic rats (4,5). Herein, we present data indicating that neoplastic transformation of the liver is accompanied by increased levels of PFK-L_2 and PFK regulatory factor. These results support the hypothesis that PFK-L_2 levels are controlled at least in part by changes in levels of the PFK regulatory factor.

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REFERENCES

1. Dunaway, G. A., Morris, H. P., and Weber, G. (1972) Life Sci. 11, 909-918.
2. Dunaway, G. A., Morris, H. P., and Weber, G. (1974) Cancer Res. 34, 2209-2216.
3. Dunaway, G. A., Trahan, L. J.-P., Morris, H. P., and Weber, G. (1974) Biochem. Biophys. Res. Commun. 59, 508-512.
4. Dunaway, G. A., and Segal, H. L. (1976) J. Biol. Chem. 251, 2323-2329.
5. Dunaway, G. A., Leung, G. L.-Y., Thrasher, J. R., and Cooper, M. D. (1978) J. Biol. Chem. 253, 7460-7463.
6. Kurata, N., Matsushima, T., and Sugimura, T. (1972) Biochem. Biophys. Res. Commun. 48, 473-479.
7. Kirby, W., Morris, H. P., and Taylor, C. B. (1974) Europ. J. Cancer 10, 629-631.
8. Knox, W. E. (1972) Enzyme Patterns in Fetal, Adult and Neoplastic Rat Tissues, S. Karger, New York.
9. Weinhouse, J. B. and Weinhouse, S. (1978) in Morris, H. P. and Criss, W. E. ed. Morris Hepatomas Mechanisms of Regulation, p. 44, Plenum Press, New York.