MASTER

The Distribution of Malate NADP Dehydrogenase in Adult, Fetal
and Neoplastic Tissues of the Rat¹

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Malate dehydrogenase (EC 1.1.1.40)

Key words. Mammary gland

Tumors

Abstract. The malate NADP dehydrogenase activities (units/g) of normal adult rat tissues range from 11.7 in lactating mammary gland to 0.81 in male liver and 0.15 in lung. All fetal tissues fall below this range. Unlike other enzymes with such low fetal concentrations, malate NADP dehydrogenase is relatively high in neoplastic tissues (0.23 to 1.82 units/g).

The absolute activity of an enzyme depends on the assay conditions used. However, it has been possible to assess from the literature the relative distribution of several enzymes in rat tissues because many of the reports include values for liver, a suitable standard tissue [4]. For malate NADP dehydrogenase (EC 1.1.1.40, "malic enzyme") there are few comparable values in the literature and this study presents the wide range of activity exhibited by normal and neoplastic tissues of the rat. Such a comprehensive distribution study identifies the tissues in which the activity is minimal and possibly without metabolic significance. More importantly, it singles out the tissues with exceptionally high activity which should be studied for purposes of identifying the physiological role of the enzyme.

The enzyme assay was based on a method by Ochoa [6]. Reaction mixtures of 3.0 ml volume contained a final concentration of 25 mM tris-HCl buffer, pH 7.4; 1 mM $MnCl_2$; 1 mM L-malic acid; 0.25 mM NADP; and 0.01 ml tissue supernatant (20% homogenate in 0.15 M KCl, centrifuged for 45 min at $100,000 \times g$). Activity determinations, to be expressed in units (μ moles of substrate metabolized/min at $25^\circ C$) per g tissue, were made from the increase in optical density (340 μm) due to formation of NADPH.

Table I shows that lactating mammary gland has the highest content: it starts to rise during pregnancy, reaches a maximum during late lactation and decreases precipitously during involution (*i.e.*, after the removal of the litter on the 21st day of life). Among the other normal adult tissues, liver, heart, intestine and kidney contain significant activities. The sex difference in adult liver [5] and the decrease in livers of tumor-bearing rats [3] have been noted previously. Since less than 0.1 units

per g cannot be measured with confidence by the present assay, activities in the fetal tissues are borderline. The equally low value in 5 day old liver fits the observation that malate NADP dehydrogenase begins to accumulate in the liver in the third postnatal week [7]. In kidney, as seen from the 5 day old value, accumulation may start earlier than in liver.

Since malate NADP dehydrogenase emerges in the liver shortly before weaning (i.e., with transition from a high fat to a high carbohydrate diet), Vernon and Walker [7] postulated that its role is to produce NADPH which is necessary for fatty acid synthesis. During the suckling period this liver function is dispensable because of the high fat content of milk. In the mammary gland the enzyme increases in parallel with the capacity for milk production (see Table I) suggesting that in this organ too the enzyme subserves the physiological function of lipid synthesis. The emergence of malate NADP dehydrogenase in the liver (probably triggered by thyroxine [2]) of the still suckling rat and the associated capacity for fatty acid synthesis may be one of the signals which initiate the normal weaning process.

The levels of malate NADP dehydrogenase in rat tumors do not correlate with their growth rates. The enzyme is higher in some slow growing tumors (1C but not 7A) than in fast growing ones (Walker and 5A). However, the important fact is that each tumor examined contained significant levels of the enzyme while fetal tissues contained very little if any. This is a striking contrast to the general rule that neoplasms have enzymatic patterns more similar to those of immature tissues than to adult tissues [4]. Thus, malate NADP dehydrogenase is one of the few biochemical constituents (iso-enzyme III of hexokinase is another [1]) found until now which help to distinguish between tumors and the normal, undifferentiated tissues they most closely resemble.

FOOTNOTES

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REFERENCES

1. Farron, F.: The isoenzymes of hexokinase in normal and neoplastic tissues of the rat. Enzyme, in press.
2. Greengard, O. and Jawdar, S. C.: The prematurely promoted formations of liver enzymes in suckling rats. Biochim. Biophys. Acta 237: 476-483 (1971).
3. Herzfeld, A. and Greengard, O.: The dedifferentiated pattern of enzymes in livers of tumor-bearing rats. In preparation.
4. Knox, W. E.: Enzyme patterns in fetal, adult and neoplastic rat tissues. (S. Karger, Basel 1972).
5. Lockwood, E. A.; Bailey, E. and Taylor, C. B.: Factors involved in changes in hepatic lipogenesis during development of the rat. Biochem. J. 118: 155-162 (1970).
6. Ochoa, S.: Malic enzyme. Methods Enzymol. 1: 739-753 (1955).
7. Vernon, R. G. and Walker, D. G.: Adaptive behavior of some enzymes involved in glucose utilization and formation in rat liver during the weaning period. Biochem. J. 106: 331-338 (1968).

Table I. Malate NADP dehydrogenase levels in rat tissues. Rats were of the MEDH inbred strain. Adult = 60-80 days old; fetal = 20th day of gestation. Mammary glands were obtained at the 18-22nd day of gestation, 1-7 days after delivery (early lactating), 10-21 days after delivery (late lactating) and 3 days after weaning (involuting). The transplanted tumors, listed in order of decreasing growth rate, are all mammary tumors except hepatoma 7800. They have been described previously [4]. "Host liver" was from rats implanted with Walker carcinoma 14 days before assay. The activities are means ($\pm$ SD) of results with the number of animals (or the number of fetal litters providing pools of tissues) in parentheses.

Normal Adult Tissues	Activity units/g	Developing and Neoplastic Tissues	Activity units/g
Mammary gland:		Fetal liver	0.07 $\pm$ 0.09(3)
Virgin	0.50 $\pm$ 0.29(7)	Fetal kidney	0.11 (2)
Pregnant	0.82 $\pm$ 0.62(4)	Fetal brain	0.10 $\pm$ 0.02(3)
Lactating (early)	5.8 $\pm$ 0.50(3)	Fetal lung	0.11 $\pm$ 0.02(3)
Lactating (late)	11.7 $\pm$ 7.6(3)	5 day old liver	0.05 (4)
Involuting	2.2 $\pm$ 0.30(5)	5 day old kidney	0.18 $\pm$ 0.03(5)
Liver (male)	0.81 $\pm$ 0.09(9)	Tumors:	
Liver (female)	1.58 $\pm$ 0.20(4)	Walker	0.37 $\pm$ 0.14(3)
Host liver	0.54 $\pm$ 0.10(7)	5A	0.35 $\pm$ 0.10(3)
Small intestine	1.11 $\pm$ 0.21(4)	3230AC	0.74 $\pm$ 0.10(3)
Heart	1.07 $\pm$ 0.15(4)	1C	1.82 $\pm$ 0.13(5)
Kidney	0.54 $\pm$ 0.12(4)	7A	0.23 $\pm$ 0.05(4)
Skeletal muscle	0.43 $\pm$ 0.06(4)	7800 hepatoma	1.62 $\pm$ 0.36(3)
Brain	0.31 $\pm$ 0.06(4)		
Spleen	0.24 $\pm$ 0.08(4)		
Lung	0.15 $\pm$ 0.14(4)		