Esterase, Malate, and Lactate Dehydrogenases Activity in Murine Neuroblastoma

Abstract. *Mouse neuroblastoma tumors have only the fifth isozyme band (A_4) of lactate dehydrogenase, whereas this band is missing in the brain which contained four other bands of lactate dehydrogenase. The α-esterase isozyme patterns of tumors, kidney, and brain are similar except that there is an additional slowest-moving form of esterase in all tumor tissues. The malate dehydrogenase pattern is not altered in any of the tissues.*

Ever since Warburg enunciated his well-known theory concerning the importance of glycolysis for the survival of the neoplastic cells, the relationships between glycolysis and malignancy have been under continuous investigation, discussion, and controversy. Generalizing from studies in several different laboratories (*1*), it appears that a high magnitude of glycolytic activity is characteristic of most tumors. In our earlier report we suggested, supporting the findings of others (*2*), that the A type polypeptide subunits of lactate dehydrogenase (LDH) are associated with anaerobic glycolysis (*3*). Since anaerobic glycolysis seems to be more critical for the survival of neuroblastoma cells than for the non-nerve cells in culture (*4*), we studied the lactate dehydrogenase activity in neuroblastoma cells in vivo by starch gel electrophoresis. In addition, the electrophoretic patterns of α-esterase and malate dehydrogenase in these tumors were compared with that of normal tissues. The above enzymes in muscles, brain, and kidney were studied as a control for neuroblastoma tumors.

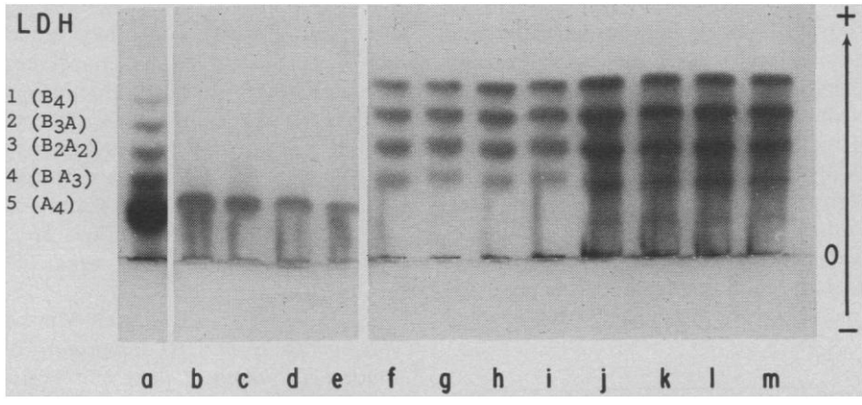

Fig. 1. Starch gel electrophoretic patterns of LDH isozymes from murine neuroblastoma (*O*, origin). Samples are (*a*) control muscle tissue, (*b*) tumor induced by TH positive clone, (*c*) tumor induced by ChA positive clone, (*d*) tumor induced by TH and ChA positive clone, (*e*) tumor induced by TH and ChA negative clone, (*f*) brain tissue from control mouse, (*g, h, i*) brain tissues of mice with neuroblastomas, (*j*) kidney tissue of control mouse, (*k, l, m*) kidney tissues of mice with neuroblastomas.

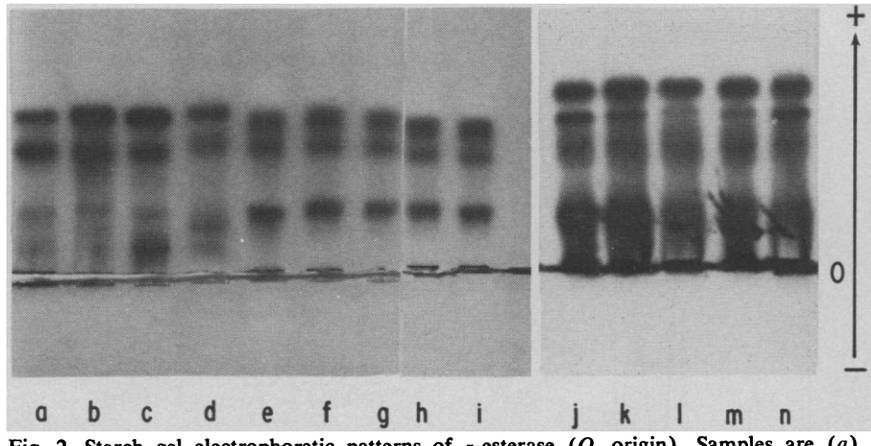

Fig. 2. Starch gel electrophoretic patterns of α-esterase (*O*, origin). Samples are (*a*) tumor induced by TH positive clone, (*b*) tumor induced by ChA positive clone, (*c*) tumor induced by TH and ChA positive clone, (*d*) tumor induced by TH and ChA negative clone, (*e*) brain tissue of control mouse, (*f, g, h, i*) brain tissues of mice with neuroblastoma, (*j*) kidney tissue of control mouse, (*k, l, m, n*) kidney tissues of mice with neuroblastoma.

Prasad *et al.* (*5*) have established four major types of clone having different neuronal properties. These include: (i) clone with tyrosine hydroxylase (TH), but without choline acetyltransferase (ChA); (ii) clone with ChA but without TH; (iii) clone without TH or ChA; and (iv) clone with both TH and ChA. In this study cells of the first three types of clone and an uncloned line of cells with both TH and ChA were injected subcutaneously into male A/J mice (6 weeks of age) to produce tumors. These tumors were then used for the enzyme studies. Each clone maintains its characteristic with respect to the basal level of neural enzyme when allowed to replicate in vivo (*5*).

Extracts of tumor, brain, and kidney tissues were prepared for the starch gel electrophoresis as previously described (*3*). Electrophoresis of the supernatant was done on vertical starch gels, and gel slices were stained for lactate and malate dehydrogenase as previously described (*6*). To stain for α-esterase, 1 percent naphthyl acetate solution was made in 50 ml of tris buffer (0.5*M*, *p*H 7.0) and 50 ml of acetone.

Five isozymes of LDH are resolved by electrophoresis of extracts of muscle and kidney tissues (Fig. 1), although the LDH-5 activity in kidney tissues is not as high as in muscle tissues. In brain tissue homogenates of mice with neuroblastoma as well as of control mice, there are only four bands of LDH. The fifth band (A_4), or the muscle type LDH, as it is also called, is absent in the brain tissues. This muscle type LDH is the only band which is expressed in the tumor tissues. The other four isozymes of LDH are absent in the tumors. All four clones of neuroblastoma cells show the same pattern of this enzyme.

The α-esterase patterns of tumor, brain, and kidney tissues seem to be similar, except that there is an addi-

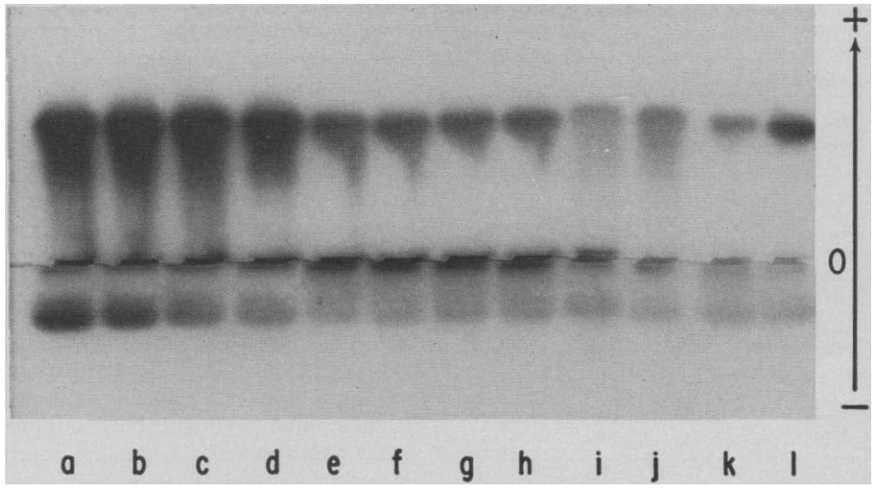

Fig. 3. Starch gel electrophoretic patterns of malate dehydrogenase isozymes (*O*, origin). Samples are (*a*) kidney tissue of control mouse, (*b, c, d*) kidney tissues of mice with neuroblastoma, (*e*) brain tissue of control mouse, (*f, g, h*) brain tissues of mice with neuroblastomas, (*i*) tumor induced by TH and ChA negative clone, (*j*) tumor induced by TH and ChA positive clone, (*k*) tumor induced by ChA positive clone, (*l*) tumor induced by TH positive clone.

tional slowest-moving band of esterase in all the tumor tissues (Fig. 2). The malate dehydrogenase pattern is not altered in any of the tissues observed (Fig. 3). Tumor, brain, kidney and muscle tissues all have the same type of distribution of malate dehydrogenase. All four clones of neuroblastoma cells have the same pattern of α-esterase and malate dehydrogenase.

The muscle type isozyme of lactate dehydrogenase (LDH-5) is the major form found in tissues exhibiting a high rate of glycolysis and is not inhibited by pyruvate (*7*). Thus the presence of only LDH-5 in neuroblastoma tumor cells may suggest that these neoplastic cells require as high a rate of glycolysis in vivo as they do in vitro. As there seems to be a significant correlation between the growth rates of some tumors (like hepatomas) and their glycolytic activity (*1*), the distribution pattern of LDH isozymes could also be significant in characterizing the growth of other types of tumors.

The significance of the presence of the extra esterase band in the tumors is not apparent. It could be speculated, however, that the extra activity of esterase is related to the anaerobic glycolysis in the sense that it helps liberate more glycerol from the fats. Glycerol is then utilized for the synthesis of either fructose or glucose, which in turn undergoes glycolysis.

Usually the B subunit (LDH-1) is high in brain and heart tissue (*8*). As neuroblastoma cells are also of neural origin one would expect the presence of a tissue-specific enzyme. The absence of B units and the presence of A units in the tumor cells suggest that expression of murine neuroblastoma is associated with a loss of specific neural enzymes and a replacement by other types which are normally either low or absent in the brain. Such phenomena of simultaneous "induction-repression" have also been observed in some hepatoma tumors (*9*). It has been shown that in early rat embryogenesis B polypeptide subunits of LDH are usually absent (*10*). Thus the absence of B units in neuroblastoma tumors and its presence in brain tissues suggest that certain embryonic properties reexpress in the neoplastic cells. This is consistent with the general observation that the malignant cells reexpress many embryonic features.

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Inherited Renal Cysts in Rats

Abstract. *A strain of rats that form renal cysts has been developed. The number of visible cysts increases with age after animals are 20 days old. Micropuncture studies indicate that the cystic fluid has a variable sodium concentration, but that the ratios of inulin concentration in tubular fluid to that in plasma are high.*

The maturation of renal function in neonatal rats has been studied by micropuncture technique in this laboratory (*1, 2*). Pregnant mothers were obtained from a commercial source (Simonsen, Gilvary, California), and micropuncture experiments were done on pups 15 to 55 days after birth. During these experiments, it was noticed that certain litters had kidneys with cysts visible on the kidney surface. Three sister-brother pairs were mated to determine if the cyst-bearing animals could be developed into a strain carrying cystic disease which could be an animal model for human polycystic disease (*3, 4*). Initial data reported here indicate that it has been possible to develop such a strain, and that further study could make contributions to our knowledge of cyst formation.

In the matings of two sets of parents (two litters each from pairs A and C),