fluids in leaded jugs, and the use of improperly glazed earthenware and leaded paints and cosmetics. The high concentrations found in our antique population most likely reflect a greater ingestion of lead than would be expected for contemporary populations, with the exception perhaps of ghetto children, persons drinking illicit alcohol, or special industrial populations whose exposure to potential sources of contamination is unusually high. Thus, the lower lead content in human hair in our contemporary population is probably a result of greater precautions in the use of lead in spite of a general increase in atmospheric concentrations (*2*).

No significant differences in the lead content of hair between populations of children from Philadelphia and a group from Michigan's western Upper Peninsula were found (Table 2). Hammer *et al.* (*6*) have shown a good correlation between the lead content of children's hair and the lead exposure ranking in five selected cities. Their values for cities with a low lead exposure ranking are similar to our values for both children from Philadelphia and children from the western Upper Peninsula. Goldsmith (*1*, p. 62) reported that no significant correlation could be established between atmospheric lead and blood lead concentrations when the atmospheric lead concentrations were below 2 $\mu g/m^3$. The atmospheric concentrations in Philadelphia and Michigan's Upper Peninsula are probably below this level; this may account for the fact that differences between these populations could not be demonstrated.

The significantly lower lead content observed in adult hair compared with that of children (Table 1) is consistent with other reports (*15*) and reflects a generally higher metabolic level in children and the fact that absorption decreases with age (*3*).

In this study we do not intend to minimize the effect of environmental lead on man's health. However, we have demonstrated a decrease in the lead content of human hair between 1871–1923 and 1971 in spite of an increase in atmospheric lead.

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Expression of Lactate and Malate Dehydrogenases in Tumors Induced by SV40 and 7,12-Dimethylbenz(a)anthracene

Abstract. *Isozyme patterns of lactate and malate dehydrogenases were studied in tumors induced by SV40 and 7,12-dimethylbenz(a)anthracene and in established cultures of cells from these tumors. The expression of B polypeptide subunits of lactate dehydrogenase is suppressed similarly by both agents. This may be due to inactivation of the gene at the locus determining the B polypeptide subunit. Malate dehydrogenase isozyme patterns are not changed significantly by the virus or the carcinogen.*

Neoplastic transformation in vitro and in vivo by oncogenic viruses (*1*) and carcinogenic hydrocarbons (*2*, *3*) is well documented. We investigated the expression of lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) in tumors induced by SV40 and by 7,12-dimethylbenz(*a*)anthracene (DMBA). The tumor cells grown in tissue culture were also studied.

Extracts from normal muscle tissues (adjacent to tumors) in hamsters show five distinct bands of LDH after starch gel electrophoresis (Fig. 1). Each of these proteins is a tetramer composed of four polypeptide units (*4*), and the polypeptide subunits can be of two different kinds, A and B (sometimes called M and H). The two distinct polypeptide subunits are determined by separate gene loci (*5*).

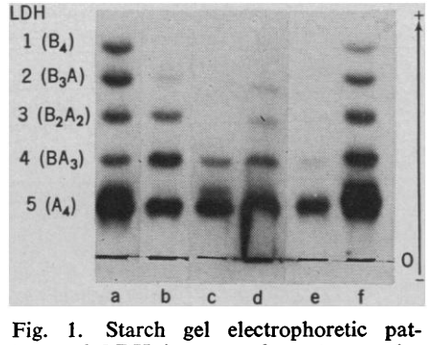

Fig. 1. Starch gel electrophoretic patterns of LDH isozymes from tumors induced by SV40 and DMBA and from corresponding transformed cells in culture (*O*, origin). Samples are (a) control muscle tissue, (b) DMBA-induced tumor, (c) DMBA-transformed cells in culture (passage 10), (d) SV40-induced tumor, (e) SV40-transformed cells in culture, and (f) control muscle tissue.

Electrophoresis was performed on homogenates of tumor tissues, control muscle tissues, and transformed cells; tissue was either freshly prepared or had been stored at −20°C. The tissues were washed three times in 0.9 percent saline containing $6.6 \times 10^{-4} M$ ethylenediaminetetraacetic acid (EDTA) and twice in deionized water to remove erythrocytes. Homogenates were prepared by grinding the tissues in a glass homogenizer with deionized water (2 cm^3 of water per gram of tissue). The crude extract was centrifuged for 30 minutes at 11,000 rev/min. Samples of supernatant, either full strength or diluted with deionized water, were used for electrophoresis.

The virus-induced tumor studied, HCO_2clI, is a clone derived from H-50 IS cells, which had been derived from a tumor induced by SV40 in a newborn hamster (*6*). The DMBA cell lines were derived from a tumor induced by DMBA. Tumors were induced by injecting 2.3 mg of DMBA in 0.1 ml of acetone into each adult hamster, as described by Lausch and Rapp (*7*). We

studied the enzyme patterns in HCO_2clI cells and in DMBA-induced tumor cells, both of which were passaged once or twice in vivo and 5 to 40 times in vitro.

Samples of transformed cells were prepared according to the method of Peterson *et al.* (*8*), with little modification. Tumor cells were released from the glass surface with trypsin (0.125 percent) and washed three times with saline containing EDTA and twice with deionized water. The cells were ruptured by freezing and thawing, and cell debris was removed by centrifugation.

Electrophoresis of the supernatant was done on vertical starch gels, and the gel slices were stained for LDH and MDH as described (*9*).

Five isozyme bands of LDH are resolved by electrophoresis of extracts of control muscle tissue (Fig. 1). These isozymes, termed 1 to 5 according to electrophoretic mobility, are assumed to possess the following combinations of subunits: B_4, A_1B_3, A_2B_2, A_3B_1, and A_4. Neither the virus nor the carcinogen affects the MDH pattern significantly, although the activity in MDH subbands is reduced or absent in tumors and their cultured cells as compared to the control tissue (Fig. 2). In contrast to the minimal effect on MDH patterns, the LDH isozyme patterns are affected significantly (Fig. 1). In tumors induced by both SV40 and DMBA, only four distinct bands appear. Also, when tumors are established in cell culture only two bands (slow-migrating) are seen. However, in the early subcultures, three or four such bands are distinct (Fig. 3). This is perhaps because in vivo cells placed in culture must acquire some different characteristics before they are adapted to their new environment. In studies with polyoma virus (*10*) and polycyclic hydrocarbons (*3*), changes in morphological characteristics were found in the early stages of cell culture. After a certain number of tissue culture passages, the transformed cells acquired the property of continuous growth. We found that LDH isozymes 3, 4, and 5 may be present in early subcultures (up to 8 to 14 passages) but

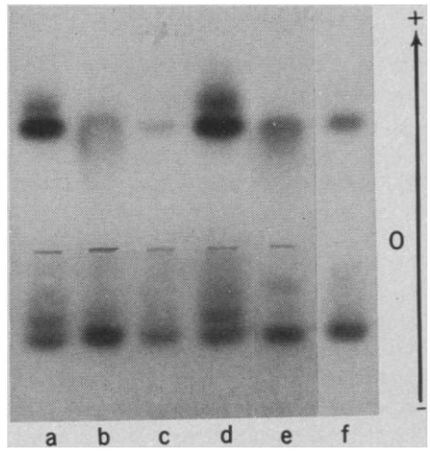

Fig. 2. Starch gel electrophoretic patterns of MDH isozymes from tumors induced by SV40 and DMBA and from corresponding transformed cells in culture (*O*, origin). Samples are (a) control muscle tissue (for DMBA tumor), (b) DMBA-induced tumor, (c) DMBA-transformed cells (passage 10), (d) control muscle tissue (for SV40 tumor), (e) SV40 tumor, and (f) SV40-transformed cells (passage 15).

in later passages only isozymes 4 and 5 are expressed. These two bands remain present for as long as 40 passages.

The expression of LDH in tumors and established cell cultures is affected similarly by SV40 and DMBA. The absence of some LDH isozymes in these cells suggests one of the following: (i) the gene determining the B subunit is inactivated; (ii) the isozymes are catabolized soon after their production; or (iii) subunit B is altered so that its electrophoretic properties no longer correspond to those of the control subunit B.

The patterns of isozyme present in a particular tissue are probably related to the metabolic characteristics of that tissue. Malignant tissue has a high capacity for anaerobic glycolysis (*11*), and subunit A is preponderant in tissues that tend to be subjected to periods of oxygen deficiency (*12*). Therefore, it is more likely that in tumor tissues and culture cells where subunit A is involved in glycolysis, the gene at the locus determining the subunit B is altered or inactivated. As a result, LDH proteins that are composed of B polypeptide subunits are not expressed in tumors and cultured cells. When the transformed cells are reinoculated into the host animals, the gene determining the subunit B is activated again, and all five bands of LDH are detected (*13*). But as the tumor reaches maturity, the gene is inactivated again, affecting the distribution of isozymes.

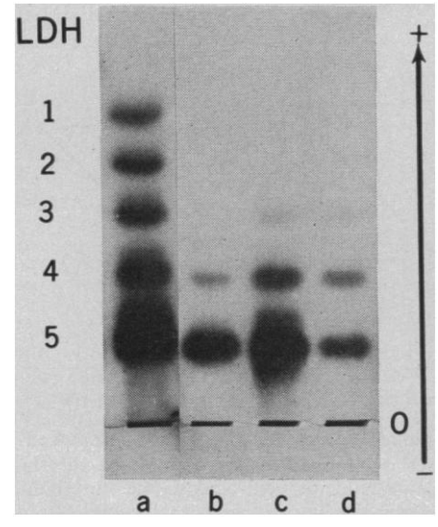

Fig. 3. Starch gel electrophoretic patterns of LDH isozymes from SV40-transformed cells in culture (*O*, origin). Samples are (a) control muscle tissue (in vivo), (b) SV40-transformed cells (passage 15), (c) SV40-transformed cells (passage 1), and (d) SV40-transformed cells (passage 2).

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