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Remote Tumor Effects on Lactic Dehydrogenase of Mouse Muscle Fibers with Different Glycolytic–Oxidative Metabolic Potentials[1] (41244)

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Abstract. The total lactic dehydrogenase (LD) activity and the isoenzyme pattern were studied in muscles of mice during the progressive growth of a distally transplanted methylcholanthrene-induced tumor. The thigh muscles as a group (a mixture of fibers with different oxidative–glycolytic metabolic potentials), the gastrocnemius muscle (primarily glycolytic), the soleus muscle (predominantly oxidative), the heart (purely oxidative), and the diaphragm (primarily oxidative) were evaluated. The total LD activity increased in muscles with a high glycolytic metabolic potential. In such muscles a significant increase of the muscle type and a significant decrease of the heart type of LD were observed. After 3 weeks of tumor growth the tumor was resected, and the LD activity and isoenzyme distribution returned toward normal values 2 weeks later.

A shift in the lactic dehydrogenase (LD, EC 1.1.1.27) isoenzyme distribution of tumor-free muscles of mice toward the isoenzyme pattern of a distally growing, nonmetastasizing, transplanted mammary carcinoma has been reported by our laboratories (1). The anterior thigh muscles of the mouse were studied as a whole, and this group included muscles with a varying predominance of fiber types with different glycolytic and oxidative metabolic potentials.

The purpose of the present study was twofold: (A) to determine if a carcinogen-induced tumor has an effect on the LD of thigh muscles in mice comparable to that of the mammary carcinoma, and (B) to investigate whether such an effect is more selective for those muscles with a predominantly oxidative or predominantly glycolytic metabolic potential. A correlation between the remote tumor effect on LD and the specific glycolytic–oxidative metabolic profile of the muscle fibers has been observed in this study.

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Materials and Methods. Female C3H/Hej mice (Jackson Laboratories, Bar Harbor, Maine), 6 to 10 weeks old, were used. Within a given experiment, mice of a similar age were used. They were fed Purina Laboratory Chow and water *ad libitum* and were kept under standardized environmental conditions (lights on at 06.00 hr, off at 18.00 hr, room temperature of 25.5 ± 0.5°).

Methylcholanthrene (MCA) tumor was induced by injecting the carcinogen in the hindleg muscles of C3H/Hej mice. The tumor underwent several sc transplantations prior to these experiments. This tumor grows locally and kills the animals by inducing cachexia. Animals started dying at the fourth week after tumor transplantation and most of them were dead by the sixth week. However, an adequate number of mice were still alive for the 6-week study period. Eight tumor-bearing mice were studied at biweekly intervals. Eight control mice were used in each experiment. The animals were killed by dislocation of the cervical spine. The experiment was repeated and the data reported represent the combined data of the two experiments with 16 control and 16 tumor-bearing mice at each study period.

For the present study, tumor cells were teased out of the tumor into RPMI 1640 cell culture medium (GIBCO, Grand Island,

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N.Y.), and 10^6 cells were injected sc onto the back of the recipient while under ether anesthesia. The control animals received normal saline injection under the same anesthesia.

The anterior thigh muscles, including the vastus lateralis, vastus medialis, rectus femoris, and gluteus superficialis, were removed as a group, frozen in dry ice, and then homogenized in ice-cold saline (1:200, w/v) using a Potter homogenizer. The gastrocnemius, soleus, heart, diaphragm, and the tumor were removed separately and processed as above. Each sample was homogenized individually, except for the diaphragm. Diaphragms from four mice were pooled together and treated as one specimen. The homogenate was centrifuged at 4° and 5000*g* for 5 min to remove cellular debris. All homogenates were analyzed for total LD activity and isoenzyme distribution within a few hours after preparation.

The muscles assayed were chosen to represent different combinations of oxidative–glycolytic metabolic patterns. The heart tissue is purely oxidative (2). The mouse diaphragm has a primarily oxidative metabolism with minimal glycolytic metabolic potential (3). The soleus is predominantly oxidative, but up to 50% of its fibers (depending on the enzymes concerned with release of energy studied) also may have a glycolytic metabolism (4, 5). The lateral aspect of the gastrocnemius is composed almost completely of fibers which are mainly glycolytic in metabolism, with only a small oxidative potential (4, 5).

After 3 weeks of tumor growth, the tumor was resected from a group of 10 animals. Two weeks later the group of thigh muscles was resected, and the LD activity and its isoenzymes were studied.

Using the method of Hanson and Freier (6), the total LD activity was assayed and expressed as international units per gram of tissue. Lactic dehydrogenase isoenzymes were separated on agarose gel plates in barbital buffer (pH 8.6) in a Beckman microzone electrophoretic cell (7). The patterns were then stained and scanned, and the area under each isoenzyme peak was estimated as a percentage of the total LD activity. The proportions of the two subunits, H (heart) type and M (muscle) type, of LD were calculated from their percentage presence in the five electrophoretically separated isoenzymes according to the following formulas:

$$\text{subunit H} = \text{LD1} + \tfrac{3}{4}\,\text{LD2} + \tfrac{1}{2}\,\text{LD3} + \tfrac{1}{4}\,\text{LD4},$$

$$\text{subunit M} = \text{LD5} + \tfrac{3}{4}\,\text{LD4} + \tfrac{1}{2}\,\text{LD3} + \tfrac{1}{4}\,\text{LD2}.$$

The data were analyzed, using the two-sample Student's *t* test. All the comparisons of tumor-bearing groups to controls having a P value of <0.05 were considered statistically significant.

Results. The progressive changes observed in the total LD activity and in the distribution of its M and H types in the different muscles of tumor-bearing mice are presented in Table I. Six weeks after transplanting the tumor, the total LD activity increased by 35.7% in the gastrocnemius and by 28% in the group of thigh muscles. No changes in total LD activity ($P > 0.05$) were observed in the soleus, diaphragm, and heart.

During the 6-week tumor growth, the M type increased from 78.2, 71.2, and 68.5% to 91.8, 85.4, and 73.2% in the gastrocnemius, thigh muscles, and soleus, respectively. This change is statistically significant ($P < 0.05$). The M/H ratio increased from 3.6, 2.5, and 2.2 in controls to 11.1, 5.8, and 2.6 in the above muscles, respectively. An increase in the M and a decrease in the H were noted in the diaphragm, but the M/H ratio was not statistically different from the controls. No significant change in the percentage distributions of the M and H types was observed in the heart.

The malignant tissue showed an increase in its total LD activity from an average of 620 IU/g after 2 weeks to 692 IU/g after 4 weeks. This was followed by a decrease to 319 IU/g after 6 weeks. This decrease could be the result of necrotic tissue present in the tumor at the time. On the other hand, during the tumor growth there was a progressive increase in the M type (the predominant LD type in the malignant tissue) and a decrease of the H type. The M/H ratio

TABLE I. TOTAL LD ACTIVITY AND DISTRIBUTION IN M AND H SUBTYPES IN MUSCULAR AND MALIGNANT TISSUES OF TUMOR-BEARING AND CONTROL MICE

Tissue	Weeks after tumor transplantation	Total LD (IU/g)	Percentage		M/H
			M	H	
Thigh muscles	0 (Control)	406 ± 17[a]	71.2 ± 2.0	28.8 ± 1.5	2.5 ± 0.1
	2	453 ± 10*	78.2 ± 1.5*	21.8 ± 1.5*	3.6 ± 0.1*
	4	474 ± 17*	82.2 ± 3.0*	17.8 ± 2.0*	4.6 ± 0.2*
	6	520 ± 16*	85.4 ± 2.0*	14.6 ± 1.6*	5.8 ± 0.2*
	2 Weeks after resection of a 3-week tumor	461 ± 12	74.0 ± 2.0	26.0 ± 1.7	2.8 ± 0.1
Gastrocnemius	0 (Control)	560 ± 13	78.2 ± 1.9	21.8 ± 1.5	3.6 ± 0.2
	2	597 ± 17*	88.0 ± 2.0*	12.0 ± 1.0*	7.3 ± 0.1*
	4	613 ± 17*	91.0 ± 2.7*	9.0 ± 0.5*	10.1 ± 0.1*
	6	760 ± 23*	91.8 ± 1.0*	8.2 ± 0.7*	11.1 ± 0.1*
Soleus	0 (Control)	467 ± 16	68.5 ± 2.0	31.5 ± 1.7	2.2 ± 0.1
	2	460 ± 12	69.6 ± 1.5	30.4 ± 2.0	2.3 ± 0.1
	4	461 ± 11	72.2 ± 2.0*	27.8 ± 1.9*	2.6 ± 0.2*
	6	470 ± 16	73.2 ± 2.0*	26.8 ± 2.0*	2.6 ± 0.2*
Diaphragm	0 (Control)	223 ± 55	55.7 ± 1.8	44.3 ± 1.3	1.3 ± 0.1
	2	200 ± 31	57.0 ± 2.0	43.0 ± 2.0	1.3 ± 0.2
	4	219 ± 16	59.0 ± 2.0*	41.0 ± 2.7*	1.4 ± 0.1
	6	213 ± 27	59.0 ± 3.0*	41.0 ± 4.0*	1.4 ± 0.2
Heart	0 (Control)	367 ± 37	6.2 ± 0.1	93.8 ± 0.1	0.07 ± 0.01
	2	356 ± 21	6.2 ± 0.1	93.8 ± 0.2	0.07 ± 0.01
	4	359 ± 16	6.2 ± 0.2	93.8 ± 0.1	0.07 ± 0.01
	6	366 ± 23	6.2 ± 0.2	93.8 ± 0.2	0.07 ± 0.01
Tumor	2	620 ± 22	92.0 ± 3.0	8.0 ± 1.0	11.5 ± 0.3
	4	692 ± 17	93.0 ± 4.0	7.0 ± 1.5	13.5 ± 0.5
	6	319 ± 34	96.5 ± 3.0	3.5 ± 1.0	27.6 ± 2.0

[a] Mean ± SD. The mean was computed from 16 control and at each study period from 16 tumor-bearing mice.
* $P < 0.05$.

of the tumor increased from 11.5 at 2 weeks after transplantation to 27.6 in 6 weeks.

Discussion. Tumor-free mouse muscles with varying metabolic profiles responded differently to the growth of a distally transplanted methylcholanthrene-induced tumor. Muscles with a high glycolytic metabolic potential were affected more than those with predominantly oxidative metabolism, and the former demonstrated a significant progressive increase in the total LD activity with an increase of the M type and a decrease of the H type. An increase of total LD activity in tumor-free muscles of mice bearing a methylcholanthrene-induced sarcoma was also reported by Lundholm *et al.* (8).

The M type is the predominant LD type in tumor tissue, and the increase in M type in the highly glycolytic muscles would indicate a metabolic shift of the tissue toward the metabolic pattern of the tumor. In skeletal muscle, the H type appears first during fetal development, and the M type is considered the mature tissue type (2). Both types, M and H, are located on separate genes in the cell (2). The observed increase in the M type in tumor-free tissues of MCA-bearing mice is in contrast to Lehmann's concept that the presence of the tumor stimulates the neosynthesis of fetal enzymes and isoenzymes in muscle and liver of the host (9). The transplanted tumor apparently induces the appropriate gene to produce more of the mature muscle M type and actually suppresses the synthesis of the fetal muscle H type, resulting in metabolic changes toward the glycolytic pathway.

Of interest is our finding that the M/H ratio returned toward control values after the resection of the tumor (Table I). This, of course, is evidence for a remote tumor effect on the host.

An increase in the isoenzymes containing the M type has been described before in tissues adjacent to a tumor (1). Again, in this study, we observed the change in the LD isoenzyme pattern in distant tumor-free muscles of tumor-bearing mice. Parina *et al.* (10) had observed an increase in the LD4 and LD5 isoenzymes in the pectoral muscle of both chicks and adult hens after their infection with the Rous sarcoma virus. An increase in the ratio of isoenzyme V over isoenzyme I has been observed by Shapot and co-workers in tumor-unaffected uteri of patients with cancer (11). However, Fountain *et al.* (12) studied the LD isoenzymes in Ehrlich–Lettre ascites tumor-bearing Swiss mice and found no difference in the LD isoenzyme patterns of abdominal muscles between the tumor-bearing and normal mice. This is in disagreement with our results and remains unexplained, although it may be due to the difference in the glycolytic–oxidative metabolic potential of the muscles studied.

Although none of the examined muscles in our study can be considered as exclusively glycolytic or oxidative tissue, it is evident that muscles with high glycolytic capacities appear to be more susceptible to induction of LD changes. These changes indicate a shift in the metabolism of the tissue toward the glycolytic pathway of energy production. The changes were observed at 2 weeks after transplanting of the tumor when it is still small and there is no evidence of necrosis, nutritional changes, or other complications. Such changes in tumor-free tissues of the host may explain the recent observations by Holroyde *et al.* (13) that the lactate production increased in patients with colorectal cancer, although the magnitude of the increase correlated poorly with the size of the tumor.

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