Potter, LePage, and Klug (3) have shown that in homogenates of kidney, brain, and muscle kept at 0° C. for 24 hours, considerable losses occur in the ability to utilize oxygen. When the ischemic tissues are kept at 30° C. in situ, the losses are greater, occur earlier, and are apparent in heart and liver as well as in the tissues mentioned above. Oxidative enzyme activity in the muscle, brain, and kidney approach the low activity seen in tumor tissue. The authors point out, however, that it is unlikely that the loss in oxidative enzyme activity can be attributed to the accumulation of lactic acid, because the lactic acid in normal tissues can build up to values greater than those found in tumor tissues without decreasing the activities of these enzyme systems. Nevertheless, it seemed possible that some product of tumor metabolism might be responsible for the low enzyme activity and that, if this hypothetical product were of low molecular weight, it might diffuse into the "normal" (noncancerous) tissue surrounding the tumor, with the result that a lowered oxidative enzyme activity would be found there. Conceivably, this could be a "conditioning mechanism" by which normal tissue might be made vulnerable to tumor invasion.1 A test of this hypothesis is the basis of the present paper.

EXPERIMENTAL

Female rats from our own colony, between the ages of 60 and 90 days at the beginning of the experiment, were used. Tumors were induced by injecting 8 mg. of methylcholanthrene in 0.925 ml. of corn oil into the posterior muscles of the left mid-thigh at an intramuscular depth of about 1 cm. On an average, 160 days elapsed between the time of injection and the appearance of the first palpable tumors. Thereafter, when the circumference of the leg at the site of the tumor had increased 2–3 times, the rats were killed by decapitation. Tissues were removed and weighed as quickly as possible, and homogenates made in chilled all-glass homogenizers as described by Potter and Elvehjem (2), with the reaction mixture described below as the suspending medium.

For enzyme studies in tumors, firm tissue was selected. "Adjacent" tissue was taken as close to the tumor as possible without including any but apparently "normal" muscle. Muscle from the corresponding site of the opposite leg was taken for control studies. Samples of tissues comparable to those used for enzyme studies were taken for histopathologic examination.

Enzyme studies were done in duplicate with a conventional Warburg apparatus at 37° C., with 0.2 ml. of 2 N NaOH in the central well, and with air as the gas phase. All solutions and flasks were kept in ice until the flasks were attached to the manometers. An equilibration time of 10 minutes was used, and the homogenates were observed for 40 minutes thereafter.

The 3.0-ml. reaction medium was a modification (1) of the one described by Potter, LePage, and Klug (3) and was made up as follows: 0.5 ml. containing sodium adenosine triphosphate (1 mg.)-sodium diphosphopyridine nucleotide (0.3 mg.), 0.5 ml. of a 12.5 per cent homogenate of tissue in the reaction mixture, 0.3 ml. of either 0.09267 M sodium succinate or 0.09267 M sodium oxalacetate, and 1.7 ml. of reaction mixture. The reaction mixture was made up from 40 ml. of 0.5 M KCl, 10 ml. of 0.1 M MgCl₂, 10 ml. of 0.1 M potassium phosphate buffer at pH 7.4, 10 ml. of 4 × 10⁻⁴ M cytochrome c, and 100 ml. water.

Inasmuch as the reaction medium employed had been used previously with only oxalacetate as added substrate, it was necessary to try succinate as a substrate in the same medium. Homogenates of both muscle and liver from normal rats were supported by a grant from the National Cancer Institute, National Institutes of Health, U.S.P.H.S., Bethesda.

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1 V. R. Potter, personal communication.
used. Because the homogenates were made up in the reaction mixture, it was only necessary to vary the amounts of homogenate and adjust the quantity of added reaction mixture to a total of 3.0 ml. The only variable, then, was the amount of tissue present. With both tissues it was found that, with 0.2-ml., 0.4-ml., and 0.5-ml. quantities of homogenate per Warburg flask, activity was proportional to the amount of tissue and, therefore, enzyme present. Activity without any added substrate was about one-third of that when succinate was added.

In these experiments, tumors were produced in the left thigh. To measure the change in enzyme activity in the tumor and in the surrounding muscle, enzyme activity in the contralateral muscle was measured as a standard; and, to establish the validity of this procedure, the enzyme activity of the muscle of the left thigh was compared with that of the right thigh in fifteen normal rats. When oxalacetate was used as the substrate, the oxygen consumption (mean ± S.D.) of the left thigh muscle was 101 (S.D. ± 25) per cent of that for the right. When succinate was used as the substrate, the value for the left thigh muscle was 93 (S.D. ± 17.4) per cent of that for the right.

RESULTS

Chart 1 shows the results of two experiments; oxalacetate (OA) was used as a substrate in one, and succinate (S) was used as a substrate in the other. In both experiments, it will be seen that the activity of the apparently normal muscle adjacent to the tumor (AM) falls approximately midway between that of the contralateral muscle of the opposite leg (OL) and that of the tumor (T). The two experiments presented in Chart 1 are illustrative only and are not intended to represent mean values.

Fifteen experiments were performed with oxalacetate as the substrate. As shown in Table 1, the rate of oxygen utilization was determined for either or both the tumor tissue and the adjacent muscle tissue. The results are expressed in terms of per cent of the activity in the contralateral muscle. All data were derived from readings made 90 minutes after the closing of the Warburg flasks. A 10-minute equilibration period had preceded this. The activity of tumor tissue is seen to be 16 ± 20 per cent (mean ± S.D.) of that of the muscle of the opposite leg. The oxidation of oxalacetate in muscle adjacent to the tumor is seen to be 50 ± 30 per cent (mean ± S.D.) of that of the opposite leg. In eleven of the twelve rats in which adjacent tissue was assayed, the activity was less than 65 per cent, and in two rats the activity was less than 20 per cent. In nine of the ten experiments in which both adjacent tissue and tumor tissue were assayed concurrently, the adjacent tissue showed greater activity than did the tumor tissue.

Five of the nine rats used for the study of succinate oxidation bore tumors that were more advanced than those of rats in which oxalacetate oxidation was studied. In these five animals, comparable "normal" adjacent tissue could not be found in adequate quantity for analysis. Because of the small number of assays and the differences in results, these experiments are not represented graphically. However, in the four instances in which adjacent tissue was assayed, the results were, respectively, 52, 0, 116, and 151 per cent of the activity of tissue from the opposite leg. In each

<table>
<thead>
<tr>
<th>Tissue</th>
<th>No. determinations</th>
<th>Per cent (mean ± S.D.)</th>
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</thead>
<tbody>
<tr>
<td>Contralateral muscle</td>
<td>15</td>
<td>100</td>
</tr>
<tr>
<td>Tumor</td>
<td>15</td>
<td>16 ± 20</td>
</tr>
<tr>
<td>Adjacent muscle</td>
<td>15</td>
<td>60 ± 30</td>
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</table>
of these four animals, the tumor was small and firm. There were no apparent differences in the tumors or in the adjacent tissue to suggest any explanation for this variation in metabolic activity. In the tumor tissue itself (nine rats), the oxidation of succinate was reduced in all but one case. With respect to the opposite leg, these values ranged from 8 to 102 per cent, with a mean of 44 per cent. Six of the nine values were below the mean.

Histologic examination was made of a block of tissue contiguous with that used in the preparation of the homogenate of tumor, of adjacent "normal" muscle, and of muscle from each rat at the corresponding site of the opposite leg. Portions of these blocks were fixed in Zenker's fluid, in 10 per cent formalin, and in absolute alcohol whenever material was sufficient. Paraffin-embedded tissue sections were stained with hematoxylin-eosin, Mallory's aniline blue, and Mallory's phosphotungstic acid-hematoxylin. Celloidin-embedded sections were stained by the Best's carmine method to demonstrate glycogen.

Most tumors were well differentiated, fairly well circumscribed fibrosarcomas, some of the larger of which exhibited central necrosis and hemorrhage. A few tumors were more anaplastic and pleomorphic; some had scattered giant cells with large single, multinucleated, or multiple nuclei, and bizarre mitotic figures. Such tumors tended to fuse with and infiltrate the surrounding skeletal muscle. Inflammatory reaction with lymphocytes, plasma cells, histiocytes, and occasionally neutrophils was minimal to moderate within both the tumor tissue and the immediately contiguous skeletal muscle. In comparison with the uniform-appearing fibers of the opposite leg muscle, the fibers of the apparently "normal" muscle adjacent to a smaller tumor appeared essentially similar. Those adjacent to a larger tumor showed considerable variation in size, turgidity, and structural features, revealing atrophy of some fibers, swelling and homogeneity of other fibers, and distortion or loss of striations and myofibrils. Demonstrable glycogen was minimal and patchy in most sections.

DISCUSSION

Although two animals showed a stimulation of the enzyme activity when succinate was added to a homogenate of the tissue adjacent to tumors, the other fourteen animals showed depressions in the activity when either the succinate or the oxalacetate substrate was added. It is entirely possible that this difference can be explained on the basis of variations in the distance from the tumor that the "adjacent" samples were taken; or, if the effect is produced by a metabolic product of tumor metabolism, on differences in the rate of production or differences in the rate of diffusion of this metabolic product in different animals. It is possible also that, before destruction of enzyme activity has advanced, a small concentration of the hypothetically metabolic product might stimulate the activity of these enzyme systems. Thus, during the transition between the phase of stimulation and the phase of inhibition or destruction, any degree of activity between that of maximal stimulation and complete inhibition might be found.

SUMMARY

1. The oxidation of succinate and of oxalacetate as added substrates in homogenates of methylcholanthrene-induced tumors in the thigh muscles of rats and in the apparently "normal" muscle surrounding the tumors has been compared with the oxidation of the same substrates in homogenates of the corresponding muscle of the opposite leg.
2. The oxidation of both substrates was reduced in methylcholanthrene-induced tumors.
3. The oxidation of both substrates in apparently normal muscle adjacent to methylcholanthrene-induced tumors was highly variable but was usually reduced to values between that of the tumors and that of the corresponding normal muscle of the opposite leg.

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Oxygen Utilization by Methylcholanthrene-induced Tumors, Adjacent Muscle, and Normal Muscle of Rats

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