Cytochrome Oxidase in the Liver of the C3H Mouse with Carcinoma.* (29954)

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An increase in the cytochrome oxidase activity in liver homogenates or mitochondria from livers of rats bearing Walker carcinoma 256 has been shown by Greene and Haven (1). This observation was confirmed by Greene(2) who showed that the increase in enzyme activity could not be attributed to an uncoupling of oxidative phosphorylation in the liver of the tumor-bearer. To extend the primary observation to a tumor and species other than the Walker carcinoma 256 in the rat, the cytochrome oxidase activity of the liver of female mice of the C3H strain bearing spontaneous or transplanted breast carcinoma has been determined and compared with similarly determined values for tumor-free mice and for Sprague-Dawley rats bearing the Walker carcinoma 256. The results are reported here.

Materials and methods. Animals. Female mice of the C3H/HeJ strain obtained from the Jackson Memorial Laboratory, Bar Harbor, Maine, were used for growth of both spontaneous and transplanted tumors. For the latter, mice 3 to 6 months of age received transplants of spontaneous carcinoma subcutaneously by trochar. Sprague-Dawley rats of both sexes were used; male rats received subcutaneous transplants of Walker carcinoma 256. Both mice and rats were maintained on Purina laboratory chow.

Preparations and assay. Normal and tumor-bearing mice were weighed, then sacrificed by decapitation and drained of as much blood as possible. The liver was quickly removed and the wet weight obtained. Tumors were removed and weighed. One gram of liver from the normal mouse and 1.1 g from the tumor-bearer, respectively, were used for preparation of homogenates. By equal reduction of the amounts of all reagents the same ratio of tissue to final volume was maintained as in the preparation of rat liver homogenates (1). The cytochrome oxidase activity of the homogenates was assayed in phosphate buffer with hydroquinone as substrate as previously described(1). The dry weight of the samples was determined after dialysis on four 1-ml aliquots of each homogenate. The remainder of the liver, not used in preparation of the homogenate, was weighed, lyophile-dried, and the weight of the dry tissue obtained.

Results. The growth of spontaneous breast carcinoma in C3H mice decreased the cytochrome oxidase activity of the liver so that every value except one was below the average of 182 μ 10_2/hr/mg dialyzed dry wt for the tumor-free mice. Although the magnitude of the decrease varied directly with tumor size expressed as percentage of total body weight (B.W.) (carcass plus tumor) (Fig. 1), the average value of 153 $\pm$ 14 μ 10_2/hr/mg dialyzed dry wt, differed significantly from the average in tumor-free mice ($p<0.001$). Essentially the same results were obtained for the liver of mice growing transplanted tumor (av = 154 $\pm$ 16; $p<0.001$). This decrease in cytochrome oxidase activity for mice with tumor was opposite to the results obtained in both Rochester(1) and Sprague-Dawley rats (Table I) in which the growth of Walker carcinoma 256 increased the cytochrome oxidase activity ($p<0.01$). The activity of this enzyme was found to be higher in the mouse than in the rat of either strain (1; Table I).

Discussion. The *decrease* in cytochrome

TABLE I. Cytochrome Oxidase Activity of Liver in Sprague-Dawley Rats.

Group	μ 10_2/hr/mg dry wt
Male (11)*	90 $\pm$ 17†
Male with Walker carcinoma 256 (12)‡	114 $\pm$ 18
Female (6)	64 $\pm$ 7

* No. of animals. † S.D. ‡ Tumors comprised 39.9% total B.W.

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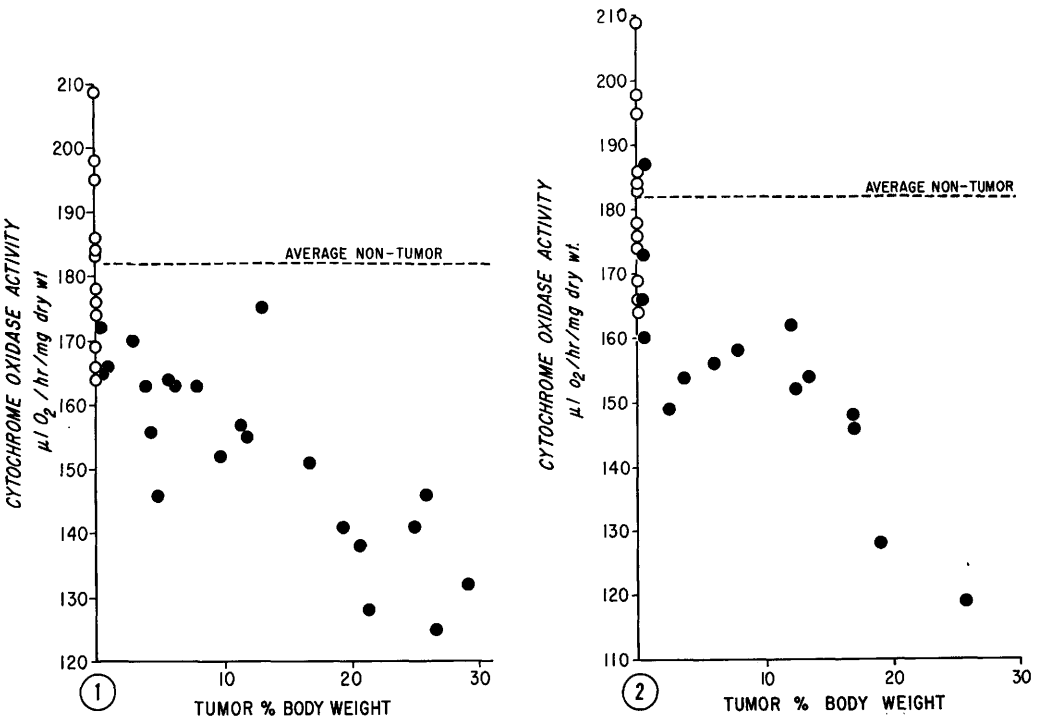

FIG. 1. Cytochrome oxidase activity of liver against spontaneous breast carcinoma as percentage of total body weight in C3H mice. ○ = non-tumor mouse; ● = tumor mouse. Each point = average of duplicate determinations on one liver. Av μ 10_2/hr/mg dialyzed dry wt for non-tumor mice = 182 ± 14; for tumor mice = 153 ± 14 ($p = <0.001$); av tumor % B. W. = 12.2; av liver % B.W. = 5.0 ± 0.5 for tumor-free *vs* 5.9 ± 0.8 for tumor mice ($p < 0.01$; >0.001); dry liver as % wet = 34.6 ± 0.6 for tumor-free *vs* 32.3 ± 1.6 for tumor mice ($p < 0.001$).

FIG. 2. Cytochrome oxidase activity of liver against transplanted breast carcinoma as percentage of total body weight in C3H mice. ○ = non-tumor mouse; ● = tumor mouse. Each point = average of duplicate determinations on one liver. Av μ 10_2/hr/mg dialyzed dry wt for non-tumor mice = 182 ± 14; for tumor mice = 154 ± 16 ($p < 0.001$); av tumor % B. W. = 9.1; av liver % B. W. (tumor-free, see Fig. 1 legend) 5.3 ± 0.8 ($p < 0.3$; >0.2); av dry liver as % wet (tumor-free, see Fig. 1) 32.5 ± 1.9 ($p < 0.01$; >0.001).

oxidase activity in the liver of the C3H mouse with tumor as opposed to the *increase* previously found for the rat growing Walker carcinoma 256(1) emphasizes the danger of generalizations regarding tumor-host relationships. In the Sprague-Dawley rat the progressive growth of tumor brought about an even greater increase in hepatic cytochrome oxidase activity than in the Rochester rat (1), probably owing to the fact that tumors comprise about 40% of the total B.W. in the former (Table I) as compared with about 34% in the latter. Clearly, the difference between the mouse and the rat may not be attributed to the effect of a spontaneous *vs* a transplanted tumor since both methods of tumor growth produced the same results in the mouse.

The higher cytochrome oxidase activity of the liver in the tumor-free mouse as compared to the rat confirms the work of Kunkel and Campbell(3) who showed that the activity of this hepatic enzyme was inversely related to body size. Jánsky(4) confirmed this relationship for total cytochrome oxidase activity of animals, including the mouse and the rat, and linked the enzymatic activity to the basal metabolism.

In contrast to the results obtained for cytochrome oxidase activity some other effects of the tumor on the host were similar in the mouse to those previously shown for the rat(5). Enlargement of the liver occurred; liver as percentage B.W. was significantly greater than normal in mice with spontaneous tumors (Fig. 1—legend). Also, the dry

weight of the liver decreased significantly with the growth of tumor (Fig. 1 and 2—legends). Finally, careful examination of the carcass of the tumor mouse revealed the loss of stored fat so characteristic of tumor growth.

The growth of tumor in mice and rats differed in at least one respect which might account for the species difference in the effect on cytochrome oxidase activity. The tumor rats of Table I were sacrified at about 2½ months after receiving transplants, when tumors comprised about 40% of total B.W. On the other hand, mice that were given transplants had grown barely palpable tumors in the same length of time. Spontaneous tumors, once they appeared, grew at approximately the same rate as the transplanted. Conceivably, the stress of rapid tumor growth in the rat with Walker carcinoma 256, a tumor which has been shown to increase the caloric requirements of the host(6,7), may account for the increased activity of the terminal oxidative enzyme, cytochrome oxidase; whereas, in the mouse, in which tumor grew slowly, the degree of stress may not have been sufficient to bring about increased enzymatic activity. Further work is necessary in order to explain the decrease in cytochrome oxidase activity in the liver of the tumor mouse.

Summary. In C3H female mice growing either spontaneous or transplanted breast carcinoma the cytochrome oxidase activity of liver homogenates decreased as the tumor attained an increasingly larger percentage of total body weight. On the other hand, confirming previous results with Rochester rats, an increase in the activity of this enzyme was found in liver homogenates from Sprague-Dawley rats bearing Walker carcinoma 256. In tumor-free animals, mice had significantly higher cytochrome oxidase activity than rats of either strain.

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Transmission of Sindbis Virus by *Aedes aegypti* to a Chick Embryo Cell Culture System.* (29955)

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Sentinel animals commonly are used for surveillance of the arthropod transmission of viral agents. Viruses have been demonstrated in endemic and epizootic environments by the use of non-immune monkeys(1), mice(2), and chickens(3). There are, however, certain limitations which restrict the usefulness of this approach. Difficulties in transportation, maintenance, and protection are inherent in the use of animals. Furthermore, except where virus transmission is indicated by obvious disease, the sentinel animal must be tested for presence of virus or for antibody conversion. To detect a low order of virus transmission, the investigator is faced with the task of processing numerous specimens. The results of such tests often reflect past activity and fail to stimulate expanded investigative or control efforts at a time when

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