

Succinoxidase Activity of the Liver of Rats Bearing Walker Carcinoma 256.* (30507)

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An increase in cytochrome oxidase activity in liver homogenates or mitochondria from livers of rats bearing Walker carcinoma 256 was found by Greene and Haven(5) and confirmed by Greene(4) for Rochester rats, and by Haven *et al*(6) for Sprague-Dawley rats. Since the concentration of the enzyme was expressed as activity per mg dry weight the increase could be accounted for either by an actual increase in the amount of enzyme, or by a loss of protein more labile than the enzyme. Determination of the activity of another liver enzyme would tell whether such protein loss had occurred. To this end, the activities of succinoxidase as well as cytochrome oxidase were determined simultaneously on each liver of pairs of tumor-free and tumor-bearing rats. The results are reported here.

Materials and methods. Animals. Male Sprague-Dawley rats were housed singly in basket-type cages with free access to water and Purina laboratory chow. When approximately 5 weeks of age rats that were to grow tumor received Walker carcinoma 256 subcutaneously on the back by the trocar technique.

Preparations and assay. At an average of 5 weeks after transplantation of tumor, normal and tumor-bearing rats were sacrificed by decapitation and drained of as much blood as possible. One normal and one tumor-bearing rat were killed at the same time for assay of the two enzymes. Livers were removed and weighed, aliquots were taken, and homogenates were prepared as previously described (5), except that the homogenate was filtered through medium then through fine cheese cloth before the final homogenization with the tight-fitting pestle and the dilution to volume with phosphate buffer of pH 7.1. The

dry weight of the samples was determined after dialysis on four 1-ml aliquots of each homogenate. Nitrogen determinations were carried out on dialyzed 1-ml samples of the homogenates by a semi-micro Kjeldahl method with a copper-selenium catalyst. Each homogenate, prepared from a single liver, was assayed for both succinoxidase and cytochrome oxidase activities, which were based on the same value for dialyzed dry weight.

The succinoxidase assay was carried out at 25°C by the manometric procedure described by Schneider and Potter(7). The rate of oxygen consumption was linear with time and proportional to the amount of homogenate used. The enzyme activity (based on the average of duplicate determinations and corrected for endogenous uptake) was expressed as $\mu\text{l O}_2/\text{hr}/\text{mg}$ dialyzed dry weight. The cytochrome oxidase activity of the homogenates was assayed in phosphate buffer with hydroquinone as substrate as previously described(5). The data were subjected to the "t" test of Fisher for statistical analysis(3).

Results and discussion. In the livers of the tumor-bearing rats no change in the succinoxidase activity occurred, while the cytochrome oxidase activity increased significantly (Table I). This was true whether the enzyme activities were expressed per mg of dialyzed dry weight or per total liver. The results confirm those of Andrews(2) for Rochester rats with this tumor and of Aboobaker(1) for succinoxidase activity of tumor-bearing mice. Since the succinoxidase activity of the tumor-bearers did not change, the increase in cytochrome oxidase was not accounted for by a loss in protein more labile than the enzyme. Substantiation of this conclusion comes from the work of Wainio *et al* (8) who found that inanition increased the activity of cytochrome oxidase per mg of nitrogen, but decreased the activity per total liver because of a decrease in liver weight.

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TABLE I. Succinoxidase and Cytochrome Oxidase Activity of Liver Homogenates from Normal (N) and Tumor-Bearing* (TB) Rats.†

Group	$\mu\text{l O}_2/\text{hr}/\text{mg}$ dialyzed dry wt				$\mu\text{l O}_2 \times 10^{-3}/\text{hr}/\text{total liver}$			
	Succinoxidase		Cytochrome oxidase		Succinoxidase		Cytochrome oxidase	
	N	TB	N	TB	N	TB	N	TB
Mean	23	36	120	146	67	82	242	343
Standard deviation	4	7	11	12	10	12	28	52
Significance of difference	$p < .4; > .3$		$p < .001$		$p < .4; > .3$		$p < .05; > .02$	

* Avg tumor % total body wt = 41.

† 8 pairs.

Although anorexia is characteristic of rats growing Walker carcinoma 256, the concomitant increase in size of the liver caused an increase rather than a decrease in cytochrome oxidase activity per total liver.

A differential loss in weight upon dialysis of the liver homogenate from the tumor-bearer as compared with the normal would not account for the increase in cytochrome oxidase of the former because the unchanged activity of the succinoxidase and the increased activity of the cytochrome oxidase were both based on the same dialyzed dry weight. For additional confirmation of this point and because such enzyme activities are frequently based on nitrogen rather than on dialyzed dry weight, dialyzed samples of liver homogenates from normal and tumor-bearing rats were ashed and the nitrogen content determined. This averaged $77.7 \pm 2.4\%$ of theoretical (dialyzed dry wt $\times 0.1666$) for the normal rats, and $78.3 \pm 2.9\%$ for the tumor-bearers. The difference was not statistically significant. Thus, as shown by identical percentage of nitrogen, the composition of the dialyzed homogenate was qualitatively the same for normal rats and tumor-bearers. Moreover, if the enzyme activity had been based on nitrogen instead of on dialyzed dry weight, the tumor-bearers would still have been higher than normal in cytochrome oxidase activity.

Summary. The succinoxidase activity of the liver of rats with Walker carcinoma 256 remained at the normal level while the cytochrome oxidase increased. Thus this increase did not result from a differential loss of protein from the liver of the tumor-bearer. On analysis, dialyzed samples of liver homogenate, upon which enzyme activities are based, gave identical percentages of nitrogen. Hence, whether cytochrome oxidase activity is based on dialyzed dry weight or on nitrogen content, the level in the tumor-bearer would still have been higher than normal.

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