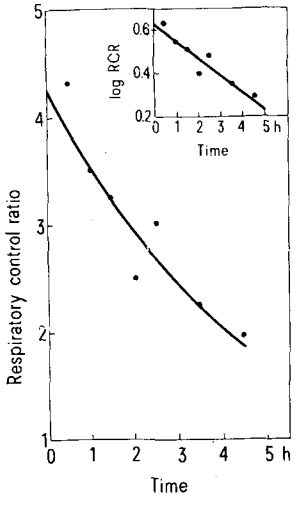
Respiratory Control in Liver Mitochondria of Rats Hosting the Walker 256 Carcinoma Tumor

It has been reported that there are ultrastructural changes which occur in the liver mitochondria of rats hosting a tumor[1,2]. The most notable change is swelling of a magnitude which usually indicates an uncoupling of mitochondria[3]. GREENE[4], however, was unable to detect any diminution in oxidative phosphorylation of mitochondria isolated from the liver of rats which were hosting the Walker 256 carcinoma tumor. In the latter experiments oxidative phosphorylation was measured by monitoring oxidation manometrically and determining ATP formation by loss in inorganic phosphorus. Subsequent to GREENE's study the measurement of respiratory control ratios[5] was introduced for determining the capability of mitochondria to carry out oxidative phosphorylation. This method allows one a more sensitive gauge of the capability of these organelles to synthesize ATP[3,6]. Because of our interest in determining if malignancies directly affect energy metabolism in the non neoplastic tissues of their hosts, we decided to reinvestigate the capability of mitochondria to carry out ATP synthesis by measuring respiratory control ratios. In this study we offer an explanation for the apparent inconsistency between the ultrastructural alterations seen in liver mitochondria from tumor bearing animals[1,2] and the results of GREENE[4] which suggest no change in the potential of these mitochondria to synthesize ATP. We also outline a method for quantitatively determining the rate of loss of respiratory control, a procedure which will have utility in any study of mitochondrial functional integrity.

Materials and methods. Male Sprague-Dawley rats (approximately 200 g) were injected s.c. on the back with 100,000–200,000 viable Walker 256 tumor cells according to the technique described by COLE[7]. Both injected and uninjected control animals were fed Purina rat chow ad libitum and the amounts eaten daily were recorded. The weights of the animals were measured every 3 to 5 days and immediately before sacrifice. Rat liver mitochondria were prepared as described by SCHNEIDER[8]. Respiratory control ratios (RCR) of the mitochondria were determined as described by SORDAHL et al.[9]. Protein determinations were made by the method of GORNALL et al.[10].



Loss in respiratory control of rat liver mitochondria versus time of incubation at 8 °C. Rat liver mitochondria were prepared at 0 °C. Immediately after preparation the mitochondria were incubated in 0.25 *M* sucrose at 8 °C, with their concentration being adjusted to 14.5 mg/ml within 30 min after the incubation had begun. The RCR measurements were carried out at the times indicated. The insert is the same data plotted as log RCR vs time. The curve and line shown were obtained from a linear regression analysis of log RCR vs time. The correlation coefficient for first order decay = 0.94.

Results and discussion. In this investigation we were particularly concerned about the nutritional status and overall health of the tumor bearing animals, since it has been suggested by investigators[11] characterizing cell organelles of Morris hepatomas that changes which occur may be due in part to the nutritional deficiences and/or degenerative processes which accompany tumor development. In the present experiments the overall health of the animal was judged by its weight gain and food uptake. The growth rate and average daily food uptake of tumor bearing and control animals were essentially the same, which suggests that the nutritional status of these animals was stable throughout the development of the tumors. During this period the animals hosting neoplasms developed large tumors with necrotic centers. The tumor sizes ranged from 4 to 6 cm in diameter.

The respiratory control ratios of the liver mitochondria prepared from the above animals were measured over a 5-hour period during which time the organelles were incubated at 8 °C. By measuring the loss in respiratory control over a period of time we were able to determine if there was any obvious defect in liver mitochondria from tumor bearing animals which would cause them to rapidly lose their ability to catalyze oxidative phosphorylation when they were removed from their cellular environment. Any structural alteration initiated by the tumor might cause them to lose ATP synthesizing capability at a faster rate than would mitochondria from animals not bearing a neoplasm. The loss of respiratory control in rat liver mitochondria prepared from one representative tumor bearing animal is shown in the Figure. The insert in which log respiratory control ratio is plotted against time demonstrates that the loss in this mitochondrial property is a first order process. In 7 preparations the correlation coefficients obtained from a linear regression analysis of log RCR vs time ranged from 0.91 to 0.97, while in 2 preparations it was 0.85 and 0.73, respectively.

In the Table both the initial respiratory control ratios of freshly prepared mitochondria and the first order rate constants for loss of respiratory control are reported. The data demonstrate that there is no significant difference in either the initial respiratory control ratios or the rate of loss of respiratory control at 8 °C when mitochondria from tumor bearing animals are compared with those of

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Initial respiratory control ratio values and first order rate constants for loss of respiratory control

Animal type	Initial RCR		k
Tumor bearing	4.25 ± 0.88	(4)[a]	$-0.143/h \pm 0.035$
Control	4.57 ± 0.71	(5)[b]	$-0.147/h \pm 0.043$[c]

The tumor bearing animals were treated as described in the methods section. Measurements were carried out on the isolated mitochondria as described in the Figure. The initial RCR and k values reported are the means obtained from linear regression analyses carried out for the data obtained from each animal.
[a] Values reported are ± SD. The number of animals are indicated in parenthesis. [b,c] No significant difference between values obtained from control rats and tumor bearing animals at the 50% level, [b] p 0.56, [c] p 0.88.

control rats. These results indicate that in apparently healthy animals with well advanced tumors there are no adverse changes occurring in mitochondria which affect their ability to produce ATP. Furthermore, the absence of any significant difference in the rate of loss of respiratory control between mitochondria from tumor hosts and those from control animals suggests that the fragility seen in morphological studies on mitochondria from tumor bearing animals[1,2] is not due to a direct effect mediated by the tumor. This conclusion is supported by respiratory control measurements on liver mitochondria from a tumor bearing animal which was judged to be near death[2]. The respiratory control ratio was 1.0, which indicated that the mitochondria could not catalyze ATP synthesis[5]. This observation coupled with the information in the Table indicates that the morphological changes which indicate damage in mitochondria[1,2] are probably related more to the dying process than to any direct effect mediated by the tumor.

This investigation underscores the necessity of using tumor bearing animals in which the degenerative processes associated with dying have not become pronounced. This is especially critical when comparing the properties of cellular organelles from animals, whether they are obtained from a neoplasm or non-neoplastic tissue. Failure to take the overall health of the animal into consideration may complicate interpretation of results since the overall physiological status of the animal may be more responsible for the changes observed than is the presence of the neoplasm.

Summary. The decrease in the respiratory control ratio of mitochondria is a first order process when these organelles are incubated in isotonic sucrose. Furthermore, the initial respiratory control ratios and the rates of loss in respiratory control in liver mitochondria from rats hosting the Walker 256 carcinoma are not significantly different from the same properties of mitochondria from untreated animals.

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Ferritin Synthesis by Splenic Tumor Tissue of Hodgkin's Disease

Elevated amounts of ferritin have been demonstrated in both splenic tumor nodules and serum of patients with Hodgkin's disease[1-4]. Large quantities of ferritin in Hodgkin's tumor tissue could be due to either a) passive uptake and storage of serum ferritin, or b) cellular ferritin synthesis. To differentiate between these alternatives, we have studied ferritin synthesis by incubating Hodgkin's disease splenic tumor tissue and splenic tissue distinct from tumor nodules with ^{14}C-labelled amino acid followed by double immunodiffusion and radioautography.

A 450 g spleen was removed at staging laparotomy from an 18-year-old male who presented with left neck and mediastinal involvement but no systemic symptoms. Cervical node biospy revealed Hodgkin's disease, nodular sclerosing type. Grossly, the splenic parenchyma was dark red with multiple pale nodules measuring up to 1.5 cm in diameter. Microscopically, there was focal obliteration by pleomorphic cellular tumor infiltrates with giant cells of Reed Sternberg type. Splenic hilar and paraaortic lymph nodes were also involved by tumor, but liver and bone marrow was normal. Final pathology was Stage IIIA.

Approximately 350 mg of slices from each tissue were incubated in Krebs Ringers Phosphate buffer pH 7.4 containing 20 µCi L-leucine-^{14}C for 8.5 h at 37 °C. The slice-buffer mixture was homogenized, 100 µg of purified human spleen ferritin was added to provide carrier ferritin, centrifuged and clear supernatant was dialyzed against isotonic saline containing unlabeled DL-leucine. Dialyzed material was concentrated to one-fifth its original volume and used for immunodiffusion against rabbit antiserum developed against purified human spleen ferritin (center well). Immunodiffusion plates were washed in distilled water, dried and placed against Kodak no-screen industrial type x-ray film for 8–12 weeks.

Double immunodiffusion of incubated patient splenic tissue extracts and purified ferritin from human spleen and liver against rabbit antisera to human splenic ferritin resulted in a single continuous precipitin line which stained for both protein and iron (Figure, left). Immuno-electrophoresis of these same components also resulted in a single precipitin arc in the α_2-region. Radioautography of these double immunodiffusion patterns (Figure, right) revealed that Hodgkin's disease splenic tumor incubated with ^{14}C-leucine always resulted in a strong positive radioautograph indicating incorporation of ^{14}C-amino acid into ferritin. Under identical experimental conditions 2 of 3 incubations of normal appearing spleen tissue distinct

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