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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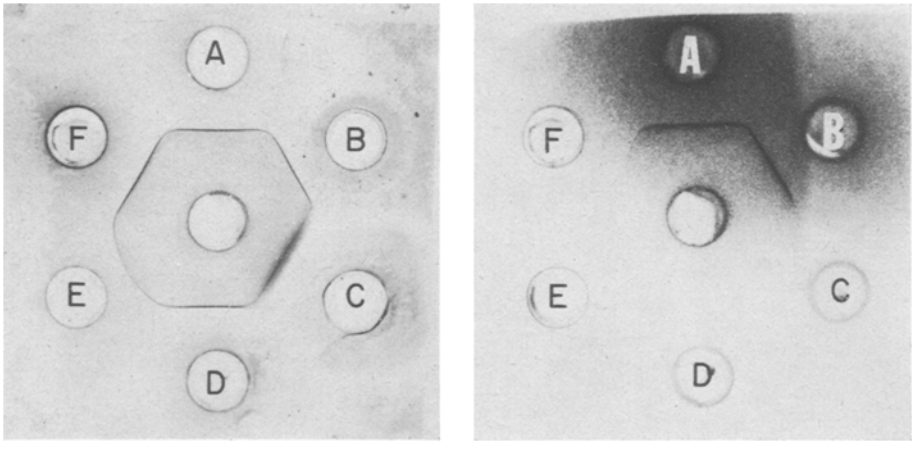

Double immunodiffusion patterns in agar (left) and radioautograph (right) obtained by incubating Hodgkin's diseae splenic tumor tissue (wells A and B) and normal appearing spleen tissue distinct from tumor nodules from the same patient (wells E and D). Wells (F) and (C) contained unlabelled human ferritin from spleen and liver respectively.

from tumor nodules did not produce demonstrable radioactive labelling of ferritin. In one such incubation, however, a trace amount of labelled ferritin was detected. This could be due to either a low level of ferritin synthesis by normal spleen/tissue or synthesis of ferritin by small foci of tumor cells which had infiltrated the normal splenic tissue area used for incubation. In support of the latter explanation, microscopic examination of sections of normal appearing spleen tissue distinct from tumor nodules did reveal varying degrees of tumor infiltration, although some areas were tumor free.

These results demonstrate increased ferritin synthesis by Hodgkin's disease splenic tumor tissue, and suggest that this is the cause of the elevated tumor and serum ferritin concentration found in patients with Hodgkin's disease.

Summary. Increased ferritin synthesis by Hodgkin's disease splenic tumor tissue was demonstrated by incorporation of ^{14}C-leucine and radioautography. This suggests that elevated tumor and serum ferritin concentrations found in patients with Hodgkin's disease is derived from tumor tissue per se.

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Patterns of Membrane Organization in Toad Bladder Epithelium: a Freeze-Fracture Study[1]

The urinary bladders of amphibians have been widely used as models of the collecting tubules of the mammalian kidney in respect to the transport of sodium, water and the hydrogen ion[2–4]. In its upper part, the collecting tubule is composed of two cell-types, the 'dark' and the 'clear' cells. In a recent freeze-fracture study[5], characteristic plasma membrane differentiations in each of these cell-types were defined: the membrane of the dark cell contains rod-shaped particles, whereas that of the clear cell exhibits square arrays of small particles. Using the freeze-fracture technique in a similar study of the toad bladder, we present evidence that the plasma membrane of one cell-type of this epithelium, the mitochondria-rich cell, also contains rod-shaped particles, while another cell-type, the granular cell, displays numerous large particles luminally in the B-fracture face of its plasma membrane. These membrane features differ sharply from the general membrane pattern seen in most other cells, as revealed by this technique[6].

Material and methods. Toads (*Bufo marinus*) were obtained from Mogul-Ed Co., Oshkosh, Wisconsin, USA. After doubly pithing the toads, we excized the urinary bladders and mounted them in glass chambers. The serosal side of the epithelium was exposed to a Ringer's solution of standard composition[7] and the mucosal side to the same solution diluted 10 times. After exposure to these solutions for various periods, the tissues were quickly removed from the chambers and fixed with a 2% glutaraldehyde solution containing 0.1 *M* phosphate buffer. For freeze-fracture studies, small pieces of epithelium cut from the original sheet were soaked in a phosphate-buffered 30% glycerol solution, then freeze-fractured in a Balzers BAF 301 unit[8]. For conventional thin-sectioning, pieces of glutaraldehyde-fixed epithelium were postfixed in phosphate-buffered osmium tetroxide, dehydrated, and embedded in Epon. Freeze-fracture replicas and thin sections, deposited on coated copper grids, were examined in a Philips EM 300 electron microscope.

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