Uncoupler-stimulated Adenosine Triphosphatase Activity

DEFICIENCY IN INTACT MITOCHONDRIA FROM MOLLIS HEPATOMAS AND ASCITES TUMOR CELLS

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SUMMARY

1. Well coupled mitochondria isolated from six hepatomas differing widely in growth rate and degree of differentiation were found to exhibit little or no 2,4-dinitrophenol-stimulated ATPase activity in sharp contrast to normal liver mitochondria. Well coupled mitochondria from L1210 and Ehrlich ascites tumor cells were also found to have markedly reduced uncoupler-stimulated ATPase activity when compared with normal liver mitochondria.

2. When intact mitochondria from one of the hepatomas (7800) were studied in greater detail, it was found that normal uncoupler-dependent ATPase activity could not be demonstrated under a variety of conditions. These included (a) the use of uncouplers more potent than 2,4-dinitrophenol; (b) alteration of osmotic and ionic conditions; (c) variation of temperature between 20 and 40°C; (d) variation of pH between 6.9 and 9.5; and (e) inclusion of either defatted bovine albumin or oxidizable substrate in the assay medium.

3. Neither forward nor reverse energy-dependent reactions in hepatoma 7800 mitochondria are altered, nor is their sensitivity to uncoupling agents. In the forward direction, hepatoma mitochondria were found to exhibit normal acceptor control and P: O ratios sensitive to uncouplers, and to exhibit uncoupler-stimulated respiration. In the reverse direction, the ATP-Pi exchange rate was found to be the same in hepatoma 7800 and control liver mitochondria and inhibited almost completely in both cases by 2,4-dinitrophenol. In addition, the capacity for ATP-supported Ca2+ accumulation was similar for hepatoma 7800 and control liver mitochondria.

4. Unlike hepatoma mitochondria, mitochondria isolated from regenerating liver 24, 48, and 72 hours after partial hepatectomy exhibit normal levels of uncoupler-stimulated ATPase activity.

5. These results are interpreted most simply by assuming that hepatoma mitochondria, and perhaps mitochondria from L1210 and Ehrlich ascites tumor cells, contain two principal sites of action for uncoupling agents, one of which may become expressed during the normal to neoplastic transition.

The effect of 2,4-dinitrophenol and other uncoupling agents on mitochondrial energy-linked activities has been studied extensively. At low concentrations, these agents inhibit oxidative phosphorylation and usually stimulate both respiration and ATPase activity (1, 2). Similar to their effects on oxidative phosphorylation, uncoupling agents inhibit other energy-linked activities including exchange reactions (3, 4), divalent cation uptake (5, 6), and reverse electron flow (7). These effects are explained most simply within the framework of current hypotheses of energy coupling by assuming that the initial high energy state resulting from electron transport is dissipated by uncoupling agents (8, 9).

In a brief communication concerned with the effect of uncoupling agents on mitochondria from hepatomas of the Morris variety, Devlin and Pruss (10) reported that mitochondria from several of these hepatomas have an atypically low uncoupler-stimulated ATPase activity. Consistent with these observations, we reported in a recent preliminary communication (11) that intact mitochondria from Morris hepatoma 7800, which exhibit normal uncoupler-inhibited ATP formation and normal uncoupler-stimulated respiration, are markedly deficient in uncoupler-stimulated ATPase activity.

Since these latter observations were in sharp contrast to what is usually observed for mitochondria from normal mammalian tissue, we extended our earlier studies with the following objectives in mind. First, we wanted to ascertain to what extent mitochondria from hepatomas of different growth rates are deficient in uncoupler-stimulated ATPase activity. Secondly, we wanted to determine whether uncoupler-stimulated ATPase activity could be explained by suboptimal assay conditions. Third, we wanted to determine the sensitivity of uncoupling agents of forward and reverse energy-linked activities in hepatoma mitochondria. Fourth, it seemed important to examine mitochondria from regenerating liver to ascertain whether the deficiency of uncoupler-stimulated ATPase activity was simply a consequence of rapid cell growth. Finally, for com-
parative purposes we wanted to assay mitochondria from some tumor cells of nonhepatic origin for uncoupler-stimulated ATPase activity. Experiments relevant to each of these objectives are described below.

**Experimental Procedure**

**Materials**

Adult male Sprague-Dawley albino rats and adult male mice (CD1 strain) were obtained from the Charles River Breeding Laboratories (Wilmington, Mass.). Female mice (BDF1 strain) were obtained from Buckberg (Rockland, N. Y.). Male rats bearing the following hepatomas (listed in order of increasing growth rate (12, 13) were obtained from Dr. H. P. Morris, Howard University: 9618A, 16, 7794A, 7800, 7777, and 3924A. L1210 and Ehrlich ascites tumor cells were obtained from Dr. A. L. Lehninger of this department. ATP and NADH were purchased from P-L Biochemicals. Phosphoethanolamine, Heps,1, lactic dehydrogenase (bovine heart, type III), and hexokinase (yeast, type C-300) were obtained from Sigma. Sucrose and n-glucose were obtained from the J. T. Baker Chemical Co. Pyruvate kinase (rabbit muscle, grade A) was purchased from Calbiochem. Phenylalanine-2',4-dinitrophenylhydrazone (FCCP) was generously donated by Dr. P. G. Heytler of Dupont, and the salicylaldehyde derivative (Compound XIII) was a gift from the Monsanto Co. (St. Louis, Mo.). Nagarse was obtained from the Enzyme Development Corp., and thin layer sheets of polyethyleneimine-impregnated cellulose (Polygram Cel P1) were obtained from Brinkmann, Inc. All other reagents used in these studies were of the highest commercial purity available.

**Methods**

**Isolation of Hepatomas and Ascites Tumor Cells—**Hepatomas were allowed to grow in the hind legs of rats for the following time periods after inoculation: hepatoma 9618A (6 to 9 months), hepatoma 16 (6 months), hepatoma 7794A (1.5 months), hepatoma 7800 (1 month), hepatoma 7777 (2 weeks), and hepatoma 3924A (1 to 2 weeks). Immediately after decapsulation of five hepatoma-bearing rats, livers and hepatoma tissues were removed. Hepatoma tissues in 0.25 ml of 2.5 M HCl were purchased from the New England Nuclear Corp. and heated 3 hours prior to use in 0.1 N HCl in a boiling water bath. 2,4-Dinitrophenol was purchased from Eastman, crystalline bovine albumin from Pentex, Inc. (Kankakee, Ill.), and d-(-)-mannitol from Schwarz-Mann. FCCP was generously donated by Dr. P. G. Heytler of Dupont, and the salicylaldehyde derivative (Compound XIII) was a gift from the Monsanto Co. (St. Louis, Mo.). Nagarse was obtained from the Enzyme Development Corp., and thin layer sheets of polyethyleneimine-impregnated cellulose (Polygram Cel P1) were obtained from Brinkmann, Inc. All other reagents used in these studies were of the highest commercial purity available.

**Acceptor Control and P:O Ratios—**Acceptor control and P:O ratios were determined as described by Chance and Williams (16). Respiratory measurements were carried out at 25°C in a closed 3-ml reaction vessel equipped with a Clark oxygen electrode. The respiration medium contained, in a final volume of 3 ml, 220 mM n-mannitol, 70 mM sucrose, 2.5 mM potassium phosphate, 2 mM Hepes, 0.5 mM EDTA, 1.7 mM MgCl2, 2 mg of defatted bovine albumin, 10 mM sodium o-p-hydroxybutyrate or 5 mM sodium succinate, and when added, 152 phi ADP. The final pH was 7.4.

**ATPase Assay—**ATPase activity was assayed in two different systems. In the first (ATPase assay system 1), the amount of phosphate released was measured. Incubation before assay was for 4 min at 30°C in a medium containing, in a total volume of 0.45 ml, 11 mM imidazole, 55 mM sucrose, and 0.05 mg of mitochondrial phosphoenolpyruvate, and 5 mg of mitochondria, pH 6.9. Where indicated in legends to figures and tables, MgCl2, pH 7.3. In the second assay system (ATPase assay system 2), the release of 32P-labeled orthophosphate from 32P-labeled ATP was followed in the assay medium. ATPP was followed in the assay for ATP-Po exchange noted below. [32P]ATP labeled with K+ in the y position by the procedure of Cllynn and Chappell (18) was purchased from the New England Nuclear Corp. and used in the exchange assay medium.

**ATPase Assays—**ATPase activity was assayed in two different systems. In the first (ATPase assay system 1), the amount of phosphate released was measured. Incubation before assay was for 4 min at 30°C in a medium containing, in a total volume of 0.45 ml, 11 mM imidazole, 55 mM sucrose, and 0.05 mg of mitochondrial phosphoenolpyruvate, and 5 mg of mitochondria, pH 6.9. Where indicated in legends to figures and tables, MgCl2, pH 7.3. In the second assay system (ATPase assay system 2), the release of 32P-labeled orthophosphate from 32P-labeled ATP was followed in the assay medium.
and ATP was carried out as follows. Aliquots of 5.0 μl were spotted on thin layer sheets of polyethyleneimine-substituted cellulose together with 40 nmol of P, ATP and P, were separated in a solvent system containing 1.9 M formic acid-0.1 M LiCl. P, spots were detected by spraying with the ammonium molybdate-containing mixture described by Hance and Ischerwood (10). ATP spots were detected with ultraviolet light and radioactivity was assessed as described below.

Calcium Uptake—ATP-supported Ca$^{2+}$ uptake was followed as described by Lehninger et al. (5) in a medium containing, in a final volume of 2 ml, 10 mM Tris, 10 mM MgCl$_2$, 80 mM NaCl, 2 mM potassium phosphate, 15 mM ATP, 2 μg of mitochondrial protein, 2 mM CaCl$_2$, and 0.10 μCi of $^{45}$CaCl$_2$, pH 7.4. After a 5-min incubation period at 30°, the reaction mixture was centrifuged immediately in polypropylene tubes at 13,000 × g in a Sorvall SM 24 rotor (0–10). After dissolving the pellet fraction in 1.0 ml of concentrated formic acid, the radioactivity of the formic acid-pellet mixture and the above supernatant were assessed as described below.

Partial Hepatectomy—This operation was performed as described by Higgins and Anderson (20). Rats were anesthetized with ether and given 10% (w/v) glucose for a day after operation. They were maintained on a normal diet until killed. An appropriate sham operation was carried out simultaneously with each partial heptectomy performed.

Measurement of Radioactivity—Radioactivity was routinely determined using a liquid scintillation spectrometer of the Beckman 100 series. Samples adsorbed to solid thin layer supports were counted in 15 ml of scintillation mixture consisting of 4.0 g of 2,5-diphenyloxazole, 0.1 g of 1,4-bis[2-(5-phenyloxazolyl)]benzene, and 1 liter of scintillation grade toluene. Samples from calcium uptake experiments were counted in 15 ml of a scintillation cocktail containing, in 1 liter of 1,4-dioxane, 50 g of naphthalene, 7.0 g of 2,5 diphenyloxazole, and 50 mg of 1,4 bis (5 phenyloxazolyl) benzoic acid.

Protein Determination—Protein was estimated by the biuret reaction using crystalline bovine albumin as standard.

Significance of Data—All experiments summarized in this report were carried out in duplicate at least four times. Data presented in each table represent the average results of such experiments. The standard deviation is less than 10% in all cases.

The biochemical intactness of mitochondria as assessed by acceptor control properties was also considered in evaluating the data. This was important since uncoupler-stimulated ATPase activity, the principal measurement made in these studies, is a mitochondrial property that depends to a large degree on mitochondrial integrity (2).

All mitochondria employed in these studies exhibited acceptor control of respiration as defined by Chance and Williams (16) with the exception of mitochondria from hepatomas 7777 and 7794A. Acceptor control ratios were near or slightly above 5.0 with succinate as substrate (see respiration medium above) in the case of mitochondria from hepatoma 9618A and its liver control. The hepatoma lines are tabulated in the table in order of decreasing growth rate (12, 13).

**TABLE I**

<table>
<thead>
<tr>
<th>Mitochondrial source</th>
<th>Respiration rate$^a$</th>
<th>ATPase activity$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without DNP</td>
<td>With DNP</td>
</tr>
<tr>
<td>Control liver (succinate)</td>
<td>19.6</td>
<td>120</td>
</tr>
<tr>
<td>Hepatoma 3924A</td>
<td>20.0</td>
<td>107</td>
</tr>
<tr>
<td>Hepatoma 7777</td>
<td>5.3</td>
<td>40.1</td>
</tr>
<tr>
<td>Hepatoma 7800</td>
<td>24.4</td>
<td>120</td>
</tr>
<tr>
<td>Hepatoma 7794A</td>
<td>7.1</td>
<td>48.0</td>
</tr>
<tr>
<td>Hepatoma 16</td>
<td>9.6</td>
<td>105</td>
</tr>
<tr>
<td>Control liver (β-hydroxybutyrate)</td>
<td>2.8</td>
<td>30.0</td>
</tr>
<tr>
<td>Hepatoma 9618A</td>
<td>5.5</td>
<td>32.0</td>
</tr>
</tbody>
</table>

$^a$ Nanomoles of oxygen consumed X min$^{-1}$ X mg$^{-1}$.

$^b$ Nanomoles of P, formed X min$^{-1}$ X mg$^{-1}$.

Effect of 2,4-Dinitrophenol on Respiration and ATPase Activity of Liver and Hepatoma Mitochondria—Initial experiments were carried out to compare liver and hepatoma 7800 mitochondria with respect to their rate of respiration and ATPase activity in the presence of 2,4-dinitrophenol. Results summarized in Fig. 1 show that freshly isolated mitochondria from this tumor have normal acceptor control and P:O ratios, and similar rates of respiration in the presence and absence of DNP. In both cases about 7.5 μM uncoupler is necessary to give half-maximal stimulation of respiration (Fig. 1, inset).

An entirely different set of results is obtained when the effect of DNP on ATPase activity is studied. As shown in Table 1, concentrations of DNP that maximally activate respiration and ATPase activity in control liver mitochondria (first entry in the Table) are essentially without effect on ATPase activity of hepatoma 7800 mitochondria (fourth entry in the table, 12, 13).
Column 2). Data also presented in Table I show that a number of other types of hepatoma mitochondria (3924A, 7777, 7794A, 16, and 9618A), all of which have markedly enhanced rates of respiration in the presence of DNP, also exhibit little or no DNP-activated ATPase activity.

Mitochondria from hepatomas 9618A, 16, and 7800 were assayed for ATPase activity over a wide range of DNP concentrations (Fig. 2). At all concentrations of uncoupler examined, hepatoma mitochondria exhibit little or no uncoupler-activated ATPase activity. A slight stimulation is observed in hepatoma mitochondria at very low uncoupler concentrations (10 to 25 μM), but in all cases this amounts to less than 20% of the maximal activity that can be activated above base-line levels in control liver mitochondria.

These results clearly indicate that under conditions where both the rate of respiration and ATPase activity of liver mitochondria are activated by DNP, only the rate of respiration is markedly activated by the uncoupler in hepatoma mitochondria. ATPase Activity of Hepatoma Mitochondria Assay under Variability of Conditions—In order to determine whether the lack of uncoupler-activated ATPase activity in hepatoma mitochondria was due to assay conditions, a variety of experimental conditions were employed. First, FCCP and Compound XIII, uncoupling agents considerably more potent than DNP (21), were included in the assay system (ATPase assay system 1, see "Methods"). As shown in Table II, concentration of these highly potent uncoupling agents (15 μM) sufficient to stimulate ATPase activity about 3-fold in control liver mitochondria has no effect on ATPase activity in hepatoma 7800 mitochondria, and essentially no effect on the ATPase activity of other hepatoma mitochondia as well.

Results presented in Fig. 3 and Table III summarize a number of other experimental variations employed in an attempt to elicit uncoupler-stimulated ATPase activity in hepatoma 7800 mitochondria, the hepatoma mitochondria used in the remaining part of these studies. As shown in Fig. 3, wide variations in KCl concentration, temperature, and pH are essentially without effect on ATPase activity of hepatoma mitochondria in the presence of DNP. Results described in Table III show that combinations of uncouplers and MgCl₂, or inclusion of succinate, defatted bovine albumin, and an ATP-regenerating system separately in the standard assay together with DNP, also fail to markedly activate ATPase activity in hepatoma mitochondria.

Additional experimental variations, the results of which are not presented here, but which also fail to elicit ATPase activity in the presence of uncoupler are as follows: variation of ATP concentration from 0.3 to 20 mM, variation of protein concentration from 0.16 to 1.6 mg per ml, variation of assay time from 1 to 10 min, omission of succinate from the assay medium, elimination of the standard 4.0-min preincubation, and assay under isosmotic conditions.

Taken together, these results show that intact hepatoma mitochondria, which have high acceptor control and P:O ratios sensitive to uncoupling agents, are markedly defective in uncoupler-stimulated ATPase activity. ATPase Activity was assayed in ATPase assay system 1 as described under "Methods." Mitochondrial protein, 0.5 mg, was present in each assay and, where indicated, either 16 μM FCCP or 16 μM Compound XIII was also included. All mitochondrial preparations exhibited uncoupler-stimulated respiration comparable to that summarized in Table I. The hepatoma lines are tabulated in order of decreasing growth rate (12, 13).
chondria have rates of ATP-Pi exchange comparable to those
uncoupling agents as in normal liver mitochondria (3-6).

Assay variations employed in attempt to elicit uncoupler-stimulated
ATPase activity in hepatoma 7800 mitochondria

ATPase activity was measured in ATPase assay system 1 as
described under "Methods" with the additions indicated: Experi-
ment 1, 6.7 mM MgCl₂, 166 pM DNP, 16.6 mM FCCP, and 16.6 mM
Compound XIII; Experiment 2, an ATP regenerating system
consisting of 3.3 mM phosphoenolpyruvate, and 11.4 µg of pyruvate
kinase (specific activity = 268 moles of pyruvate formed X min⁻¹
X mg⁻¹), and 166 µM DNP; Experiment 3, 160 µM DNP, 2.0 mg of
defatted bovine albumin, and 6.7 mM MgCl₂; and Experiment 4,
mitochondria suspended in 50 mM succinate, pH 7.5, prior to
assay. In all experiments, 0.6 mg of either control liver or hepa-

toma 7800 mitochondria exhibiting acceptor control ratios near
5.0 with succinate was present in the assay.

| Experiment | Additions | ATPase activity
| Control liver | Hepatoma 7800 |
| --- | --- | --- |
| 1 | None | 86.5 | 72.0 |
| | + MgCl₂ | 198 | 141 |
| | + MgCl₂ + DNP | 535 | 78.1 |
| | + MgCl₂ + FCCP | 546 | 68.5 |
| | + MgCl₂ + Compound XIII | 606 | 80.5 |
| 2 | None | 114 | 100 |
| | + ATP regenerating system | 106 | 100 |
| | + ATP regenerating system + DNP | 495 | 126 |
| 3 | None | 70.5 | 52.6 |
| | + DNP + bovine albumin | 298 | 47.0 |
| | + DNP + bovine albumin + Mg²⁺ | 308 | 99.5 |
| 4 | None | 91.6 | 65.5 |
| | + Succinate | 107 | 35.6 |
| | + Succinate + DNP | 302 | 47.5 |

* Nanomoles of Pᵢ formed X min⁻¹ X mg⁻¹.

Table IV

Effect of 2,4-dinitrophenol and oligomycin on ATP-Pᵢ exchange and
ATPase activity of control liver and hepatoma 7800 mitochondria
when assayed under identical conditions

ATP-Pᵢ exchange and ATPase activity were assayed exactly as
described under "Methods." ATPase assay system 2 was em-
ployed. Mitochondrial protein, 1.0 mg, and where indicated, 1.0
µg of oligomycin and 160 µM FCCP were present in the assay.

<table>
<thead>
<tr>
<th>Mitochondrial source</th>
<th>ATP-Pᵢ exchange activity*</th>
<th>ATPase activity*</th>
</tr>
</thead>
</table>
| Without inhibitor | With oligo-
mycin | Without inhibitor | With oligo-
mycin |
| Control liver | 43.6 | 1.7 | 0 | 55.0 | 0 | 200 |
| Hepatoma 7800 | 48.0 | 0 | 0 | 34.0 | 0 | 28.8 |

* Nanomoles of Pᵢ exchanged X min⁻¹ X mg⁻¹.

on the basis of these results it does not appear that a simple
noncarcinogenically induced alteration of the growth rates of
liver hepatocytes is sufficient to alter the response of mitochon-
drial ATPase to uncoupling agents. Thus, it would appear
catalyzed by normal liver mitochondria. Complete inhibition
of this exchange in both control liver and hepatoma mitochondria
is given by 160 µM DNP. Results also presented in Table IV
(Column 3) show that under identical conditions to the ATP-Pᵢ
exchange assay, hepatoma 7800 mitochondria have little un-
coupler-stimulated ATPase activity, a finding consistent with
other assay results summarized in this report (Tables I, II, and
III, and Fig. 2).

Results summarized in Table V show that ATP-supported
Ca²⁺ uptake of hepatoma mitochondria is somewhat higher
than that of normal liver mitochondria, an observation noted in
a number of different experiments. In both cases the amount
of uncoupler employed, 5.0 µM FCCP, was sufficient to inhibit
uptake by more than 80%.

These results show that in addition to catalyzing forward
energy-dependent reactions at a normal rate, hepatoma 7800
mitochondria catalyze reverse energy-dependent reactions at
normal or near normal rates as well.

Uncoupler-stimulated ATPase Activity of Mitochondria
Prepared from Regenerating Liver—The hepatomas employed
for studies described previously in this report are fairly represen-
tative of hepatomas throughout the growth rate spectrum of
hepatomas established by Morris and his colleagues (12, 13).
Thus, hepatomas 3924A and 7777 are two of the more rapidly

growing tumors in this spectrum, hepatomas 16 and 9618A are
two of the more slowly growing tumors, and hepatomas 7800
and 794A are intermediate in growth rate. Since mitochondria
from all of these tumors are defective in their capacity to
express normal levels of uncoupler-stimulated ATPase activity
(Tables I, II, III, and IV, and Figs. 2 and 3), it was of interest
to get some indication as to whether this defect was a general
property of mitochondria from this class of tumors or simply
the result of rapid cell growth and division. For this reason,
mitochondria were isolated from regenerating liver cells at vari-
ous times after partial hepatectomy and assayed for ATPase
activity in the presence of uncoupling agents.

Results of experiments presented in Fig. 4 show that mito-
chondria isolated from regenerating rat liver at 0, 24, 48, and
72 hours after partial hepatectomy have high levels of DNP-
stimulated ATPase activity which is almost indistinguishable
from values obtained with control liver mitochondria. Similar
results were obtained when the more potent uncouplers, FCCP
and Compound XIII, replaced DNP in the assay (Fig. 4, B and
C).

On the basis of these results it does not appear that a simple
noncarcinogenically induced alteration of the growth rates of
liver hepatocytes is sufficient to alter the response of mitochon-
drial ATPase to uncoupling agents. Thus, it would appear

TABLE III

Table V

Effect of FCCP and oligomycin on ATP-supported Ca²⁺ uptake in
control liver and hepatoma 7800 mitochondria

| Mitochondrial source | Calcium uptake
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Control liver</td>
<td>With FCCP</td>
<td>Without inhibitor</td>
</tr>
<tr>
<td>Hepatoma 7800</td>
<td>231</td>
<td>91.0</td>
</tr>
<tr>
<td>384</td>
<td>168</td>
<td>69.2</td>
</tr>
</tbody>
</table>

* Nanomole Ca²⁺ X mg⁻¹ protein.
that the low uncoupler-stimulated ATPase activity observed in mitochondria isolated from hepatomas of the Morris series may be a characteristic property of the hepatomas.

Uncoupler-stimulated ATPase Activity of Mitochondria from L1210 and Ehrlich Ascites Tumor Cells—Experiments described in this report have dealt primarily with mitochondria isolated from a variety of hepatomas, and have shown that all of these hepatomas are defective in uncoupler-stimulated ATPase activity. Additional experiments were carried out to determine whether intact mitochondria from other commonly used experimental tumor cells also have a low ATPase activity in the presence of uncoupling agents.

Data presented in Fig. 5 summarize results of experiments on both L1210 and Ehrlich ascites tumor mitochondria in which the effects of DNP on both respiration and ATPase activity were studied. As indicated in Fig. 5A, respiration of both types of mitochondria is markedly enhanced by 100 μM DNP to about the same levels as those observed with mouse liver mitochondria.

In contrast to its ability to activate respiration, however, DNP was much less effective in enhancing ATPase activity. Ehrlich ascites tumor mitochondria could be activated no more than 30% of maximal liver values, whereas L1210 ascites tumor mitochondria were activated to about 35% of maximal liver values. Neither FCCP nor Compound XIII were more effective than DNP in eliciting uncoupler-stimulated ATPase activity in these tumor mitochondria.

These results show that the low uncoupler-stimulated ATPase activity of tumor mitochondria is not only a property of Morris hepatomas.

Mg++-ATPase Activity of Liver, Regenerating Liver, and Tumor Mitochondria—The relationship between uncoupler- and Mg++-activated ATPase activity in mitochondria is not well understood. Whereas optimal uncoupler-stimulated ATPase activity is observed only in intact mitochondrial systems, optimal Mg++-ATPase activity is observed only when the structural integrity of mitochondria is destroyed by some rather harsh treatment such as sonication (2). Presumably, the two activities share a common site, perhaps the catalytic site, since both are inhibited by oligomycin (23). Experiments were carried out therefore to establish whether the tumor mitochondria studied, all of which have low uncoupler-activated ATPase activities, have correspondingly low Mg++-ATPase activities.

Results presented in Table VI show that sonicated tumor mitochondria assayed in the presence of 6.7 mM MgCl₂ exhibit different degrees of Mg++-ATPase activity. Mitochondria from the two slow growing hepatomas 9618A and 16 have about 30%
the activity of control liver mitochondria; mitochondria from
the two intermediate growth rate hepatomas have between 60
to 65% of control activities and mitochondria from the two
rapidly growing hepatomas have activities between 65 to 78% of
control value. Mitochondria isolated from regenerating liver
cells at 24, 48, and 72 hours after partial heptectomy have
normal Mg\textsuperscript{2+} ATPase activities. The two ascites tumor mito-
chondria studied have widely different Mg\textsuperscript{2+} ATPase activities;
the L1210 mitochondria have 74% the activity of liver mito-
chondria, whereas the Ehrlich mitochondria exhibit only 33% of
liver activity.

The results in Table VI are of considerable interest in that
they suggest that the Mg\textsuperscript{2+} ATPase activity of tumor mitochondria
may be inhibited to varying degrees, perhaps by an inhibitor protein similar to that described by Pullman and Monroy (24).
However, no direct correlation can be made between Mg\textsuperscript{2+}-
activated ATPase activity and the consistently low uncoupler-
activated ATPase activity of these tumor mitochondria.

**DISCUSSION**

Results of experiments summarized here show that intact
mitochondria isolated from six hepatomas of the Morris series,
as well as intact mitochondria isolated from L1210 and Ehrlich ascites tumor cells are markedly deficient in their capacity to
catalyze ATPase activity in the presence of a variety of potent
uncoupling agents. These findings are consistent with our
earlier studies on mitochondria from hepatoma 7800 (11) and
with the more recent studies of LaNoie et al. (25) on mito-
chondria from hepatomas 7800, 7777, and 16. In addition, they
confirm and extend the earlier observations of Devlin and
Pruss (10), that phosphorylating mitochondria from hepatomas
3924A and 3083 are deficient in uncoupler-activated ATPase activity.

Additional experiments described here were carried out to
determine whether uncoupler-sensitive, energy conservation
reactions are impaired in tumor mitochondria. In these ex-
periments, hepatoma 7800 was studied in greatest detail because
this hepatoma reaches harvestable size in only 3 to 4 weeks, and
because a high yield of intact mitochondria (acceptor control
ratios \(>5\)) can be obtained from this tumor. Results of these
experiments show that such energy-linked activities as oxidative
phosphorylation, respiration, ATP-P\textsubscript{i} exchange, and ATP-
supported Ca\textsuperscript{2+} uptake are catalyzed at normal or near normal
rates by hepatoma 7800 mitochondria. Moreover, each of these
processes responds in the usual manner to uncoupling agents.
Thus, experiments summarized in this report indicate that
forward and reverse energy conservation reactions are not
impaired in hepatoma mitochondria, while uncoupler-stimulated
ATPase activity is markedly deficient.

A simple hypothesis can be proposed which is consistent with
the data presented in this paper. Such a hypothesis assumes
that, unlike normal liver mitochondria, which are usually thought
to have only one principal site of action for uncoupling agents
(8, 9, 26), hepatoma mitochondria may have an additional site
of action. Thus, in hepatoma mitochondria, uncouplers may
act not only to dissipate the "initial high energy state" as is
usually postulated (8, 9, 27), but may also act at the level of
\(X \sim I\) or \(X \sim P\) (see scheme below).

```
Electron transport
\begin{array}{c}
\text{initial high energy state}

\end{array}
\begin{array}{c}
X
P_i
ADP
ATP
\end{array}
```

Alternatively, the second site of uncoupler action may lie at
the level of entry of ATP. Such a hypothesis accounts for the
lack of uncoupler-stimulated ATPase activity in hepatoma
mitochondria, and the essentially complete uncoupler sensitivity
of energy conservation reactions involving the ATPase.

Regardless of the mechanism by which uncoupler-stimulated
ATPase activity is impaired in tumor mitochondria, the data
presented here seem to indicate that this impairment is a fairly
widespread characteristic of mitochondria from a variety of
tumors of different growth rates, degrees of differentiation, and
induced by different carcinogens. These findings, together
with the additional observation reported here that mitochondria
isolated from regenerating liver cells have normal uncoupler-
stimulated ATPase activity, would tend to suggest that the low
activity found in tumors may represent a common alteration of
the mitochondria during development of neoplasia. It is not
known if this low activity is a result or a consequence of neoplasia.

It is possible that a low uncoupler-stimulated ATPase activity
is advantageous to the cancer cell. For example, many tumors
may either generate or accumulate one or more agents with
potential uncoupler-like properties, and therefore require a low
uncoupler-activated mitochondrial ATPase activity so that
glycolytically produced ATP, the principal energy source of
many tumors (28), can be preserved for anabolic reactions. Such
a suggestion is not entirely without precedence, since it is well
established that the isolation media for the preparation of well
coupled mitochondria from many tumors require bovine albumin
presumably to bind one or more agents deleterious to mitochon-
drial integrity (29–31).

Along these same lines, it should perhaps be noted that mito-
chondria from some normal mammalian tissues, e.g. brain and
adrenal cortex, have a very low uncoupler-activated ATPase
activity (<100 nmoles of Pi per min per mg) (32–34). Inter-
restingly these two tissues, similar to many tumors, exhibit a
high glycolytic rate (26). In contrast to brain and adrenal
cortex, those tissues with a low or normal glycolytic capacity,
"e.g. liver, kidney, heart, and resting muscle" (28), all contain
mitochondria which catalyze high uncoupler-activated ATPase
activity (35–37).

Results of studies summarized in this report represent a con-
tinuing effort (11, 14, 38–40) by this laboratory to define those
molecular lesions in tumor mitochondria that arise either to-
gether with, or as a consequence of the normal to neoplastic
transition. Of those mitochondrial defects we have noted to
date, we find as indicated in this report that a low uncoupler-
stimulated ATPase activity is by far the most common. Future
efforts will be directed at determining the molecular basis of this
defect by studying the interaction of uncoupling agents with
the ATPase (41–43) and ATP transport system of liver and
hepatoma mitochondria.

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Note Added in Proof—ATPase activity response patterns in the presence of DNP are not identical for mitochondria isolated from tumor cells grown in ascites form. More recent studies have shown that mitochondria from an azo dye induced hepatoma grown in ascites form (AS 3011, obtained from Dr. A. C. Griffin, The University of Texas M. D. Anderson Hospital and Tumor Institute at Houston, Texas) exhibit a fairly typical DNP-stimulated ATPase activity response pattern below 0.1 mM DNP. In addition, mitochondria isolated from a strain of Ehrlich ascites cells (also obtained from Dr. Griffin) different from the one used in this study exhibit an intermediate DNP-stimulated ATPase activity response pattern similar to that of mitochondria from L1210 ascites cells (Fig. 5). Differences in responses cannot be easily ascribed to obvious differences in the quality of the mitochondria since they have all been isolated by similar procedures and have within experimental error the same acceptor control ratios.

REFERENCES

Uncoupler-stimulated Adenosine Triphosphatase Activity: DEFICIENCY IN INTACT MITOCHONDRIA FROM MORRIS HEPATOMAS AND ASCITES TUMOR CELLS
Peter L. Pedersen and Harold P. Morris


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