THE DISTRIBUTION OF FUMARASE ACTIVITY IN
MOUSE LIVER HOMOGENATES

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Previous studies (1-3) have demonstrated that the mitochondrial frac-
tion of several mammalian tissues can utilize fumarate as a substrate for
oxidation. While these findings presumably indicate that at least a por-
tion of the fumarase activity of the cells in question is associated with the
mitochondria, a complete investigation of the intracellular distribution of
this enzyme has not been reported hitherto.

This paper presents the results of experiments designed to determine the
distribution of fumarase activity in mouse liver homogenates fractionated
by differential centrifugation. A major portion of the total activity was
found in the mitochondrial fraction. Data are also reported relating to
the physical state of this enzyme within the mitochondrion and to the pos-
sibility of distributional artifacts arising from adsorption of enzyme by the
particulate components of the cell.

EXPERIMENTAL

Fractionation of Homogenates in 0.25 M Sucrose—Livers of female C3H
strain 3 months of age (killed by cervical dislocation) were perfused in
situ with ice-cold 0.145 M sodium chloride followed by cold 0.25 M sucrose,
then forced through a perforated stainless steel disk, and thereafter ho-
monized in 0.25 M sucrose (4). Centrifugal fractionation of the homo-
genates into nuclear (Nw), mitochondrial (Mw2), submicroscopic particu-
late (P), and supernatant (Ss) fractions was carried out as described
previously (5). Disruption of mitochondria suspended in 0.25 M sucrose
was achieved by exposure of the suspension to sonic vibrations for 30
minutes at 0° in the 9 kc. Raytheon type R-22-3 oscillator (6). After such
exposure, the suspensions (Mw2S) always contained a small number of in-
tact mitochondria, which were removed by centrifugation at 24,000 × g
for 10 minutes (Sd1). The particulate fragments (Sd2) and the soluble
fraction (Ss) of the disrupted mitochondria were then separated by centri-
fugation at 110,000 × g for 1 hour in the SW-39 horizontal rotor of the
Spinco model E ultracentrifuge (6).

Fractionation of Homogenates in Calcium-Sucrose Mixtures—The nuclear
fraction was isolated as previously described (7) from filtered homogenates
prepared in 0.0018 M calcium chloride-0.25 M sucrose. In the further fractionation of these homogenates, both the mitochondria and the submicroscopic particles were included in a particulate fraction separated from the final supernatant fluid by centrifugation at 110,000 × g for 1 hour.

**Analytical Methods**—Fumarase activity was determined spectrophotometrically at 25° and pH 7.4 (8), with L-malate (Eastman Kodak Company) as substrate. The reaction rate was zero order over a 5 minute period and linearly proportional to enzyme concentration, provided that the enzyme was exposed to distilled water for a period of 3 to 5 minutes before buffer and substrate were added. An extinction coefficient for fumaric acid of 2.11 × 10⁶ sq. cm. per mole (9) was used in calculating the rates on a molar basis. Total nitrogen was determined colorimetrically after acid digestion (10).

All values reported in Tables I to IV are for 100 mg. of perfused liver or an equivalent amount of each fraction.

**Results**

**Distribution of Fumarase Activity in Fraction Obtained in 0.25 M Sucrose**—As shown in Table I, approximately 55 per cent of the total fumarase activity of the homogenate was recovered in the mitochondrial fraction, about 9 per cent each in the nuclear and soluble fractions, and the remainder in the submicroscopic particulate fraction. Only the mitochondrial fraction contained an enzyme concentration greater than that in the homogenate.

**Distribution of Fumarase Activity within Mitochondrion**—Previous studies (cf. (11)) have demonstrated that several enzymes associated primarily with mitochondria are released into solution upon disruption of the particles. A similar investigation of the distribution of fumarase activity in preparations of disrupted mitochondria is reported in Table II. Exposure of intact mitochondria to sonic vibrations resulted in a 25 per cent decrease in the fumarase activity. Of the remaining activity, 74 per cent was in a soluble state, and only 19 per cent was associated with particulate fragments having a specific activity one-half that of the disrupted mitochondria (MwS).

**Adsorption of Fumarase Activity by Mitochondria and Submicroscopic Particles**—The finding that a significant portion of the fumarase activity of the homogenate was associated with submicroscopic particulate material showing a relatively low specific activity (Table I) suggested that adsorption of the enzyme on these particles might have occurred during preparation of the homogenate. Since the soluble fraction of the disrupted mitochondria (Table II) provided a source of soluble fumarase, adsorption experiments similar to those reported previously (12) were carried out. The results are shown in Table III. Both types of particle lost a small
proportion of their total nitrogen on exposure to the soluble fraction. The mitochondria adsorbed essentially no fumarase activity. The submicroscopic particles, however, adsorbed 45 per cent of the activity of the soluble fraction and showed an almost 2-fold increase in specific activity. These results are compatible with the possibility that some or all of the fumarase

**Table I**

*Distribution of Fumarase Activity in Fractions Obtained from C3H Mouse Liver Homogenates in 0.25 M Sucrose*

<table>
<thead>
<tr>
<th>Liver fraction</th>
<th>Total nitrogen</th>
<th>Fumarase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg.</td>
<td>µM per hr.</td>
</tr>
<tr>
<td>Homogenate</td>
<td>2.62</td>
<td>612</td>
</tr>
<tr>
<td>Nw</td>
<td>0.397</td>
<td>57.1</td>
</tr>
<tr>
<td>Mw2</td>
<td>0.714</td>
<td>334</td>
</tr>
<tr>
<td>P</td>
<td>0.804</td>
<td>166</td>
</tr>
<tr>
<td>S1</td>
<td>0.950</td>
<td>55.8</td>
</tr>
</tbody>
</table>

**Table II**

*Fumarase Activity of Isolated C3H Mouse Liver Mitochondria Subjected to Disintegration by Sonic Vibrations*

<table>
<thead>
<tr>
<th>Liver fraction</th>
<th>Total nitrogen</th>
<th>Fumarase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg.</td>
<td>µM per hr.</td>
</tr>
<tr>
<td>Mw2</td>
<td>0.734</td>
<td>358</td>
</tr>
<tr>
<td>Mw2S</td>
<td>0.717</td>
<td>258</td>
</tr>
<tr>
<td>Sd1</td>
<td>0.060</td>
<td>21.7</td>
</tr>
<tr>
<td>Sd2</td>
<td>0.264</td>
<td>49.3</td>
</tr>
<tr>
<td>S2</td>
<td>0.415</td>
<td>191</td>
</tr>
</tbody>
</table>

activity associated with the submicroscopic particles of Table I may have represented enzyme present originally in a soluble state within the cell or released in soluble form from other cellular structures when the cells were disrupted. The data lessen the probability that the association of fumarase activity with the mitochondria was the result of adsorption.

Fumarase Activity of Nuclear Fraction—Less than 4 per cent of the total fumarase activity was recovered in the nuclear fraction isolated in calcium chloride-sucrose solution and only slightly contaminated with mitochondria and intact liver cells (Table IV). Some of this remaining activity may
have been associated with erythrocytes (13) which escaped perfusion and were included in the nuclear fraction.

**TABLE III**

Adsorption of Fumarase Activity by Mitochondria and Submicroscopic Particles from Soluble Fraction (S₂) of C3H Mouse Liver Mitochondria

Mixtures of the soluble fraction of disrupted mitochondria with an equal volume of a suspension of mitochondria or of submicroscopic particles were prepared. The mitochondria were recovered from the mixture by centrifugation at 24,000 × g for 10 minutes, and the submicroscopic particles by centrifugation at 110,000 × g for 1 hour.

<table>
<thead>
<tr>
<th>Liver fraction</th>
<th>Total nitrogen</th>
<th>Fumarase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg.</td>
<td>Total Activity</td>
</tr>
<tr>
<td>Mw₂</td>
<td>0.620</td>
<td>330</td>
</tr>
<tr>
<td>P</td>
<td>0.716</td>
<td>138</td>
</tr>
<tr>
<td>S₂</td>
<td>0.334</td>
<td>190</td>
</tr>
<tr>
<td>Mw₂A*</td>
<td>0.536</td>
<td>338</td>
</tr>
<tr>
<td>S₂A†</td>
<td>0.373</td>
<td>189</td>
</tr>
<tr>
<td>PA‡</td>
<td>0.649</td>
<td>223</td>
</tr>
<tr>
<td>S₂B§</td>
<td>0.390</td>
<td>104</td>
</tr>
</tbody>
</table>

* Mitochondria recovered from mixture of Mw₂ + S₂.
† Supernatant fluid recovered from mixture of Mw₂ + S₂.
‡ Submicroscopic particles recovered from mixture of P + S₂.
§ Supernatant fluid recovered from mixture of P + S₂.

**TABLE IV**

Distribution of Fumarase Activity in Fractions Obtained from C3H Mouse Liver Homogenates Prepared in Calcium Chloride-Sucrose Solution

<table>
<thead>
<tr>
<th>Liver fraction</th>
<th>Total nitrogen</th>
<th>Fumarase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg.</td>
<td>Total Activity</td>
</tr>
<tr>
<td>Homogenate</td>
<td>2.66</td>
<td>600</td>
</tr>
<tr>
<td>Nuclear fraction</td>
<td>0.262</td>
<td>23.5</td>
</tr>
<tr>
<td>Particulate fraction</td>
<td>1.51</td>
<td>550</td>
</tr>
<tr>
<td>Supernatant fluid</td>
<td>1.07</td>
<td>40.5</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The fumarase activity of mouse liver appears to be associated mainly with the mitochondrial fraction. It is at present impossible to draw any conclusion as to whether the bulk of the extramitochondrial fumarase exists...
in the intact cell as a soluble enzyme or is attached to the submicroscopic particles. It would appear unlikely that the association of fumarase activity with the isolated mitochondria is the result of adsorption. The possibility exists, but cannot be verified experimentally, that an even larger proportion of the fumarase activity was associated with the mitochondria in the intact cell, but was extracted from the mitochondria during the cell fractionation procedure.

The present findings are in apparent contrast to those in yeast (14) in which, after disruption and fractionation of the cells in 0.1 M phosphate buffer of pH 7.3, 90 per cent of the fumarase activity failed to sediment upon centrifugation at 31,000 × g for 1 hour. Only 10 per cent of the total activity was associated with the particulate fraction obtained by this centrifugation and said to correspond, probably, to the mitochondria and microsomes.

SUMMARY

A study is reported of the distribution of fumarase activity among the fractions obtained by differential centrifugation from mouse liver homogenates. A major portion of the enzyme activity was recovered in the mitochondrial fraction. When the mitochondrial membranes were disrupted by exposure to sonic oscillations, most of the activity was released into solution. Nuclei exhibited little fumarase activity. Submicroscopic particles were capable of adsorbing large amounts of soluble fumarase activity, and therefore the probable intracellular localization of the extra-mitochondrial activity could not be ascertained. In homogenates, less than 10 per cent of the activity was in soluble form.

BIBLIOGRAPHY

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