THE INTRACELLULAR LOCALIZATION OF URICASE*

BY ARNOLD H. SCHEIN, ESTELLE PODBER, AND ALEX B. NOVIKOFF

(From the Departments of Biochemistry and Pathology, College of Medicine, University of Vermont, Burlington, Vermont)

(Received for publication, December 20, 1959)

Lan (1, 2) has measured the uricase activity of rat liver homogenate and liver cell nuclei. He found the nuclear activity to be 30 to 46 per cent higher than that of the homogenates when both were computed on a dry weight basis.

In connection with our studies of purine metabolism we have investigated the intracellular distribution of uricase activity. Differential centrifugation was employed to prepare cell fractions from rat liver homogenates suspended in hypertonic sucrose and in distilled water. Uricase activity was estimated by following uric acid destruction at 45°. The results indicate that most of the uricase activity of the homogenate is concentrated in the large granules (mitochondria).

EXPERIMENTAL

Materials and Methods

Preparation of Rat Liver Homogenates (10 Per Cent)—Livers were obtained from young adult, Sprague-Dawley, male rats and were cooled with finely chopped ice. The chilled livers were rapidly weighed on a Roller-Smith balance and transferred to a cold homogenization tube of the Potter-Elvehjem type (3). All subsequent operations were conducted in a cold room or refrigerated centrifuge (International, model PR-1) at a temperature of 1–5°. Homogenates were prepared with 9 volumes of either ice-cold hypertonic (0.88 M) sucrose or with distilled water.

Fractionation of Rat Liver Homogenate in 0.88 M Sucrose (4) and in Distilled Water—30 ml. of homogenate in 0.88 M sucrose were centrifuged for 10 minutes at 600 × g. (The radial distance for calculation of g was from the center of the centrifuge tube.) The supernatant solution was carefully removed and the sediment suspended in the same volume of sucrose solution and recentrifuged as above. This washing procedure was repeated once more. The final sediment consisting of nuclei, together with some

* A preliminary report has appeared (Federation Proc., 9, 224 (1950)).

This investigation was supported in part by a research grant from the National Cancer Institute, National Institutes of Health, United States Public Health Service.

331
free mitochondria (particularly of the larger sizes), unbroken liver cells, and red blood cells, was evenly redistributed in 30 ml. of sucrose solution.

The supernatant fluid and washings from the nuclear fraction were centrifuged at 24,000 × g for 20 minutes and washed twice with small portions of sucrose solution. The precipitate, consisting mainly of mitochondria and some microsomes (visible by phase microscopy), was resuspended in 30 ml. of sucrose solution.

The supernatant fluid and washings from the mitochondrial fraction were combined, diluted to a concentration of 0.44 M sucrose, and centrifuged at 24,000 × g for 120 minutes. The resulting pellets, containing the major portion of the microsomes, were redistributed in 30 ml. of 0.88 M sucrose solution, and the supernatant fluid was made up to 60 ml. with 0.44 M sucrose. This "supernatant fluid" fraction contained no particles visible by phase microscopy; yet higher speeds (140,000 × g) (Spinco centrifuge) did bring down particulate matter. In addition to this fine particulate matter the fraction contains soluble materials of the cell.

By this procedure, each ml. of the nuclear, mitochondrial, and microsomal fractions contained essentially the same amount of specific particulate matter as 1 ml. of the original 10 per cent homogenate. On the other hand, the material in the "supernatant fluid" was one-half as concentrated.

In the case of the water homogenates, both the speed and time of centrifugation were varied so that the calculated particle size (5) of the various fractions approximated those of the hypertonic sucrose homogenates. Otherwise the fractionation procedure was carried out exactly as above with distilled water substituted for sucrose solutions.

Measurement of Uricase Activity—The incubating medium, consisting of 10 ml. of a borate-buffered (pH 9.3) lithium urate solution containing the equivalent of 1000 γ of uric acid and 2 drops of catalase (Vita-Zyme Laboratories, Inc.), was equilibrated at 45° in a 100 ml. volumetric flask. Suitable aliquots of the tissue fractions were added (usually two levels of tissue were employed) and the flasks shaken for 30 minutes in a Dubnoff metabolic incubator. The reaction was stopped by the addition of 0.5 ml. 5 per cent NaCN and the reactants diluted to 100 ml. If less than 500 γ of tissue-derived nitrogen was present, its removal was unnecessary, since protein, peptides, or amino acids do not inhibit color development of uric acid with the reagents used when the reaction is "swamped" by glycine (6). The turbidities at the color development stage caused by the presence of larger concentrations of protein were avoided by adding 0.2 ml. quantities of 10 per cent sodium tungstate and $\frac{3}{8}$ N H$_2$SO$_4$ before making up to volume. The solutions were filtered before use and neutralized. Residual uric acid determinations were performed on 5 ml. aliquots
of the clear filtrates by a modification of the Benedict method.\textsuperscript{1} The quantity of uric acid destroyed served as an index of uricolytic activity. Uric acid destruction was directly proportional to the amount of tissue extracts and homogenates over a wide range of concentration.

Warburg respirometry was employed as a check on our method. Uric acid destruction of the flask contents was measured by our procedure\textsuperscript{1} and compared to values calculated from the $O_2$ uptake. Equivalent results were obtained in all cases. These experiments are not reported here.

Other Determinations—Nitrogen was determined by micro-Kjeldahl digestion, followed by nesslerization, desoxypentose nucleic acid (DNA) and pentose nucleic acid (PNA) by the procedure of Schneider (7), and succinoxidase activity by the method of Schneider and Potter (8). The nucleic acid and succinoxidase measurements served as checks on the reproducibility of the fractionation and also as a basis for comparison of our fractions with those of other workers.

Results

From Table I it may be seen that there is a similar pattern of distribution of nitrogen, uricase activity, succinoxidase activity, PNA, and DNA in the cell fractions obtained by fractionation in hypertonic sucrose and in distilled water.

Uricase—The uricase activity of the mitochondrial fractions is approximately 75 per cent that of the homogenates with both sucrose and distilled water. The specific activities (micrograms of uric acid oxidized per microgram of N) of the mitochondria are from 2 to 3 times that of the homogenates. The specific uricase activities of the nuclear fractions and microsomes are approximately half that of the homogenates. The uricase activities and especially the specific activities of both supernatant fluids are extremely low.

Succinoxidase—The distribution of succinoxidase activities is quite similar to that reported by Hogeboom et al. (4). 70 to 75 per cent of the total activity is concentrated in the mitochondria. The nuclear fractions and microsomes, with sucrose and with water, contain from 4 to 14 per cent of the homogenate activity. The supernatant fluid from water homogenates has an appreciably higher succinoxidase activity (8 per cent) than from sucrose homogenates (0.4 per cent). The reason for this difference has not been investigated.

PNA and DNA—The nucleic acid distribution is essentially like that reported by Price et al. (9). PNA is found in all of the cell fractions. DNA is present exclusively in the nuclear fraction; this is the only fraction

\textsuperscript{1} Schein, A. H., and Rice, E., unpublished work.
### Table I

**Distribution of Nitrogen, Uricase Activity, Succinoxidase Activity, PNA, and DNA in Rat Liver Fractions**

Average values; the range of values is included in parentheses. The results represent the amount per 100 mg. of fresh tissue or its equivalent.

<table>
<thead>
<tr>
<th>Material</th>
<th>Nitrogen</th>
<th>Uricase activity*</th>
<th>Succinoxidase activity†</th>
<th>PNA</th>
<th>DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Homogenate</td>
<td>Total</td>
<td>Homogenate</td>
<td>Total</td>
</tr>
<tr>
<td>Homogenate</td>
<td>3220</td>
<td>100</td>
<td>3460</td>
<td>100</td>
<td>1.08</td>
</tr>
<tr>
<td>Nuclear fraction</td>
<td>550</td>
<td>17</td>
<td>260</td>
<td>7</td>
<td>0.48</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>960</td>
<td>14</td>
<td>2540</td>
<td>73</td>
<td>2.7</td>
</tr>
<tr>
<td>(740–1100)</td>
<td>(26–35)</td>
<td>(200–3080)</td>
<td>(67–78)</td>
<td>(2.2–3.8)</td>
<td>(2.2–3.8)</td>
</tr>
<tr>
<td>Microsomes</td>
<td>450</td>
<td>14</td>
<td>290</td>
<td>8</td>
<td>0.61</td>
</tr>
<tr>
<td>(230–660)</td>
<td>(8–19)</td>
<td>(110–570)</td>
<td>(3.5–17)</td>
<td>(0.43–0.92)</td>
<td>(0.43–0.92)</td>
</tr>
<tr>
<td>Supernatant fluid</td>
<td>1170</td>
<td>39</td>
<td>75</td>
<td>2</td>
<td>0.08</td>
</tr>
<tr>
<td>(890–1530)</td>
<td>(33–42)</td>
<td>(30–115)</td>
<td>(1–3)</td>
<td>(0.08–0.13)</td>
<td>(0.08–0.13)</td>
</tr>
<tr>
<td>Recovery</td>
<td>96</td>
<td>6</td>
<td>92</td>
<td>(3–2)</td>
<td>(3–2)</td>
</tr>
<tr>
<td></td>
<td>2720</td>
<td>100</td>
<td>3660</td>
<td>100</td>
<td>1.35</td>
</tr>
<tr>
<td>----------------------</td>
<td>-------</td>
<td>------</td>
<td>-------</td>
<td>------</td>
<td>-------</td>
</tr>
<tr>
<td></td>
<td>(2630-2800)</td>
<td>(3000-4230)</td>
<td>(1.2-1.5)</td>
<td>(2160-2460)</td>
<td>(1.9-2.9)</td>
</tr>
<tr>
<td>Homogenate</td>
<td>320</td>
<td>12</td>
<td>200</td>
<td>5</td>
<td>0.60</td>
</tr>
<tr>
<td>(270-370)</td>
<td>(10-13)</td>
<td>(120-280)</td>
<td>(4-7)</td>
<td>(60-150)</td>
<td>(2-6)</td>
</tr>
<tr>
<td>Nuclear fraction</td>
<td>680</td>
<td>25</td>
<td>2730</td>
<td>75</td>
<td>4.1</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>340</td>
<td>13</td>
<td>170</td>
<td>5</td>
<td>0.51</td>
</tr>
<tr>
<td>(320-360)</td>
<td>(11-14)</td>
<td>(135-210)</td>
<td>(4-5)</td>
<td>(9-12)</td>
<td>(260-280)</td>
</tr>
<tr>
<td>Microsomes</td>
<td>1240</td>
<td>46</td>
<td>60</td>
<td>2</td>
<td>0.08</td>
</tr>
<tr>
<td>(1190-1280)</td>
<td>(45-46)</td>
<td>(30-90)</td>
<td>(0.7-3.0)</td>
<td>(165-190)</td>
<td>(7-8)</td>
</tr>
<tr>
<td>Supernatant fluid</td>
<td>95</td>
<td>85</td>
<td>85</td>
<td>85</td>
<td>0.07-0.08</td>
</tr>
<tr>
<td>(91-98)</td>
<td>(84-85)</td>
<td>(88-95)</td>
<td>(100-101)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Micrograms of uric acid destroyed in 30 minutes.
† Microliters of O₂ in 60 minutes.
‡ Homogenate taken as 100 per cent. Average values calculated from individual determinations.
§ Negligible.
in which nuclei (free or in unbroken cells) are found by microscopic examination.

**DISCUSSION**

Although approximately 75 per cent of the liver uricase activity is concentrated in the mitochondria, a small but significant proportion of the activity is found in both the nuclear and microsome fractions. It is not possible to state whether the activity is intrinsic to the nuclei or whether the uricase activity of the nuclear fraction is derived from mitochondrial contamination. Phase microscopy always revealed some free mitochondria and unbroken cells in this fraction. The uricase activity of the microsome fraction might result from such factors as the possible presence of small mitochondria, absorption of uricase upon microsomes, or the uricase content of microsomes themselves. Until more is known of the morphological and functional relations between small mitochondria and microsomes, little can be gained from speculations concerning the uricase activity of the microsome fraction.

It is of interest that the uricase activity of mitochondria is essentially the same whether these particles are isolated from hypertonic sucrose or from distilled water, despite the marked differences in their size and shape in these two media (4, 10).

The evidence for the localization of uricase in mitochondria fulfills the criteria proposed by Hogeboom and Schneider (11) for ascribing a biochemical property to a given structural component of the cell; namely, the cell fraction is cytologically defined in terms of the intracellular component it represents, the validity of the method of assay for the biochemical property is established, and the biochemical property is concentrated to a greater extent in the cell fraction than in the original whole tissue. Thus uricase belongs in the group of enzymes including succinoxidase, cytochrome oxidase (12), and octanoxidase (13), whose activities are associated to a large degree with mitochondria.

**SUMMARY**

1. A method for measuring uricase activity has been described.

2. Approximately 75 per cent of the uricase activity of rat liver was found in the mitochondrial fractions obtained by differential centrifugation of 0.88 M sucrose and distilled water homogenates.

**BIBLIOGRAPHY**

THE INTRACELLULAR LOCALIZATION OF URICASE
Arnold H. Schein, Estelle Podber and Alex B. Novikoff

J. Biol. Chem. 1951, 190:331-337.

Access the most updated version of this article at http://www.jbc.org/content/190/1/331.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/190/1/331.citation.full.html#ref-list-1