An Endogenous Uncoupling and Swelling Agent in Liver Mitochondria and Its Enzymic Formation*

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Physical or chemical disruption of the structure of liver mitochondria usually leads to preparations with considerable respiratory activity, but with little or no activity in coupling phosphorylation to respiration. Under carefully controlled conditions, however, submitochondrial preparations with considerable phosphorylating activity may be obtained (1-5). At least some of the loss of phosphorylating activity which ordinarily accompanies mitochondrial disruption may be caused by uncoupling factors which are released or become active as mitochondrial structure is altered. In a search for such endogenous factors, two distinct activities of this general type were observed (6). The first, R factor, is a protein-containing factor which releases respiration of mitochondria or mitochondrial subfragments from its normal dependence on the presence of a phosphate acceptor system, but which does not uncouple phosphorylation (6). The second agent, here designated as U factor, which is heat-stable in nature and readily extracted from mitochondrial fractions with isooctane and other solvents, has been found to cause uncoupling phosphorylation, release respiration from control by adenine diphosphate, and cause the swelling of mitochondria. These effects of U factor may be prevented by the presence of bovine serum albumin. This paper is concerned with the assay, activity, general properties, and enzymic formation of U factor in rat liver mitochondria. The possible identity of U factor with the active principles of the mitochondrial uncoupling preparations of Pullman and Racker (7), Polis and Shmukler (8), and Hülsmann et al. (9) is also pointed out.

EXPERIMENTAL AND RESULTS

Rat liver mitochondria were isolated from sucrose homogenates as described before (10) and were washed 3 times; these served as starting material for the preparation of U factor and the phosphorylating digitonin fragments (10) used to assay the U factor activity.

Preparation of U Factor—A standard preparation of U factor in isooctane solution was used for many of the experiments described below. It was prepared as follows. The washed mitochondrial fractions were suspended in 60 gm. of rat liver were suspended in 0.03 M KCl to make a total volume of 60 ml. The suspension was subjected to sonic oscillation for 30 minutes at 4° in the cavity of a 9 kc. Raytheon magnetostriction oscillator. The sonically disrupted mitochondria were then incubated at 33° for 2 hours. The suspension was brought to pH 3.0 with 5 M HCl and extracted 3 times with equal volumes of isooctane (2,2,4-trimethylpentane, “spectro” grade, Eastman). The emulsions formed were broken by freezing and thawing followed by centrifugation. The clear, colorless isooctane extracts were combined and stored at 0°, with no change in activity for months. This extract, was assayed as described below, contained approximately 2.5 units of U factor per ml.

Assay of U Factor Activity—The ability of U factor to uncouple oxidative phosphorylation was used as a basis for a semiquantitative assay. Ordinarily, U factor was first extracted from aqueous mitochondrial suspensions or extracts by shaking 3 times with equal volumes of isooctane, following the general procedure described above. Usually the aqueous phase was first brought to pH 3 to 4 with HCl prior to extraction, although essentially complete extraction was also observed at pH 6.5. The combined isooctane extracts were made to a standard volume.

The enzymic assay of U factor present in isooctane extracts was carried out as follows. Small aliquots of the isooctane extract, between 0.1 to 5.0 ml, were placed in 20-ml beakers of the type used in the Dubnoff shaker. These extracts were then evaporated to dryness by placing them in a large desiccator attached to a water aspirator. The isooctane-extractable material was thus deposited in a thin, invisible layer in the beaker. The components of the enzymic test system were then pipetted directly into the prepared beakers and the medium shaken in the beaker for 15 minutes at 20° before the addition of the enzyme to dissolve or suspend the uncoupling factor.

The enzyme test system contained 0.01 m m-β-hydroxybutyrate, 0.0024 m ADP, and 0.08 m phosphate buffer, pH 6.5, labeled with P32 (≈ 2 × 10^6 c.p.m.) . A water suspension of phosphorylating digitonin fragments from rat liver (10), containing about 200 μg total N, was added last to complete the system which had a total volume of 2.0 ml. Incubation was carried out at 20° for 15 to 20 minutes. The reaction was stopped with trichloroacetic acid and the phosphate uptake and acetate formation measured as described before (10); the data were used to calculate the P:2e ratios. The control P:2e ratios, obtained in the absence of added U factor, were between 1.5 and 2.0.

U factor activity was expressed in terms of arbitrary units; 1 unit was defined as the amount required to lower the P:2e ratio in the above test system to 50 per cent of its control value. Ordinarily two or three levels of each sample of U factor were

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Uncoupling of phosphorylation and respiratory release by U factor; action of serum albumin

The test system for oxidative phosphorylation contained 0.01 M dl-β-hydroxybutyrate, 0.0024 M ADP, 0.01 M histidine pH 6.5, 0.03 M phosphate buffer pH 6.5 (labeled with $^{32}P$), ~1 x 10$^4$ c.p.m., 0.01 M glucose, 1.0 mg. hexokinase preparation; 0.001 M EDTA, digitonin fragments (215 μg N), and U factor equivalents as shown below in total volume of 2.0 ml. Serum albumin was added at 4.0 mg. per vessel as shown. The test system for release of respiration contained only 0.01 M β-hydroxybutyrate, 0.01 M histidine pH 6.5, 0.001 M EDTA, digitonin fragments (215 μg N), and U factor as shown to total volume of 2.0 ml. All vessels were incubated 20 minutes at 20°. Aliquots of U factor solution in isocetane (2.2 units per ml.) were evaporated in reaction vessels as shown.

<table>
<thead>
<tr>
<th>U factor</th>
<th>Serum albumin</th>
<th>Acetocacetate formed</th>
<th>P:2e</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mmoles</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No acceptor</td>
<td>Pls acceptor</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>mmoles</td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>—</td>
<td>120</td>
<td>324</td>
</tr>
<tr>
<td>0.3</td>
<td>—</td>
<td>178</td>
<td>318</td>
</tr>
<tr>
<td>0.5</td>
<td>—</td>
<td>218</td>
<td>328</td>
</tr>
<tr>
<td>0.7</td>
<td>—</td>
<td>258</td>
<td>334</td>
</tr>
<tr>
<td>1.0</td>
<td>—</td>
<td>290</td>
<td>326</td>
</tr>
<tr>
<td>1.5</td>
<td>+</td>
<td>308</td>
<td>332</td>
</tr>
<tr>
<td>2.0</td>
<td>+</td>
<td>104</td>
<td>338</td>
</tr>
<tr>
<td>6.0</td>
<td>+</td>
<td>94</td>
<td>338</td>
</tr>
<tr>
<td>6.0</td>
<td>+</td>
<td>122</td>
<td>306</td>
</tr>
</tbody>
</table>

**Table II**

Effect of U factor on ATP exchange reactions and ATPase

The test system for the ATPase and ATP-P$_{32}$ exchange reaction contained 0.006 M ATP pH 6.5, 0.0004 M phosphate labeled with $^{32}P$ (1.22 x 10$^4$ c.p.m.) and digitonin fragments (200 μg N) in a total volume of 2.0 ml. A 1.5-ml aliquot of U factor in isocetane (5 units) was evaporated in the vessel beforehand. The incubation period was 20 minutes. At 20°.

The ATP-ADP exchange was measured in a system containing 0.004 M ATP-C$_{32}$ (14,000 c.p.m.), 0.006 M ATP, pH 6.5, and digitonin fragments (55 μg N) (aged 72 hours at 0° (11)) in total volume of 0.5 ml.; 1.75 units of U factor were added as shown. The incubation period was 20 minutes at 20°. Serum albumin was added as shown at a level of 2.0 mg. per ml.

**Action of U Factor on Oxidative Phosphorylation and on Respiratory Control by ADP**—The typical data collected in Table I show that preparations of U factor extracted from sunically disrupted and aged rat liver mitochondria as described previously cause uncoupling of phosphorylation and release of respiration from its dependence on the presence of a phosphate acceptor system, when tested with suspensions of digitonin fragments from rat liver mitochondria. Both the uncoupling and release of respiration are dependent on concentration of U factor; at higher levels tested uncoupling and release of respiration were essentially complete. Furthermore, it is seen that the release of respiration by U factor resembles that given by dinitrophenol (6) in that the extent of release of respiration in the absence of acceptor is parallel and roughly proportional to the extent of uncoupling when acceptor is present. The action of U factor thus is different from that of R factor, the latter causes respiratory release but does not uncouple phosphorylation significantly (6).

The data in Table I also show that bovine serum albumin completely prevents the respiratory-releasing activity and also the uncoupling activity of the highest level of U factor added to the test systems. When great excesses of U factor were added in the absence of serum albumin, substantially greater than required to uncouple phosphorylation completely, the respiration was severely inhibited; a typical experiment is shown in Table I. This effect is also prevented by serum albumin.

**Effect of U factor on ATPase and ATP Exchange Reactions**—U factor also causes the inhibition of the ATP-P$_{32}$ exchange reaction and the stimulation of ATPase activity of the digitonin fragments as shown by the data in Table II. In these respects U factor also resembles the action of dinitrophenol. These effects of U factor are completely prevented by an excess of bovine serum albumin as shown by the data. Great excesses of U factor in the absence of serum albumin caused substantial inhibition of ATPase; its action is therefore biphasic as is its action on respiration.

The data in Table II show, however, that the ATP-ADP exchange reaction as it occurs in aged digitonin fragments (11) is not inhibited by U factor. Under these conditions dinitrophenol likewise has no effect on this exchange (11).

From these findings it appears likely that U factor at the concentration levels tested is an uncoupling agent that has effects very similar to those of dinitrophenol, suggesting that it acts at the same point in the coupling mechanism, presumably near the carrier level (11-13). U factor thus differs in its action from other uncoupling agents such as gramicidin, arenaite, and azide which have characteristically different action on the ATP exchange reactions and ATPase activity (11, 12).

**Mitochondrial Swelling Caused by U Factor**—U factor causes a great acceleration of the swelling of rat liver mitochondria (cf., 14-16) suspended in a medium of 0.125 M KCl-0.02 M Tris buffer at pH 7.4, as is shown in Fig. 1. Approximately 0.2 unit of U factor caused swelling of a 50 mg. of tissue equivalent of rat liver mitochondria, equal to the rate produced by 1 x 10$^{-4}$ M 1-thyroxine (14) or 3 x 10$^{-4}$ M sodium oleate. As seen in Fig. 1, the swelling produced by U factor was completely prevented by the presence of 1.0 mg per ml. of bovine serum albumin in the test medium.

**Effect of Sodium Oleate** Sodium oleate solutions appear to be capable of duplicating all of the effects of U factor with respect to uncoupling, inhibition of the ATP-P$_{32}$ exchange, stimulation

1 The abbreviations used are: EDTA, ethylenediaminetetraacetate; P$_i$, inorganic orthophosphate; Tris, tris(hydroxymethyl)-aminomethane.
of ATPase activity, release of respiration from its dependence on the presence of a phosphate acceptor system and mitochondrial swelling. The uncoupling activity of a 1.0 unit of U factor could be reproduced by approximately 0.03 µmole of sodium oleate. Similarly the mitochondrial swelling activity of a 1.0 unit of U factor could be produced by about 0.03 µmole of sodium oleate.

Enzymic Formation of U Factor—Data in Table III show that freshly prepared intact mitochondria or sonically disrupted mitochondria contain very little or no U factor activity when extracted and assayed according to the procedures described above. However, when such preparations are incubated for 1 to 2 hours at 37° a large increase in extractable U factor activity occurs. Such formation of U factor activity is accompanied by a virtually complete loss of phosphorylation activity of the mitochondria, suggesting that formation of the factor causes uncoupling of the phosphorylation mechanism. Similar experiments in Table III show that U factor is formed on incubation of sonically disrupted mitochondria at about the same rate as in intact mitochondria. The formation of U factor does not occur, however, if the mitochondria are first heated for 5 minutes in a boiling water bath prior to incubation. The formation of U factor thus appears to be enzymic in nature. The formation of U factor in suspensions of sonically treated mitochondria is a function of pH, being optimum between pH 5.6 and 6.5, as shown by the data in Table IV.

Effect of Cofactors and Inhibitors—The rate of enzymic formation of U factor in sonically disrupted mitochondria is affected by the presence of various agents. Data in Table V show that the formation of U factor is not inhibited by 0.001 M sodium cyanide, 0.001 M sodium azide, 0.01 M arsenate, or 0.001 M CaCl₂. On the other hand, 0.02 M sodium fluoride inhibited U factor formation 50 per cent and 0.001 M HgCl₂ inhibited it about 80 per cent.

The data in Table V also show that inorganic phosphate is apparently not a requirement for the formation of U factor. Similarly the presence of ATP, ADP, DPN, or Mg²⁺ had no effect. However, it is highly significant that the presence of ATP and CoA during the incubation of the sonically disrupted mitochondria often greatly reduced the amount of U factor extractable with isooctane. The inhibition under these circumstances approached 80 per cent, particularly with short reaction periods. CoA added alone was generally ineffective. Oxidized CoA was ineffective even when added with ATP. The possible significance of these observations is discussed below.

Mitochondrial Components Required for Formation of U Factor—Some simple fractionation experiments were carried out to establish which mitochondrial components are involved in the formation of U factor. Intact mitochondria were incubated for 2 hours at 37°, chilled, subjected to sonic treatment for 15 minutes, and then centrifuged at 120,000 × g for 30 minutes in the Spinco model L ultracentrifuge to separate the sonically disrupted mitochondria into a soluble supernatant fraction and an insoluble pellet, which was resuspended in 0.03 M KCl. Each fraction, as well as the unfractionated suspension, was separately extracted with isooctane and the location of U factor determined by the uncoupling assay. The data in Table VI show that U factor formed in intact mitochondria on aging is recovered almost entirely from the particulate residue which presumably consists of fragments of the mitochondrial membrane with only a small portion present in the soluble protein fraction, which in turn presumably originates largely in the mitochondrial matrix.

![Fig. 1. Swelling action of U factor on rat liver mitochondria.](http://www.jbc.org/)

**TABLE III**

Formation of U factor and decline of P:2e ratio during incubation of mitochondria

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Type of preparation</th>
<th>U factor activity (units per tube)</th>
<th>P:2e ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before incubation</td>
<td>After incubation</td>
<td>Before incubation</td>
</tr>
<tr>
<td>1</td>
<td>Intact mitochondria</td>
<td>2</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>Intact mitochondria</td>
<td>2</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>Boiled intact mitochondria*</td>
<td>2</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>Sonicated mitochondria*</td>
<td>5</td>
<td>60</td>
</tr>
</tbody>
</table>

* Tubes were placed in boiling H₂O bath for 5 minutes before incubation at 37°.

Experiments were then carried out to determine the location of the enzyme capable of forming U factor. Freshly prepared sonically disrupted mitochondria were centrifuged and separated into a soluble fraction and a particulate residue. These fractions were incubated at 37° for 2 hours, separately or combined as
The formation of U factor was restored. The experiment suggests that the particulate fraction contains a heat-stable substance, presumably involved in the formation of U factor, are not located in the same fraction.

When the supernatant fraction was boiled and incubated with unboiled particulate fraction, full activity in formed. Conversely, when the boiled particulate fraction was combined with unboiled supernatant fraction, full activity in the formation of U factor was restored. The experiment suggests that the particulate fraction contains a heat-stable sub-

<table>
<thead>
<tr>
<th>pH</th>
<th>U factor formed</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.8</td>
<td>34</td>
</tr>
<tr>
<td>5.6</td>
<td>78</td>
</tr>
<tr>
<td>6.5</td>
<td>70</td>
</tr>
<tr>
<td>7.4</td>
<td>50</td>
</tr>
<tr>
<td>8.5</td>
<td>20</td>
</tr>
</tbody>
</table>

When the supernatant fraction was boiled and incubated with unboiled particulate fraction, full activity in formed. Conversely, when the boiled particulate fraction was combined with unboiled supernatant fraction, full activity in the formation of U factor was restored. The experiment suggests that the particulate fraction contains a heat-stable sub-

Table IV

Effect of pH on formation of U factor

Each tube contained sonic-treated mitochondria derived from 5.0 gm. of whole rat liver in 10.0 ml. 0.03 M KCl, buffered at pH 4.8 with 0.01 M acetate, at pH 5.6 and 6.5 with 0.02 M histidine, and at pH 7.4 and 8.5 with 0.01 M Tris. The tubes were incubated 1 hour at 37°, acidified, extracted with isooctane, and the U factor formed was determined as described in the text.

Table V

Effect of inhibitors and cofactors on formation of U factor

Assay carried out on sonic-treated mitochondria exactly as in Table IV, using a medium of 0.03 M KCl-0.01 M Tris buffer, pH 7.4. Additions to the medium were made as shown.

<table>
<thead>
<tr>
<th>Additions</th>
<th>U factor formation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>units</td>
</tr>
<tr>
<td>1. None</td>
<td>105</td>
</tr>
<tr>
<td>0.001 M sodium cyanide</td>
<td>100</td>
</tr>
<tr>
<td>0.001 M sodium azide</td>
<td>95</td>
</tr>
<tr>
<td>0.01 M sodium arsenate</td>
<td>105</td>
</tr>
<tr>
<td>0.01 M calcium chloride</td>
<td>100</td>
</tr>
<tr>
<td>0.02 M sodium fluoride</td>
<td>50</td>
</tr>
<tr>
<td>2. None</td>
<td>70</td>
</tr>
<tr>
<td>0.001 M HgCl₂</td>
<td>10</td>
</tr>
<tr>
<td>0.003 M ATP</td>
<td>65</td>
</tr>
<tr>
<td>0.003 M ADP</td>
<td>75</td>
</tr>
<tr>
<td>0.003 M DPN</td>
<td>75</td>
</tr>
<tr>
<td>0.001 M MgCl₂</td>
<td>80</td>
</tr>
<tr>
<td>0.01 M phosphate</td>
<td>75</td>
</tr>
<tr>
<td>3. None</td>
<td>55</td>
</tr>
<tr>
<td>0.005 M ATP</td>
<td>45</td>
</tr>
<tr>
<td>0.005 M CoA-SH</td>
<td>50</td>
</tr>
<tr>
<td>0.005 M ATP + 0.005 M CoA-SH</td>
<td>10</td>
</tr>
<tr>
<td>0.005 M ATP + 0.005 M CoA (---SS---)</td>
<td>40</td>
</tr>
</tbody>
</table>

The data in Table VI show that neither fraction possesses the entire activity of the whole suspension. The particulate fraction alone forms only a small amount of U factor, less than 15 per cent of that formed by the whole suspension. The soluble fraction alone shows only about 30 per cent of the activity of the whole suspension. When the two fractions were boiled before incubation, each lost its activity completely (Table VI). These findings indicate that the substrate and enzyme, or cofactors, presumably involved in the formation of U factor are not located in the same fraction. When the supernatant fraction was boiled and incubated with unboiled particulate fraction, relatively little U factor was formed. Conversely, when the boiled particulate fraction was combined with unboiled supernatant fraction, full activity in the formation of U factor was restored. The experiment suggests that the particulate fraction contains a heat-stable sub-

Table VI

Fractionation experiments on formation of U factor

In Experiment 1, 20.0 ml. of a suspension of rat liver mitochondria derived from a 12.0 gm. whole rat liver in 0.03 M KCl-0.02 M Tris, pH 7.4, was incubated 1 hour at 37°. It was then treated sonically 15 minutes at 4°. A 10-ml. aliquot was centrifuged at 105,000 X g. for 30 minutes to yield a supernatant fraction and the pellet, which was resuspended in 10 ml. 0.03 M KCl. After acidification, each fraction and the whole suspension was extracted with isooctane and assayed for U factor activity.

In Experiment 2, 10.0 ml. aliquots of freshly prepared sonic suspension, supernatant fraction, and resuspended residue, prepared as above and derived from fresh, unincubated mitochondria isolated from 6.0 gm. of whole rat liver, were incubated for 2 hours at 37°. Some of the tubes were first heated 5 minutes in a boiling H₂O bath before incubation, as indicated below.

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Fraction incubated</th>
<th>Fraction analyzed</th>
<th>U factor formed units</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Intact mitochondria</td>
<td>Sonic-treated mitochondria</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>Supernatant fraction</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Residue fraction</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Supernatant fraction plus</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td></td>
<td>residue</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fraction incubated and assayed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Whole sonic suspension</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Whole sonic suspension boiled</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Supernatant fraction</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Supernatant fraction boiled</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Residue fraction</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Residue fraction boiled</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Boiled residue + unboiled supernatant</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Boiled supernatant + unboiled residue</td>
<td>12</td>
<td></td>
</tr>
</tbody>
</table>
which freshly prepared digitonin fragments were employed. Data in Table VIII demonstrate that freshly prepared digitonin fragments contain a small but significant amount of material extractable with isooctane and capable of uncoupling phosphorylation. Extracts of the aged digitonin fragments in benzene-soluble, acidified, and thus less inhibitory to phosphorylation. The formation of U factor in aging digitonin fragments is enzymic was shown by an experiment with washed digitonin fragments in which no increase in isooctane extractable, uncoupling factor occurred during incubation over 72 hours at 0°C. Further evidence for the identity of the factor formed in aged digitonin fragments was obtained by washing aged digitonin fragments with serum albumin solution. The serum albumin solution was then extracted with isooctane which was found to contain the uncoupling factor.

These experiments show that the phosphorylating digitonin fragments contain the components necessary for the formation of U factor, although only a small fraction of the total capability of intact mitochondria in forming the factor is present.

**DISCUSSION**

The evidence summarized in this paper indicates that a potent uncoupling agent of oxidative phosphorylation is formed in intact mitochondria or in mitochondrial fractions on incubation in the absence of oxidizable substrates or adenosine triphosphates by an enzymic reaction in which the substrate or a heat-stable cofactor is contributed largely by the insoluble particulate fraction, presumably derived from the membranes, and a heat-labile component is contributed largely by the soluble fraction.

The properties of the uncoupling agent suggest that it may be a long-chain fatty acid. The U factor is heat-stable and readily extractable from aqueous phases by isooctane, diethyl ether, or benzene, at pH 6.5 or below, but not extractable above pH 8. It is already known that fatty acids uncouple oxidative phosphorylation (19) and inhibit certain processes dependent on it, such as fatty acid oxidation (20). In addition fatty acids have profound effects on ATPase activity (19). Enzymic formation of free fatty acids from endogenous lipides of mitochondria appears to be a possible mechanism for the formation of an endogenous uncoupling agent, since the mitochondrial fraction of liver homogenates is known to have some phospholipase activity and may be at least partly responsible for the observation of Mc-Murray and Lardy (17) that additions of CoA-SH increase or stabilize the P:2e ratio in suspensions of mitochondrial fragments obtained by sonic treatment of mitochondria. This effect of ATP + CoA-SH on the formation of U factor was somewhat variable and never produced complete inhibition of U factor formation.

The factor described here may be identical with the active principle of the mitochondrial uncoupling material described by Pullman and Racker (7) and may also be present in the mitochondria of Dictyostelium discoideum (cf. (21)), which could offer considerable potential substrate for the formation of free fatty acid. Lastly, identity of U factor as a fatty acid is suggested by the ability of serum albumin to prevent its uncoupling action; serum albumin also prevents uncoupling by fatty acids such as oleate presumably because of its avid capacity to bind fatty acids.

Additional suggestive evidence that U factor may be a fatty acid comes from the finding that the presence of ATP and CoA inhibits its enzymic accumulation. Mitochondria contain activating enzymes that cause the formation of fatty acyl-CoA esters from free fatty acids (22). Enzymic conversion of free fatty acids into the CoA esters could be expected to result in a form of the fatty acid which is more soluble in water and less readily adsorbed, and thus less inhibitory to phosphorylation. Furthermore, the CoA esters would not be expected to pass into isooctane from an aqueous phase. Enzymic formation of the relatively less "toxic" CoA esters from the toxic free fatty acids may be at least partly responsible for the observation of Mc-Murray and Lardy (17) that additions of CoA-SH increase or stabilize the P:2e ratio in suspensions of mitochondrial fragments obtained by sonic treatment of mitochondria. This effect of ATP + CoA-SH on the formation of U factor was somewhat variable and never produced complete inhibition of U factor formation, however.

**Table VII**

Reactivation of aged digitonin fragments by serum albumin

<table>
<thead>
<tr>
<th>Age of digitonin fragments</th>
<th>ATP-Pi exchange, ATP formed</th>
<th>Oxidative phosphorylation P:2e</th>
</tr>
</thead>
<tbody>
<tr>
<td>hrs.</td>
<td>-SA*</td>
<td>+SA</td>
</tr>
<tr>
<td>0</td>
<td>122</td>
<td>148</td>
</tr>
<tr>
<td>24</td>
<td>5</td>
<td>124</td>
</tr>
</tbody>
</table>

* SA*, serum albumin.

**Table VIII**

Formation of U factor on aging digitonin fragments

<table>
<thead>
<tr>
<th>Age of digitonin fragments</th>
<th>U factor per mg. enzyme N</th>
</tr>
</thead>
<tbody>
<tr>
<td>hrs.</td>
<td>units</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>24</td>
<td>8</td>
</tr>
<tr>
<td>48</td>
<td>12</td>
</tr>
<tr>
<td>72</td>
<td>18</td>
</tr>
<tr>
<td>72 (boiled)</td>
<td>1</td>
</tr>
</tbody>
</table>

A suspension of digitonin fragments in H2O (1.24 mg. N per ml.) was aged at 2°C. Aliquots, 4.0 ml., were taken at the times shown, acidified, extracted with isooctane, and U factor activity of the extracts assayed.
cell or mitochondrial structure and subsequent lipolysis to produce free fatty acid, and that this mechanism has no particular physiological significance, such a conclusion seems premature. It appears necessary first to establish the identity of U factor by more certain, and further work is now being carried out to purify the iso-octane-extracted, mitochondrial factor by chromatographic means. Although many long-chain fatty acids have uncoupling activity, it is possible that the bulk of the endogenous U factor activity may be contributed by one specific type of fatty acid which may not be one of the more common or abundant fatty acids of animal tissues. Also, free or unesterified fatty acids are normally found in the blood and tissues as a transport form in significant concentrations (25). Furthermore, a physiologically uncoupling function of free fatty acids has been postulated by Langdon to explain some features of the metabolic integration of fatty acid and pyruvate oxidation in mitochondria (26).

Promotion of mitochondrial swelling by U factor suggests that it is an important endogenous or intracellular factor in swelling, together with the other intracellular swelling agents, such as thyroxine, Ca++, phosphate, and glutathione (cf. (27)). It is significant that serum albumin causes substantial potentiation of the action of ATP and Mg++ in reversing mitochondrial swelling (27), suggesting that the serum albumin combines with U factor.

**SUMMARY**

In incubation of intact or sonically disrupted rat liver mitochondria at 37° causes the loss of the ability to couple phosphorylation to respiration and the simultaneous formation of an iso-octane-extractable, heat-stable uncoupling agent designated as U factor. This factor also stimulates respiration in the absence of a phosphate acceptor system, inhibits the adenosine triphosphate (ATP)-inorganic orthophosphate-P32 exchange reaction, and stimulates ATPase activity. U factor also causes swelling of rat liver mitochondria. These actions of U factor are abolished by the presence of serum albumin.

Enzymic formation of U factor in sonically disrupted mitochondria is heat-labile and has an optimal pH of about 6.0. It is not affected by inhibiting respiration with cyanide or azide, by the addition of ATP, adenosine diphosphate, Mg++, Ca++, diphosphoryridine nucleotide, or arsenate, but is inhibited by fluoride and Hg++. The presence of both ATP and the reduced form of coenzyme A greatly inhibits the accumulation of iso-octane-extractable U factor, and this may account for some of the beneficial action of coenzyme A in oxidative phosphorylation.

The enzymic formation of U factor requires a heat-stable component, possibly a lipide, which is present largely in the particular fraction from sonic-treated mitochondria, plus a heat-labile component which is present in the soluble fraction of sonicated mitochondria. Enzymic formation of U factor also occurred in phosphorylating digitonin fragments derived from mitochondrial membranes.

U factor has properties consistent with those of higher fatty acids and its enzymic formation from endogenous lipides may be responsible for the rapid inactivation of oxidative phosphorylation that occurs when mitochondria are disrupted. A physiological role for U factor appears possible.

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**REFERENCES**

An Endogenous Uncoupling and Swelling Agent in Liver Mitochondria and Its Enzymic Formation
Albert L. Lehninger and LeMar F. Remmert


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