Reversible Inhibition of Adenine Nucleotide Translocation by Long Chain Fatty Acyl Coenzyme A Esters in Liver Mitochondria of Diabetic and Hibernating Animals*

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SUMMARY

The sluggish respiratory response to ADP in liver mitochondria of alloxan diabetic rats and hibernating ground squirrels was overcome by addition of uncouplers of oxidative phosphorylation, suggesting that poor penetration of ADP prevented maximum oxygen consumption. The in vivo depression of adenine nucleotide translocation through the inner mitochondrial membrane was confirmed directly by measurements of [14C]ADP translocase and 32P-ATP exchange reactions. The inhibition of adenine nucleotide penetration was considered to be a consequence of increased hepatic lipid content, particularly long chain acyl-CoA esters, which occur in diabetes and hibernation. Demonstrated changes in the pattern of mitochondrial long chain fatty acids may be responsible for the altered spectral characteristics of the cytochromes observed in these animals. The inhibition of adenine nucleotide translocation could be reproduced in vitro with normal rat liver mitochondria by production of, or upon addition of certain esters of long chain fatty acids. Simultaneous addition of pyruvate or α-ketoglutarate with the free fatty acid prevented formation of the acyl-CoA ester and inhibition of translocation. Selective reversal of the inhibition by carnitine provided strong evidence implicating the acyl-CoA ester rather than the free fatty acid as the inhibitory agent. From these results, it is postulated that certain long chain acyl-CoA esters, acting as natural effectors of adenine nucleotide translocation, can perform a physiological role in the regulation of mitochondrial metabolism.

MATERIALS AND METHODS

Chemicals—Alloxan and d,L-carnitine hydrochloride were purchased from Schwarz-Mann; [14C]ADP from Schwarz BioResearch; 32P inorganic phosphate from New England Nuclear; [14C]ATP and NCS Solubilizer from Amerham-Scarce; acyl-CoA derivatives from P-L Biochemicals; cytochrome c, type III, and bovine serum albumin-fatty acid-free, type F, from Sigma; and atractyloside from Calbiochem. Salicylanilide XIII, 5-Cl, 3-t butyl, 2'-Cl, 4'-NO2-salicylanilide, was kindly donated by Dr. Henry A. Lardy. All other reagents were of the highest grade commercially available.

Experimental Animals—Male rats (Badger Research Corp.,
Reversible Inhibition of Adenine Nucleotide Translocation

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RESULTS

Mitochondrial Respiration in Livers of Diabetic and Hibernating Animals—Initial studies compared respiratory control in liver mitochondria of diabetic and hibernating animals with their control counterparts. In contrast to the control, ADP added to mitochondria of the experimental animal respiring in the presence of substrate and inorganic phosphate, i.e. State 4, did not maximally stimulate oxygen consumption (Fig. 1). The depressed response to external addition of ADP was consistently more pronounced in the hibernator than in the diabetic animals. In the diabetic rat the depression which appeared to be directly proportional to the severity of the diabetes could be at least partially eliminated by extensively washing the mitochondria. In both the diabetic and the hibernator, the low oxygen consumption during the State 3 to 4 cycle was stimulated by the addition of Safecylamide XII, a potent uncoupler of oxidative phosphorylation (14). These results indicate that the mitochondria were not uncoupled but that the penetration of ADP through the inner mitochondrial membrane was decreased.

\[ ^2P_7\text{-ATP Exchange Activity and} ^{[\text{C}]\text{ADP Translocation in Liver Mitochondria of Diabetic and Hibernating Animals—In order to examine the penetrability of the adenine nucleotides directly,} ^{[\text{C}]\text{ADP and}} ^{[\text{C}]\text{ATP translocation experiments were performed. In addition, the}} ^2P_7\text{-ATP exchange activity, an index of oxidative phosphorylation, was also measured. This exchange reaction, which is dependent upon the ability of exogenously supplied ATP to penetrate the inner mitochondrial membrane, should parallel that of adenine nucleotide translocation. Results of these experiments as shown in Tables I and II are consistent with the limited ADP stimulation of mitochondrial respiration in diabetic and hibernating animals. In all cases,} ^2P_7\text{-ATP exchange activity and}} ^{[\text{C}]\text{ADP translocation were lower in the experimental groups. The more pronounced decrease exhibited by the diabetic monkey and hibernating ground squirrel could represent species difference, but is more likely a result of the intensity of the metabolic condition. Carnitine, which facilitates oxidation of acetyl-CoA esters through the carnitine acyl transferase enzyme (or enzymes), and \( \alpha \)-ketoglutarate, which by preferentially utilizing CoA through the \( \alpha \)-ketoglutarate dehydrogenase complex prevents activation of endogenous fatty acids, revert the decreased} ^{2P_7\text{-ATP exchange and}} ^{[\text{C}]\text{ADP translocase activities toward normal. However,}} \] restored by the method of Sealy (13). Acetoacetate was prepared according to the method of Sealy (13).
TABLE I

**32P**-**ATP** exchange activity in liver mitochondria of normal and diabetic animals, and hibernating ground squirrels

The basic incubation mixture contained 10 mM ATP, 10 mM **32P** (20,000 cpm), 75 mM Tris-Cl, pH 7.0, and 45 mM sucrose in a volume of 1.0 ml. The reaction was initiated with 2.5 mg of mitochondrial protein and incubated at 20°C for 15 min.

<table>
<thead>
<tr>
<th>Additions</th>
<th>Rat</th>
<th>Monkey</th>
<th>Ground Squirrel</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Diabetic</td>
<td>Normal</td>
</tr>
<tr>
<td><strong>32P</strong>-<strong>ATP</strong> cpm/mole ATP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>452</td>
<td>360</td>
<td>320</td>
</tr>
<tr>
<td>± 10.8 ± 8.2 ± 9.2 ± 5.4 ± 6.5 ± 2.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DL-Carnitine, 5.0 mm</td>
<td>463</td>
<td>443</td>
<td>330</td>
</tr>
<tr>
<td>± 11.2 ± 9.4 ± 8.5 ± 6.3 ± 9.5 ± 2.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Ketoglutarate, 5.0 mm</td>
<td>469</td>
<td>432</td>
<td>398</td>
</tr>
<tr>
<td>± 12.0 ± 11.6 ± 9.5 ± 6.5 ± 8.4 ± 3.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KCN, 1.0 mm</td>
<td>341</td>
<td>270</td>
<td>280</td>
</tr>
<tr>
<td>± 7.3 ± 7.0 ± 6.1 ± 3.2 ± 7.3 ± 1.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KCN + α-Ketoglutarate</td>
<td>445</td>
<td>363</td>
<td>315</td>
</tr>
<tr>
<td>± 8.5 ± 11.0 ± 12.2 ± 4.8 ± 8.1 ± 4.8</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Standard error (five to six animals in each group).

**TABLE II**

[^14C]ADP translocation in liver mitochondria of normal and diabetic animals, and hibernating ground squirrels

The basic reaction mixture of 40 mM Tris-HCl, pH 7.4, 100 mM KCl, 1.0 mM MgCl₂, and 2.5 mg of mitochondrial protein in a volume of 1.0 ml was incubated for 4 min at 25°C. The reaction was then initiated by the addition of 0.08 mM [^14C]ADP (40,000 cpm). After 2 min, the reaction was terminated with atractylside.

<table>
<thead>
<tr>
<th>Additions</th>
<th>Rat</th>
<th>Monkey</th>
<th>Ground Squirrel</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Diabetic</td>
<td>Normal</td>
</tr>
<tr>
<td>cpm X 10⁻¹⁵/particle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>292</td>
<td>157</td>
<td>263</td>
</tr>
<tr>
<td>± 6.7 ± 21.4 ± 18.6 ± 9.5 ± 4.6 ± 1.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DL-Carnitine, 5.0 mm</td>
<td>280</td>
<td>255</td>
<td>258</td>
</tr>
<tr>
<td>± 2.0 ± 9.5 ± 17.1 ± 14.1 ± 4.2 ± 9.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin, 15 mg.</td>
<td>270</td>
<td>238</td>
<td>261</td>
</tr>
<tr>
<td>± 2.3 ± 10.4 ± 8.5 ± 17.1 ± 3.7 ± 1.7</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Standard error (five to six animals in each group).

previously reported by Boyer et al. (6) to minimally inhibit **32P**-**ATP** exchange would appear to act in the present experimental system by preventing complete oxidation of endogenous long chain fatty acyl-CoA esters, which in turn decrease penetration of exogenously added ATP. The more intense inhibition upon addition of KCN, as well as reversal of the inhibition of α-ketoglutarate and albumin, tends to support this contention.

Inhibition of **32P**-**ATP** Exchange Activity and[^14C]ADP Translocation in Normal Rat Liver Mitochondria by in Vitro Addition of Long Chain Fatty Acids and Their CoA Esters—An in vitro system was used to study the condition of increased hepatic fatty acid oxidation which occurs during hibernation and diabetes (9). **32P**-**ATP** exchange activity and translocation of[^14C]ADP were measured in rat liver mitochondria in the presence of fatty acids and cyanide at concentrations too low to produce uncoupling of oxidative phosphorylation and stimulation of ATPase activity. In the presence of KCN to permit accumulation of CoA esters, shorter chain fatty acids represented by butyric and octanoic acids had no effect on **32P**-**ATP** exchange and[^14C]ADP translocase activities. In contrast, myristic, palmitic, and oleic acids showed strong inhibition in the presence of KCN. α-Bromomyristic acid, which is a relatively poor substrate for fatty acid activation to the acyl-CoA ester (15), did not cause inhibition. Stearic and erucic, long chain saturated and unsaturated fatty acids showed strong inhibition in the presence of KCN.
esters. As shown in Table IV, [14C]ADP translocation was inhibited 50 and 92% at concentrations of 3 and 30 μM, respectively, of α-keto acids, 5.0 mM; and of d, l-carnitine, 5.0 mM. Preliminary incubation with oleic acid was for 10 min.

<table>
<thead>
<tr>
<th>Additions</th>
<th>Exchange activity</th>
<th>Translocase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cpm/mole ATP</td>
<td>cpm X 10⁻⁶/10⁻⁶/pellet</td>
</tr>
<tr>
<td>None</td>
<td>415</td>
<td>276</td>
</tr>
<tr>
<td>Myristic acid</td>
<td>415</td>
<td>276</td>
</tr>
<tr>
<td>Myristic acid + KCN</td>
<td>103</td>
<td>140</td>
</tr>
<tr>
<td>Myristic acid + α-ketoglutarate</td>
<td>398</td>
<td>273</td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>438</td>
<td>283</td>
</tr>
<tr>
<td>Palmitic acid + KCN</td>
<td>256</td>
<td>100</td>
</tr>
<tr>
<td>Palmitic acid + α-ketoglutarate</td>
<td>449</td>
<td>287</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>460</td>
<td>280</td>
</tr>
<tr>
<td>Oleic acid + KCN</td>
<td>93</td>
<td>104</td>
</tr>
<tr>
<td>Oleic acid + α-ketoglutarate</td>
<td>424</td>
<td>291</td>
</tr>
<tr>
<td>Oleic acid + pyruvate</td>
<td>396</td>
<td></td>
</tr>
<tr>
<td>Oleic acid + acetoacetate</td>
<td>101</td>
<td></td>
</tr>
<tr>
<td>Oleic (previously incubated) + KCN + α-ketoglutarate</td>
<td>106</td>
<td></td>
</tr>
<tr>
<td>Atractyloside</td>
<td>35</td>
<td>18</td>
</tr>
<tr>
<td>Atractyloside + α-ketoglutarate</td>
<td>41</td>
<td>22</td>
</tr>
<tr>
<td>Atractyloside + carnitine</td>
<td>30</td>
<td>20</td>
</tr>
</tbody>
</table>

Additions | Acyl-CoA concentration | Exchange activity | Translocase activity |
-----------|------------------------|-------------------|----------------------|
None       | 452                    | 401               | 141                  |
Butyroyl-CoA | 30                    | 401               | 141                  |
Stearyl-CoA | 30                    | 393               | 39                   |
Stearyl-CoA + carnitine | 30 | 440 | 141 |
Myristoyl-CoA | 30                  | 399               | 132                  |
Myristoyl-CoA + carnitine | 30            | 399               | 132                  |
Palmitoyl-CoA | 30            | 390               | 131                  |
Palmitoyl-CoA + carnitine | 30        | 390               | 131                  |
Oleoyl-CoA | 30                    | 378               | 127                  |
Oleoyl-CoA + carnitine | 30 | 378 | 127 |
Oleoyl-CoA + α-ketoglutarate      | 30                | 60                 | 15                   |

![Fig. 2. Difference spectra for the cytochrome components of active and hibernating ground squirrel liver mitochondria. Mitochondrial protein from active ground squirrel, 27 mg, and from hibernator, 37.8 mg were diluted to 3.0 ml with 0.25 M sucrose and 0.05 M Tris-HCl, pH 7.4. Oxidized (potassium ferriyamide) minus reduced (sodium hydrosulfite). Note—The fourth number from the left on the abscissa should be 500 rather than 500.](http://www.jbc.org/)

Fig. 2. Difference spectra for the cytochrome components of active and hibernating ground squirrel liver mitochondria. Mitochondrial protein from active ground squirrel, 27 mg, and from hibernator, 37.8 mg were diluted to 3.0 ml with 0.25 M sucrose and 0.05 M Tris-HCl, pH 7.4. Oxidized (potassium ferriyamide) minus reduced (sodium hydrosulfite). Note—The fourth number from the left on the abscissa should be 500 rather than 500.
a parallel manner. Reversal of long chain acyl-CoA ester inhibition by carnitine is more complete in mitochondria from normal animals than those from the diabetic and hibernators. Livers from these animals contain high concentrations of endogenous acyl-CoA esters which may not be completely converted to acyl carnitine. Guinea pig liver and rat heart mitochondria responded similarly to addition of long chain acyl-CoA esters, indicating a rather general species and tissue effect.

**Difference Spectra and Cytochrome Oxidase Activity in Liver Mitochondria** In a previous study (1) we observed a striking alteration of the cytochrome spectra from hibernating ground squirrel mitochondria. In order to verify this interesting and somewhat surprising phenomena (Fig. 2), special care was taken to allow a sufficient number of control animals to become active after hibernation. Studies were also carried out on diabetic and normal rat liver mitochondria (Fig. 3) to determine whether the spectral changes were related to factors common to the metabolism of both hibernation and diabetes. In the diabetic rat as well as the hibernating ground squirrel mitochondria, the peak height of $a_{43}$ decreased compared with control animals. The spectral change occurred consistently in liver mitochondria from all hibernators tested, whether Citillus lateralis or C. tridecemlineatus and was never observed in animals allowed to awaken from hibernation and become active. The extent of the reduction of the $a_{43}$ peak height was much greater in the hibernator than in the diabetic. However, the spectral changes in diabetic rat liver mitochondria were not always observed and were most evident in severely ketotic animals. Following treatment with insulin, diabetic rats always had normal spectra. Difference spectra (not shown) of heart mitochondria from diabetic and normal rats were identical. Pyridine hemochromogen determinations for total mitochondrial cytochromes revealed an average value for the hibernator which was half that for the active animal. Although values for the diabetic were lower than normal, the differences were not significant. The specific activity of cytochrome oxidase assayed enzymatically was lower in both the hibernator and diabetic, although again the extent of the decrease was much greater in the hibernator (Table V).

**Fatty Acid Patterns in Liver Mitochondria from Hibernating and Diabetic Animals**—While the functional significance is not completely understood, the altered cytochrome spectra might reflect changes in structural components of liver mitochondrial membranes brought about by abnormal fatty acid and phospholipid metabolism. The fatty acid patterns of liver mitochondria from ground squirrels and rats are shown in Table VI. Diabetic rat liver mitochondria contain a higher percentage of palmitic acid and lower percentages of arachidonic acid and total polyunsaturated acids as compared to the normal control rat. This result is consistent with a defect in the oxidative de

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**TABLE V**

*Cytochrome oxidase activity of liver mitochondria from diabetic animals and hibernating ground squirrels*

Assay temperature: 25°C. Details given in text.

<table>
<thead>
<tr>
<th>Experimental animal</th>
<th>Cytochrome oxidase activity (μmol ferricytochrome c oxidized/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rat</td>
<td>3.1 ± 0.15 ±</td>
</tr>
<tr>
<td>Diabetic rat</td>
<td>2.4 ± 0.2</td>
</tr>
<tr>
<td>Active ground squirrel</td>
<td>2.8 ± 0.1</td>
</tr>
<tr>
<td>Hibernating ground squirrel</td>
<td>1.2 ± 0.04</td>
</tr>
</tbody>
</table>

* Standard error (five to six animals in each group)

---

**TABLE VI**

*Fatty acid composition of liver mitochondria from control and diabetic rats and from active and hibernating ground squirrels*

<table>
<thead>
<tr>
<th>Experimental animal</th>
<th>10:0</th>
<th>18:0</th>
<th>18:1</th>
<th>18:2</th>
<th>20:4</th>
<th>22:6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control rat (26)</td>
<td>17.6 ± 0.37 ±</td>
<td>20.6 ± 0.57 ±</td>
<td>11.1 ± 0.19 ±</td>
<td>23.8 ± 0.51 ±</td>
<td>23.9 ± 0.31 ±</td>
<td>3.0 ± 0.78 ±</td>
</tr>
<tr>
<td>Diabetic rat (10)</td>
<td>21.4 ± 1.19 ±</td>
<td>19.9 ± 0.92 ±</td>
<td>12.3 ± 1.07 ±</td>
<td>25.1 ± 1.01 ±</td>
<td>17.5 ± 1.04 ±</td>
<td>3.8 ± 0.82 ±</td>
</tr>
<tr>
<td>Active ground squirrel (5)</td>
<td>21.4 ± 1.2 ±</td>
<td>19.6 ± 0.78 ±</td>
<td>18.1 ± 1.16 ±</td>
<td>25.6 ± 0.8 ±</td>
<td>9.5 ± 0.67 ±</td>
<td>5.8 ± 1.33 ±</td>
</tr>
<tr>
<td>Hibernating ground squirrel (9)</td>
<td>19.3 ± 0.9 ±</td>
<td>16.6 ± 0.05 ±</td>
<td>28.7 ± 0.0 ±</td>
<td>24.0 ± 0.05 ±</td>
<td>11.4 ± 0.06 ±</td>
<td>1.0 ± 0.53 ±</td>
</tr>
</tbody>
</table>

* Fatty acids are designated by carbon chain length (e.g. 16:0 = palmitic acid) and by number of unsaturated bonds (e.g. 18:1 = oleic acid). The values are expressed as the mean percentage of the total fatty acids ± the standard error.

* Number in parentheses indicates number of animals.

* $p < 0.01$.

* $p < 0.05$. 

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**Fig. 3.** Difference spectra for the cytochrome components of normal and diabetic rat liver mitochondria. Mitochondrial protein from normal animals, 19.2 mg, and from diabetic animals, 19.2 mg, were diluted to 1.0 ml in a solution containing 0.2% sodium deoxycholate and 20 mM potassium phosphate buffer, pH 7.4. Oxidized (potassium ferricyanide) minus reduced (sodium hydrosulfite).
steady state of phosphorylation, the rate of ATP formation in-
number of cytochrome oxidase molecules (25); and (c) in the
percentage of polyunsaturated fatty acids than the active ani-
rat, those from hibernating ground squirrel contained a lower
in alloxan diabetic rats. Like liver mitochondria of the diabetic
acid, which had been previously observed by Mercuri et al. (17)
saturation of linoleic to y-linolenic acid and hence, arachidonic
(b) the number of adenine nucleotide-binding sites equals the
relationship based upon extensive experimental data shows: (a) the
primary focus for metabolic control in viva. A close interrela-
tion of electron transport-linked phosphorylation of ADP in rat
mitochondria incubated with palmitoyl-Cob but not with
and uncouple oxidative phosphorylation (18, 19). The isolation
membrane is due to the acyl-CoA ester rather than the free fatty
acids were uncoupled as a result of detergent effects of
mitochondria were uncoupled as a result of detergent effects of
free fatty acids, could be reinterpreted in light of the present
studies carried out in rabbit liver mitochondria, Davis and Gib-
son (38) have suggested that elevated levels of long chain free
fatty acids in liver could enhance gluconeogenesis from certain
amino and keto acids by partially uncoupling oxidative phos-
phorylation. A subsequent increase in the rate of flow of inter-
carbohydrate and lipid metabolism with adenine nu-
adenine nucleotide translocation. The possible inter-relation-
sionships of carbohydrate and lipid metabolism with adenine nu-
acid to stimulate ATPase activity, certain long chain saturated and
unsaturated fatty acids inhibited the 32Pi-ATP exchange activ-
ty in rat liver mitochondria. More recently, Wojtczak and
Zaluska (7) reported the inhibition of translocation of adenine
nucleotides through rat liver mitochondrial membranes by
oleic acid. In the present experiments, inhibition of 32Pi-ATP
exchange and [14C]ATP translocase activities by long chain fatty
acids could be overcome by preferentially utilizing endogenous
CoA to metabolize α-keto acids. These results, together with
the carnitine reversal of inhibition by long chain fatty acyl-
CoA esters, indicate that the acids must be activated either at
the outer mitochondrial membrane or intermembrane space (22)
to be effective, and that the inhibition which occurs at the inner
membrane is due to the acyl-CoA ester rather than the free fatty
acid. Additional support for this proposal comes from the study
of Pande and Blanchaer (23), who observed a reversible inhibition
of electron transport-linked phosphorylation of ATP in rat
heart mitochondria incubated with palmitoyl-CoA but not with
palmitoyl carnitine.

The adenine nucleotide translocase which is closely associated
with energy-linked mitochondrial respiration might serve as a
primary focus for metabolic control in vivo. A close interrela-
tionship based upon extensive experimental data shows: (a) the
ADP specificity of oxidative phosphorylation results from the
nucleotide specificity of the adenine nucleotide translocase (24);  
(b) the number of adenine nucleotide-binding sites equals the
number of cytochrome oxidase molecules (25); and (c) in the
steady state of phosphorylation, the rate of ATP formation in-
side the mitochondria equals the rate of ATP transport out of
the mitochondria (26). Because of the central role of the
adenine nucleotide translocase in mitochondrial metabolism,
any natural inhibitors would serve as potential regulators.
Atractyloside (16) and bongkrekic acid (27) inhibit adenine
nucleotide translocation; however, these compounds are not
normally found in mammalian tissues. Long chain acyl-CoA
esters are normal constituents of animal tissues and thus could
serve as natural in vivo inhibitors of the translocase. In support
of this hypothesis are the present results showing marked de-
pression of [14C]ADP translocase and 32Pi-ATP exchange ac-
tivities in liver mitochondria from diabetic and hibernating
animals. Hibernation (28, 29) and diabetes (30, 31) are ex-
amples of physiological and pathophysiological states characte-
ized by enhanced rates of fatty acid oxidation and glucone-
genesis. An increased content of long chain fatty acids and
their corresponding CoA esters in whole liver (32, 33) and
isolated mitochondria (34) has been consistently observed in fasted
and alloxan diabetic rats. Matsukura and Toshio (35) showed
an inverse relationship between mitochondrial respiratory re-
sponse to ATP and the levels of mitochondrial fatty acids in
severe diabetic rats. Their conclusion, that diabetic rat liver
mitochondria were uncoupled as a result of detergent effects of
free fatty acids, could be reinterpreted in light of the present
experiments to indicate that apparent uncoupling was a result of
poor penetration of exogenously supplied ADP.

Although fatty acid stimulation of gluconeogenesis in rat liver
is well documented (36, 37) postulated mechanisms of action
such as an effect on the oxidation-reduction state of the cell and
provision of excess acetyl-CoA to inhibit pyruvate oxidase and
stimulate pyruvate carboxylase are still inconclusive. Based on
studies carried out in rabbit liver mitochondria, Davis and Gib-
son (38) have suggested that elevated levels of long chain free
fatty acids in liver could enhance gluconeogenesis from certain
amino and keto acids by partially uncoupling oxidative phos-
phorylation. A subsequent increase in the rate of flow of inter-
mediates to oxalacetate and stimulation of GTP production
would result from substrate level phosphorylation coupled to the
oxidation of α-ketoglutarate. In the guinea pig liver, fatty
acids apparently have no stimulatory effect and may even be
inhibitory for the conversion of alanine or lactate to glucose
(39). Walker et al. (40) suggested that the majority of reducing
equivalents for reduction of oxalacetate to malate in rat liver
mitochondria could be supplied by the oxidation of fatty acids
either directly or possibly through an energy linked reversal of
electron transport. Long chain acyl-CoA esters may, by
inhibiting adenine nucleotide translocation and inducing a trans-
ition from State 3 to State 4 respiration, serve in this capacity.
This metabolic condition would be particularly effective in regu-
ulating the ADP:ATP ratio and in generating reducing equiva-
Ients through reverse electron transfer. During active glu-
coneogenesis carbon and hydrogen would be shunted from the
mitochondria to the cytosol for synthesis of glucose. Mitoch-
dondia from other organs particularly heart, respond in a
similar manner to long chain fatty acyl-CoA esters, thus indi-
cating a more general metabolic effect of reversible inhibition
of adenine nucleotide translocation. The possible inter-rela-
tionships of carbohydrate and lipid metabolism with adenine nu-
eleotide translocase activity in cardiac tissue is now under in-
vestigation.
Acknowledgments—We wish to express our appreciation to Professor H. A. Lardy for his continued interest and support.

REFERENCES

Corrections

Vol. 247 (1972) 1513-1519

In Lerner, Edith, Austin L. Shug, Charles Elson, and Earl Shrago. Reversible Inhibition of Adenine Nucleotide Translocation by Long Chain Fatty Acyl Coenzyme A Esters in Liver Mitochondria of Diabetic and Hibernating Animals

The date received for publication was Oct. 12, 1971, not Oct. 12, 1970.

Vol. 246 (1971) 6019-6023

In Marshall, Thomas H., and Ay Arqun. The Specificity of Porcine Elastase and α-Chymotrypsin. Effect of Fatty Acid Chain Length in a Homologous Series of Nitrophenyl Esters

Page 6021, Table I, the third entry under “Chymotrypsin-catalyzed hydrolysis at pH 7.8, 25°C” should read:
n-Butyrate 13 0.711 0.625 11 0.580

Vol. 247 (1972) 2095-2101


Page 2099, Equation 5 should have a superscript 2 outside the brackets

\[ \frac{\pi}{E_0} = \left( \frac{k_2}{k_2 + k^a} \right)^2 \]

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Edith Lerner, Austin L. Shug, Charles Elson and Earl Shrago


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