The high concentration of lipide material in mitochondria (1–3) suggests that the enzyme activity in the mitochondria may be particularly associated with the presence of lipide. Burr and Beber (4) have reported that animals fed a fat-deficient diet possess a significantly higher metabolic rate than comparable controls. In previous studies of the rôle of essential fatty acids in metabolism, Kunkel and Williams (5) found that both cytochrome oxidase and endogenous respiration of the livers of fat-deficient rats were significantly higher than normal, although succinoxidase was unaffected by the deficiency. Recently Klein and Johnson (6) reported that liver mitochondria from fat-deficient rats esterify less high energy phosphate per mole of oxygen taken up during the oxidation of certain intermediates of the tricarboxylic acid cycle. It was suggested that the uncoupling of oxidative phosphorylation in essential fatty acid (EFA) deficiency is restricted to a certain step in the electron transport system, at or near the level of DPN reduction or oxidation. Uncoupled phosphorylation (7) could thus account for the high metabolic rate (4), the high endogenous respiration, and the high cytochrome oxidase of EFA-deficient rats (5).

Tulpule and Patwardhan (8) have observed a reduction in liver glutamic dehydrogenase activity of EFA or vitamin Be-deficient rats, which was further decreased by a double deficiency of these dietary factors. On the other hand succinic and butyric dehydrogenase activities, as measured by methylene blue reduction, were unaffected by a vitamin Be deficiency; however, EFA or EFA plus vitamin Be deficiencies caused some reduction in these dehydrogenases.

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1 The abbreviations used throughout this paper are as follows: EFA, essential fatty acids; DPN, diphosphopyridine nucleotide; DPNH, reduced DPN; AASF, antimycin A-sensitive factor.
The present studies were undertaken to investigate further the metabolic defects caused by a deficiency of EFA. Since the severity of gross symptoms produced by EFA deficiency is markedly accentuated by a superimposed vitamin Be deficiency (9), a study of the relationship of vitamin Be to EFA deficiency has been included. The enzymes selected for study were succinic oxidase, the antimycin A-sensitive factor (AASF) (10), cytochrome oxidase, DPNH oxidation and associated phosphorylation, and reduced cytochrome c oxidation and associated phosphorylation. The first three systems were chosen to obtain as complete a picture as possible of the electron transport through a non-DPN-linked mitochondrial system. The DPNH and reduced cytochrome c systems were selected to study electron transport and coupled phosphate esterification through a DPN-linked system, unencumbered by consideration of the initial dehydrogenation at the normal substrate (e.g., malate and α-ketoglutarate) level.

EXPERIMENTAL

Male albino rats (Holtzman Rat Company, Madison) weighing 70 to 80 gm. were used as experimental animals. The rats were separated into six groups of eight rats each. The dietary regimens of the six groups were as follows: Group I, the basal diet2 plus 670 mg. per cent of methyl linoleate and 0.25 mg. per cent of vitamin Be (complete diet) for 6 weeks ad libitum; Group II, the complete diet for 4 weeks with daily food consumption limited to one-third that of Group I (restricted food intake), followed by 2 weeks of the complete diet ad libitum; Group III, the basal diet for 4 weeks with restricted food intake, followed by 2 weeks of the complete diet ad libitum; Group IV, the same as Group III, except that for the last 2 weeks the basal ration was fed ad libitum plus 0.25 mg. per cent of vitamin Be; Group V, the same as Group IV, except that 670 mg. per cent of methyl linoleate were included for the last 2 weeks in place of vitamin Be; and Group VI, the basal diet for 4 weeks with restricted food intake, followed by 2 weeks of the basal diet ad libitum. Thus, after the 6 week feeding periods, Groups I to III served as controls, Group IV as the EFA-deficient group, Group V as the vitamin Be-deficient group, and Group VI as the combined EFA and vitamin Be-deficient group. The initial food restriction technique employed in the present experiments has been shown by Barki et al. (13) to precipitate an EFA deficiency more efficiently than ad libitum feeding of a fat-deficient diet.

* The basal diet, consisted of the following components in per cent: vitamin-free casein 20, salts IV (11) 4, vitamin mix (12) with pyridoxine omitted 2, and sucrose 74. Vitamins A, D, E, and K in 90 per cent ethanol were administered to each rat orally once a week.
After the feeding periods, the animals were sacrificed by stunning. The livers were quickly removed and chilled, and weighed portions were homogenized in the appropriate suspending media. Succinic oxidase was assayed by the method of Schneider and Potter (14); AASF by a method suggested by Potter and Reif (10), in which succinic oxidase activity for a given amount of tissue was measured *versus* antimycin A concentration; cytochrome oxidase by the method of Schneider and Potter (14); DPNH oxidation and coupled phosphorylation, with 100 mg. of whole rat liver homogenate in place of isolated mitochondria by the method of Lehninger (15); and reduced cytochrome c oxidation, coupled with phosphorylation, by a modification of the method of Lehninger *et al.* (16). In the last method, phosphate uptake and oxidation of cytochrome c reduced with ascorbic acid were measured at 37° for 40 minutes with 100 mg. of whole rat liver homogenate in place of isolated mitochondria. A correction was applied to account for ascorbic acid autoxidation by incubating separate flasks with ascorbic acid, other components of the reaction mixture, and boiled liver homogenate (100° for 15 minutes). Inorganic phosphate was estimated by the method of Bell and Doisy (17), as modified by Briggs (18).

**Results**

*Succinic Oxidase, Antimycin A-Sensitive Factor, and Cytochrome Oxidase*—From the results of these experiments presented in Table I, it can be seen that succinic oxidase was unaffected by deficiencies of EFA, B₆, or both in combination. This confirms previous results from this laboratory and elsewhere (5, 6).

Although succinic oxidase activity was unaffected, it is interesting to note that AASF, which denotes a hypothetical electron transport component between succinic dehydrogenase and cytochrome c (10), was significantly increased. This increase was most pronounced with the double deficiency of EFA and vitamin B₆, although it was increased by each deficiency individually. Another interesting observation is that the preliminary restricted feeding of the EFA-vitamin B₆-deficient diet caused an increase in AASF, which was not reversed by *ad libitum* feeding of the complete diet for 2 additional weeks (Group III). No explanation can be offered for this lack of reversal at present.

Cytochrome oxidase was increased to the greatest extent when the animals were made deficient in both EFA and vitamin B₆. When only vitamin B₆ was supplemented (Group IV), no reversal of the high activity was obtained. Linoleate supplementation to vitamin B₆-deficient rats, how-

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*The authors wish to thank Dr. F. M. Strong of this department for a generous sample of antimycin A.*
### Table I

**Effect of Essential Fatty Acid and Vitamin B<sub>1</sub> Deficiencies on Activity of Succinic Oxidase, Antimycin A-Sensitive Factor, and Cytochrome Oxidase**

<table>
<thead>
<tr>
<th>Group No.*</th>
<th>Succinic oxidase activity, μl. O&lt;sub&gt;2&lt;/sub&gt; per hr. per mg. dry liver</th>
<th>AASF titer, molarity X 10&lt;sup&gt;6&lt;/sup&gt; of antimycin A per mg. dry liver</th>
<th>Cytochrome oxidase activity, μl. O&lt;sub&gt;2&lt;/sub&gt; per hr. per mg. dry liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Control 1)</td>
<td>45.1 ± 2.7‡</td>
<td>2.5 ± 0.1</td>
<td>241 ± 7</td>
</tr>
<tr>
<td>II (&quot; 2)</td>
<td>42.7 ± 2.6</td>
<td>3.0 ± 0.2</td>
<td>235 ± 5</td>
</tr>
<tr>
<td>III (&quot; 3)</td>
<td>47.2 ± 5.0</td>
<td>3.3 ± 0.2</td>
<td>236 ± 5</td>
</tr>
<tr>
<td>IV (EFA-deficient)</td>
<td>46.6 ± 3.6</td>
<td>3.3 ± 0.2</td>
<td>269 ± 9</td>
</tr>
<tr>
<td>V (Vitamin B&lt;sub&gt;1&lt;/sub&gt;-deficient)</td>
<td>42.9 ± 2.3</td>
<td>3.5 ± 0.2</td>
<td>250 ± 8</td>
</tr>
<tr>
<td>VI (EFA- and vitamin B&lt;sub&gt;1&lt;/sub&gt;-deficient)</td>
<td>42.6 ± 3.0</td>
<td>3.9 ± 0.1</td>
<td>277 ± 9</td>
</tr>
</tbody>
</table>

* Eight rats in each group. See the text for a complete description of dietary regimens.

† The final concentration of antimycin A to give one-half maximal inhibition of succinic oxidase.

‡ Standard error of the mean.

### Table II

**Effect of Essential Fatty Acid and Vitamin B<sub>1</sub> Deficiencies on Phosphate Esterification Coupled with Oxidation of DPNH and Reduced Cytochrome c**

The values are given in microatoms.

<table>
<thead>
<tr>
<th>Group No.*</th>
<th>No. of animals</th>
<th>DPNH oxidation</th>
<th>Reduced cytochrome c oxidation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ΔP</td>
<td>ΔO</td>
</tr>
<tr>
<td>I (Control 1)</td>
<td>6</td>
<td>7.40</td>
<td>6.79</td>
</tr>
<tr>
<td>II (&quot; 2)</td>
<td>7</td>
<td>7.55</td>
<td>6.76</td>
</tr>
<tr>
<td>III (&quot; 3)</td>
<td>7</td>
<td>7.19</td>
<td>7.03</td>
</tr>
<tr>
<td>IV (EFA-deficient)</td>
<td>8</td>
<td>5.49</td>
<td>6.81</td>
</tr>
<tr>
<td>V (Vitamin B&lt;sub&gt;1&lt;/sub&gt;-deficient)</td>
<td>8</td>
<td>6.96</td>
<td>7.90</td>
</tr>
<tr>
<td>VI (EFA- and vitamin B&lt;sub&gt;1&lt;/sub&gt;-deficient)</td>
<td>7</td>
<td>4.72</td>
<td>7.67</td>
</tr>
</tbody>
</table>

* See the text for a complete description of dietary regimens.

† Standard error of the mean.

ever, decreased the activity somewhat (Group V), although it was still higher than the comparable controls (Group III).

**Oxidative Phosphorylation**—From the results in Table II, it can be seen
that deficiencies of EFA or vitamin B₆ alone significantly depressed phosphate esterification coupled either with DPNH or reduced cytochrome c oxidation. The combined deficiencies produced a greater depression in the P:O ratio for DPNH oxidation than either deficiency alone. It should be noted that all of these experiments were carried out with whole liver homogenates so that phosphatase activity would be considerably higher than if isolated mitochondria were used. Thus the P:O ratios, even for the control groups, were considerably lower than theoretical (2 for DPNH oxidation and 1 for reduced cytochrome c oxidation). However, the significance of the differences among the various groups can easily be observed. Especially noteworthy is the fact that phosphorylation coupled to reduced cytochrome c oxidation was almost completely removed by either the single or the combined deficiencies.

The single or combined deficiencies lowered the P:O ratios for both substrates by approximately the same amount; i.e., a decrease of 0.3 to 0.5. Because of the phosphatase activity of the systems, this decrease probably approaches a loss of 1 in the P:O ratio. Since the decrease in the P:O ratio associated with the single step oxidation (reduced cytochrome c → cytochrome c) approaches 1, while the P:O ratio for the two-step reaction (DPNH → DPN and reduced cytochrome c → cytochrome c) was also decreased by the same value, the effect of the EFA deficiency can probably be localized at the phosphate esterification coupled with the oxidation of reduced cytochrome c.

**DISCUSSION**

In these studies it has been confirmed that the oxidation of succinate, as measured by oxygen uptake, is not changed in EFA deficiency (5, 6). Similarly, the oxidation per se of DPNH is unchanged in EFA-deficient rat liver. An interesting problem is encountered, however, in that the titer of the antimycin A-sensitive factor and cytochrome oxidase activity are increased in EFA deficiency, although the actual oxidation of succinate or DPNH is unchanged. Thus the rate-limiting step, at least in succinate oxidation, probably lies at a point different from either AASF or cytochrome oxidase. With DPNH oxidation the explanation is more obscure, since the oxidation of DPN-linked substrates is only partially inhibited by antimycin A, depending on the type of tissue studied (19).

The fact that a vitamin B₆ deficiency accentuates the effects of an EFA deficiency is evident from the present experiments. It is interesting to note that the observed enzyme changes were not completely reversed, even when methyl linoleate was supplied in ample amounts to EFA-vitamin B₆-deficient rats (Group V). This suggests that vitamin B₆ is necessary for the utilization of linoleate, particularly in maintaining the phosphate esterification system associated with the oxidation of reduced cytochrome c.
ESSENTIAL FATTY ACIDS AND ENZYMES

SUMMARY

1. The effects of essential fatty acids (EFA) and vitamin B_6 deficiencies on certain rat liver enzymes have been studied. Vitamin B_6 appears to be necessary for the proper utilization of linoleate in maintaining the activity of those enzymes affected by EFA deficiency.

2. Dietary deficiency of EFA produces an increase in the titer of the antimycin A-sensitive factor and cytochrome oxidase of rat liver. Liver succinic oxidase is unaffected, however.

3. A marked decrease in the ratio of phosphate esterified to oxygen consumed was observed for the oxidation of both reduced DPN and reduced cytochrome c in EFA deficiency. Quantitative consideration of these studies indicates that the main site of action of EFA is the phosphate esterification system, coupled with the oxidation of reduced cytochrome c.

BIBLIOGRAPHY

STUDY OF THE ROLE OF ESSENTIAL FATTY ACIDS IN LIVER METABOLISM
P. G. Tulpule and J. N. Williams, Jr.


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