BIOTIN-LIKE ACTIVITY OF POSITIONAL AND STEREO-ISOMERS OF OCTADECENOIC ACIDS

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A number of microorganisms have been investigated which require some particular fatty acid in addition to biotin as a growth factor (1-6). On the other hand, a large group of organisms are able to utilize certain fatty acids in place of biotin in the medium (7-11). Axelrod and coworkers (12, 13) reported that the saponifiable fraction from blood plasma possesses high biotin activity. This extract was later shown (Axelrod et al. (14)) to be composed of oleic, linoleic, arachidonic, and saturated acids; the latter exhibited no biotin-like activity for microorganisms but greatly enhanced the response to the unsaturated fraction. Trager (15) found that this lipide fraction of plasma, when injected into chicks, reduces the severity of dermatitis caused by a biotin deficiency induced by feeding a diet high in raw egg white. This biotin-sparing activity of the plasma lipide could not be duplicated by the injection of oleic acid, nor could it be brought about by the oral administration of the fat-soluble material from plasma. Trager (16) later reported that the larvae of the mosquito, Aedes aegypti, require biotin in their medium; however, oleic acid and the lipides of plasma possess biotin-sparing activity for this organism. Several studies have demonstrated that simple replacement of biotin by fatty acids has not occurred, since powerful biotin inhibitors in the experiment reported have not prevented the growth of organisms in which biotin has been replaced by aspartic acid and certain lipides (17-19).

The present investigation was undertaken to determine the relative biotin-like activity of the positional isomers and stereoisomers of oleic acid

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as demonstrated by an effect on acid production by *Lactobacillus arabinosus*. Oleic acid isomers are known to be produced on hydrogenation of the vegetable oils and may, under certain conditions, be present in considerable amounts. Determinations of the biotin-like potency of several samples of mixed fatty acids from such hydrogenated fats and from limpid vegetable oils are also included.

EXPERIMENTAL

Procedure for Microbiological Assays—The biotin activity was determined by measuring the acid formation, by the use of *L. arabinosus* 8014 with a few modifications being employed (20). To prepare the medium, 7.8 gm. of a special biotin-free medium was dissolved in 88 ml. of distilled water, and 12 ml. of 10 per cent vitamin-free casein hydrolysate were added. In carrying out the assays, 5 ml. of the above medium were added to each culture tube and the final volume was adjusted to 10 ml. The stock culture of the organism was maintained on micro tomato agar (Difco) stabs. The inoculum was prepared by transferring the organisms from a 24 hour stab on tomato juice agar to a micro inoculum broth (Difco) and incubating at 30–32°. After 24 hours, the organisms were washed and suspended in normal saline.

The media with or without biotin were autoclaved at 15 pounds pressure for 15 minutes. After the tubes had cooled to room temperature, sterilized fatty acids alone or sterilized fatty acid biotin mixtures to be tested were added, aseptically dissolved in volumes ranging from 0.03 to 0.1 ml. of redistilled 95 per cent alcohol. Previous investigations (14, 21), as well as our own control tests, demonstrated that 0.1 ml. of alcohol per tube has no effect on the growth of our organism whether stimulated by biotin or by oleic acid.

The microbiological assays were performed by the usual multiple assay procedures. The assay was carried out over a range of growth-stimulating concentrations that gave approximately straight line curves when dosage of the test material was plotted against acid formation. The biotin-like activity of the various lipides was calculated from a standard curve which was simultaneously determined and expressed as millimicrograms of biotin.

1 American Type Culture Collection number.

2 H. M. Chemical Company, Ltd., Los Angeles, California. Each ml. had the following composition in mg. (includes added casein hydrolysate): glucose 49.6, sodium acetate 24.8, casein hydrolysate 12.4, L-cystine 0.24, DL-tryptophan 0.24, K$_2$HPO$_4$·3H$_2$O 1.24, KH$_2$PO$_4$ 1.24, MgSO$_4$·7H$_2$O 0.48, FeSO$_4$·7H$_2$O 0.024, MnSO$_4$·4H$_2$O 0.024, adenine sulfate 0.012, guanine hydrochloride 0.012, uracil 0.012, xanthine 0.012, thiamine hydrochloride 0.0024, niacin 0.0024, calcium pantothenate 0.0024, riboflavin 0.0024, pyridoxine hydrochloride 0.0046, p-aminobenzoic acid 0.00024.

3 Nutritional Biochemicals Corporation.
The dosages of the fatty acids used for each general study were 0, 12, 20, 32, 40, 50, 70, 100, 160, 200, 300, and 400 μg per tube. The straight part of the curve usually fell within the 12 to 70 μg level. The study of the effect of biotin on the activity of the fatty acid was accomplished by making concentrated solutions of the acid plus biotin and diluting so that for each level of acid there was a constant ratio of biotin to acid. The ratio was 1,000,000 parts of acid to 5 parts of biotin; thus, at 100 μg acid concentration, the amount of biotin was 0.5 mg/mm. The values reported are averages of the stimulating activities within the straight line part of each curve. More than one assay was carried out on different days for each acid tested, except for cis-8-octadecenoic acid with biotin, in which case only one multiple level assay was performed. As a final step, the tubes were inoculated with 1 drop of the saline suspension of the organism. Tubes were well mixed by shaking and were incubated at 30–32°C for 48 hours.

**Sterilization of Fatty Acids**—The fatty acids to be tested were sterilized by filtration through ultrafine sintered glass. In cases in which fatty acids were tested with biotin, the solution of the mixture of the two was sterilized by the same process. The glass filters were washed with the solvent until the original volume of solution was recovered.

**Sources and Properties of Fatty Acids**—Most of the samples of cis- and trans-octadecenoic acids were prepared by Dr. W. Frederick Huber of The Procter and Gamble Company. All the cis acids were synthetic, except for cis-6- and cis-9-octadecenoic acids, while all the trans acids were prepared from the corresponding cis compounds by selenium elaidinization. The method of synthesis and the physical properties of these acids have been reported by Huber (22). The samples of cis-9-octadecenoic acid (oleic) and cis-9,10-, cis-12,13-octadecadienoic acid (linoleic) were obtained from The Hormel Foundation.

The fatty acid mixtures, prepared from natural and hydrogenated fats, were furnished us by members of the Research Laboratories of The Best Foods, Inc. A comparison of the saponification and acid values of the original triglycerides and of the fatty acids prepared from them indicated that complete saponification had been effected.

**Results**

The biotin-like activities of various cis acids tested alone and with supplementary biotin are listed in Table I. Corresponding values for the trans acids are given in Table II.

No great differences in biotin-like activity were noted for any of the cis-octadecenoic acids tested, although the cis-8 acid possesses a statistically higher activity than any of the other acids. In confirmation of the
results of Axelrod et al. (14), our linoleic acid showed less response than did oleic acid and gave approximately the same potency reported by these earlier workers. The responses following the administration of biotin with the fatty acids are additive; that is, the biotin plus the fatty acid, at the various levels fed, was equivalent in activity to the sum of the activities

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>M.p. °C.</th>
<th>Biotin equivalent (mg/gm.) per mg. fatty acid</th>
<th>Biotin equivalent (mg/gm.) per mg. fatty acid when mixed with biotin</th>
</tr>
</thead>
<tbody>
<tr>
<td>cis-6-Octadecenoic acid</td>
<td>29.8*</td>
<td>11.2 (8); 11.8 (5); 11.9 (7)</td>
<td>13.2 (4); 11.6 (6)</td>
</tr>
<tr>
<td>cis-8-Octadecenoic acid</td>
<td>22.7-23.8*</td>
<td>15.3 (8); 14.1 (8)</td>
<td>14.7</td>
</tr>
<tr>
<td>cis-9-Octadecenoic acid</td>
<td>13.0</td>
<td>9.9 (7); 10.5 (6); 10.5 (7)</td>
<td>9.7 (8); 10.8 (4)</td>
</tr>
<tr>
<td>cis-11-Octadecenoic acid</td>
<td>13.0-14.0*</td>
<td>10.8 (6); 12.7 (5); 12.5 (6)</td>
<td>12.4 (6); 11.2 (5); 10.5 (7)</td>
</tr>
<tr>
<td>cis-12-Octadecenoic acid</td>
<td>26.8-27.6*</td>
<td>10.9 (5); 10.9 (7); 10.6 (7)</td>
<td>13.4 (7); 13.4 (7)</td>
</tr>
<tr>
<td>cis-9,10-, cis-12,13-Octadecadienoic acid</td>
<td>5</td>
<td>5.0† (8); 5.8† (8); 5.2† (8)</td>
<td>8.6 (7); 6.6 (8); 5.6 (8)</td>
</tr>
</tbody>
</table>

The figures in parentheses indicate the number of levels averaged for each multiple level assay.

* Data from Huber (22).
† Average of all values. The response, calculated to a common dilution, decreased uniformly from lowest to highest concentrations.

of the two supplements when administered separately. Some synergistic stimulation was observed with the cis-12-octadecenoic acid and biotin supplement, as well as with the linoleic acid and biotin supplement.

The trans-octadecenoic acids (Table II) reacted quite differently. The trans-9-octadecenoic acid (elaidic) possessed the greatest biotin-like effect (11.4 and 9.6 mg/gm. per mg of acid for two samples of independent origin). From position 9, at which the unsaturation is moving on either side toward the end carbon atoms, the biotin-like potency of the trans acids...
decreased in step-like fashion until no or very little measurable activity remained.

**Table II**

*BIOTIN-LIKE EFFECT OF TRANS-ODECENOIC ACIDS ALONE AND WITH BIOTIN; L. ARABINOSUS ASSAY*

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>M.p. °C.</th>
<th>Individual tests</th>
<th>Average</th>
<th>Individual tests</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>trans-6 Octadecenoic acid</td>
<td>53</td>
<td>0 (8); 0 (8); 0.7 (7); 0.9 (6)</td>
<td>0.4</td>
<td>3.2 (8); 4.2 (8)</td>
<td>3.7</td>
</tr>
<tr>
<td>trans-7-Octadecenoic acid</td>
<td>43.5-44.5</td>
<td>2.5 (8)<em>; 2.9 (7)</em></td>
<td>2.7</td>
<td>14.0 (6); 16.3 (5)</td>
<td>15.2</td>
</tr>
<tr>
<td>trans-8-Octadecenoic acid</td>
<td>51.5-52.3</td>
<td>2.9 (5)<em>; 2.8 (7)</em></td>
<td>2.8</td>
<td>8.9 (8); 9.3 (7)</td>
<td>9.1</td>
</tr>
<tr>
<td>trans-9-Octadecenoic acid</td>
<td>44.5-45.5</td>
<td>11.4 (5); 11.5 (7); 11.5 (5)</td>
<td>11.5</td>
<td>11.3 (7); 9.5 (6)</td>
<td>10.4</td>
</tr>
<tr>
<td>trans-9-Octadecenoic acid</td>
<td>41.5-42.5</td>
<td>9.4 (4); 9.7 (6)</td>
<td>9.6</td>
<td>9.6 (4); 10.0 (7)</td>
<td>9.8</td>
</tr>
<tr>
<td>trans-10-Octadecenoic acid</td>
<td>52.0-52.6</td>
<td>8.9 (7)<em>; 6.0 (8)</em>; 3.5 (8)*</td>
<td>6.1</td>
<td>11.6 (6); 10.2 (6); 11.2 (6)</td>
<td>11.0</td>
</tr>
<tr>
<td>trans-11-Octadecenoic acid</td>
<td>43.5-44.5</td>
<td>2.6 (8); 2.7 (8)</td>
<td>2.6</td>
<td>5.7 (7); 8.8 (7)</td>
<td>7.3</td>
</tr>
<tr>
<td>trans-11-Octadecenoic acid (vaccenic acid)†</td>
<td>39</td>
<td>1.7 (6); 1.3 (7)</td>
<td>1.5</td>
<td>6.6 (5); 7.8 (5)</td>
<td>7.2</td>
</tr>
<tr>
<td>trans-12-Octadecenoic acid</td>
<td>52.0-53.0</td>
<td>2.5 (8)<em>; 2.9 (7)</em></td>
<td>2.7</td>
<td>14.0 (6); 16.3 (5)</td>
<td>15.2</td>
</tr>
<tr>
<td>17-Octadecenoic acid</td>
<td>55.5-56.1</td>
<td>0 (8); 0 (8)</td>
<td>0.0</td>
<td>1.5 (8); 2.1 (8)</td>
<td>1.8</td>
</tr>
</tbody>
</table>

The figures in parentheses indicate the number of levels averaged for each multiple level assay.

* Average of all values. The response, calculated to a common dilution, decreased uniformly from lowest to highest concentrations.

† Samples from The Best Foods, Inc., prepared by hydrogenation of tung oil. The remaining samples were supplied by The Procter and Gamble Company. The melting points of the latter samples are those of Huber (22).

The almost equal biotin-like activities of elaidic and oleic acids are not in agreement with the results of Axelrod and coworkers (13, 14), who obtained a ratio of 5:1 in one case and 6:1 in the other for oleic over elaidic, using the same organism that we employed but with a somewhat different medium. Our tests were made on two samples of elaidic acid from two
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sources with approximately equal effects. The value for vaccenic acid (trans-11-octadecenoic) is in much closer agreement with the ratio of activity reported by Axelrod et al., 5:1.2 and 6:1.2 (13, 14), for oleic over vaccenic. Our ratio was 4:1. Trans-7-, -8-, -10-, and -12-octadecenoic acids showed progressively increasing "toxic" effects for the microorganism as the dosages increased; responses calculated to a common dilution decreased uniformly from lowest to highest concentrations of these trans acids. When biotin was given with these acids, the inhibitory effects of

![Diagram](http://www.jbc.org/)

**Fig. 1.** Biotin-like activity for cis-6- (left) and trans-11-octadecenoic acids (right). The solid curve represents the response for graded doses of biotin when added to the medium and incubated with *L. arabinosus* for 48 hours. ○, response to graded doses of fatty acids without biotin. ○, response to graded doses of fatty acids incubated simultaneously with graded doses of biotin at a constant ratio; however, the values plotted are for the fatty acids moiety after subtraction of the activity contributed by the biotin moiety as determined from the biotin curve.

higher concentrations of the acids were not apparent. Others (6, 8, 11) have pointed to the tendency of unsaturated fatty acids to display toxic effects at concentrations similar to those required for growth.

The administration of biotin concurrently with the trans-octadecenoic acids (except elaidic acid) and with 17-octadecenoic acid reveals a considerable degree of synergism. Maximum synergistic effects were noted with the trans-7 and trans-12 acids, both of which had exhibited, in the absence of biotin, inhibition of microbiological growth with increasing dosage. In fact, the most pronounced synergism between the trans acids and biotin occurred with those acids which are most "toxic" when employed in the absence of biotin.

The variations in response of the cis- and trans-octadecenoic acids, when
added with or without biotin, are graphically illustrated in Fig. 1. The
data presented for cis-6- and trans-11-octadecenoic acids are typical for

**TABLE III**

*Biotin-Like Effect of Fatty Acids Obtained from Various Natural and Hydrogenated Oils Alone and with Biotin, *L. arabinosus* Being Used*

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Biotin equivalent (mg/mg) per mg. fatty acid</th>
<th>Biotin equivalent (mg/mg) per mg. fatty acid when mixed with biotin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source</td>
<td>Iodine value</td>
<td>Individual tests</td>
</tr>
<tr>
<td>Hydrogenated coconut oil</td>
<td>0.3</td>
<td>0; 0</td>
</tr>
<tr>
<td>Coconut oil</td>
<td>9.3</td>
<td>1.8 (6); 2.1 (7)</td>
</tr>
<tr>
<td>Cottonseed oil (winterized)</td>
<td>115.4</td>
<td>10.1 (9); 9.1 (6); 8.5 (5); 9.1 (6)</td>
</tr>
<tr>
<td>Olive oil</td>
<td>85.6</td>
<td>12.4 (6); 12.7 (6); 10.9 (7)</td>
</tr>
<tr>
<td>Soy bean oil</td>
<td>138.5</td>
<td>7.7 (6); 7.8 (6); 7.7</td>
</tr>
<tr>
<td>Butter oil</td>
<td>40.0</td>
<td>8.7 (8); 8.7 (7); 10.0</td>
</tr>
<tr>
<td>Margarine oil*</td>
<td>76.4</td>
<td>11.0 (7); 12.7 (7)</td>
</tr>
<tr>
<td>Shortening A†</td>
<td>76.0</td>
<td>10.2 (7); 10.4 (4); 10.4 (6)</td>
</tr>
<tr>
<td>&quot; B†</td>
<td>76.0</td>
<td>12.9 (7); 10.8 (5); 10.6 (6); 12.0 (9)</td>
</tr>
<tr>
<td>&quot; C‡</td>
<td>62.8</td>
<td>8.2 (7); 8.2 (9); 8.1 (9)</td>
</tr>
<tr>
<td>&quot; D§</td>
<td>61.2</td>
<td>10.8 (8); 10.4 (6)</td>
</tr>
</tbody>
</table>

The figures in parentheses indicate the number of levels averaged for each multiple level assay.

* A blend of cottonseed and soy bean oil in about equal quantities; hydrogenated under selective conditions (25). Melting point of blend = 34.4°.
† Soy bean oil shortening; hydrogenated under non-selective conditions (25). M.p. = 42.2°.
‡ Cottonseed oil shortening; hydrogenated under non-selective conditions. M.p. = 42.8°.
§ Cottonseed oil shortening; hydrogenated under selective conditions. M.p. = 39.4°.

the other cis and trans acids, with the exception of trans-9-octadecenoic acid.

A study of the biotin-like activities of the fatty acids obtained from various natural and hydrogenated oils (Table III) does not reveal any appreciable differences among those obtained from butter, cottonseed, margarine, olive and soy bean oil, as well as from four shortenings. How-
ever, coconut oil had a very low activity, while saturated coconut oil possessed no potency. It is known that a reduction in biotin-like activity of the fatty acids accompanies increased saturation. Axelrod et al. (14) demonstrated reduced activity or complete inactivity with saturation of the double bond by hydrogenation such as we obtained with saturated

<table>
<thead>
<tr>
<th>Fatty acids from</th>
<th>Composition of the total mixed fatty acids* Spectrophotometric</th>
<th>Biotin equivalent of mixed fatty acids</th>
<th>Synergistic effect of saturated acids</th>
<th>Biotin equivalent of mixed fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saturated</td>
<td>Oleic</td>
<td>Linoleic</td>
<td>Found</td>
</tr>
<tr>
<td></td>
<td>per cent</td>
<td>per cent</td>
<td>per cent</td>
<td>per cent</td>
</tr>
<tr>
<td>Coconut oil ....</td>
<td>92</td>
<td>6</td>
<td>2</td>
<td>2.0</td>
</tr>
<tr>
<td>Cottonseed oil.</td>
<td>26</td>
<td>22</td>
<td>52</td>
<td>9.2</td>
</tr>
<tr>
<td>Olive oil .......</td>
<td>12</td>
<td>81</td>
<td>7</td>
<td>12.0</td>
</tr>
<tr>
<td>Soy bean oil ....</td>
<td>13</td>
<td>25</td>
<td>62</td>
<td>7.7</td>
</tr>
<tr>
<td>Butter fat ......</td>
<td>59</td>
<td>37</td>
<td>3</td>
<td>9.1</td>
</tr>
<tr>
<td>Margarine fat...</td>
<td>21</td>
<td>74</td>
<td>5</td>
<td>11.8</td>
</tr>
<tr>
<td>Shortening C</td>
<td>38</td>
<td>55</td>
<td>7</td>
<td>8.2</td>
</tr>
<tr>
<td>&quot;D ...</td>
<td>32</td>
<td>66</td>
<td>2</td>
<td>10.6</td>
</tr>
</tbody>
</table>

* Linolenic acid content of edible oils need not be considered in calculating the biotin equivalent of the mixed fatty acids derived from such oils. Not only is linolenic acid absent from most edible oils (viz., cottonseed, peanut, and corn), but also its biotin-like activity is relatively small, about one-fourth that of oleic acid (14). Furthermore, hydrogenated oils for margarine or shortening, even those made with soy bean oils, contain no linolenic acid.

† (d) = (e)/(c) × 100.

‡ Based upon the composition of the fatty acids with respect to the percentages of oleic, linoleic, and saturated fatty acids present. See the text for the formula.

§ (d) = (g)/(f) × 100.

|| Including 7 per cent of linolenic acid.

coconut oil. Biotin used in conjunction with the mixed fatty acids causes in all cases some reduction in activity of the fatty acids. The activity was completely abolished in the case of coconut oil.

In contrast to the effects of complete hydrogenation, partial hydrogenation of the vegetable oils results in no decrease in the biotin-like activity of the mixed fatty acids. This finding was unexpected, inasmuch as the iodine values of the mixed fatty acids of the hydrogenated vegetable oils were considerably less than those of the fatty acids from the limpid oils.
Further studies, however, have demonstrated that conversion during hydrogenation of linoleic to oleic acids, and the higher biotin-like activity of the latter plus augmentation of the microbiological response by the increase in saturated fatty acids, more than compensate for the decrease in unsaturation of the mixed fatty acids.

In Table IV are presented typical results obtained by spectrophotometric assays (23) of the oil samples following alkali isomerization to convert the non-conjugated fatty acids to their light-absorbing conjugated forms. Calculations of the oleic acid and saturated acid contents were made according to the method described by Beadle (24). Based upon a 10.3:5.3 ratio for the biotin-like activity of oleic versus linoleic acid (Table I), and assuming that the isooleic acids formed from linoleic acids are as active as oleic acid itself, calculations have been made of the anticipated biotin-like potencies of the oils. In every case the values found for biotin-like activity were definitely greater than those anticipated from the fatty acid composition. The percentage deviation of the found from the anticipated values in general increased as the mixed fatty acids contained more of the saturated fatty acids. Calculations indicated that on the average, for each per cent of saturated fatty acid, the biotin-like activity of the mixed fatty acids increased by 2.3 per cent over that anticipated from the type and amounts of unsaturated fatty acids present. This finding of an augmentation of the microbiological response by saturated fatty acids, which in themselves are inactive (viz., in assays of saturated coconut oil), is confirmatory of the findings by others (11, 14). The results of the present study go further in permitting calculation of the biotin-like activity of the fatty acids of limpid and hydrogenated oils from their fatty acid composition according to the following formula.

\[
\text{Biotin equivalent, as millimicrograms of biotin per mg. of fatty acids equals} \]

\[
\frac{\text{% oleic} \times 10.3 + \text{% linoleic} \times 5.3}{100} \times \left(1.00 + 2.3 \times \frac{\text{% saturated fatty acid}}{100}\right)
\]

The agreement between values thus calculated and those found is surprisingly good, considering the precision of the methods employed in determining the fatty acid composition of the samples and the accuracy of the microbiological assay for estimating biotin-like activity.

DISCUSSION

The biotin-like activity of fatty acids is associated not only with the degree of saturation of the acid, but also with the position of the double bond in the carbon chain. The stereochemical configuration assumed by the bond is likewise of prime importance. There is no correlation between
the biotin-like activities of the acids studied and the melting points. The increase in activity for all the trans acids tested, except for elaidic acid (trans-9-octadecenoic acid), when biotin was also added to the medium is surprising, especially since a greater effect was obtained for trans-7, -10, and -12 acids than was obtained for oleic acid with or without biotin present. We cannot rule out the possibility that the synergistic effects noted are due to enhanced activity of the biotin through the agency of the acids rather than the reverse. This increased activity for acids with double bonds remote from the 9 position casts some doubt on the theory that biotin is involved in oleic acid synthesis. Potter and Elvehjem (26) reported that biotin appears to cause synthesis of aspartic acid, and perhaps may be involved in that of oleic acid, but, if that is so, the mechanisms would probably be different. Williams et al. (8) suggest that biotin may be involved in the synthesis of oleic acid, since some Lactobacilli do not appear to require biotin when oleate and aspartate are present in the medium. Trager (15, 16) also suggested that biotin must be important in the synthesis of lipides. The evidence obtained in the present study, however, does not support the hypothesis that biotin is specifically concerned with oleic acid synthesis.

It would appear from the results obtained with the trans-octadecenoic acids with and without biotin that biotin may be involved either in isomerization of the trans acids or in the enhancement of the absorption of the acid by the organism.

From the results obtained in assays of the mixed fatty acids derived from hydrogenated vegetable oils, the conclusion is justified that the isooleic acids formed still exhibit a biotin-like effect, comparable to that of oleic acid itself. In further support of this conclusion are the findings tabulated in testing a considerable number of oleic acid isomers. Attention is directed particularly to the results obtained in assays of trans-9-octadecenoic (elaidic), cis-12-octadecenoic, and trans-12-octadecenoic acids, the more likely isoooleic acids formed as a result of hydrogenation of limpid vegetable oils. The first two isomers are fully as active as oleic acid, while the latter is only one-fourth as active. Moreover, there is no evidence that any acids possessing an antimetabolite function are formed during hydrogenation, nor do the mixed fatty acids derived from such fats show any tendency to inhibit growth of L. arabinosus at the dosage levels employed.

SUMMARY

A number of positional and stereoisomeric octadecenoic acids have been tested for biotin-like activity with Lactobacillus arabinosus. Strikingly different results were obtained for cis and for trans compounds, except for
elaidic and oleic acids in which the double bond is located at position 9. Whereas the cis-octadecenoic acids are more or less equally effective in promoting a biotin-like response, the trans acids, with the exception of elaidic acid, are less active. From position 9, at which the unsaturation is moving on either side toward the end carbon atoms, the biotin-like potency of the trans acids decreases in step-like fashion until no, or very little, measurable activity remains. Biotin exhibits synergistic effects with the trans-octadecenoic acids of limited activity. With the more active trans-9 acid (elaidic) and with the cis-octadecenoic acids tested, the responses following the dual administration of biotin and the fatty acids are additive. Oleic acid isomers formed by hydrogenation of vegetable oils still exhibit a biotin-like effect comparable to that of oleic acid itself. The isooleic acids are not antimetabolites. The conversion during hydrogenation of the linoleic acid in vegetable oils to oleic acids, and the higher biotin-like activity of the latter plus augmentation of the microbiological response by the increase in saturated fatty acids, more than compensate for the decrease in unsaturation of the mixed fatty acids. An equation has been given to permit calculation of the biotin-like activity of the fatty acids of limpid and hydrogenated oils from their fatty acid composition.

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