ERUCIC ACID AS THE FACTOR IN RAPE OIL AFFECTING ADRENAL CHOLESTEROL IN THE RAT

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In a previous communication it was reported that feeding rape oil to rats for a month as 25 per cent by weight of their diet caused a 3- to 4-fold increase in the amount of cholesterol in their adrenals (1). A similar effect has been obtained by feeding large amounts of turnip seed oil, nasturtium seed oil, or a weed seed oil obtained chiefly from species of Brassica. Other fats and oils have generally produced a definite, but much smaller, increase in adrenal cholesterol.

The oils which cause the greatest increases in adrenal cholesterol in the rat are characterized by their high content of erucic acid, and experiments described in the present paper on the fractionation of rape oil indicate that erucic acid is chiefly responsible for its effect on adrenal cholesterol. In a further series of experiments, a number of individual fatty acids or their esters have been fed to rats and observations made on changes in adrenal cholesterol.

EXPERIMENTAL

Fractionation of Rape Oil—Rape oil was saponified by refluxing 600 gm. of the oil with 480 cc. of aqueous potassium hydroxide (10 gm. per 20 cc.) and 480 cc. of alcohol. The mixture was then poured into 7 liters of water and the non-saponifiable material (4.2 gm.) was extracted with ether. The fatty acids were separated by acidification of the aqueous solution and converted to methyl esters by refluxing with 4 volumes of methyl alcohol containing 0.5 per cent of concentrated sulfuric acid. These were fractionated by distillation in a Todd column (2) at approximately 1 mm. pressure.

Methyl oleate and methyl erucate were prepared from the corresponding acids (Eastman Kodak) by the method described above and purified by fractionation in the Todd column. The methyl oleate boiled at 170–174° at 1 to 2 mm. pressure (iodine value 89.4, neutral equivalent 305), the

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† The author is indebted to Yocum Faust, Ltd., London, Ontario, for generous supplies of refined rape oil.
methyl erucate at 198–202° (iodine value 70.9, neutral equivalent 352). Pure erucic acid (m.p. 32–34°) was prepared by saponification of the methyl erucate, followed by recrystallization from alcohol.

The purified methyl erucate was also used as a starting point for the synthesis of nervonic acid. It was reduced to erucyl alcohol in 90 per cent yield with lithium aluminum hydride (3), and the remainder of the synthesis followed the method of Hale, Lycan, and Adams (4), with some modifications suggested by Müller (5).

The average over-all yield from methyl erucate was 40 per cent of theoretical. The crude nervonic acid was recrystallized once from 7.5 volumes of alcohol at -16° and the resulting mixture of cis and trans isomers melting at 40–50° was used in some of the feeding experiments. The mixture of isomers was easily separated by recrystallization from 20 volumes of alcohol, first at 0° at which most of the trans isomer and some of the cis isomer precipitated and then at -16°, when pure cis isomer (m.p. 39–41°, iodine value 68.4) was obtained. The yield of cis isomer was increased by repeating the recrystallization on the mixture obtained at 0°. Brassidic acid was obtained by treating erucic acid with sodium nitrite and nitric acid and purified by recrystallization from alcohol (6).

Young male rats of the Sprague-Dawley strain, weighing 80 to 90 gm., were used in the feeding tests. The individual fatty acids or rape oil fractions were intimately mixed with the regular diet of Masters' fox chow. In the experiments in which erucic and nervonic acids were fed (Table II), a synthetic diet of the following composition was used: casein, 20 per cent by calories; fatty acid, percentage by weight as indicated; salt mixture, 2.5 per cent by weight; glucose, to make 100 per cent. To these were added an adequate vitamin supplement and 5 gm. per 100 gm. of diet of Celluflour.

Results

As a result of careful studies in a number of laboratories (7–9), the chemical constitution of rape oil is quite well known. It contains less than 1 per cent of non-saponifiable matter and the major component fatty acids are oleic 16, linoleic 15, linolenic 9, eicosenoic 12, and erucic 40 per cent. In order to identify one or more of these components as the factor affecting adrenal cholesterol, various fractions of rape oil were fed to rats, with the results shown in Table I.

It is evident that the activity can be destroyed by hydrogenation and can be concentrated by fractional distillation into the higher boiling fatty acid fraction. The non-saponifiable fraction appears to be inactive, even

2 Salts 40 (Steenbock, H., and Nelson, E. M., J. Biol. Chem., 56, 355 (1923)).
when fed in a high fat diet. These facts suggested that either eicosenoic or erucic acid is responsible for the observed effect on adrenal cholesterol.

**Table I**

*Effect of Rape Oil Fractions on Rat Adrenal Cholesterol*

<table>
<thead>
<tr>
<th>Fraction No.</th>
<th>Iodine value</th>
<th>Per cent in diet</th>
<th>Days fed</th>
<th>No. of rats</th>
<th>Body weight (gm.)</th>
<th>Adrenal weight (mg.)</th>
<th>Total adrenal cholesterol (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. None</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Rape oil</td>
<td>103</td>
<td>25</td>
<td>28</td>
<td>24</td>
<td>189</td>
<td>157</td>
<td>30.2</td>
</tr>
<tr>
<td>3. Hydrogenated rape oil</td>
<td>50</td>
<td>25</td>
<td>28</td>
<td>6</td>
<td>189</td>
<td>157</td>
<td>28.9</td>
</tr>
<tr>
<td>4. Methyl esters of fatty acids (b.p. 154–175°)</td>
<td>129</td>
<td>15</td>
<td>21</td>
<td>3</td>
<td>170</td>
<td>157</td>
<td>17.9</td>
</tr>
<tr>
<td>5. Methyl esters (b.p. 175–194°)</td>
<td>80</td>
<td>15</td>
<td>21</td>
<td>3</td>
<td>173</td>
<td>157</td>
<td>5.42</td>
</tr>
<tr>
<td>6. Non-saponifiable fraction + 25% corn oil</td>
<td>0.4</td>
<td>26</td>
<td>5</td>
<td>174</td>
<td>29.7</td>
<td>2.38</td>
<td>2.38</td>
</tr>
<tr>
<td>7. 25% corn oil (control for (6))</td>
<td>26</td>
<td>5</td>
<td>179</td>
<td>27.2</td>
<td>2.28</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The hydrogenation was kindly performed by Procter and Gamble, Ltd., Hamilton, Ontario.

**Table II**

*Effect of Fatty Acids on Rat Adrenal Cholesterol*

All diets were fed for 21 days.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Per cent in diet</th>
<th>No. of rats</th>
<th>Rat weight (gm.)</th>
<th>Adrenal weight (mg.)</th>
<th>Total adrenal cholesterol (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oleic acid</td>
<td>20</td>
<td>6</td>
<td>178</td>
<td>26.2</td>
<td>2.30</td>
</tr>
<tr>
<td>Eicosenoic acid, methyl ester*</td>
<td>20</td>
<td>2</td>
<td>160</td>
<td>27.2</td>
<td>2.28</td>
</tr>
<tr>
<td>Erucic acid, methyl ester*</td>
<td>10</td>
<td>3</td>
<td>168</td>
<td>35.1</td>
<td>4.15</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2</td>
<td>193</td>
<td>35.4</td>
<td>1.61</td>
</tr>
<tr>
<td>Nervonic acid</td>
<td>15</td>
<td>2</td>
<td>170</td>
<td>38.1</td>
<td>4.65</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>2</td>
<td>162</td>
<td>34.2</td>
<td>3.08</td>
</tr>
<tr>
<td></td>
<td>10†</td>
<td>2</td>
<td>157</td>
<td>33.6</td>
<td>2.52</td>
</tr>
</tbody>
</table>

* The author is grateful to Dr. B. M. Craig, Prairie Regional Laboratory, National Research Council, Saskatoon, for the methyl esters of eicosenoic and erucic acids which were purified by distillation in a Podbielniak column (9).

† cis-Nervonic acid was used in this experiment.

and experiments with purified methyl esters of these acids showed that erucic acid is the active compound (Table II).

Methyl erucate purified by distillation in the Todd column contained
approximately 1 per cent of dienoic acid as measured by its absorption in the ultraviolet region after alkali isomerization (10), and it was necessary to eliminate the possibility that the dienoic acid was actually the active substance. This was accomplished by partial hydrogenation of the methyl erucate in which the diene content was reduced to 0.05 per cent without destroying the activity.

Feeding tests have also been carried out with a number of individual fatty acids, and in most cases only a small increase in adrenal cholesterol was observed. The results of feeding oleic acid are shown in Table II, and similar results were obtained with palmitic, stearic, and linoleic acids. Nervonic acid, a higher homologue of erucic acid, gave a response comparable to that of erucic acid and considerably greater than that of the C18 fatty acids.

**DISCUSSION**

The evidence that has been presented indicates that erucic acid is capable of producing marked increases in rat adrenal cholesterol, and this compound appears to be mainly responsible for the effect of rape oil on adrenal cholesterol. The commonly occurring fatty acids are much less effective than erucic, and this confirms previous results of feeding fats and oils containing major proportions of these acids (1). Further experiments on feeding oils such as tung oil, castor oil, and carrot seed oil suggest that the less common fatty acids, elaeostearic, ricinoleic, and petroselenic, likewise have little effect on adrenal cholesterol.

It is of interest to compare the relative activities of the monounsaturated acids homologous (Table III) with erucic acid. Oleic acid and eicosenoic acid, the C18 and C20 members of the series, cause small increases in adrenal cholesterol of similar magnitude, while the C22 member, erucic acid, is much more active, and the activity appears to be maintained in the C24 member, nervonic acid. Although no tests were made with pure samples of ximenic acid and lumequic acid, the C26 and C30 members of the same series, ximenia caffra oil, which contains 10 per cent of the former and 11 per cent of the latter, had very little effect on adrenal cholesterol. Direct comparison of the results obtained on the basis of amounts of the acids fed is not justified because absorption of fatty acids from the gut decreases with increasing chain length. When fed at the 10 per cent level in the diet, 85 per cent of oleic acid, 55 per cent of erucic acid, and only 25 per cent of nervonic acid

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3 The author is indebted to Dr. N. H. Grace and his associates at the National Research Council, Ottawa, for performing the hydrogenation and determining the diene content.

4 Personal communication from S. P. Ligthelm, University of Cape Town, South Africa. The author is indebted to Dr. Ligthelm for the ximenia caffra oil used in this experiment.
were absorbed (unpublished observations). Thus, the difference in activity between the C₁₈ and C₂₄ members of the series is perhaps greater than would appear from the results. The naturally occurring acids are in the cis form, and in a feeding test brassidic acid, the trans form of erucic acid, showed no evidence of activity, but brassidic acid is absorbed to a much smaller extent than erucic acid is. A test on a single rat with a purified sample of trans-nervonic acid also showed little activity.

The finding that nervonic acid causes a marked increase in adrenal cholesterol is particularly interesting, since this compound has been isolated from cerebrosides (11) and also from sphingomyelin of brain, though not from that of spleen and lung tissue (12). The possibility that nervonic acid may play a physiological rôle in the metabolism of cholesterol will be the subject of further investigation.

Adrenal enlargement with an increase in adrenal cholesterol has been reported recently following the administration of small amounts of a substituted desoxybenzoin, amphenone B (13). This compound also produced thyroid enlargement, an effect which was not observed in our experiments with fatty acids.

**TABLE III**

<table>
<thead>
<tr>
<th>Homologous Monounsaturated Fatty Acids</th>
<th>CH₃(CH₂)₇CH=CH(CH₃)₂COOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oleic acid</td>
<td>CH₃(CH₂)₇CH=CH(CH₃)₂COOH</td>
</tr>
<tr>
<td>Eicosenoic acid</td>
<td>CH₃(CH₂)₇CH=CH(CH₃)₂COOH</td>
</tr>
<tr>
<td>Erucic acid</td>
<td>CH₃(CH₂)₇CH=CH(CH₃)₂COOH</td>
</tr>
<tr>
<td>Nervonic acid</td>
<td>CH₃(CH₂)₇CH=CH(CH₃)₂COOH</td>
</tr>
<tr>
<td>Ximenic acid</td>
<td>CH₃(CH₂)₇CH=CH(CH₃)₂COOH</td>
</tr>
<tr>
<td>Lumequic acid</td>
<td>CH₃(CH₂)₇CH=CH(CH₃)₂COOH</td>
</tr>
</tbody>
</table>

The author wishes to thank Dr. R. L. Noble and Dr. J. B. Collip for the advice and the interest they have shown in this work. He is also indebted to Miss Carolyn Drulard, Mrs. J. Arnold, Mr. E. Andersen, and Mr. G. M Carpenter for valuable technical assistance.

**SUMMARY**

The increase in adrenal cholesterol resulting from feeding rape oil to rats has been shown to be produced by erucic acid. Among other fatty acids tested, only nervonic acid, a component of cerebrosides and sphingomyelin, gave a response comparable to that of erucic acid.

The author wishes to thank Dr. R. L. Noble and Dr. J. B. Collip for the advice and the interest they have shown in this work. He is also indebted to Miss Carolyn Drulard, Mrs. J. Arnold, Mr. E. Andersen, and Mr. G. M Carpenter for valuable technical assistance.

**BIBLIOGRAPHY**

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