On the Protection against Infarction by Corn Oil

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Knowledge that corn oil affords protection against infarction in rats that are maintained on an experimental diet has led to new studies on the composition of corn oil. Fresh and unsaponified corn oil has been used to avoid possible losses of constituents. Four previously unrecognized tocopherol-like compounds have been discovered. Biosynthetic precursors of the tocopherols were not found but are not necessarily absent. The presence of large amounts of sterol esters of ferulic acid was observed. The content of ubiquinone-9 in fresh corn oil is comparable to that of commercial oil. Hexahydroubiquinone-4 is stable in commercial oil for up to seven months.

Existing and new knowledge on the constituents of corn oil may contribute towards elucidation of the protection which this oil affords rats against infarction.

The therapy of heart disease is one of the most challenging tasks of biomedical research. Studies over recent years have emphasized the connection between heart disease and certain types of diets. Investigations\(^1\) have revealed positive correlations between the cardiovascular death rate and the intake of total calories and animal fat, and negative correlations between this death rate and the intake of vegetable fat.

Cardiovascular and renal diseases are the number-one cause of death in countries with a high standard of living.\(^2\) Experimentally produced arterial lesions in laboratory animals, similar to human arteriosclerosis, were first described\(^3\) in 1913, and atheromatous-like lesions were produced in rabbits by feeding a cholesterol-rich diet. Many investigators over the following years confirmed such lesions, but only very few\(^4,5\) reported occlusive arterial thrombosis and infarction of heart, kidneys, or other organs.

Since 1957, Hartroft, Thomas, and O'Neal\(^6-11\) have reported continuing studies of a practical dietary method for producing thrombosis and myocardial infarction in rats. They postulated that the various atherogenic diets previ-
ously used, all with high cholesterol content and comparatively little fat, favored the production of lesions of the arterial wall, but did not have any effect on the thrombogenesis and thrombolysis of the blood. They, therefore, combined a previously used atherogenic diet with 40% saturated fat, and with this new diet they were able to develop myocardial infarction regularly in 25—35% of the rats. When corn oil instead of saturated fat was used in the diet, the rats were completely protected against infarction. The biochemical basis for the protective effect of corn oil against infarction has not been established, and this is the objective of our study.

Fats from various natural sources differ considerably in their fatty acid and glyceride composition. Usually, aquatic organisms have a more complicated fat mixture than terrestrial organisms. Animal fat of aquatic origin contains a wide range of fatty acids mainly of the unsaturated series. The fat from higher land animals contains both unsaturated and saturated fatty acids; the main unsaturated fatty acid is almost always oleic acid, and the main saturated acid is palmitic acid. Vegetable fats generally contain the same fatty acids as animal fats. Palmitic and oleic acids are dominant in the fats of land plants together with considerable amounts of linoleic acid. This latter acid is present in only minute quantities in animal fat.

The fatty acid composition of corn oil differs depending on where the corn has been grown. Generally, the oil is very rich in unsaturated fatty acids. Oil from corn grown in the U.S.A. is reported to contain 10—15% saturated fatty acids (palmitic and stearic acid), 20—30% oleic acid, and 50—60% linoleic acid. The iodine value is reported to be about 125 compared to about 143 for safflower seed oil, which has one of the highest known content of unsaturated fat. The glycerides of corn oil were investigated by their segregation into 19 glyceride fractions by crystallization from acetone. It was found that di- and trilinoleoglycerides constitute 84% of the total fat.

Certain unsaturated fatty acids like linoleic acid are essential to animals. Young rats that are given saturated fat as the only dietary fat source developed skin lesions, ceased to grow, and had reduced caloric efficiencies. Human infants on a low-fat diet developed similar symptoms, which were alleviated when the diet was augmented with linoleic acid. There is evidence that the function of linoleic acid is to act as a precursor for other unsaturated fatty acids and that certain tetra- and hexenoic acids promote the growth of rats better than linoleic acid.

Corn oil is reported to contain 0.3—0.7 mg of tocopherol/g of oil. An indication that the tocopherol content of corn oil may possibly be a contributing factor in the protection of rats against infarction was reported by Chalvardjian and Hartroft but the significance of tocopherol is inconclusive.

They reported that “a lower incidence of thrombi and infarcts in rats fed tocopherol plus lard than in rats fed lard without tocopherol fell just short of statistical significance.” The production of thrombi and infarcts with a diet containing 40% stripped corn oil was considered “of considerable importance.” Further, “the incidence of both cardiac thrombi and infarcts in E-deficient rats given lard was significantly higher than in those fed (stripped) corn oil.”

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Some data are available concerning the effects of corn oil on thrombosis in humans. Hansen et al.\textsuperscript{27} reported that a group of elderly patients fed one-half of their intake of fat as corn oil or soybean oil had statistically fewer cases of thrombosis than a control group receiving an ordinary hospital diet. Apparently, the patients have to be on this diet for a period longer than one month to be protected against thrombosis. There are other studies on vegetable oils for treatment of human diseases. Milhorat et al.\textsuperscript{28} reported that when fresh wheat germ was given to patients suffering from dermatomyositis and progressive muscular dystrophy, the creatininuria was reduced. Corn oil may contain the same constituents as wheat germ, and similar activity may therefore be expected.

Knowledge is incomplete yet about the biosynthetic precursors of the tocopherols which might also be present in corn oil; such knowledge might be pertinent to this study of the protection against infarction. The biosynthesis of tocopherol is currently attracting considerable interest.\textsuperscript{29–35} Pennock et al.\textsuperscript{30,31} have suggested that \(\delta\)-tocotrienol (I) could be a precursor to \(\alpha\)-tocopherol (II) by hydrogenation to \(\delta\)-tocopherol or be methylated to \(\beta\)-, \(\gamma\)-, or \(\alpha\)-tocotrienol and then hydrogenated to the tocopherols. All the four tocotrienols and the four tocopherols have been found in palm oil\textsuperscript{30} and in \textit{Hevea} latex\textsuperscript{32} which suggests that there could be a biosynthetic pathway to \(\alpha\)-tocopherol (II) through \(\delta\)-, \(\gamma\)-, and \(\alpha\)-tocotrienol. They also found\textsuperscript{31} that (Me-\textsuperscript{14}C)-methionine labelled \(\alpha\)-, \(\beta\)-, and \(\gamma\)-tocotrienol but not \(\delta\)-tocotrienol. This supports the suggested pathway from \(\delta\)- to \(\alpha\)-tocotrienol. Whistance et al.\textsuperscript{33} have reported that maize shoots incorporate \textsuperscript{14}C-labelled skimmic acid into vitamin K\textsubscript{1}, \(\alpha\)-tocopherylquinone, 2-nonaprenylphenol, plastoquinone, ubiquinone (Q), \(\alpha\)- and \(\gamma\)-tocopherol. In a similar experiment using \(p\)-hydroxybenzoic acid-U-\textsuperscript{14}C, only Q was labelled. The side chain of the tocopherols is presumed to arise from mevalonic acid\textsuperscript{29,34} although Threlfall and Goodwin\textsuperscript{35} failed to demonstrate its radioactive incorporation into any of the terpenoids in \textit{Euglena gracilis}.

2-Decaprenylphenol is an intermediate in the biosynthetic sequence from \(p\)-hydroxybenzoic acid (HBA) to Q.\textsuperscript{36} If HBA is a precursor to tocopherol, as supported by the observation that tocopherol is labelled by radioactive skimmic acid, it is reasonable to assume that the first step could be a phytlation of HBA (III) similar to the first step in the biosynthetic sequence from

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Volume (liters)</th>
<th>Compounds isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane</td>
<td>1</td>
<td>Dimers of tocopherols,\textsuperscript{44} (\alpha)-Tocopherol</td>
</tr>
<tr>
<td>3 % Ether in hexane</td>
<td>11</td>
<td>Ubiquinone-9</td>
</tr>
<tr>
<td>5 % Ether in hexane</td>
<td>4</td>
<td>Fatty acid esters</td>
</tr>
<tr>
<td>10 % Ether in hexane</td>
<td>4</td>
<td>Pericolic esters of sterols</td>
</tr>
<tr>
<td>25 % Ether in hexane</td>
<td>6</td>
<td>Orange colored material</td>
</tr>
<tr>
<td>50 % Ether in hexane</td>
<td>4</td>
<td>Orange colored material</td>
</tr>
<tr>
<td>Ether</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

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HBA to Q, and 2-phytylphenol (V) could result from decarboxylation of IV. 2-Tetraprenylphenol (VI) has been isolated from \textit{R. rubrum}. Although any supposed 2-tetraprenyl precursor of a tocopherol such as VI could be biologically reduced to its corresponding phytol derivative (V), it has seemed possible that the phytol side chain in the precursors of the tocopherols might be introduced directly, since it is known that phytol exists in plant tissue. The conversion of 2-phytylphenol (V) into $\alpha$-tocopherol (II) involves only subsequent reactions which are identical, in principle, to those already elucidated for the biosynthesis of ubiquinone from 2-multiprenylphenol.\textsuperscript{36}

Corn oil is one of the richest sources of ubiquinone known.\textsuperscript{39} We have found the content of Q-9 in fresh corn oil to be comparable to that reported for industrial Mazola. The stability of hexahydrobiocinone-4 in Mazola has been determined since this oil has been used as a vehicle in the administration of hexahydrobiocinone-4 to children having an anemia of protein-calorie malnutrition.\textsuperscript{2,40}

Fresh and unsaponified corn oil was used in our experimental studies, because of the possible losses of constituents that might occur in the commercial production of the oil, or by procedures including saponification. Our studies have led to the discovery of four tocopherol-like compounds, (Table 2) and to the recognition of the presence of large amounts of sterol esters of ferulic

### Table 2. Properties of isolated tocopherol-like compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chromatography Phenol Color Reactions</th>
<th>UV $\lambda _{\text{max}}$ (m$\mu$)</th>
<th>$\delta_{\text{CCL}_4}$ (cm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R_F$ $^a$</td>
<td>DSA $^b$</td>
<td>Gibb $^c$</td>
</tr>
<tr>
<td>I</td>
<td>0.80</td>
<td>red</td>
<td>black</td>
</tr>
<tr>
<td>II</td>
<td>0.67</td>
<td>no reaction</td>
<td>black</td>
</tr>
<tr>
<td>III $^e$</td>
<td>0.67</td>
<td>red</td>
<td>black</td>
</tr>
<tr>
<td>IV</td>
<td>0.60</td>
<td>red</td>
<td>black</td>
</tr>
<tr>
<td>$\gamma$-Tocopherol</td>
<td>0.30</td>
<td>red</td>
<td>black</td>
</tr>
</tbody>
</table>

$^a$ On Silica gel developed in ether:hexane (1:9).

$^b$ Diazotized sulphanilic acid.

$^c$ See Ref. 41.

$^d$ See Ref. 42.

$^e$ Has unsaturated side chain.

$^l$ (sh) = shoulder.
acid (VII) in corn oil. Existing and new knowledge about the constituents of corn oil may be oriented towards an elucidation of the biochemical mechanism of the protection which corn oil affords rats against infarction.

**MATERIALS AND METHODS**

*General comments.* The ultraviolet absorption spectra were measured with a Cary Model 14 M spectrophotometer, and the infrared absorption spectra were measured with a Beckman-IR5A spectrophotometer. Thin layer chromatography was performed using silica gel G plates of 0.3 or 1 mm thickness. The plates were activated at 130° for 1.5 h and were stored in a dry atmosphere until used. Freshly distilled hexane was used throughout.

*Preparation of fresh corn oil.* Twelve kg fresh corn was ground to a flour and then extracted with redistilled hexane in a Soxhlet apparatus. After evaporation of the solvent, the residual turbid, orange-colored oil weighed 378 g (3.15%).

*Chromatography.* To isolate phenolic and other acidic substances, the oil was dissolved in 800 ml of hexane and placed on a column of 500 g of aluminum oxide (Alumina, Merck 71707). The column was first eluted with 1500 ml of hexane to remove neutral fat and then with 3000 ml of ether/methanol (3:1). After evaporation, the second eluate gave 30 g of a dark red oil. This was chromatographed on a column of 800 g of silica gel, eluted with ether/hexane mixtures of increasing polarity, and the eluate was collected in 1-liter fractions. Each fraction was scanned for phenols by spraying a thin layer chromatogram with diazotized sulfanilic acid, Gibb's reagent, or Emmerie-Engel reagent. The fractionation and its results are summarized in Table 1.

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Search for and recovery of 2-phytylphenol. Ten g of fresh corn oil and 10 mg of synthetic 2-phytylphenol were dissolved in 100 ml of hexane and the mixture was placed on a column of 15 g of aluminium oxide (Alumina, Merck 71707). The column was first eluted with 100 ml of hexane and then with 100 ml of ether/methanol (3:1). The ether/methanol eluate was evaporated and chromatographed on a 1 mm thick silica gel G plate in ether/hexane (1:9) with synthetic 2-phytylphenol as reference. The area corresponding to the RF of 2-phytylphenol was removed from the plate and eluted with ether. Ultraviolet measurement of an aliquot of this preparation at 273 μm revealed that 90% of the added 2-phytylphenol was recovered from the oil. This showed that phenolic compounds could be isolated from the corn oil by this procedure. When the main portion of corn oil was fractionated in the same way, no phytylphenol or other related alkylphenols were detected.

Isolation of sterol ferulates. From fractions eluted from the silica gel column with ether/hexane (1:3), approximately 10 g of white crystalline material was isolated. A thin layer chromatogram of the product, developed in chloroform, showed one phenolic spot at RF 0.6 (blue color with Gibb's reagent; dark red with diazonized sulfanilic acid; positive Emmerie-Engel reaction). After recrystallization from hexane it melted at 153–155°C and exhibited λ\text{max} ^\text{hexane} 231, 280 (sh), 290, and 314 μm; λ\text{RHO} ^\text{max} 235, 298 (sh), and 324 μm; and ε\text{CHCl}_{3} ^\text{max} 3500 cm\text{ }^{-1} (OH), 1690 cm\text{ }^{-1} (unsaturated acid ester carbonyl), and 1630 cm\text{ }^{-1} (conjugated –C=С–). These data are in agreement with those reported for dihydro-γ-sitosterylferulate isolated from wheat germ oil, which showed m.p. 155–156°C; UV: λ\text{max} ^\text{hexane} 231, 275–80 (sh), 290, 314.5 μm; and λ\text{RHO} ^\text{max} 235, 298–9 (sh), and 324 μm.

Determination of CoQ in corn oil. A mixture of 10.0 g of corn oil, 5 g of pyrogallol, and 25 ml of 50% potassium hydroxide solution dissolved in 300 ml of 95% ethanol was refluxed for 30 min and then cooled by adding 300 g of crushed ice. The dark brown solution was then extracted with three 300 ml portions of hexane. The hexane extract was washed with three 250 ml portions of water and then evaporated. The residue was chromatographed on a 1 mm thick silica gel G plate in ether/hexane (2:3) with CoQ_{10} as reference. The area corresponding to the RF of CoQ_{10} was removed from the plate and eluted with ether. The content of CoQ was determined by a modified Craven’s assay on an aliquot of this eluate.

Determination of the stability of hexahydrocoenzyme Q_{1} in corn oil. Hexahydrocoenzyme Q_{1} (H_{6}CoQ_{1}) was dissolved in corn oil (Mazola) to give solutions of different concentrations. The solutions were analyzed as described above for H_{6}CoQ_{1} on the first day and then again after storage in the dark at 4°C for different periods of time. The values are presented in Table 3. No decrease in concentration of H_{6}CoQ_{1} was found and the variation of the values are within the limits of error reported for this method.

RESULTS AND DISCUSSION

Many compounds with established or potential physiological activity have been isolated from corn oil (Table 4). Hartroft and his colleagues have reported that corn oil can protect laboratory animals fed a thrombogenic

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Concentration found first day (mg/ml)</th>
<th>Time of storage (months)</th>
<th>Concentration found after storage (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>4.5</td>
<td>27</td>
</tr>
<tr>
<td>2</td>
<td>27</td>
<td>7</td>
<td>27</td>
</tr>
<tr>
<td>3</td>
<td>110</td>
<td>6.5</td>
<td>98</td>
</tr>
<tr>
<td>4</td>
<td>270</td>
<td>6.5</td>
<td>275</td>
</tr>
</tbody>
</table>

Table 3. Stability of hexahydrocoenzyme Q_{1} in corn oil.

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Table 4. Compounds, other than fatty acid esters, present in corn oil.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Amount found</th>
<th>Amount reported</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A</td>
<td>—</td>
<td>Not reported</td>
<td>53, 54</td>
</tr>
<tr>
<td>Bixin</td>
<td>—</td>
<td>Not reported</td>
<td>55</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>—</td>
<td>Not reported</td>
<td>53</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>0.45 mg/g</td>
<td>0.3—0.7 mg/g</td>
<td>25</td>
</tr>
<tr>
<td>Sterols</td>
<td>—</td>
<td>Not reported</td>
<td>47</td>
</tr>
<tr>
<td>Squalene</td>
<td>—</td>
<td>0.16—0.42 mg/g</td>
<td>56</td>
</tr>
<tr>
<td>Sterol ferulates</td>
<td>25 mg/g</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Ubiquinone</td>
<td>0.185 mg/g</td>
<td>0.12—0.21 mg/g</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>0.285 mg/g</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Mazola)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Diet against thrombosis and heart infarction. It is not known whether this prophylactic activity is exerted by one or more of the compounds listed in Table 1 or by some other component.

Four tocopherol-like compounds with approximately the same polarity as synthetic 2-phytylphenol were isolated. The structure of these compounds will be subsequently described. In the fractionation of phenolic and acidic components we were unable to demonstrate the presence of 2-phytylphenol or any other phenolic compounds that could be considered apparent precursors of tocopherol. A fractionation procedure for 2-phytylphenol was used which permitted recovery of 90% of the added synthetic reference compound.

Of interest is the recognition of large amounts of sterol esters of ferulic acid VII (Table 1). Eisner and Firestone reported that β- and γ-sitosterol and stigmasterol are present in corn oil in the ratio 89:10:1. They also found two more unidentified sterols in trace quantities. The sterol ferulate had a melting point and ultraviolet absorption spectrum which were in agreement with those properties reported for dihydro-γ-sitosterylferulate. It is likely that the isolated white crystalline material consists of several very similar sterol ferulates, since five different sterols have been found in corn oil. Ferulic acid is widely distributed in plants, and it has been found to be a growth factor for certain fungi. Some reports in the literature indicate that the hypercholesterolemic activity of corn oil is associated with sterols, but other workers have been unable to demonstrate this effect.

Corn oil was found to be a usable vehicle for pharmaceutical preparations of hexahydrocoenzyme Q₄. Such formulations have been used in the first clinical evaluation of hexahydrocoenzyme Q₄ which was the positive hematological response of children with protein-calorie malnutrition and macrocytic anemia. These oil solutions were stable for at least seven months.

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