EVALUATION OF THE HEALTH ASPECTS OF HYDROGENATED SOYBEAN OIL AS A FOOD INGREDIENT

1976

Prepared for

Bureau of Foods
Food and Drug Administration
Department of Health, Education, and Welfare
Washington, D.C.

Contract No. FDA 223-75-2004
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Life Sciences Research Office
Federation of American Societies
for Experimental Biology
9650 Rockville Pike
Bethesda, Md. 20014
NOTICE

This report is one of a series concerning the health aspects of using the Generally Recognized as Safe (GRAS) or prior sanctioned food substances as food ingredients, being made by the Federation of American Societies for Experimental Biology (FASEB) under contract no. 223-75-2004 with the Food and Drug Administration (FDA), U. S. Department of Health, Education, and Welfare. The Federation recognizes that the safety of GRAS substances is of national significance, and that its resources are particularly suited to marshalling the opinions of knowledgeable scientists to assist in these evaluations. The Life Sciences Research Office (LSRO), established by FASEB in 1962 to make scientific assessments in the biomedical sciences, is conducting these studies.

Qualified scientists were selected as consultants to review and evaluate the available information on each of the GRAS substances. These scientists, designated the Select Committee on GRAS Substances, were chosen for their experience and judgment with due consideration for balance and breadth in the appropriate professional disciplines. The Select Committee's evaluations are being made independently of FDA or any other group, governmental or nongovernmental. The Select Committee accepts responsibility for the content of each report. Members of the Select Committee who have contributed to this report are named in Section VII.

Tentative reports are made available to the public for review in the Office of the Hearing Clerk, Food and Drug Administration, after announcement in the Federal Register, and opportunity is provided for any interested person to appear before the Select Committee at a public hearing to make oral presentation of data, information, and views on the substances covered by the report. The data, information, and views presented at the hearing are considered by the Select Committee in reaching its final conclusions. Reports are approved by the Select Committee and the Director of LSRO, and subsequently reviewed and approved by the LSRO Advisory Committee (which consists of representatives of each constituent society of FASEB) under authority delegated by the Executive Committee of the Federation Board. Upon completion of these review procedures the reports are approved and transmitted to FDA by the Executive Director of FASEB.

While this is a report of the Federation of American Societies for Experimental Biology, it does not necessarily reflect the opinion of all of the individual members of its constituent societies.

C. Jelleff Carr
Ph. D., Director
Life Sciences Research Office
FASEB
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I. INTRODUCTION

This report concerns the health aspects of using hydrogenated soybean oil as a food ingredient. It has been based partly on the information contained in a scientific literature review (monograph) furnished by FDA (1), which summarizes the world's scientific literature from 1920 through 1973.* To assure completeness and currency as of the date of this report this information has been supplemented by searches of over 30 scientific and statistical reference sources and compendia that are generally available; use of new, relevant books and reviews and the literature citations contained in them; consideration of current literature citations obtained through computer retrieval systems of the National Library of Medicine; searches for relevant data in the files of FDA; and by the combined knowledge and experience of members of the Select Committee and the LSRO staff. In addition, an announcement was made in the Federal Register of January 28, 1977 (42 FR 5425 and 5426) that opportunity would be provided for any interested person to appear before the Select Committee at a public hearing to make oral presentation of data, information and views on the health aspects of using hydrogenated soybean oil as a food ingredient. The Select Committee received no requests for such a hearing on hydrogenated soybean oil.

As indicated in the Food, Drug, and Cosmetic Act [21 USC 321 (s)], GRAS substances are exempt from the premarking clearance that is required for food additives. It is stated in the Federal Regulations (2) [21 CFR 170.3] that GRAS means general recognition of safety by experts qualified by scientific training and experience to evaluate the safety of substances on the basis of scientific data derived from published literature. This section of the Regulations also indicates that expert judgment is to be based on the evaluation of results of credible toxicological testing or, for those substances used in food prior to January 1, 1958, on a reasoned judgment founded in experience with common food use, and is to take into account reasonably anticipated patterns of consumption, cumulative effects in the diet, and safety factors appropriate for the utilization of animal experimentation data. FDA recognizes further [21 CFR 170.30] that it is impossible to provide assurance that any substance is absolutely safe for human consumption.

*The document (PB-228 557/5) is available from the National Technical Information Service, U.S. Department of Commerce, P.O. Box 1553, Springfield, Virginia 22161.
The Select Committee on GRAS Substances of LSRO is making its evaluations of these substances in full recognition of the foregoing provisions. In reaching its conclusions on safety the Select Committee, in accordance with FDA's guidelines, is relying primarily on the absence of substantive evidence of, or reasonable grounds to suspect, a significant risk to the public health. While the Select Committee realizes that a conclusion based on such reasoned judgment is expected even in instances where the available information is qualitatively or quantitatively limited, it recognizes that there can be instances where, in the judgment of the Select Committee, there are insufficient data upon which to base a conclusion. The Select Committee, aware that biological testing is dynamic, bases its conclusions on information now available; it cannot anticipate the results of experiments not yet conducted or those of tests that may be reconducted, using new technologies. These conclusions will need to be reviewed as new or better information becomes available.

In this context, the LSRO Select Committee on GRAS Substances has reviewed the available information on hydrogenated soybean oil and submits its interpretation and assessment in this report, which is intended for the use of FDA in determining the future status of this substance under the Federal Food, Drug, and Cosmetic Act.

II. BACKGROUND INFORMATION

Hydrogenated soybean oil is generally recognized as safe (GRAS) under the provisions of the Federal Regulations (2) as a substance migrating to food from cotton and cotton fabrics used in dry food packaging [21 CFR 182.70]. Hydrogenated soybean oil also has unpublished approval as GRAS as a direct food ingredient (3) and this is its major use in the United States. Title 21 of the Federal Regulations permits the use of edible vegetable oils, and hence hydrogenated soybean oils, in "Food Dressings and Flavorings" under Part 169, and in "Margarine," under Part 166. This report concerns the evaluation of the health aspects of hydrogenated soybean oil in these indirect and direct food applications.

Soybean oil is obtained from the seeds of the soybean or soya bean, Glycine max. (L) Merrill. Most of the soybeans processed in the United States are extracted with hexane, a mixture of petroleum hydrocarbons, to remove the oil; some continuous screw press operations are used to express the oil and produce special meal products (4). The composition of soybean oil varies according to climate and other conditions of growth; the range in fatty acid content reported for the fresh oil is given in Table I (5). The fatty acids are present mostly as triglycerides; more than half of them are polyunsaturated, 85 percent of which are diene. As extracted from the bean, soybean oil
<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Chemical formula</th>
<th>Percent of total fatty acids&lt;sup&gt;a&lt;/sup&gt;</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Average</td>
<td>Range</td>
</tr>
<tr>
<td>Saturated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myristic</td>
<td>C&lt;sub&gt;14&lt;/sub&gt;H&lt;sub&gt;28&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0.2</td>
<td>0.1-0.3&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Palmitic</td>
<td>C&lt;sub&gt;16&lt;/sub&gt;H&lt;sub&gt;32&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>10.6</td>
<td>9.0-11.6</td>
</tr>
<tr>
<td>Stearic</td>
<td>C&lt;sub&gt;18&lt;/sub&gt;H&lt;sub&gt;36&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>4.1</td>
<td>3.0-5.0</td>
</tr>
<tr>
<td>Arachidic</td>
<td>C&lt;sub&gt;24&lt;/sub&gt;H&lt;sub&gt;48&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0.3</td>
<td>0.1-0.5&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Behenic</td>
<td>C&lt;sub&gt;22&lt;/sub&gt;H&lt;sub&gt;44&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0.3</td>
<td>0.2-0.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Unsaturated-monoenoic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palmitoleic</td>
<td>C&lt;sub&gt;16&lt;/sub&gt;H&lt;sub&gt;30&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0.2</td>
<td>0.2-0.3&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Oleic</td>
<td>C&lt;sub&gt;18&lt;/sub&gt;H&lt;sub&gt;34&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>24.5</td>
<td>20.7-29.0</td>
</tr>
<tr>
<td>Eicosenoic</td>
<td>C&lt;sub&gt;20&lt;/sub&gt;H&lt;sub&gt;36&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0.3</td>
<td>0.1-0.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Unsaturated-dienoic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linoleic</td>
<td>C&lt;sub&gt;18&lt;/sub&gt;H&lt;sub&gt;32&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>53.2</td>
<td>50.3-55.9</td>
</tr>
<tr>
<td>Unsaturated-trienoic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linolenic</td>
<td>C&lt;sub&gt;18&lt;/sub&gt;H&lt;sub&gt;30&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>7.4</td>
<td>5.0-9.6</td>
</tr>
</tbody>
</table>

<sup>a</sup>Average and range of values reported for 22 samples except as noted in<sup>b</sup>, <sup>c</sup>, and<sup>d</sup>.

<sup>b</sup>Average and range for the 5 samples for which the fatty acid was reported.

<sup>c</sup>Average and range for the 7 samples for which the fatty acid was reported.

<sup>d</sup>Average and range for the 8 samples for which the fatty acid was reported.
contains small percentages (1.5 to 2.5) of nonglyceridic substances commonly removed in subsequent processing. Included are phosphatides, carbohydrates and carbohydrate derivatives, protein fragments, sterols and tocopherols. Processing of soybean oil consists of a series of unit operations: degumming, alkali refining, bleaching and deodorization. The result of these operations is a product which is nearly all triglycerides (6).

In the hydrogenation of soybean oil, hydrogen added at the double bonds of the unsaturated fatty acids reduces the degree of unsaturation of the oil, imparts improved flavor stability and, at higher degrees of hydrogenation, plastic properties. In commercial practice most soybean oil is only partially hydrogenated under conditions in which hydrogen is selectively added to linolenic and linoleic acids. However, not only are double bonds saturated but a large number of geometric and positional isomers are formed in the resultant dienoic and monoenoic acids. Selectivity of hydrogenation and extent of isomerization vary depending upon hydrogenation conditions particularly with respect to catalyst and its concentration, pressure of hydrogen, temperature and agitation (6).

The extent to which soybean oil is hydrogenated depends upon the intended use of the product. Iodine value (I.V.), a measure of the number of double bonds present, may be about 110 for a hydrogenated soybean oil used as a salad or cooking oil as compared to 130 for the unhydrogenated oil (4). These oils are "winterized" after hydrogenation; this consists of a cold treatment to remove high melting (saturated) components that would form a cloud in the oil at refrigerator temperatures. The oils are referred to as "hydrogenated winterized" soybean oil (4), or as "specially processed" soybean oil (7). Average fatty acid composition of three such oils analyzed by Carpenter et al. (7) is given in Table II. At this degree of hydrogenation the content of polyunsaturates is reduced about 30 percent, monounsaturates are about doubled but saturated fatty acids are essentially unchanged. As compared to the all cis form in the natural oil, about 11 percent of the fatty acids of the hydrogenated oil are trans-isomers, most of which are monoenoics. Hydrogenation results in migration of the positions of double bonds; the data of Carpenter et al. (7) shows that the double bonds in 24 percent of the monoenoates are at positions other than carbon atoms 9 and 12, the positions in the natural oil. Scholfield et al. (8) found that the double bonds in the monoenoic fatty acids of a hydrogenated winterized soybean oil were distributed through the Δ6 to Δ14 positions.

Hydrogenated soybean oil is a major component of many of the margarines and shortenings currently marketed. In order to obtain the desired plasticity and fatty acid composition, margarines are commonly formulated from vegetable oils hydrogenated to different degrees, as well as liquid (unhydrogenated) oil. Cottonseed oil, liquid or partially hydrogenated, and
TABLE II

Fatty Acid Composition of Hydrogenated Soybean Salad and Cooking Oil*

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Percent of total fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Saturated</strong></td>
<td></td>
</tr>
<tr>
<td>Palmitic</td>
<td>9.7</td>
</tr>
<tr>
<td>Stearic</td>
<td>4.6</td>
</tr>
<tr>
<td><strong>Monenoic</strong></td>
<td></td>
</tr>
<tr>
<td>cis</td>
<td>35.3</td>
</tr>
<tr>
<td>trans</td>
<td>9.3</td>
</tr>
<tr>
<td><strong>Dienoic</strong></td>
<td></td>
</tr>
<tr>
<td>cis, cis</td>
<td>36.1</td>
</tr>
<tr>
<td>cis, trans</td>
<td>2.0</td>
</tr>
<tr>
<td>trans, trans</td>
<td>0</td>
</tr>
<tr>
<td><strong>Trienoic</strong></td>
<td>2.5</td>
</tr>
</tbody>
</table>

* Hydrogenated winterized soybean oil, I.V. approximately 110. Average of analyses for three oils reported by Carpenter et al. (7)

Liquid corn, safflower and soybean oils are used in blends with hydrogenated soybean oil. A cube or stick margarine spreadable over a wide temperature range is prepared by blending soybean oil hydrogenated to two or three different iodine values or degrees of hardness. Margarines with a higher content of polyunsaturated fatty acids are formulated by incorporation of unhydrogenated oils and may consist of 50 to 70 percent liquid vegetable oil and 30 to 50 percent hydrogenated soybean oil of about 60 I.V. or another vegetable oil of equivalent hardness. Tub margarines, softer than cube and stick margarines, usually contain 75 to 85 percent liquid oil and a hardened oil hydrogenated to an I.V. of 60 to 65. Soft margarines may also be made from a cube or stick margarine blend of hard and soft hydrogenated components with 50 percent liquid oil added (9). The range in formulations of the fat component of margarines is reflected in their fatty acid composition as shown in Table III which is limited to margarines in which liquid or partially hydrogenated soybean oil is the major component. Based on
<table>
<thead>
<tr>
<th>Sample description</th>
<th>Fat ingredients</th>
<th>16:0</th>
<th>18:0</th>
<th>18:1</th>
<th>18:2</th>
<th>18:3</th>
<th>20:0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cube and stick margarine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>a</td>
<td>9.6</td>
<td>5.5</td>
<td>67.6</td>
<td>11.0</td>
<td>0.7</td>
<td>0.4</td>
</tr>
<tr>
<td>B</td>
<td>a</td>
<td>8.7</td>
<td>5.2</td>
<td>67.2</td>
<td>10.6</td>
<td>0.9</td>
<td>0.3</td>
</tr>
<tr>
<td>H</td>
<td>a</td>
<td>12.2</td>
<td>7.9</td>
<td>67.3</td>
<td>12.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>I</td>
<td>a</td>
<td>13.4</td>
<td>6.6</td>
<td>62.4</td>
<td>17.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>J</td>
<td>ea</td>
<td>11.5</td>
<td>7.6</td>
<td>50.3</td>
<td>27.3</td>
<td>3.2</td>
<td>-</td>
</tr>
<tr>
<td>Whipped margarine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>a</td>
<td>9.2</td>
<td>5.7</td>
<td>62.6</td>
<td>15.3</td>
<td>1.1</td>
<td>0.3</td>
</tr>
<tr>
<td>M</td>
<td>a</td>
<td>12.4</td>
<td>7.4</td>
<td>60.7</td>
<td>19.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Soft tub margarine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>bc</td>
<td>9.2</td>
<td>5.6</td>
<td>55.8</td>
<td>24.2</td>
<td>1.3</td>
<td>0.3</td>
</tr>
<tr>
<td>BB</td>
<td>a</td>
<td>9.9</td>
<td>5.8</td>
<td>52.0</td>
<td>25.8</td>
<td>1.8</td>
<td>0.4</td>
</tr>
<tr>
<td>F</td>
<td>bd</td>
<td>8.2</td>
<td>5.0</td>
<td>40.2</td>
<td>43.3</td>
<td>1.4</td>
<td>0.3</td>
</tr>
<tr>
<td>K</td>
<td>ae</td>
<td>13.5</td>
<td>6.9</td>
<td>41.8</td>
<td>34.4</td>
<td>3.3</td>
<td>-</td>
</tr>
<tr>
<td>L</td>
<td>ea</td>
<td>11.4</td>
<td>6.4</td>
<td>35.8</td>
<td>41.0</td>
<td>4.4</td>
<td>-</td>
</tr>
</tbody>
</table>

1 Letters indicate brands; AA and A, BB and B were made by the same company. Samples A through F were collected in July, 1970 (10); H through M in March, 1974 (11).

2 Ingredients are listed in order of amounts present; the letters designate fats as follows:
   a. partially hydrogenated soybean and cottonseed oils
   b. partially hydrogenated soybean oil
   c. partially hydrogenated and liquid cottonseed oils
   d. liquid safflower oil
   e. liquid soybean oil

TABLE III
Fatty Acid Composition of Soybean Oil Margarines (10, 11)
the palmitic acid content, several of these margarines, i.e., A, B, C, AA, and BB, appear to be formulated almost completely from soybean oils; the stearic acid content is little more than that of the unhydrogenated oil indicating that hydrogenation has created little saturated acids in margarine oils.

Polyunsaturates in the margarines listed in Table III ranged from one-fifth to four-fifths that of liquid soybean oil, and monounsaturates were two to three times that of unhydrogenated oil. As shown in Table IV, content of trans-monoenues ranged from about 10 percent in a tub margarine (F) to 28 percent in a cube (A) or stick product; trans-dienes (cis, trans and trans, cis) varied from 2.3 to 4.3 percent and conjugated dienes from 0.4 to 1.9 percent; content of both types of dienes appears unrelated to the type of margarine. The monoenues contained positional isomers with the double bond located at carbon atom 6 through carbon atom 12. In contrast, the diene fraction contained only Δ9 and Δ12 isomers (10).

Solid shortenings differ from margarines in that 5 to 15 percent of more highly hydrogenated fat (hard fat) is generally incorporated in the formulation to extend the plastic or melting range of the product, a property desirable for bakery use. High stability shortenings for frying, for example, may be formulated from partially hydrogenated soybean oil, I. V. about 70, and 5 percent hard fat. An all-purpose shortening with a wider plastic range may be prepared from partially hydrogenated soybean oil, I. V. about 88, and 10 to 15 percent of a hard fat. In addition to the solid shortenings, a number of fluid and pourable type formulations have been developed for frying purposes. Although hydrogenated soybean oil is the principal component in shortening formulations, other vegetable oils and animal fats also are used and the composition of a particular product will depend on the specific oils employed (9). Mattson et al. (12) reported that the values given in Table V are typical of 95 percent of the vegetable shortenings sold through retail stores in the United States. Compositions of vegetable fat shortenings used in the food industry are given in Table VI.

For comparison with the composition of hydrogenated vegetable oil products Table VII gives the composition of the food fats, butter, lard and tallow. These food fats have been replaced or extended by vegetable oil products.

Refined and hydrogenated vegetable oils contain small amounts of unsaponifiable compounds which include sterols and tocopherols. McOsker et al. (14) reported 0.8 percent unsaponifiables in partially hydrogenated winterized soybean oil, I. V. 109, and also in hydrogenated soybean oil, I. V. 100, and in a hydrogenated blend of cottonseed and soybean oils, I. V. 76. The major sterols in vegetable oils — campesterol, stigmasterol and β-sitosterol— appear to be unchanged in the hydrogenation of margarine oils. Total sterol content of several margarines and hydrogenated vegetable oils of
TABLE IV
Trans and Conjugated Fatty Acid Composition of Margarines (10)

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>AA</th>
<th>BB</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monoene (18:1) trans</td>
<td>27.7</td>
<td>26.2</td>
<td>26.3</td>
<td>20.6</td>
<td>15.6</td>
<td>10.4</td>
</tr>
<tr>
<td>Diene (18:2) trans</td>
<td>2.4</td>
<td>4.3</td>
<td>3.6</td>
<td>2.3</td>
<td>4.2</td>
<td>2.3</td>
</tr>
<tr>
<td>Total conjugation</td>
<td>1.4</td>
<td>1.4</td>
<td>1.9</td>
<td>1.6</td>
<td>1.6</td>
<td>0.4</td>
</tr>
</tbody>
</table>

* For type of margarine see sample descriptions in Table III.

TABLE V
Fatty Acid Composition of Household Vegetable Shortenings*(12)

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Percent of total fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated</td>
<td>25</td>
</tr>
<tr>
<td><strong>Monounsaturated</strong></td>
<td></td>
</tr>
<tr>
<td>cis</td>
<td>33</td>
</tr>
<tr>
<td><strong>trans</strong></td>
<td>14</td>
</tr>
<tr>
<td><strong>Polyunsaturated</strong></td>
<td></td>
</tr>
<tr>
<td>cis, cis</td>
<td>23</td>
</tr>
<tr>
<td>cis, trans</td>
<td>5</td>
</tr>
<tr>
<td>trans, trans</td>
<td>trace</td>
</tr>
</tbody>
</table>

*From 0.1 to 0.3 percent of total fatty acids are conjugated dienes.
### TABLE VI

**Fatty Acid Composition of Shortenings Used Commercially**<sup>a</sup>(13)

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Percent of total fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plastic type</td>
</tr>
<tr>
<td>Saturated</td>
<td>15-40</td>
</tr>
<tr>
<td>Unsaturated</td>
<td></td>
</tr>
<tr>
<td>Monoenoic</td>
<td>45-76</td>
</tr>
<tr>
<td>Dienoic</td>
<td>2-30</td>
</tr>
<tr>
<td>Trienoic</td>
<td>0-1.7</td>
</tr>
</tbody>
</table>

<sup>a</sup> Animal-vegetable fat blends are not included.

### TABLE VII

**Fatty Acid Composition of Butterfat, Lard and Tallow** (13)

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Percent of total fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Butterfat&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Saturated</td>
<td>67</td>
</tr>
<tr>
<td>Unsaturated</td>
<td></td>
</tr>
<tr>
<td>Monoenoic</td>
<td>30</td>
</tr>
<tr>
<td>Dienoic</td>
<td>2</td>
</tr>
<tr>
<td>Trienoic</td>
<td>0.3</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean value of the range given in reference 13.
unspecified oil composition ranged from 0.07 to 0.34 percent (15). Sterol content of four unhydrogenated soybean oil samples varied from 0.15 to 0.38 percent (16). Total tocopherol content of margarines A, B, C, AA and BB listed in Table III ranged from 0.017 to 0.059 percent (10).

Trace amounts of oxidation products having undesirable flavors and odors may be formed in partially hydrogenated vegetable oils when exposed to air as a result of the formation of peroxides and their decomposition to aldehydes (17). Partially hydrogenated soybean and linseed oils develop a characteristic flavor on storage which has been referred to as a "hardening" flavor; the rate of development is slower in the dark. The flavor has been shown to be principally due to 6-trans-nonenal. The 6-trans and the 6-cis-isomers are produced by decomposition of peroxides of 9,15- and/or 8,15-octadecadienoic acids (18).

The carbonyl compounds of the volatiles of partially hydrogenated oxidized soybean oil were separated into methyl ketones, saturated aldehydes, 2-enals and 2,4-dienals but individual compounds were not identified. These compounds and other volatiles would be largely removed in deodorization of the hydrogenated oil (19).

There is no Federal standard of identity for hydrogenated soybean oil. It is not listed in the Food Chemicals Codex (20). The Codex Alimentarius Commission defines the source and specifies acceptable ranges of density, refractive index, saponification and iodine values and maxima for acid and peroxide values, additives and contaminants such as arsenic, metals and insoluble materials. No specifications are given in terms of fatty acid composition (21). Hydrogenated oil appears to be covered by the FAO/WHO recommended general standard for edible fats and oils not covered by individual Codex standards (22). The Codex Alimentarius recommendations still await official ratification by members of the Commission.

III. CONSUMER EXPOSURE DATA

In 1974, 6.125 billion pounds of soybean oil, hydrogenated and unhydrogenated, were used for food purposes. Of this about 1.45 billion pounds went into margarines, 1.85 billion pounds into shortenings and 2.8 billion pounds into cooking and salad oil. Change in per capita use of soybean oil in these products from 1964 to 1974 was from 5.8 to 6.9 pounds for margarine, 7.3 to 8.8 pounds for shortening and 5.7 to 13.3 pounds for salad and cooking oil and reflected the trend in overall consumption of these products. Soybean oil constituted about 75 percent of the fats used in margarine production, 50 percent of those used in shortenings and 70 percent of the fats going into salad and cooking oils (23).
No direct data are available on the proportions of hydrogenated and unhydrogenated soybean oil that go into margarine, shortening, salad and cooking oil. In 1970 Sgoutas and Kummerow (24) estimated 3 billion pounds of soybean oil, or about one-half of the 5.8 billion pounds used was hydrogenated. Assuming the same proportion was hydrogenated in 1974, about 3.2 billion pounds of hydrogenated oil were consumed. This would represent a total daily per capita consumption of about 19 g. The trans-fatty acid content of the fat in the American diet, largely contributed by hydrogenated vegetable oils, has been estimated to be about 8 percent (25).

A National Research Council subcommittee (26) surveyed manufacturers in 1970 concerning the level of addition of GRAS substances to foods and estimated the possible average daily intake of these substances for various age groups. Although hydrogenated soybean oil was not included on the list of substances for which information was requested, three or fewer companies volunteered data on the level of addition of soybean oil to several food product categories. No distinction between hydrogenated and unhydrogenated oil was made nor were data on quantities used reported. Because of the limited data base, the NRC tabulations are not included in this report.

IV. BIOLOGICAL STUDIES

The biological properties of hydrogenated vegetable oils have been extensively studied. As discussed in the Background Information section of this report, hydrogenation results in the formation of saturated fatty acids and unnatural fatty acid isomers. The extent to which these products are formed depends on the degree and conditions of hydrogenation but significant quantities of unnatural isomers are present in most hydrogenated vegetable oil products and the content of saturated acids is significantly increased in most shortenings. Although hydrogenation offers advantages such as controlled product consistency and improved flavor stability, questions have been raised about the nutritional and biological effects of the saturated fatty acids and unnatural isomers formed. In the following sections the literature relating to these questions is reviewed in some detail.

Absorption and metabolism

A digestibility of 95 percent in rats was reported for a margarine formulated from a 50:50 blend of hydrogenated cottonseed-soybean oils containing 16.4 percent saturated, 69.5 percent monoenoic, and 9.7 percent dioenoic fatty acids of which 35 percent were trans-isomers (27). In studies with human subjects, digestibility of a vegetable oil margarine was found to be 96.7 percent (28). For comparison, a value of 97 percent was found for unhydrogenated soybean oil in rats (29).
Several studies have been made on the metabolism of the geometric isomers of oleic and linoleic acids which may be formed in the hydrogenation of vegetable oils. Ono and Fredrickson (30) administered $^{14}$C-labeled oleic, elaidic, cis, cis-linoleic and trans, trans-linoleic acids as the non-esterified fatty acids with simultaneously administered $^3$H-palmitic acid as a "metabolic" marker. No difference was found in intestinal absorption of the isomeric pairs as determined from rates of appearance, relative to palmitic acid, in thoracic duct lymph of cannulated adult male rats administerd the fatty acids by stomach tube in 0.5 ml olive or corn oil. There were no differences between cis and trans-isomers in their distribution within classes of chyle lipids nor was isomerization during absorption detected. Rate of excretion of $^{14}$CO$_2$ in air after intravenous injection of the albumin-bound labeled fatty acids in adult rats was the same for the four isomers which would indicate that there is no discrimination of the isomers in their transport from plasma and in the pathways and fatty acid pools involved in oxidation. All unsaturated isomers were removed at similar rates from the plasma of dogs in which the labeled compounds had been intravenously injected. $^{14}$C-labeled isomeric pairs administered intravenously as non-esterified fatty acids in 0.5 ml plasma, each in combination with $^3$H-palmitic acid, to 19-to 20-day pregnant Sprague-Dawley rats, were transferred across the placental membrane to the fetus at the same rate.

Coots (31) compared the metabolism of $^{14}$C-labeled cis, cis-linoleic, trans, trans-linoleic and a mixture of cis, trans- and trans, cis-linoleic acids fed in a liquid diet to young adult rats as components of randomly rearranged soybean oil. The diet contained 27 percent fat, 16 percent sucrose and 18 percent non-fat dry milk supplemented with vitamins and minerals. Initial rate of excretion of labeled CO$_2$ by rats fed cis, cis-linoleic acid was the same as the rate of excretion by the groups fed soybean oil containing the geometrical isomers of linoleic acid. After 10 hours, however, the rate from the cis, cis-group fell behind and at 51 hours, 64 percent of the recovered carbon-14 had been eliminated in the CO$_2$ of the cis, cis-linoleic acid group while 72 percent of the recovered carbon-14 had been eliminated in the CO$_2$ of the groups fed either the trans, trans-or the mixture of cis, trans- and trans, cis-isomers. The difference, statistically significant at the 90 percent confidence level, was attributed by the author to the lack of essential fatty acid activity of the trans-isomers which would make them more available than the cis, cis-isomer as an energy source. Overall absorption, as indicated by residual carbon-14 in the gastrointestinal tract in the animals at 51 hours and in the feces collected, exceeded 98 percent for all groups.

Coots (32) also compared the metabolism of $^{14}$C-labeled oleic, elaidic, palmitic and stearic acids; each was fed to young adult rats as a component of randomly rearranged soybean oil in a diet similar to that for the linoleic acid isomers. All acids were more than 96 percent absorbed. Rate and extent to which elaidic acid was excreted in respiratory CO$_2$ were essentially the same as those of oleic acid.
A number of studies have demonstrated that the cis, trans- and trans, trans-isomers of linoleic acid do not have essential fatty acid (EFA) activity (33-35). Mattson (36) confirmed that these isomers fed as the ethyl esters at 50 mg (about 200 mg per kg body weight) per day do not have EFA activity for 12-week-old EFA-deficient rats; he also showed that neither these linoleate isomers nor ethyl elaidate interfered with the activity of cis, cis-linoleic acid when fed at equal levels (50 mg per day). Alfin-Slater et al. (37) found that elaidic acid fed to EFA-deficient rats as hydrogenated triolein (33 percent trans) in combination with methyl linoleate at daily intakes of 500 mg (about 2 g per kg) and 20 mg (80 mg per kg) respectively, had no antimetabolic activity as indicated by growth rates.

Anabolism or elongation of the trans-linoleic isomers appears to be selective. Blank and Privett (38) and Privett and Blank (39) fed supplements of methyl esters of cis, trans-(200 mg per day-800 mg per kg) and trans, trans-(400 mg per day-1.6 g per kg) linoleic acids to EFA-deficient young male rats for 17-19 days. Analysis of the fatty acid composition of liver, kidney, epididymal and plasma lipids showed that in animals fed cis, trans-linoleic acids the content of an isomer of arachidonic acid, believed to be cis-5, cis-8, cis-11, trans-14 eicosatetraenoic acid, was increased as a result of elongation of the cis, trans-linoleic acid. This elongated derivative, however, apparently does not have the functional characteristics of the elongated cis, cis-isomer in respect to essential fatty acid activity. The arachidonic acid content was not increased in the group fed trans, trans-linoleic acid, and no conversion of this isomer to higher polyunsaturated fatty acids appeared to take place. In animals receiving methyl linolelaidate (methyl trans-9, trans-12-octadecadienoate), a relatively higher amount of 18:2 fatty acid was deposited in liver sterol esters as compared to the other supplemented groups. Structural analysis of the 18:2 acids of the epididymal fat of the group fed methyl linolelaidate showed 79.5 percent to be linolelaidic acid.

Deposition of trans fatty acids in animal tissues. Several investigators have studied the incorporation of dietary trans-acids into animal tissues and their effect on fatty acid distribution. Sinclair and Chipman (40) found that the liver lipids of adult rats fed for 4 weeks a diet containing 48.9 percent elaidin (about 25 g per kg body weight) and 2 percent cholesterol (about 1 g per kg body weight) contained a higher percentage of trans-fatty acids in the cholesterol esters (43 percent) than in the triglycerides (31 percent). The serum of young male New Zealand rabbits fed 6 g (about 3 g per kg) elaidinized linoleic acid daily for 84 days contained 13.2 percent trans-isomers in the fatty acids of the cholesterol esters, 11.3 percent in the triglycerides and 2.5 percent in the phospholipids (41).

Decker and Mertz (42) determined the fatty acid composition of the epididymal fat pad, liver mitochondria and erythrocyte stroma of weanling male albino rats fed a diet containing either 8 percent native olive oil or elaidinized olive oil (55 percent trans-acids, or about 4 g trans-acids per kg
body weight) supplemented with 2 percent cod liver oil to provide essential fatty acids. At the end of 6 weeks during which the growth rate and general well-being of rats fed the two diets were not different, the animals were killed and the tissues analyzed. Epididymal fat contained about twice the concentration of trans-isomer as compared to other tissues. Molar concentrations were 0.55, 0.27, 0.18 and 0.15 in elaidinized olive oil, epididymal fat, mitochondria and red cell stromata, respectively. Concentrations of both palmitic and oleic acids were depressed in fat pads, unchanged in red cell membranes and increased in mitochondria by elaidic acid supplementation as compared to control animals fed olive oil.

Schrock and Connor (43) compared the fatty acid composition of serum lipids, lipoproteins and adipose tissues of male New Zealand rabbits fed a "cis" or a "trans" high fat (40 percent of calories) diet. The fats were similar in content of saturated fatty acids (30 percent), polyunsaturates (12 to 15 percent) and monounsaturates except that fat in the "cis" diet contained 51.8 percent oleic whereas the "trans" diet contained 18.8 oleic and 37.6 percent elaidic acid. Previously starved for 3 weeks to deplete adipose tissue stores, the rabbits regained their original weights after 3 weeks of ad libitum feeding and were sacrificed at that time. The trans-diet led to 21.7 percent elaidic acid in adipose tissue fat, 15.4 percent in the serum triglycerides, 10.4 percent in the serum phospholipids, and 6.0 percent in the serum cholesterol esters. Concentrations of other serum fatty acids also were changed by feeding trans-fatty acids. Cholesterol esters of the trans-fed rabbits contained more linoleic (42.1 vs. 33.7 percent) and less oleic plus elaidic (25.7 vs. 38.1 percent) than the cholesterol esters of the cis-fed rabbits. Elaidic acid was incorporated into all lipoproteins. Triglycerides of the very low density (VLDL) and low density lipoproteins (LDL) had 15.6 and 14.1 percent elaidic acid but the high density lipoproteins (HDL) triglycerides contained only 9.8 percent of total fatty acids as elaidic acid.

Emken et al. (44) determined the rate and extent of incorporation of two deuterium-labeled fatty acids, elaidate-d₈ (El-d₈) and oleate-d₆ (Ol-d₆) in plasma and red cell neutral and phospholipid fractions of a human subject fed the fatty acids simultaneously as a mixture of trielaidin-d₈ and triolein-d₁₂. A mixture (36.5 g) containing 17.8 g of trielaidin-d₈ and 18.7 g of triolein-d₁₂ was blended in water with 50 g calcium caseinate, 50 g maltose-dextrose and 25 g sucrose. The mixture was fed to a 23-year-old male weighing 160 pounds (73 kg) in place of breakfast after an overnight fast. Blood samples were drawn at intervals from 0 to 72 hours after feeding. Gas chromatographic-mass spectral analysis of plasma neutral lipids showed extensive incorporation of deuterated fat into triglycerides and free fatty acids but low incorporation into cholesterol esters. Maxima of 54.9, 64.2 and 4.1 percent were reached at 6, 6, and 12 hours, respectively. Percentages of Ol-d₆ and El-d₈ in plasma triglyceride samples were the same as in
the mixture fed; more El-\textsubscript{d\textsubscript{2}} than Ol-\textsubscript{d\textsubscript{4}} was incorporated in the 6 hour free fatty acid sample but it was cleared more rapidly than Ol-\textsubscript{d\textsubscript{4}}, and was lower than Ol-\textsubscript{d\textsubscript{4}} in 12 and 24 hour samples. Deuterated fat cleared rapidly and was less than 1 percent in triglycerides and free fatty acids at 48 and 72 hours. Deuterated oleate and elaidate were incorporated in all plasma phospholipids; extent of incorporation varied from a maximum of 61.8 percent in phosphatidyl ethanolamine to 11 percent in sphingomyelin. There was preferential incorporation of El-\textsubscript{d\textsubscript{2}} over Ol-\textsubscript{d\textsubscript{4}} in phosphatidyl ethanolamine and in phosphatidyl choline. Analysis of the fatty acids in the 1-position of plasma phosphatidyl choline showed preferential incorporation of El-\textsubscript{d\textsubscript{2}} in this position; moreover, the analysis indicated that elaidate replaced unlabeled monoene and not saturates. The triglyceride fraction of the red blood cells had a maximum of 23 percent deuterated fat in the 8 hour sample. No preferential selection of El-\textsubscript{d\textsubscript{2}} or Ol-\textsubscript{d\textsubscript{4}} occurred during incorporation and removal of the labeled fats. Cholesterol esters, free fatty acids and phospholipid fatty acids incorporated less than 5 percent deuterated fat.

Tissues of 24 human subjects examined at autopsy in 1957 showed 2.4 to 12.2 percent trans-fatty acids in adipose tissue, 4.0 to 14.4 percent in liver, 4.6 to 9.3 in heart, 2.3 to 8.8 in aortic tissue, and 2.3 to 8.8 in atheroma from those subjects who had died of atherosclerosis (45).

Selectivity of action of enzymes in vitro. Selectivity of action of liver microsomal enzymes on geometric and position isomers of fatty acids in the synthesis and hydrolysis of neutral lipids and phospholipids has been the subject of several investigations. Sgoutas (46) found that the cholesterol esterases in the microsomal and soluble fractions of rat liver hydrolyzed \textsuperscript{14}C-labeled cholesterol esters of cis- and trans octadecenoic and octadecadienoic acids at different rates. Unsaturated cholesterol esters were hydrolyzed faster than saturated esters by the soluble enzyme fractions. Esters with cis-double bonds were hydrolyzed more rapidly than those with trans structure. Order of hydrolysis rates was: cis-unsaturated > trans-unsaturated saturated cholesterol esters. A similar order was observed when the microsomal fraction was used as an enzyme source.

Lands (47) compared stearyl-CoA, oleyl-CoA and elaidyl-CoA as acyl donors in synthesizing lecithin from 1- and 2-acylglycerol-3-phosphorylcholine (acyl-GPC) using acyl CoA:phospholipid acyltransferases from pig liver microsomes and rat liver mitochondria. Rates of esterification at the 2-position of acyl-GPC were about equal for the oleate and elaidate. However, the enzymes which acted at the 1-position discriminated markedly between the cis-and trans-isomers; the rate of esterification with elaidate (42 \textmu moles per min per mg protein) was comparable to that observed for the more linear saturated stearate (28 \textmu moles per min per mg protein) and much greater than that for oleate (1.0 \textmu moles per min per mg protein).
The position of the double bond in cis-octadecenoates has been shown in in vitro experiments to affect the activity of acyl CoA:cholesterol O-acyltransferase from rat liver microsomes. Rates of esterification of cholesterol by labeled fatty acyl-CoA esters were in the order: Δ9-, 550; Δ11-, 440; Δ6-, 327; and Δ15-isomer, 217 μ moles per min per mg microsomal protein. Rate was highest for the double bond at carbon-9 and a difference of two carbon atoms in the position of the double bond in either direction caused a reduction in the reaction rate (48).

Rate of hydrolysis of cholesterol esters of 16 positional isomers of cis-octadecenoic acid with rat liver hydrolase was greatest for the Δ9-isomer, 460 μ moles of ester hydrolyzed per hour per mg protein, and decreased approximately linearly to 190 for the Δ2-isomer and to 150 for the Δ-17 isomer. The authors point out that the relatively lower rate for the positional isomers may partly account for the enrichment of hepatic cholesterol esters of these fatty acid isomers observed in other studies. They note, however, that fatty acid composition of liver cholesterol esters is controlled by several additional factors including the nature of the fatty acid pool (49).

Acyl-CoA:phospholipid acyltransferases from rat and pig liver acting at the 1- and 2-positions of acyl-glycero-3-phosphorylcholine were found to discriminate between acyl-CoA isomers in a study of 16 positional isomers of cis-octadecenoic acid. The microsomal enzymes from both animals had very similar acyltransferase specificities. In both species, the Δ5-, Δ9- and Δ12-isomers had rates greater than their adjacent isomers when being transferred to the 2-position of glycerophosphorylcholine. Relative transfer rates were 26, 98, and 41, respectively. The Δ5-, Δ8- and Δ12-isomers had rates (17, 105, and 121, respectively) faster than their adjacent isomers in transfer to the 1-position. The authors suggest that the acyl transfer activities can be used as an index to the amount of a given fatty acid which will be found in the 1- and 2-positions of liver diacylglycerophosphorylcholine (50).

Discrimination in the deposition of the Δ8- to Δ12-isomers of cis-octadecenoic acid into egg yolk lipids was found by Mounts (51). Laying hens were orally administered the tritium-labeled positional isomers in mixture with carbon-14 labeled oleic acid as an internal reference standard. All fatty acids were administered as the methyl esters. Incorporation into neutral lipids was in the order: Δ9>Δ8; Δ9>Δ10; Δ9>Δ11; Δ9>Δ12. For the phospholipids, the order was Δ9>Δ8; Δ9>Δ10; Δ9<Δ11; Δ9<Δ12. Incorporation of the isomeric acids in the 2-positions of the phospholipids was in nearly the same ratios as for the total phospholipids except for the Δ12-isomer which was decreased, indicating a preference of acyl CoA:1-acyl glycerol phosphatidyl transferase for oleic acid. However, the greater incorporation of the position isomers in the 1-position of the phospholipids showed that the acyl CoA:2-acylglycerol
phosphatide acyltransferase preferred the positional isomers relative to oleic acid. Greatest specificity was for the Δ11 - and Δ12 - isomers as found by Reitz et al. (50) with rat and pig liver microsomal enzymes.

Effect of trans acids on membrane properties. A comparison of membrane properties of erythrocytes and liver mitochondria from the rats fed native and elaidinized olive oil (42) was reported by Decker and Mertz (52). Liver mitochondria from elaidic acid supplemented rats exhibited 2-to 3-fold greater initial rates of swelling in hypotonic swelling media containing inorganic phosphate. Erythrocytes containing trans-fatty acids hemolyzed at a rate 5 times greater than that of the control cells when incubated with α-lecithinase. Resistance to osmotic stress in saline solutions and to other hemolytic agents including chenodeoxycholate, glycerol, diethylene glycol, thiourea and copper ions showed only slight differences. The cells from elaidic acid-supplemented rats appeared somewhat more resistant to osmotic stress and to chenodeoxycholate, and less resistant to glycerol than their controls.

Chapman et al. (53) studied the effect of trans-double bonds on the surface properties of phospholipids by comparing the surface pressure-area curves of monomolecular films of phosphatidylethanolamines and phosphatidylcholines containing elaidic or oleic acid in the 2-position. Monolayers of the oleoyl phospholipids were considerably more expanded, i.e., had a greater surface area per molecule, than the corresponding elaidyl compounds; the oleoyl isomers also interacted with cholesterol in the monolayer films over a wider range of surface pressures. The authors proposed that these differences in film properties of the cis- and trans-fatty acids could be reflected in differences in their behavior in biological membranes.

Short-term feeding studies

Thomasson et al. (54) fed groups of 24 male and 24 female rats for a period of 12 weeks nutritionally adequate diets in which 54 percent of the calories came from the experimental fat and 6 percent came from sunflower seed oil as a source of essential fatty acids. Three commercially hydrogenated soybean oils were fed and compared with unhydrogenated soybean oil as a control. Fatty acid composition of the oils was as follows:

<table>
<thead>
<tr>
<th>Fatty Acid, Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated</td>
</tr>
<tr>
<td>cis</td>
</tr>
<tr>
<td>Soybean oil (control)</td>
</tr>
<tr>
<td>Hydrogenated soybean oil (L₆)</td>
</tr>
<tr>
<td>Hydrogenated soybean oil (L₁₅)</td>
</tr>
<tr>
<td>Hydrogenated soybean oil (L₄₀)</td>
</tr>
</tbody>
</table>
Mean intake of dietary fat per animal during the 12 weeks was 305 g for males and 245 g for females, which is estimated to range from about 27 to 15 g per kg over the 12 week period. Mean gain in weight in 12 weeks was 258 g for males and 137 for females. No significant differences were found among experimental groups in feed consumption or weight gain. Fat excreted in the feces was less than 5 percent of that eaten and was within the limits of normal excretion for all groups. No indications of abnormal changes were found in food intake, body weight, fat content of feces, water consumption, rectal temperature, kidney function (aspartate transaminase test), liver function (alanine transaminase and alkaline phosphatase tests), blood (hemoglobin, hematocrit, hemolysis, white blood cells, coagulation, and platelet adhesiveness), body fat composition or in findings on post mortem examination. Body fat composition reflected the composition of the dietary fat.

**Long-term studies**

Performance of rats (Wistar derived strain) in a multigeneration study in which the animals were fed a ration containing 9.2 percent margarine oil, 35 percent trans-fatty acids, as essentially the sole source of fat in the diet has been reported through the 60th generation. Other diet components were ground whole wheat, skim milk powder, and sodium chloride. With the exception of females during lactation the rats received, once weekly, a supplement of 5 g lean meat and 5 g lettuce. The margarine oil in the diet until the 44th generation was a 1:1 blend of hydrogenated soybean and cottonseed oils which contained 19.4 percent saturated fatty acids, 71.3 percent monoenoic, 4.9 percent dienoic (3.7 percent essential fatty acids by biological assay) and 35.3 percent trans-fatty acids. Generations 44 to 60 were fed a similar product having the composition: 16.4 percent saturated fatty acids, 69.5 percent monoenoic, 9.7 percent dienoic (6.9 percent essential fatty acids) and 35 percent trans-acids. Margarine oil was fed to generations 1-26; generations 27-60 received whole margarine at an equivalent fat level (27). Growth of rats as evidenced by weight gain was generally uniform; deviations for some generations were attributed to seasonal variations and the presence at times of a chronic respiratory infection. Tibia length at 90 days, selected as a further index of growth, was 3.72 cm for the 40th generation and 3.74 cm for the 60th as compared to 3.52 cm for the 20th generation. Fertility of rats was maintained throughout the 60 generations. Through the 5th generation, all females bred cast litters. A minimum response was obtained in the 13th generation in which only 8 of 18 (44 percent) were fertile. From 25th through the 46th generation, 87 percent of females bred (20 to 35 per generation) had successful pregnancies and cast an average of 7.4 pups per litter which reached an average weight of 34.1 g at weaning. Values for the 60th generation were not reported but levels were maintained for the 65th, 70th and 74th
generations which were fed the same quantity of fat but one which contained more linoleic (27 percent) and less trans-acids (20 percent). Average weaning weight in beginning stock rats was 31.1 g; that of both the 25th and 46th generations was 34.1 g indicating satisfactory lactation performance. Results of four longevity experiments on rats fed the hydrogenated margarine oils were reported: 50 percent survival of one group of males of the 27th generation was 97 weeks compared to 99 weeks for a control group fed a stock diet; 50 percent survival of one group of males of the 34th generation was 80 weeks and that of females was 111 weeks; that of the 75th generation, males and females was 80 weeks. Respiratory disease was stated as the cause for reduced longevity. In another longevity experiment rats were fed the hydrogenated fat at 3, 9, 18 and 30 percent levels; 50 percent survival times for males were 112, 111, 109 and 100 weeks, respectively. No indication of mutagenicity, teratogenicity or carcinogenicity was reported in the multigeneration and longevity studies (27, 55, 56).

Diets containing 12.5 percent margarine or 11.5 percent margarine fat were fed to control and test animals in 2-year rat feeding tests of the chronic toxicity of isopropyl citrate and stearyl citrate. Composition of the margarine was the same as that fed to the first 43 generations in the multigeneration test described above (2 g trans-fatty acids per kg body weight). Other components of the diet were casein, 20 percent; sucrose, 55.5 percent; yeast, 8 percent; and salt mixture, 4 percent. At the end of two years, 50 percent of 20 male and 50 percent of 20 female rats survived. Histopathological sections of liver, kidney, stomach, small intestine, large intestine, spleen, heart, gonads, brain and adrenals of the survivors were examined and it was concluded that although the animals showed some diseased tissue attributable to old age, no changes traceable to diet were observed (27, 57).

Soybean oil and hydrogenated soybean oil, I.V. 108, were included in a 2-year rat chronic toxicity test of commercially used frying fats. Each fat was fed at the 15 percent level in a semipurified diet to 50 male and 50 female Sprague-Dawley weanling rats. Growth as evidenced by weight gain at 2, 12 and 21 months did not differ for the unhydrogenated and hydrogenated oils; feed efficiency also did not differ for the two oils. Histological examination of ten rats of each sex for each diet after 2 years feeding showed no difference attributable to diet in incidence of fatty liver, chronic pyelonephritis, kidney tubule mineralization, adrenal telangiectasis, alveolar foam cells, mammary or other tumors. Liver cholesterol and kidney sodium concentrations were statistically higher for the unhydrogenated soybean oil diet (29).
Vles and Gottenbos compared soybean oil, three hydrogenated soybean oils, butterfat and coconut oil in long-term feeding studies of mice (58) and rats (59). In both studies the animals were fed diets in which fat supplied a relatively large part (60 percent) of total calories; 54 calorie percent coming from the experimental fat, and 6 calorie percent from soybean oil to provide essential fatty acids. Compositions of the three hydrogenated soybean oils fed in the mice experiments are given in Table VIII.

**TABLE VIII**

Fatty Acid Composition of Hydrogenated Soybean Oils Fed to Mice (58)

<table>
<thead>
<tr>
<th></th>
<th>16:0</th>
<th>18:0</th>
<th>18:1</th>
<th>18:2</th>
<th>18:3</th>
<th>Trans</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSO-1</td>
<td>10.4</td>
<td>4.3</td>
<td>40.1</td>
<td>39.8</td>
<td>2.6</td>
<td>15</td>
</tr>
<tr>
<td>HSO-2</td>
<td>10.8</td>
<td>7.5</td>
<td>41.4</td>
<td>35.9</td>
<td>2.8</td>
<td>19</td>
</tr>
<tr>
<td>HSO-3</td>
<td>10.9</td>
<td>10.2</td>
<td>68.5</td>
<td>8.4</td>
<td>1.1</td>
<td>61</td>
</tr>
</tbody>
</table>

The values in Table VIII are averages of the different batches of hydrogenated oils of each type that were prepared during the feeding study. Each diet was fed ad libitum to groups of 40 male and 40 female newly weaned SPF-Swiss mice. At the end of the 18 month experiment all surviving mice were decapitated and autopsies performed. No significant differences in mortality were found between the different groups. Weight gain of the male rats on the HSO-2 diet was significantly less than that on the unhydrogenated soybean oil diet at 52 weeks but not at 78 weeks. The mice fed the HSO-3 diet (61 percent trans) had significantly heavier livers than the animals on the other soybean oil diets but histopathological examination showed no abnormalities. No significant differences in total tumor frequency were found between the groups nor were there obvious differences in the type and site of neoplasms. Incidence of non-neoplastic diseases and changes were randomly distributed among the groups.

In the long-term rat experiments of Vles and Gottenbos (59) the diets were fed ad libitum to groups of 39 to 40 each of male and female newly weaned SPF-Wistar rats. Compositions of the hydrogenated soybean oils were similar to those fed in the mice studies (Table VIII). Differences in growth between the various dietary groups were not significant for males or for females. At 50 percent mortality in the butterfat group (males, 762 days--; females 737 days), percentage mortality in the soybean oil, HSO-1, HSO-2,
HSO-3 and coconut oil groups was 28, 33, 38, 28 and 41 percent in the males and 18, 43, 40, 30 and 28 percent in the females, respectively. Differences in the total population of neoplastic tumor bearers (males plus females) between dietary groups were not significant. However, among the males, the HSO-1 group had a significantly higher number of tumor bearers whereas among females, the group fed the HSO-1 diet had a significantly lower number of tumors (P<0.05). Other pathological changes were not significantly different among the groups.

**Special studies—atherosclerosis**

Epidemiological studies of the prevalence of atherosclerotic diseases gave rise to the concept of "risk factors" that reflect personal characteristics associated with a higher than average risk of clinical disease in addition to the recognized consequences of aging (60, 61). These studies, including the seven country International Cooperative Study on the Epidemiology of Cardiovascular Disease (62), the U.S. National Cooperative Pooling Project (63) and the International Atherosclerosis Project (64) have demonstrated significant correlations between coronary heart disease rates and intake of saturated fat, serum cholesterol level and hypertension. Other studies show a correlation between per capita cigarette consumption and coronary heart disease (61, 63). These factors are considered major risk factors for first coronary events. Other possible risk factors include sedentary living habits, personality-behavior patterns, marked obesity and positive family history of coronary heart disease (61-63).

Identification of saturated fat intake as a risk-factor in coronary heart disease has led to concern about the level and composition of fat and fat products in the American diet. Several groups have made recommendations for dietary modifications in this regard (63, 65, 66). Others have recommended caution in changing to a higher proportion of polyunsaturated fats in the diet, pointing out that there is no conclusive evidence that this would reduce the incidence of coronary heart disease (67). A recent review article notes some uncertainties associated with a diet high in polyunsaturated fats (68). In the following sections animal and human studies relating to the effect of hydrogenated soybean oil on serum lipid levels and development of atherosclerosis will be reviewed.

**Human studies.** Controlled feeding experiments on human subjects utilizing a variety of natural fats and oils showed that isocaloric substitution of the saturated fatty acids, lauric, myristic or palmitic, for mixed carbohydrates in the diet caused an elevation of serum cholesterol levels; no change was produced by substitution of monounsaturated glycerides, and a depression in serum cholesterol resulted from the isocaloric substitution of polyunsaturated fatty acid glycerides (69-71). Stearic acid and short chain
fatty acids (<12 carbons) in natural fats were found to have little or no effect on cholesterol levels (70-73); they were also less hypertriglyceridemic than carbohydrate but more so than palmitic acid (73). However, experiments (74) conducted with a series of semisynthetic fats prepared by interesterification of triglycerides indicated that stearic acid was hypercholesterolemic and had a coefficient in regression equations for serum cholesterol about half those for palmitic and myristic acid. The authors considered that this discrepancy may have resulted from the random distribution of stearic acid among all three positions of the triglyceride molecule in the interesterified fats as contrasted to the location of stearic acid almost exclusively in the 1- and 3-positions in most natural fats.

From the viewpoint of current commercial hydrogenated soybean oil products, production of hypercholesterolemic saturated fatty acids appears to be of minor importance since hydrogenation is selective and little saturated fatty acid is formed in margarines and salad and cooking oils; stearic acid, which is formed in shortening oils, appears to be neutral or low in hypercholesterolemic activity. Content of the 14:1 and 16:1 fatty acids in soybean oil is essentially zero which eliminates the possibility of formation of myristic and palmitic acids. Trans-acids are formed, however, and several studies have been reported on the effect of hydrogenated oils on serum lipid levels in man and on the development of atherosclerosis in animals. In this connection it may be noted that although the unsaturated fatty acids synthesized in animal tissues have the cis-configuration (75), fat of ruminant animals (beef cattle, sheep, deer) contains 5 to 10 percent trans-acids, presumably formed by the action of rumen microorganisms (76, 77). Position isomers of oleic acid also have been reported in bovine body fat and butterfat (78).

The experiments of Beveridge and Connell (79) who investigated the response in university students to eight commercial margarines containing 12.0 to 47.8 percent trans-fatty acids, did not indicate a hyperlipemic effect of these acids. In one experiment 78 men and 19 women were fed a fat-free liquid formula diet for 8 days. They were then divided into 10 groups and continued for 8 days on rations modified by the substitution of 45 percent of calories from fat (eight margarines, corn oil and butter) for an equicaloric amount of carbohydrate. Blood samples were taken before breakfast on days 0, 4, 8, 12 and 16. Corn oil had a significant hypocholesterolemic effect (P<0.05) and butter, a significant hypercholesterolemic response (P<0.01). Only three margarines led to a significant increase (P<0.05) in serum cholesterol compared to the level obtained on the fat-free ration. Average trans- and saturated fatty acid contents of these three margarines were the same as those of the other five but content of 18:2 acids was somewhat less (10.2 vs. 16.1 percent).

Anderson, Grande and Keys (80) reported three experiments, each involving 23 to 27 men, in which a comparison was made of the serum lipid response to diets containing hydrogenated and unhydrogenated oils. In two
experiments the men were first fed, for 21 days, a diet similar to a typical American diet providing about 40 percent of the total calories from fat. In the first experiment the men were then assigned to either of two groups, one of which received 30 g (0.5 g per kg body weight) of selectively hydrogenated safflower oil (32 percent saturated, 55 percent monoenoic, and 13 percent polyenoic fatty acids; 35 percent trans-acids) in place of 68 g of carbohydrate in the initial diet; in the diet of the other group 30 g of unhydrogenated safflower oil were substituted for carbohydrate; fat provided 46 percent of total calories for both groups. After 21 days the diets of the two groups were interchanged for another 21 day period after which both groups were returned to the initial diet. In the second experiment, the men were divided into 4 groups after 21 days on the American type diet; two received diets which compared hydrogenated and natural safflower oil and the other two, hydrogenated and natural corn oil. Each oil (100 g) provided about 30 percent of total calories in the diet and was added to a low-fat basal diet which provided 100 g protein and 39 g fat consisting of 4 g vegetable fats, 12 g butterfat and 23 g of mainly beef and pork fats. Composition of the hydrogenated corn oil was 26 percent saturated, 68 percent monoenoic, 6 percent polyenoic fatty acids; trans-fatty acid content was 37 percent.

In the first experiment described above, hydrogenated safflower oil in the diet produced a significantly higher (P<0.001) serum cholesterol level than an equal amount of the natural oil. The difference (10 mg per 100 ml) however, corresponded closely to that predicted (11 mg per 100 ml) by the equation of Keys, et al. (69) which relates change in serum cholesterol level to differences in the content of saturated and polyunsaturated fatty acids in the diets. This equation was based on experiments with natural fats; hence the experiment detected no difference between the effect of the unnatural isomers in the hydrogenated safflower oil and that expected from the natural isomers. In the second experiment, the serum cholesterol level was considerably higher (P<0.001) when hydrogenated oil was in the diet as compared to the natural oil. However, the difference was less than that predicted by Keys' equation suggesting a hypocholesterolemic effect of the unnatural isomers (80).

In a third experiment, Anderson et al. (80) fed as test fats, one in which 98.5 g of a selectively hydrogenated corn oil was added to the same low-fat basal diet as used in experiment 2, and another in which the supplementary fat (100 g) had the same content of saturated, monoenoic and dinoenoic fatty acids as the hydrogenated corn oil but contained no unnatural isomers. The monoenoic fraction of the hydrogenated corn oil contained 20.1 percent cis-isomer and 36.1 percent trans-isomer based on the total sample; on the same basis, the dinoenoic fatty acids were 12.8 percent cis, 2.0 percent cis, trans and 5.5 percent trans, trans-nonconjugated isomers, and 1.7 percent cis, trans and 5.5 percent trans, trans-conjugated isomers. The hydrogenated corn oil diet produced a significantly higher serum cholesterol level
(209 ± 9.0) than the diet containing the supplementary fats (188 ± 6.8 mg per 100 ml) which had no unnatural isomers. The authors tentatively suggested that the increase was associated with the relatively large percentage of trans-monoenoic fatty acids in the corn oil, but it may be noted that the content of trans, trans-non-conjugated and conjugated diene isomers was much greater than that reported for margarines (Table III), shortenings (Table V), and hydrogenated winterized soybean salad and cooking oils (Table II). The hydrogenated corn oil diet also produced significantly greater increases in serum phospholipids and triglycerides compared to its natural fat analog.

Grasso et al. (81) compared the effect of a hydrogenated soybean oil (I.V. 107, 20 percent trans-fatty acids) with a blend of vegetable oils having the same fatty acid composition (I.V. 107. 3 percent trans) on the serum lipid levels of two patients, a 51 year old male diabetic and a 49 year old female with moderate residual hypothyroidism. Both received diets containing 100 g coconut oil for an initial 41 day period after which the test fats were fed alternately for successive 3 to 4 week periods. In both subjects, the hydrogenated soybean oil produced a significant depression of the plasma cholesterol and phospholipid levels reached on the coconut oil diet. Cholesterol and phospholipid levels in the male subject did not differ, but triglycerides were higher, on the diets containing hydrogenated soybean oil compared to the vegetable oil blend; the hydrogenated soybean oil diet produced a higher cholesterol level (277 vs. 241 mg per 100 ml) in the female patient but did not affect phospholipid or triglyceride levels.

McOsker et al. (14) evaluated the effect of seven dietary fats upon the serum cholesterol levels of human subjects. The fats included cottonseed oil, I.V. 114; 2 partially hydrogenated soybean oils, I.V. 109 and 100, respectively; two blends of partially hydrogenated cottonseed and soybean oils, I.V. 95 and 76, respectively; butter; and a mixture of animal and vegetable fats, I.V. 61, which was a composite representative of all fats, both visible and invisible, present in the average U.S. diet in 1955. Total trans-acids ranged from 15.2 to 20.6 percent in the hydrogenated fats. The fats were fed as a component of a liquid formula diet consisting of egg white, dextrose, fat, salt and water; fat provided 41 percent of the daily calories. Each fat was administered to four different groups of 6 male subjects each during the 32 weeks of feeding. The subjects were in good health with no known metabolic disorders and ranged in age from 25 to 44 years. No significant differences were found in serum cholesterol levels produced by the hydrogenated oils and the unhydrogenated cottonseed oil. All serum cholesterol levels except that given by the hydrogenated fat, I.V. 76, were lower than that produced by the animal-vegetable fat mixture and all, including the I.V. 76 fat, were lower than the level given by butter.

The response of serum cholesterol and triglyceride levels in human subjects to a hydrogenated fat containing a relatively high level of trans-fatty
acids was investigated by Mattson et al. (12) by feeding two liquid formula
diets, one containing the hydrogenated fat, the other alike in fatty acid com-
position, fat, protein, carbohydrate, and cholesterol content and caloric density
but containing only cis-unsaturated fatty acids. Composition of the fats is
given in Table IX. Test fats constituted 18.4 percent of dry solids in the diets
and provided 35 percent of the calories; other constituents were dried egg white,
dried egg yolk, dextrose, sodium chloride and flavoring. Subjects selected
for the test were 37 males, 24 to 41 years old, who had an average plasma
cholesterol level of 187.8 and triglyceride level of 108.4 mg per 100 ml. After
33 of these subjects completed an initial 21 days on the high cis-diet, 17 sub-
jects continued on this diet, and the remaining 16 participants received the
high trans-diets for four weeks. Blood samples were drawn twice weekly
after an overnight fast. No significant differences were found in plasma cho-
lesterol levels on shifting from the high cis-(186.2 ± 5.6 mg per 100 ml) to
the high trans-(189.4 ± 6.9) diet. Plasma triglyceride levels also did not
differ significantly. Absence of a time effect was shown by the almost iden-
tical values for cholesterol and triglycerides in the group receiving the high
cis-diet during both test periods (12).

TABLE IX

Fatty Acid Composition of Diets Fed by Mattson, et al. (85)

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>High cis fat</th>
<th>High trans fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>12.0</td>
<td>10.9</td>
</tr>
<tr>
<td>18:0</td>
<td>13.0</td>
<td>15.5</td>
</tr>
<tr>
<td>18:1</td>
<td>58.4</td>
<td>53.6</td>
</tr>
<tr>
<td>18:2</td>
<td>16.3</td>
<td>20.0</td>
</tr>
</tbody>
</table>

Isomeric Fatty Acids

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>cis cis, cis cis, trans, trans</th>
</tr>
</thead>
<tbody>
<tr>
<td>18:1</td>
<td>58</td>
</tr>
<tr>
<td>trans</td>
<td>--</td>
</tr>
<tr>
<td>18:2</td>
<td>16</td>
</tr>
<tr>
<td>cis, trans</td>
<td>--</td>
</tr>
<tr>
<td>trans, trans</td>
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</tr>
</tbody>
</table>
Vergroesen (82) reported that elaidic acid in the presence of cholesterol has a hypercholesterolemic effect compared with oleic acid. He fed a liquid formula diet with 40 percent dietary energy provided by fat for 4 weeks to groups of 12 male and female volunteers. Experimental fats were prepared by mixing hardened and unhardened olive oil with coconut and safflower oils. Dried egg yolk was added to obtain a diet containing 115 mg cholesterol per 1000 kcal. After 4 weeks on a dietary fat containing 14 percent palmitic and stearic acids, 34 percent elaidic, 41 percent oleic and 10 percent linoleic acids, total cholesterol was about 200 mg per 100 ml as compared to about 189 mg per 100 ml from a fat similar in composition except the 18:1 acid was all oleic acid. Both were lower than the level on the pretreatment diet, 207 mg per 100 ml. Composition of the pretreatment diet was not given nor were standard deviations of the values reported. Increasing the linoleic acid in these dietary fats from 10 to 34 percent at the expense of 18:1 acids caused a slight hypercholesterolemic effect. Vergroesen stated that in a previous experiment elaidic acid in a liquid formula diet providing 40 percent dietary energy from fat (including one egg yolk per day) increased serum cholesterol even more than palmitic acid at the same concentration (35 percent of fat). However, in the absence of cholesterol, elaidic acid had the same effect as oleic acid. Details of these experiments were not given.

Mishkel and Spritz (83) fed six patients triglycerides of trans-linoleic acid (trans-LLL) containing approximately 60 percent of the trans, trans-isomer and 40 percent of the cis, trans- and trans, cis-isomers. Trans-LLL was fed in liquid formula diets in amounts varying from 25 to 100 percent of the dietary fat (40 percent of total calories), the remainder being made up with corn oil or lard. Serum triglyceride concentration increased in all subjects at the highest level fed (75 or 100 percent of the dietary fat) as compared to their respective control levels on corn oil (three subjects) cis-LLL (two subjects) and 75 lard, 25 cis-LLL (one subject), and also in one subject (lard-cis-LLL control) when fed at a lower level (25 percent of the fat). Serum cholesterol response was less consistent. Increase occurred in three subjects when trans-LLL comprised 75 or 100 percent of their dietary fat as compared to their corn oil control levels; an increase occurred in one subject when trans-LLL was at the 100 percent level, but not in a second subject as compared to their levels on cis-LLL control diets; and an increase occurred relative to the lard-cis-LLL control when trans-LLL comprised 25 percent of the dietary fat but a decrease resulted when the dietary fat was all trans-LLL. In another experiment, three subjects were fed fat-free and trans-LLL (40 percent of calories) diets for 15-day periods. Triglyceride level was higher on the trans-LLL diet in one subject, about the same in another, and lower in the third subject. Some, but not all, subjects complained of severe leg pain which began during the trans-LLL diet and remitted when this fat was discontinued.
Animal studies

Comparison of the effect of corn oil (I.V. 130) and hydrogenated corn oil (I.V. 72) on serum cholesterol levels and development of atheroma in Dutch Belted rabbits was reported by Kritchevsky et al. (84). Groups of 10 to 15 rabbits were fed rabbit chow diets containing 9 percent (about 2.5 g per kg body weight) of the experimental fat with or without 3 percent cholesterol (about 1 g per kg). Inspection of aortas for atherosclerotic lesions after two months feeding showed on a scale of 0 to 4 the following degrees of atheroma: control chow diet, 0.06; corn oil, 0.10; hydrogenated corn oil, 0.10; chow and cholesterol, 3.80; hydrogenated corn oil and cholesterol, 3.71; and corn oil and cholesterol, 2.71. Thus, neither hydrogenated corn oil nor corn oil induced atherosclerosis in the absence of cholesterol in the diet; in the presence of cholesterol, corn oil reduced the severity of atherosclerosis much more than hydrogenated corn oil did.

The effect of elaidic acid on the development of atherosclerosis in rabbits was investigated by McMillan et al. (85) who fed 3 to 4 month-old male, New Zealand rabbits daily, for 84 days, 60 g food rations containing 53 g rabbit feed pellets, 1 g cholesterol (about 500 mg per kg) and 6 g of either natural or elaidinized olive oil. Trans-fatty acid content of the total fat in the latter diet was about 45 percent (about 1 g per kg body weight). Some residual nitro addition compounds remained in the olive oil as a result of the elaidinizing treatment with dilute nitric acid and sodium nitrite. The elaidinized olive oil diet resulted in significantly higher free, esterified and total serum cholesterol levels, and slightly more atherosclerosis of the aortic arch, but no more visible atherosclerosis of the thoracic or abdominal portions of the aorta than control rabbits. Similar results were reported by Weigensberg et al. (86) in feeding tests of free elaidic and oleic acids in the diets of rabbits.

Effect of feeding a mixture of geometric isomers of linoleic acid on serum lipid levels and development of atherosclerosis in rabbits was reported by Weigensberg and McMillan (87). Twenty male, New Zealand rabbits about 3 months old were fed a daily food ration containing 1 g cholesterol (about 500 mg per kg body weight) and 6 g elaidinized linoleic acid mixed with 53 g of feed pellets. The elaidinized linoleic acid was a mixture of geometric isomers which provided daily 1526 mg cis, cis-, 1170 mg cis, trans-, 1080 mg trans, cis- and 1440 mg trans, trans-linoleic acid. On a bodyweight basis, intake of these isomers was about 750, 580, 500 and 700 mg per kg at the beginning of the 84-day feeding period. A second group of 23 rabbits was fed 6 g linoleic acid in place of the elaidinized acid. A third group received cholesterol but the fatty acid supplement was replaced with additional feed pellets. The elaidinized linoleic acid gave no higher concentration of free cholesterol, cholesterol ester, triglyceride or phosphatides
in their serum than the control rabbits. The rabbits fed elaidinized linoleic acid showed little, or no more, atherosclerosis of the aortic arch, no more atherosclerosis in the thoracic and abdominal aorta, and not significantly higher cholesterol content in their aortas than did the rabbits fed cis, cis-linoleic acid. The authors concluded that the 84-day-diet of cholesterol and linoelaidic acid was not significantly more atherogenic to rabbits than a diet of cholesterol and linoleic acid and that both of these diets have about the same degree of atherogenicity as a diet containing cholesterol but without these fatty acid supplements.

No effect on serum cholesterol and phospholipid levels was found in three to four-month-old swine (11 per group of Yorkshire and Landrace-Yorkshire cross breeds) fed margarine or butter in a pig grower ration for 4 weeks as compared to a control group which received only the grower ration (2 percent fat). Butter and margarine content were increased from 20 percent of the calories at the beginning to 40 percent at the end of the test period. Composition of the margarine was not stated. Some atherosclerosis was found in swine on the low-fat control ration; the butter diet caused considerable increase while margarine as a fat source caused little increase in the amount of atherosclerosis (88).

Kummerow et al. (89) reported results of a study in which six-month old swine were fed a hydrogenated fat that contained less than 1 percent C 18:2 and 40 percent trans-fatty acids. Other test fats included beef tallow, corn oil and butterfat; 20 percent test fat was added to a basal ration which provided 14.3 percent protein and 3 percent corn oil. After eight months, total serum lipids and cholesterol were highest on the hydrogenated fat diet (388 ± 120, 138 ± 9 mg per 100 ml) as compared to beef tallow (331 ± 13, 124 ± 5), butterfat (332 ± 15, 120 ± 7) and corn oil (276 ± 21, 104 ± 7). Percent of total area of aorta showing atherosclerotic involvement was 10.0, 5.2, 7.3, and 5.0, respectively, for these diets. Intake of essential fatty acids was marginal in these feeding tests and in this respect it may be noted that Hill et al. (90) found that rigid exclusion of essential fatty acids from the diet of eight young swine from one to five days after birth led to skin lesions in one and aortic lesions in five animals at or before 98 days of experiment.

V. OPINION

Hydrogenated soybean oil is a major food fat in the American dietary. Food uses of hydrogenated soybean oil are in the production of margarine, shortening, and salad and cooking oils. These products are not formulated from completely hydrogenated vegetable oils; soybean oils for margarines
and salad and cooking oils are only partially hydrogenated and the saturated fatty acid content is increased little by the process. The content of polyunsaturates in a soft tub margarine or salad and cooking oil may be two to three times that of the saturated fatty acids. Shortenings of the plastic type have a higher saturated fatty acid content than margarines but are no higher in this respect than the animal fats they have replaced, and generally have a higher content of polyunsaturated fatty acids.

Geometric and position isomers of the unsaturated fatty acids formed in hydrogenation have physical properties different from the isomers present in the natural oil. Presence of these unnatural isomers has led to extensive investigation of their nutritional and biological properties. The major geometric isomer formed is elaidic acid, the trans-isomer of oleic acid, although cis, trans-dienoic acids also are produced. It is estimated that the trans fatty acid content of the fat in the average American diet, largely contributed by hydrogenated vegetable oil products, is about 8 percent.

Digestibility of partially hydrogenated soybean oils is high and comparable to that of the unhydrogenated oil. No difference has been found in the intestinal absorption and rates of disappearance from the plasma of the trans-isomers of oleic and linoleic acid as compared to the cis-isomers. Rates of excretion as respiratory carbon dioxide are essentially the same for the trans- and cis- isomers.

The trans-isomers of linoleic acid do not have essential fatty acid activity, but the content of the cis-cis-isomer in hydrogenated soybean oil products is as high or higher than is found in butterfat, lard or tallow. Presence of either the trans-isomer of oleic acid or the trans-isomers of linoleic acid does not interfere with the utilization of linoleic acid as an essential fatty acid and there is no problem of essential fatty acid deficiency posed to consumers of commercial hydrogenated soybean oil products.

Many animal studies have demonstrated that the deposition of dietary trans-fatty acids in tissues and tissue lipids is selective. In vitro studies with liver mitochondrial and microsomal enzymes have demonstrated selectivity of action among fatty acid geometric and position isomers in the synthesis and hydrolysis of phospholipids and cholesterol esters. In a human subject labeled oleic and elaidic acids were rapidly exchanged with the fatty acids in the serum lipids. Analysis of human tissues at autopsy reflected the presence of trans-fatty acids in the diet.

In vitro experiments indicate that membrane functions can be affected by the incorporation of trans-fatty acids in experimental diets. Liver mitochondria isolated from rats fed elaidic acid exhibited two to threefold greater initial rates of swelling in hypotonic swelling media compared to
those from control rats. Erythrocytes containing trans-fatty acids hemolyzed at a rate five times greater than that of control cells when incubated with α-lecithinase. However, a long-term rat feeding test of a hydrogenated soybean-cottonseed oil containing 35 percent trans-acids showed no histopathology attributable to diet. Feeding hydrogenated oil of this composition to rats for 60 generations produced no adverse effects on fertility, litter size, weight at weaning, growth at 90 days, and longevity. A two-year rat feeding test of a hydrogenated soybean cooking oil and a control soybean oil revealed no differences in histopathology attributable to diet.

Considerable experimental evidence shows that polyunsaturated fatty acids in dietary fat as triglycerides from natural sources have a hypocholesterolemic effect, monounsaturated fatty acids are neutral and saturated fatty acids, particularly those 12 to 16 carbons in chain length, are hypercholesterolemic when they replace mixed carbohydrates on an isocaloric basis. Soybean oil, however, contains only traces of the 12 to 16 carbon unsaturated acids which can be converted to the corresponding saturated acids by hydrogenation, and it is in only some of the industrial shortenings that the stearic acid content is increased by hydrogenation. Although results of studies on the effect of trans-isomers on serum lipid levels in humans are not definitive, the weight of evidence indicates that trans-monoenoic acids, the principal geometric isomers present in hydrogenated soybean oil, are not hypercholesterolemic. Similarly, the results of animal experimentation indicate that trans-acids of hydrogenated soybean oil are not atherogenic at normal dietary levels.

In the light of the foregoing, the Select Committee concludes that:

There is no evidence in the available information on hydrogenated soybean oil that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when it is used as a direct or indirect food ingredient at levels that are now current or that might reasonably be expected in the future.
VI. REFERENCES CITED


11. Memorandum (on composition of margarines) dated March 27, 1974, from R. J. Dimler, U.S. Department of Agriculture, Northern Regional Research Laboratory, Peoria, Ill., to F. R. Senti, U.S. Department of Agriculture, Washington, D.C.


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