Biological studies on the nutritional value of edible fats.
Part III. Influence of selected fats on the morphological changes in various organs

by Swiatoslaw Ziemlanski, Teresa Opuszynska, and Stefan Krus

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Swietoslaw Ziemlanski, Teresa Opuszynska, Stefan Krus

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Biological studies on the nutritional value of edible fats. Part III. Influence of selected fats on the morphological changes in various organs.

From the Institute of physiology and biochemistry of nutrition in Warsaw. Manager: Assist. Prof. S. Ziemlanski M.D.

From the Department of pathological anatomy of the Institute of Biostructure, Academy of Medicine in Warsaw. Manager: Assist. Prof. St. Krus MD.

Authors' English abstract

In 300 Wistar rats in long-term experiments (7 and 12 months) the effects of rapeseed oil — liquid as well as hardened (orna) and experimental margarines (MA, MB, MC and MD) of different composition were studied (Table I). The content of erucic acid in these margarines ranged from 18% (MA) to 33% (MB). In liquid rapeseed oil it was 47.6% and in "orna" fat it was 37.1%. The animals received ad libitum experimental diets containing 15% by weight of one of these fats. One group of rats was kept on granulated laboratory chow "Bacutil" containing only 7% kcal of fat.

Gross and histological examinations of the liver, kidneys, spleen, adrenals, testicles, pancreas, myocardium and small intestine were carried out. The degree of fatty degeneration of these organs was studied, staining specimens with oil red O, fat red TB, and sudan III. The degree of fatty degeneration of the liver was determined using Thaler's staging system. No pathological changes were found in the organs examined, with the exception of the heart. In the myocardium of animals receiving liquid or hardened rapeseed oil and experimental margarines with higher contents of erucic acid in diet focal infiltrations with histiocytes and fibrosis were found. These changes increased in intensity after 12 months of the experiment (Table IV), e.g. in the group receiving liquid rapeseed oil after 7 months on diet in 8 of 9 rats (88.9%) cardiac changes were found, and after 12 months changes developed in 17 of 19 (89.5%) rats in this group. Such changes are an evidence of microfocal myocardial necrosis.

*Translator's note: the number in the right margin is the page number of the original text.
In long term experiments with rats of the variety Wistar, investigations were made into the effect on the occurrence of morphological changes in various organs produced by applying liquid and solidified rape oil and experimental margarines of different raw material composition and different contents of erucic acid. Occurrence of focal histiocytic infiltrations in the heart muscle and its focal fibroses was established.

In the first and second part of this work (1,2), general and methodical principles of the investigations conducted and the results of some physiological-biochemical and histo-chemical experiments, as well as changes of fractions of fats in the blood serum and the content of fatty acids and their trans-isomers in the lipids of the tissue of selected organs were presented. The purpose of this third part of the paper is to give the results of macroscopic and microscopic anatomo-pathological investigations of various organs and tissues of animals to which rape seed oil liquid or hydrogenated (fat "Oma") and also experimental margarines containing various quantities of rape seed oil were administered in their diet.

As follows from the results of investigations by many authors (3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18), rape seed oil administered to various experimental animals in their diet brings about a number of functional and structural changes in determined organs, in particular in the heart muscle.
Investigations of Roine et al. of 1960 (6), confirmed 7 years later by Rocquelin and Cluzan (8) and subsequently by Abdellatif and Vles (10, 11, 12) have shown that administering major quantities of rape seed oil to animals in the diet causes pathological changes in the heart muscle. These changes are characterized by the occurrence of microfocal histiocytic infiltrations and fibroses. Disorders of this kind are evidence of the forming of microfocal necrosis of the heart muscle.

The changes described are preceded by a fatty degeneration of the heart muscle. The intensity of the fatty degeneration is particularly great in very young rats. Fatty degeneration of the heart was found also in ducklings, guinea pigs, hamsters and rabbits (10, 11, 12).

Other anatomical disorders due to consumption of larger quantities of rape seed oil in the diet are: transient fatty degeneration of skeleton muscles in young rats, occurrence of vacuolation of the heart muscle, hydropericardium and occurrence of cirrhosis of the liver in young ducklings, splenomegaly in guinea pigs and widening of kidney ducts in rats. These changes occur when the content of rape seed oil amounts to about 50-60% of the total caloricity of the diet.

A lower content of rape seed oil in the diet does not result in such drastic deviations from the normal condition in experimental animals, nevertheless changes in the heart appear after application of not more than 30% kcal from rape seed oil in the diet.
Methods of investigation

A detailed description of the methods of investigation was presented in the first part of this paper (1,2). Long-term investigations of 7 and 12 months were conducted with Wistar rats of our own breeding.

Four kinds (A, B, C, D) of experimental margarines of different raw material composition (Table I) and different content of fatty acids were used for the investigations. Parallel, year-long studies were conducted with liquid rape seed oil and 6 months' experiments with solidified rape seed oil, i.e. the fat "Oma". The content of erucic acid in the margarines investigated varied from 18% (MA) to 33% (MB). In liquid rape seed oil, the content of erucic acid was 47.6% whereas in the fat "Oma" it was 37.1%.

TABLE I.
Composition of experimental margarines

<table>
<thead>
<tr>
<th>Type of margarine</th>
<th>Sunflower oil</th>
<th>Rape seed oil solidified</th>
<th>Palm oil hydrogenated</th>
<th>Fish oil hydrogenated</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of erucic acid</td>
<td>% of erucic</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>high-</td>
<td>low-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Margarine A (18.4%)</td>
<td>30</td>
<td>-</td>
<td>70</td>
<td>-</td>
</tr>
<tr>
<td>Margarine B (33.0%)</td>
<td>30</td>
<td>70</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Margarine C (20.3%)</td>
<td>30</td>
<td>40</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>Margarine D (22.9%)</td>
<td>30</td>
<td>40</td>
<td>-</td>
<td>30</td>
</tr>
</tbody>
</table>

The animals received ad libitum experimental diets containing 15% by weight of fat from margarines, liquid rape seed oil, the "Oma" fat, or butter as control fat. One group of animals obtained only the breeding granulated diet of the
"Bacutil" Co containing 7% kcal from fat.

The determination of the degree of fatty degeneration of the organs studied was performed by dyeing with a 0.5% solution of Oil Red 0 in 60% triethylphosphate. The time of dyeing the scraps was 10 min. at room temperature. The fat-Red 7B and Sudan III also were used. The degree of fatty degeneration of the liver was determined by the scale of Thaler (19).

According to Thaler, three degrees of fatty degeneration of the liver are distinguished: I - scattered fattening, with small drops of fat appearing in separate foci of cells on the background of the unchanged parenchyma; II - zone fattening, occupying a part of the lobules or whole lobules of the liver; III - diffusive fattening of the whole liver parenchyma.

The total sum of fatty degeneration of all the animals of the given experimental group was divided over the number of the specimens examined, thus obtaining the index of fatty degeneration of the liver.

Macroscopic and microscopic anatomo-pathological investigations included the following organs: liver, kidneys, spleen, supra-renal glands, testicles, pancreas, heart muscle and the small intestine.

The microscopic preparations for inspection were made following the commonly accepted method of dyeing 6μ paraffin strips with hematoxylin and eosin.
Results of investigations.

The analysis of the weight of the selected organs expressed as percentage of the body weight of the rat indicates differences between groups of animals receiving margarines and rape seed oil in the diet, in comparison with the control group receiving butter. Changes in the weight of the heart deserve particular attention. In the group MA, MB and that of liquid rape seed oil, after both seven and twelve months of experimenting the average values of weight of the heart were much higher as compared to the butter-fed control group.

After 12 months, the mean weight of the heart in the group MA was 1.64 g (% of body weight - 0.33), in group MB - 1.71 g (% of body weight - 0.36), in the group of rape seed oil - 1.62 g (% of body weight - 0.32) and in the butter-fed group 1.49 g (% of body weight - 0.31).

The weight of the kidneys also increased in the groups of animals MA, MB and in those receiving rape seed oil. In the group MA - 3.67 g (% of body weight - 0.78); in the group MB - 3.50 g (% of body weight - 0.75); in the group of rape seed oil - 3.72 g (% of body weight - 0.75) and in the butter-fed group - 3.27 g (% of body weight - 0.68). The weight of testicles showed distinct increase in the groups of animals receiving margarines A and B, after both 7 and 12 months. These changes were not found in the group fed rape seed oil. The average weight of testicles in the group MA was 3.58 g.
(% of body weight - 0.76), in the group MB - 3.52 g (% of body weight - 0.74) and correspondingly in the butter-fed group - 3.12 g (% of body weight - 0.65). In the groups MD and "Oma", underdevelopment of the spleen was established.

**Microscopic anatomo-nathological investigations**

Deviations from the normal condition in the microscopic structure of the kidneys, spleen, supra-adrenal glands, pancreas and in the skeleton muscles have not been established. Microscopic inspections of the liver (with the exception of cirrhotic changes in one rat of the group MC) did not show any deviations from the normal state.

Investigation of fatty degeneration of the liver with application of the Oil Red 0, fat Red 7B and Sudan III have proven that all the types of margarines investigated caused fatty degeneration changes in the liver; their intensity depends on both the type of fat administered in the diet and the length of duration of the experiment.

After 7 months of administering the experimental diets in the groups MA, MB and "Oma", the fatty degeneration of the liver was minimum and, in the majority of the animals studied, remained within the limits of values obtained for bred rats (MA - 0.53, MB - 0.93 and "Oma" - 0.96). Although the extension of feeding the diet to 12 months increased the
degree of liver fattening in animals of the groups MA and MB, the values obtained were still the lowest in comparison with the other types of the fats studied (1.46 and 1.33).

The margarines C and D, just like butter, caused after 7 months of experiments somewhat greater changes of fatty degeneration which became intensified after 12 months of administering the diet. This intensification of the changes in proportion to the length of the experiment becomes manifest with particular distinction in the group MD (1.33 after 7 months, 2.4 after 12 months).

The greatest changes, after both 7 and 12 months of experimenting, were determined in the group of animals obtaining liquid rape seed oil in the diet (index 2.5 and 2.1). A comparison of the established changes of fatty degeneration are shown graphically in Fig.1.

![Graph](image)

**Fig.1.** Fatty degeneration of the liver in experimental animals.
These data are also illustrated in Tables II and III.

Microscopic inspections of the heart muscle have shown distinct deviations from the normal condition. In the heart, the occurrence of focal changes having the character of granulomas, i.e. histiocytic infiltrations and focal fibroses, in animals fed various kinds of experimental margarines was established. The greatest changes were determined in the group fed liquid rape seed oil and in the group MB containing in the diet the largest quantities of erucic acid. These changes can be interpreted as conditions of inflammatory and necrotic character.

Table IV and examples of photos of microscopic preparations (Fig. 2, 3, 4) illustrate the deviations from the normal state found in the heart muscle of the animals studied.
Table II

Fatty degeneration of the liver in experimental animals after 7 and 12 months

<table>
<thead>
<tr>
<th>Type of fat</th>
<th>7 months</th>
<th></th>
<th></th>
<th></th>
<th>12 months</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>number of rats</td>
<td></td>
<td></td>
<td></td>
<td>number of rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>with fatty degeneration of liver</td>
<td></td>
<td></td>
<td></td>
<td>with fatty degeneration of liver</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>in the limits of group control</td>
<td></td>
<td></td>
<td></td>
<td>in the limits of group control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Masło Butter</td>
<td>15</td>
<td>14</td>
<td>5</td>
<td>8</td>
<td>2</td>
<td>1,73</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Margaryna A</td>
<td>15</td>
<td>7</td>
<td>14</td>
<td>1</td>
<td>—</td>
<td>0,53</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>Margaryna B</td>
<td>14</td>
<td>9</td>
<td>10</td>
<td>4</td>
<td>—</td>
<td>0,93</td>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td>Margaryna C</td>
<td>15</td>
<td>14</td>
<td>5</td>
<td>8</td>
<td>2</td>
<td>1,03</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Margaryna D</td>
<td>15</td>
<td>11</td>
<td>6</td>
<td>9</td>
<td>—</td>
<td>1,33</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Olj rzepakowy</td>
<td>9</td>
<td>9</td>
<td>2</td>
<td>1</td>
<td>6</td>
<td>1,50</td>
<td>19</td>
<td>19</td>
</tr>
</tbody>
</table>

Note: The table shows the number of rats and the degree of fatty degeneration of the liver in the liver of experimental animals after 7 and 12 months of the experiment.
TABLE III

% of established cases of II and III degree of fatty degeneration of the liver in rats receiving various fats in their diet.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Animals</th>
<th>% of fatty degeneration of II and III degree after 7 months</th>
<th>Number of Animals</th>
<th>% of fatty degeneration of II and III degree after 12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Margarine A</td>
<td>15</td>
<td>7%</td>
<td>15</td>
<td>47%</td>
</tr>
<tr>
<td>Margarine B</td>
<td>14</td>
<td>7%</td>
<td>15</td>
<td>53%</td>
</tr>
<tr>
<td>Margarine C</td>
<td>15</td>
<td>8%</td>
<td>15</td>
<td>80%</td>
</tr>
<tr>
<td>Margarine D</td>
<td>15</td>
<td>60%</td>
<td>15</td>
<td>100%</td>
</tr>
<tr>
<td>&quot;Oma&quot; *</td>
<td>29</td>
<td>31%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Butter</td>
<td>15</td>
<td>67%</td>
<td>15</td>
<td>87%</td>
</tr>
<tr>
<td>Rape seed oil</td>
<td>9</td>
<td>78%</td>
<td>19</td>
<td>74%</td>
</tr>
</tbody>
</table>

* data for the group of animals receiving in their diet the fat "Oma" were obtained after 6 months.

TABLE IV

Frequency of occurrence of granulomas in the heart of rats obtaining fats at the level of 30% kcal in the diet.

<table>
<thead>
<tr>
<th>Group</th>
<th>Total number of animals</th>
<th>Number of animals with changes after 7 months</th>
<th>Total number of animals</th>
<th>Number of animals with changes after 12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breeding</td>
<td>15</td>
<td>0</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>Butter</td>
<td>14</td>
<td>2 (14.3%)</td>
<td>24</td>
<td>2 (8.3%)</td>
</tr>
<tr>
<td>Margarine A</td>
<td>15</td>
<td>1 (6.7%)</td>
<td>25</td>
<td>8 (32.0%)</td>
</tr>
<tr>
<td>Margarine B</td>
<td>15</td>
<td>7 (46.7%)</td>
<td>22</td>
<td>11 (50.0%)</td>
</tr>
<tr>
<td>Margarine C</td>
<td>14</td>
<td>4 (28.6%)</td>
<td>24</td>
<td>11 (45.8%)</td>
</tr>
<tr>
<td>Margarine D</td>
<td>15</td>
<td>1 (6.7%)</td>
<td>23</td>
<td>11 (47.0%)</td>
</tr>
<tr>
<td>Rape seed oil</td>
<td>9</td>
<td>8 (88.9%)</td>
<td>19</td>
<td>17 (89.5%)</td>
</tr>
<tr>
<td>&quot;Oma&quot;</td>
<td>29</td>
<td>22 (75.9%)</td>
<td>not investigated</td>
<td></td>
</tr>
</tbody>
</table>

The data obtained indicate that the greatest changes occurred in the group of animals receiving liquid and solidified rape seed oil. After 7 months of experiments, in
as many as 8 out of 9 animals fed 15% by weight of rape seed oil in the diet, occurrence in the heart muscle of numerous small granulomas, i.e. 88.9% was noted; in the group of animals receiving the fat "Oma", in 22 animals (75.9%) of 29 animals examined, changes of varying intensity in the heart having the character of granulomas were established. In the group of animals obtaining margarine B, the same changes were found in 7 out of 15 rats examined, i.e. 46.7%. In the remaining groups of animals these changes occurred to a lesser degree; in the group MC in 7 out of 14 rats i.e. 28.6%, in the groups MD and MA one case among 15 animals of each group was determined i.e. 6.7%.

*Translator's note: 28.6% not clear to me.*
Fig. 2. Heart muscle. Dark sections of the fibers in the upper top of the picture are proof of necrosis or necrobiosis of the muscle. Histiocytes are accumulated in the vicinity. H.E. magn. 300 x.

Fig. 3. Granuloma composed of histiocytes among muscle fibres. H.E. magn. 300x.

Fig. 4. Fibrosis of the heart muscle (centre of picture), remains of histiocyte infiltration (centre of picture on left). H.E. magn. 300x.

The histopathological picture becomes somewhat intensified after 12 months of administering experimental margarines or rape seed oil. The greatest intensification of the changes (89.5%) was noted in the group of animals obtaining rape seed oil. These data are illustrated in Table IV.

In the control group, i.e. the breeding group, pathological changes were not observed after experiments
lasting 7 and 12 months.

In the group of animals obtaining butter, 2 cases of occurrence of small granulomas for 14 rats examined (i.e. 14.3%) were established after 7 months, and 2 cases for 24 rats (i.e. 8.3%) after 12 months.

In many animals of all the groups studied, occurrence of changes in the mucous membrane of the intestine was noted; these consisted in shortening and widening of the villi, infiltration of acid-absorbing lymphocytes and granulocytes in the stroma of the villi, and fibrosis of the villi. These changes became intensified after 12 months of experiments. This problem requires further investigation, in order to clarify whether the changes found in rats occur with age, or whether the quantity and quality of the fat and the kind of diet have a direct influence on the occurrence of this kind of disorders.

Discussion of the results

It follows from the macroscopic and microscopic anatomo-pathological examinations of various organs of rats, to which experimental margarines of various composition as well as liquid and solidified rape seed oil were administered in the diet, that the most substantial deviations from the normal conditions were established in the heart muscle. There is a strict relationship between the occurrence of morphological changes in this organ and the content of erucic acid in the diet.

*Translator's note:
page 693 - Russian abstract - omitted.
English abstract - see first page of translation.
The occurrence in the heart muscle of focal necrotic changes should be connected with the impossibility of a regular metabolic conversion of the erucic acid. Investigations of many authors (3,6,10,11,12,17,18) and our own (unpublished data) indicate the occurrence of fatty degeneration of the heart and accumulation of erucic acid in this organ. These changes appear in the first place in young animals. It is worth noting that Beare-Rogers (18) achieved fatty degeneration of the heart muscle in young animals also after application of larger quantities of fish fat, the so called herring oil, in the diet. This fat is characteristic by its large content of monounsaturated long-chain fatty acids. However the fatty degeneration of the heart never reaches as high intensity as after administering rape seed oil in the diet.

The appearance in the heart muscle of microfocal granulomas, similar to those which occur after application of rape seed oil in the diet, was determined by us in our investigations after administering to the animals in the diet a major quantity of the theobroma oil-like fat used in some countries as a substitute for cocoa butter in the manufacture of chocolate products.

One can conclude hence that microfocal necrotic changes of the heart muscle may occur as a result of inferior metabolic conversion of certain fats and oils. The mechanism of these changes is the subject of further investigations.
Literature

(For translated items see below)


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