In a previous paper (1) it was reported that butter fat homogenized into mineralized skim milk gave better growth of weanling rats than certain vegetable oils homogenized into skim milk and fed under the same conditions with ample carotene, irradiation, \(\alpha\)-tocopherol and minerals added in all cases. The superiority of butter fat for growth was not found to be due to factors contained in the non-saponifiable fraction of butter or to be due to compounds such as lecithin, choline, sphingomyelin or sphingosine (2). It was then thought advisable to separate the fatty acids of butter into various fractions and feed the glycerol esters of these fractions along with a vegetable oil in concentrations approximately equal to that found in butter fat.

**EXPERIMENTAL**

The fatty acid fractions of the butter fat were prepared as follows: Five hundred grams of melted butter fat were poured into 1000 cc. of 20 per cent alcoholic potassium hydroxide solution and heated on the steam bath for about one-half hour. The alcohol was evaporated off in an open dish, the soaps dissolved in water and the separations made similar to a procedure described by Hilditch and Jones (3). The solution was neutralized with sulfuric acid and steam distilled until about 5 liters of distillate were collected. The volatile acids were extracted from the distillate by 5 successive extractions with ether. The non-volatile fraction was washed thoroughly with hot water and dissolved in 2000 cc. of alcohol. The alcohol solution was heated almost to boiling and 200 gm. of lead acetate crystals added. The solution was allowed to cool and the insoluble lead soaps filtered off and recrystallized from alcohol. After removing, by vacuum distillation, the alcohol from the soluble lead soaps, which constitutes most of the unsaturated fatty acids, the material was washed with hot water and the free fatty acids regenerated by adding dilute sulfuric acid until the solution was distinctly acid to congo red. The acids were separated from the water and precipitated lead sulfate, washed with hot water and taken up in ether to remove the last traces of lead sulfate. The insoluble lead soaps which constitute most of the saturated acids but still contaminated...
with some of the unsaturated acids were treated in exactly the same manner as the unsaturated acids and finally taken up in ether to remove the last traces of lead sulfate. The ether was removed from the various fractions under vacuum. Five hundred gm. of butter fat yielded 36.5 gm. of volatile acids, 180 gm. of the unsaturated acid fraction and 200 gm. of the saturated fraction.

The triglycerides were synthesized as follows (4). The fatty acids from each fraction were placed in a suitable round bottom distilling flask and the theoretical amount of glycerol added as calculated from the fatty acid composition of each fraction (3). The flask containing the fatty acids and glycerol was placed in an oil bath and heated to 200° C. for six hours with a fine stream of carbon dioxide passing through the mixture. The glycerides of each fraction were then mixed with corn oil (Mazola) in the following proportions:

<table>
<thead>
<tr>
<th>Triglycerides of volatile acids</th>
<th>corn oil</th>
<th>Total</th>
<th>Corn oil plus</th>
<th>unsaturated</th>
<th>unsaturated</th>
<th>saturated</th>
<th>saturated</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 parts</td>
<td>93 parts</td>
<td>100</td>
<td>60</td>
<td>40</td>
<td>60</td>
<td>45</td>
<td></td>
</tr>
</tbody>
</table>

These are approximately the proportions of the different fractions isolated from the butter.

Weanling rats about 20 days old and weighing 30 to 35 gm. were used. Six rats, 3 males and 3 females, were placed on each fraction to be tested and kept in individual cages. Five groups were set up and fed as follows, ad libitum:

<table>
<thead>
<tr>
<th>Group</th>
<th>Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Butter fat milk</td>
</tr>
<tr>
<td>II</td>
<td>Corn oil milk</td>
</tr>
<tr>
<td>III</td>
<td>Corn oil plus volatile fraction milk</td>
</tr>
<tr>
<td>IV</td>
<td>&quot; &quot; unsaturated &quot; &quot;</td>
</tr>
<tr>
<td>V</td>
<td>&quot; &quot; saturated &quot; &quot;</td>
</tr>
</tbody>
</table>

The fats were homogenized into fresh skim milk with a small hand homogenizer and all milks were made up to contain 4 per cent fat. Twenty micrograms of carotene were added to each gram of fat and 100 micrograms of \( \alpha \)-tocopherol acetate\(^2\) were given to each rat every week. All rats were irradiated 10 minutes each day. All milks were mineralized with iron, copper and manganese so that each 100 ce. of milk contained 1.5 mg. of iron, 0.15 mg. copper and 0.15 mg. of manganese.

The results obtained are illustrated in figures 1 and 2 (Experiment 17) and figures 3 and 4 (Experiment 19). The animals on corn oil plus the saturated fraction of butter fat (Group V) grew faster than the animals on butter fat and considerably faster than the animals on corn oil, or on the volatile or unsaturated fractions. The data in figures 1 and 2 do not

\(^2\) We are indebted to Hoffmann LaRoche, Inc., Nutley, New Jersey, for generous supplies of dl-\( \alpha \)-tocopherol acetate.
include the unsaturated fraction because shortly after mixing the tri-glycerides with corn oil the mixture began to thicken and develop an odor somewhat like paint. The animals on this fraction failed to grow. In the second trial, Experiment 19, figures 3 and 4, the fractions from butter were mixed with corn oil just before being homogenized into the milk. The males on butter fat in Experiment 17, figure 2, made an average gain of 76 gm., on corn oil 65 gm., on the volatile fraction 57 gm., and on the saturated fraction 80 gm. in three weeks. The females on butter fat in this experiment made an average gain of 63 gm., on corn oil 52 gm., on the vola-
tile fraction 56 gm., and on the saturated fraction 68 gm. in 3 weeks. The males on butter fat in Experiment 19, figure 4, made an average gain of 70 gm., on corn oil 63 gm., on the volatile fraction 48 gm., on the unsaturated fraction 63 gm., and on the saturated fraction 82 gm. gain in 3 weeks. The females on butter fat gained an average of 66 gm., on corn oil 47 gm., on the volatile fraction 51 gm., on the unsaturated fraction 55 gm., and on the saturated fraction 73 gm. in 3 weeks. The general appearance and condition of the fur coat of the animals on the saturated fraction was better during the first two or three weeks on the experiment than the other groups.
However, after this time there was very little difference between the groups in appearance.

Food consumption records indicate that the animals on the saturated fraction did not make more efficient gains than animals on butter. That is, growth paralleled consumption very closely. However, we have found that the faster-growing animals usually made slightly more efficient gains than animals growing at a slower rate and these differences appeared between groups of rats on butter fat and vegetable oils during the first two or three weeks of the experiment.

DISCUSSION

The results obtained indicate that the superiority of butter fat fed in the milks is due to fatty acids contained in the saturated fraction of butter fat. It also appears that the superiority is due to long chain saturated fatty acids. The saturated fraction was found to have an iodine number of 9.2, showing that some unsaturated fatty acids still remained. However, if the superiority of growth was due to unsaturated fatty acids then it appears that corn oil or the unsaturated fraction of butter fat should have supplied the necessary fatty acids. Hilditch and Jones (3) found the saturated fatty acid fraction of butter fats to have an iodine number of 10 and believed that the unsaturated fatty acids in the saturated fraction were only those carried over in the separation. However, no final proof can be given that an unsaturated fatty acid, being insoluble as the lead soap, is not responsible for the superiority until the saturated fraction is completely hydrogenated and fed.

No final explanation can be offered for the observation that better growth was obtained with the saturated butter fraction and corn oil as compared with butter alone. It is probable that the fatty acids which are responsible for the superiority of butter over vegetable oils are present in larger amounts in butter fat, and in the mixture of corn oil and the saturated fraction from butter fat an increased amount of saturated fatty acids of high molecular weight made possible the accelerated growth.

CONCLUSIONS

1. The fatty acids responsible for the superior growth of young rats obtained on butter fat as compared with certain vegetable oils homogenized into skim milk with all of the known essential fat soluble vitamins added, apparently lie in the saturated fraction of butter fat.

2. When the fatty acids of butter fat were separated into the volatile acids by steam distillation and into the unsaturated and saturated acids as lead soaps and the triglycerides of these fractions fed in corn oil in approximately the composition found in butter the saturated fraction with
corn oil was found to be a little superior to butter fat while the other two fractions compared favorably with corn oil.

REFERENCES


