THE INFLUENCE OF INGESTED MINERAL OIL UPON THE DEVELOPMENT OF AN ESSENTIAL FATTY ACID DEFICIENCY IN THE RAT

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FIVE FIGURES

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Scientific literature contains many references to the influence of ingested mineral oil upon the absorption of certain of the vitamins. As early as 1927, Dutcher et al. showed that a mixture of mineral oil with butterfat was ineffective in curing vitamin A-deficient rats, and additional work by this group (Dutcher et al., '33, '34) attributed this result to the solvent action of mineral oil on carotene. This effect of mineral oil on carotene utilization has been confirmed with both rats and man by a number of other workers (Alexander et al., '47; Andersen, '38; Collison et al., '29; Curtis and Kline, '39; Curtis and Ballmer, '39; Jackson, '31, '34a; Mahle and Patton, '47; Mitchell, '33; Moore, '29; With, '39, '40, '42; Burns et al., '51).

1 Portions of the material contained in this paper have been taken from a thesis submitted by E. Kyle Bacon in partial fulfillment of the requirements for the degree of Master of Science in Biochemistry and Nutrition, and from a dissertation submitted by Samuel M. Greenberg in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biochemistry and Nutrition, in the Graduate School of the University of Southern California.

Contribution 303 from the Department of Biochemistry and Nutrition, University of Southern California, Los Angeles.
In the case of vitamin A itself (in alcohol or ester form) somewhat conflicting results have been obtained. Moness and Christiansen ('29) could find no difference in the vitamin A activity of a cod liver oil concentrate whether administered in mineral oil or in olive oil. Alexander and associates ('47) likewise failed to show any adverse action of mineral oil on the absorption of vitamin A. Other workers have, however, presented considerable evidence that the absorption of vitamin A is impaired by mineral oil ingestion in both the rat and man, although to a lesser degree than is the case with carotene (Andersen, '39a, b; Curtis and Horton, '40; Hawk et al., '29; Smith and Spector, '40a; With, '39, '40).

Vitamin D has received less attention. The reports of earlier workers (Dutcher et al., '27; Jackson, '34b) showed no adverse action; however, studies by Smith and Spector ('40a, b, c) have indicated that in both rats and dogs mineral oil interferes markedly with the absorption of this vitamin. Elliott et al. ('40) and Lassen et al. ('48) have noted an accelerating action of ingested mineral oil upon the development of a vitamin K deficiency in rats. A similar observation was made by Barnes ('42) with mice, while Javert and Macri ('41) observed the same action in man. No reports have been made concerning the effect of ingested mineral oil on vitamin E, although Smith and Spector ('40a) did report an impairment of fertility in rats receiving 10% mineral oil in the diet. Although these workers did not so conclude, this impairment may have been due to a vitamin E deficiency, since Lassen and associates ('48) found evidence of a possible vitamin E deficiency in adult male rats receiving as little as 5% mineral oil in their ration.

Since the effect of mineral oil upon the absorption of some fat-soluble vitamins seems well established, it was thought of interest to extend these studies to determine whether or not mineral oil also interferes with the absorption of fatty acids. If so, it is conceivable that mineral oil could be used to accelerate the development of an essential fatty acid deficiency. Such a procedure would be highly desirable in view of the long depletion period required at present. Burr and co-workers
were able to produce an essential fatty acid deficiency in weanling rats fed a fat-free diet, in from three to 5 months, with skin symptoms developing in 70 to 90 days but without cessation of weight gain in some cases until the animals were 5 months of age (Brown and Burr, '36; Burr and Burr, '29, '30; Burr et al., '30-'31, '32; Burr and Beber, '37). Other workers have obtained satisfactory depletion in terms of cessation of growth in somewhat shorter periods (Bailey, '43; Martin, '39; McKibbin et al., '39; Quackenbush et al., '39; Quackenbush and Steenbock, '42; Quackenbush et al., '42; Sinclair, '40). Deuel and collaborators ('50) obtained a plateau of weight with male rats after depletion periods of 10 to 12 weeks on a diet very low in fat. Repeated efforts to reduce the last traces of fat in the diet by extraction of the casein, which constituted the only source of fat contamination, failed to reduce the depletion time, indicating that the trace of fat in the diet was not the limiting factor in the appearance of the deficiency symptoms.

The experiments reported below were carried out for the purpose of determining the effect which mineral oil might have on the development of an essential fatty acid deficiency in rats.

METHODS

The composition of the diets is given in table 1. Supplements of the fat-soluble vitamins were given weekly by intraperitoneal injection. In series A to D, vitamins A, D, and E were administered in an ethyl laurate solution and menadione was injected subcutaneously in propylene glycol. All vitamins were given intramuscularly in a propylene glycol solution in series E. The daily dosages were as follows: vitamin A alcohol, 14 µg; vitamin D₂, 0.11 µg; vitamin E, 0.4 mg; and vitamin K (2-methyl-1,4-naphthoquinone), 0.075 mg.

The rats were housed in metabolism cages having wire bottoms so arranged that urine and feces could be collected separately. Analyses were made of the feces only in series A for the first three weeks of the tests. The feces were collected daily and stored in a refrigerator under anhydrous ethyl
### Table 1

**Percentage composition of diets**

<table>
<thead>
<tr>
<th>COMPONENTS</th>
<th>DIET 1 (basal)</th>
<th>DIET 2</th>
<th>DIET 3</th>
<th>DIET 4</th>
<th>DIET 5</th>
<th>DIET 6</th>
<th>DIET 7</th>
</tr>
</thead>
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<tr>
<td>Vitamin-test casein (1)</td>
<td>20.0</td>
<td>20.0</td>
<td>20.0</td>
<td>20.0</td>
<td>20.0</td>
<td>20.0</td>
<td>20.0</td>
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<tr>
<td>Sucrose</td>
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<td>71.0</td>
<td>69.0</td>
<td>67.5</td>
<td>66.0</td>
<td>63.5</td>
<td>61.0</td>
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<tr>
<td>Cellulose (3)</td>
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<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
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<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
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<tr>
<td>Water-soluble vitamins (5)</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Sulfasuxidine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Streptomycin (6)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Mineral oil</td>
<td></td>
<td>2.0</td>
<td>3.5</td>
<td>5.0</td>
<td>7.5</td>
<td>10.0</td>
<td></td>
</tr>
</tbody>
</table>

1 General Biochemicals, Inc.
2 Casein replaced with spray-dried casein hydrolysate and fortified with 0.5% L-tryptophan in diet 1a.
3 Cellulose obtained from Chicago Dietetic Supply House.
4 Osborne-Mendel salt mixture.
5 The water-soluble vitamin mixture had the following percentage composition:

- Series A and C — choline and inositol, each 12.0; para-aminobenzoic acid, 6.0; thiamine chloride hydrochloride, 0.72; calcium pantothenate, 0.67; niacin, 0.60; riboflavin and pyridoxine, each 0.27; folic acid, 0.10; biotin, 0.02; and sucrose, 67.6%.
- Series B and D — choline, 12.0; inositol, 5.0; para-aminobenzoic acid, 2.0; riboflavin and thiamine chloride hydrochloride, each 0.72; calcium pantothenate, 0.67; pyridoxine, 0.27; folic acid and niacin, each 0.10; biotin, 0.01%; and sucrose, 78.41%.

In series E, the amounts of water-soluble vitamins were double those used in series A and C.

We wish to thank Hoffmann-La Roche, Inc., for the biotin, Lederle Laboratories for the folic acid, and Merck and Co., Inc. for the other synthetic vitamins.

6 Kindly supplied by Parke-Davis and Company.

Weanling male albino rats, obtained from our stock colony, were used for the tests. The experiments were continued for as long as 15 weeks in some cases, but in most instances they had a considerably shorter duration. A commercial mineral oil \(2\) (heavy California liquid petrolatum) was used.

2 Water consumption was likewise determined in two experiments (series A and C).

2 Formula 3A (anhydrous ethyl alcohol denatured with 5% methyl alcohol).

2 Squibb.
RESULTS

Effect on growth

The results of the 5 series of tests are recorded in table 2. Although there was no shortening of the period required for depletion in essential fatty acids when 2 or 3.5% of mineral oil was incorporated in the diet (groups 2 and 3), the time required was appreciably shortened when the diet contained 5% of mineral oil (group 4). The usual period of 11 to 12 weeks necessary to produce the essential fatty acid deficiency in the present tests was lowered to three to 4 weeks in series A, but it was 6 to 8 weeks in series C (group 11) and 10 to 11 weeks in the case of series E (group 20). It is believed that these variations may be ascribed to variations in the body stores of essential fatty acids at the beginning of the experiment. When the level of mineral oil was increased to 7.5% (diet 5), the period of depletion was approximately 4 weeks (series D, group 14, and series E, group 22). When the mineral oil was increased to 10%, the depletion period had a duration of only two to three weeks.

Figure 1 gives typical growth curves with the several levels of mineral oil (series B), while figure 2 records the results of series E.

Symptoms of deficiency

The symptoms obtained on diets 1, 2, and 3 were typical of those previously observed in essential fatty acid deficiency. Additional symptoms were, however, observed with diets 4, 5, and 6. These were symptoms ordinarily associated with a biotin deficiency. For example, in the case of group 11, in addition to the failure to grow, the rats exhibited an alopecia, particularly on the head, and priapism was present in 9 of the 10 rats in the group. There were likewise brown, crusty skin lesions, particularly on the paws, and a “spectacle eye” condition. These conditions are illustrated in figures 3 and 4.

Another symptom, which was particularly pronounced in the rats on the high levels of mineral oil, was increased water consumption. Hove and Harris ('46) have shown that in-
### Table 2

**The effect of mineral oil in causing depletion of essential fatty acids**

<table>
<thead>
<tr>
<th>SERIES</th>
<th>GROUP NO.</th>
<th>DIRT NO.</th>
<th>MINERAL OIL</th>
<th>NUMBER OF RATS</th>
<th>LINOLEATE GIVEN DAILY</th>
<th>CESSION OF GROWTH</th>
<th>REMARKS</th>
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<td>A</td>
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<td>1</td>
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<td>(20) *</td>
<td>11-12</td>
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<tr>
<td></td>
<td>2</td>
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<td>10</td>
<td>0</td>
<td>11-12</td>
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</tr>
<tr>
<td></td>
<td>3</td>
<td>3</td>
<td>3.5</td>
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<td>11-12</td>
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<tr>
<td></td>
<td>4</td>
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<td>5.0</td>
<td>10</td>
<td>0</td>
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<tr>
<td>B</td>
<td>5</td>
<td>4</td>
<td>5.0</td>
<td>9</td>
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<td></td>
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<td></td>
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<tr>
<td></td>
<td>7</td>
<td>6</td>
<td>10.0</td>
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<td>0</td>
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<td>7.5</td>
<td>9</td>
<td>50</td>
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<tr>
<td>E</td>
<td>19</td>
<td>1</td>
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<td>10</td>
<td>0 (50)</td>
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<td></td>
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<tr>
<td></td>
<td>20</td>
<td>4</td>
<td>5.0</td>
<td>10</td>
<td>0</td>
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<td>21</td>
<td>4</td>
<td>5.0</td>
<td>10</td>
<td>50</td>
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</tr>
<tr>
<td></td>
<td>22</td>
<td>5</td>
<td>7.5</td>
<td>10</td>
<td>0</td>
<td>4-5 *</td>
<td></td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>5</td>
<td>7.5</td>
<td>10</td>
<td>50</td>
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</table>

*Administered orally unless stated otherwise.
*Given after depletion to 4 rats, with partial resumption of growth and cure of skin symptoms.
*Multiple deficiency symptoms (both essential fatty acid and "biotin").
*Group 11, after depletion, divided into two new groups and fed new diets without mineral oil.
*Intraperitoneally.
creased water consumption parallels the appearance of external symptoms of fatty acid deficiency in the rat. The amount of water consumed rose sharply when mineral oil levels higher than 2% were included in the diet. Group 4, receiving 5% of mineral oil in the diet, consumed 350 ml of water per day per kilogram body weight at the end of the second week, while at the same time group 3, receiving 3.5% mineral oil, had a water intake of 227 ml per day per kilogram. On the other hand, the
rats on the fat-free basal diet, and those in group 2 on the 2% mineral oil diet, which had not developed deficiency symptoms at this early period, had approximately equal water consumptions of about 160 ml per day per kilogram. A comparison of

Fig. 2 Mean weight gain of groups of animals in series E, showing the effect of linoleate supplements. The group number is shown on the chart. No. 19 — no mineral oil, linoleate (50 mg) started at the end of 7 weeks. No. 20 — 5% mineral oil in diet. No. 21 — 5% mineral oil in diet, supplemented with 50 mg methyl linoleate per day. No. 22 — 7.5% mineral oil in diet. No. 23 — 7.5% mineral oil in diet, supplemented with 50 mg methyl linoleate per day.

water intake in rats on the fat-low basal diet without and with 5% mineral oil is illustrated in figure 5. The effect of the injection of methyl linoleate on the water consumption of group 12, which also received the 5% mineral oil diet, is also illustrated. Since the food consumption is not significantly changed
Fig. 3  This illustrates the "spectacle eye" condition which occurred in practically all of the rats receiving 5% or more of mineral oil in the diet.

Fig. 4  This photograph illustrates the denuded condition of the skin and the priapism. The brown, crusty skin lesions are not clearly evident except in color.
by the inclusion of mineral oil in the diet, and since the effect on water consumption is reversed by linoleate, this effect seems to be specifically related to the essential fatty acid deficiency.

Fig. 5  The average water consumption of male rats (expressed in milliliters per kilogram per day) on fat-low basal diet (group 10), on 5% mineral oil diet (group 11), and on 5% mineral oil diet but also receiving 50 mg methyl linoleate by intraperitoneal injection (group 12) over 12 weeks. Group numbers are indicated on the chart.

Effect of mineral oil on composition of feces

In order to obtain information as to the effect of the inclusion of mineral oil in the diet on the lipid composition of the feces, studies were made of the unsaponifiable fraction, total fatty acids and iodine number of the fatty acids of samples of feces collected during the first three weeks of the experiment in series A.

Composite group samples of the rat feces, representing the total feces excreted by 10 rats over a period of one week, were
dried, ground, and saponified with 0.5 N alcoholic KOH for 30 minutes. The saponified mixture was filtered and the unsaponifiable fraction separated by repeated extractions with petroleum ether (B.P., 30 to 60°C.). The residue was then acidified and the liberated fatty acids were extracted with petroleum ether. The solvent was removed from both fractions at low temperature under nitrogen and the residues were weighed. The data are recorded in Table 3.

**DISCUSSION**

The results reported in the above experiments demonstrate that mineral oil, added to a fat-low diet such as is usually used to produce an essential fatty acid deficiency, accelerates the development of the deficiency syndrome. This acceleration was particularly noticeable when the fat-low basal diet con-
tained 5% or more of mineral oil, while at levels of 2% and 3.5% mineral oil the effect was less noticeable.

The water consumption, measured in experiments A and C, showed that the groups receiving mineral oil in amounts of 5% had an abnormally high water consumption early in the experiments, before other deficiency symptoms were observed. This suggests that a rapid quantitative method might be worked out, based upon water consumption as a criterion of incipient essential fatty acid deficiency. Such a method would require that due regard be given to the humidity and temperature of the environment.

The results of the analysis of the rat feces in experiment A (table 3) show that, as the level of mineral oil in the fat-low diet is increased, the amount of metabolic fat (expressed as fatty acids) lost with the feces also increases. In addition, the metabolic fat lost with the feces by the group receiving 5% mineral oil in the diet (group 4) was more unsaturated than in the case of the group receiving the fat-low diet (group 1). From this, it appears that one action of the mineral oil is that of removing a greater proportion of unsaturated fatty acids from the rats. However, the results obtained in experiments C and E, using the oral or parenteral route, in preventing the growth-inhibiting action of mineral oil, would indicate that the solvent action of mineral oil cannot provide an adequate explanation of the effect. Furthermore, these results and the failure of sulfasuxidine and streptomycin to speed up the onset of the essential fatty acid deficiency (experiment B) may indicate that no significant amount of essential fatty acids is synthesized by the intestinal flora.

While typical essential fatty acid deficiency symptoms were observed in these experiments with mineral oil, there were other symptoms (the "spectacle eye," alopecia, the humped posture, and the spastic gait) which suggested also a biotin deficiency. An attempt to correct this deficiency by doubling the biotin content of the diet was made in experiment E, and in fact the entire B complex vitamin content was doubled in order to eliminate the possibility of any shortage of the other
B vitamins. This addition did not, however, prevent the appearance of, or alleviate any of, these biotin-like deficiency symptoms. Inasmuch as the amount of biotin given these rats is many times that usually contained in a normal rat diet, we may conclude that they were not biotin deficiency symptoms, or that the mineral oil contained in the diet interferes with the rat's utilization of biotin. That the second of these assumptions is the more probable is indicated by the work of Mackay and Barnes ('41), who reported an apparent sparing action of corn oil fatty acids upon biotin. Our experiment also indicates that there is a relationship between biotin and the essential fatty acids. It has been pointed out by a reviewer that our diets are almost certainly low in B12. This may also have contributed to the development of multiple deficiency symptoms.

**SUMMARY**

Mineral oil, at levels of 2, 3.5, 5, 7.5 and 10%, was added to a fat-low diet which under ordinary conditions will result in a fatty acid deficiency and cessation of growth in 11 to 12 weeks. Below 5%, mineral oil did not result in cessation of growth earlier than 11 to 12 weeks, but the higher levels of mineral oil resulted in growth failure after a shorter period (two to three weeks on 10% mineral oil). In addition to earlier cessation of growth, other symptoms of fatty acid deficiency appeared, including increased water consumption. The symptoms could be prevented by the inclusion of 50 mg of linoleate per day.

The fecal excretion of total fatty acids was found to be increased by the presence of mineral oil in the diet, and the iodine number of the fecal fatty acids was increased by mineral oil. A purely solvent action of mineral oil apparently cannot be accepted, however, since the deficiency symptoms were prevented by either oral or intraperitoneal linoleate.

In addition to typical symptoms of essential fatty acid deficiency, the animals on diets containing 5% or more mineral oil developed symptoms suggestive of a biotin deficiency. It
was not possible to correct these symptoms by increasing the biotin content of the diet in the absence of essential fatty acids.

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