hydrogenated peanut oil or hydrogenated whale oil or with no fat in the diet. The animals in these groups also had the slowest growth rate.

5. The increased calorie intake could not be due merely to an increased evaporation of water in the animals reared on hydrogenated oil diets or fat-free diets.

6. The external signs of the animals fed hydrogenated peanut oil or hydrogenated whale-oil diets resembled the classical skin signs seen in animals on fat-free diets. Haematuria was found on the hydrogenated peanut-oil as well as on the fat-free ration.

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The Role of Fat in the Diet of Rats

4. Influence of Supplementation with Raw Skim Milk, Linoleic Acid or both on Growth

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In continuation of previous studies, it was the purpose of the experiments reported here to study further the growth-promoting effect of raw skim milk, especially in connexion with hydrogenated dietary fat (Aaes-Jørgensen & Dam, 1954*a*, *b*), and further to compare the effect of raw skim milk with dietary supplements of linoleic acid. Young female rats were used because they require a smaller amount (about 20 mg) of linoleic acid/animal/day (Greenberg, Calbert, Savage & Deuel, 1950), whereas the optimum level of linoleate required by fat-depleted male rats exceeds 200 mg/day (Deuel, Greenberg, Anisfeld & Melnick, 1951). The latter workers stated further that the requirements of essential fatty acids are apparently increased with the concomitant ingestion of fat.

EXPERIMENTAL

Newly weaned female rats were distributed in twenty-four groups of six animals. In Table 1 is shown the composition of the diets used together with the drinking fluid given *ad lib.* throughout the 18 weeks of the experiment. A transparent aqueous suspension of vitamins A and D (Decamin aquosum, Ferrosan Ltd., Copenhagen) was given as two drops three times a week, supplying 1160 i.u. vitamin A and 17 i.u. vitamin D/animal/week. Some of the animals received weekly supplements of 150 mg linoleic acid.

Table 1. *Drinking fluids and percentage composition of diets of the rats*

Group	31	32	33	34	35	36	37	38	39	40	41	42
Sucrose	67	67	67	67	46	46	46	46	67	67	67	67
Extracted casein*	20	20	20	20	20	20	20	20	20	20	20	20
Salt mixture†	5	5	5	5	5	5	5	5	5	5	5	5
Vitamin mixture‡	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5
Choline chloride	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5
Lard	7	7	7	7	28	28	28	28	0	0	0	0
Peanut oil	0	0	0	0	0	0	0	0	7	7	7	7
Hydrogenated peanut oil (m.p. 40–42°)§	0	0	0	0	0	0	0	0	0	0	0	0
Linoleic acid‖ (mg/animal/week)	0	150	0	150	0	150	0	150	0	150	0	150
Drinking fluid	W	W	R	R	W	W	R	R	W	W	R	R
Group	43	44	45	46	47	48	49	50	51	52	53	54
Sucrose	46	46	46	46	67	67	67	67	46	46	46	46
Extracted casein*	20	20	20	20	20	20	20	20	20	20	20	20
Salt mixture†	5	5	5	5	5	5	5	5	5	5	5	5
Vitamin mixture‡	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5
Choline chloride	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5
Lard	0	0	0	0	0	0	0	0	0	0	0	0
Peanut oil	28	28	28	28	0	0	0	0	0	0	0	0
Hydrogenated peanut oil (m.p. 40–42°)§	0	0	0	0	7	7	7	7	28	28	28	28
Linoleic acid‖ (mg/animal/week)	0	150	0	150	0	150	0	150	0	150	0	150
Drinking fluid	W	W	R	R	W	W	R	R	W	W	R	R

W = water, R = raw skim milk.

* 'Vitamin test casein', Genatosan, Loughborough, England.

† McCollum's salt mixture no. 185, supplemented with 13·5 mg KI, 139 mg $CuSO_4.5H_2O$ and 556 mg $MnSO_4.4H_2O$ per 100 g.

‡ 0·5 g of the mixture consisted of: biotin 0·05 mg, folic acid 0·05 mg, *p*-aminobenzoic acid 35 mg, thiamine hydrochloride 5 mg, inositol 15 mg, pyridoxin hydrochloride 5 mg, calcium pantothenate 5 mg, nicotinic acid 8 mg, inositol 15 mg, ascorbic acid 5 mg, DL-α-tocopherol acetate (Ephynal, Roche Products Ltd.) 5 mg, dicalcium salt of 2-methyl-1:4-naphthohydroquinone diphosphoric acid (Synkavit, Roche Products Ltd.) 1 mg, and sucrose to 500 mg.

§ Dansk Soyakagefabrik Ltd., Copenhagen.

‖ F. Hoffmann-La Roche & Co. Ltd., Basle, Switzerland.

RESULTS

In Table 2 are shown the average weights of the animals in the different groups at the end of the experiment (18 weeks) and their daily intakes of linoleic acid in the diet and as supplements*.

With a 7% dietary fat level and water as drinking fluid the growth rate of the animals on lard (group 31) or peanut oil (group 39) was significantly better than that of the animals on hydrogenated peanut oil (group 47) (series A, Table 2). Supple-

* Calculated from the average daily food intake of a rat through the last 6 weeks of the experiment. These data are given in the next paper (Aaes-Jørgensen & Dam, 1954*c*). The linoleic-acid contents were determined by F. Engel by means of the alkali-isomerization method described in the Report of the Spectroscopy Committee (Stillman, 1949).

Table 2. *Mean values for daily intake of linoleic acid for groups of six rats during 18 weeks of the experiment, and for their initial and final weights*

Series	Group no.	Diet characteristics	Linoleic-acid intake: Diet (mg)	Linoleic-acid intake: Supplement (mg)	Weight: Initial (g)	Weight: Final (value with its standard error) (g)
A	31	7 % lard, water	51	—	44·8	190·3 ± 4·7
	39	7 % peanut oil, water	214	—	44·8	208·0 ± 11·0
	47	7 % hydrogenated peanut oil, water	—	—	44·8	156·5 ± 5·3
B	32	7 % lard, linoleic acid, water	49	21·4	45·2	185·2 ± 7·8
	40	7 % peanut oil, linoleic acid, water	243	21·4	45·2	212·5 ± 9·7
	48	7 % hydrogenated peanut oil, linoleic acid, water	—	21·4	45·0	181·7 ± 4·5
C	33	7 % lard, raw skim milk	38	—	44·6	214·0* ± 5·2
	41	7 % peanut oil, raw skim milk	166	—	44·8	228·8 + 8·5
	49	7 % hydrogenated peanut oil, raw skim milk	—	—	45·2	203·2 ± 13·0
D	34	7 % lard, linoleic acid, raw skim milk	31	21·4	45·0	227·0 ± 11·4
	42	7 % peanut oil, linoleic acid, raw skim milk	182	21·4	45·0	222·3 ± 9·8
	50	7 % hydrogenated peanut oil, linoleic acid, raw skim milk	—	21·4	44·8	206·7 ± 12·1
E	35	28 % lard, water	173	—	45·0	237·0 ± 14·5
	43	28 % peanut oil, water	742	—	45·0	260·2 ± 14·6
	51	28 % hydrogenated peanut oil, water	—	—	45·0	134·2 ± 7·5
F	36	28 % lard, linoleic acid, water	191	21·4	45·0	244·2 ± 17·1
	44	28 % peanut oil, linoleic acid, water	665	21·4	45·2	215·3 ± 10·2
	52	28 % hydrogenated peanut oil, linoleic acid, water	—	21·4	45·2	196·5 ± 6·6
G	37	28 % lard, raw skim milk	156	—	45·0	246·2 ± 10·5
	45	28 % peanut oil, raw skim milk	596	—	45·0	254·0 ± 12·8
	53	28 % hydrogenated peanut oil, raw skim milk	—	—	45·4	180·2 ± 8·7
H	38	28 % lard, linoleic acid, raw skim milk	135	21·4	45·0	239·3 ± 10·2
	46	28 % peanut oil, linoleic acid, raw skim milk	596	21·4	45·2	256·0 ± 14·4
	54	28 % hydrogenated peanut oil, linoleic acid, raw skim milk	—	21·4	45·0	212·5 ± 4·8

* Five animals only.

mentation with 21·4 mg linoleic acid/animal/day (series B) caused almost no difference in the growth rate obtained with lard (group 32) or hydrogenated peanut oil (group 48). On peanut oil (group 40) the growth rate was remarkably higher than on hydrogenated peanut oil (group 48). Comparison of series A and B showed a significant increase of growth rate on hydrogenated peanut oil supplemented with 21·4 mg linoleic acid/animal/day (groups 47 and 48). Raw skim milk as drinking fluid (series C) instead of water (series A) stimulated growth with all three types of fat. The effect was especially high with the hydrogenated peanut-oil diet (groups 47 and 49). The effect on growth rate of raw skim milk as drinking fluid (series C) was at least as good as that of supplementation with linoleic acid at the 7% dietary fat level, irrespective of the type of fat.

With a 28% dietary fat level and water (series E) hydrogenated peanut oil (group 51) caused significantly less growth than that obtained with lard (group 35) and peanut oil (group 43). Rats on the lard diet supplemented with 21·4 mg linoleic acid/animal/day (series F, group 36) grew better than those on the peanut-oil diet similarly supplemented (series F, group 44). It is difficult to explain this finding. Deuel *et al.* (1951) reported toxicity to male rats of a very high level of methyl linoleate, but we observed no such effect in the other series with 28% peanut oil (Table 2). Comparison of series E and F showed that the animals on hydrogenated peanut oil (group 51) grew significantly better when the diet was supplemented with 21·4 mg linoleic acid/animal/day, but still not so well as the groups on the lard (group 36) and peanut-oil diets (group 44). Evans & Lepkovsky (1932), Burr (1942) and Deuel *et al.* (1951) suggest that increased dietary fat requires an increased amount of linoleic acid. In our experiments the growth rate of the animals in group 52 (28% hydrogenated peanut oil) was even better than that of those in group 48 (7% hydrogenated peanut oil) when both diets were supplemented with 21·4 mg linoleic acid/animal/day. Further, the growth rate on the respective unsupplemented diets (groups 47 and 51) was markedly lower on 28% (group 51) than on 7% hydrogenated peanut oil (group 47). This, possibly, indicates a deleterious effect of hydrogenated peanut oil. Experiments with a dose higher than 21·4 mg linoleic acid/animal/day are in progress in this laboratory.

Raw skim milk as drinking fluid (series G) instead of water (series E) increased significantly the growth rate on hydrogenated peanut oil (groups 53 and 51). Supplementation with linoleic acid and with raw skim milk as drinking fluid (series H) increased significantly the growth rate of the animals on hydrogenated peanut oil (group 54) compared with the unsupplemented diet (series G).

Comparing the two levels of fat in the diets, 7 and 28%, respectively, the following statements can be made. Series A and E, Table 2: increase of the amount of the lard or of peanut oil (water as drinking fluid) increased the growth rate (groups 31 and 35). With hydrogenated peanut oil as the fat component, increase of the fat level from 7 to 28% resulted in a pronounced decrease in growth rate (groups 47 and 51). This finding is in agreement with previous results (Aaes-Jørgensen & Dam, 1954*b*).

With diets containing 7 or 28% of the three fats, supplemented with linoleic acid, series B and F, Table 2, it was found that increase of the fat content caused an increase in the growth rate of all groups. (The special complication in connexion with group 44, series F, has been discussed earlier in this paper.)

With 7 and 28% fat and raw skim milk (series C and G) increase of the level of lard and peanut oil resulted in an increase in the growth rate, but on the hydrogenated peanut-oil diet the growth on the 28% level was somewhat slower than on the 7% level supplemented with raw skim milk (groups 49 and 53).

When the diets were supplemented with linoleic acid and raw skim milk was given as drinking fluid, as in series D and H, there was no statistically significant difference in the growth rate between the 7 and 28% fat levels.

Comparison of series A (diets with 7% fat level) with series F, G and H (28% fat, supplemented with linoleic acid, raw skim milk or both) showed that the growth rate was always better in the groups with 28% than in those with 7% fat when supplements of linoleic acid, raw skim milk or both were given. Thus the growth-depressing effect of 28% hydrogenated oil was overcome to about the same degree by supplementing the diet with linoleic acid as by changing the drinking fluid from water to raw skim milk.

Comparison of series B (diets with 7% fat supplemented with linoleic acid) with series G and H (diets with 28% fat and supplemented with raw skim milk or raw skim milk with linoleic acid) showed that the diets with 28% fat gave a growth response better than, or at least as good as, the 7% fat diets supplemented with linoleic acid. With diets containing 28% hydrogenated peanut oil, raw skim milk (group 53) gave a growth effect equal to that of a diet with 7% hydrogenated peanut oil supplemented with linoleic acid (group 48), but not as good as that obtained with diets containing 28% lard or peanut oil supplemented with raw skim milk (groups 37 and 45) or supplemented with raw skim milk and linoleic acid (groups 38 and 46).

In all these experiments food and fluid consumption and urine production were measured.

The results are reported in the following paper (Aaes-Jørgensen & Dam, 1954*c*).

DISCUSSION

Deuel *et al.* (1951) stated that administration of hydrogenated coconut oil has a depressing effect on the growth of rats fed a low-fat régime, in proportion to the level fed; this depressing effect could be completely counteracted by adequate supplementation with methyl linoleate. However, in their experiments much smaller quantities of dietary fat (250–500 mg/animal/day) were used than in ours. Further, the total calorie intake was not reported. In our experiment 21·4 mg linoleic acid/day given to animals reared on 7 or 28% hydrogenated peanut oil diets (series B and F) resulted in a higher growth rate of the animals given 28% hydrogenated peanut oil. When the animals fed on the unsupplemented diets (series A and E) are given linoleic acid it is seen that the addition increased the weight at the end of the experiment from $156 \cdot 5 \pm 5 \cdot 3$ (group 47) to $181 \cdot 7 \pm 4 \cdot 5$ g (group 48) on the 7% dietary fat level, and from $134 \cdot 2 \pm 7 \cdot 5$ g (group 51) to $196 \cdot 5 \pm 6 \cdot 6$ g (group 52) on the 28% dietary fat level. The observation that supplementation with 21·4 mg linoleic acid resulted in a higher growth increase at the 28% hydrogenated peanut oil level than at the 7% dietary level seems not to support the theory that increase of dietary fat leads to an increased requirement of essential fatty acids. The assumption that female rats require about 20 mg linoleic acid/day (Greenberg *et al.* 1950) is based on experiments with rats previously depleted of essential fatty acids from the time of weaning by rearing on a low-fat diet. It seems to us interesting that raw skim milk supplied as drinking fluid instead of water resulted in a growth-promoting effect that paralleled the effect of supplementing the same diet with linoleic acid when water was the drinking fluid. The effect was most pronounced at the 7% dietary fat level. These results suggest that on the experimental diets raw skim milk and the supplement of 21·4 mg linoleic acid were of about the same value for growth. As noted previously (Aes-Jørgensen & Dam, 1954*a*, *b*), it is impossible for raw skim milk in the quantities drunk by the rats to have supplied 20 mg of linoleic acid daily. Nor does it seem possible to explain the effect by the extra supplement of pyridoxin. Skim milk contains about 1·5–2·5 mg pyridoxin/l. (Espe, 1946). The rats never drank more than about 40 ml. raw skim milk/animal/day and 10 g of the diet contained 0·5 mg. The daily requirement is about 10–50 μg (Griffith & Farris, 1942).

We have so far no explanation of these findings. Perhaps some relation between fat and protein metabolism is involved. The higher growth rate on the lard and peanut-oil diets was not a result of an increased calorie intake, as will be shown in the next paper (Aaes-Jørgensen & Dam, 1954*c*).

SUMMARY

1. Female rats were reared on diets with lard, peanut oil or hydrogenated peanut oil at two different levels. These diets were supplemented with linoleic acid, raw skim milk or both. The growth rates were recorded throughout an experimental period of 18 weeks.

2. Increase of the dietary fat level from 7 to 28% increased the growth rate of female rats when lard or peanut oil was used, but decreased the growth rate when hydrogenated peanut oil was used.

3. Raw skim milk as drinking fluid caused a significant increase in the growth rate on 7 or 28% hydrogenated peanut-oil diets, compared with that on the same diets with water as drinking fluid.

4. Supplementation of the diets containing 7 or 28% hydrogenated peanut oil with 150 mg linoleic acid/animal/week increased the growth rate significantly, compared with that on the unsupplemented diet with water as drinking fluid.

5. The effect of supplementing the hydrogenated peanut-oil diet with linoleic acid or giving raw skim milk instead of water as drinking fluid was of almost the same magnitude, at least at the 7% dietary fat level.

6. No clear-cut additive effect on growth rate was seen on supplementing the diet with linoleic acid and at the same time giving raw skim milk as drinking fluid instead of water.

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