The Role of Fat in the Diet of Rats

1. Influence of Lard, Hydrogenated Peanut Oil and Absence of Dietary Fat on Growth, Food and Fluid Consumption and Urine Production

By E. AAES-JØRGENSEN and H. DAM

Department of Biochemistry and Nutrition, Polytechnic Institute, Copenhagen

(*Received 27 February* 1954)

Evans & Burr (1926–7) showed that rats reared on a fat-free diet, consisting of fat-free casein and yeast, purified sucrose, salts and three drops of cod-liver oil daily, failed to grow properly. Later, Burr & Burr (1929) described in rats a fat-deficiency syndrome characterized by retardation of growth, development of scaly skin and caudal necrosis, kidney lesions, haematuria and early death. Burr & Burr (1930) also noticed impaired fertility, increased water consumption and diminished urine production. Linoleic acid had a curative effect on the syndrome. Since the early work of Evans & Burr and of Burr & Burr, different aspects of the fat-deficiency syndrome have been studied; the results have been frequently reviewed, e.g. by Burr (1940, 1942), Burr & Barnes (1943), Daubert (1949), Deuel & Greenberg (1950), Deuel (1950) and Stangl (1951).

The present experiments were designed to compare the effects of diets containing a fat with low linoleic-acid content, namely hydrogenated peanut oil, or containing lard or no fat at all.

EXPERIMENTAL

Newly weaned male rats were distributed in seven groups with six animals in each. The diets as well as the drinking fluids were given *ad lib.* throughout 14 weeks. Food and fluid consumption were measured for all the groups during the last 10 weeks of the experiment. Urine output was measured simultaneously in animals placed individually in special cages over funnels. Faeces were separated from the urine by a glass ball suspended below the stem of the funnel. The urine was collected under xylene. The animals were weighed weekly; at the end of the experiment they were killed with chloroform.

Table 1 shows the composition of the diets used and the kind of drinking fluid given to the different groups. Vitamins A and D were dissolved in pure oleic acid and given separately as drops three times weekly, supplying 1160 i.u. vitamin A and 19 i.u. vitamin D_2/animal/week. The whey was prepared by coagulating raw skim milk with commercial rennet.

RESULTS

The average growth rates are shown in Table 2. From these results it is seen that on lard diets (groups 1–3) growth rates were significantly higher than on hydrogenated peanut-oil diets (groups 4–6) or on the fat-free diet (group 7). With raw skim milk as

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

Group	1	2	3	4	5	6	7
Sucrose	67	67	67	67	67	67	74
Extracted casein*	20	20	20	20	20	20	20
Salt mixture†	5	5	5	5	5	5	5
Vitamin mixture‡	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
Lard	7	7	7	0	0	0	0
Hydrogenated peanut oil (m.p. 35–37°)§	0	0	0	7	7	7	0
Drinking fluid	W	R	Wh	W	R	Wh	W

W = water, R = raw skim milk, Wh = whey.

* '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, riboflavin 5 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.

Table 2. *Mean values for daily gain in weight and food, fluid and calorie intake, and for efficiency of utilization of calories for groups of six rats in the last* 10 *weeks of the experiment*

Group no.	Diet characteristics	Food intake (g)	Fluid intake (ml.)	Total intake of calories (*a*) (Cal.)	Daily gain in weight (*b*) (g)	Efficiency of utilization of calories*	Mean final weight with its standard error
1	Lard, water	14·3	17·3	58·9	2·24	3·74	270·0 ± 11·3
2	Lard, raw skim milk	13·4	30·2	64·7	2·65	4·10	314·5 ± 7·9
3	Lard, whey	13·5	24·0	58·5	2·37	4·05	278·9 ± 10·6
4	Hydrogenated peanut oil, water	12·6	28·8	51·5	1·38	2·68	183·4 ± 5·5
5	Hydrogenated peanut oil, raw skim milk	10·5	38·9	54·8	1·54	2·81	202·0 ± 6·8
6	Hydrogenated peanut oil, whey	10·2	31·5	47·7	1·36	2·85	181·9 ± 6·4
7	Fat-free, water	17·4	27·2	63·7	1·36	2·14	184·3 ± 9·0

* $(b \div a) \times 100$.

drinking fluid (groups 2 and 5) the growth rate was higher than with water (groups 1 and 4). Further, the growth rate of group 1, on a lard diet with water, was still significantly higher than that of group 5 on a hydrogenated peanut-oil diet with raw skim milk as drinking fluid. With whey as drinking fluid (groups 3 and 6) the growth rate was about the same as with water (groups 1 and 4).

Growth rate on the fat-free diet (group 7) was of the same order as on the hydrogenated peanut-oil diets with either water or whey (groups 4 and 6), but not as high as that of the animals in group 5, on the hydrogenated peanut-oil diet with raw skim milk as drinking fluid.

Food and fluid consumptions of the different groups in the last 10 weeks of the experiment are presented in Table 2.

Animals in groups 1–3 (lard diets) ate a little more food than did those in groups 4–6 (hydrogenated peanut-oil diets); for fluid intake the reverse was found. Further, with raw skim milk as drinking fluid on both the lard and the hydrogenated peanut-oil diets (groups 2 and 5) the animals ate less food and consumed daily from 10 to 13 ml. more fluid than those on the same diets with water as drinking fluid (groups 1 and 4). When whey was the drinking fluid (groups 3 and 6), the tendency was the same as with raw skim milk (groups 2 and 5), but to a lesser degree. Animals in group 7, on the fat-free ration with water, showed an increase in food as well as in fluid intake compared with group 1 fed on the lard diet with water.

Utilization of food was much less efficient on the hydrogenated peanut-oil diets (groups 4–6) and still lower on the fat-free diet (group 7) than on the lard diets (groups 1–3).

In the last part of the experimental period the urine production of three animals (chosen at random) from each group was measured for 2-day periods. Results of these measurements are presented in Table 3.

Table 3. *Mean values for daily food and fluid intake and urine production for 2-day periods of three rats randomly selected from each group*

Group no.	Diet characteristics	Average weight at time of measurement (g)	Food intake (g)	Fluid intake (ml.)	Urine production (ml.)	Total intake of calories (Cal.)	Ratio, fluid intake:urine production
1	Lard, water	254	15·5	21·0	6·7	64	3·1
2	Lard, raw skim milk	305	13·2	36·4	13·2	66	2·8
3	Lard, whey	274	14·5	33·3	11·3	67	2·9
4	Hydrogenated peanut oil, water	189	14·3	34·0	5·0	59	6·8
5	Hydrogenated peanut oil, raw skim milk	181	10·6	37·9	11·0	57	3·4
6	Hydrogenated peanut oil, whey	173	12·7	35·3	5·4	59	6·5
7	Fat-free, water	182	15·5	31·2	3·0	58	10·4

The figures given for the amounts of urine/animal/day do not represent absolute values, because of evaporation from the funnel, but they are considered usable for comparisons between the different groups. Water intake was higher and urine production lower in group 7 (fat-free) than in group 1 (lard). The figures for water intake and urine production in group 4 (hydrogenated peanut-oil) showed a similar tendency to those in the fat-free group. Table 3 further shows the ratio fluid intake : urine output, which was found to be 3·4, 6·5 and 10·4 for groups 1, 4 and 7, respectively.

With raw skim milk as drinking fluid (groups 2 and 5) fluid intake as well as urine output were higher than with water. The ratios fluid intake : urine output were 2·8 and 3·4, respectively. With whey as drinking fluid (group 3) the same effect was found

as with raw skim milk (group 2) on the lard diet, whereas no such effect was seen when the dietary fat was hydrogenated peanut oil (group 6).

Weekly inspection of the animals revealed in none of the groups any skin signs characteristic of animals reared on a fat-free diet.

DISCUSSION

It is interesting to note that animals on a diet with 7% hydrogenated peanut-oil (group 4) showed the same growth rate as did those on a fat-free diet (group 7).

There seem to be several ways of explaining this observation: (1) The animals' depots of essential fatty acids were not used up during the 14-week experimental period. (2) There are no essential fatty acids left in the hydrogenated peanut oil, or they are present in such small quantities that they have no growth-promoting effect. (3) The hydrogenated peanut oil contains some inhibiting or toxic compound.

A further possibility, namely that the effect of the hydrogenated peanut oil was due to a smell or taste that the animals did not like, seems to be incompatible with the figures for food consumption in the different groups (Table 2). It is known that hydrogenated fats with melting points below 50° are well digested (Deuel & Greenberg, 1950), and accordingly there were no cases of diarrhoea throughout the experimental period in any group.

The greater growth with raw skim milk instead of water as drinking fluid (groups 2 and 5, and groups 1 and 4) was seen on the lard diet as well as on the hydrogenated peanut-oil diet. It cannot, therefore, be due to a small amount of essential fatty acids in raw skim milk. Substituting whey (groups 3 and 6) for water (groups 1 and 4) as drinking fluid did not improve growth. This may indicate that the growth-promoting effect of raw skim milk is to be found in its casein.

No signs, such as scaly tail, scaliness of the paws, or dandruff, were seen in the groups on the fat-free diet or on that containing hydrogenated peanut oil. This was in accordance with the experience of Evans & Lepkovsky (1932) and Brown & Burr (1936), who found that the appearance of these signs depends greatly on the humidity of the air. The high humidity (about 80–90%) in our animal rooms when these experiments were carried out apparently delayed the appearance of these signs.

SUMMARY

1. Male rats reared on diets containing 7% fat grew significantly better on lard than on hydrogenated peanut oil, irrespective of whether the drinking fluid was water, raw skim milk or whey.

2. Growth was better when the drinking fluid consisted of raw skim milk instead of water, irrespective of whether the diet contained lard or hydrogenated peanut oil.

3. The improvement of growth resulting from using raw skim milk as drinking fluid was less than that resulting from replacement of the hydrogenated peanut oil with lard.

4. Rats on a fat-free ration grew at the same rate as rats on the hydrogenated peanut oil.

5. The use of raw skim milk instead of water resulted in a decrease of the quantity of food eaten; the total calorie intake was only slightly increased.

6. Urine production decreased and water uptake increased when hydrogenated peanut oil or no fat was given in place of lard.

7. The use of raw skim milk to drink in place of water increased urine production and fluid uptake on diets with lard as well as on diets with hydrogenated peanut oil.

REFERENCES

Brown, W. R. & Burr, G. O. (1936). *J. biol. Chem.* **114**, xvi.
Burr, G. O. (1940). In *Chemistry and Medicine*, p. 102. [M. B. Visscher, editor.] Minnesota: University Press.
Burr, G. O. (1942). *Fed. Proc.* **1**, 224.
Burr, G. O. & Barnes, R. H. (1943). *Physiol. Rev.* **23**, 256.
Burr, G. O. & Burr, M. M. (1929). *J. biol. Chem.* **82**, 345.
Burr, G. O. & Burr, M. M. (1930). *J. biol. Chem.* **86**, 587.
Daubert, B. F. (1949). *J. Amer. Oil Chem. Soc.* **26**, 466.
Deuel, H. J. Jr. (1950). *J. Amer. diet. Ass.* **26**, 255.
Deuel, H. J. Jr. & Greenberg, S. M. (1950). *Fortschr. Chem. org. Naturst.* **6**, 1.
Evans, H. M. & Burr, G. O. (1926–7). *Proc. Soc. exp. Biol., N.Y.*, **24**, 740.
Evans, H. M. & Lepkovsky, S. (1932). *J. biol. Chem.* **96**, 143.
Stangl, E. (1951). *Int. Z. Vitaminforsch.* **23**, 164.

The Role of Fat in the Diet of Rats

2. Influence of Dietary Fats on Growth

By E. AAES-JØRGENSEN and H. DAM

Department of Biochemistry and Nutrition, Polytechnic Institute, Copenhagen

(*Received 27 February* 1954)

The need for a certain amount of fat for optimal growth, development and reproduction of rats is well known (Deuel & Greenberg, 1950). According to Deuel, Meserve, Straub, Hendrick & Scheer (1947) diets containing 20–40% cottonseed oil or margarine gave optimal growth. These authors stated that the effect was in part, but not entirely, attributable to a greater calorie intake on diets containing fat. However, comparison of the nutritive values of different fats can be misleading when based on one dietary level only. Barki, Collins, Elvehjem & Hart (1950) found optimal effect of various dietary fats, such as butterfat, maize oil, soya-bean oil and coconut oil, at different levels. Similar results were found by von Beznák, von Beznák & Hajdu (1943), who concluded that the differences in nutritive value of different fats (butterfat, horse fat, soya-bean oil, lard, oleomargarine, linseed oil and rapeseed oil) are dependent not only on the essential fatty acids and fatty acids with six to fourteen C atoms, but also even more on the ratios of different fatty acids present.

In continuation of previous investigations (Aaes-Jørgensen & Dam, 1954) with lard and hydrogenated peanut oil the experiments recorded here were designed to study further the influence on growth of qualitative and quantitative changes in the dietary fat components.