The Role of Fat in the Diet of Rats

3. Influence of Kind and Quantity of Fat on Food and Fluid Consumption and Urine Production

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In the early work of Burr & Burr (1930) it was found that water consumption was increased, whereas calorie intake and urine production were unchanged, in rats reared on a fat-free diet. Wesson & Burr (1931) investigated the metabolic rate of fat-deficient rats and found an increase in basal metabolic rate. The basal respiratory quotient was normal (0·91), but in the first hours after a carbohydrate feed it was often well above unity, clearly indicating the formation of fat from carbohydrate by rats in various stages of the fat-deficiency disease. As no relief was obtained from the signs of the fat-deficiency disease by the fat thus formed from carbohydrate, it was concluded that the curative linoleic and linolenic acids were formed by the rat neither from the carbohydrate nor from the fat. Burr & Beber (1937) did not find the fat-deficient rats more active than their controls. Their metabolic rates calculated as Cal./sq.m of surface were much higher than those of the control animals. Ramalingaswami & Sinclair (1951) found increased water consumption and normal or slightly diminished urinary output and no haematuria in fat-deficient rats. The water contents of the tissues showed that the excess water was not retained.

In the experiments to be described the effects of various fats on food and fluid intake and urine production were measured. Growth rates obtained by feeding these diets have already been described (Aaes-Jørgensen & Dam, 1954*b*).

EXPERIMENTAL

The animals and diets used in this experiment were the same as previously reported (Aaes-Jørgensen & Dam, 1954*b*).

Food and fluid consumption were measured for all groups through the last 13 weeks of the experiment. Urine was collected as described earlier (Aaes-Jørgensen & Dam, 1954*a*). Each of the animals was left over the collecting funnel for 48 h, during which time urine production and food and fluid consumption were measured.

RESULTS AND DISCUSSION

Table 1 shows the average food and fluid consumption for the different groups through the last 6 weeks of the experiment.

Fluid intake per sq.m body surface per day. From Table 1 it is seen that on diets with 7 % lard, peanut oil or coconut oil as dietary fat and water as drinking fluid (groups 10, 14 and 26, respectively) the water consumption per sq.m surface was fairly constant.

An increase in the level of extracted casein in the lard diet from 20 to 30% (groups 10 and 20) did not influence water consumption.

With increasing amounts of lard (7, 14 and 28%, groups 10, 16 and 18) a decrease in water intake was observed.

Table 1. *Mean values for weight, surface area, food, fluid and calorie intake for groups of six rats during the last 6 weeks of the experiment*

Group no.	Diet characteristics	Weight (g)	Surface area* (sq.cm)	Fluid intake/day ml.	Fluid intake/day ml./sq.m	Food intake/day (g)	Total calorie intake/day Cal.†	Total calorie intake/day Cal./sq.m
10	7% lard, water	188	379	25·5	673	10·6	43·4	1145
11	7% lard, raw skim milk	191	383	36·5	953	6·8	40·3	1052
12	7% hydrogenated peanut oil, water	162	343	34·4	1003	11·2	45·8	1335
13	7% hydrogenated peanut oil, raw skim milk	174	360	40·3	1119	8·0	48·7	1353
14	7% peanut oil, water	192	385	24·3	631	10·6	43·4	1127
15	7% peanut oil, raw skim milk	233	438	40·6	927	9·4	52·3	1194
16	14% lard, water	187	378	22·4	593	10·8	47·9	1267
17	14% hydrogenated peanut oil, water	160	341	34·3	1006	11·7	52·0	1525
18	28% lard, water	195	389	20·5	527	7·9	43·1	1108
19	28% hydrogenated peanut oil, water	139	310	32·6	1052	8·9	45·9	1481
20	7% lard, 30% casein, water	191	383	25·9	676	10·6	43·6	1138
21	7% hydrogenated peanut oil, 30% casein, water	175	362	32·9	909	10·9	44·8	1238
22	No fat, 10% casein, water	96	242	21·4	884	10·1	37·7	1558
23	No fat, 20% casein, water	153	330	30·5	924	13·1	49·3	1494
24	No fat, 30% casein, water	158	338	31·9	944	11·4	43·0	1272
25	7% hydrogenated whale oil, water	163	345	32·2	933	11·4	46·9	1359
26	7% coconut oil, water	188	379	25·5	673	11·1	45·4	1198

* The surface area was calculated by the formula: surface area (sq. cm) = $11·36\sqrt[3]{W^2}$ (where W = weight in g) (Harte, Travers & Sarich, 1948; Brody, 1945).

† Calculated by assuming that protein and carbohydrate yield 4 Cal./g and fat 9 Cal./g.

When hydrogenated peanut oil was fed at a 7% level (group 12), water intake was greater than with 7% lard (group 10). This is in accordance with the results described earlier (Aaes-Jørgensen & Dam, 1954*a*). A similar increase was seen when the group receiving 7% hydrogenated whale oil (group 25) was compared with the 7% lard group (group 10). Increase of the casein content from 20 to 30% in a diet with 7% hydrogenated peanut oil decreased water consumption somewhat (groups 21 and 12).

Increase of hydrogenated peanut oil in the diet from 7 to 14 and 28% (groups 12, 17 and 19) led to only small variations in water intake. The fluid intake was very high in all three groups.

On fat-free diets (groups 22–24) water intake per sq.m. surface increased perhaps slightly with increasing casein content in the diet. It was clearly higher than that found for animals on the 7% lard diet (group 10), but a little lower than that of animals on hydrogenated peanut-oil diets (groups 12, 17 and 19).

Fluid consumption was always higher with raw skim milk in place of water—group 11 compared with group 10 (lard diets), group 13 compared with group 12 (hydrogenated peanut-oil diets) and group 15 with group 14 (peanut-oil diets). Further, of the animals given raw skim milk those on the hydrogenated peanut-oil diet (group 13) consumed the greatest quantities of fluid.

From Table 1 it is seen that with raw skim milk instead of water, food intake, calculated as grams of food eaten, decreased. These observations, like those of Harte *et al.* (1948), confirm the general assumption that animals consume as much solid and liquid food as is necessary for maintenance of a normal metabolic rate.

It should be emphasized that there were fairly large variations between individual groups in the total calorie intake calculated as calories per surface unit per animal. These variations will be further discussed in relation to the results presented in Table 2.

Urine production in relation to the various diets and drinking fluids is shown in Table 2. The results are the mean values of measurements on individual animals in each group for 2-day periods during the last 6 weeks of the experiment.

The experimental error of the measurements in Table 2 was greater than that of the corresponding figures in Table 1 because the figures in Table 2 are averages of measurements for only 48 h per animal, whereas the figures in Table 1 represent average values for the different groups for 6 weeks. However, comparison of Tables 1 and 2 suggests the same general patterns of response.

Urine production per sq.m body surface per day. From Table 2, it is seen that urine production was about the same on diets with 7% lard, peanut oil or coconut oil (water as drinking fluid) (groups 10, 14 and 26). Increase in the casein content from 20 to 30% on the lard diet (group 20) seemed to have increased the urine production a little compared with that of group 10. The water uptake was also about the same in these groups.

On diets with increasing contents of lard (from 7 to 14 and 28%, groups 10, 16 and 18), urine production became lowest in group 18, and water intake was simultaneously decreased in this group.

With hydrogenated peanut oil or hydrogenated whale oil at a level of 7% (groups 12 and 25), and water as drinking fluid, a marked decrease in urine production was seen compared with that of group 10 (7% lard). At the same time there was a clear-cut increase in water consumption. On a diet with 7% hydrogenated peanut oil and 30% casein (group 21) the same tendency was seen, but not as markedly.

On diets with 7, 14 and 28% hydrogenated peanut oil and water (groups 12, 17 and 19) urine production decreased with increasing dietary fat level. Water intake was remarkably increased compared with that of the corresponding lard groups (groups 10, 16 and 18).

In the groups on the fat-free diets and water (groups 22–24) urine production was markedly decreased, compared with that of group 10 (7% lard). The water consumption was extraordinarily high in the fat-free groups.

Table 2. *Mean daily values for gain in weight, fluid and calorie intake and urine production for 2-day periods during the last 6 weeks of the experiment calculated from individual measurements on all rats in each group*

Group no.	Diet characteristics	Urine production ml.	Urine production ml./sq.m	Fluid intake ml.	Fluid intake ml./sq.m	Difference: fluid intake − urine production (ml./sq.m)	Total calorie intake (from Table 1) (*a*) (Cal./sq.m)	Calories used for evaporation of water* (*b*) (Cal./sq.m)	Calories used for growth (*a* − *b*) (Cal./sq.m)	Weight gain (g)	Calorie cost of weight gain (Cal./sq.m/g)
10	7% lard, water	7·8	206	23·4	617	411	1145	250	895	0·53	1689
11	7% lard, raw skim milk	19·0	496	40·5	1057	561	1052	341	711	0·47	1513
12	7% hydrogenated peanut oil, water	4·2	122	32·8	956	834	1335	507	828	0·37	2238
13	7% hydrogenated peanut oil, raw skim milk	10·9	303	47·8	1328	1025	1353	624	729	0·30	2430
14	7% peanut oil, water	6·8	177	21·5	558	381	1127	232	895	0·57	1570
15	7% peanut oil, raw skim milk	25·2	575	51·0	1164	589	1194	358	836	0·82	1020
16	14% lard, water	9·7	257	24·6	651	394	1267	240	1027	0·46	2232
17	14% hydrogenated peanut oil, water	2·0	59	30·1	883	824	1525	501	1024	0	—
18	28% lard, water	3·5	90	16·1	414	324	1108	197	911	0·57	1598
19	28% hydrogenated peanut oil, water	1·6†	52	27·0	871	819	1481	498	983	0·04	24575
20	7% lard, 30% casein, water	9·7	253	25·3	661	408	1138	248	890	0·30	2967
21	7% hydrogenated peanut oil, 30% casein, water	5·0	138	27·5	760	622	1238	378	860	0·13	6615
22	No fat, 10% casein, water	2·4†	99	27·1	1120	1021	1558	621	937	0·18	5206
23	No fat, 20% casein, water	3·4	103	34·8	1055	952	1494	579	915	0·15	6100
24	No fat, 30% casein, water	4·8	142	35·8	1059	917	1272	558	714	0·02	35700
25	7% hydrogenated whale oil, water	2·4	70	32·1	930	860	1359	523	836	0·07	11943
26	7% coconut oil, water	8·4	222	23·8	628	406	1198	247	951	0·56	1698

* 608·5 Cal./kg for evaporation of water at 29°.

† Some of the animals in these groups showed haematuria and results from them were not used in making the calculations.

Raw skim milk as drinking fluid increased urine production when compared with that of the groups on the corresponding diets and water. Of all the groups given raw skim milk the one on the diet with 7% hydrogenated peanut oil (group 13) had the lowest urine production and the highest intake of raw skim milk.

Ramalingaswami & Sinclair (1951) found, like Burr & Burr (1930), a normal or a slightly diminished urinary output in fat-deficient rats. In the present experiment urine production was remarkably low on diets containing hydrogenated fat and on fat-free diets. Coconut oil gave the same picture as lard and peanut oil in this respect. Raw skim milk as drinking fluid generally increased urine production, but again the animals on the hydrogenated peanut-oil ration had a lower urine output than animals fed lard or peanut oil.

These results showed that the urine production of the animals on diets with hydrogenated peanut oil, hydrogenated whale oil and fat-free rations was decreased alongside with an increase in fluid intake compared with the animals on lard, peanut or coconut-oil diets, i.e. the difference between fluid intake and urine output was large in these groups (Table 2). When raw skim milk was given instead of water (groups 11, 13 and 15) this difference was somewhat greater on the diets containing lard and peanut oil (groups 11 and 15). On hydrogenated peanut oil the difference was exceedingly high.

A parallelism between growth rate and difference in fluid intake and urine output is found on comparing the data from Tables 1 and 2. Slow growth was always found when the difference between fluid intake and urine production was great. These findings indicate that the excess fluid was eliminated from the body.

Further, the animals on the lard, peanut-oil and coconut-oil diets had about the same calorie intake. The calorie intake per surface unit per animal per day in the groups fed hydrogenated peanut oil, hydrogenated whale oil or the fat-free diets was remarkably increased. This increase in the calorie intake paralleled poor growth (Table 1), increased fluid intake and decreased urine production (Table 2).

The figures in the last columns of Table 2 represent an approximate calculation of the calories used per gram of weight gain, after subtraction of the calories needed for evaporation of the water (found as difference between fluid intake and output) from the total calorie intake. It was assumed that all the 'reduced' calories were used for growth. Sinclair (1952) assumed that the increased calorie intake in fat deficiency could be explained by the increased need of calories for evaporation of water. The figures in Table 2 indicate that the increased evaporation of water did not wholly explain the fact that animals on hydrogenated-oil or fat-free diets ate much and grew slowly.

Urines from the individual animals in all the groups of this experiment were examined for specific gravity, occurrence of blood, protein, ketone bodies and sugar; the pH was measured and the colour recorded.

When urine production was low, there was always an increase in its specific gravity, and the colour was darker (more yellow-brown) than normal. In some of the animals from groups 19 and 22 (28% hydrogenated peanut oil and a fat-free diet, respectively) haematuria occurred in the last weeks of the experiment. Animals in the groups receiving raw skim milk had a slight galactosuria, but the specific gravity increased only little or not at all, probably because urine production in these animals was somewhat increased. Protein and ketone bodies were found in only few animals and were not limited to any particular group. The pH ranged from about 5·60 to 6·90. The principal impression was that the urine from the animals receiving raw skim milk was less acid (pH 6·20–6·90) than that from the animals producing the more concentrated urine (around pH 5·60–5·90).

During the experimental period the animals were inspected weekly. In the preliminary experiment (Aaes-Jørgensen & Dam, 1954*a*) we did not find the classical skin signs of essential fatty-acid deficiency in the animals reared on a fat-free diet. We assumed that this was due to the high humidity of the animal room, as shown by Brown & Burr (1936). Before beginning the present experiment, control of temperature and humidity was established. During the experimental period the temperature in the animal room varied from 23 to 25° and the relative humidity from 45 to 55%.

Animals on the fat-free rations (groups 22–24) showed scaly tail and dry and scaly skin, especially of the hind-legs. The fur was dishevelled and felt moist or greasy, particularly in the animals from group 22 (fat-free diet with 10% casein) and to some extent in those from group 23 (fat-free diet with 20% casein). Some of the animals from group 24 (fat-free diet with 30% casein) had small wounds, especially in the back of the neck and on the shoulders.

Animals on diets with hydrogenated peanut oil (groups 12, 17 and 19) or hydrogenated whale oil (group 25) and water showed signs similar to those in the animals reared on fat-free diets.

No signs resembling the fat-deficiency syndrome were found in any of the animals in the other groups.

The relation of lack of essential fatty acids to skin and histological kidney lesions is discussed in a paper being prepared for publication elsewhere.

SUMMARY

1. Female rats were reared on diets with various amounts of fats during an experimental period of 16 weeks. Food and fluid consumption and urine output were measured throughout the last 13 weeks of the experiment.

2. Water intake was higher on diets with hydrogenated peanut oil or hydrogenated whale oil, and in absence of dietary fat than on diets with lard, peanut oil or coconut oil. Change of drinking fluid from water to raw skim milk resulted in a general increase of the fluid consumption, and the fluid intake was also greatest for the animals on hydrogenated peanut oil.

3. Urine production was lower on diets with hydrogenated peanut oil or hydrogenated whale oil or no fat than on diets with lard, peanut oil or coconut oil. Changing the drinking fluid from water to raw skim milk increased the diuresis generally. Again, the animals on the hydrogenated peanut-oil diet showed the lowest output of urine.

4. The greatest calorie intake per surface unit per day occurred in the animals on

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)