# The role of fat in the diet of rats

## 12. Effect on rats of type and quantity of dietary fat with and without linoleate supplementation*

By J. P. FUNCH, E. AAES-JØRGENSEN AND H. DAM

*Department of Biochemistry and Nutrition, Polytechnic Institute, Copenhagen*

(*Received* 18 *May* 1957)

Earlier results (Aaes-Jørgensen & Dam, 1954*b*, *c*; Aaes-Jørgensen, 1954) have demonstrated a decrease in body-weight of rats with increasing amounts of hydrogenated fat in the diet. The experiment reported here was undertaken to investigate whether these results were solely due to an increase in the requirement of linoleic acid with the concomitant ingestion of fat as suggested by Burr (1942) and Deuel, Greenberg, Anisfeld & Melnick (1951), or whether isomers of unsaturated fatty acids formed during hydrogenation were partly responsible for accentuation of the fat-deficiency symptoms.

### EXPERIMENTAL

Newly weaned male rats were distributed over fifteen groups of six animals each. The compositions of the diets, calculated on a percentage-by-weight basis, and the amounts of ethyl-linoleate supplement are shown in Table 1. The ethyl linoleate was given orally three times a week. Vitamins A and $D_2$ were given as drops of an aqueous colloidal solution (Decamin aquosum, Ferrosan Ltd, Copenhagen), supplying 120 i.u. vitamin A and 18 i.u. vitamin $D_2$/animal/week. Food and water were given *ad lib*. The food consumption was estimated daily from the 10th to the 13th week of experiment. The daily calorie intake per sq.m. body surface and the efficiency of utilization of calories for growth during the same period were calculated. The animals were weighed and inspected weekly and were killed with chloroform at the end of the experimental period, which lasted 26 weeks. Autopsies were performed, and testes, kidneys, skin specimens, liver, and adrenals were examined histologically. Blocks of tissue were fixed in 10% commercial formalin (4% formaldehyde). Paraffin sections were stained routinely with haematoxylin and eosin. Part of the material from the kidneys was stained for connective tissue with van Gieson's stain, and material from all the livers was cut on a freezing microtome and stained for fat with Sudan black.

### RESULTS AND DISCUSSION

#### *Growth rates*

In Table 2 are listed the mean values for weights at the beginning and the end of the experiment, as well as daily gain in weight, calorie intake and efficiency of utilization of calories for growth from the 10th to the 13th week of the experiment. It will be seen that 7% trilaurin (group 200) or 7% hydrogenated arachis oil (group 209) gave

* Paper no. 11: *Brit. J. Nutr.* (1957), **11**, 298.

Table 1. *Composition of the diets of the rats*

| Group no. | Dietary fat | Ethyl linoleate* (mg/rat/day) | Vitamin Test Casein† (%) | Sucrose (%) | Salt mixture‡ (%) | Vitamin mixture‡ (%) | Choline chloride (%) |
|---|---|---|---|---|---|---|---|
| 200 | 7% trilaurin§ | — | 20 | 67 | 5 | 0·5 | 0·5 |
| 201 | 7% trilaurin | 20 | 20 | 67 | 5 | 0·5 | 0·5 |
| 202 | 7% trilaurin | 100 | 20 | 67 | 5 | 0·5 | 0·5 |
| 203 | 28% trilaurin | — | 20 | 46 | 5 | 0·5 | 0·5 |
| 204 | 28% trilaurin | 20 | 20 | 46 | 5 | 0·5 | 0·5 |
| 205 | 28% trilaurin | 100 | 20 | 46 | 5 | 0·5 | 0·5 |
| 206 | None | — | 20 | 74 | 5 | 0·5 | 0·5 |
| 207 | None | 20 | 20 | 74 | 5 | 0·5 | 0·5 |
| 208 | None | 100 | 20 | 74 | 5 | 0·5 | 0·5 |
| 209 | 7% hydrogenated arachis oil‖ | — | 20 | 67 | 5 | 0·5 | 0·5 |
| 210 | 7% hydrogenated arachis oil | 20 | 20 | 67 | 5 | 0·5 | 0·5 |
| 211 | 7% hydrogenated arachis oil | 100 | 20 | 67 | 5 | 0·5 | 0·5 |
| 212 | 28% hydrogenated arachis oil | — | 20 | 46 | 5 | 0·5 | 0·5 |
| 213 | 28% hydrogenated arachis oil | 20 | 20 | 46 | 5 | 0·5 | 0·5 |
| 214 | 28% hydrogenated arachis oil | 100 | 20 | 46 | 5 | 0·5 | 0·5 |

* From the Hormel Foundation, Minnesota, U.S.A. Prepared by urea complex-repeated crystallization and vacuum fractional distillation. Iodine value (Wijs): 163·3 (theoretical value: 164·0). Conjugated poly-unsaturated constituents (from ultraviolet-absorption data) expressed as percentage of methyl esters of $C_{18}$ fatty acids: dienoic, not more than 0·18%; trienoic, not more than trace; tetraenoic, none.

† From Genatosan Ltd, Loughborough, England.

‡ Cf. Aaes-Jørgensen & Dam (1954*a*).

§ From Dansk Sojakagefabrik Ltd, Copenhagen, Denmark. Prepared by Aage Jart, M.Sc., as glyceryl trilaurate from a technical lauric acid, the composition of which was: 7% capric acid ($C_{10}$), 92% lauric acid ($C_{12}$), and 1% myristic acid ($C_{14}$). The analytical values for the glyceryl trilaurate used in the present experiment were: m.p. 39·5°; saponification value 264; iodine value (Wijs) 1·1; free acids (calculated as lauric acid) 0·1%.

‖ From Aarhus Oliemølle A/S, Aarhus, Denmark; m.p. 40–42°.

equal growth, which was a little better than that of the rats receiving either 28% trilaurin (group 203) or no fat (group 206). The animals on diets with 28% trilaurin grew at much the same rate as those receiving no fat, but the growth rate of the rats on 28% hydrogenated arachis oil (group 212) was to a marked degree less than that of the animals receiving 7% hydrogenated arachis oil (group 209) or no fat in the diet (group 206) (statistically significant at 1% level of probability). This finding is in agreement with the results of an earlier experiment (Aaes-Jørgensen & Dam, 1954*b*).

Supplementation with 20 or 100 mg ethyl linoleate/animal/day improved growth considerably over that obtained on the corresponding unsupplemented diets. A supplement of 20 mg linoleate did not ensure growth on hydrogenated arachis oil (groups 210 and 213) equal to that obtained on the corresponding trilaurin diets (groups 201 and 204), but rats given a supplement of 100 mg linoleate showed growth rates on the diets with hydrogenated arachis oil (groups 211 and 214) equal to those obtained on the corresponding amounts of trilaurin (groups 202 and 205).

Table 2. *Mean weight for each group of six rats before and after an experimental period of 26 weeks; growth period and weight at cessation of growth; daily gain in weight, calorie intake and efficiency of utilization during the 10th to the 13th week of experiment*

| Group no. | Dietary fat | Ethyl linoleate (mg/rat/day) | Initial weight (g) | Cessation of growth: Week | Cessation of growth: Weight of rat (g) | Final weight (value with its standard error) (g) | 10th–13th week: Daily gain in weight (g) | 10th–13th week: Total calorie intake/day: Cal.* | 10th–13th week: Total calorie intake/day: Cal./sq.m.† | 10th–13th week: Efficiency of utilization of calories‡ |
|---|---|---|---|---|---|---|---|---|---|---|
| 200 | 7 % trilaurin | — | 39·3 | 18th | 203 | 200 ± 17·0 | 0·20 | 47·3 | 1204 | 0·4 |
| 201 | 7 % trilaurin | 20 | 39·3 | — | — | 309 ± 13·0 | 0·98 | 51·8 | 1131 | 1·9 |
| 202 | 7 % trilaurin | 100 | 39·5 | — | — | 344 ± 11·3 | 1·08 | 55·1 | 1113 | 2·0 |
| 203 | 28 % trilaurin | — | 39·7 | 17th | 184 | 183 ± 5·8§ | 0·51 | 52·6 | 1465 | 1·0 |
| 204 | 28 % trilaurin | 20 | 39·5 | — | — | 327 ± 22·0 | 1·31 | 64·5 | 1405 | 2·0 |
| 205 | 28 % trilaurin | 100 | 39·2 | — | — | 385 ± 11·9 | 1·68 | 62·4 | 1238 | 2·7 |
| 206 | None | — | 39·3 | 18th | 204 | 187 ± 10·2 | 0·54 | 51·9 | 1327 | 1·0 |
| 207 | None | 20 | 39·5 | — | — | 299 ± 8·9 | 1·30 | 53·4 | 1153 | 2·4 |
| 208 | None | 100 | 39·7 | — | — | 326 ± 8·9 | 1·37 | 57·2 | 1163 | 2·4 |
| 209 | 7 % hydrogenated arachis oil | — | 39·3 | 17th | 199 | 202 ± 13·1 | 0·37 | 53·8 | 1408 | 0·7 |
| 210 | 7 % hydrogenated arachis oil | 20 | 39·3 | — | — | 271 ± 9·2 | 0·87 | 58·4 | 1358 | 1·5 |
| 211 | 7 % hydrogenated arachis oil | 100 | 39·3 | — | — | 345 ± 12·5 | 1·32 | 61·2 | 1275 | 2·2 |
| 212 | 28 % hydrogenated arachis oil | — | 39·2 | 10th | 174 | 153 ± 5·7 | 0 | 59·9 | 1687 | 0 |
| 213 | 28 % hydrogenated arachis oil | 20 | 39·3 | — | — | 292 ± 14·1 | 0·76 | 61·9 | 1385 | 1·2 |
| 214 | 28 % hydrogenated arachis oil | 100 | 39·2 | — | — | 395 ± 18·9 | 1·68 | 69·1 | 1334 | 2·4 |

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

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

‡ (Daily gain in weight (g)/total calorie intake/day) × 100.

§ Two animals of this group died before the end of the experiment. One died after 15 weeks, cause of death unknown. One died after 21 weeks, cause of death volvulus.

Cessation of growth occurred earlier and at a lower level on the diet containing 28% hydrogenated arachis oil as the sole dietary fat than on the other unsupplemented diets, which agrees with findings in previous studies with hydrogenated fats (Aaes-Jørgensen, 1954; Aaes-Jørgensen, Funch, Engel & Dam, 1956*b*). In the former publication it was shown that cessation of growth occurred earlier with hydrogenated fats produced from oils with a high content of unsaturated fatty acids (e.g. herring, seal and whale oil) than from oils with a low content of unsaturated fatty acids (e.g. coconut oil).

The calorie intake per surface unit was higher and the efficiency of utilization of calories for growth was lower on the unsupplemented diets than on the corresponding diets supplemented with ethyl linoleate. This may be explained by the results of Wesson & Burr (1931) who found an increase in basal metabolic rate of fat-deficient rats, findings confirmed and extended by Burr & Beber (1937) and by Panos, Finerty & Wall (1956). The latter authors showed that supplementation of a fat-free diet with methyl palmitate produced no change in oxygen-consumption rate, but daily supplements of 100 mg methyl linoleate or 200 mg cottonseed oil ensured a normal energy metabolism.

The results reported here seem to indicate that the growth-depressing effect of 28% hydrogenated arachis oil cannot be explained solely by assuming that increase of dietary fat level results in an increase of the daily requirement of linoleic acid (Burr, 1942; Deuel *et al.* 1951). A further aggravation of the lack of essential fatty acids might be provoked by the presence in hydrogenated fat of isomers of the unsaturated fatty acids formed during hydrogenation. This suggestion would be in accordance with the findings of Holman & Aaes-Jørgensen (1956), who have shown that the *trans* isomers of linoleic acid inhibited growth, worsened the skin condition and were unable to induce recovery of the testes of animals severely depleted of essential fatty acids.

The difference in effect of high dietary levels of trilaurin and hydrogenated arachis oil on the thriving of rats might also be explained by assuming that increased amounts of long-chain dietary fatty acids increase the requirement for essential fatty acids more than do increased amounts of medium-chain acids.

Further experiments are needed to decide between these possibilities.

### *Clinical signs, and results of histological studies*

The animals fed on the unsupplemented diets with and without fat showed skin signs characteristic of animals reared on fat-free diets.

A summary of the histopathological findings in testes, kidneys, skin, liver and adrenals is presented in Table 3.

*Testes.* The changes were essentially identical with those previously described for rats fed on diets with 28% hydrogenated arachis oil for 18 or 19 weeks (Aaes-Jørgensen, Funch, Engel & Dam, 1956*b*; Aaes-Jørgensen, Funch & Dam, 1957). The degree of degeneration of the spermatogenic epithelium was assessed from a scale described in detail in an earlier paper (Aaes-Jørgensen, Funch, Engel & Dam, 1956*b*). It can be seen from Table 3 that the feeding of diets containing no fat, trilaurin or hydrogenated arachis oil without linoleate supplementation for 26 weeks caused degeneration of the spermatogenic epithelium, but to varying degrees. Diets containing 7 or 28%

Table 3. *Histological changes in the rats after 26 weeks on experiment*

| Group no. | Dietary fat | Ethyl linoleate (mg/rat/day) | Testes, mean degree of degeneration* (see text, p. 429) | Kidneys: Mean degree of degeneration† Cortex | Kidneys: Mean degree of degeneration† Papilla | Kidneys: Mean occurrence of calculi at cortico-medullary border‡ | Epidermis, no. of cell layers: Stratum granulosum | Epidermis, no. of cell layers: Stratum Malpighii | Derma: Sebaceous glands | Derma: Cell infiltration | Liver, mean value for fat accumulation§ | Adrenals |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 200 | 7 % trilaurin | — | 2·5 | 1 | 2·3 | 1·8 | 3 | 4 | Hypertrophy, detritus and some atrophy | Slight | 1·2 | Normal |
| 201 | 7 % trilaurin | 20 | 0 | 0·2 | 0·2 | 2·2 | 1–2 | 1–2 | Normal | Normal | 2·7 | Normal |
| 202 | 7 % trilaurin | 100 | 0 | 0 | 0 | 0·5 | 1–2 | 1–2 | Normal | Normal | 2·0 | Normal |
| 203 | 28 % trilaurin | — | 1·8 | 1·5 | 1·5 | 1·5 | 3–4 | 4 | Hypertrophy and detritus | Slight | 0 | Normal |
| 204 | 28 % trilaurin | 20 | 0 | 0 | 0 | 0·3 | 1–2 | 1–2 | Normal | Normal | 0·2 | Normal |
| 205 | 28 % trilaurin | 100 | 0 | 0 | 0 | 0 | 1–2 | 1–2 | Normal | Normal | 0·2 | Normal |
| 206 | None | — | 4·5 | 1·8 | 3·6 | 1·3 | 3 | 4–5 | Hypertrophy, detritus and some atrophy | Slight | 1·2 | Normal |
| 207 | None | 20 | 0 | 0 | 0 | 0 | 1–2 | 1–2 | Normal | Normal | 2·8 | Normal |
| 208 | None | 100 | 0 | 0 | 0 | 0·7 | 1–2 | 1–2 | Normal | Normal | 1·3 | Normal |
| 209 | 7 % hydrogenated arachis oil | — | 4·5 | 1·3 | 3 | 0·3 | 3 | 4 | Detritus and atrophy | Slight | 1·3 | Normal |
| 210 | 7 % hydrogenated arachis oil | 20 | 0·7‖ | 0 | 0·2 | 0·2 | 1–2 | 1–2 | Normal | Normal | 1·0 | Normal |
| 211 | 7 % hydrogenated arachis oil | 100 | 0 | 0 | 0 | 0 | 1–2 | 1–2 | Normal | Normal | 1·5 | Normal |
| 212 | 28 % hydrogenated arachis oil | — | 5 | 1·3 | 3·5 | 0 | 3–4 | 4 | Some hypertrophy, detritus and atrophy | Moderate | 0·7 | Normal |
| 213 | 28 % hydrogenated arachis oil | 20 | 0 | 0 | 0·5 | 0 | 1–2 | 1–2 | Normal | Normal | 0 | Normal |
| 214 | 28 % hydrogenated arachis oil | 100 | 0·8¶ | 0 | 0·2 | 0 | 1–2 | 1–2 | Normal | Normal | 0 | Normal |

* Assessed from a scale graduated from 0 (no degeneration) to 5 (total degeneration) (see Aaes-Jørgensen, Funch, Engel & Dam, 1956*b*).
† Assessed from a scale graduated from 0 (no degeneration) to 5 (severe degeneration with necrosis and calcification).
‡ Assessed from a scale graduated from 0 (no calculi) to 5 (abundance of calculi).
§ Assessed from a scale graduated from 0 (no visible fat) to 5 (diffuse accumulation of fat).
‖ Five animals had degree 0; one animal had degree 4.
¶ Five animals had degree 0; one animal had degree 5.

trilaurin caused the least degeneration. Diets containing no fat or 7% hydrogenated arachis oil produced a degeneration of the spermatogenic epithelium that was assessed in half the animals as degree 4 and in the others as degree 5. In all rats given 28% hydrogenated arachis oil a severe degeneration assessed as degree 5 had occurred. Whereas the rats fed on the fat-free diet or the diets with hydrogenated arachis oil as the sole dietary fat all had severe degeneration of the spermatogenic cells, the unsupplemented trilaurin-containing diets showed great variation in impairment of spermatogenesis. In some rats an almost normal pattern of spermatogenesis was seen; others showed moderate to severe degeneration. The average degree of degeneration was a little lower in the rats given 28% trilaurin than in rats given 7% of the same fat, but the difference cannot be considered significant because of the great individual variations.

In an earlier experiment (Aaes-Jørgensen, Funch, Engel & Dam, 1956*b*), 20 mg linoleic acid/animal/day was found capable of preventing the deleterious effect of hydrogenated arachis oil on spermatogenesis. In agreement with this finding, supplementation of the fat-free diet and the diets containing 7 or 28% of either trilaurin or hydrogenated arachis oil with 20 or 100 mg ethyl linoleate/animal/day ensured, in the experiment reported here, a normal histological pattern of the testes in all animals except two, which displayed a severe degeneration of the seminiferous tubules. One of these rats was given 7% hydrogenated arachis oil supplemented with 20 mg linoleate/day, the other 28% of the same fat supplemented with 100 mg linoleate/day (cf. Table 3). The degeneration of testes in these two rats probably bears no relationship to the diets fed, because otherwise the rats had no signs of deficiency of essential fatty acids and grew throughout the experimental period. In our experience an accidental degeneration of the testes may occur even in rats fed on a sufficient diet containing a fat mixture rich in linoleic acid.

In this 26 weeks' experiment the fat-free diet caused severe testicular degeneration, in contrast with the results of an earlier experiment of 18 weeks' duration (Aaes-Jørgensen, Funch, Engel & Dam, 1956*b*) in which spermatogenesis was only slightly affected by a similar diet. These findings seem to indicate that a profound degeneration of the testes does not occur before the animals are severely depleted of essential fatty acids.

*Kidneys.* The pathological findings in the kidneys in this experiment were essentially the same as those described previously (Aaes-Jørgensen, Funch, Engel & Dam, 1956*a*, *b*; Aaes-Jørgensen, Funch & Dam, 1956). Together with the earlier findings the results of this experiment seem to indicate that there is no clear correlation between the occurrence of calculi at the cortico-medullary border and deficiency of essential fatty acids. However the degeneration in the cortex and the papilla seems to be part of the syndrome of deficiency of essential fatty acids. In the groups given no fat (group 206) or 28% hydrogenated arachis oil (group 212) severe degeneration with necrosis and dystrophic calcification of the papilla had occurred in some rats. The pathological change in the cortical tubules was that of a nephrosis, including degeneration, dilatation, dystrophic calcification and compensatory regeneration. Primary interstitial nephritis, glomerulonephritis or fibrous infiltration was not observed.

Supplementation with linoleate lessened or prevented the degenerative changes in the cortex and papilla.

*Skin.* Previous histological studies of the skin of female rats reared on fat-free diets, or on diets containing 28% hydrogenated arachis oil as the sole dietary fat, disclosed thickening of the epidermis and other features characteristic of rats fed on a fat-deficient diet (Aaes-Jørgensen, Funch, Engel & Dam, 1956*b*). In the present experiment with male rats the skin specimens were taken from the interscapular region of the dorsum. It will be seen from Table 3 that diets containing no fat, or diets with 7 or 28% trilaurin or hydrogenated arachis oil without linoleate supplementation, produced nearly the same histological alterations in the epidermis and derma.

The stratum granulosum included three to four layers of cells densely packed with irregularly shaped granules of keratohyalin. The Malpighian layer was composed of four to five layers of cells with distinct intercellular spaces. The stratum corneum was also increased in thickness, and we found a keratotic plugging of the opening of the hair follicles, as described in fat-deficient rats by Ramalingaswami & Sinclair (1953). In agreement with these authors we found a hypertrophy of the sebaceous glands in most animals fed on the diets without linoleate supplementation, and the enlarged sebaceous glands often contained a detritus of fine granular basophilic material which seems to be a degeneration product of the acini. In some rats fed on the unsupplemented diets, especially in those given 7 or 28% hydrogenated arachis oil, the sebaceous glands appeared atrophic and in advanced stages of dissolution; in several places only remnants were present. This seems to support the hypothesis put forward by Ramalingaswami & Sinclair (1953) that the hypertrophy is only an earlier stage of a degenerative process caused by obstruction to the outflow of sebum by the keratotic plugs. There was slightly greater infiltration of cells in the derma of the animals fed on the unsupplemented diets than in those supplemented with ethyl linoleate. Moreover in the rats not receiving linoleate the subcutaneous fat was almost completely reduced, only a few fat cells being present between the derma and the muscular layer. Supplementation of the fat-free diets with 20 or 100 mg linoleate/animal/day resulted in a moderate amount of subcutaneous fat, and addition of the same amount of linoleate to the diets containing trilaurin or hydrogenated arachis oil ensured an abundance of subcutaneous fat. The rats fed on the diets supplemented with 20 or 100 mg linoleate showed the same histological pattern of the skin as that found by us in rats reared on a diet containing 28% untreated arachis oil (Aaes-Jørgensen, Funch, Engel & Dam, 1956*b*).

There seems to be no doubt that the histological changes in the skin of fat-deficient animals described by several authors are not due to lack of fat *per se*, but to the lack of essential unsaturated fatty acids, since the disorder, which is produced when even large amounts of a saturated fat are fed, is completely prevented by supplementation with small amounts of linoleate.

*Liver.* Frozen sections stained with Sudan black showed a moderate accumulation of fat in the livers of the rats given no fat, 7% trilaurin, or 7% hydrogenated arachis oil, with or without linoleate supplementation (cf. Table 3). This fat was found to be in the form of small droplets located primarily in cells in the region of the portal

triad. The nuclei of the fat-laden cells showed no pathological changes. The rats given 28% trilaurin or 28% hydrogenated arachis oil with or without linoleate supplementation had no or only a slight accumulation of stainable fat in the liver.

*Adrenals.* No significant abnormalities were observed.

## SUMMARY

1. The purpose of the experiment reported here was to investigate the influence of increasing dietary levels of trilaurin or hydrogenated arachis oil on the requirement for essential fatty acids in male rats as evidenced by growth and by the histological picture of testes, kidneys, skin, liver and adrenals. The rats were distributed over fifteen groups of six animals each and received the experimental diets for a 26-week period from weaning.

2. An increase in the dietary levels of fat depressed growth to a larger extent when hydrogenated arachis oil was fed instead of trilaurin.

3. Hydrogenated arachis oil damaged the testes, kidneys and skin to a higher degree than trilaurin at corresponding dietary levels.

4. The deleterious effect of hydrogenated arachis oil could be partly due to the presence of isomers of the unsaturated fatty acids formed during hydrogenation. The noxious effect of this fat was counteracted by supplementation with 20 mg ethyl linoleate/animal/day and completely prevented by 100 mg/animal/day.

5. The difference in chain length of the fatty acids present in the two kinds of fat as a possible influence on linoleate requirement remains to be elucidated.

## REFERENCES

Aaes-Jørgensen, E. (1954). The Role of Fat in the Diet of Rats. 6. Influence on Growth of Various Fats in Ordinary Refined State and after Hydrogenation or Polymerization. Copenhagen: Store Nordiske Videnskabsboghandel.
Aaes-Jørgensen, E. & Dam, H. (1954*a*). *Brit. J. Nutr.* **8**, 281.
Aaes-Jørgensen, E. & Dam, H. (1954*b*). *Brit. J. Nutr.* **8**, 285.
Aaes-Jørgensen, E. & Dam, H. (1954*c*). *Brit. J. Nutr.* **8**, 296.
Aaes-Jørgensen, E., Funch, J. P. & Dam, H. (1956). *Brit. J. Nutr.* **10**, 317.
Aaes-Jørgensen, E., Funch, J. P. & Dam, H. (1957). *Brit. J. Nutr.* **11**, 298.
Aaes-Jørgensen, E., Funch, J. P., Engel, P. F. & Dam, H. (1956*a*). *Brit. J. Nutr.* **10**, 32.
Aaes-Jørgensen, E., Funch, J. P., Engel, P. F. & Dam, H. (1956*b*). *Brit. J. Nutr.* **10**, 292.
Brody, S. (1945). *Bioenergetics and Growth.* New York: Reinhold Publishing Corp.
Burr, G. O. (1942). *Fed. Proc.* **1**, 224.
Burr, G. O. & Beber, A. J. (1937). *J. Nutr.* **14**, 553.
Deuel, H. J., Jr., Greenberg, S. M., Anisfeld, L. & Melnick, D. (1951). *J. Nutr.* **45**, 535.
Harte, R. A., Travers, J. J. & Sarich, P. (1948). *J. Nutr.* **36**, 667.
Holman, R. T. & Aaes-Jørgensen, E. (1956). *Proc. Soc. exp. Biol., N.Y.*, **93**, 175.
Panos, T. C., Finerty, J. C. & Wall, R. L. (1956). *Proc. Soc. exp. Biol., N.Y.*, **93**, 581.
Ramalingaswami, V. & Sinclair, H. M. (1953). *Brit. J. Dermatol.* **65**, 1.
Wesson, L. G. & Burr, G. O. (1931). *J. biol. Chem.* **91**, 525.