We are grateful to Dr E. C. Owen for suggesting the experiment and the basal diet used in it, and to Dr C. Higginbottom for growing *Bacterium cadaveris*. Miss S. McLaughlan and Mr T. Hutchison provided skilled technical assistance.

REFERENCES

Agrawala, I. P., Duncan, C. W. & Huffman, C. F. (1953). *J. Nutr.* **49**, 29.
Chanda, R., Clapham, H. M. & Owen, E. C. (1955). *Biochem. J.* **60**, 391.
Duncan, C. W., Agrawala, I. P., Huffman, C. F. & Luecke, R. W. (1953). *J. Nutr.* **49**, 41.
Gale, E. F. (1945). *Biochem. J.* **39**, 46.
Gale, E. F. (1948). *Eiweiss-Forsch.* **1**, 145.
Gale, E. F. & Epps, H. M. R. (1944). *Biochem. J.* **38**, 232.
Holmes, P., Moir, R. J. & Underwood, E. J. (1953). *Aust. J. biol. Sci.* **6**, 637.
Hubbell, R. B., Mendel, L. B. & Wakeman, A. J. (1937). *J. Nutr.* **14**, 273.
Kon, S. K. & Porter, J. W. G. (1947–8). *Nutr. Abstr. Rev.* **17**, 31.
Loosli, J. K., Williams, H. H., Thomas, W. E., Ferris, F. H. & Maynard, L. A. (1949). *Science*, **110**, 144.
McDonald, I. W. (1944). *J. Physiol.* **107**, 21*P*.
McElroy, L. W. & Goss, H. (1940). *J. Nutr.* **20**, 527.
McNaught, M. L. (1951). *Biochem. J.* **50**, i.
McNaught, M. L. & Smith, J. A. B. (1947–8). *Nutr. Abstr. Rev.* **17**, 18.
Mendel, L. B. (1921). *J. Franklin Inst.* **192**, 7.
Morris, S. & Wright, N. C. (1933*a*). *J. Dairy Res.* **4**, 177.
Morris, S. & Wright, N. C. (1933*b*). *J. Dairy Res.* **5**, 1.
Owen, E. C., Smith, J. A. B. & Wright, N. C. (1943). *Biochem. J.* **37**, 44.
Schram, E., Dustin, J. P., Moore, S. & Bigwood, E. J. (1953). *Analyt. chim. acta*, **9**, 149.
Smith, J. A. B. & Baker, F. (1944). *Biochem. J.* **38**, 496.

The role of fat in the diet of rats

9. Influence on growth and histological findings of diets with hydrogenated arachis oil or no fat, supplemented with linoleic acid or raw skim milk, and of crude casein compared with Vitamin Test Casein

BY E. AAES-JØRGENSEN, J. P. FUNCH, P. F. ENGEL AND H. DAM

Department of Biochemistry and Nutrition, Polytechnic Institute, Copenhagen

(*Received* 16 *April* 1956)

We are reporting here the results of experiments carried out in continuation of previous investigations (Aaes-Jørgensen, Engel, Funch & Dam, 1955) into the effect of supplementing with 1 mg linoleic acid/animal/day diets containing no fat or hydrogenated arachis oil. The linoleic acid is roughly equivalent to the amount of essential fatty acids consumed daily by animals reared on 'fat-free' diets containing 20% crude casein or receiving raw skim milk instead of water as drinking fluid. Another diet was prepared to contain only 0·24% arachis oil as the sole fat constituent: this quantity corresponds to the level of total fat in 'fat-free' diets containing 20% crude casein. Such a diet supplies the animals with not more than 6–10 mg linoleic acid/animal/day. The controls were fed on diets containing 28% arachis oil. Histological studies were performed on various organs of all the animals.

EXPERIMENTAL

Newly weaned male and female rats were distributed over twenty groups of nine animals each. Food and drinking fluid were given *ad lib.* for 18 weeks. Percentage compositions of the diets are shown in Table 1. Supplementary linoleic acid was given as drops (Table 1), as were vitamins A and D. The suspension of Decamin aquosum (Ferrosan Ltd, Copenhagen) supplied 130 i.u. vitamin A and 20 i.u. vitamin D_2/animal/week. The Decamin aquosum also contained a minute amount of arachis oil, supplying each animal with about 0·00066 mg linoleic acid per day. The animals were weighed and inspected weekly; at the end of the experiment they were killed with chloroform.

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

Group no. ...	151	152	153	154	155	156	157	158	159	160
Vitamin Test Casein*	20	—	20	—	20	20	20	20	20	20
Crude casein†	—	20	—	20	—	—	—	—	—	—
Sucrose	74	74	46	46	46	46	46	74	46	73·76
Salt mixture‡	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
Choline chloride	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5
Hydrogenated arachis oil‖ (m.p. 40–42°)	—	—	28	28	28	28	28	—	—	—
Arachis oil‖	—	—	—	—	—	—	—	—	28	0·24
Linoleic acid (mg/animal/day)¶	—	—	—	—	—	1	20	1	—	—
Drinking fluid	W	W	W	W	R	W	W	W	W	W

W = water, R = raw skim milk.

* From Genatosan Ltd, Loughborough, England. (Precipitated from skim milk with acetic acid, washed successively with water, alcohol and ether, and dried.)

† From Dansk Mejeri Industri and Export Kompagni, Stege, Denmark. (Precipitated from skim milk by acidification with acid starter at 30–35°, heated to 60–65°, washed with water and dried.)

‡ McCollum & Simmonds's (1918) 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-tocopheryl acetate (Ephynal, Roche Products Ltd) 5 mg, dicalcium salt of 2-methyl-1:4-naphthohydroquinone diphosphoric acid ester (Synkavit, Roche Products Ltd) 1 mg, vitamin B_{12} 1·5 μg and sucrose to 500 mg.

‖ From Dansk Soyakagefabrik Ltd, Copenhagen.

¶ From F. Hoffman-La Roche and Co. Ltd, Basle, Switzerland.

Post-mortem. Kidneys, liver and small intestine of all rats, testes of all males and ovaries and abdominal-skin specimens of all females were examined histologically. Blocks of tissue of a thickness not greater than 0·5 cm were fixed in 10% commercial formalin (4% formaldehyde). Dehydration was accomplished in multiple changes of diluted, undiluted and absolute ethanol, by means of an automatic machine (Autotechnicon). The tissues were then cleared in xylene and embedded in a mixture of 94% paraffin and 6% bees-wax (m.p. of mixture 58°). Sections of 5 μ were cut, and three selected sections of each block were stained routinely with Hansen's haematoxylin and eosin.

Part of the material from the testes was stained with van Gieson's stain to demonstrate myofibrils and connective tissue, or cut on a freezing microtome and stained with oil red O for study of fat content.

RESULTS AND DISCUSSION

Growth rates

The mean weights of the animals at the beginning and end of the experiment are shown in Table 2. The quantities of fat and essential fatty acids consumed daily by rats eating 10 g of the various diets are recorded in Table 3.

Fat-free diets. Male rats grew better on crude casein than on Vitamin Test Casein (groups 152 and 151). In the females no such effect was noted in this experiment, but it has been observed previously (Aaes-Jørgensen *et al.* 1955).

The crude-casein diet furnished each rat with about 1 mg of essential fatty acids/day (Table 3). It was therefore compared with Vitamin Test Casein diets supplemented with 1 mg linoleic acid/day, given by dropper. The results (groups 152 and 158) were similar, suggesting that the effect obtained with crude casein may be due, at least in part, to its content of linoleic acid.

The total fat content of the crude-casein diet is about 0·24% (Table 3). A comparison was therefore also made with a diet containing Vitamin Test Casein and 0·24% of another fat, namely arachis oil, which contains much more linoleic acid than the fat in the crude casein.

The content of linoleic acid in the diet with 0·24% arachis oil was about 0·06%, which gave a daily intake of linoleic acid/rat/day of about 6–10 mg (equal to 10–15 g of diet). Such a diet (group 160) gave better growth in males than could be obtained with 1 mg linoleic acid/animal/day (group 158) or with crude casein (group 152); the females also responded (group 160). This shows that, in so far as the fat content of the crude casein accounts for its favourable effect on growth, it is not the content of total fat that is of importance, since a similar amount of fat with a higher content of linoleic acid gave better results. The results (groups 152, 158 and 160) were always inferior to that obtained with 28% arachis oil (group 159).

Compared with that on the diet containing Vitamin Test Casein (group 151) the growth period of the animals of both sexes was prolonged by crude casein (group 152), by supplementing with 1 mg linoleic acid/animal/day (group 158) or by supplementing with 0·24% arachis oil (group 160). In the last group the animals grew through the entire experiment, as did the controls (group 159).

Diets containing 28% hydrogenated arachis oil. As in our earlier experiments, the diets containing 28% hydrogenated arachis oil (groups 153 and 154) produced much poorer growth than the corresponding fat-free diets (groups 151 and 152) (Table 2). In the males growth was somewhat better on 28% hydrogenated arachis oil with crude casein than with Vitamin Test Casein; in females no such difference was noted (groups 153 and 154). A supplement of 1 mg linoleic acid/rat/day (group 156) gave results in the same direction as those obtained by similar supplementation of the fat-free diet, though much less marked. A significant improvement of growth was

Table 2. *Mean initial and final weights, growth period and weight at cessation of growth, and number of animals in each group alive at the end of the experimental period of* 18 *weeks*

(Nine animals/group)

Diet characteristics	Group no.	Males: Initial weight (g)	Males: Cessation of growth: Week	Males: Cessation of growth: Weight of rat (g)	Males: Final weight (value with its standard error) (g)	Females: Initial weight (g)	Females: Cessation of growth: Week	Females: Cessation of growth: Weight of rat (g)	Females: Final weight (value with its standard error) (g)	No. of animals alive after 18 weeks: Male	No. of animals alive after 18 weeks: Female	Time and cause of death
No fat, Vitamin Test Casein	151	52·0	13th	210	210± 9·2	51·0	11th	159	160± 8·0	9	9	—
No fat, crude casein	152	53·0	17th	242	243± 9·8	51·0	16th	163	161± 3·3	9	9	—
28 % hydrogenated arachis oil, Vitamin Test Casein	153	53·0	10th	178	166± 10·5	51·0	10th	147	138± 4·0	8	9	♂ no. 22 died after 17 weeks; tympanitis
28 % hydrogenated arachis oil, crude casein	154	53·0	13th	190	181± 4·1	51·0	11th	140	141± 5·4	7	8	♂ no. 50 died after 14 weeks; tympanitis. ♂ no. 38 died after 16 weeks; rupture of the stomach. ♀ no. 27 died after 14 weeks; perihepatitis and peritonitis
28 % hydrogenated arachis oil, Vitamin Test Casein, raw skim milk	155	53·0	15th	210	209± 10·2	51·0	13th	171	172± 12·4	9	7	♀ no. 38 died after 6 weeks; lung haemorrhage. ♀ no. 41 died after 6 weeks; pneumonia
28 % hydrogenated arachis oil, Vitamin Test Casein, 1 mg linoleic acid/animal/day	156	53·0	9th	183	174± 9·7	51·0	9th	139	141± 6·3	9	6	♀ no. 51 died after 11 weeks; pneumonia. ♀ no. 42 died after 12 weeks; unknown. ♀ no. 44 died after 16 weeks; unknown
28 % hydrogenated arachis oil, Vitamin Test Casein, 20 mg linoleic acid/animal/day	157	53·1	—	—	271± 8·3	51·2	—	—	190± 7·8	9	9	—
No fat, Vitamin Test Casein, 1 mg linoleic acid/animal/day	158	53·1	15th	235	235± 6·8	51·1	14th	162	161± 5·6	9	9	—
28 % arachis oil, Vitamin Test Casein	159	53·0	—	—	364± 17·9	51·0	—	—	232± 17·4	8	9	♂ no. 90 died after 10 weeks; otitis media
0·24 % arachis oil, Vitamin Test Casein	160	53·3	—	—	263± 8·5	51·9	—	—	182± 5·2	9	9	—

Table 3. *Approximate content of fat and of linoleic, linolenic and arachidonic acids in the diets (mg/10 g)*

	Group no. 151				152				153				154				155			
	Fat	Linoleic acid	Linolenic acid	Arachidonic acid	Fat	Linoleic acid	Linolenic acid	Arachidonic acid	Fat	Linoleic acid	Linolenic acid	Arachidonic acid	Fat	Linoleic acid	Linolenic acid	Arachidonic acid	Fat	Linoleic acid	Linolenic acid	Arachidonic acid
Dietary fat*	—	—	—	—	—	—	—	—	2800	0·56	—	—	2800	0·56	—	—	2800	0·56	—	—
Vitamin Test Casein†	1·8	0·03	—	—	—	—	—	—	1·8	0·03	—	—	—	—	—	—	1·8	0·03	—	—
Crude casein‡	—	—	—	—	24	0·4	0·2	0·2	—	—	—	—	24	0·4	0·2	0·2	—	—	—	—
Raw skim milk§	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	25	0·5	0·2	0·1
Linoleic-acid supplement	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Total amount/animal/day‖	1·8	0·03	—	—	24	0·4	0·2	0·2	2824	0·6	—	—	2802	1	0·2	0·2	2827	1·1	0·2	0·1

	Group no. 156				157				158				159				160			
	Fat	Linoleic acid	Linolenic acid	Arachidonic acid	Fat	Linoleic acid	Linolenic acid	Arachidonic acid	Fat	Linoleic acid	Linolenic acid	Arachidonic acid	Fat	Linoleic acid	Linolenic acid	Arachidonic acid	Fat	Linoleic acid	Linolenic acid	Arachidonic acid
Dietary fat*	2800	0·56	—	—	2800	0·56	—	—	—	—	—	—	2800	748	25	—	24	6·4	0·2	—
Vitamin Test Casein†	1·8	0·03	—	—	1·8	0·03	—	—	1·8	0·03	—	—	1·8	0·03	—	—	1·8	0·03	—	—
Crude casein‡	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Raw skim milk§	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Linoleic-acid supplement	1	1	—	—	20	20	—	—	1	1	—	—	—	—	—	—	—	—	—	—
Total amount/animal/day‖	2803	1·6	—	—	2822	20·6	—	—	2·8	1	—	—	2802	748	25	—	26	6·4	0·2	—

* The arachis oil contained 26·7 % linoleic acid and 0·9 % linolenic acid. The hydrogenated arachis oil contained (at most) 0·02 % linoleic acid.

† Vitamin Test Casein contained 0·09 % fat.

‡ Crude casein contained 1·2 % fat with 1·7 % linoleic acid, 0·7 % linolenic acid and 1 % arachidonic acid.

§ The animals drank at most 50 ml./day, i.e. about 25 mg fat with 1·9 % linoleic acid, 0·7 % linolenic acid and 0·5 % arachidonic acid.

‖ Vitamin A and vitamin D_2 solution supplied the animals with 0·00266 mg fat and about 0·00066 mg linoleic acid/rat/day. These amounts are considered negligible and are not included in the total amounts of fat and fatty acids.

obtained by giving 20 mg linoleic acid/day (group 157), to which both sexes responded. The growth of this group was almost equal to that of the animals given 0·24% arachis oil (group 160); in both groups, however, the growth rate was significantly lower than that of the controls given 28% arachis oil (group 159) (Table 2).

A remarkable improvement in growth was also obtained by giving raw skim milk instead of water as drinking fluid (group 155), in accordance with our previous findings. The skim milk furnished each rat with about 1 mg/day of essential fatty acids (Table 3); however, the effect obtained either by direct supplementation with 1 mg linoleic acid, or on diets containing crude casein instead of Vitamin Test Casein, was much less than that obtained with raw skim milk, and is thus not sufficient to explain the effect of skim milk on growth.

On the diets containing hydrogenated arachis oil growth in both sexes ceased earlier than on the corresponding diets without hydrogenated fat (Table 2). Further, decline in weight after growth had ceased occurred in several of the groups on hydrogenated fat. Cessation of growth was somewhat delayed by the use of raw skim milk as drinking fluid (group 155 compared with 153) and of crude casein (group 154 compared with 153), but not by supplementation with 1 mg linoleic acid/animal/day (group 156 compared with 153). With a supplement of 20 mg linoleic acid/animal/day (group 157) the animals grew through the entire experiment.

In a previous study with hydrogenated fats (Aaes-Jørgensen, 1954) 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 deleterious effect of hydrogenated fat as evidenced by growth rate and histological findings, may be due to a deficiency of essential fatty acids, which may be particularly marked in the presence of isomers of the unsaturated fatty acids formed during hydrogenation.

Clinical signs and results of autopsy

The accepted skin signs of essential fatty-acid deficiency were noticed in all animals except those of group 157 (28% hydrogenated arachis oil plus 20 mg linoleic acid/animal/day) and group 159 (28% arachis oil). The animals in group 160 (0·24% arachis oil) showed a slight degree of scaliness of the tail as the only skin sign.

The fur of the animals given hydrogenated arachis oil was particularly thin. These animals also showed the highest degree and most frequent occurrence of dandruff, whereas scaliness of the legs and tail often appeared much later and to a lesser degree than in the animals reared on 'fat-free' diets. Some of the females from groups 159 and 160 showed a yellow-brown pigmentation of the fur, especially on and between the shoulders.

In several of the animals with skin signs it was noticed that the signs that had persisted for some time tended to fade out more or less without any changes in the diet or experimental conditions. In previous experiments a similar phenomenon has been observed now and then. Barki, Nath, Hart & Elvehjem (1947) obtained similar effects with mature rats after depletion, that is to say, giving the fat-free diet *ad lib.* for a sufficiently long time led to spontaneous disappearance of all signs. The reason is not

yet understood. Skin signs do not always seem to be a well-defined criterion of experimental essential fatty-acid deficiency.

A severe kyphosis was seen in many of the animals of both sexes given hydrogenated arachis oil (groups 153, 154 and 156), but only in a single male from group 155 (skim milk as drinking fluid) and in none in group 157 (supplementation with 20 mg linoleic acid). Whether the kyphosis was caused by the hydrogenated fat or was simply related to the poor condition of these animals we do not know.

At autopsy the liver and kidneys from the animals given either hydrogenated arachis oil or no fat were frequently a little lighter in colour and somewhat larger than those of the controls. The kidneys often had an uneven surface. (One or two bladder-worms encapsuled in the liver were found in animals from various groups.) The testes from the animals reared on diets containing hydrogenated arachis oil were small, weighing only 0·5–0·75 g each, whereas those from the animals on the fat-free ration or from the controls weighed on the average 1·2 g. The absolute weights of heart and kidneys were lower, but in relation to body-weight the weights were higher, than those of the control group. Similar observations of the weights of the kidneys have been described earlier from this laboratory (Aaes-Jørgensen, 1954).

Histological studies

A summary of the histopathological findings in the kidneys, liver, intestine, testes, ovaries and skin is provided in Table 4.

Kidneys. Calcified tubules (calculi) near the cortico-medullary border were present to a higher degree in females than in males. In accordance with our earlier findings (Aaes-Jørgensen *et al.* 1955), the animals on the fat-free diets had more calculi than the animals fed on diets containing 28% hydrogenated arachis oil. The animals given 28% arachis oil (group 159) had few or no calculi. However, calculi may not be a sign of deficiency of essential fatty acids. Various problems connected with the appearance of calculi have been discussed previously (Aaes-Jørgensen, Funch, Engel & Dam, 1956).

A dilatation of cortical tubules was seen in some groups, especially in the males. Various degrees of degeneration of the cells of the papillary duct were noted in all the groups except the control group getting 28% arachis oil (159). In the groups with moderate or abundant degeneration of the papilla, necrosis and calcification also occurred in some rats. Calcification in the papilla appeared to follow cellular degeneration, but there was no clear evidence that degenerative changes preceded calculus formation near the cortico-medullary border.

Liver and small intestine. No significant abnormalities were observed.

Testes. A preliminary note on some of the changes in the testicular tissue has been presented earlier (Aaes-Jørgensen & Dam, 1955). It can be seen from Pls. 1 and 2 and from Table 4 that the rats showed a great variation in impairment of spermatogenesis.

The impairment was graded from 0 to 5 as follows:

Degree 0: no damage.

Degree 1: the number of spermia in the lumen of seminiferous tubules is diminished. Maturation of the spermia seems to be protracted, because the number of maturing spermia attached to Sertoli cells is somewhat increased. The rest of the seminiferous

Table 4. *Histopathological changes in the experimental rats*

Group no.	Diet characteristics	Kidneys: Mean occurrence of calculi* Males	Kidneys: Mean occurrence of calculi* Females	Kidneys: Dilatation of tubules in cortex† Males	Kidneys: Dilatation of tubules in cortex† Females	Kidneys: Degeneration in papilla† Males	Kidneys: Degeneration in papilla† Females	Testes, mean degree of injury (see text, p. 298)	Ovaries, occurrence of 'wheel nuclei'†	Epidermis of females: No. of cell layers in stratum spinosum and stratum basale	Epidermis of females: No. of layers of keratohyaline in stratum granulosum	Epidermis of females: Intercellular spaces in stratum spinosum†
151	No fat, Vitamin Test Casein	0·4	2·1	+	+	++	±	0·9	±	7–8	3	+
152	No fat, crude casein	0·8	2·0	—	+	++	+	0·7	±	5–6	2–3	±
153	28 % hydrogenated arachis oil, Vitamin Test Casein	0	0·5	++	+	+++	++	4·0	++	6–7	3	+
154	28 % hydrogenated arachis oil, crude casein	0	0·4	+	—	++	++	3·1	+	6–7	3	+
155	28 % hydrogenated arachis oil, Vitamin Test Casein, raw skim milk	0	0·3	+	—	+	++	4·1	±	5–6	2–3	±
156	28 % hydrogenated arachis oil, Vitamin Test Casein, 1 mg linoleic acid/animal/day	0	0·3	+	—	++	+	1·8‡	+	5–6	3	±
157	28 % hydrogenated arachis oil, Vitamin Test Casein, 20 mg linoleic acid/animal/day	0	0·6	—	—	±	±	0	±	2–3	1–2	—
158	No fat, Vitamin Test Casein, 1 mg linoleic acid/animal/day	0·1	2·7	—	—	±	±	0·8	±	5–6	2–3	±
159	28 % arachis oil, Vitamin Test Casein	0	0·3	—	—	—	—	0	—	2	1	—
160	0·24 % arachis oil, Vitamin Test Casein	0	3·4	—	—	±	±	0	—	2–3	1	—

* Assessed from a scale graduated from 0 (no calculi) to 5 (abundance of calculi).
† The sign ± indicates traces; +, ++ or +++ indicates slight, moderate or marked changes.
‡ Three animals had a mean score of 4·7, and six one of 0·5.

epithelium appears normal. The epididymis is characterized by a diminished number of spermia and the presence of desquamated degenerating spermatogenic cells.

Degree 2: there are few mature spermia. The spermatids are undergoing degeneration. Hyperchromatosis of the nuclear wall gives the nucleus a vesicular appearance. Often the chromatin particles have become condensed at one side of the nucleus. In the epididymis the number of spermia is low and the number of degenerating spermatogenic cells is increased.

Degree 3: mature spermia are absent. The degeneration of the spermatids proceeds. Karyolysis or pyknosis of the nucleus and a plasmolysis of the surrounding cytoplasm takes place. Multinucleated large round cells are formed by fusion of degenerating spermatids. Large dense masses of acidophilic precipitates and spermatogenic cells showing all degrees of pyknotic and autolytic changes appear in many of the ducts of the epididymis.

Degree 4: spermatids are absent. The epithelium of the seminiferous tubules is greatly reduced, containing only one or two layers of germinal cells. The multinucleated cells are scarce or in advanced stages of dissolution. At this stage degeneration and desquamation of the spermatocytes and spermatogonia are conspicuous. The epididymis usually contains some acidophilic material and a few degenerating cells.

Degree 5: at this advanced stage of degeneration the intertubular tissue appears oedematous, and the atrophic tubules contain only the syncytium of Sertoli cells with a few scattered spermatogonia. The Sertoli-cell nuclei show no degenerative changes, but the syncytial cytoplasm appears vacuolized and more fibrous than normal. The ducts of the epididymis are usually empty.

From the above description it is seen that the spermatogenic cells degenerate in the inverse order of origin. Sections of the seminiferous tubules of the same testis may show various different stages of degeneration, because some tubules seem more resistant to degeneration than others. One degree of degeneration, however, is usually predominant.

In advanced stages of degeneration the interstitial tissue had a tendency to accumulate in irregular clumps, and owing to atrophy of the tubules there was a relative increase of the interstitial tissue, but as far as could be determined no actual hypertrophy or hyperplasia had taken place. No fibrous or fatty infiltrations of the testes were noted.

Fat-free diets with Vitamin Test Casein or crude casein (groups 151 and 152) caused no typical degeneration of the spermatogenic tissue, as illustrated in Pl. 1, 1. However, in the epididymis the number of spermatozoa was reduced and many degenerating spermatogenic cells appeared (Pl. 1, 2,3). Supplementation with 1 mg linoleic acid/rat/day (group 158) seemed to have no influence on the pattern in the epididymis, but with 28 or even 0·24% arachis oil these changes were completely prevented (groups 159 and 160).

Hydrogenated arachis oil, whether in diets containing Vitamin Test Casein or crude casein or in the former supplemented with raw skim milk (groups 153, 154 and 155), caused a moderate to severe degeneration of the spermatogenic cells (Pl. 1, 4,5). Often only a few scattered spermatogonia and the syncytium of Sertoli cells were found in the

seminiferous tubules, and the testes were small. Supplementation with 1 mg linoleic acid/rat/day (Pl. 2, 1) caused an almost normal pattern of spermatogenesis in the testes of six of the animals from this group but three rats had severe degeneration of the spermatogenic cells. The deleterious effect of hydrogenated arachis oil on spermatogenesis was completely prevented by 20 mg linoleic acid (group 157).

As shown in Pl. 1, 6, the ducts of the epididymis of the rats given 28% hydrogenated arachis oil were empty. Supplementation with 1 mg linoleic acid/rat/day, which in six out of nine animals resulted in almost normal seminiferous tubules (Pl. 2, 1), gave a picture in the epididymis (Pl. 2, 2) similar to that seen in rats on fat-free diets (Pl. 1, 2), i.e. many degenerating spermatogenic cells and a decreased content of spermatozoa. Whether the spermatozoa had been alive and fertile is unknown. Studies on these questions are in progress.

The fact that typical degeneration of the spermatogenic tissue was not seen on fat-free diets agrees with findings of Greenberg & Ershoff (1951) and of Mackenzie, Mackenzie & McCollum (1939), but is in contrast to those of Evans, Lepkovsky & Murphy (1934) and of Panos & Finerty (1954). The two last-mentioned groups of workers found spermatogenesis arrested at the stages of spermatocytes; they also found numerous multinucleated giant cells and large vacuoles in the seminiferous tubules of rats on fat-free diets. Our findings clearly show the aggravating effect of hydrogenated fat on the deficiency of essential fatty acids, markedly illustrated by the damage of the normally active spermatogenic tissue. The deleterious effect of hydrogenated fat as evidenced by growth rate and histology could be due to the isomers of unsaturated fatty acids formed during hydrogenation. Linoleic acid counteracted the effect.

Ovaries. Interstitial cells containing 'wheel nuclei', similar to those described in fat-deficient rats by Panos & Finerty (1953), were present, but to varying degrees, in the groups fed on the fat-free or hydrogenated arachis oil diets. These authors suggest that 'wheel nuclei' may indicate a deficiency of pituitary secretion of luteinizing hormone. The presence of 'wheel nuclei' (cf. Table 4 and Pl. 2, 3) was most conspicuous in group 153 (28% hydrogenated arachis oil). The ovaries of this group apparently contained a larger amount of interstitial tissue than those of the other groups. In some of the ovaries of the rats given hydrogenated arachis oil the interstitial cells showed widespread cellular changes resembling stages of degeneration. The cytoplasm stained faintly, and there was a shrinkage of the nucleus and a condensation of the chromatin into an irregular lump, bearing some resemblance to a thorn-apple, and apparently a more advanced stage of degeneration than 'wheel nuclei' (Pl. 2, 3,4).

Skin. Skin specimens from the abdomens of the female rats were taken for histological examination. The skins of the males were not investigated. As will be seen from Table 4, histological alterations in the epidermis were prominent when the fat-free diets or diets containing hydrogenated arachis oil were given. Moreover, the rats receiving these diets had cellular infiltration in the dermis to a higher degree than those given 28% arachis oil. The changes found were essentially the same as those described in fat-deficient rats by Ramalingaswami & Sinclair (1953) and by Panos &

Finerty (1953, 1954). Supplementation with 1 mg linoleic acid/animal/day did not ameliorate this condition on diets free of fat or containing hydrogenated arachis oil, whereas a normal pattern was seen in the animals supplemented with 20 mg linoleic acid/animal/day (group 157, receiving hydrogenated arachis oil) and in those reared on 0·24% arachis oil (group 160) or 28% arachis oil (group 159).

SUMMARY

1. The purpose of the experiments reported here was to study the influence of supplements of small amounts of linoleic acid, of two kinds of casein, of hydrogenated arachis oil, of raw skim milk and of arachis oil on the growth and histology of male and female rats receiving the experimental diets for an 18-week period from weaning. Kidneys, liver, small intestine of all animals, testes with epididymis of males and ovaries and epidermis of females were examined.

2. On fat-free diets and on diets containing 28% hydrogenated arachis oil males grew to somewhat greater final weights with crude casein than with Vitamin Test Casein. A similar effect was not seen in the females. The period of growth on crude casein was longer for both sexes.

3. On fat-free diets containing Vitamin Test Casein an effect on growth of males similar to that produced by crude casein could be obtained with a daily supplement of 1 mg linoleic acid, an amount approximately corresponding to the essential fatty-acid content of the crude casein. On diets containing 28% hydrogenated arachis oil the effect of 1 mg linoleic acid was less marked.

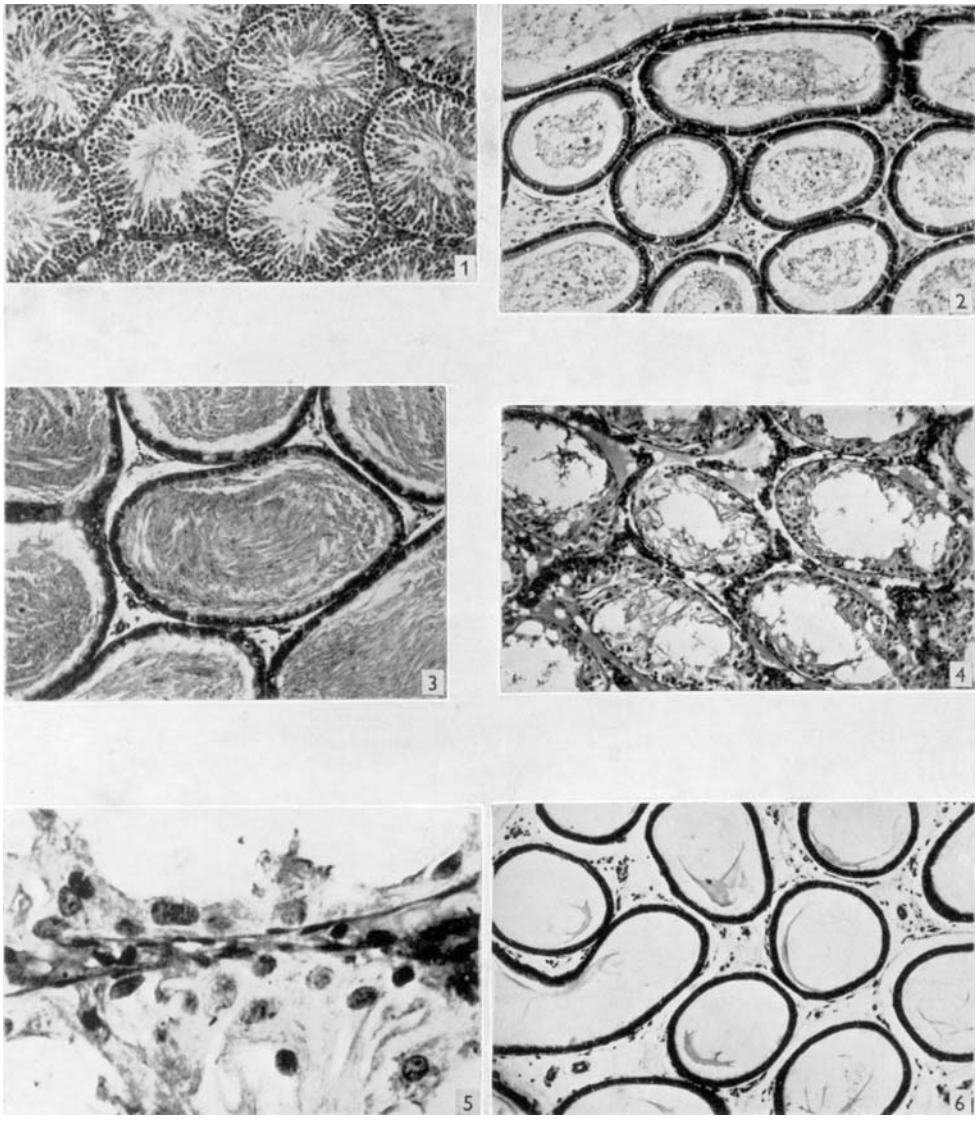
4. On diets containing 28% hydrogenated arachis oil, replacement of water as drinking fluid by raw skim milk improved growth. On the assumption that the skim milk furnished each rat with 1 mg essential fatty acids per day, these acids in the skim milk did not account for all of its growth-improving effect.

5. Cessation of growth occurred earlier on diets containing 28% hydrogenated arachis oil than on those with no fat. With hydrogenated arachis oil, growth declined after having reached a maximum, whereas without fat a steady level was reached. The maximum reached on hydrogenated fat was lower than the steady level reached on fat-free diets.

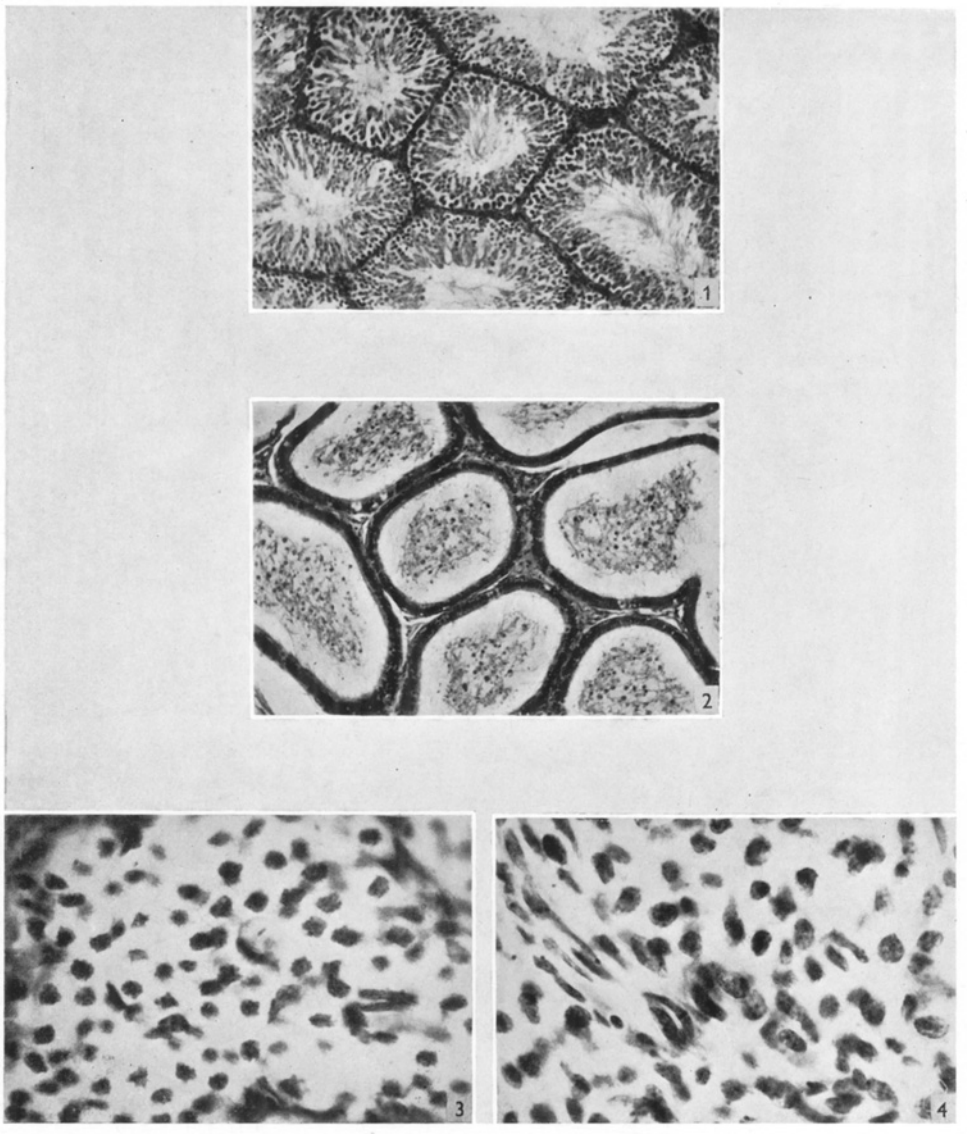
6. A supplement of 20 mg linoleic acid/rat/day greatly improved growth of both sexes on diets containing 28% hydrogenated arachis oil and enabled the rats to grow throughout the experimental period. The weight reached was, however, far below that obtained with 28% arachis oil instead of hydrogenated arachis oil.

7. The addition of 0·24% arachis oil to a fat-free diet containing Vitamin Test Casein brought about improved growth in both sexes and enabled the animals to grow throughout the experimental period. This effect is ascribed to the linoleic acid (60 mg/100 g diet) furnished by the arachis oil, since an approximately equal amount of fat with a lower content of essential fatty acids, namely the milk fat in the crude casein, had a smaller effect on growth.

8. In the kidneys calcified tubules (calculi) occurred mostly in the females and most frequently on fat-free diets and on such diets supplemented with 0·24% arachis oil



(*Facing p.* 302)



or with 1 mg linoleic acid/rat/day. The frequency of calculi was reduced by 28% arachis oil, as well as by 28% hydrogenated arachis oil. It is therefore likely that this abnormality was not due to deficiency in essential fatty acids. Dilatation of tubules in the cortex occurred on fat-free diets and especially on diets containing hydrogenated arachis oil. Papillary degenerations were most severe and most frequent in the males and more on diets with hydrogenated fat than on those without fat. This sign was prevented by linoleic acid.

9. No changes were found in liver and small intestine in any of the groups.

10. Small degenerated testes were found in the males receiving hydrogenated arachis oil. The spermatogenic tissue was severely damaged. As a rule the seminiferous tubules only contained the syncytium of Sertoli cells and a few scattered spermatogonia. Supplementation with linoleic acid counteracted the effect.

11. The females, especially those receiving 28% hydrogenated arachis oil, showed an increased number of 'wheel nuclei' in the interstitial tissue of the ovaries. Sometimes the interstitial cells showed widespread cellular changes, resembling stages of degeneration. The changes were largely counteracted by linoleic acid.

12. Histological changes in the epidermis of female rats were seen on fat-free diets and on those containing hydrogenated arachis oil. On the latter diets a supplement of 20 mg linoleic acid/rat/day largely prevented such changes, and on the fat-free diet 0·24% arachis oil secured normal development.

REFERENCES

Aaes-Jørgensen, E. (1954). *The Role of Fat in the Diet of Rats.* 6. *Influence of Various Fats in Ordinary and Refined State and after Hydrogenation or Polymerization.* Copenhagen: Store Nordiske Videnskabsboghandel.

Aaes-Jørgensen, E. & Dam, H. (1955). *Congr. int. Biochim.* III. *Bruxelles. Résumés des Communications*, p. 117.

Aaes-Jørgensen, E., Engel, P. F., Funch, J. P. & Dam, H. (1955). *Brit. J. Nutr.* **9**, 42.

Aaes-Jørgensen, E., Funch, J. P., Engel, P. F & Dam, H. (1956). *Brit. J. Nutr.* **10**, 32.

Barki, V. H., Nath, H., Hart, E. B. & Elvehjem, C. A. (1947). *Proc. Soc. exp. Biol., N.Y.*, **66**, 474.

Evans, H. M., Lepkovsky, S. & Murphy, E. A. (1934). *J. biol. Chem.* **106**, 445.

Greenberg, S. M. & Ershoff, B. H. (1951). *Proc. Soc. exp. Biol., N.Y.*, **78**, 552.

McCollum, E. V. & Simmonds, N. (1918). *J. biol. Chem.* **33**, 55.

Mackenzie, C. G., Mackenzie, J. B. & McCollum, E. V. (1939). *Biochem. J.* **33**, 935.

Panos, T. C. & Finerty, J. C. (1953). *J. Nutr.* **49**, 397.

Panos, T. C. & Finerty, J. C. (1954). *J. Nutr.* **54**, 315.

Ramalingaswami, V. & Sinclair, H. M. (1953). *Brit. J. Dermatol.* **65**, 1.

EXPLANATION OF PLATES

PLATE 1

1. Testis from an animal fed on a fat-free diet (group 151). Haematoxylin-eosin. ×96.
2. Epididymis from the same animal. Haematoxylin-eosin. ×96.
3. Epididymis from a normal animal fed on the diet with 28% arachis oil (group 159). Haematoxylin-eosin. ×96.
4. Testis from an animal fed on the diet with 28% hydrogenated arachis oil (group 153). Haematoxylin-eosin. ×96.
5. As no. 4. ×605.
6. Epididymis from the same animal. Haematoxylin-eosin. ×96.

PLATE 2

1. Testis from an animal fed on the diet with 28 % hydrogenated arachis oil supplemented with 1 mg linoleic acid/animal/day (group 156). Haematoxylin-eosin. ×96.
2. Epididymis from the same animal. Haematoxylin-eosin. ×96.
3. Ovarian interstitial tissue from an animal fed on the diet with 28 % hydrogenated arachis oil (group 153). Nuclei with 'thorn-apple' appearance will be noticed. Haematoxylin-eosin. ×960.
4. Ovarian interstitial tissue from a normal animal fed on the diet with 28 % arachis oil (group 159). Haematoxylin-eosin. ×960.

Nutrition of the cat

1. A practical stock diet supporting growth and reproduction

BY CECILIA D. DICKINSON AND PATRICIA P. SCOTT

Department of Physiology, Royal Free Hospital School of Medicine, London, W.C. 1

(*Received 25 April 1956*)

In order to investigate problems of breeding and rearing cats in laboratory conditions, an experimental colony was established in 1950 at the Royal Free Hospital School of Medicine, where it has been in continuous existence for 6 years.

The amounts of food and the methods of feeding described in this paper have proved adequate for the maintenance in good health of thirty breeding females, their kittens, three males and about thirty other adult cats at any one time.

EXPERIMENTAL

Animal management

The methods of animal husbandry used in the maintenance of this colony have been described by Cornelius (1952) and Scott (1956), with some recent improvements detailed below. The room used for breeding and rearing was 9 × 22 × 8 ft., with two wire-mesh partitions from floor to ceiling, supplied with doors dividing it into three parts. A continuous-extraction ventilation system afforded about five changes of air per hour, and the room was maintained at about 70° F. Apart from artificial lighting between 3.30 p.m. and 5 p.m. during the winter months, the colony was subjected only to seasonal changes in the length of daylight.

Usually fifteen breeding female cats (queens) with one male (tom) were maintained in both end divisions of the breeding room, and fully weaned kittens were allowed to run in the centre division until mature or removed for experiments. The males were normally allowed to run with the females except for two short periods (in the autumn and winter months of 1953 and 1955) when they were used in mating experiments. As pregnant females neared their expected delivery date (*c.* 65 days after mating) they were confined individually in specially designed cages (Scott, 1952) placed on racks or on the floor. Litters, whose individual members were identified shortly after birth, were kept together in these cages until the end of the lactation period of 7–8 weeks,