Gray, F. V., Pilgrim, A. F., Rodda, H. J. & Weller, R. A. (1952). *J. exp. Biol.* **29**, 57.
Gray, F. V., Pilgrim, A. F. & Weller, R. A. (1951). *J. exp. Biol.* **28**, 74.
Hungate, R. E., Dougherty, R. W., Bryant, M. P. & Cello, R. M. (1952). *Cornell Vet.* **42**, 423.
Hunt, C. H., Burroughs, E. W., Bethke, R. M., Schalk, A. F. & Gerlaugh, P. (1943). *J. Nutr.* **25**, 207.
Kiddle, P., Marshall, R. A. & Phillipson, A. T. (1951). *J. Physiol.* **113**, 207.
Lardinois, C. C., Mills, R. C., Elvehjem, C. A. & Hart, E. B. (1944). *J. Dairy Sci.* **27**, 579.
McClymont, G. L. (1951). *Aust. J. agric. Res.* **2**, 92.
McNaught, M. L. (1951). *Biochem. J.* **49**, 325.
Masson, M. J. (1951). *Research*, **4**, 73.
Monroe, C. F. & Perkins, A. E. (1939). *J. Dairy Sci.* **22**, 983.
Moyle, V., Baldwin, E. & Scarisbrick, R. (1948). *Biochem. J.* **43**, 308.
Olson, T. M. (1941). *J. Dairy Sci.* **24**, 413.
Phillipson, A. T. (1942). *J. exp. Biol.* **19**, 186.
Phillipson, A. T. (1952). *Brit. J. Nutr.* **6**, 190.
Phillipson, A. T. & McAnally, R. A. (1942). *J. exp. Biol.* **19**, 199.
Popják, G., French, T. H. & Folley, S. J. (1951). *Biochem. J.* **48**, 411.
Schambye, P. & Phillipson, A. T. (1949). *Nature, Lond.*, **164**, 1094.
Troy, H. C. & Sharp, P. F. (1935). *Mem. Cornell agric. Exp. Sta.* no. 179.

The role of fat in the diet of rats

11. Influence of a small amount of ethyl linoleate on degeneration of spermatogenic tissue caused by hydrogenated arachis oil as the sole dietary fat*

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In earlier experiments (Aaes-Jørgensen, Funch, Engel & Dam, 1956; Aaes-Jørgensen, Funch & Dam, 1956) weanling rats reared on diets containing hydrogenated arachis oil as the only source of fat showed severe testicular degeneration and were sterile. The present experiment was undertaken to study the possible curative influence of a relatively small dose of ethyl linoleate (20 mg/male rat/day). This dose is about the same as the daily dose of linoleic acid (20 mg) that was sufficient for prophylaxis against testicular degeneration but insufficient for optimal growth in the previous experiments.

EXPERIMENTAL

Animals and their management. Six newly weaned male rats were fed on a diet consisting of 28% hydrogenated arachis oil (m.p. 40–42°),† 20% Vitamin Test Casein,‡ 46% sucrose, 5% salt mixture,§ 0·5% vitamin mixture,§ and 0·5% choline chloride. An aqueous colloidal solution containing vitamins A and D_2‖ was given by

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† From Dansk Sojakagefabrik Ltd, Copenhagen.
‡ From Genatosan Ltd, Loughborough, England.
§ See Aaes-Jørgensen & Dam (1954).
‖ Decamin aquosum, kindly furnished by Ferrosan Ltd, Copenhagen.

pipette, 0·1 ml. twice a week, and supplied the animals with 120 i.u. vitamin A and 18 i.u. vitamin D_2/week. Food and water were given *ad lib.* The animals were weighed and examined weekly.

Orchidectomy. After 19 weeks of experimental feeding orchidectomy of the right testis was performed on all animals under ether anaesthesia. From the day of operation and during the rest of the experiment the animals were given supplements of 20 mg ethyl linoleate/animal/day.

Biopsy of left cauda epididymidis. After 27 weeks of experimental feeding, biopsy was performed in the left cauda epididymidis of all the animals. The animals were lightly anaesthetized with ether and the cauda epididymidis was palpated through the scrotal skin. By means of a thin glass tube, of the thickness of a hypodermic needle and fitted with a rubber tube, a small quantity of the epididymal content was removed and blown out into a drop of 0·9% NaCl which was examined for spermatozoa.

Mating experiment. After 13 weeks of ethyl-linoleate supplementation (5 weeks after biopsy of the cauda epididymidis) the animals were mated with 15-week-old females, each male being placed with two females for 2 weeks. After the mating period the males were killed with chloroform. Autopsy was performed and gonads, kidneys, liver, adrenals, heart and small intestine were examined histologically. The female mating partners were weighed and inspected daily, and the oestrous cycles were followed by the vaginal-smear technique (Long & Evans, 1922) every day from 1 week before the mating period began until it ended. This procedure was partly to ensure that all the virgin females had a normal oestrous cycle and partly to obtain an indication of copulation and pregnancy. During the mating period and the next 3 weeks the females, which were raised on a normal stock diet, were offered the same diet as the males, but with no ethyl-linoleate supplementation. At the end of this period, the females were killed with chloroform, autopsies were performed and ovaries and uterus examined histologically.

RESULTS AND DISCUSSION

Growth. Table 1 shows that except for rats nos. 14 and 22 growth ceased after 12 weeks on the unsupplemented diet. Body-weights of some of the animals decreased a little in the subsequent weeks. Orchidectomy of the right testis was not followed by complications other than a slight inflammation around the operation wound, which was cured by a single application of penicillin ointment and did not interfere with the growth of the animals. Supplementation with ethyl linoleate at once increased the growth rate. Biopsy of the left cauda epididymidis had no effect on the growth rate except perhaps in animal no. 8. Mating of the animals resulted in a temporary decrease in weight in all except nos. 13 and 14.

Macroscopic skin signs. Table 2 shows that the skin signs were maximal at about the 15th week of the experiment, that is before the beginning of the ethyl-linoleate supplementation. This finding is in accordance with earlier results (Aaes-Jørgensen, Funch, Engel & Dam, 1956; Aaes-Jørgensen, Funch & Dam, 1956). In this connexion it should be noted that almost no skin signs were seen on the tail or fore-legs throughout

Table 1. *Weights (g) of rats during the experiment*

Week of experiment	Rat no. 8	10	13	14	22	23	Treatment
0	39	41	40	40	42	38	None, basal diet only
4	108	131	111	113	119	102	
8	134	164	134	135	145	130	
12	139	176	144	137	153	140	
16	140	170	142	147	161	144	
19	137	164	142	150	164	144	
20	143	174	141	151	166	148	At the end of the 19th week the right testis was removed, and supplementation of the diet with 20 mg ethyl linoleate/rat/day began
21	137	181	150	154	171	153	
22	161	190	153	160	177	159	
23	170	196	159	168	178	165	
24	177	207	164	173	187	168	
25	184	218	171	181	197	173	
26	192	227	181	189	207	181	
27	194	233	183	189	211	184	
28	189	236	187	195	217	191	At the end of the 27th week biopsy of cauda epididymidis was performed
29	206	241	192	202	224	195	
30	212	248	199	204	232	203	
31	218	256	204	208	240	208	
32	222	262	208	206	248	202	
33	220	259	211	207	243	194	Mating took place after the 32nd week
34	222	265	221	207	256	198	

Table 2. *Mean score of skin signs* in the rats*

Rat no.	No. of weeks since beginning of experiment 5	10	15	19	21	23	25	30	34
8	0·3	1·3	1·8	1·8	1·3	0·8	0·5	0·3	0
10	0·5	1·5	2·0	1·3	2·3	1·0	0·5	0	0
13	0·3	2·0	2·0	1·5	1·3	1·0	0·5	0	0
14	0	1·0	2·0	1·8	1·5	1·0	0·8	0·3	0·5
22	0·8	1·8	2·3	1·5	1·3	1·0	0·8	0·5	0
23	0·3	1·3	2·3	2·0	1·8	1·0	0·8	0·3	0·5

* Mean score for tail, hind-legs, fore-legs and appearance of fur (including dandruff). 0 indicates normal; 1 indicates dryness; 2 indicates slight, 3 moderate and 4 marked changes.

the whole experiment. However, the scales of the tail began to develop a yellowish colour, especially around the edges, at about the 13th–15th week. The colour increased in intensity as well as in area during the rest of the experiment. The scales did not fall off, as in deficiency of essential fatty acids. The skin signs observed were dandruff and scaliness of the hind-legs. Dandruff was the dominating sign and persisted longer after the beginning of the ethyl-linoleate supplementation than the other signs. The hair, mainly on the back, became loose and fell out in tufts, bearing masses of adherent yellowish skin scales (Pl. 1). The curative effect of the ethyl-linoleate supplementation was evident after a few weeks (Table 2). At the end of the experiment the animals had almost no skin signs, although most of them had a somewhat sparse fur. Further, during the last weeks, i.e. after the ethyl-linoleate supplementation, a yellowish brown pigmentation developed on the skin of the back. Since the coloured material could be scraped off, it was thought to be due to discoloration, possibly caused by autoxidation, of the sebum, rather than pigment located in the cells of the skin. A somewhat

Table 3. *Weight of, and histopathological changes in, right ablated testis and epididymis of the rats after 19 weeks on experimental diet*

	Testis				Epididymis	
	Weight					
Rat no.	g	As percentage of body-weight	Histopathological findings	Degree of degeneration*	Smear from cauda	Histopathological findings
8	0·338	0·24	Most tubules with Sertoli cells only; a few with spermatogonia, spermatocytes and spermatids	4	No living spermatozoa, a few dead, with large heads; plenty of cell debris	In all animals the corpus epididymidis was empty or nearly so, and the cauda epididymidis was filled with degenerating cells and acidophilic material; some proliferation of the intertubular connective tissue and of the epithelium of the corpus epididymidis had taken place
10	0·398	0·24	Sertoli cells only	5	No living spermatozoa, a few dead, with large heads; plenty of cell debris	
13	0·400	0·28	Sertoli cells only	5	Many spermatozoa with enlarged heads, none alive	
14	0·471	0·31	Most tubules with Sertoli cells only; a few with spermatogonia, spermatocytes and spermatids; some multinucleated giant cells	4	Few spermatozoa, all with enlarged heads, none alive; plenty of cell debris	
22	0·482	0·29		4	Many spermatozoa, enlarged heads, none alive; cell debris scanty	
23	0·394	0·27		4	Few spermatozoa, enlarged heads; none alive; some cell debris	

* For key to evaluation see Aaes-Jørgensen, Funch, Engel & Dam (1956), p. 298.

common sign in ill-thriving animals is a prolapse of the penis. It also occurred in some of our animals before the ethyl-linoleate supplementation but was completely cured by it.

Testes. Orchidectomy after 19 weeks of experiment showed that the weight of the right testis from all the animals was very low. It ranged between 0·24 and 0·31% of body-weight (Table 3). On gross examination all the testes appeared small and soft. Scrapings from the lumen of the cauda epididymidis showed no living spermatozoa. Many dead spermatozoa were sometimes present. All of these spermatozoa had large heads. Large amounts of cell debris were usually present.

Table 3 shows also the results of the histological examination of the right testis from each of the six animals. It was found that the spermatogenic tissue was severely damaged to the point of total degeneration. The corpus epididymidis was empty. The cauda epididymidis contained large amounts of degenerating cells and acidophilic material. Some proliferation of the intertubular connective tissue and of the epithelium of the corpus epididymidis had taken place.

The nuclei of the Leydig cells of severely damaged testes stained more homogeneously than those of normal testes. In normal testes the chromatin of the nuclei of the Leydig cells appears in distinct, sharply outlined, coarse granules, but in severely damaged testes it appears as fine granules uniformly dispersed in the nucleus. The cytoplasm of normal Leydig cells appears homogeneous or finely granulated, but in damaged testes that of many Leydig cells was coarsely granulated, and vacuoles were sometimes present.

Biopsy of left cauda epididymidis. At autopsy no scar from the biopsies performed 8 weeks after orchidectomy of the right testis and the beginning of ethyl-linoleate supplementation was found either in the scrotum or on the cauda epididymidis. No spermatozoa were found, which seems to indicate that a supplement of 20 mg ethyl linoleate/animal/day for 2 months given to animals in which spermatogenesis is severely impaired is not sufficient to cure the previously damaged spermatogenic tissue. In earlier studies (Aaes-Jørgensen, Funch, Engel & Dam, 1956) we have found that even a very small amount of linoleic acid (1 mg/animal/day) given from weaning as a supplement to a similar diet almost prevented testicular degeneration over a period of 18 weeks in six of nine animals. Similar results were obtained with rats on a diet containing 14% hydrogenated arachis oil and 20% crude casein (Aaes-Jørgensen, Funch & Dam, 1956).

Histological findings in male rats. All the males had abundant abdominal fat. On gross examination the solitary follicles of the Peyer's patches of the intestine were large and prominent in all. The weights and microscopic descriptions of the left testes are given in Table 4. In most of the rats the ethyl-linoleate supplementation stimulated the growth of the whole animal more than the growth of the testis. However, in rat no. 8 a considerable, and in rat no. 22 a slight, increase in weight, expressed as a percentage of body-weight, of the left testis over that of the ablated right testis had occurred. If we assume that the degeneration of the left testis was of the same degree as that of the right testis before ethyl-linoleate supplementation, a comparison of the results of the histological examination of the right and left testes (Tables 3 and 4)

Table 4. *Weight of, and histopathological changes in, left testis and epididymis of the rats at autopsy after 34 weeks on experimental diet*

	Testis				Epididymis	
	Weight					
Rat no.	g	As percentage of body-weight	Histopathological findings	Degree of degeneration*	Smear from cauda	Histopathological findings
8	0·859	0·39	The seminiferous epithelium appeared normal; some normal and many immature spermatozoa were present	1	Living spermatozoa present	Filled with spermatozoa of normal or immature appearance; some degenerating cells present
10	0·562	0·21	Sertoli cells only	5	No spermatozoa	No spermatozoa; some degenerating cells
13	0·417	0·19	Most tubules with Sertoli cells only; some tubules with normal seminiferous epithelium	3	No living spermatozoa	Filled with degenerating cells; a few immature spermatozoa present
14	0·382	0·19	Almost all tubules with Sertoli cells only; a few tubules with normal seminiferous epithelium	4	No spermatozoa	Almost empty; a few degenerating cells
22	0·757	0·30	Most tubules appeared normal; a few tubules with Sertoli cells only	2	Plenty of living spermatozoa	Some spermatozoa and plenty of degenerating cells
23	0·435	0·22	Almost all tubules with Sertoli cells only; a few with degenerating spermatogenic cells	4	No spermatozoa	Almost empty; a few degenerating cells

* For key to evaluation see Aaes-Jørgensen, Funch, Engel & Dam (1956), p. 298.

shows that a remarkable recovery of the spermatogenic tissue had taken place in animals nos. 8 and 22 during the period of ethyl-linoleate supplementation. These two animals had living spermatozoa in the scrapings from the lumen of the cauda epididymidis, which, too, may represent an improvement in the condition.

In animal no. 13 regeneration of some tubules had occurred, and in animals nos. 14 and 23 some regeneration of a few tubules had taken place, whereas severe degeneration of all the tubules was still evident in animal no. 10.

Histological examination of the kidneys revealed no calculi at the cortico-medullary border and, apart from a faint dilatation of some of the tubules, no abnormal changes were observed in the renal cortex. In the papillas, however, severe degeneration and dystrophic calcification were found in all animals.

The liver, adrenals, small intestine and heart were normal.

Mating experiment. Vaginal smears taken daily from 1 week before and throughout the entire mating period of 2 weeks showed a normal oestrous cycle in all the animals, but spermatozoa were not detected in any of the smears, and no young were born. Changes in the weight of the females during the mating period and the following 3 weeks gave no indication of either pregnancy or gestation-resorption. Further, placental signs were not observed, and no implantation scars were found at autopsy in the uterus of any of the animals. At autopsy all the females appeared normal, and histological examination of ovaries and uteruses revealed no abnormalities.

SUMMARY

1. Degeneration of the spermatogenic tissue, induced by feeding six weanling rats on a diet containing 28% hydrogenated arachis oil for 19 weeks, was partly cured by a supplement of 20 mg ethyl linoleate/animal/day during the following 15 weeks. This conclusion is based on histological evidence and evidence from biopsy and mating tests.

2. Although living spermatozoa were found in two animals at the end of the experiment, none of the four mating partners offered were made pregnant by these animals, which may have been due to inability of the spermatozoa to fertilize the eggs or to lack of sexual desire in the males.

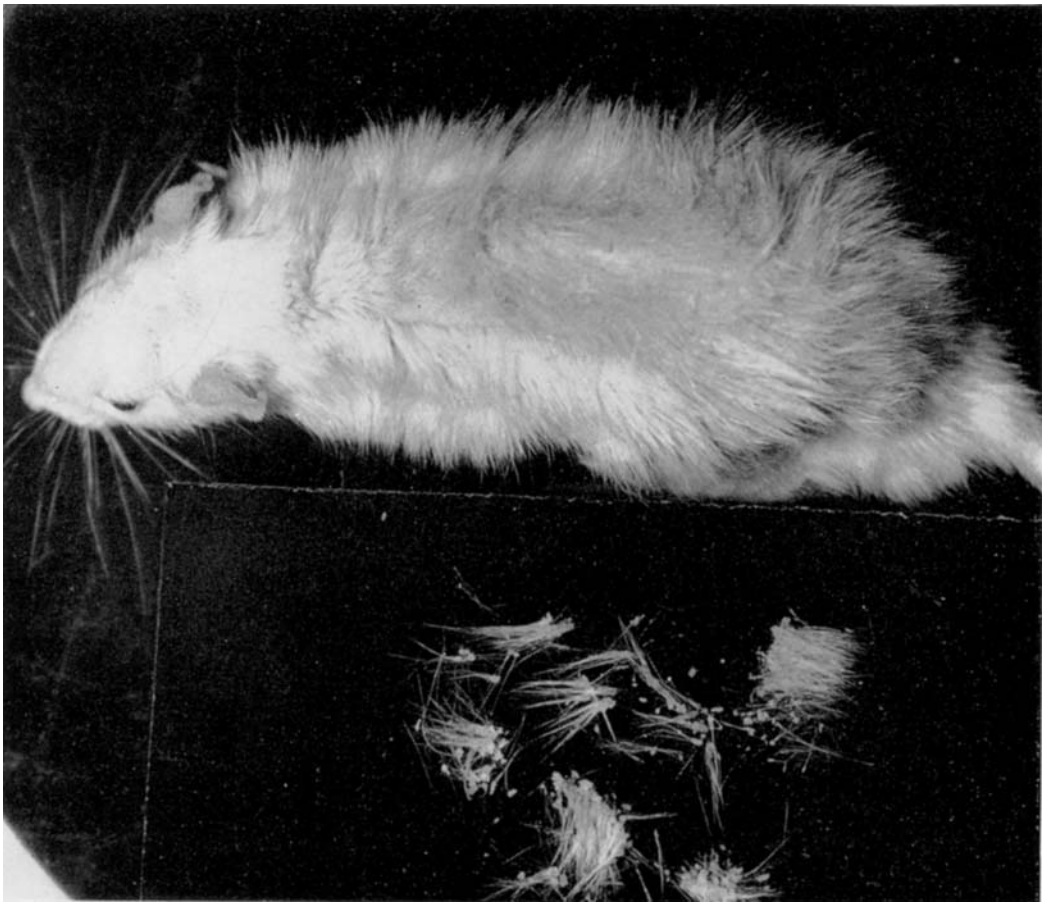
3. During the period of ethyl-linoleate supplementation the animals developed a yellowish brown pigmentation on the back. The pigment may have been produced by autoxidation of the sebum of the skin.

REFERENCES

Aaes-Jørgensen, E. & Dam, H. (1954). *Brit. J. Nutr.* **8**, 281.
Aaes-Jørgensen, E., Funch, J. P. & Dam, H. (1956). *Brit. J. Nutr.* **10**, 317.
Aaes-Jørgensen, E., Funch, J. P., Engel, P. F. & Dam, H. (1956). *Brit. J. Nutr.* **10**, 292.
Long, J. A. & Evans, H. M. (1922). *Mem. Univ. Calif.* **6**, 1.

EXPLANATION OF PLATE

Male rat fed, from weaning, for 19 weeks on a diet containing 28% hydrogenated arachis oil without ethyl-linoleate supplementation. The fur is thin. On handling the animal the hair fell out in tufts, bearing masses of adherent yellowish skin scales.



(*Facing p.* 304)