

The role of fat in the diet of rats

10. Influence on reproduction of hydrogenated arachis oil as the sole dietary fat

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It is known that essential fatty-acid deficiency involves irregular ovulation and impaired reproduction (Burr & Burr, 1929, 1930; Evans, Lepkovsky & Murphy, 1934*a*; Maeder, 1937; Mackenzie, Mackenzie & McCollum, 1939).

Quackenbush, Kummerow & Steenbock (1942) and Kummerow, Pan & Hickman (1952) showed that normal reproduction ensued when female rats grown to maturity on a fat-free diet were given a supplement of linoleic acid or arachidonic acid, or of a fat containing one or both of these acids. Panos & Finerty (1953) found the oestrous cycle in rats distinctly irregular after from 18 to 20 weeks on a fat-free diet. In the interstitial tissue of the ovaries they noticed a large number of 'deficiency' nuclei ('wheel nuclei'). Follicles and corpora lutea appeared normal. Quantitative analysis of pituitary-cell types revealed a significant decrease in percentages of acidophiles and an increase in basophiles. Because of these observations the authors suggested that one of the important consequences of fat deficiency in females is an ovary-pituitary hormonal imbalance.

Burr & Burr (1930) showed that male rats on a fat-free diet will usually not mate, and those that do mate cannot sire normal litters. Male sterility was studied further by Evans, Lepkovsky & Murphy (1934*b*) in rats reared up to 5 months on a fat-free diet. The addition of small amounts of essential fatty acids cured as well as prevented this sterility and restored sex interest. Studies on the effect of fat-free diets on the spermatogenic tissue have also been made by Mackenzie *et al.* (1939), Greenberg & Ershoff (1951) and Panos & Finerty (1954). Their findings are discussed in an earlier paper (Aaes-Jørgensen, Funch, Engel & Dam, 1956) in which we reported a severe degeneration of the spermatogenic tissue in male rats, resulting in empty epididymal ducts, when 28% hydrogenated arachis oil was the sole dietary fat for 18 weeks. In the same experiment a remarkable effect of small amounts of essential fatty acids was seen on the spermatogenic tissue. However, the low supplement of linoleic acid led to a marked decrease in the content of spermatozoa and to large numbers of degenerating spermatogenic cells in the epididymal ducts. Whether the animals were fertile was not studied directly.

This paper deals with the reproductive capacity of rats of both sexes reared on a crude-casein diet containing 14% hydrogenated arachis oil, so that it was relatively high in fat but low in essential fatty acids.

EXPERIMENTAL

Newly weaned male and female rats were reared for 20 weeks on a diet composed of 60% sucrose, 20% crude casein*, 14% hydrogenated arachis oil (m.p. 40–42°)†, 5% salt mixture‡, 0.5% vitamin mixture§ and 0.5% choline chloride. The diet and water were given *ad lib*. Vitamins A and D₂ were given as drops of an aqueous colloidal solution (Decamin aquosum, Ferrosan Ltd, Copenhagen) which supplied the animals with 120 i.u. vitamin A and 20 i.u. vitamin D₂/week. The animals were weighed and inspected weekly. After 15 weeks on experiment they were mated with mature normal animals. The experiment was begun in February and mating took place in the middle of May. The mating partners had been reared on a common stock diet, but were given the diet containing 14% hydrogenated arachis oil from the beginning of the mating period and throughout the rest of the experiment. The mating animals were separated after 2 weeks.

After 1 week from the beginning of the mating period the females were weighed and inspected daily for the next 4 weeks. At the end of the experiment (20 weeks), all the animals and their mates were killed with chloroform. Autopsies were performed and various tissues were studied histologically.

RESULTS AND DISCUSSION

The weight changes of the weanling males are shown in Table 1. It will be seen that the mating period began just when most animals had passed their maximal weight. The time for cessation of growth and the subsequent decline in weight agree well with the results of previous work in which hydrogenated arachis oil and crude casein had been used (Aaes-Jørgensen *et al.* 1956).

The results given in Table 1 show also that scaliness occurred in the skins of all six males.

The normal, mature stock females, numbered 1–6 in Table 1 and used as mating partners for the males on test, grew somewhat in the first 2 weeks after mating; growth then ceased and the weight declined a little in the following weeks except in no. 5 which was the only female also to show the placental sign, i.e. blood in the vagina. She gave birth to ten living young, all of which died within 6 days, for no known reason. The increase in weight of the other five females just after mating seems to have been due to normal growth rather than to pregnancy. The subsequent decrease in weight of these animals is thought to have been due to the inadequate diet (14% of hydrogenated arachis oil), rather than to resorption-gestation or to abortion, since no placental sign occurred in any of them; further, the results of the autopsy showed that scars in the uterus from implantations had occurred in female no. 5 only. The kidneys, ovaries, uterus, liver and mammae of all the six females appeared normal on gross examination. Female no. 4 had otitis media and otitis interna in the right ear.

* From Dansk Mejeri Industri and Export Kompagni Ltd, Stege, Denmark.

† From Dansk Sojakagefabrik Ltd, Copenhagen.

‡ McCollum & Simmonds's (1918) salt mixture no. 185, supplemented with 13.0 mg KI, 139 mg CuSO₄.5H₂O and 556 mg MnSO₄.4H₂O/100 g.

§ Cf. Aaes-Jørgensen & Dam (1954), Table 1.

Table 1. Growth and skin signs of male rats reared on a diet with 14% hydrogenated arachis oil, with information about their normal female partners and results of mating

Rat no.	Initial weight (g)	Males				Female mating partners														
		Growth				Appearance of placental sign after beginning of mating					Histology of kidneys									
		Week	Weight of rat (g)	Weight at mating (15th week) (g)	Final weight (20th week) (g)	Mean score of skin signs* at beginning of mating period (15th week)	Rat no.	At mating (g)	1 (g)	2 (g)	3 (g)	4 (g)	5 (g)	None	of beginning of mating period	No. of young born	Still-born	No. of scars in uterus	Occurrence of calculi†	
45	52	14th	183	179	162	1.3	1	236	247	250	247	244	237	None	0	0	0	2	Normal	Slight round-cell infiltration
46	40	11th	140	132	105	1.8	2	289	302	312	306	301	298	None	0	0	0	3	Slight degeneration	Slight dilatation, much blood round-cell
48	44	14th	140	138	132	1.3	3	238	249	250	246	237	241	None	0	0	0	1	Slight degeneration	Normal
52	48	14th	178	172	165	1.5	4	232	249	252	255	249	241	None	0	0	0	3	Slight degeneration	Normal
53	52	14th	196	194	173	1.8	5	254	269	289	319	278	288	17th-22nd	10†	0	9	0	Normal	Slight round-cell infiltration
54	48	14th	189	177	158	1.8	6	262	278	300	291	280	269	None	0	0	0	0	Slight degeneration	Slight round-cell infiltration

* Mean score for tail, hind-legs, fore-legs and appearance of fur (including dandruff). 0 indicates normal; 1 indicates slight; 2 indicates dryness; 3 moderate and 4 marked changes.
 † Assessed from a scale graduated from 0 (no calculi) to 5 (abundance of calculi).
 ‡ One died 1 day, two 3 days, two 4 days, one 5 days and four 6 days after birth.

Histological studies of the kidneys of the females showed that calculi occurred in most of the kidneys (Table 1). However, the calculi were located more in the cortex than on the cortico-medullary border. Otherwise the kidneys showed only small abnormalities. The other tissues (i.e. ovaries, liver and adrenals) appeared normal.

The results of the autopsy and of histological examination of various organs from the males given 14% hydrogenated arachis oil for 20 weeks are shown in Table 2. At autopsy blood was noticed in the urine of nos. 48 and 54. Further, in all these animals the kidneys were more or less impaired, and in some the testes appeared small and soft.

Degeneration of the spermatogenic tissue occurred, especially in no. 54, and to some degree in no. 46 (Table 2). Except in no. 54, spermatozoa were found in the epididymis of all these rats, although in reduced amounts and often with a large number of degenerating spermatogenic cells. The reason for the failure of the male rats to fertilize five out of six females might be that they had lost sexual desire, or that the spermatozoa found in the epididymis had degenerated or died. Further studies in this field are in progress.

Histological examinations of the kidneys of the males (Table 2) showed severe degeneration and necrosis of the papilla. The cortex showed some degeneration and dilatation, with an occasional occurrence of calculi on the cortico-medullary border. These findings are in accordance with our earlier results (Aaes-Jørgensen *et al.* 1956); together with the data in Table 1 they emphasize the poor condition of the males. Liver, adrenals and seminal vesicles were found to be normal.

Weight changes of the weanling females given 14% hydrogenated arachis oil for 20 weeks are shown in Table 3. These females were mated with normal mature males after having been on the diet containing hydrogenated fat for 15 weeks. From Table 3 it will be seen that the growth of the females, which was rather poor, had ceased at about the time of mating (15 weeks) and that typical skin signs of fat deficiency were present in all the animals at the beginning of this period.

After 1 week from the beginning of the mating period the females were weighed and inspected daily for the following 4 weeks (Table 3). Two of them, nos. 51 and 53, became pregnant, but no living young were born. Female no. 51 died 29 days after the beginning of the mating period during parturition. Female no. 53 had four still-born young 24 days after the beginning of the mating period and died from pneumonia before the end of the experiment (Table 3).

Two females, nos. 42 and 47, showed the placental sign (Table 3), but failed to become pregnant. Conception may have occurred in female no. 42, as judged from the increased weight found just after mating. The subsequent loss in weight suggests a gestation-resorption. At autopsy, however, the uterus appeared infantile and no implantation scars could be detected (Table 4). Female no. 47 lost weight from the 14th week to the end of the experiment. It is not known why these two animals showed the placental sign for 7 days. The ovaries of female no. 47 did not look normal on gross examination (Table 4).

In two females, nos. 45 and 52, no placental sign occurred, little change in weight occurred after the beginning of the mating period, no litter resulted (Table 3) and at autopsy the uterus appeared infantile (Table 4).

Table 2. Observations at autopsy and histopathological changes in male rats reared on a diet with 14% hydrogenated arachis oil

Rat no.	Observations at autopsy		Histopathological findings											
	Testes	Kidneys	Bladder	Testes					Epididymis			Kidneys		
	Normal size	Lighter coloured than normal	Empty	Spermatogonia	Spermatocytes	Spermatids	Spermatozoa	Degree of degeneration*	Spermatozoa	Polynucleated cells	Other degenerating cells	Occurrence of calculi†	Papilla	Cortex
45	Normal size	Lighter coloured than normal	Empty	++	+++	+++	++	1	++	0	+	0	Severe degeneration and necrosis	Some degeneration, slight dilatation and round-cell infiltration
46	Small and soft	Enlarged, many small whitish spots on the surface; papilla necrotic	Empty	++	++	++	+	2	+	+	+++	2	Severe degeneration and necrosis	Some degeneration, dilatation and calculi
48	Normal size	Surface uneven	Bloody urine	+++	+++	++	++	1	++	+	+++	0	Severe degeneration and necrosis	Some degeneration, dilatation and round-cell infiltration
52	Smaller than normal	Darker coloured than normal	Empty; large gelatinous coagulum	++	+++	++	+++	0	++	0	+	0	Severe degeneration and necrosis	Slight degeneration, round-cell infiltration
53	Normal size	Enlarged, many yellow spots on the surface of the cut	Empty	+++	+++	++	++	1	++	+	+	2	Severe degeneration	Slight degeneration, dilatation, round-cell infiltration
54	Small and soft	Enlarged, small whitish spots on the surface, papilla necrotic	Bloody urine	+	(+)	0	0	5	0	0	0	1	Severe degeneration with necrosis and casts	Some degeneration, dilatation, round-cell infiltration and calculi

* Assessed from a scale graduated from 0 (no degeneration) to 5 (total degeneration) (cf. Aaes-Jørgensen *et al.* 1956).
 † Assessed from a scale graduated from 0 (no calculi) to 5 (abundance of calculi).

Table 3. *Growth of female rats reared on a diet with 14% hydrogenated arachis oil, and results of mating with normal male rats*

Rat no.	Initial weight (g)	Growth					20 (final) (g)	Mean score of skin signs† at beginning of mating period (15th week)	Appearance of placental sign (days after beginning of mating period)	No. of young		No. of implantation scars in uterus
		Cessation of growth		Weight after weeks						Born alive	Still-born	
		Week	Weight of rat (g)	16 (g)	17 (g)	18 (g)						
42	54	14	110	109	111	117	105	104	105	0	0	0
45	64	15	149	149	144	145	144	149	145	0	0	0
47	46	14	115	112	109	109	103	105	105	0	0	0
51	50	15	166	166	163	170	181	193	†	†	0	†
52	46	13	155	155	150	155	152	144	152	0	0	0
53	57	15	148	148	144	149	154	123	§	0	4	§

* Mated with normal, mature stock males.

† 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.

‡ Died during parturition; one foetus in the parturient canal, four foetuses in the left and five foetuses in the right horn of the uterus; all foetuses were apparently fully developed.

§ Died from pneumonia 8 days after parturition and was not found until pronounced post-mortem changes had occurred.

Table 4. *Observations at autopsy and histopathological changes in female rats reared on a diet with 14% hydrogenated arachis oil*

Rat no.	Observations at autopsy				Histopathological findings			
	Ovaries	Uterus	Kidneys	Mammae	Occurrence of calculi*	Papilla	Kidneys	Ovaries
42	Normal	Infantile	Pale	Very small	1	Some degeneration	Slight degeneration, some round-cell infiltration	Abundance of 'wheel nuclei'
45	Normal	Normal	Pale, surface finely pitted	Very small	2	Some degeneration and necrosis	Some degeneration, dilatation and round-cell infiltration	Abundance of 'wheel nuclei', many with 'thorn-apple' appearance
47	Each consisted of three big reddish lumps	Infantile	Pale	Normally developed	1	Some degeneration and casts	Slight degeneration and dilatation	Three very large corpora lutea; abundance of 'wheel nuclei'
51	Normal	†	Pale, with whitish specks	---	3	Severe degeneration	Severe degeneration and some dilatation	Abundance of 'wheel nuclei'
52	Normal	Infantile	Normal	Very small	1	Severe degeneration	Normal	Abundance of 'wheel nuclei', many with 'thorn-apple' appearance

53†

* Assessed from a scale graduated from 0 (no calculi) to 5 (abundance of calculi).

† Rat died during parturition; one foetus in the parturient canal, four foetuses in the left and five foetuses in the right horn of the uterus; all foetuses were apparently fully developed.

‡ Died from pneumonia 8 days after parturition and was not found until pronounced post-mortem changes had occurred.

At autopsy of the females reared on the diet containing 14% hydrogenated arachis oil most of the kidneys were found to be very light in colour and were severely damaged. Calculi were more numerous than in the males (Tables 2 and 4). The mammae appeared very small and undeveloped in females nos. 42, 45 and 52 (Table 4).

In the ovaries, the cytoplasm of the interstitial tissue stained faintly (with eosin), and many 'wheel nuclei' were present. In some ovaries the 'wheel nuclei' had a 'thorn-apple' appearance, as described in the earlier work by Aaes-Jørgensen *et al.* (1956). The liver and adrenals were normal.

The results show that the reproductive capacity of the female rats was severely impaired by feeding hydrogenated arachis oil for 15 weeks. The normal mature males used as mating partners for these females (Tables 3 and 4) were found to be normal, both on autopsy and on histological examination of their testes, kidneys, adrenals and liver. It should be noticed that these stock males differed from the stock females in having no calculi in their kidneys, but in our experience kidney calculi appear more often in females than in males, and are not related simply to lack of essential fatty acids.

SUMMARY

1. Two groups of weanling rats (six males and six females) were reared on a diet containing 14% hydrogenated arachis oil and 20% crude casein for 15 weeks. They were then mated with six females and six males from the stock colony.

2. Only two of the six females reared on the experimental diet became pregnant, and living young were not born.

3. Only one of the six males reared on the same diet sired young, and all these young died within 6 days.

4. After a period of 20 weeks from weaning all the rats reared on the experimental diet as well as their mating partners from the stock colony were killed and autopsied. Testes, ovaries, kidneys, liver and adrenals were examined histologically. The results for the rats reared on 14% hydrogenated arachis oil were essentially the same as those found earlier with diets containing 28% hydrogenated arachis oil. Spermatogenesis was impaired and 'wheel nuclei' appeared in the ovaries. The gonads of the stock animals appeared normal.

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