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FISH-INDUCED ANEMIA IN RATS

By

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Helgebostad & Martinsons (1958) fed large amounts of raw coalfish (Gadus virens) to mink kits and observed that anemia and "cotton fur" were induced in a large number of the experimental animals. When boiled coalfish substituted the raw fish, the frequency and extent of the anemia and of the "cotton fur" were greatly reduced and the animals appeared almost normal. Similar symptoms were induced by feeding raw whiting and haddock, (Gadus merlangus and Gadus aeglefinus).

Just after weaning, and during the first part of their growth period, the mink kits exhibited normocytic, hypochromic anemia. This condition gradually developed into a microcytic anemia indicating an iron deficiency (*Helgebostad et al.* 1961).

Parenterally administered iron had both a prophylactic and a therapeutic effect. *Martinsons* (1960) states that iron absorption from ferrous chloride was normal in mink fed raw coalfish. The anemia, however, was not cured by oral administration of ferrous sulphate (*Helgebostad* 1961; *Helgebostad & Ender* 1961).

Stout et al. (1960 a, 1960 b) fed raw hake and whiting to mink kits and observed symptoms similar to those observed by *Helgebostad & Martinsons* (loc. cit.).

It is a reasonable assumption that raw fish of the Gadus species contains some substance which renders the iron unavailable to mink. This substance is destroyed, inactivated or removed by boiling the fish. The author of an unsigned paper entitled "Fish and unavailable iron" in Nutrition Reviews (1961) hopes

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"that this type of study will be extended to other animals in an effort to determine whether mink are unique in developing an anemia when fed these species of fish."

Unpublished experiments by *Helgebostad* indicate that the "raw fish diet" produces only minor anemic symptoms when fed to foxes. The present paper deals with a long term feeding experiment with albino rats. The mother animals were fed a coalfish diet continously for 10 months. Their litters were raised on the same ration.

Raw coalfish induced an anemia in the young growing animals. The anemia was partly, but not totally prevented by feeding boiled fish. Irrespective of raw or boiled coalfish being used in their diets, the adult mothers showed no signs of anemia at the conclusion of the experiment.

MATERIALS AND METHODS

The albino rats were of the Institute's own strain, where the stock animals are fed a commercial pellet diet (Felleskjøpet's Diet for Rats and Mice) fortified with brewer's yeast suspended in whole fresh milk.

Nine contemporaneously born female triplets (age 24 days) were earmarked and distributed in three groups. All animals were given the same ration (stock diet) in a pre-experimental period which lasted 10 days. The experimental animals were fed the different diets when they were 34 days old. Group I, the control group, continued with the standard stock ration. Group II was fed a "raw fish diet" and group III was fed a "boiled fish diet". The different diets and water were given ad libitum from tinned cans and from drinking bottles.

The experimental animals were mated when they were 84, 171 and 250 days old. The male breeders were removed after 14 days of rotative service.

The female rats were weighed weekly until the first mating, and they were later weighed immediately before each mating period. The young rats from all litters were weighed 32 days after birth. Blood samples were taken from all male young rats at the same age. The female young rats were not examined. The male young rats were anesthetized with ether, and blood samples were taken from the jugular vein. Sodium oxalate was used as an anticoagulant.

Hemoglobin concentrations were determined by means of a

Zeiss haemometer formerly calibrated with rat blood according to *Hawk et al.* (1947). The cell volumes were measured in Wintrobe tubes at 3200 r.p.m. for 15 minutes.

The feeding experiment lasted 10 months. Hemoglobin levels and cell volumes of the mother animals were determined at the conclusion of the experiment, and the rats were then killed.

Group I (the control group) was fed a commercial pellet diet (Felleskjøpet's Diet for Rats and Mice) which had the following composition:

Whole barley, coarse flour	25 %
Whole wheat, coarse flour	20 %
Wheat bran	10 %
Grass meal	5 %
Herring meal	10 %
Dried skimmilk	15 %
Soya flour	14 %
Sea-weed meal	0.2 %
"Stjernegranulat" (vitamin preparation containing vitamin A and D)	0.2 %
Sodium chloride	0.6 %

Dried brewer's yeast suspended in fresh whole milk was given twice weekly. The weekly addition per rat was 3.5 g yeast and 16.5 ml. milk.

Group II (raw fish) was fed a paste diet:

Gutted, raw coalfish (heads included)	90.5 %
Dried skimmilk	2.3 %
Wheat starch	7.2 %

Group III (boiled fish) was fed a paste diet with the same composition as the one used for group II, except that the raw fish was replaced by the same amount of boiled fish.

Vitamins were added to both fish diets. The additions of vitamins per kg paste diet were:

Vitamin A	6800 I.U.
Vitamin D	900 I.U.
Vitamin E	9.0 mg
Thiamine	2.5 „
Riboflavin	1.7 „
Calcium panthothenate	6.8 „
Nicotinic acid	8.6 „
Pyridoxine	0.5 „
Folic acid	0.2 „
Biotin	0.01 „
Inositol	25.0 „
p-aminobenzoic acid	50.0 „

The pellet diet differed distinctly from the pasty fish diets, both as regards water contents and other components. The contents of water, protein and iron in the three diets are shown in Table 1.

Table 1. A comparison between the three experimental diets.

Diet	Per cent of water in diet	g protein per 100 g dry matter	mg iron per 100 g dry matter
Pellets	9.3	25.4	27.9
Raw fish	79.1	55.1	12.5
Boiled fish	80.4	58.9	11.9

RESULTS

The feeding experiment started when the female rats were 34 days old. The animals which were fed the pellet diet, gained weight more rapidly than those which were fed the fish rations. This difference, however, became less pronounced at increased age. Immediately before the second mating period, when the mother animals were 250 days old, the animals in group II (raw fish diet) actually weighed more than the animals in group I (pellet diet). Weight curves for the experimental animals until the first mating are shown in Fig. 1.

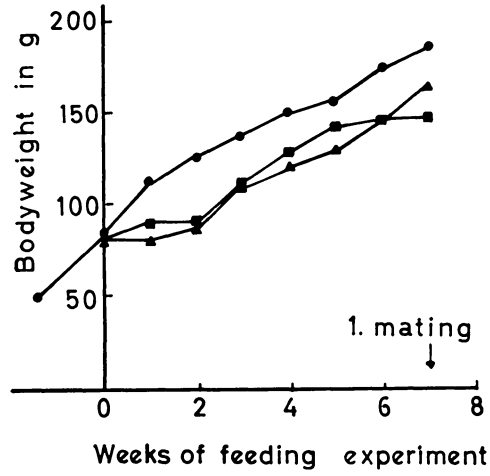

Fig. 1. Growth curves of female rats until 1. mating. The feeding experiment was started when the rats were 34 days old.

• control group, ▲ raw fish diet, ■ boiled fish diet.

Table 2. Growth and blood values of mother animals.

Diet	Average body weights of mother animals						At conclusion of experiment	
	at weaning (age 24 d.)	at start of experiment (age 34 d.)	before 1st mating (age 84 d.)	before 2nd mating (age 171 d.)	before 3rd mating (age 250 d.)	at conclusion of experiment (age 320 d.)	g hemoglobin per 100 ml	Cell volume
Pellets	51	82	186	226	242	261	14.7	48
Raw fish	52	78	164	224	252	255	15.7	57
Boiled fish	50	78	147	217	242	244	14.9	52

Table 3. Fertility data.

	Conception rate Pregnant/total Mating			Number of young, total Mating			Number of young, stillborn Mating			Live young after 32 days Mating			Live male young after 32 days Mating		
	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
Pellets	6/9	6/9	6/8 a)	66	61	59	0	0	0	40	41	56	18	19	30
Raw fish	7/9	8/9	7/8 a)	50	66	62	1	2	17 c)	32	38	42	14	22	18
Boiled fish	7/9	9/9	6/8 b)	55	65	44	6	11 c)	13 c)	35	30	30	18	14	15

a) One mother animal killed, labyrinthitis.
b) One mother animal died during labour, after second mating period.
c) Embryos at full term, whose mothers died during labour, are included.

Table 4. Examination of litters. The table comprises 168 males and 176 females.

Diet	Male young examined at the age of 32 d.									Female young, body weight 32 d. from mating		
	Average body weight in g from mating			g hemoglobin per 100 ml from mating			Cell volume in per cent from mating					
	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
Pellets	77 ± 1.3	86 ± 0.9	74 ± 1.7	12.0 ± 0.16	11.4 ± 0.13	11.4 ± 0.10	37 ± 0.4	39 ± 0.5	36 ± 0.3	73 ± 1.0	77 ± 1.3	70 ± 1.7
Raw fish	34 ± 0.7	53 ± 1.1	51 ± 1.9	6.2 ± 0.10	7.4 ± 0.09	6.4 ± 0.09	24 ± 0.3	32 ± 0.4	28 ± 0.4	32 ± 0.9	51 ± 1.4	43 ± 1.8
Boiled fish	47 ± 1.6	47 ± 2.4	50 ± 2.3	9.0 ± 0.27	8.9 ± 0.46	9.5 ± 0.43	28 ± 1.0	32 ± 1.3	34 ± 1.1	43 ± 1.8	51 ± 1.6	45 ± 1.7

The ± values represent the probable error of the mean. The numbers of young rats in the different groups are shown in Table 3.

Table 5. Tripara mother animals. Male young 32 days after birth.

Diet	Mother animals nos.	Number of live male young 32 d. after birth, Litter			Average body weight in g, Litter			g hemoglobin per 100 ml, Litter			Cell volume in per cent, Litter		
		1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
Pellets	153, 154, 157	8	8	9	81	89	94	12.2	11.3	11.9	36	40	36
Raw fish	164, 166, 168, 169	8	12	15	34	49	48	6.3	7.2	6.4	24	31	28
Boiled fish	173, 177, 178, 179	13	7	11	45	54	52	9.3	10.3	10.6	30	35	36

At the conclusion of the experiment the rats were 320 days old. Hemoglobin and cell volume were determined in the individual blood samples. — Table 2 shows averages of the body weights of the mother animals throughout the experiment.

The conception rates and the development of the litters from birth to 32 days post partum were observed for the three groups. The observations are summarized in Table 3.

The litter members were examined 32 days after birth. The young rats from mothers on a "raw fish" regimen were evidently anemic. Their ears, noses and toes were pale. Their eyes looked colourless and dull. Their livers were pale, and the spleens were in some cases enlarged. In some of the rats a fur condition resembling the "cotton fur" condition in mink was observed. Litters from mothers fed a "boiled fish" ration exhibited similar symptoms, but in a considerably lesser degree.

Average body weights, and hemoglobin levels and cell volumes of blood samples drawn from the male young rats are shown in Table 4.

Only a limited number of rats (11 out of 27) gave birth to three litters. These mother animals represented all three groups. Data of the male young rats from tripara mothers are presented in Table 5.

DISCUSSION

Helgebostad and his co-workers have in several papers demonstrated that heavy feeding with raw fish of Gadus species produced anemic symptoms in mink. The harmful effect was most pronounced during the growth period before and just after weaning. The anemia was more or less eliminated if the fish was boiled. The incidence of anemia was further decreased if inorganic iron was added to the boiled fish ration.

The described feeding experiment on rats comprised three groups, each consisting of 9 females. The control group was fed a commercial pellet diet fortified with brewer's yeast and whole milk. Group II and III were fed fish diet which contained large amounts of raw and boiled coalfish. The young female rats exhibited retarded growth when they were fed the high protein fish diets. The growth inhibition during the early growth was larger with "raw fish diet" than with "boiled fish diet". The adult female rats, however, appeared to thrive better on a raw fish diet than when the fish was boiled. After puberty the rats

managed well on the fish rations, both of which sustained pregnancy and lactation.

Young rats are usually weaned at the age of 21 days. Litters from rats which were fed the fish diets, appeared unable to manage on their own so early. — In the experiments described in this paper the mothers were not removed from their litters until 32 days after birth. The young rats started nibbling at their mothers' diet when they were about 14 days old, but continued to suck their mothers as long as milk was produced.

At the age of 32 days litter members from the fish diet groups weighed considerably less than those from the pellet group. The observed anemia, caused by the raw fish diet, might possibly be explained as a hunger anemia. The remedial effect of boiling the fish, however, had little or none effect on the body weights.

There were apparently some thermolabile factors in the raw fish, which interfered with the iron metabolism of the rats. The interference was effective during prepuberty. Adult female rats coped well with the raw fish diet. The extra iron demand of the female rats during pregnancy and lactation was evidently satisfied by both fish rations.

Repeated gestation periods of animals which were fed fish diets did not result in increased anemia symptoms in the litters. Nor did the repeated gestation periods result in anemic mother animals. The experiment included 27 mother animals, and three mating periods resulted in 62 conceptions. Three parturient rats, one from raw fish diet and two from boiled fish diet, died during birth at full term. This might indicate that the fish diets have in these few cases interfered with the reproductive mechanisms. But in an unpublished experiment which has been performed at this institute, we have fed "raw fish diet" to female rats continously through 10 generations. None of the parturient rats died during this ten generation experiment. In the ninth generation some lactation disturbances occurred, and some of their litters died before weaning. The surviving young rats exhibited a retarded growth until puberty, but the adult females of the tenth generation were non-anemic, normal and healthy.

CONCLUSION

Undiversified feeding with a raw coalfish diet to rat females during gestation and lactation, and to their litters during weaning and after-weaning, resulted in evident anemic symptoms in the

young animals. If boiled fish substituted the raw fish, the anemic symptoms were reduced, but not eliminated. Neither the raw nor the boiled fish diets produced anemic symptoms in the adult females.

REFERENCES

Hawk, P. B., B. L. Oser & W. H. Summerson: Practical physiological chemistry. 12th ed. The Blakiston Company, Philadelphia 1947.

Helgebostad, A.: Fortsatte studier over foringsbetinget anemi hos mink. Norsk Pelsdyrblad 1961, *24*, 1—16.

Helgebostad, A. & F. Ender: Nursing anemia in mink. Acta vet. scand. 1961, *2*, 236—245.

Helgebostad, A., B. Gjønnes & R. R. Svenkerud: Foringsanemi hos mink. Symptomer, patologi og terapi. Nord. Vet.-Med. 1961, *13*, 593—603.

Helgebostad, A. & E. Martinsons: Nutritional anemia in mink. Nature (Lond.) 1958, *181*, 1660—1661.

Martinsons, E.: Relasjoner mellom serumjern, organjern, serumproteiner og hematologiske verdier hos mink. Nord. Vet.-Med. 1960, *12*, 471—489.

Stout, F. M., J. E. Oldfield & J. Adair: Nature and cause of the "cotton fur" abnormality in mink. J. Nutr. 1960 a, *70*, 421—426.

Stout, F. M., J. E. Oldfield & J. Adair: Aberrant iron metabolism and the "cotton fur" abnormality in mink. J. Nutr. 1960 b, *72*, 46—52.

Unsigned paper: Fish and unavailable iron. Nutr. Rev. 1961, *19*, 143—144.

SUMMARY

The described feeding experiment on rats comprised three groups, each consisting of 9 females. The control group was fed a commercial pellet diet fortified with brewer's yeast and whole milk. Groups II and III were fed pasty fish diets which contained 90.5 % of undehydrated raw or boiled coalfish. The feeding experiment started when the rats were 34 days old. The rats were mated three times during the experiment.

Undiversified feeding with a raw coalfish diet to rat females during gestation and lactation, and to their litters during weaning and after-weaning, resulted in evident anemic symptoms in the young animals. If boiled fish substituted the raw fish, the anemic symptoms were reduced, but not eliminated.

Repeated gestation periods of mother animals, fed fish diets, did not result in increased anemic symptoms in their litters. Nor did the repeated gestation periods result in anemic mother animals.

The anemic young rats, when continously fed the same fish diets, gradually improved their health state. The resulting adult animals were non-anemic and appeared normal as to growth and development.

ZUSAMMENFASSUNG

Fisch-induzierte Anämie bei Ratten.

Der Fütterungsversuch umfasste drei Gruppen mit je 9 Weibchen. Die Kontrollgruppe wurde mit einem kommerziellen, pelletierten Futter, welches durch Bierhefe in Vollmilch ausgerührt bereichert war, gefüttert. Den Gruppen II und III wurde eine Futtermischung, die 90,5 % rohe oder gekochte Sei (Gadus virens) enthielt, verabreicht. Bei Beginn des Fütterungsversuches waren die Ratten 34 Tage alt. Die Ratten wurden während des Versuches dreimal gepaart.

Wo die rohe Sei-Diät den Ratten während der Trächtigkeit und Laktationszeit und den Jungen während und nach der Entwöhnungszeit gegeben wurde, zeigten sich deutliche anämische Zustände bei den jungen Tieren. Wurde der rohe Fisch mit gekochtem Fisch ersetzt, waren die anämischen Zustände vermindert aber nicht eliminiert.

Wiederholte Trächtigkeitsperioden mit ununterbrochener Fischfütterung führten nicht stärkere Anämiesymptome in den Würfen mit sich. Ebenso verursachten wiederholte Trächtigkeitsperioden keine anämischen Zustände bei den Muttertieren.

Anämische Jungtiere die weiter mit den Fischdiäten gefüttert wurden, wurden allmählich gesund. Als ausgewachsene Tiere waren diese Tiere nicht anämisch und sie schienen normal in bezug auf Wachstum und Entwicklung.

SAMMENDRAG

Fisk-indusert anemi hos rotter.

Foringsforsøket omfattet tre grupper, hver på 9 hunner. Kontrollgruppen ble foret med en kommersiell pelletert kost forsterket med ølgjær utrørt i helmelk. Gruppe II og III ble foret med forblandinger som inneholdt 90,5 % rå eller kokt sei. Foringsforsøket ble startet da rottene var 34 dager, og rottene ble parret tre ganger i løpet av forsøket.

Når den rå sei-dietten ble foret til rottene under drektighet og diegivning, og til kullene under avvenningsperioden og i tiden etter denne, så oppsto der tydelige anemiske tilstander hos ungdyrene. Dersom den rå fisken var erstattet med kokt fisk, var de anemiske symptomer redusert, men ikke eliminert.

Gjentatte drektighetsperioder med uavbrutt fiskeforing førte ikke til sterkere anemisymptomer i kullene. Gjentatte drektighetsperioder førte heller ikke til anemiske tilstander hos mordyrene.

Anemiske ungdyr som fortsatt ble foret med fiskediettene, ble etterhvert friske. De resulterende voksne dyr var ikke anemiske, og de syntes normale med hensyn på vekst og utvikling.

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