ABERRANT IRON METABOLISM AND THE COTTON FUR ABNORMALITY SYNDROME IN MINK

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INTRODUCTION

Mink (Mustela vison) are indigenous to the North American continent and abound in suitable environs in the wild state. They have been reportedly raised in Canada in various degrees of confinement since the latter half of the nineteenth century; however mink raising as an agricultural enterprise is relatively new, having expanded from a small dispersed origin in the 1930's. Today mink ranching is widespread in the United States and Canada and has extended to other areas of the world, especially the Scandinavian countries, where climatic conditions are favorable. The production of mink kits in the United States was in excess of five million in 1958; of this Oregon produced slightly over 200,000 animals. This represents approximately a $5,000,000 industry within this state alone, which ranks only eighth in total production of mink in the United States.

Unlike most of man's other livestock collections, mink are naturally carnivorous, feeding primarily on rodents, birds, frogs and fish. This peculiarity has presented those raising mink commercially with problems of nutrition quite unlike those of other livestock

enterprises. In feeding mink it is impractical, because of availability and cost, to mimic this wild type diet; therefore it is necessary to supply a more or less artificial type ration. Early, such rations were based largely on horsemeat, but with a decreasing supply of horses and an increasing competition for their meat, it has virtually vanished from the ration. Other types of feeds that have been used are largely by-products, and include offal from the meat packing industry, fish wastes and otherwise non-used fishes from the fishing industry and more recently waste resulting from processing of poultry.

Fish, because of their availability, have composed the bulk of the mink's ration, especially in coastal areas. However, such rations because of certain objectionable characteristics are not in all cases suitable mink feeds. Various fresh water fish species, for example, contain an enzyme, thiaminase, which is causative of Chastek's paralysis in mink fed these fish. The paralysis results from a thiamine deficiency created through the action of this enzyme in splitting the dietary thiamine molecule into its two constituent, biologically inactive, parts. Fish containing this enzyme can be rendered harmless and become a valuable feed merely by cooking prior to including them in the ration. Similarly, in Norway, an abnormal pelt condition, known as "Bomullspels" (cotton pelt), in

which the underfur of the affected mink fails to pigment, was developed as a result of feeding rations high in marine fish.

Here in the United States this same abnormal pelt condition has been recognized since the wild mink days, before mink ranching became common, and then, as now, cotton animals were practically worthless from the point of view of pelt sales. Through the years, numbers of cotton animals have increased until they constitute a serious problem of the fur industry.

This thesis deals with the elucidation of this problem, its cause and its prevention. Results have been generally gratifying and some of the pertinent practical aspects answered. During the course of the work, several related facts have come to light which undoubtedly will have merit in other phases of mink nutrition. It is also believed that some points of a basic nature concerning the metabolism of iron have been disclosed and bear closer examination.

REVIEW OF LITERATURE

History of the Cotton Fur Abnormality

"Among the curious and worthless freaks that have no reference to season, place, or age, is the Cotton Mink, distinguished from the normal mink by having the underfur of flimsy texture, and drab or white instead of dark brown or warm grey" -- so the cotton mink was described by Ernest Thompson Seton in 1929 (35, p. 520). In an earlier publication (1924) cited by Seton (35, p. 520) the abnormality was considered "the result of an obscure disease... the under fur, which, according to the stage of their sickness, may be gray, light, and finally white; not unlike cotton in colour and texture. The outer fur never ...shows any perceptible evidence of the condition of the under fur...".

This anomalous condition is widespread throughout the United States and cotton mink were reported in Iowa and Illinois as early as 1924 (21, p. 30). Numerous mink ranches in Oregon, Washington and Idaho experienced the condition in the spring of 1939 (21, p. 30). In Connecticut a ranch that annually produces 3000 mink kits produced 600 "cottons" during the 1954 season (39, p. 1). A national survey was conducted by the Oregon State College Experimental Fur Farm in 1954 to assess the seriousness of

several fur abnormalities which occur in mink (2, p. 16-17). From 1117 returned questionaires, 22.6 per cent indicated production of cotton mink on their ranches. Fur farms in the Great Lakes region, because of dense concentration in this area, reported the greatest numbers of 'cottons'; however the area with the greatest percentage of farms reporting cottons included the Western and Pacific Coast states.

That cotton mink are not uncommon in the wild state is indicated by Seton (35, p. 520). In certain sections of Oregon (and probably elsewhere) trapping mink has been abandoned, as a large majority of the animals trapped are "cottons".[1]

Experimental work on cotton mink was initiated in 1940 at Washington State College to determine if the condition were hereditary and if any peculiar blood characteristics were associated with affected animals (21, p. 30-34). Heredity experiments with cotton animals obtained from various mink ranches in Washington and Oregon and fed a "standard" mink ration were directed primarily toward concentrating the cotton tendency by mating cotton males to cotton females. Such mating produced only kits with normal fur, and line breeding these offspring back to the

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[1] Personal communication with Darrell I. Gretz, Assistant District Agent, Branch of Predator and Rodent Control, United States Fish and Wildlife Service.

original stock failed to produce cotton animals. Blood studies, including hemoglobin determinations, red and white blood cell counts and differential counts showed no differences between the original cotton and normal mink.

More recently, experiments carried out in Norway have shown that young silver foxes placed at weaning on diets high in marine fishes or fish products developed a fur characterized by a lack of pigmentation; however fox pups fed exclusively raw meat showed normal fur without signs of greying (7, p. 409-412). From these observations the authors postulated a deficiency associated with the fish diets. Supplementation with rice starch and glucose, Fe, Cu, Co, Zn, and Mn; vitamins A, D, E, K, thiamine, pantothenic acid, and vitamin C proved ineffective in preventing achromotrichia. A tyrosine deficiency was obviated as fish in comparison with meat had been shown to be a relatively good source of this amino acid. Similarly, a lack of lysine as a causative factor was considered, but subsequently was assumed unimportant, as cod roe (which prevented symptoms of achromotrichia in foxes) is relatively low in this amino acid.

Further experiments (16, p. 11-12) showed that considerable protection against achromotrichia could be effected by feeding a supplementary mixture of all known synthetic B vitamins or substances rich in these factors,

such as cod roe, animal liver and brewers' yeast. No curative effect was noted if greying of the fur was well advanced prior to supplementation.

Later the experiments were extended to include mink (17, p. 11-12) and the symptoms characteristic of cotton fur occurred when these animals were fed diets primarily composed of "coal fish" (Gadus virens). The cotton fur condition appeared to be intensified by the presence of large amounts of marine fats in the feed, which led these writers to conclude that fat peroxides acted in vivo to destroy certain B vitamins that are necessary for normal fur pigmentation.

It is generally believed by those concerned with the fur trade that the cause of cotton mink is associated with the animal's nutrition. In the United States, cotton mink have often been associated commercially with the feeding of whiting, Merluccius bilinearis (39, p. 1). In Oregon an unexpectedly large proportion of cotton mink resulted when attempts were made to utilize Pacific hake, Merluccius productus, (a marine species seldom used commercially) as a mink feed source (1, p. 3).

Achromotrichia in Relation to Nutritional Deficiencies of Folic Acid, Lysine and Copper

Hair pigments are of one of two types, melanin or pheomelanin; the former representing the black-brown pigments and the latter, the yellow-red pigments (8, p. 255-256). The concentration and distribution within the hair shaft of these two chemically-distinct hair pigments which are of a granular nature, definite shape and measurable size, accounts for the wide variety of hair colors observed in mammals. Both pigments are elaborated by the melanocyte which is the only site of melanogenesis in the mammal (4, p. 109-113). Melanogensis involves conversion of the colorless amino acid, tyrosine, to the insoluble, colored, structurally-undefined-polymer, melanin, through the catalytic activity of the enzyme, tyrosinase. Biochemical reactions involved in this conversion were established by Raper (34, p. 253-278) and reviewed by Fitzpatrick *et al.* (8, p. 260-262) in the light of recent advances. Metabolic pathways of the pheomelanin pigment are separate but possibly interrelated to that of melanin (8, p. 256).

Greying of hair, or the failure of the melanocyte to deposit pigment granules within the hair shaft during hair growth, in mammals has been attributed to various causes,

including heredity, emotional strains, endocrine disturbances, chronic diseases and nutritional deficiencies. Reports prior to 1948 of greying in relation to nutritional deficiencies have been adequately reviewed by Frost (10, p. 368-382) and the literature has not increased rapidly since this time.

Achromotrichia has been at some time or other linked nutritionally with deficiencies of these members of the vitamin B-complex: pantothenic acid, para-amino-benzoic acid, folic acid, biotin and choline; deficiencies of the amino acids, lysine and cystine, and deficiencies of the minerals, copper and zinc (10, p. 368-382). Concomitant with achromotrichia, deficiencies of folic acid, lysine and copper give rise to symptoms of anemia in various animal species (25, p. 405-410; 23, p. 55; 37, p. 372-376). Since anemia, as will be presented later, is an integral part of the cotton fur syndrome in mink, the relation of these factors to melanogenesis has been reviewed.

First to attribute a chromotrichial action to folic acid per se was Martin (30, p. 353-355) in 1942. He fed black laboratory rats purified rations supplemented with all known B vitamins except folic acid and in some instances biotin; to these rations he added sulfaguanidine at levels of one and two per cent. The rats took on an initial

dusty appearance and later appeared silvery. The marked symmetrical greying apparent at five months was cured in two months time by supplementing with either yeast or liver. Folic acid administered at the rate of 3 mg. per day cured three rats completely and two rats partially within a month. Rats supplemented with either calcium pantothenate or para-amino-benzoic acid persisted in the grey state. Likewise, Wright and Welch (46, p. 55-66) noticed greying in black male rats fed succinylsulfathiazole on purified rations supplied with adequate pantothenic acid. Greying was prevented with the administration of folic acid.

A high incidence of greying in dogs associated with an anemic condition was produced by feeding a synthetic diet containing adequate amounts of pantothenic acid, biotin, inositol, choline, para-amino-benzoic acid, copper, and zinc by Frost and Dann (11, p. 355-362). Achromotrichia, the onset of which varied from two to eleven months, appeared at the nape of the neck and at the base of the tail. Severity of greying also showed considerable variation. Animals supplemented with either liver or brewers' yeast showed clear-cut cures of achromotrichia within six weeks, paralleled by improvements in the blood picture, appetite, and weight gain.

The same authors together with McIntire (12, p. 65-69) in 1946 using black Leghorn chicks designed a ration to contain all previously recognized chromotrichial factors with the exclusion of the L. casei factor (folic acid). This factor was injected five times weekly to supply chicks with either 1.0, 2.5, 5.0 or 10.0 micrograms per day. Pigmentation was lacking and feather growth poor at the lower levels, the 5 microgram level allowed for fairly good feathering but not for proper pigmentation and at the 10 microgram level both feathering and pigment formation were comparable to chicks receiving 10 per cent of brewers' yeast.

A year later, Lillie and Briggs (29, p. 475-477) using New Hampshire chicks published their observations of the efficiency of folic acid in preventing abnormal feather pigmentation. Chicks fed purified diets containing 150 micrograms or more of folic acid per 100 grams ration were completely protected from achromotrichial manifestations. Below 100 micrograms of folic acid per 100 grams ration, the incidence of achromotrichia was inversely proportional to the folic acid content of the ration.

Wright and Welch (45, p. 426-427; 46, p. 55-66) have inferred an interrelationship between pantothenic and folic acid, as based on liver levels of calcium pantothenate in rats, both with and without folic acid

administration. Previously sufficient amounts of dietary calcium pantothenate failed to be normally stored in the liver when succinylsulfathiazole was added to a purified diet, and rats greyed. Further increase in dietary pantothenic acid levels was without effect in reversing achromotrichia, as was subcutaneous administration of pantothenic acid. Folic acid administration cured the greying and raised the liver storage of pantothenic acid to equal that of rats fed an adequate diet. The authors concluded that folic acid was not a chromotrichial factor, but had an indirect influence on pigmentation through its effect on pantothenic acid utilization.

Earlier work (1940) by Dimick and Lepp (5, p. 413-426) indicated that crude substances such as rice bran concentrates gave complete protection against achromotrichia in rats, whereas addition of pantothenic acid only incompletely cured greying. Frost et al. (13, p. 507-511) reported that rats supplemented with liver extracts containing as little as 40 micrograms of pantothenic acid per day were completely cured of induced achromotrichia, but others fed much larger amounts of pure calcium pantothenate showed only limited response. However, adding calcium pantothenate to liver extracts low in this factor increased their anti-greying potency. The implication is,

then, that natural feedstuffs contain a factor (probably folic acid) which acts synergistically with pantothenic acid to prevent greying.

Fritz et al. (9, p. 387-396) reported that bronze turkey poults raised on diets high in protein concentrates of a plant origin frequently developed a whitening of the primary and secondary wing feathers. This depigmentation was transitory in nature, being most evident during the first four to five weeks and disappeared as the bird aged, even though the same ration was fed. Crystalline lysine, or protein concentrates high in lysine, added so that the ration contained approximately 1.2 per cent of lysine was sufficient to prevent achromotrichia. Supplementation with pantothenic acid was ineffective. A similar depigmentation of feathers occurred in two weeks when Kratzer et al. (27, p. 285-292) fed poults low lysine-containing rations. Increasing the protein level from 10 to 35 per cent increased the level of lysine required to prevent depigmentation. Vohra and Kratzer (41, p. 1145) demonstrated that lysine deficiency likewise precipitated greying in rats. Later, these same authors (42, p. 1096-1098) supplemented lysine deficient rations with excesses of copper, but were unable to prevent depigmentation in turkeys.

A macrocytic, hypochromic anemia with associated achromatosis of feathers was observed by Klain et al. (25, p. 405-410) in 4 week old chicks fed a low lysine diet based on sunflower seed oil meal. Since the type of anemia and the appearance of greying were similar to those of chickens receiving a folic acid-deficient ration, the authors suggested a close relationship between lysine and folic acid in production of feather pigment. Further work (26, p. 317-328) demonstrated that the amino acid content of depigmented feathers was the same as that of normal feathers, but the tyrosinase activity of depigmented feathers was less than in normal feathers, as measured by pigment production on incubation with tyrosine.

A very interesting observation made recently by Vohra and Kratzer (43, p. 1249) suggests that lysine or a moiety of it may actually be incorporated into the melanin molecule since radioactive melanin was isolated from feathers of poults injected with dl-lysine 2-C^{14}.

In 1931, Keil and Nelson (23, p. 55) noted that when black or black hooded rats were fed exclusively whole milk diets they developed a silvery-grey coat in conjunction with anemia. Supplementation with copper caused the fur to return to a normal color. Depigmentation of young and adult rats and rabbits and young cats was produced by Gorter (14, p. 185) on various rations. Of 15 different

minerals supplied only copper would cure and prevent the depigmentation. Cattle on copper deficient pastures developed depigmentation and anemia as reported by Sjollema (37, p. 372-376). Achromotrichia was also observed by Muir in cattle grazing on pastures containing excesses of molybdenum which acts to induce a copper deficiency. Such greying could be cured by copper sulfate treatment (8, p. 286). Smith and Ellis (38, p. 81-88) found that the syndrome of achromotrichia, alopecia and dermatitis produced in Dutch rabbits fed a copper deficient ration was a more sensitive indication of copper deficiency than was anemia.

The work of Singer and Davis (36, p. 472-473) indicates that copper and pantothenic acid may be closely related in function. Black rats fed a synthetic, copper-deficient diet for 7 weeks developed a depigmentation typical of pantothenic acid deficiency. Restoration of pigment occurred after supplementing with either copper or pantothenic acid. This relationship was further emphasized when Hundley and Ing (22, p. 385) analyzed skin samples of two groups of black rats fed pantothenic acid-adequate and deficient rations. The average skin copper level of normally pigmented rats on an adequate diet was 38.3 micrograms per gram and that of depigmented rats on a pantothenic acid deficient diet was approximately 5

times this level, indicating a blockage of copper utilization by a pantothenic acid deficiency. Dick's (40, p. 99) evidence suggests that of the various systems requiring copper, that of pigment formation is the most sensitive to a copper deficiency, as depigmentation occurs at copper levels satisfactory to prevent other signs of copper deficiency. Administration and withdrawal of copper produced alternate bands of pigmented and depigmented areas on wool of black sheep.

The role of copper in melanogenesis is evident since it is contained as a prosthetic group in the enzyme tyrosinase which is required for the oxidation of tyrosine to melanin. Utilization of copper seems to be directly associated with pantothenic acid, for which the exact chromotrichial function in unknown.

Although it has been amply demonstrated that folic acid, lysine and copper are of consequence in the biochemistry of hair pigmentation, their mode of action (especially that of folic acid and lysine) has not been established. The role of copper seems to be direct in that it is actually concerned in the catalytic production of melanin. Evidence is not conclusive, but it indicates that the lysine molecule or a portion of it is structurally incorporated into the melanin polymer. The functional status of folic acid in melanogenesis is only speculative,

but it has been inferred to mediate utilization of pantothenic acid, which in turn is concerned with proper use of copper. Even though the observed facts intimate a relationship of these various chromotrichial factors considerably more research of a basic nature will be required to establish correctly their functional roles.

EXPERIMENTAL

The principal objectives of this research on the cotton fur abnormality problem were twofold -- (1) to determine its cause and (2) to develop effective preventive measures. Because of limited previous experimental work, definition of this malady had progressed little beyond the note of underfur depigmentation and the unthrifty appearance of afflicted animals. Therefore in order to make a more logical attack on the primary problems, an early approach was to characterize the condition more specifically, both grossly and physiologically.

There was good reason to believe that cotton fur had a nutritional basis in that Hartsough (39, p. 1) observed the association between it and the feeding of whiting to mink; also the Norwegians, Helgebostad and Ender (17, p. 11), had linked cotton fur with high fish diets, unsaturated fats and deficiency of the B-complex vitamins. From this information and from analysis of components of rations which had previously produced cotton mink at the Oregon State College Experimental Fur Farm, animal feeding trials were designed in an attempt to determine the general cause of cotton mink.

Progress with this phase was successful and with the knowledge of how to produce cotton mink experimentally,

the task of determining preventive measures was much more easily accomplished. In addition, this information made it possible to measure the effect of certain other factors, such as genetic background, on the incidence of cotton mink. Later, experimentation was turned primarily to elucidation of the specific nutritional basis of the problem, utilizing an approach which at times was largely of an exploratory nature, making use both of available facts and logical methods and materials. The work was greatly accelerated by an observation which had been made earlier in the experimental program, concerning the physiological state of the animal. This was used to advantage in assessing the effect of certain purified nutrients on the abnormality.

Methods and Materials

The series of experiments, herein reported, were conducted from 1956 through 1959 and primarily involve feeding trials with more than 1000 mink from the Oregon State College herd. In view of the large numbers of trials involved, specific information on experimental groups, treatments and rations is tabulated in Appendix A, Part I. Composition of rations supplements is located in Appendix B and further details and methods peculiar to individual group treatments are listed in Appendix C, Part I. Methods

presented below are general and where deviations from these methods occur, specific mention is made.

General Methods

Young males and females of the standard dark type made up the large majority of the animals, however a few trials involved either pastel (light brown) or sapphire (grey-blue) animals. With the exception of the 1959 experimental trials and a few indicated cases in 1958, all animals were selected for experimental treatment at random except that groups were balanced for litter size and two males or two females from the same litter were not placed within the same experimental group. Within a given year animals received a similar pre-weaning ration and were placed at weaning on one of the experimental rations given in Appendices A and B.

Fresh fish, horsemeat, liver and tripe were frozen as soon as received and refrigerated at -15 degrees C. until ready for mixing. All fish in rations were eviscerated in 1956; thereafter, with the few noted exceptions, they were included whole. Daily ration preparation involved grinding, weighing and mixing the wet ingredients to which was added the dry (supplementary) portion of the ration. This was remixed thoroughly and sufficient water added to obtain a desirable consistency (containing approximately

30 per cent dry matter) for feeding.

Animals were housed individually in standard "pelter" cages of woven galvanized wire (12 x 14 x 36 inches in size) and fed on the top wire of the pen. Feed remaining from the previous feeding was picked up and the wire brushed thoroughly before new feed was put out. Feed levels were group controlled and increased when the majority of the group indicated readiness for larger amounts.

The experimental period began after weaning in early July and continued until mid or late December when the animals were slaughtered and their pelts removed, although in some cases certain animals were maintained for further experimentation. Pertinent information on trials conducted after the completion of main trials is given in Part II of Appendix A and methods peculiar to treatments used in Part II of Appendix C. Weights, obtained by enclosing the mink within a trap and weighing on a Chatillon, model 4700, gram scale, were recorded at monthly intervals.

Specific Methods

Various methods of blood collection were tried and many difficulties encountered. The method generally used which gave quite satisfactory results in terms of adequacy of sample and ease and safety of collection involves the following procedure: The animal is restrained and anesthetized with an ether cone by an assistant. With the

animal lying on its back, feet up and head to the right, the heart is palpated with the left hand. A 1½ inch, 20 gauge needle on a syringe previously rinsed with a saturated sodium citrate solution is inserted between the ribs into the heart and the blood withdrawn. This blood is emptied from the syringe into tubes containing 0.1 cc. of potassium oxalate (evaporated to dryness) per cc. of blood collected.

Hemoglobin values were determined using a Spencer Hemoglobinometer, model 1000 (American Optical Company). Erythrocyte counting was accomplished by methods outlined by Wintrobe (44, p. 248-253) using isotonic saline as a diluent. Hematocrit values were obtained following Wintrobe's methods (44, p. 242-244); however centrifugation was made at 2400 r.p.m. for one hour.

Determinations of total iron were accomplished using the method given by Kennedy (24, p. 385-391) with several modifications of procedure. Preparation of the standard iron solution was according to specifications listed in Hawk, Oser and Summerson (15, p. 564). The color development and extraction procedure used were as follows: 5 milliliters of sodium sulfocyanate were added to the sample, which was shaken vigorously and allowed to stand for approximately 30 minutes. Exactly 10 milliliters of iso-amylic alcohol were added to the flask, the contents

shaken vigorously and left to stand for 7 hours (the time required for color development). This time appears to be influenced by the alcohol used, since color development was much faster in a subsequent lot of iso-amylic alcohol. Readings were made on a Coleman spectrophotometer (model 14) at 490 millimicrons using a number 14-214 filter.

Results

The experimental observations and results following are presented by development of specific aspects of the problem and are not necessarily related to the chronological sequence followed in experimentation. Experimental work presented within individual sections is by no means exclusive to that section and where results are applicable they are reported in several sections. Furthermore, certain initial trials with hake, with which the author was not personally involved, were directed to testing its suitability as a replacement mink feed; subsequently its relation to the cotton fur syndrome was established. Since some of the information obtained in these trials was directly concerned with the cotton problem, it has been included.

The Nature of the Cotton Fur Abnormality

The lack of pigment in the underfur is the most striking symptom of a large syndrome characterizing cotton mink.

Development of cotton fur coincides with the winter furring cycle and is first evident in the new underfur that appears in late September and early October in this region. In every case noted or reported the longer guard hairs are normally pigmented. Figure 1 depicts a cotton fur and a normally pigmented fur for comparison. The degree to which the underfur is depigmented is not constant in all cotton mink, as an array of animals from those possessing an underfur almost devoid of pigment to those approaching a normal fur color have been observed (Figure 2). In many cases the depigmented underfur covers only the sides, leaving the area of the back normally colored. Individual fur fibers of the cotton animal appear to be of a finer, less substantial structure; however little quantitative work on this aspect has been accomplished. Isolated cases were observed where the quality as measured by depth, density and coverage of the depigmented fur was excellent.

The external appearance of cotton mink, aside from the characterizing light-colored underfur, varies with individuals, but quite often mink so affected are small and emaciated and possess a roughened fur coat; some cotton mink, however, appear to be in a healthy condition. Growth curves (Figure 3) attest to the fact that cotton mink (shown as CF mink on the graph) are smaller than their normal counterparts (shown as basal), indicating

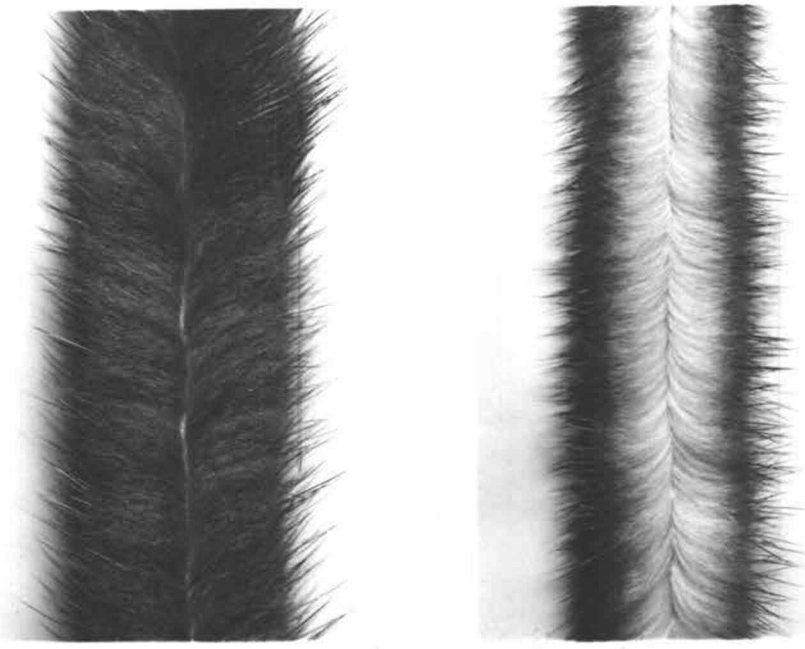


Figure 1. Pelts of normal (left) and cotton mink, parted to show underfur. Note that longer guard fur remains normally pigmented.

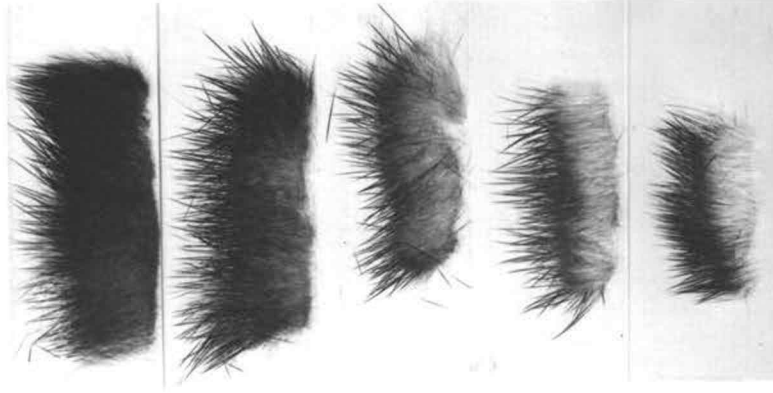


Figure 2. Fur samples illustrating variation in degree of achromotrichia encountered in mink affected with cotton fur. (Normal pelt (left) for comparison.)

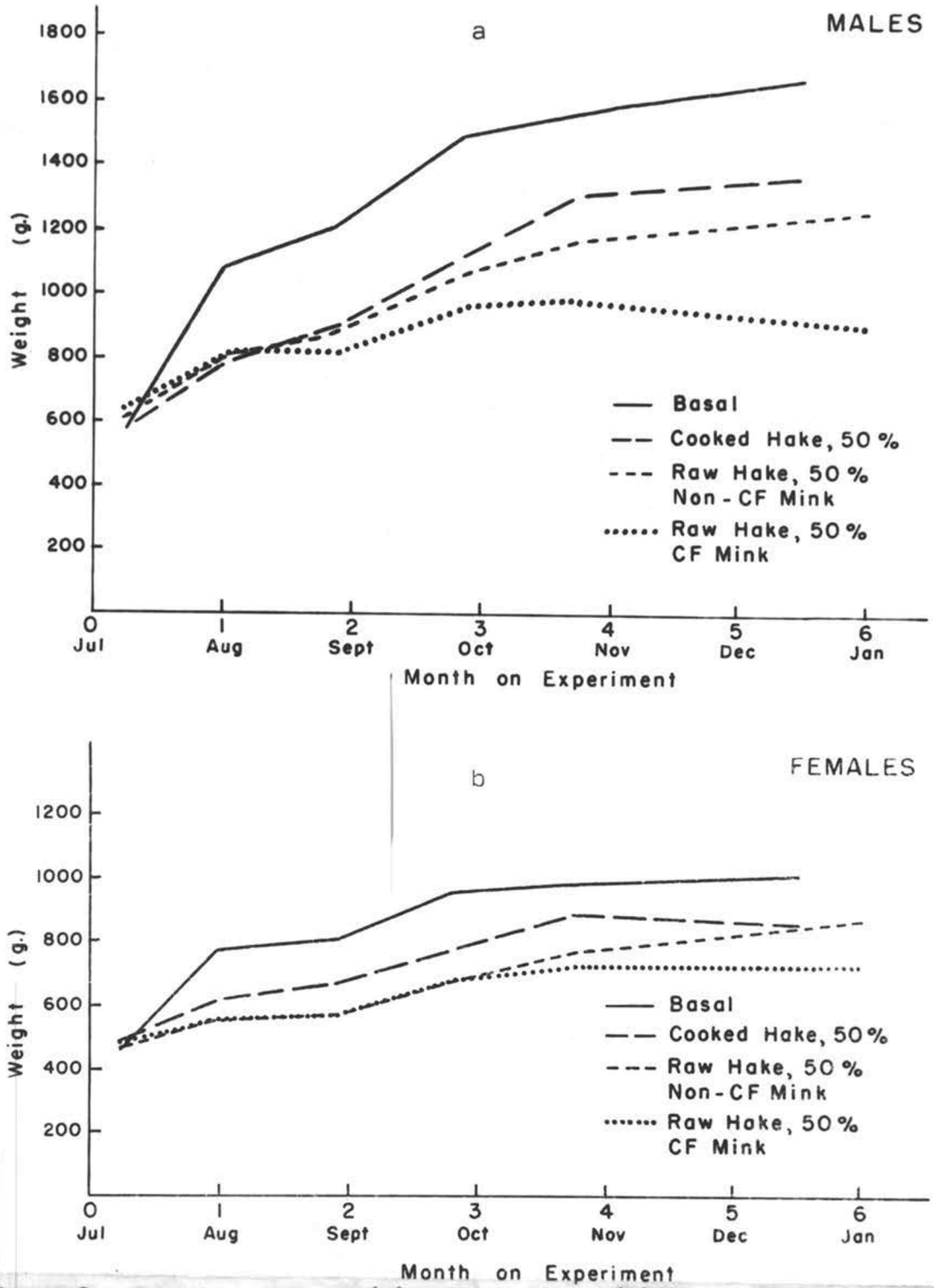


Figure 3. Growth curves of (a) male mink and (b) female mink fed basal (77), raw hake (61) and cooked hake (20) diets, with a comparison of cotton (CF) and non-cotton mink fed the raw hake diet. (Numbers of mink per diet shown within parentheses)

presence of further anomalous conditions that affect growth. These curves include weight data of 28 cotton mink and 77 normal mink taken in 1958. Mature cotton males weigh on an average 60 per cent as much as normal males fed an adequate control ration; a few exceed this figure, but the upper limit is usually 70 per cent. Female cotton mink average 86 per cent of the weight of normal females. Photographs showing the typical size relationships between cotton and normal male mink are presented in Figure 4.

Early mortality is very high among cotton mink. Autopsy reveals that they are extremely small and thin, almost snake-like in appearance. Figure 5 compares a cotton mink which died from this condition and the carcass of a normal animal which was killed for pelting. The skinned carcass of the dead animal is generally characterized by an almost entire absence of subcutaneous and internal fat deposits (Figure 6).

It was noted during pelting operations in 1956 that carcasses from cotton mink could be readily identified by their pale appearance as compared to normal mink (Figure 7) and, further, that blood from cottons had a watery consistancy. Hemoglobin determinations made on two cotton and 5 normal mink verified the former's anemic state. Blood studies conducted in 1957 and 1958 are summarized

Figure 4. Normal (left) and cotton male mink showing typical size relationships.

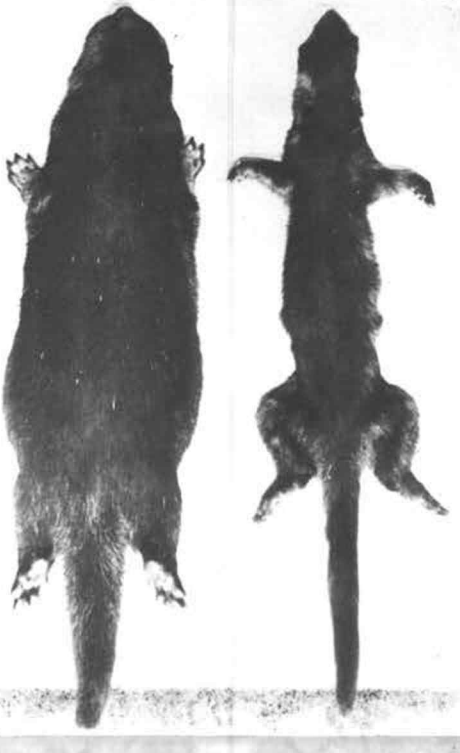
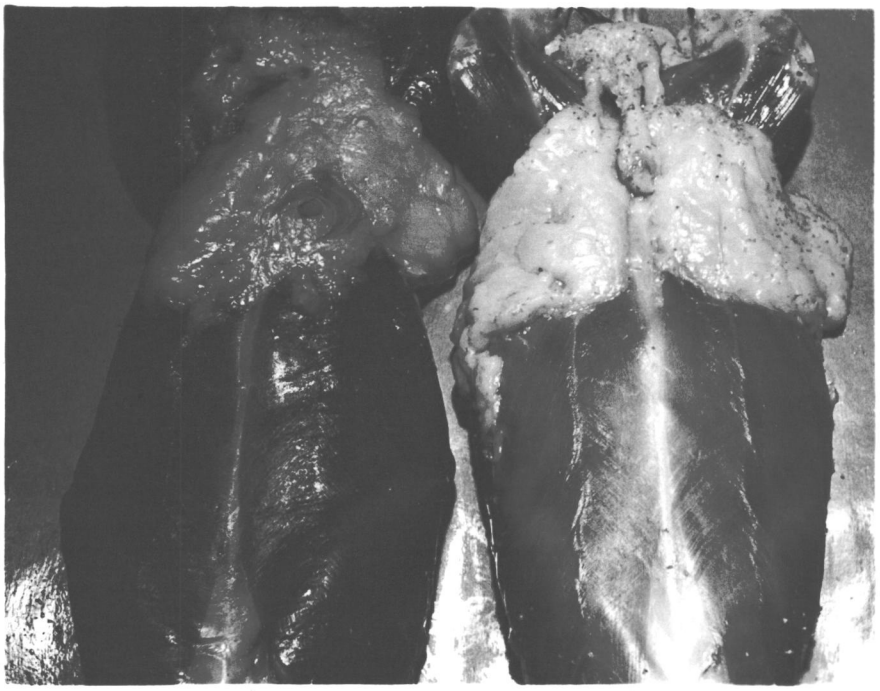


Figure 5. Normal male mink compared with "cotton" that died from this abnormality. Extreme emaciation is typical in fatal cases.

normal (left) vs. cotton mink illustra
areas of [illegible] fat deposits in fat



ure 7. Skinned [illegible] of normal (left) and co
illus[illegible] fat condition of the

in Table 1 illustrating blood values found for cotton as compared with normal mink.

Values listed as normal were taken from a sample of 32 standard dark mink (16 males and 16 females) chosen at random from the group fed the adequate control ration in 1958. Blood values for "cottons" represent average values of groups of from 22 to 27 experimentally produced cotton mink. Values indicate that in general cotton mink have markedly reduced hemoglobin and hematocrit values and slightly reduced numbers of erythrocytes. Index values (3, p. 62) show that the amount of hemoglobin within cells and the volume of individual cells are quite low for cotton in relation to normal mink. These conditions are characteristic of a microcytic, hypochromic anemia. Stained smears (Figures 8 and 9) showed an abundance of poikilocytes and anisocytes in the blood of cotton mink, especially in those severely affected.

Table 1
Blood values of normal and cotton mink

	Number of animals	Hemoglobin g/100 cc.	Erythrocytes million/mm.	Hematocrit %	Indexes		
					Color	Volume	Saturation
Normal	32	18.7 ± 0.6[1]	9.00 ± 0.68	45.0 ± 3.1	1	1	1
Cotton	22-27	10.8 ± 3.0	8.50 ± 2.59	28.1 ± 8.8	.61	.66	.93
% of normal		57.8	94.4	62.4			

[1] The ± values represent standard deviation.

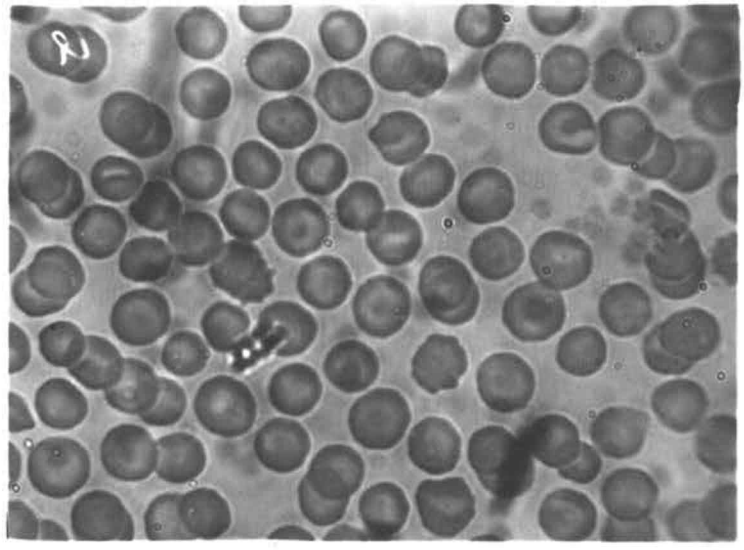


Figure 8. Blood smear from normal mink depicting regularity of size, shape and color density of red blood cells.

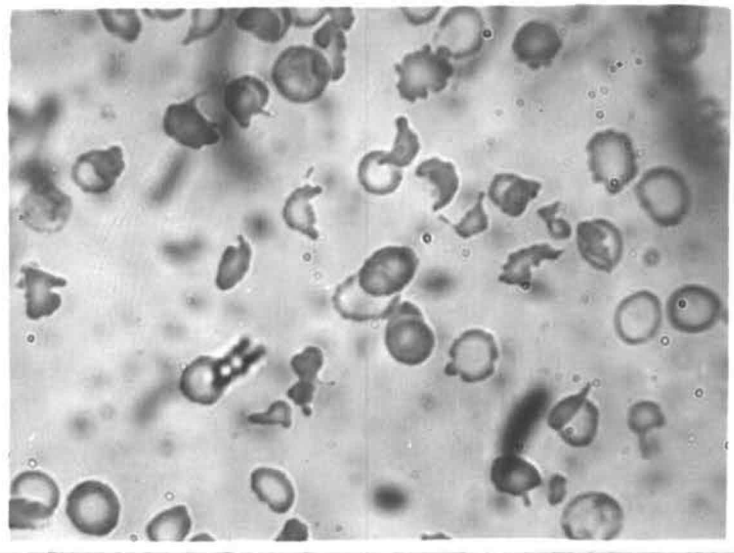


Figure 9. Blood smear from severely affected cotton mink showing presence of poikilocytosis, anisocytosis and hypochromia.

Experimental Production of the Abnormality

Cotton mink have been produced on a variety of experimental rations fed at this station, but the denominator common to all cases is not readily apparent. There was an early indication that a sudden upset of the mink's metabolism, precipitated by inclusion of spoiled fish in the diet, could cause mink to develop the cotton condition. This method of possible production was further investigated by "throwing" mink off feed by an abrupt change of a major dietary constituent. There are obvious relationships to commercial mink ranching practice in both these situations. In all, 7 diets involving 140 mink were used over a 4 year period (5 diets were fed prior to 1956) in attempts to produce cotton mink by upsetting their normal metabolic pattern; the result was three cotton mink, indicating that a sudden dietary upset as caused by feeding spoiled fish or by suddenly changing the diet is not a primary cause of cotton mink.

On the theory that the peroxides formed during rancidification of fish oils could oxidize certain essential nutrients, thereby rendering them unavailable to mink, and since Helgebostad and Ender (17, p. 11) had implicated rations containing large amounts of marine fats in the production of cotton mink, two experiments each, in 1956 and 1957, were devoted to assessing the effects of rancid

dietary fat on the incidence of cotton mink. Sixteen mink were fed the basal diet modified by adding 5 per cent of rancid sardine oil in place of a like weight of sole, and 16 additional mink received the basal diet with sufficient rancid horsemeat to supply 5 per cent of rancid horse fat in the diet. No cotton mink occurred on either treatment, although the furs produced had a brownish cast. The following year, 30 mink were fed the basal diet with 5 per cent of herring oil replacing an equivalent weight of sole. Since ionizing radiation has been mentioned (6, p. 4-12) as causing a high rate of fat peroxide formation, a sample of herring oil was subjected to radiation treatment at Arco, Idaho, and an additional 30 mink were fed the basal diet similarly supplemented with irradiated oil. Again, no cotton mink resulted in either case, but there was a brownish coloration of the fur. The results of these four trials indicate that the cotton fur condition could not be attributed to the inclusion of rancid marine or animal fats at a 5 per cent level in the diet, as used in this experiment.

Atlantic whiting had been implicated in "cotton" production by Hartsough (39, p. 1) and it was noted at the Oregon State College Fur Farm that when experimental diets containing Pacific hake were tested for nutritional adequacy as an alternative source of mink feed, many

cotton mink resulted. Data drawn from experimental trials containing Pacific hake (Merluccius productus) or Atlantic whiting (Merluccius bilinearis) in the ration clearly demonstrate that the production of cotton mink follows the feeding of these two fish species. This production appears to be directly dependent on the percentage of these fishes comprising the diet as is evident from Figure 10 where each point represents a group of 20 to 48 mink.

It was an early observation that the feeding of eviscerated hake did not result in as many cotton mink as did the feeding of whole hake. This was confirmed by further trials, as indicated by the graph (Figure 10): In two trials employing eviscerated whiting at fairly high levels no cotton mink resulted suggesting that the cotton-causing factor had been eliminated with the viscera.

When cotton mink were transferred to an adequate control ration for a six-month period (January to July), the new fur emerged normally pigmented following early summer shedding. The normal fur color was maintained in these adult animals even when they were replaced on the cotton-causative (hake-containing) ration from July through the next winter's furring period. This observation demonstrates that only young mink develop the cotton fur condition and suggests that depigmentation of the fur occurs only when

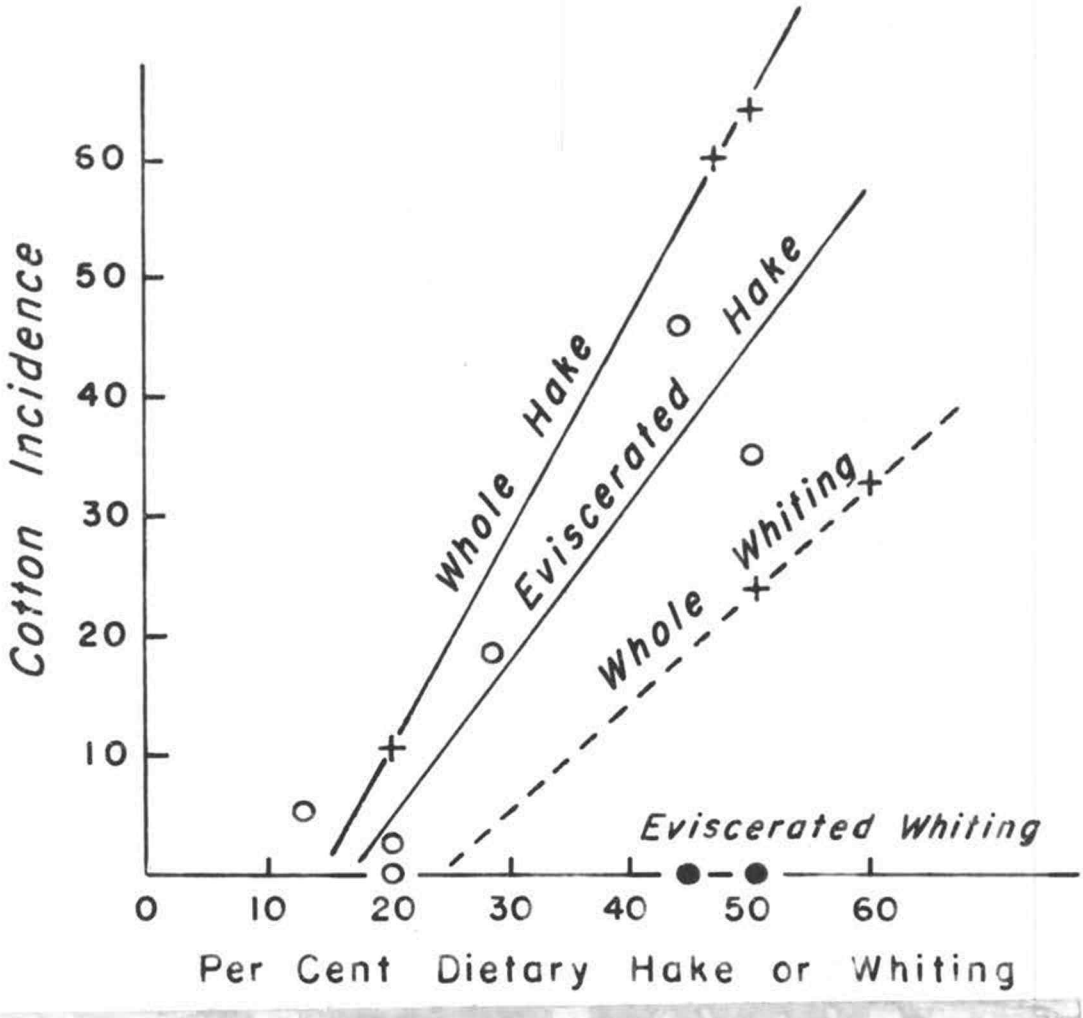


Figure 10. Relationship of cotton fur incidence to amounts of whole or eviscerated hake and whiting in the ration.

the demands for fur growth coincide with the stress of body growth.

Preventive Measures

As it had been shown that inclusion of two species of marine fishes were directly causative of cotton mink, it was thought probable that they contained either a toxic factor or were inducing a dietary deficiency of some essential nutrient. A natural deficiency in these diets seemed unlikely because the original rations employing hake which were cotton causative were heavily fortified with supplemental vitamins and minerals. The similarity between this and another case, in which certain fresh water fish contain an enzyme capable of inducing Chastek's paralysis in foxes and mink, was noted. As cooking destroys the action of this enzyme (thiaminase) a logical step was to cook the hake and assess its effect on producing cotton mink.

In 1957, 85 mink were fed a ration containing 30 per cent of whole hake, treated by cooking with a double boiler arrangement using steam as a heat source. Ten cotton mink resulted, giving an incidence of 12 per cent. This was a reduction in the 29 per cent incidence of "cottons" to be expected from feeding a similar level of raw whole hake (Figure 10), but the results were inconclusive as the treatment failed to completely eliminate

the condition.

The 1957 cooking methods were subject to question, therefore the experiment was repeated in 1958 using 20 mink and 50 per cent of whole hake. The cooking process was rigorously controlled as indicated in Appendix C and the fish heated to at least 93 degrees C. Results from this trial, measured in terms of incidence of cotton mink, growth rates, and blood picture were particularly well defined (Table 2). Mink receiving the cooked hake ration did not develop cotton fur, indicating that the hake-containing ration is adequate for development of normal pigmentation and that hake contains a heat-labile, cotton-causing factor. Likewise, cooked hake promotes superior growth to uncooked hake (Figure 3); however growth still does not equal that of animals fed the adequate control ration. Blood values are much improved over mink fed the raw hake ration.

The mortality rate on rations containing over 30 per cent raw hake is very high; in 1957, for example, there was 27 per cent mortality on a ration containing 47 per cent hake. To prevent this death loss, one ounce of raw liver in addition to their regular ration was given to 13 mink which were either losing weight or failing to gain after two and one-half months on a 50 per cent raw hake ration. Weights picked up almost immediately and at the

Table 2

Effect of feeding cooked hake on the cotton fur syndrome

Ration identification	No. of animals	"Cotton" incidence	Mortality	Final Weights		Hemoglobin
				M	F	
		%	%	grams		grams/100cc.
Adequate control (58-1)	77	0	3	1681 ± 233[1]	1006 ± 129	18.7 ± 0.6[2]
Cooked hake (58-13)	20	0	0	1374 ± 189	863 ± 83	18.0 ± 0.8
Raw hake (58-12)	20	63	20	953 ± 404	773 ± 189	14.6 ± 3.3

1 ± values are standard deviations.

2 taken from a sample of 32 randomly selected individuals.

end of the experimental period only one of these mink had died; however 11 had developed cotton fur. At the completion of furring an interesting observation was made in that the cotton portion of the fur was evident as a band of white or grey out at the tip of the underfur. That part nearest the skin was more normally colored, giving a banded appearance to the fur, indicating a preventive effect of raw liver.

Genetic Aspects

All mink fed a cotton-inducing ration do not develop cotton fur, similarly their growth rate is considerably better than those that develop the condition (Figure 3). These observations and the indication that certain families were more involved suggested that susceptibility to this condition could be under genetic control. To test this hypothesis, entire litters from female mink that had previously produced "cottons" and those from females which had produced no "cottons" on a hake-containing ration were raised in 1958 on a cotton-causing, hake-containing ration. Results, given as numbers of normal and cotton mink produced, are presented in Figure 11 and indicate that cotton mink tend to run in families and are probably genetically influenced. Presented on page 42 is Table 3 which demonstrates the differences in reaction of two strains of mink

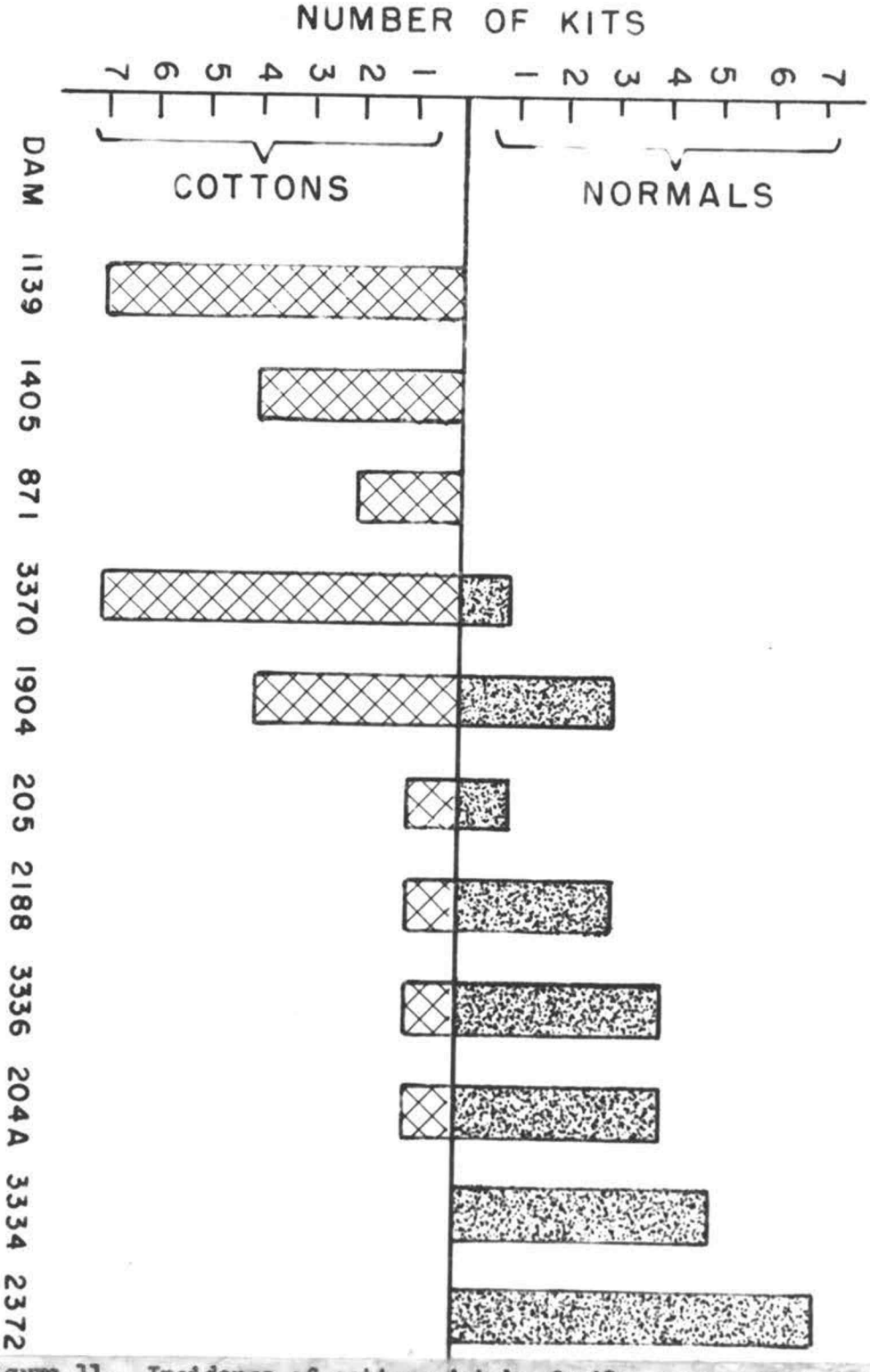


Figure 11. Incidence of cotton mink by family group. Each bar represents a litter; the stippled and cross-hatched portions indicate numbers of young within these litters which appeared normal or cotton-furred respectively.

Table 3

Performance data of 4 mink families selected for resistance and 4 selected for susceptibility to the cotton fur syndrome (all fed a cotton-causative ration)

Selected for	No. of animals	"Cotton" incidence %	Final weights (grams) M	Final weights (grams) F	Hemoglobin (g/100 cc.)	Hematocrit %
Susceptibility	21	95.2	978 ± 246[1]	677 ± 196	11.6 ± 4.0	30.1 ± 11.5
Resistance	21	9.5	1236 ± 185	896 ± 96	17.2 ± 1.7	43.0 ± 5.2

[1] ± values represent standard deviations.

to consumption of a similar cotton-causative ration. Suffice it to say that the differences are not ambiguous.

Experiments in 1959 support these findings and indicate that selection for cotton mink is effective. Selection of experimental animals in 1958 from litters chosen at random resulted in a 63 per cent "cotton" incidence when they were fed a causative diet; selection of approximately one-third of the kits from cotton females raised the "cotton" incidence to 83 per cent in 1959. The means of inheritance of susceptibility is not clear, but in general when "cottons" were mated to "cottons", the offspring were largely "cottons", as opposed to the case of normal mated to normal where both types of offspring were produced in approximately equal proportions.

Specific Cause

From the observation that the cotton fur-producing property of hake is destroyed by heat, it was deduced that a ration containing hake was not inherently deficient, but probably contained either a toxin or a substance inhibitory to the proper utilization of an essential nutrient by the animal. It appeared that this antifactor could be interfering with the metabolism of a specific B-complex vitamin since the Norwegian workers had indicated that supplementation with a mixture of all known synthetic B vitamins or raw materials rich in these factors was able to prevent

cotton fur as produced on their experimental rations (17, p. 11). Therefore, in an attempt to determine the specific nutritional basis of the cotton fur abnormality, an exploratory trial was set up, utilizing the noted anemic condition of cotton mink as an index of effect. Cotton mink, which had been produced previously on hake and whiting-containing rations, were grouped according to hemoglobin levels and injected weekly with solutions containing twice their weekly requirement of thiamine, folic acid and vitamin B_{12}, singly or in various combinations, or with crude liver extract. Results of this supplementation were largely inconclusive; however certain mink appeared to respond to either folic acid or thiamine injections. There was no response to crude liver extract. Later, two mink which had showed no improvement to vitamin supplementation were injected with 100 milligrams of ferric ammonium citrate. Of these, one died and one showed a significant increase in hemoglobin from 8.7 to 17.2 grams per 100 cc. blood.

Because a folic acid deficiency may lead, in certain animal species to a failure of normal hair pigmentation and also to anemia, and in view of the response (however inconsistent) of anemic, cotton mink to folic acid injections, a larger trial utilizing a ration containing 50 per

cent of raw hake was set up to assess the effects of supplementary folic acid (injected weekly at levels calculated to supply either 70 or 140 times the daily folic acid requirement) (32, p. 12) on prevention of the cotton fur syndrome. Results of this experiment were convincingly negative as indicated in Table 4.

Again, upon completion of the main experimental period (July to December), all available cotton mink were included in a subsequent experiment which was designed to measure the effect of an organic iron solution,[2] alone and in various combinations with folic acid and vitamin B_{12}, on hemoglobin regeneration. Anemic, cotton mink, which continued to receive the raw hake-containing ration, were arbitrarily divided into three groups according to hemoglobin levels and each group allocated to a treatment at random. All supplementation was parenteral (for further information on methods refer to Appendix C). The results are listed in Table 5.

Iron injections gave a positive and consistent response in all animals receiving them, and injections of vitamin B_{12} or folic acid had no additionaleffect on hemoglobin levels. This information helped to provide an answer to the anemia of cotton mink but gave no solution

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[2] Supplied as Armidexan, Armour Veterinary Laboratories, Chicago.

Table 4

Effect of parenterally supplied folic acid on the cotton fur syndrome

Ration identification	No. of animals	"Cotton" incidence	Mortality	Final weight M	Final weight F	Hemoglobin	Hematocrit
		%	%	grams		g/100 cc.	%
Adequate control (58-1)	77	0	3	1681±233[1]	1006±129	18.7±0.6[2]	45.0±3.1[2]
Raw hake - folic acid supplemented (58-12)	20	63	20	953±404	773±189	14.6±3.3	36.1±8.7

1 ± values represent standard deviations.

2 Taken from a sample of 32 randomly selected individuals.

Table 5

Effect on hemoglobin regeneration of supplementing anemic, cotton mink fed a 50 per cent hake ration with various nutrients (parenterally supplied)

Treatment	No. of animals	Initial hemoglobin (Dec 4) g/100 cc. of blood	Final hemoglobin (Feb 20) g/100 cc. of blood
Control (58-17)	6	12.3 ± 2.9[1]	11.3 ± 7.1
Iron (58-18)	6	11.2 ± 4.2	17.7 ± 0.2
Folic acid (58-19)	7	12.6 ± 2.2	11.0 ± 2.9
Iron + folic acid (58-20)	7	12.0 ± 2.5	16.8 ± 0.4
Iron + B_{12} (58-21)	6	12.1 ± 4.4	17.5 ± 1.0
Iron + folic acid + B_{12} (58-22)	6	12.0 ± 3.5	17.9 ± 1.5

[1] ± values represent standard deviation.

to the primary problem of failure of normal hair pigmentation.

In 1959 further attempts were made to identify the nutritional factor(s) involved, and experimental groups were devoted to testing the effect of nutrients which are known to be associated with both pigment and blood formation on prevention of the cotton fur syndrome. Mink fed a ration containing 50 per cent of raw hake were divided into groups of from 10 to 20 animals; each group received one of the following supplements: (1) a parenteral solution of 11 well-known B-complex vitamins, (2) oral lysine plus tyrosine, (3) parenteral copper and (4) parenteral iron. A fifth group, serving as a negative control, was not supplemented.

Results of supplementing mink fed the 50 per cent hake ration are presented in Table 6. Performance of mink fed an adequate control ration as well as mink fed the hake ration with no supplementation is given for comparison.

No preventive effect due to supplementation with 11 B-complex vitamins was noted. "Cotton" incidence was 83 per cent, growth was markedly subnormal and hemoglobin values were 38 per cent below normal. Supplementation with lysine and tyrosine, important intermediaries in melanin formation, similarly produced no remission of symptoms. Likewise, injection of copper, which is

Table 6

Effects of supplementing a 50 per cent raw hake ration with various nutrients

Treatment	Number of animals	Mortality %	"Cotton" incidence %	Terminal Weight[1] (g) M	F	Hemoglobin g/100 ml
Adequate Control Ration	61	0	0	1818±240[2]	1063±128	18.7±0.6[3]
Non-Supplemented Control	10	0	80	1292±284	850±114	11.9±3.4
B-complex Vitamins	20	25	83	1079±565	829±259	11.6±3.6
Lysine + Tyrosine	12	25	83	1194±252	656±418	10.1±4.1
Copper	10	50	90	1008±505	729±364	11.0±3.7
Iron	20	0	0	1621±273	975±126	16.8±0.8

1 Measured as weight off test for surviving animals and as death weight for dead animals.

2 ± values represent standard deviation.

3 Normal hemoglobin values as determined on 32 mink in 1958.

essential in both hemoglobin and melanin formation, offered no protection against the cotton fur syndrome as 9 of 10 treated mink were classified as "cottons" at the end of the experiment. Iron supplementation gave striking results as none of 20 animals receiving injected organic iron developed the depigmented condition of the underfur. Figure 12 shows pelts of representative mink fed the adequate control ration, the 50 per cent raw hake-containing ration plus parenteral iron, and the 50 per cent raw hake, non-supplemented ration. Size was significantly improved over non-supplemented mink as illustrated in Figure 13. Blood hemoglobin levels were 41 per cent higher than non-supplemented controls, but about 10 per cent below hemoglobin values of mink fed the adequate control ration. Variation of size and of hemoglobin levels was markedly reduced within the iron-supplemented group and was similar to that of the adequate control-fed group.

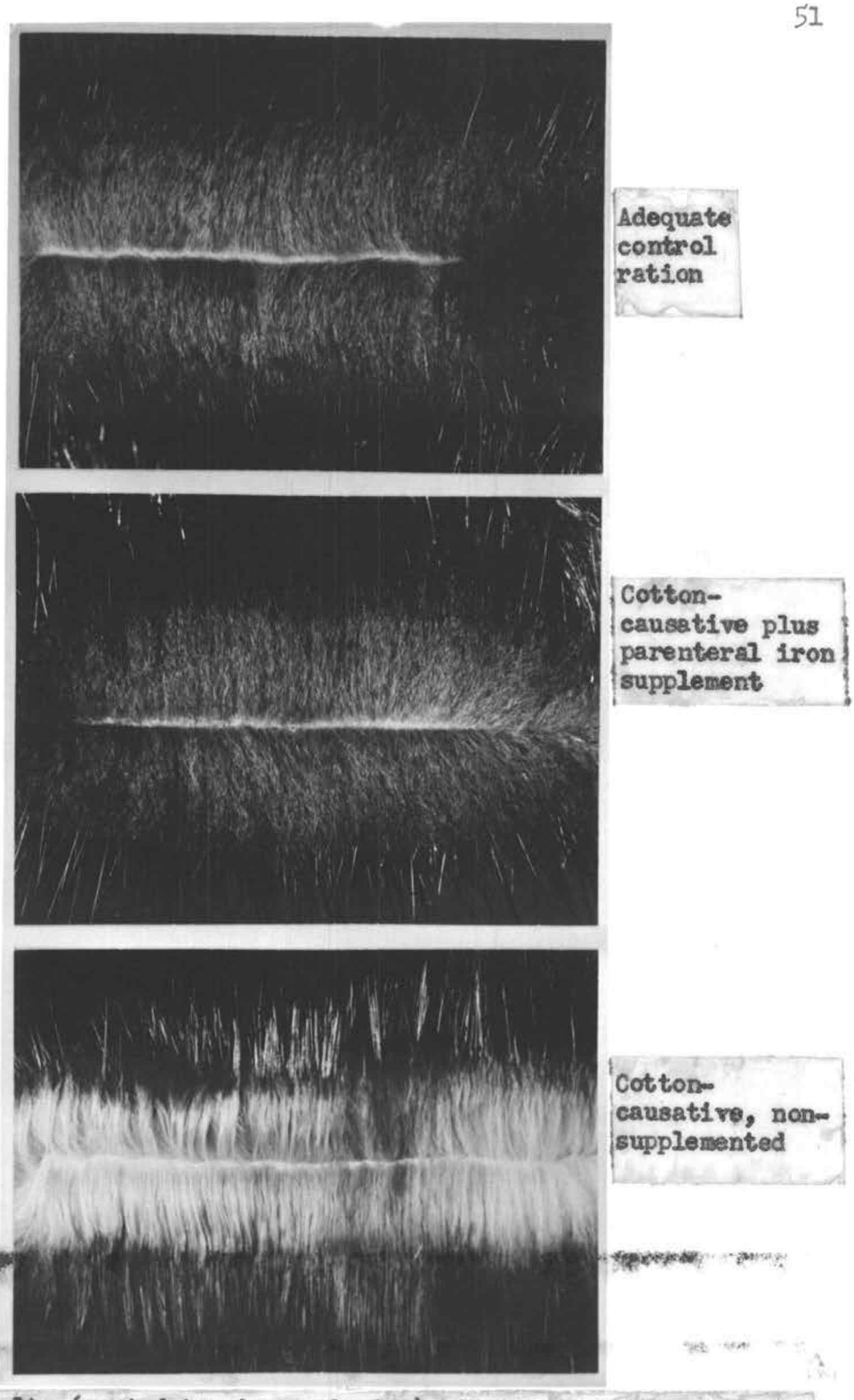

Figure 12. Pelts (parted to show underfur) of representative mink fed the diet indicated. Parenteral iron administered to young mink prior to and during the furring cycle prevented the cotton condition evident in the unsupplemented animals.

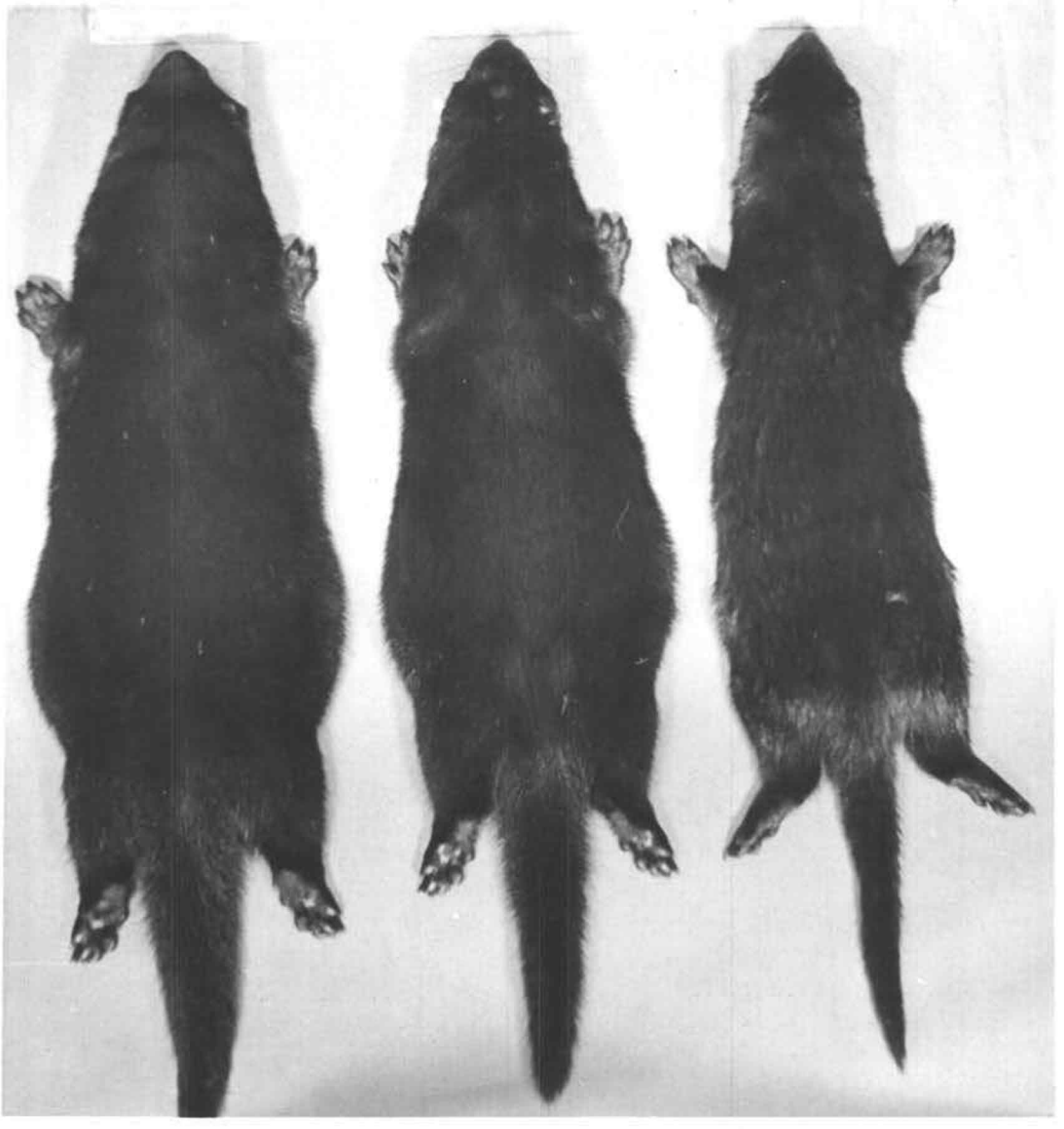

Figure 13. Effect of parenteral iron on growth. Representative males from the adequate control (left), cotton-causative plus parenteral iron supplement (center) and non-supplemented cotton-causative diet groups (right) are depicted. Note that size of the iron-supplemented animal is considerably larger than the non-supplemented animal and approaches that of the adequate control.

Further Studies with Iron

It has been shown that the hake-containing ration is adequate for promoting normal fur pigmentation and blood formation in mink, provided it has been cooked prior to inclusion in the ration. From this one can deduce that this ration contains sufficient iron to meet the normal demands of the animal; a deduction which has been borne out by analyzing the hake-containing ration for iron. There are 108 milligrams of total iron per kilogram of dry matter in the hake-containing ration and 114 milligrams of total iron per kilogram of dry matter in the adequate control ration, as fed in 1959. Since parenterally supplied iron is effective in curing the anemia associated with cotton fur and also in preventing the condition itself, it appears that dietary iron is not being effectively used by the animal as a result of the presence of an unidentified factor contained in Pacific hake. To determine the quantity of iron that this factor in hake is capable of "binding", thereby making it unavailable for the animal's normal use, the following experiments were run.

Twenty-seven cotton mink produced during the 1959 experiments were selected from those groups in which no protection against cotton fur was evident. Animals were weighed, divided by sex and stratified according to blood hemoglobin levels. Allocation of mink thus arranged was

at random within consecutive blocks of three animals. All mink were continued on the cotton-causative ration and received in addition either (1) no added dietary iron, (2) 17.6 mg. of iron (supplied as "Ferronord"[3], an iron-glycinate complex) per Kg. of ration, as fed, or (3) 88.1 mg. of iron per Kg. of ration, as fed. The first level of iron was calculated to supply the mink with his daily requirement (based on iron requirements per pound of feed listed for dogs; no data on iron are available for mink) (33, p. 1). The second level is 5 times the first. At the end of a 30 day feeding period, animals were reweighed and hemoglobin levels measured. Results are tabulated in Table 7 in terms of hemoglobin regeneration and weight changes.

It is apparent that neither of these two levels of dietary iron glycinate is capable of restoring normal hemoglobin levels to anemic, cotton mink in the presence of 50 per cent of raw hake in the ration when fed for the period of time indicated.

In a sequential trial involving the same groups of mink, the iron supplement was increased by tenfold at each level, so that mink were receiving 176 and 881 milligrams of iron per kilogram of feed on a wet basis. Results of

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[3] Nordmark Pharmaceutical Laboratories, Incorporated, Irvington, N. J.

Table 7

Effects of orally supplemented iron on weight gains and hemoglobin regeneration of anemic, cotton mink (1)

Treatment	No. of animals	Hemoglobin levels (g/100 cc.)		Weight change	
		Initial	Regeneration[1]	M	F
				(grams)	
No supplemental iron	9	10.7±5.2[2]	0.3±3.6	-18±107	-75±40
17.6 mg. iron added/Kg. ration (as fed basis)	9	10.9±5.0	0.3±1.9	-89±213	-47±66
88.1 mg. iron added/Kg. ration (as fed basis)	9	11.7±4.7	0.4±1.5	-25± 51	-95±68

1 Measured as the average increase in hemoglobin level during the 30 day iron supplementation period.

2 ± values represent standard deviation.

this trial are shown in Table 8.

Hemoglobin values show no response to the 176 mg. level of iron in the ration, but at five times this level (881 mg.) a beneficial effect on hemoglobin level was noted, especially in very anemic mink with hemoglobin levels close to 4 g. per 100 cc. The increase is not significant statistically as notable improvement was recorded only in the most anemic animals. In addition, results are somewhat confounded as the iron level in the feed was so high that the feed was moderately unpalatable to the mink, which resulted in a lowered feed intake.

Table 8

Effects of orally supplemented iron on weight gains and hemoglobin regeneration of anemic, cotton mink (2)

Treatment	No. of animals	Hemoglobin levels (g/100 cc.)		Weight change (grams)	
		Initial	Regeneration[1]	M	F
No supplemental iron	8	11.1±6.0[2]	1.6±1.4	-195±152	-77± 94
176 mg. iron added per Kg. ration (as fed basis)	8	11.4±6.2	0.3±2.4	-310±235	-100±130
881 mg. iron added per Kg. ration (as fed basis)	9	12.1±5.7	3.5±4.3	-255±216	-125± 70

1 Measured as the average increase in hemoglobin level during the 30 day iron supplementation period.

2 ± values represent standard deviation.

Effects of Feeding Hake-Containing Rations to Mice

Mink, being seasonal breeders, were limited as experimental animals in that young are available only once during the year. Consequently, mice were adapted to the experimental program in the spring of 1958 because they appeared to possess the general required characteristics, in that they are continuous breeders, are omnivorous and are available in pigmented strains. A line of black mice was developed by outcrossing albino females to a male of the wild type (agouti hair color), thus freeing the genetically suppressed pigment formation. Trials with mice were run in conjunction with the mink feeding trials, the main objective being to determine the effect of various pertinent compounds on prevention of the abnormal conditions developed through feeding raw hake.

Consumption of raw hake precipitated a syndrome in mice apparently quite different from that in mink. Black mice fed rations containing either 50, 75 or 100 per cent hake showed no signs of greying even after three months on the ration. Other symptoms, however, were quite noticeable and included partial or complete loss of the body hair, a condition which was accompanied in some cases by skin lesions as illustrated in Figure 15. A not too uncommon development was a thickening of the external ear in conjunction with a discharge from lesions on the ear

Figure 14. Development of a thickened external ear as a result of including raw hake in a ration for mice.

Figure 15. A frequent symptom observed in mice fed a ration containing 75 per cent of raw hake is loss of hair accompanied by skin lesions.

surface (Figure 14). In advanced cases the forelimbs showed a malformation of bone structure, causing the animal to walk on the inner side of the foreleg and foot. Other symptoms noted were paralysis or partial paralysis of one of the hind limbs and a keratinization of the cornea of the eye. Growth rate was markedly depressed and mortality rate was high.

To verify that the observed effects were induced by the feeding of raw hake, other groups of mice were fed rations containing 75 per cent of cooked hake. The anomalous conditions observed using the raw fish, with the exception of a thinning of hair in some animals and watering eyes in others, did not appear using the cooked fish.

Briefly these experiments revealed that either oral or parenteral supplementation of several or all B-complex vitamins produced no preventive or curative effects and further substantiate similar observations made with mink.

Discussion

It is clear from the experimental work presented that the cotton fur abnormality in mink involves considerably more than failure of the underfur to pigment properly. Associated are disrupted physiologic conditions manifested

as growth depression and anemia which are of far greater consequence to the animal. The condition is chronic in some animals, acute in others. Acutely affected animals lose weight rapidly, probably in part due to reduced feed consumption, and die in a state of extreme emaciation, having literally starved to death. Death sometimes occurs even before the characterizing symptom of white underfur is established. Bertman (35, p. 520) correctly, although without experimental basis, considered the abnormality "the result of an obscure disease". Manifestations of the abnormality, widely different though they seem, are the result of a metabolic disorder and evidently stem from the same basic cause. This is true at least for the abnormality as it is produced under experimental conditions here.

Why the underfur becomes depigmented, whereas the longer and more substantial guard fur remains normally pigmented (as has been universally noted) is an unanswered question which probably can be resolved through an increased knowledge of the physiology of hair growth. An important factor, it would seem, is the relative times of formation of these two furs. It is possible that melanin produced for deposition into the shaft of the guard hair is formed before the animal is subjected to conditions conducive to cotton fur formation. Another possibility,

which should be investigated, is that a new guard fur is not produced in cotton mink.

Experimental results reported suggest that depigmentation of fur occurs primarily in young animals during the period when metabolic demands of fur pigmentation coincide with stress of body growth, and not as Seton (35, p. 520) states without "reference to season, place or age". Helgebostad and Ender's (18, p. 15) observation that "adult animals are not to the same degree prone to develop fur anomalies ... as pups and kits" is in support of this. In addition, it has been the general observation by nutritionists that greying resulting from nutritional deficiencies usually occurs during the stage of growth (10, p. 368).

Evidence suggests that cotton mink can arise from several seemingly unrelated causes, but it is known that they occur frequently and consistently when Pacific hake (Merluccius productus) and Atlantic whiting (M. bilinearis) are contained in a ration otherwise adequate for normal fur pigmentation and blood formation. This frequency of occurrence has been closely correlated to the amount of these fishes fed, over a period of five years, which is quite remarkable in that other dietary ingredients varied markedly as did gross experimental conditions during this period.

Once, the possibility was considered that the causative factor in hake occurred in the content of the fish's gastro-intestinal tract. Experiments on localization of the factor, however, indicated its presence in both carcass and viscera of hake. It was also apparent that the entire fish is richer in the factor than its eviscerated counterpart. Different incidence of "cottons" resulting from feeding whiting and hake infers that hake contains a higher concentration of the cotton-causative factor than does whiting. This reasoning is strengthened by the observation that evisceration of whiting resulted in complete elimination of cotton fur, while evisceration of hake only reduced its occurrence. Thus it appears that the factor is entirely concentrated in the viscera of whiting. As attempts are made to concentrate and isolate this factor, very likely an individual organ such as the liver will prove to be a potent source.

Original reports by Ender and Helgebostad (7, p. 409-412; 16, p. 11-12; 17, p. 11) did not implicate any particular species of fish in production of depigmentation in foxes and mink, but stated that feeding rations consisting mainly of "salt-water fishes" was causative. In later publications (19, p. 1660-1661) Helgebostad and Martinson have referred to definite species, including coalfish (Gadus virens) and whiting (Gadus merlangus), as

being causative. These species are closely related to those species fed at the Oregon Experimental Fur Farm; hence the possibility exists that many other closely related species also contain this factor.

Helgebostad and Ender (17, p. 11) state that depigmentation of fur in foxes and mink is accentuated by addition of "relatively large amounts of marine fats to the fish (containing) diet" and speculate that the deleterious effect of these fats lies in their unsaturation, which causes in vivo, oxidative-destruction of B vitamins necessary for normal fur pigmentation. Work accomplished here with feeding rations containing rancid fish oils neither substantiates nor disproves this hypothesis. Rations which contained 5 per cent of rancid fat did not produce mink with cotton fur; however this treatment was not superimposed on a causative diet and further, since there was no measure of rancidity obtained, it is possible that oils fed had gone beyond the peroxidative stage (of deleterious effect) and were relatively stable.

Definite fish species undoubtedly are of paramount importance in causing cotton fur, but not all cases of cotton mink observed have resulted from feeding these fishes. In 1959, for example, a ration containing 25 per cent of tuna waste produced four "cottons" and in 1959 one "cotton" was found on a diet high in dry materials, which

included herring meal. Two elements have been noted which seem to be common to all cases of true cotton mink produced here during the time (four years) with which the author is familiar. First, all rations have contained some amount and type of marine fish as an ingredient and second all rations have placed some degree of stress on the animal. The first element can probably be eliminated because, although possible, it is very doubtful that all or even many unrelated species of marine fish contain the cotton-causing factor known to be present in hake and whiting. However, it is not so easy to dispel the second situation common to production of cotton mink. Stress placed on the animal by hake and whiting diets is obvious; the tuna diet was extremely rancid as determined by the TBA test for rancidity (47, p. 1-5) and caused yellow fat disease in the mink consuming it; the "high dry" ration failed to support optimum growth for one or more reasons. Specific experiments, designed early in this program to assess the effect of a disturbance of ration quality on production of cotton mink, showed that this was not a primary cause. The significant point here is not how many "cottons" were produced, but that "cottons" did occur, demonstrating that some property of these rations was cotton-causative. (These observations form part of a hypothesis to be presented later in this discussion.)

There has been a conclusive demonstration that cotton mink resulting from feeding hake-containing rations can be avoided by cooking the hake prior to its inclusion in the ration. This observation can probably be extended to whiting, although there are no actual data on this point. Destruction by heat of the cotton-causing ability of hake elucidates the following points: (1) a simple deficiency of the diet is not responsible for the observed syndrome, as was postulated by Ender and Helgebostad (7, p. 410), (2) it verifies that the fish contains the causative factor, (3) it obviously indicates that the factor is heat labile and probably proteinaceous in nature, and furthermore, (4) it demonstrates that Pacific hake, widely available and virtually unused, may have utility in mink feeding, barring other nutritional inadequacies.

Recently, Helgebostad and Martinsons (19, p. 1660-1661) have also demonstrated that symptoms of light underfur and anemia caused by diets containing large amounts of coalfish and whiting (G. merlangus) could be totally prevented by replacing the dietary raw fish with boiled fish of the same species. They postulated a heat-labile factor, probably an enzyme in the raw fish.

In the 1958 trial, supplementing mink receiving a raw hake-containing ration with beef liver significantly increased weight gains and arrested cotton fur development

as evidenced by a normally colored band at the base of the otherwise white underfur. This reversal was not conclusive, however, as the liver was given apart from the regular ration. The possibility remains that the liver was consumed exclusive of or at a time differing from the other feed and was consequently not affected by the proposed antifactor of hake.

The selection of experimental animals from parents which had or had not previously exhibited cotton fur, either directly or through their offspring, revealed that certain families may be genetically predisposed to the syndrome. The physiological state of individuals of these families clearly demonstrates that one group is able to withstand the stress of the hake-containing ration while the other group succumbs to it. Although these observations are convincing in the data presented, they require further substantiation to be conclusive. In an earlier study concerning the inheritance of the condition, Hummon and Bushnell (21, p. 30-34) showed that "no cotton mink are obtained from matings of male and female cotton parents or the interbreeding of their descendants". These conclusions were quite valid considering that a "standard" mink ration had been fed. However, had they fed a ration conducive to the formation of cotton fur, the results may have

been quite different. This exemplifies the fact that a genetically-determined susceptibility may not be apparent until environmental factors allow for expression of the genotype.

What the basic difference between susceptible and resistant mink families is and how this susceptibility is inherited is not known. One might infer that one strain of mink is more efficient in their metabolism of iron, but this appears unlikely since amounts of iron in excess of 100 times the daily allowance had to be contained in the feed before susceptible mink could apparently make use of it. A more logical hypothesis might be that the factor holds iron in a form unavailable to susceptible mink families but available to resistant families. Whatever this basic difference is, much more intensive work will be required to establish it.

Knowledge of one specific cause of cotton mink and of certain factors affecting the condition aid in determining what practical preventive measures can be employed. Avoidance of feeding fish species known to be causative is the most obvious means of prevention; however, since the causative factor in the fish is destroyed by heat, cooking these fish prior to inclusion in the ration will avoid the problem. Another likely method, not directly tested, but inferred from trials using a raw liver supplement in

conjunction with the causative ration, would be to alternate feeding the implicated fish with other non-causative feeds. Thus, all of the feed would not be exposed to the interfering action of the cotton-causing factor. A final means of prevention would be selection of breeding stock from those animals not showing a history of the abnormality.

Discovery that cotton mink were anemic provided a useful criterion for investigating the nutritional basis of this anomaly; hence blood formation, which is relatively rapid as compared with the slower, cyclical process of fur growth, was used to measure response to supplementation with purified nutrients. Using this measure, it was found that although several individual B vitamins had no effect, parenteral iron was capable of restoring blood of affected mink to almost normal values.

Supplementing the causative ration with 11 B vitamins during the growth and furring period proved ineffective in preventing or reducing incidence of cotton fur or its allied symptoms. This apparently contrasts with Norwegian work which has repeatedly stressed the importance of adding supplementary B vitamins to prevent greying of foxes and mink in connection with intensive fish feeding (16, p. 11-12; 17, p. 11; 18, p. 15-16). However, it is believed that the experimental conditions were sufficiently different so that strict comparison cannot be made. Fish

provided the sole source of protein in Norwegian rations, whereas rations here contained protein from horsemeat, meatmeal, skimmilk powder and soybean oil meal in addition to fish protein. Furthermore, their rations were composed almost entirely of fish with little or no supplementation. It is quite conceivable that such a ration could have a B vitamin deficiency superimposed on the deficiency created by the cotton-causing factor. If such is the case, rancid fish oils could easily aggravate the condition.

The ineffectiveness of parenteral copper and oral lysine and tyrosine showed that these important components of melanogenesis were not limiting. On the other hand, organic iron when supplied parenterally to mink fed the causative ration induced normal pigmentation of fur, increased weight gains immensely and resulted in essentially normal hemoglobin values. This would indicate that the symptoms of cotton fur, induced by the feeding of raw hake, are essentially those of an iron deficiency. Certainly anemia is the classical symptom of iron deficiency and anemia of cotton mink is of the microcytic, hypochromic type invariably associated with such deficiency. Depressed growth could also be easily related to iron deficiency, either indirectly as an effect of the severe anemia or directly in that iron is contained in several important enzyme systems. The relation between the

deficiency of iron and depigmentation, however, is not so readily obvious.

The causative ration contains nearly 95 per cent as much iron as does the adequate ration, yet does not supply enough iron to susceptible mink for normal growth or blood formation, and further this lack of iron interferes in some unknown way with pigment formation. When the hake portion of the causative ration is cooked, symptoms of cotton fur disappear, demonstrating that the inherent iron content of this ration is quantitatively ample to prevent symptoms of cotton fur. From these considerations it appears that raw hake and probably raw whiting contain a factor (possibly a chelating agent) which acts to render not only iron contained in the fish but also that of other ration components unavailable to the animal. Additional proof that dietary iron is largely unavailable is shown by the failure of anemic, cotton mink fed the causative ration to respond to daily oral supplementation with iron glycinate at either 1, 5 or 25 times their estimated daily allowance. Some hemoglobin regeneration was evident when iron was supplied at 125 times the daily allowance, indicating that the factor is reversible and provides a crude estimate of its strength in terms of "iron binding" capacity. In this regard Helgebostad and Martinsons (19, p. 1661) state that "oral doses of 40 mgm. iron daily for three weeks gave

some relief (increase in hemoglobin level), although it was not so successful as parenteral administration".

As iron has not been linked directly with achromotrichia in the past, the immediate cause of observed depigmentation is speculative. Since achromotrichia has been observed in mink as a symptom of several unrelated nutrient deficiencies (20, p. 160; 28, p. 147), it seems more plausible to suggest that failure of fur to pigment normally is a symptom of a non-specific dietary deficiency, rather than to assume that iron is directly concerned with pigmentation processes. Shortage of an element as vital as iron to normal body physiology undoubtedly would affect overall metabolic reactions and it is conceivable that those processes which are of least consequence to the organism's survival, such as hair pigmentation, would likely be first impaired. This implies that biochemical reactions concerned with pigmentation have low priority in relation to reactions serving more critical functions. Since hair growth and associated pigment formation occur during a very short and specific period in a given year, nutritional stress at this time results in depigmentation of the fur.

Interrupted iron metabolism is possibly not the sole consequence of feeding raw hake to animals. It was observed that when mice were fed a ration including 75 per

cent of raw hake, certain individuals exhibited a deformation of bones of the forelimbs, causing them to stand and walk on the inner side of the foreleg and foot. The similarity of this condition to that pictured by Underwood (40, p. 243) as resulting from a manganese deficiency in rabbits is remarkable. In view of the fact that an antagonism has been demonstrated between iron and manganese in several animal species (31, p. 309-317), presumably because of their common properties, it is speculated that a factor interfering with the metabolism of one could interfere similarly with the other. The action of the heat-labile factor contained in hake may, in addition to restricting normal utilization of dietary iron, also restrict manganese utilization and may, in fact, to a variable degree be restricting metabolism of certain other, similar elements.

SUMMARY

1. The cotton fur abnormality in mink has been experimentally produced and described.
2. Affected mink possess varying degrees of depigmented underfur, lack considerably in size and generally exhibit a microcytic, hypochromic anemia.
3. Inclusion of raw, whole hake (Merluccius productus) or whiting (M. bilinearis) in rations otherwise adequate for normal fur pigmentation and blood formation is causative of the cotton-fur syndrome in mink. Incidence of cotton fur increases proportionately with the amount of these fish comprising the ration.
4. Evisceration of the causative fish prior to feeding eliminated cotton fur in the case of whiting and lowered it in the case of hake.
5. Thorough cooking of hake prior to its inclusion in the ration resulted in complete elimination of cotton fur development.
6. Feeding 5 per cent of animal or marine fats, under conditions conducive to fat peroxide formation did not result in cotton pelts.
7. A genetic tendency toward susceptibility and resistance to the cotton fur syndrome has been demonstrated when known causative diets containing 50 per cent of raw

hake are fed.

8. Practical means of preventing the abnormality may be effected by either avoiding or cooking the causative fish and by selection of breeding stock for resistance.

9. Supplementing groups of mink fed a causative ration with 11 parenterally administered B vitamins, parenteral copper or oral lysine plus tyrosine did not prevent cotton fur from developing.

10. Mink fed a cotton-causative ration and supplied with parenteral iron did not develop the cotton fur syndrome.

11. Iron glycinate added to the causative ration at one, 5 or 25 times the daily recommended allowance did not restore normal hemoglobin levels to anemic, cotton mink. At 125 times the daily allowance recovery was noted in the most anemic mink.

12. It is postulated that raw hake and whiting contain a heat-labile factor, causative of cotton fur in mink, which interferes in some unknown manner with normal iron metabolism.

13. The effect of iron on pigmentation is thought to be indirect reflecting low priority of fur pigment formation in the face of nutritional stress.

14. One specific cause of the cotton fur abnormality is feeding certain fishes; however it is postulated that a more general cause is a nutritional stress which occurs during the critical period when body growth coincides with fur formation.

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A P P E N D I C E S

Appendix A (Part I)
1956 Experimental Groups and Rations

Group	Ration Description	Treatment[1]	No. and Type of Mink Dark M	Dark F	Pastel M	Pastel F	Dates Fed	Ration Composition % Horsemeat	Beef Liver	Tripe	Mixed Sole[2]	Mixed Rockfish[2]	Turbot	Hake	Lard	Sardine Oil	Whiting, eviscerated	Supplement (for composition refer to Appendix B)
56-1	Adequate Control	No Additional	22	18	-	-	Jul 5-Pelt.	7	4	14	42	25	-	-	-	-	-	8 (OSC 1)
56-5	Low Eviscerated Hake	" "	26	22	-	-	Jul 5-Sep 14	-	3	-	-	27	40	20	-	-	-	10 (OSC 9)
							Sep 15-Pelt.	-	"	-	-	24	"	"	3	-	-	" (")
56-6	Low Hake	" "	26	22	-	-	Jul 5-Sep 14	-	"	-	-	27	"	20	-	-	-	" (")
							Sep 15-Pelt.	-	"	-	-	24	"	20	3	-	-	" (")
56-7	Low Eviscerated Hake	" "	26	22	-	-	Jul 5-Sep 9	-	"	-	-	22	"	20	-	-	-	15 (OSC 35)
							Sep 10-Pelt.	-	"	-	-	17	"	"	-	-	-	20 (" ")
56-8	Adequate Control	Rancid Sardine Oil	8	8	-	-	Jul 5-Pelt.	7	4	14	37	25	-	-	-	5	-	8 (OSC 1)
56-9	" "	Rancid Horsemeat	8	8	-	-	" - "	26	"	"	23	"	-	-	-	-	-	" (")
56-10	High Eviscerated Whiting	No Additional	8	8	-	-	Jul 5-Jul 16	7	"	"	42	"	-	-	-	-	-	" (")
							Jul 17-Aug 8	22	3	5	-	-	-	-	-	-	50	20 (")
							Aug 9-Pelt.	"	"	"	-	-	-	-	-	-	"	" (OSC 35)
56-11	Med. Eviscerated Hake	" "	-	-	22	8	Jul 5-Pelt.	-	3	-	-	22	34	31	-	-	-	10 (OSC 9)
56-12	Adequate Control	Abrupt Diet Change	-	-	22	8	Jul 5-Aug 31	7	4	14	42	25	-	-	-	-	-	8 (OSC 1)
							Sep 1-Sep 19	"	"	"	-	-	-	67	-	-	-	" (")
							Sep 20-Pelt.	"	"	"	42	25	-	-	-	-	-	" (")

1 Further details of treatments are given in Appendix C, Part I.
2 All fish components of the rations were eviscerated, except where otherwise noted.

Appendix A (Part I)
1957 Experimental Groups and Rations

Group	Ration Description	Treatment[1]	No. and Type of Mink Dark M	Dark F	Pastel M	Pastel F	Dates Fed	Ration Composition % Horsemeat	Beef Liver	Tripe	Mixed Sole	Mixed Rockfish	Turbot	Hake	Whiting	Herring Oil	Supplement (for composition refer to Appendix B)
57-1	Adequate Control	No Additional	40	30	10[2]	10[2]	Jul 1-Pelt.	7	4	14	42	25	-	-	-	-	8(OSC 1)
57-5	Low Hake	Hake Cooked	40	30	10	5	" -Sep 9	7	3	-	10	15	20	30	-	-	15(OSC 44)
							Sep 10-Pelt.	-	-	-	20	15	10	30	-	-	25(")
57-7	High Whiting	No Additional	8	7	8	7	Jul 24-Oct 17	7	-	-	-	10	15	-	60	-	8(OSC 1)
							Oct 18-Pelt.	-	-	-	-	11	16	-	64	-	9(")
57-8	High Hake	" "	8	7	8	7	Jul 1-Aug 2	7	-	-	-	10	15	60	-	-	8(")
							Aug 3-Sep 4	7	10	-	-	10	15	50	-	-	8(")
							Sep 5-Sep 8	-	-	-	10	15	20	30	-	-	25(")
							Sep 9-Sep 10	-	10	-	10	15	20	20	-	-	25(OSC 44)
							Sep 11-Pelt.	-	10	-	-	15	20	30	-	-	25(")
57-9	Adequate Control	Irradiated Herring Oil	8	7	8	7	Jul 17- "	7	4	14	37	25	-	-	-	5	8(OSC 1)
57-10	" "	Upset Metabolism	8	7	8	7	Sep 3- "	"	"	"	42	"	-	-	-	-	"(")
57-11	" "	Rancid Herring Oil	10	-	-	-	Sep 13- "	"	"	"	37	"	-	-	-	5	"(")
57-12	High Fish	Tertiary Butyl Peroxide	10[3]	-	-	-	Nov 12- "	7	-	-	51	34	-	-	-	-	8(")

1 Further details of treatments are given in Appendix C, Part I.
2 Sapphire Mink 3 Adult Mink

Appendix A (Part I)
1958 Experimental Groups and Rations

Group	Ration Description	Treatment[1]	No. and Type of Mink Dark M	Dark F	Sapphire M	Sapphire F	Dates Fed	Ration Composition % Horsemeat	Beef Liver	Tripe	Mixed Sole	Mixed Rockfish	Turbot	Hake, Raw	Hake, Cooked	Hake, Eviscerated	Hake Viscera	Whiting	Supplement (for composition refer to Appendix B)
58-1	Adequate Control	No Additional	39	38	-	-	Jul 7-Aug 14	8	3	10	10	20	40	-	-	-	-	-	9(OSC 46)
							Aug 15-Sep 13	-	3	15	"	"	"	-	-	-	-	-	12(")
							Sep 14-Pelt.	10	-	"	20	"	15	-	-	-	-	-	20(")
58-9	"	Folic Acid Antagonist	13	13	-	-	Jul 7-Jul 31	8	3	10	10	20	40	-	-	-	-	-	9(OSC 46)
	"						Aug 1-Pelt.	-	3	15	25	25	20	-	-	-	-	-	12(")
58-10	High Hake	No Add'l[2]	13	15	-	-	Jul 7- "	7	-	-	-	10	15	50	-	-	-	-	18(OSC 49)
58-11	" "	" " 2	14	16	-	-	" - "	"	-	-	-	"	"	"	-	-	-	-	"(")
58-12	" "	Parenteral Folic Acid	10	10	-	-	" - "	"	-	-	-	"	"	"	-	-	-	-	"(")
58-13	" "	Cooked Hake	10	10	-	-	" - "	"	-	-	-	"	"	-	50	-	-	-	"(")
58-14	High Eviscerated Hake	No Additional	13	12	-	-	" - "	"	-	-	-	"	"	-	-	50	-	-	"(")
58-15	Hake Viscera	Hake Viscera	13	12	-	-	" - "	"	-	-	45	"	"	-	-	-	5	-	"(")
58-16	High Whiting	No Add'l	-	-	12	13	Jul 15- "	"	-	-	-	"	"	-	-	-	-	50	"(")
58-17	High Hake	" " 3	2	2	-	2[4]	Jul 14- "	"	-	-	-	"	"	50	-	-	-	-	"(")

1 Further details of treatments are given in Appendix C, Part I.

2 Mink in these experimental groups were selected on the basis of inherent resistance or susceptibility to the cotton fur syndrome.

3 These animals were adult mink which had been on the high hake ration the previous year as kits.

4 Pastel mink.

Appendix A (Part I)
1959 Experimental Groups and Rations

Group	Ration Description	Treatment[1]	No. and Type of Mink				Ration Composition %								
			Dark		Sapphire		Horsemeat	Beef Liver	Tripe	Mixed Sole	Mixed Rockfish	Turbot	Hake	Supplement (for composition refer to Appendix B)	
			M	F	M	F									
59-1	Adequate Control	No Additional	31	30	8	8	8	3	10	20	25	25	-	9	(OSC 46A)
59-9$_A$	High Hake	" " [2]	15	13	-	-	7	-	-	-	10	15	50	18	(OSC 49A)
59-9$_B$	" "	Parenteral Iron[3]	13	7	-	-	"	-	-	-	"	"	"	"	(")
59-9$_C$	" "	11 B-complex Vitamins[3]	12	8	-	-	"	-	-	-	"	"	"	"	(")
59-9$_D$	" "	Parenteral Copper[3]	6	4	-	-	"	-	-	-	"	"	"	"	(")
59-9$_E$	" "	Oral Lysine and Tyrosine[3]	7	5	-	-	"	-	-	-	"	"	"	"	(")
59-9$_F$	" "	No Additional[3]	6	4	-	-	"	-	-	-	"	"	"	"	(")

1 Further details of treatments are given in Appendix C, Part I.

2 Mink in this experimental group were selected on the basis of inherent resistance or susceptibility to the cotton fur syndrome.

3 Sixty-two per cent of the mink in these groups were selected at random. The other 38 per cent were chosen from females which were cottons in 1958.

Appendix A (Part II)

Subsequent Experimental Groups and Rations

(Basic rations fed to mink remained the same as in the main trial, however treatments imposed differed.)

Group	Ration Description	Treatment[1]	No. and Type of Mink Dark M F	Pastel M F	Dates Fed	Ration Composition % Horsemeat	Beef Liver	Mixed Rockfish	Turbot	Hake	Whiting	Supplement (for composition refer to Appendix B)
$57\text{-}7_A$	High Whiting	B Vitamin Supplement	1 2	1 -	Dec 23-Apr 15	7	-	10	15	-	60	8(OSC 1)
$57\text{-}8_A$	High Hake	B Vitamin Supplement	5 5	4 3	" - "	-	10	15	20	30	-	25(OSC44)
58-17[2]	High Hake	No Additional	3 3	- -	Dec 28-Mar 2	7	-	10	15	50	-	18(OSC 49)
58-18[2]	" "	Parenteral Iron	2 4	- -	" - "	"	-	"	"	"	-	"(")
58-19[2]	" "	Folic Acid	6 2	- -	" - "	"	-	"	"	"	-	"(")
58-20[2]	" "	Iron and Folic Acid	5 2	- -	" - "	"	-	"	"	"	-	"(")
58-21[2]	" "	Iron and Vitamin B_{12}	3 3	- -	" - "	"	-	"	"	"	-	"(")
58-22[2]	" "	Iron and Folic Acid and Vitamin B_{12}	4 2	- -	" - "	"	-	"	"	"	-	"(")
59-10[2]	High Hake	No Additional	6 3	- -	Jan 23-Feb 29	"	-	"	"	"	-	"(OSC 49A)
59-11[2]	" "	Low Oral Iron	6 3	- -	" - "	"	-	"	"	"	-	"(")
59-12[2]	" "	High Oral Iron	6 3	- -	" - "	"	-	"	"	"	-	"(")

1 Further details of treatments are given in Appendix C, Part II.

2 All mink in 1958 and 1959 experimental groups were "cottons".

Appendix B
Supplement Composition (% of total)

Ingredients	Supplement OSC 1	OSC 9
Wheat Germ	25.0	40
Cer-L-Meal[1]	25.0	20
Brewers' Yeast	18.8	15
Alfalfa Meal	18.7	15
Bonemeal	12.5	10
	100.0	100

[1] Produced by Crown Mills, Portland, Oregon.

Ingredients	OSC 35	OSC 44
Whole Rolled Wheat	15.0	15.0
Oatmeal	15.0	--
Steel Cut Oats	--	15.0
Wheat Germ Meal	5.0	5.0
Dried Skim Milk	15.0	15.0
Dried Whey, 25% Protein	5.0	5.0
Soybean Oil Meal	15.0	15.0
Herring Meal, 70% Protein	15.0	15.0
Meat Meal, 50% Protein	5.0	5.0
Molasses Dried Beet Pulp	2.5	2.5
Stabilized Beef Fat	2.5	2.5
Distillers' Solubles	2.5	2.5
Dicalcium Phosphate	1.0	1.0
Iodized Salt	0.5	0.5
Premix[2]	1.0	1.0
	100.0	100.0

[2] Premix

Ingredients	Per Pound
Calcium Pantothenate	750 mg
Riboflavin	181 "
Vitamin B-12	1.3 "
Pyridoxine	100 "
Folic Acid	45 "
Thiamine H Cl	200 "
Vitamin E	500 International Units
Vitamin A, Stabilized Dry	78,000 " "
dl-Methionine	0.15 pounds

(Dispersed in one pound of a mixture of wheat middlings, soybean oil meal and corn distillers' dried grains with solubles.)

Appendix B
Supplement Composition (% of total)

Ingredients	Supplement OSC 46	OSC 46A
Wheat Germ Meal	25.0	25.0
Brewers' Yeast	4.2	4.2
Alfalfa Meal	12.5	12.5
Dried Skim Milk	8.3	8.3
Meat Meal	16.7	16.7
Soybean Oil Meal	16.7	16.7
Flaked Corn	16.6	—
Ground Oat Groats	—	16.6
	100.0	100.0

To each ton of the above supplements the following were added:

Fortafeed 2-49c - 8 pounds
TM-10 (Terramycin) - 5 "
dl-Methionine - 1 "

Ingredients	OSC 49	OSC 49A
Wheat Germ Meal	25	25
Alfalfa Meal	13	13
Dried Skim Milk	8	8
Meat Meal	18	18
Soybean Oil Meal	18	18
Flaked Corn	18	-
Ground Oat Groats	-	18
	100	100

Appendix C (Part I)

Description of Experimental Treatments

Group Number	Treatment
56-8	Rancid Sardine Oil - Sardine oil was stored at room temperature for about 8 months and included in the ration at a 5 per cent level. No measure of rancidity was available.
56-9	Rancid Horsemeat - Horsemeat stored at -15 degrees C. for one year was included in the ration at a 26 per cent level (calculated to supply 5 per cent of horse fat). No measure of rancidity was taken.
56-12	Abrupt Diet Change - Sixty-seven per cent of hake was abruptly substituted for 42 per cent of sole and 25 per cent of mixed rockfish in the ration for a 20 day period.
57-5	Cooked Hake - Hake was cooked in open kettles with a double boiler type arrangement; heat was supplied by a steam generator. There was some indication that cooking was not entirely adequate.
57-9	Irradiated Herring Oil - Herring oil which had been subjected to ionizing radiation at Arco, Idaho, was included at the 5 per cent level in the ration. No measure of rancidity was available.
57-10	Upset Metabolism - Sole which had set at room temperature for 4 days was included in the diet for 5 consecutive days beginning Sept. 3.
57-11	Rancid Herring Oil - Five per cent of rancid herring oil (light pressed), which had been stored at 4 degrees C., was added to the ration.
57-12	Tertiary Butyl Peroxide - This synthetic oxidant was included in the ration (mixed with the wet ingredients) for a 10 day period (Nov. 13-23). Each mink received 1 g. daily.

Appendix C (Part I), continued

Group Number	Treatment
58-9	Folic Acid Antagonist - Pyrimethamine ("Daraprim", Burroughs-Wellcome and Company, Tuckahoe, N. Y.), a structural analog of folic acid, was added to an adequate control ration at either 125 or 250 mg. per Kg. dry matter. The former level was included from 29 Aug. through 22 Nov. and the latter from 22 Aug. through 22 Nov. Five hundred and 1000 mg. levels proved lethal to mink.
58-12	Parenteral Folic Acid - One half of this group (5 males and 5 females) were injected intraperitoneally with one cc. of a solution containing 1.4 mg. of folic acid per cc. at weekly intervals (14 Jul. - 15 Dec.). The other half received double this dose. These amounts were calculated to supply 70 and 140 times a mink's daily folic acid requirement (32, p. 12).
58-13	Cooked Hake - The hake was thawed, ground and cooked by one of the following procedures: 3 Jul. - 1 block autoclaved at 15 p.s.i. for 30 minutes. 10 Jul. - 8 blocks heated in steam retort until they reached 110 degrees C. in the center. 15 Jul. - 9 blocks heated in steam cooker to 93 degrees C.
58-15	Hake Viscera - The hake viscera remaining from the 50 per cent eviscerated hake ration (58-14) was included in the ration fed this group. It composed approximately 5 per cent of the ration fed.
59-9B	Parenteral Iron - An iron-dextran compound ("Armidexan", Armour Veterinary Laboratories, Chicago, Ill.) was injected intramuscularly into mink at one of two levels. Six males and 4 females received 50 mg. (1 cc.) of iron biweekly for a total of 350 mg. and 7 males and 3 females received 50 mg. monthly for a total of 200 mg. Injection period began 21 Jul.

Appendix C (Part I), continued

Group Number	Treatment
59-9C	11 B-Complex Vitamins - At weekly intervals beginning 21 Jul., the following amounts of vitamins were injected intraperitoneally into male mink: thiamine - 2.7 mg., riboflavin - 4.9 mg., pyridoxine - 2.7 mg., niacin - 24.6 mg., pantothenic acid - 19.7 mg., choline - 49 mg., inositol - 350 mg., para-amino-benzoic acid - 2.8 mg., folic acid - 0.5 mg., vitamin B_{12} - 0.5 mg. and biotin - 0.5 mg. Beginning 11 Sept. the niacin level was adjusted to 4.9 mg. and inositol to 70 mg. Female mink received one half this dosage. These vitamin solutions were prepared by carefully weighing the indicated amounts and dissolving in distilled water. 1 N NaOH was added to dissolve the riboflavin and the pH was adjusted with 1 N HCl. The solution was brought to volume and refrigerated in foil-covered storage bottles.
59-9D	Parenteral Copper - A copper glycinate solution (in peanut oil) was injected subcutaneously behind the front leg in doses of 27 mg. (0.2 cc.) at either monthly or bimonthly intervals beginning 21 Jul.
59-9E	Oral Lysine and Tyrosine - 1.8 g. of l-lysine and 0.7 g. of l-tyrosine were supplied daily in the feed from 22 Sept. to pelting. These amino acids were premixed in the dry supplement.

Appendix C (Part II)

Description of Treatments for Subsequent Experimental Groups

Group Number	Treatment
57-7A, 57-8A	B-Vitamin Supplementation - Of 21 mink retained, 12 were injected intraperitoneally with solutions of 0.2 mg./cc. of folic acid, 0.03 mg./cc. of vitamin B_{12}, 1.1 mg./cc. of thiamine (either singly or in combination) or crude liver extract (Eli Lilly and Company, Indianapolis, Ind.) at approximately weekly intervals for varying periods up to two months. Injections were initially 1 cc. and subsequently 2 cc. Two mink previously injected with (1) folic acid and vitamin B_{12} and (2) folic acid and thiamine were later injected with 100 mg. of ferric ammonium citrate.
58-18	Parenteral Iron - Mink on this treatment received weekly intramuscular injections of "Armidexan" (50 mg. iron per cc.) for 6 weeks (one half dose 5th week).
58-19	Folic Acid - Mink were intraperitoneally injected at weekly intervals with a folic acid solution containing 1.4 mg. of folic acid per cc. Total dose was 7 mg. of folic acid.
58-20	Iron and Folic Acid - These animals received weekly intraperitoneal injections of "Armidexan" and folic acid. The total dose was 275 mg. of iron and 7 mg. of folic acid.
58-21	Iron and Vitamin B_{12} - Intraperitoneal injections of 50 mg. of "Armidexan" and 0.3 mg. of vitamin B_{12} were made at weekly intervals to mink on this treatment. Total dosage was 275 mg. of iron and 1.8 mg. of vitamin B_{12}.
58-22	Iron and Folic Acid and Vitamin B_{12} - Mink received a total dose of 250 mg. of iron as "Armidexan", 8.5 mg. of folic acid and 1.8 mg. of vitamin B_{12} by weekly intraperitoneal injection.

Appendix C (Part II), continued

Group Number	Treatment
59-11	Low Oral Iron - A supplement of iron glycinate (supplied as "Ferronord", Nordmark Pharmaceutical Laboratories) was fed at 17.6 mg. iron per Kg. of feed (as fed basis). Iron glycinate was premixed with the dry supplement prior to mixing with the wet ration ingredients.
59-12	High Oral Iron - Iron glycinate ("Ferronord") supplied daily at 88.1 mg. iron per Kg. of ration (as fed basis). The iron-glycinate was premixed with the dry supplementary portion of the ration.