Aberrant Iron Metabolism and the "Cotton-Fur" Abnormality in Mink [1,2]

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The "cotton-fur" (CF) abnormality in mink fed Pacific hake and whiting has been described (Stout and associates, '60). In addition to previously listed symptoms of reduced growth and achromotrichia, presence of anemia in CF animals has been noted (Adair and Davis, '57; Helgebostad and Martinsons, '58). The observation that the development of CF could be prevented by cooking the fish studied (Stout et al., '60) suggested that an induced rather than a natural dietary deficiency was the basis of this anomaly. Other work showed considerable protection against a similar type of achromotrichia in fox pups by feeding before graying was well advanced, a supplementary mixture of synthetic B-vitamins; these included thiamine, riboflavin, nicotinic acid, *p*-aminobenzoic acid, inositol, pyridoxine, biotin, pantothenic acid, folic acid, choline and vitamin B_{12}, or substances rich in these factors, such as raw cod roe, animal liver and dried brewers' yeast (Helgebostad and Ender, '51). No protection was evident when the diet of young foxes was supplemented with rice starch, glucose, Fe, Cu, Co, Zn and Mn, vitamins A, D, E, K, thiamine, pantothenic acid and vitamin C (Ender and Helgebostad, '47).

At various times achromotrichia has been linked nutritionally with deficiencies of pantothenic acid, *p*-aminobenzoic acid, folic acid, biotin, choline, cystine and lysine, and copper and zinc (Frost, '48). Concomitant with achromotrichia, anemia has been reported to result from deficiencies of pantothenic acid (McCall et al., '46), copper (Sjollema, '38), lysine and folic acid, (Klain et al., '57).

Previous observations at this station[3] indicated that folic acid, vitamin B_{12}, thiamine (injected singly or in combination), folinic acid or a crude liver extract had no perceptible effect on restoration of blood hemoglobin and hematocrit levels in CF mink. However, intramuscular injection of organic iron restored essentially normal hemoglobin and hematocrit levels to CF mink. This latter observation has also been reported in Norway (Helgebostad and Martinsons, '58).

Results of blood studies on CF mink in relation to normal mink are reported here. Also in an attempt to identify the nutritional factor(s) involved, effects of supplementing a CF-genic ration with (1) B-complex vitamins, (2) lysine plus tyrosine, (3) copper, and (4) iron, on pigmentation, growth, mortality rate and blood formation were measured. Further, results concerning the effect of oral iron supplementation on anemia of CF mink are presented.

EXPERIMENTAL AND RESULTS

Standard dark mink (100) selected in part from litters of females which were previously "cottons" and known to be susceptible to the CF condition were fed a CF-genic ration having the following percentage composition: horsemeat, 7; mixed rockfish, 10; turbot, 15; Pacific hake, 50; and a supplement, 18.[4] Similar mink (61), although randomly selected, were used for comparison, and fed a ration,

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[2] This study was supported by a grant from the Mink Farmers' Research Foundation, Milwaukee.

[3] Unpublished data, Stout, F. M. et al., 1957, 1958.

[4] Percentage composition: wheat germ, 25; alfalfa meal, 13; skim-milk powder, 8; meatmeal, 18; soybean oil meal, 18; rolled oat groats, 18.

known to support optimum growth and furring, of this percentage composition: horsemeat, 8; tripe, 10; beef liver, 3; mixed rockfish, 25; turbot, 25; mixed sole, 20; and a supplement, 9.[5] The animals were housed and fed as indicated previously (Stout et al., '60).

Mink fed the CF-genic ration were divided into groups of 10 to 20 animals; each group received one of the following supplements: (1) B-complex vitamins, (2) lysine and tyrosine, (3) copper or (4) iron. A fifth group, serving as a negative control, was not given a supplement. B-complex vitamins were injected intraperitoneally at weekly intervals for 14 weeks into male mink of group 1 in the following milligram amounts: thiamine, 2.7; riboflavin, 4.9; pyridoxine, 2.7; niacin, 4.9; pantothenic acid, 19.7; choline, 49; inositol, 70; *p*-aminobenzoic acid, 2.8; folic acid, 0.5; vitamin B_{12}, 0.5; and biotin, 0.5. Females received one half of this dosage. Lysine and tyrosine were supplied daily to mink in group 2 in the feed as 1.8 gm of L-lysine and 0.7 gm of L-tyrosine. Copper glycinate was injected subcutaneously into mink in group 3 in doses of 27 mg either monthly or bimonthly. Iron as ferric hydroxide[6] was injected intramuscularly at two levels: 50 mg biweekly for a total of 350 mg to half of group 4, or 50 mg monthly for a total of 200 mg to the remainder.

Blood data were obtained by the following methods. Three milliliters of blood were withdrawn from anesthetized mink by cardiac puncture and immediately transferred to oxalated tubes. Hemoglobin values were determined using a Spencer Hemoglobinometer, model 1000.[7] Erythrocyte counting was accomplished by methods outlined by Wintrobe ('46) using isotonic saline as a diluent. Hematocrit values were obtained following Wintrobe's method ('46); however, centrifugation was made at 2400 rpm for one hour.

[5] Percentage composition: wheat germ, 25; alfalfa meal, 12.4; skim-milk powder, 8.2; meatmeal, 16.5; soybean oil meal, 16.5; rolled oat groats, 16.5; brewers' yeast, 4.2; Fortafeed 249-C (American Cyanamid Co.), 0.4; terramycin (TM-10, Chas. Pfizer Co.), 0.25; DL-methionine, 0.05.

[6] Armidexan, Armour Veterinary Laboratories, Chicago.

[7] American Optical Company.

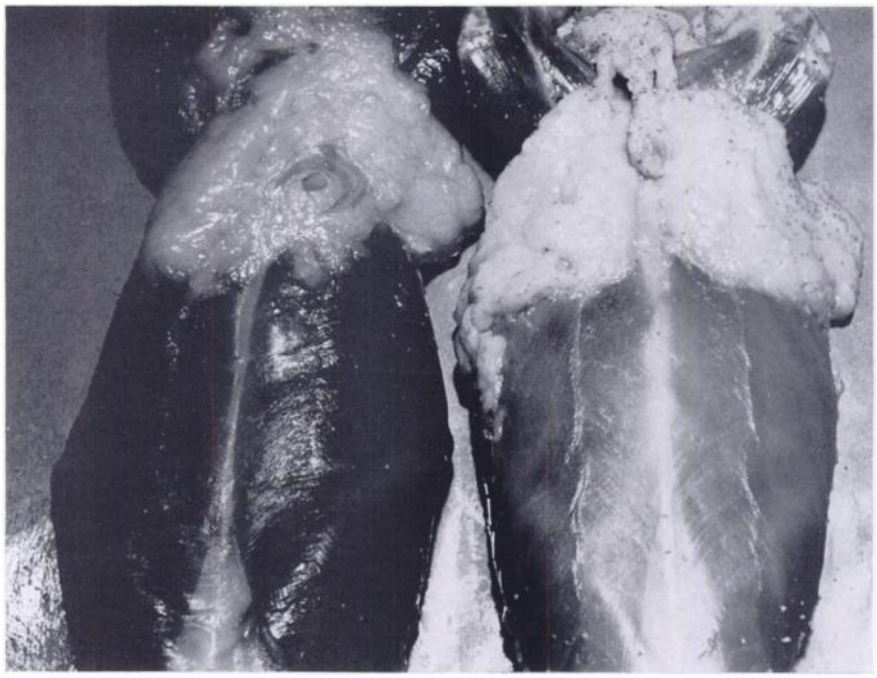

Fig. 1 Skinned carcasses of normal (left) and CF mink illustrating the anemic condition of the latter.

In a sequential trial, 27 CF mink were selected from those groups of the previous experiment in which no protection against CF 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 continued to be fed the CF-genic ration and received in addition either (1) no added dietary iron, (2) 17.6 mg of ferrous iron[8] per kg of ration, as fed, or (3) 88.1 mg of ferrous iron[9] per kg of ration as fed. At the end of a 30-day feeding period animals were reweighed and hemoglobin levels measured.

Evidence of anemia present in the CF syndrome is provided by the appearance of skinned carcasses of CF mink in relation to normal mink (fig. 1). Blood data are listed in table 1. Values shown 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. Blood values for "cottons" represent groups of 22 to 27 experimentally-produced CF mink fed the CF-genic ration. The data indicate that in general CF mink have markedly reduced hemoglobin and hematocrit values and slightly reduced total numbers of erythroctyes. Index values (Best and Taylor, '45) show that the amount of hemoglobin within and the volume of individual cells are low for CF mink in relation to normal mink. These conditions are characteristic of a microcytic, hypochromic anemia. Stained smears showed an abundance of poikilocytosis and anisocytosis in blood from CF mink, especially from those severely affected.

Results of supplementing mink fed the CF-genic ration are presented in table 2. Performance of mink fed an adequate control ration as well as those fed the CF-genic ration with no supplementation is given for comparison. No preventive effect due to supplementation with 11 B-complex vitamins was noted. "Cotton" incidence

[8] Supplied as Ferronord, Nordmark Pharmaceutical Lab., Inc., Irvington, N. J.
[9] See footnote 8.

TABLE 1

Blood values of normal and CF mink[1]

Mink	Hemoglobin	Erythrocytes	Hematocrit	Indexes		
				Color	Volume	Saturation
	gm/100 ml	*million/mm³*	%			
Normal	18.7 ± 0.6[2](32)	9.00 ± 0.68(32)	45.0 ± 3.1(32)	1	1	1
"Cotton"	10.8 ± 3.0 (27)	8.50 ± 2.59(22)	28.1 ± 8.8(27)	0.61	0.66	0.93
% of normal	57.8	94.4	62.4			

[1] Figures in parentheses show numbers of mink used in assembling data.
[2] The ± values represent standard deviation.

TABLE 2

Effects of supplementing a CF-genic ration with various nutrients

Treatment	Number of animals	Mortality	CF incidence	Terminal weight[1]		Hemoglobin
				M	F	
		%	%	*gm*	*gm*	*gm/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[4]	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] The ± values represent standard deviation.
[3] Hemoglobin values for controls determined on 32 randomly chosen animals.
[4] Supplied as Armidexan, Armour Veterinary Laboratories.

was 83%, growth was markedly subnormal and hemoglobin values were 38% below normal. Supplementation with lysine and tyrosine, important intermediaries in melanin formation, similarly produced no remission of symptoms. Likewise, injection of copper, which is essential in both hemoglobin and melanin formation, offered no protection against the CF condition as 9 of 10 treated mink were classified as "cottons" at the end of the experiment. Iron supplementation gave striking results since none of 20 animals receiving injected organic iron developed the CF condition (fig. 2). Size was significantly greater than in mink not receiving the supplement, as illustrated in figure 3. Blood hemoglobin levels were 41% higher than observed in non-supplemented controls, but about 10% below hemoglobin values of mink fed the adequate control ration. Variation in size and hemoglobin levels was markedly reduced within the iron-supplemented group and was similar to that of the adequately-fed control group.

In a subsequent trial when CF mink fed a CF-genic ration received oral supplements of two levels of iron, hemoglobin regeneration paralleled that of mink receiving no added iron (table 3). Furthermore, all three groups of mink lost weight during the experimental period.

DISCUSSION

Discovery that CF mink were anemic provided a useful criterion for investigating the nutritional basis of this anomalous condition; hence, blood formation, which is relatively rapid as compared with the slower, cyclical process of fur growth, could be 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 CF mink to almost normal values.[10]

Supplementing the CF-genic ration with 11 B-vitamins during the growth and furring period proved ineffective in preventing or reducing incidence of CF or its allied symptoms. This observation apparently is in contrast with Norwegian work which has repeatedly stressed the impor-

[10] See footnote 3.

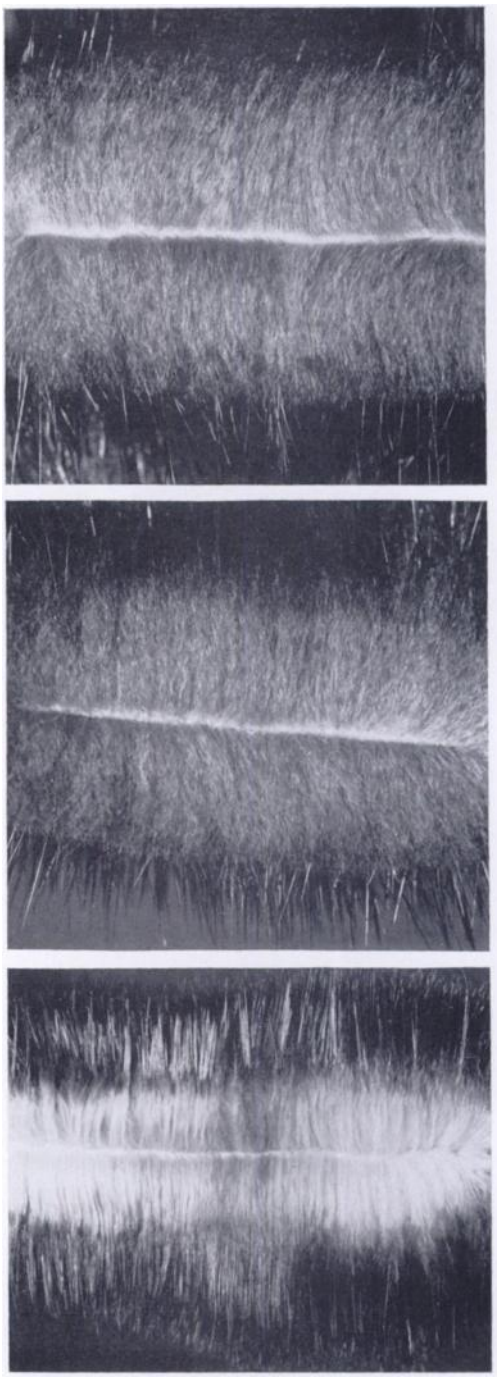

Fig. 2 Pelts (parted to show underfur) of representative mink fed the adequate control ration (top), CF-genic ration plus parenteral iron supplement (center) and CF-genic, non-supplemented ration (bottom). Parenteral iron administered to young mink prior to and during the furring cycle prevented the CF condition evident in the unsupplemented animal.

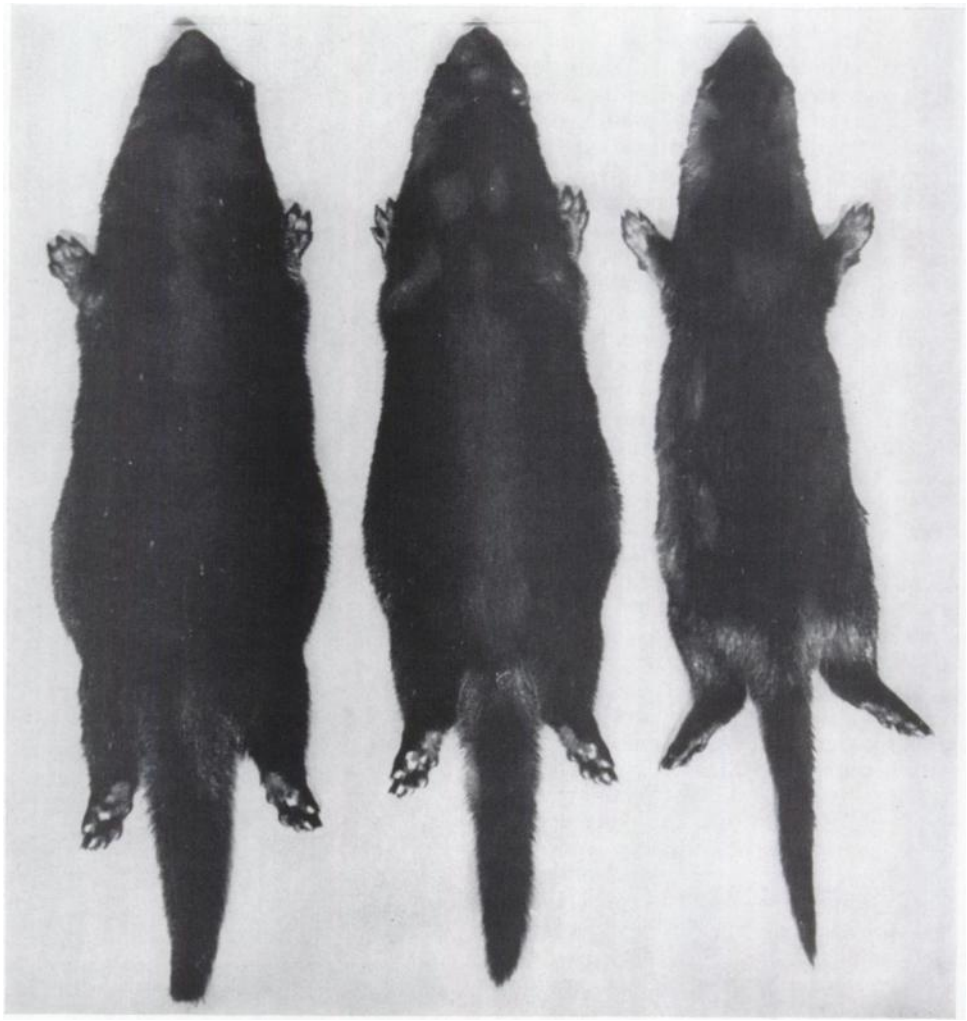

Fig. 3 Effect of parenteral iron on growth. Representative males from the adequately-fed control group (left), the group fed the CF-genic ration + parenteral iron supplement (center) and non-supplemented CF-genic diet groups (right) are shown. Note that the iron-supplemented animal is considerably larger than the non-supplemented animal and approaches the size of the adequately-fed control mink.

TABLE 3

Effects of orally-supplemented iron on weight gain and hemoglobin regeneration of anemic, CF mink

Ferrous iron[1]/ kg ration (as-fed basis)	No. of animals	Hemoglobin levels		Weight change	
		Initial	Regeneration[2]	M	F
mg		*gm/100 ml*		*gm*	*gm*
None	9	10.7 ± 5.2[3]	0.3 ± 3.6	−18 ± 107	−75 ± 40
17.6	9	10.9 ± 5.0	0.3 ± 1.9	−89 ± 213	−47 ± 66
88.1	9	11.7 ± 4.7	0.4 ± 1.5	−25 ± 51	−95 ± 68

[1] Supplied as Ferronord, Nordmark Pharmaceutical Lab., Inc.

[2] Measured as the average increase in hemoglobin level during the 30-day iron-supplementation period.

[3] The ± values represent standard deviation.

tance of adding supplementary B-vitamins to prevent greying of foxes and mink in connection with intensive fish feeding (Helgebostad and Ender, '58). However, it is believed that 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, skim-milk powder and soybean oil meal in addition to fish protein.

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 CF-genic ration induced normal pigmentation of fur, increased weight gains immensely and resulted in essentially normal hemoglobin values. This would indicate that symptoms of CF, induced by feeding raw hake, are essentially those of an iron deficiency. Certainly anemia is the classical symptom of iron deficiency, and anemia of CF mink is of the microcytic, hypochromic type invariably associated with such deficiency. Depressed growth could also be easily related to a deficiency of iron, either indirectly as an effect of the severe anemia or directly since iron is contained in several important enzyme systems. The relation between iron deficiency and depigmentation, however, is not so readily obvious.

The total iron content of the adequate control ration is 114 mg per kg (as-fed basis) and that of the CF-genic ration is 108 mg per kg (as-fed basis), determined by the method of Kennedy ('27). The CF-genic ration contains nearly 95% as much iron as the adequate ration, yet does not supply enough iron to 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 CF-genic ration is cooked, however, symptoms of CF disappear (Stout et al., '60), demonstrating that the inherent iron content of this ration is quantitatively ample to prevent CF symptoms. From these considerations it appears that raw hake and probably raw whiting contain a factor (possibly a chelating agent) which acts to render unavailable to the animal not only iron contained in the fish but also that of other ration components. Additional proof that dietary iron is unavailable is shown by failure of anemic, CF mink fed the CF-genic ration to respond to daily oral supplementation with iron glycinate (table 3).

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 (Helgebostad et al., '59; Leoschke and Elvehjem, '59), 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.

SUMMARY

1. Comparison of blood values for "cotton fur" (CF) and normal mink revealed that CF mink exhibit a microcytic, hypochromic anemia.

2. Supplementing groups of mink fed a CF-genic ration with 11 parenterally-administered B-vitamins, parenteral copper, or oral lysine plus tyrosine, did not prevent mink from developing the CF syndrome.

3. Mink fed a CF-genic ration and supplied with parenteral iron did not develop the CF syndrome.

4. Iron glycinate added to a CF-genic ration was incapable of restoring normal hemoglobin values to anemic, CF mink.

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

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LITERATURE CITED

Adair, J., and K. G. Davis 1957 1956 Mink nutrition research. Misc. paper 41, Oregon Agricultural Experiment Station, Corvallis.

Best, C. H., and N. B. Taylor 1945 The Physiological Basis of Medical Practice, ed. 4. The Williams and Wilkens Company, Baltimore.

Ender, F., and A. Helgebostad 1947 Fôringens innflytelse på pelskvaliteten hos sølvrev. Norsk Veterinoer-Tidsskr., *12:* 381.

Frost, D. V. 1948 The relation of nutritional deficiencies to graying. Physiol. Rev., *28:* 368.

Helgebostad, A., and F. Ender 1951 Fortsatte undersøkelser over fôringens innflytelse på pelskvaliteten hos sølvrev. IV B-vitaminenes betyding for normal pelsfarge. Norsk Pelsdyrblad, *7:* 1.

——— 1958 Fôrets innflytelse på pelskvaliteten. Norsk Pelsdyrblad, *1:* 1.

Helgebostad, A., and E. Martinsons 1958 Nutritional anemia in mink. Nature, *181:* 1660.

Helgebostad, A., R. R. Svenkerud and F. Ender 1959 Experimentell biotinmangel hos mink og rev. Nordisk Veterinaermedicin, *11:* 141.

Kennedy, R. P. 1927 The quantitative determination of iron in tissues. J. Biol. Chem., *74:* 385.

Klain, G. J., D. C. Hill and H. D. Branion 1957 Effect of lysine-deficiency on hematopoiesis in the chick, including observations on folic acid deficiency. Poultry Sci., *36:* 405.

Leoschke, W. L., and C. A. Elvehjem 1959 The importance of arginine and methionine for the growth and fur development of mink fed purified diets. J. Nutrition, *69:* 147.

McCall, K. B., H. A. Waisman, C. A. Elvehjem and E. S. Jones 1946 A study of pyridoxine and pantothenic acid deficiencies in the monkey (*Macaca mulatta*). J. Nutrition, *31:* 685.

Sjollema, B. 1938 Kupfermangel als ursache von tierkrankheiten, Biochem. Ztschr., *295:* 372.

Stout, F. M., J. E. Oldfield and J. Adair 1960 Nature and cause of the "cotton-fur" abnormality in mink. J. Nutrition, *70:* 421.

Wintrobe, M. M. 1946 Clinical Hematology, ed. 2. Lea and Febiger, Philadelphia.