ones[3] on arterial lesions related to cod liver oil—*renal insufficiency*. The manner in which renal insufficiency is produced (heavy metal injury, bilateral nephrectomy,[4] *Leptospira canicola*[5] is relatively unimportant, but some degree of renal damage is *essential*. The "standard diet" or kennel diet plus cod liver oil can be fed to dogs indefinitely (at least as long as a year) and no arterial lesions are ever observed until renal function is disturbed. This emphasizes the role of the kidney in the internal metabolism of the "dietary factor," which presumably is lipid in nature.

As time goes by, we gain confidence that arterial lesions can be produced with regularity in dogs by controlling 2 factors, (1) diet and (2) kidney damage; that both factors are necessary, and that only these 2 factors are involved.

[3] Agduhr, E., and Senstrom, N., *The Appearance of the Electrocardiogram in Heart Lesions Produced by Cod Liver Oil Treatment*, Uppsala, Almguist and Wiksells, 1930; Cowdry, E. V., *Arteriosclerosis*, New York, The Macmillan Co., 1933.

[4] Holman, R. L., *Am. J. Path.*, 1943, **19**, 159.

[5] Holman, R. L., unpublished data.

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Pigment Studies on the Incisor Teeth of Vitamin E Deficient Rats of the Long-Evans Strain.*

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Several investigators[1-5] have reported that vitamin E deficient rats, over a period from 45 to 222 days, lose their natural brownish yellow pigment of the maxillary incisor teeth. During the past 9 years we have had occasion to examine several hundred vitamin E deficient rats of the Long-Evans strain. Although we did not check specifically for abnormal white incisors, we feel reasonably sure that such an obvious change in the pigmentation of the teeth would not have passed entirely unnoticed. To check accurately this depigmentation phenomenon in our strain of rats, the following experiment was conducted.

Procedure. Four groups (Groups 3, 5, 9, 11) of rats were maintained on our vitamin E deficient Diet No. 5† for either 310 or 460 days. Other groups (Groups 1, 7) were given Mason's vitamin E deficient Diet No. 69‡ for 130 days. Each of these groups was compared with normal control groups fed a commercial dog biscuit diet plus bi-

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[1] Dam, H., and Granados, H., *Science*, 1945, **102**, 327.

[2] Davis, A. W., and Moore, T., *Nature*, 1941, **147**, 794.

[3] Granados, H., and Dam, H., *Science*, 1945, **101**, 250.

[4] Granados, H., and Dam, H., PROC. SOC. EXP. BIOL. AND MED., 1945, **59**, 295.

[5] Moore, T., *Biochem. J.*, 1943, **37**, 112.

† Diet No. 5:

Casein (commercial)	24.0
Cornstarch (uncooked)	35.0
Salts No. 2	5.0
Lard	20.0
Cod Liver Oil	2.0
Brewers' Yeast	10.0
Cellulose Flour	4.0

All diet ingredients, except the cod liver oil, were mixed and then allowed to stand at room temperature for 2 weeks. The cod liver oil was added just before feeding.

TABLE I.

Maxillary Incisor Pigment Observations in Vitamin E-Deficient Rats.

(All rats were placed on the experimental diets at 21 days of age. The figures in the parenthesis represented the range of pigment values within each group. The unit 10 represented the maximum amount of normal pigmentation. Variations of the groups considered significant if P = 0.05 or less.)

Group	Diet	Days on diet	No. in group	Mean degree of pigmentation	S.E.	P.
		Females.				
1	E-Deficient No. 69	130	14	6.4 (6-9)	0.26	>0.05
2	Normal	130	12	7.0 (6-8)	0.23	
3	E-Deficient No. 5	310	18	7.3 (6-10)	0.13	*<0.01*
4	Normal	310	12	8.5 (6-10)	0.17	
5	E-Deficient No. 5	460	8	6.8 (6-8)	0.14	*<0.01*
6	Normal	460	7	8.4 (6-10)	0.27	
		Males				
7	E-Deficient No. 69	130	11	7.0 (5-8)	0.27	>0.05
8	Normal	130	13	7.6 (5-8)	0.21	
9	E-Deficient No. 5	310	15	7.5 (6-9)	0.11	>0.1
10	Normal	310	10	7.8 (5-10)	0.19	
11	E-Deficient No. 5	460	4	7.2 (5-9)	0.27	>0.1
12	Normal	460	5	7.6 (6-8)	0.14	

weekly supplements of fresh lettuce. During the last 60 days of the experiment the amount of pigmentation was carefully estimated with the use of a color chart with values 1 to 10. The value 10 representing an incisor tooth with a dark brown pigmentation, and the value 1 representing a chalky white incisor devoid of any pigment.

Results. The male rats in all groups at the termination of the experiment had almost identically the same degree of pigmentation in the maxillary incisors regardless of the diet used. The female rats, however, did show some slight variation. In the deficient animals the upper incisors were slightly less pigmented than in the control group. The largest variation was found in Groups 3 and 5 (Table I). The maxillary incisors of the control group had an average reading of 8.5, 8.4 as compared to 7.3, 6.8 of the deficient animals respectively. This is a significant difference but does not remotely approach the almost complete depigmentation in E deficient albino rats held on the vitamin E free diet for only 45 days as reported by Granados and Dam.[3]

The mandibular incisors in the deficient animals of both sexes uniformly had slightly higher pigmentation readings than the control rats. The greatest difference was in the 310-day-old male animals (Groups 9, 10). The deficient male group had a reading of 3.4 while the male control group had only a 2.3 reading. These were not sig-

‡ Mason's Diet No. 69:

Casein (commercial)	20.0
Cornstarch (uncooked)	50.0
Salts (Hubbell's)	2.5
Lard	18.0
Brewers' Yeast	7.5
Cod Liver Oil	2.0

nificant differences.

Discussion. It was obvious that our strain of rats was not showing the striking depigmentation phenomenon reported by other investigators. Several factors were investigated for an explanation for our findings. At the onset of this experiment we felt that the type and amount of iron used in the different diets might account for the discrepancies, since ferric iron is the cause of the pigment color.[1,4,5] Our Diet No. 5 contained 155 mg of ferric alum citrate per 100 g of diet. Mason's Diet No. 69 contained 50 mg of ferric phosphate per 100 g of diet. Since the rats on the latter diet (Groups 1, 7) failed to develop any significant depigmentation, we concluded that neither the differences in the iron compounds nor the unequal amounts of iron consumed by the rats were the determining factors in the failure of our rats to manifest any significant depigmentation of their incisors.

The presence of highly unsaturated fatty acids in the diet is a predisposing factor for depigmentation.[1] Both of the deficient diets, however, had equal amounts of cod liver oil and only slight differences in lard content (18-20%). Diet No. 5, however, had a shelf age of 2 weeks and was, therefore, slightly rancid upon feeding. In contrast, Diet No. 69 contained only fresh lard as it was prepared weekly and fed immediately to the animals. The fluctuation in the amounts of unsaturated fatty acids due to the incipient rancidity in Diet No. 5 is probably negligible. We believe, therefore, that the failure of our vitamin E deficient rats to show marked depigmentation of the maxillary incisors is not due to any obvious diet differences. Age and sex differences in our animals and those of other workers were not determining factors since our observations were on both sexes and the duration of our experiment equaled or exceeded that of the previous investigators.

The factor responsible for this disagreement with other workers on this problem is probably due to the difference in strain of our animals. All investigators, except one, reporting this peculiar depigmentation used albino rats. (Moore[5] used both albino and piebald rats). Our rats were inbred hybrids. The strain was started by Dr. Long in 1908 from a wild male grey rat and 2 tame albino rats.[6] The inbreeding of this hybrid strain may be the determining factor as it seems to be the only important variable not already accounted for. While no data are available on the effect of vitamin E deficiency on the wild grey rat, a recent note from Granados, *et al.*,[7] claimed that Syrian hamsters showed a greatly delayed depigmentation pattern as compared to the albino rat. They also reported that the Florida cotton rat showed no loss of incisor pigment after 150-180 days on an E-deficient diet. Thus this latter "wild" rat reacted to the deficiency in much the same manner as did our hybrid "wild" strain rats. It might be possible that these "wild" strains of rodents have the capacity to either manufacture or retain greater amounts of this tooth pigment than do the long established domesticated strains. Furthermore, Irving[8] also reported no depigmentation in the incisors of 3 female rats after 167 days on an E-deficient diet. Unfortunately, however, he did not indicate the strain of rat used.

Conclusions. 1. Vitamin E deficient rats of the Long-Evans strain did not show the extensive depigmentation of the maxillary incisors as reported by the other investigators. 2. Female rats maintained on an E-deficient diet for 310 days or longer gradually developed a slight incisor depigmentation that was statistically significant. Male rats on similar diets failed to manifest a significant change. 3. A comparison of diet differences, sex and age variations in our animals and those of other workers failed to provide an adequate explanation for our contradictory results. 4. It is suggested that a genetic difference between the Long-Evans and albino strains of rats may be the explanation for the failure of the former strain to show the maxillary incisor depigmentation earlier reported in vitamin E deficient albino rats.

6 Long, J. A., personal communication.

7 Granados, H., Mason, K. E., and Dam, H. (Proc. 1946 Meeting International Association for Dental Research), *J. Dental Research*, in press.

8 Irving, J. T., *Nature*, 1942, **150**, 122.