SPECIAL ARTICLES

ROLE OF UNSATURATED FATTY ACIDS IN CHANGES OF ADIPOSE AND DENTAL TISSUES IN VITAMIN E DEFICIENCY[1,2]

IN vitamin E-deficient rats the yellow-brown discoloration of adipose tissue depends upon the presence of dietary cod liver oil,[3,4] and the depigmentation of incisor enamel, upon dietary fats.[4,5] Peroxidation of the body fat precedes and parallels discoloration of the adipose tissue, the pigment of which shows chemical properties similar to those exhibited by pigment formed in experimental liver cirrhosis.[6] Histologically this adipose tissue reveals all globules which appear similar to, if not identical with, the pigment in other tissues of the same animals.[7] The possibility that highly unsaturated fatty acids might be responsible for these abnormal changes was tested in the following experiments.

First, the effects of fatty acids and the non-saponifiable fraction of cod liver oil were compared.[8] Two female albino rats were mated with normal males and maintained on a stock diet until delivery. Then, one female with her young (Group 1) was maintained for a 41-day period on a vitamin E-deficient diet in which the fatty acids obtained from an equivalent of 20 per cent. of cod liver oil were incorporated.[9] The other female with her young (Group 2) was reared on the vitamin E-deficient diet in which was incorporated the non-saponifiable fraction obtained from the same amount of oil.[10] Since changes in the adipose and dental tissues appeared in animals receiving the fatty acids (Group 1), the effects of three different fatty acid fractions were compared. Low, middle and highly unsaturated fractions were obtained by the procedure of Brown and Stoner[11,12] and given in vitamin E-deficient diets in amounts corresponding to that contained in 20 per cent. cod liver oil. Seven normal pregnant females were divided into 3 groups and from the time of delivery reared on experimental diets as follows: Two females with their young (Group 3) were given the low unsaturated fraction; one female with her young (Group 4) was fed the middle unsaturated fraction, and 4 females with their young (Group 5) the highly unsaturated fraction. Peroxide values were determined, and the intensity of the incisor pigment was recorded. Individual experimental results from the offspring of each group are presented in Table 1.

TABLE 1

Group no.	Days on experiment from birth	Peroxide value milliequivalents per 1000 gm*	Color of adipose tissue	Dental pigment†	Fraction of cod liver oil in per cent. of diet, equivalent to 20 per cent. cod liver oil
1	8		0		Fatty acids, total, 19.0
"	16		0	0/0	" " " "
"	22		0	0/0	" " " "
"	28	0	0	0/0	" " " "
"	38	234	+++	0/0	" " " "
"	41	184	+++	0/0	" " " "
"	41	26	++	0/0	" " " "
2	8		0		Non-saponifiable 0.28
"	16		0		" " "
"	22		0		" " "
"	28		0	7/4	" " "
"	38	0	0	8/5	" " "
"	41	0	0	9/4	" " "
3	10				Fatty acid fract. 1 2.9
"	21		0		" " " " "
"	27	0	0	7/3	" " " " "
"	38	0	0	8/6	" " " " "
"	48	0	0	10/7	" " " " "
"	58	0	0	10/6	" " " " "
4	10		0		Fatty acid fract. 2 10.1
"	21		0		" " " " "
"	27	0	0	1/0	" " " " "
"	38	9.9	0	5/3	" " " " "
"	48	0	0	7/4	" " " " "
"	59	0	0	6/4	" " " " "
"	75	0	0	7/4	" " " " "
5	10		0		Fatty acid fract. 3 6.0
"	21		0		" " " " "
"	27		0	0/0	" " " " "
"	33	100	+	2/2	" " " " "
"	42	94	++	3/2	" " " " "
"	44	152	+++	0/2	" " " " "
"	46	281	+++	0/3	" " " " "

* Because of the scarcity of adipose tissue during the first 3 or 4 weeks of life, peroxide values were not determined during these periods.

† First figures indicate intensity of pigment in maxillary incisors; second figures refer to pigment of mandibular incisors.

[1] From the Departments of Biochemistry and Anatomy and Division of Dental Research.

[2] Aided by grants from Wyeth Incorporated of Philadelphia and the Eastman Dental Dispensary of Rochester.

[3] H. Dam, *Jour. Nutrition*, 27: 193, 1944.

[4] H. Granados and H. Dam, SCIENCE, 101: 250, 1945.

[5] *Idem, Proc. Soc. Exp. Biol. and Med.*, 59: 295, 1945.

[6] *Idem, Acta Physiologica Scandinavica.* In press.

[7] H. Dam and K. E. Mason, *Federation Proc.*, 4: 153, 1945.

[8] The cod liver oil was saponified at room temperature by the usual procedure, using ether, KOH and methanol, dilution and removal of the non-saponifiable by shaking with ether many times. The fatty acids were obtained by further dilution of the soap solution, acidification with HCl and shaking with ether. Both fractions were rinsed with water, dried with Na_2SO_4, evaporated and stored *in vacuo* at low temperature.

[9] Sucrose, 45 gm; casein, alcohol-extracted, 20 gm; dried yeast, ether-extracted, 10 gm; salt mixture no. 2, U.S.P., 5 gm; tetrasodium salt of 2-methyl-1-4-naphthohydroquinone diphosphoric acid, 1 mg; vitamins A and D in oleic acid, 2 drops weekly per animal; fatty acids from cod liver oil, 23.6 gm, added to 100 gm of mixture of the preceding substances.

[10] The same basic diet as above with 65 gm of sucrose instead of 45 gm. To 100 gm of this diet was added 0.28 gm of the non-saponifiable fraction of cod liver oil.

[11] J. B. Brown and G. Stoner, *Jour. Am. Chem. Soc.*, 59: 3, 1937.

[12] The fractions were characterized as follows: Fraction 1 crystallized from a 10 per cent. acetone solution at minus 20° C, solid at room temperature, iodine value 13.4, amount 15 per cent. of total fatty acids. Fraction 2 crystalized from the filtrate of fraction 1 at minus 75° C, light yellow oil at room temperature, i.v. 99.4, amount 53 per cent. of total fatty acids. Fraction 3 remained in solution at minus 75° C, light yellow oil at room temperature, i.v. 283, amount 32 per cent. of total fatty acids.

Group 1 exhibited considerable peroxidation and marked brown discoloration of the adipose tissue, changes which were accompanied by inhibition of enamel pigment deposition. The non-saponifiable fraction (Group 2) totally failed to produce such changes. There was no essential difference in the growth curves of the two groups, although both were somewhat subnormal.

Growth in Groups 3 and 4 was essentially normal, in contrast to the marked retardation of growth in Group 5. All offspring in this group died between 10 and 46 days of age following the development of anemia and diarrhea. This is interpreted as a toxic manifestation of the highly unsaturated fraction when given separately. The fact that animals receiving the highly unsaturated fraction (Group 5) developed marked fat peroxidation and discoloration about the same time as those receiving the total fatty acid fraction (Group 1) leaves no doubt that the highly unsaturated fatty acids are responsible for these changes. In Group 4 (middle unsaturated fraction) only one animal showed low fat peroxidation without discoloration at the time marked peroxidation and discoloration appeared in Groups 1 and 5. These changes appeared simultaneously with the rapid increase in fat deposition which was apparent at the beginning of the second month of life.

The deposition of the dental pigment took place at a normal rate in Group 3, whereas in Group 4 the deposition of the pigment was slower and did not reach the same intensity as compared with that of Group 3. This could be interpreted as a slight inhibition of the full development of the pigment due to the middle unsaturated acids or more likely to a small amount of highly unsaturated acids in the middle fraction. In Group 5 the inhibition of the pigment deposition was not so complete as that in Group 1, which received the total acids, for there was a slight and retarded deposition of pigment from the 33rd day to the 42nd, at which time the pigment disappeared completely from the upper incisors. The mandibular incisors of the animals which reached the 46th day of age were still slightly pigmented, a fact which confirms previous observations of a prolonged ability of the mandibular enamel organ to deposit the pigment.[4,13] This experiment demonstrates that the highly unsaturated fatty acids are responsible for the enamel depigmentation. Qualitative differences in the fatty acids of different fats, namely cod liver oil and lard, may account for the fact that both fats produce enamel depigmentation but only cod liver oil causes discoloration of the adipose tissue.[5] The question whether the lack of the antioxidant effect of vitamin E is responsible not only for the peroxidation and discoloration of fat but also for the dental depigmentation is still not clear.

Our examination of the chemical nature of the enamel pigment has eliminated the possibility of its being a lipochrome, porphyrin or melanin. It does not fluoresce and contains iron in the ferric form, which dissolves readily in 10 per cent. HCl. When exposed to a weaker acid (2 per cent. HCl) the pigmented layer comes off as a finely granular and fibrous film or layer. Incineration of the tooth in a gas flame separates the enamel from the dentin and leaves the pigmented layer a black color. After burning away the carbon with ammonium nitrate the yellow-brown color of the outer surface of the ashes from the enamel reappears, indicating that inorganic iron alone accounts for the total color of the pigment, which in the intact tooth appears to be embedded in or loosely combined with an organic matrix. Depigmented enamel of vitamin E-deficient rats failed to give reactions for iron with potassium ferro- or ferric-cyanide; when subjected to incineration it developed only a slight gray color, which turned again white after burning away the carbon with ammonium nitrate. Exposure of depigmented teeth to 2 per cent. HCl resulted in the splitting off of a much thinner, less compact and colorless film from the outer surface of the enamel. It is, therefore, also demonstrated that the highly unsaturated fatty acids in absence of vitamin E act on the enamel organ in such a way as to inhibit the deposition of the normal iron-containing layer, resulting in white appearance of the enamel surface.

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[13] H. Granados, K. E. Mason and H. Dam, *Jour. Dental Research* (Proc. 23rd general meeting International Assoc. for Dental Research), 1945. In press.

ARTERIAL ANASTOMOSIS IN DOGS EMPLOYING VEIN GRAFTS FROM CHICKENS AND TURKEYS

As a part of extended studies directed towards devising a ready source of veins for arterial replacement in animals and humans, vein grafts from young healthy chickens and turkeys were transplanted to bridge defects in the arteries of dogs. These experiments were planned on the basis of the findings of Copley that chicken thromboplastin prepared from brain and skin is ineffective or only slightly effective in activating thrombin in recalcified oxalate plasma of mammals.[1] We have since found that chicken thromboplastin as contained in vein juice is effective with whole dog's blood in arterial segments *in vivo* for the production of coagulation thrombosis.[2] Since

[1] A. L. Copley, *Am. Jour. Physiol.*, 137: 178–186, 1942.
[2] A. L. Copley and P. L. Stefko. To be published.