LIPID PEROXIDATION IN TISSUES OF VITAMIN E DEFICIENT RATS

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The polyunsaturated fatty acids (PFA) of lipids tend to peroxidize in tissues of rats on vitamin E deficient diets (Dam and Granados, 1945). However, Bernhard (1958) claimed that vitamin E had no effect on PFA levels in either brain or liver. Lipid peroxides can cause much structural and metabolic damage. Horgan et al (1957) have demonstrated the toxicity of injected lipid peroxides as well as those peroxides induced by radiation (Ottolenghi et al, 1955). The function of rat and rabbit mitochondria are affected by peroxidation (Ottolenghi, 1959; Tappel and Zalkin, 1959) and these aberrations are reducible by vitamin E. Ascorbic acid synthesis in liver homogenates was reduced by the presence of lipid peroxides (Carpenter et al, 1959). Recently Tsen and Collier (1960) in this laboratory have found evidence that lipid peroxides are responsible for the susceptibility of erythrocytes from E deficient rats to hemolysis by dialuric acid and oxygen.

The present investigation has confirmed the presence of lipid peroxides in certain tissues (brain, heart, liver, spleen, lung, kidney, adrenals, intestine) of normal rats (Cole, 1956; Kibrick et al, 1959) and has shown that the tissue peroxide content and susceptibility to peroxidation increased on diets low in vitamin E. The results also indicate that, while the level of PFA is reduced by vitamin E deprivation, the tendency of the remaining PFA to form lipid peroxides is greatly enhanced in most tissues. In brain and heart the most active sub-cellular area for lipid peroxide formation was located in a microsomal supernatant.

Methods Rats were fed a commercial vitamin E deficient diet (Nutritional Biochemicals Corp., Cleveland, Ohio) supplemented with 30 % w/w torula yeast (Rhinelander Paper Co., Rhinelander, Wisconsin). All animals were allowed water ad libitum. Only male rats of the Wistar strain were used. In comparisons

between control and deficient animals littermates were employed.

Tissues were homogenized in ice-cold 0.05 M-Tris buffer, pH 7.4 and the resulting homogenates were adjusted to a concentration of 100 mg per ml with buffer.

Lipid peroxides were determined by a slight modification of the thiobarbituric acid reaction (TBA) (Ottolenghi, 1959; Tappel and Zalkin, 1959;Kenaston et al, 1955). The enzymic procedure of MacGee (1959) for the estimation of PFA was used. Absorbance was measured by a Beckman DK 2 recording spectrophotometer.

Results The PFA levels of heart, liver, adrenals and plasma were reduced by E deficiency (Table I). It has been found that feeding corn oil to rats on E deficient diets partly prevented the fall in PFA content. The accumulation of PFA in tissues of animals fed PFA-diets has been demonstrated (Dam et al, 1958; Horwitt et al, 1959; Klein, 1957) and the present results tend to support these findings.

TABLE I

DECREASE IN POLYUNSATURATED FATTY ACID CONTENT OF TISSUES FROM VITAMIN E-DEFICIENT RATS[1]

Tissue	Percent Decrease
Brain	0
Heart	53
Liver	61
Adrenals	75
Plasma	57

1. Group of 16 animals on deficient diet for 62 days

All the tissues tested possessed some amount of lipid peroxide in vivo (0 time TBA) but, with the exception of adrenals, there were no significant differences between control and deficient animals in the in vivo peroxide content when measured as TBA units per 100 mg tissue. However, on incubation of tissue homogenates in air, all tissues from E deficient rats, except those from brain and intestine, showed much more peroxidation than their controls. The tissue response in order of decreasing activity was adrenal, lung, spleen, heart and liver. Brain tissue had a very high peroxide level in vivo and a great increase in vitro (after incubation) but there was no

difference between the two diets. This agrees with the report of Bieri (1959). Intestine possessed little innate peroxide and showed very slight susceptibility to peroxidation. Boiled homogenates (100° for 10 min.) of heart and brain showed responses similar to unheated tissue.

When the peroxide content was related to the tissue PFA level (Table II) definite differences between control and deficient tissues were noted *in vivo*. Brain was the exception. Heart and adrenal seemed to have a very high susceptibility to peroxide formation. It appears, therefore, that, while most tissues of E deficient rats have a decreased content of PFA, the remaining PFA possess an increased susceptibility to peroxidation. This is probably

TABLE II

LIPID PEROXIDATION IN NORMAL AND VITAMIN E-DEFICIENT RAT TISSUES[1,2]

Tissue	Type	TBA Units Per 100 mg PFA	
		Before Incubation	After 240 min. of incubation
Brain	Control	165 (5)[3]	450 (3)
	Deficient	141 (5)	444 (3)
Heart	Control	77 (6)	382 (7)
	Deficient	230 (6)	1418 (6)
Liver	Control	26 (2)	138 (3)
	Deficient	59 (2)	319 (3)
Adrenals	Control	122 (4)	869 (4)
	Deficient	254 (4)	5805 (4)

1. Rats on diets for 50 to 60 days
2. Tissue homogenized in 0.05 M-Tris buffer, pH 7.4 and incubation was done at 37°
3. Brackets indicate number of animals in group

a result, at least in part, of a decrease in the level of natural tissue antioxidant (vitamin E).

The sub-cellular fraction of brain and heart which possessed the greatest relative amount of peroxides was located in the supernatant fluid (Table III) isolated from homogenates that had been centrifuged at 30,000 x g for 90 minutes. This supernatant contains microsomal material. Carpenter *et al* (1959) have noted a somewhat similar occurrence in liver homogenates. Comparison between

the supernatants of control and deficient hearts showed a much greater difference than comparison between the whole homogenates (Table IV).

TABLE III

PEROXIDE FORMATION IN SUB-CELLULAR FRACTIONS OF NORMAL RAT HEART AND BRAIN HOMOGENATES

Sub-cellular Fraction	Brain		Heart	
	TBA Units Per 100 mg PFA			
	Before Incubation	120 min. Incubation	Before Incubation	120 min. Incubation
Mitochondria	115	254	62	103
Microsomes	174	334	40	79
Supernatant	913	2452	1438	5753

TABLE IV

PEROXIDE FORMATION IN SUPERNATANT FRACTION OF NORMAL AND VITAMIN E-DEFICIENT RAT HEART AND BRAIN HOMOGENATES

Tissue	Percent Increase in TBA Units Per 100 mg tissue	
	Before Incubation	After 180 min. of incubation
Brain	110	140
Heart	480	4500

Summary Since several major tissues have lowered PFA levels in vitamin E deficient rats, it appears that lack of this vitamin causes the loss of PFA as a whole and probably the essential fatty acids in particular. The experimental results tend to indicate that this loss is caused by lipid peroxidation.

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