Effect of Storage on Tissue Tocopherols*

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IN PROCEDURES involving chemical or biologic evaluation of tocopherols in tissues of man or lower animals there often are variable and unavoidable intervals of time between acquisition and analysis of tissue samples. In the case of human tissues there are also variable intervals between death and necropsy. There appear to be no recorded observations on the rate and extent of loss of tissue tocopherols under such conditions. The studies reported here, representing a phase of an extensive exploration of the tocopherol content of human tissues,[1,2] are concerned with the effects of postmortem storage at 4° C and at −20° C upon tocopherols in certain human tissues.

METHODS AND RESULTS

Tocopherols were determined by the macrochemical procedure of Quaife and Harris[4] as modified for fresh animal tissues by Quaife and Dju[5] and calculated as mg per cent (mg total tocopherols mg/ 100 g fresh tissue). Test experiments with pure tocopherols added to fresh liver, muscle and lard gave recovery values ranging from 92 to 101 per cent indicating the degree of accuracy of the procedure. The tissues employed and conditions of storage are given in the outline of individual experiments.

Storage at 4° C for Eight Days

Samples of five tissues of four premature infants were taken at necropsy, and again after storage of the corpse for two, four, or eight days in a refrigerator maintained at about 4° C (Table I). Tocopherol loss was greatest in adipose tissue, amounting to about 30 per cent after only four days as compared to losses of 32 and 22 per cent in liver after eight days. Tocopherols of skeletal muscle and brain decreased somewhat less, whereas those of lung tissue suffered but little change after eight-day storage.

TABLE I

Tocopherol Loss in Tissues of Infants During Storage at 4° C

Tissue	Storage days	Tocopherol, *mg/100 g*		Loss, %
		Fresh	Stored	
Muscle	2	0.74	0.68	8.1
Muscle	4	0.52	0.43	17.3
Adipose tissue	4	2.80	1.95	30.0
Muscle	8	0.45	0.38	15.5
Liver	8	1.38	0.93	32.2
Brain	8	0.15	0.13	13.3
Lung	8	0.72	0.69	4.1
Liver	8	1.51	1.18	21.8
Brain	8	0.36	0.29	19.4
Lower extremity		Skin left on	Skin removed	Difference, %
Muscle	8	0.58	0.52	10.3
Adipose tissue	8	1.71	1.23	28.1

The skin was removed from the lower extremity of a premature infant and the other extremity left intact. Both were stored for eight days under the same conditions. The tocopherol values for muscle and adipose

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A preliminary report of these studies[3] was presented before the American Institute of Nutrition, at Cleveland, Ohio, in 1951.

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tissue from the skinned extremity were, respectively, 10 and 28 per cent less than for the same tissues from the other extremity (Table I), indicating that exposure to air is also a factor causing diminution of tissue tocopherols during storage.

Storage at −20° C for Eight Weeks

Samples of fresh tissues from a 28-year-old female, the victim of a brain tumor at the eighth month of pregnancy, were analyzed before and after eight weeks of deep-freeze storage at about −20° C. The tissues were tied in plastic bags from which excess air had been excluded. Tocopherol loss was again highest in adipose tissue, and appreciably less in liver, skeletal muscle, and kidney (Table II).

TABLE II

Tocopherols of Adult Human Tissues Analyzed at Time of Autopsy and Again after Eight Weeks Storage at −20° C

Tissue	Lipid, %	Total tocopherols, mg/100 g fresh tissue: At autopsy	Lipid, %	After storage for 8 weeks	Loss of tocopherols, %
Para-renal fat	50.46	8.59	50.08	5.19	39.5
Mesenteric fat	63.03	11.89	62.30	7.90	33.5
Liver	3.20	1.81	3.67	1.44	20.4
Muscle	2.96	0.71	2.10	0.58	18.3
Kidney	3.20	0.51	3.43	0.44	13.7

Storage at −20° C for Periods up to 109 Weeks

Large samples of adipose tissue and liver from a 62-year-old male, whose death resulted from coronary occlusion, were frozen in liquid nitrogen and ground in the usual manner. The frozen ground material was divided into 48 aliquots for each tissue, such that four aliquots could be used for the initial analyses and for each of 11 subsequent analyses after storage intervals of 1, 2, 4, 7, 9, 11, 19, 24, 34, 52, and 109 weeks. The aliquots were placed in extraction thimbles enclosed in plastic bags from which excess air was excluded prior to deep-freeze storage. At each interval, the lipid extracts obtained were also characterized as to iodine value and peroxide value both prior to and following concentration of tocopherols by molecular distillation.

The data on tocopherol levels, expressed as mg per cent and as per cent of initial values, are summarized in Figures 1 and 2. The ordinates for the two tissues recorded in Figure 1 serve to indicate that, per unit weight of fresh tissue, tocopherol values for adipose tissue were approximately ten times those for liver; however, when expressed in terms of mg/g of fat, tocopherol values for adipose tissue were only about one-third those of liver. There may also be noted an unexplained increase in tocopherol values for adipose tissue during the first week, and for liver during the first two weeks of storage.*

It is apparent from the graphs that the tocopherol content of adipose tissue was significantly lowered by the 4th week of storage, to about 79 per cent of the original value; subsequently there was a progressive decline to 66, 55, 52, 55 and 50 per cent at 9, 11, 19, 24, and 34 weeks, and to 33 and 24 per cent after 52 and 109 weeks, respectively. The tocopherol of liver was reduced more gradually, especially during the first 24 weeks (Fig. 1 and 2), such that at 4, 9, 11, 24, 34, 52, and 109 weeks, respectively, the values were 93, 78, 74, 60, 54, 37, and 34 per cent of those prior to storage.

Tocopherols expressed as mg/g of fat showed essentially the same relative decline, which may be correlated with the fact that the extractable fat of both tissues was remarkably constant at all phases of storage (mean value of 82.45 ± 1.43 per cent for adipose tissue; 3.76 ± 0.25 per cent for liver) during the 2-year period. The lipid extract of tissue samples analyzed at each interval up to 52 weeks, determined both before and after molecular distillation, gave mean iodine values of 69.1

* Since subsequent analyses of other adipose tissue and of liver treated in like manner failed to show any increase of tocopherol values during the early weeks of storage, the increased levels recorded are of questionable significance; they may be related to differences inherent in the aliquots employed or to limitations of the analytical procedure employed, or to both.

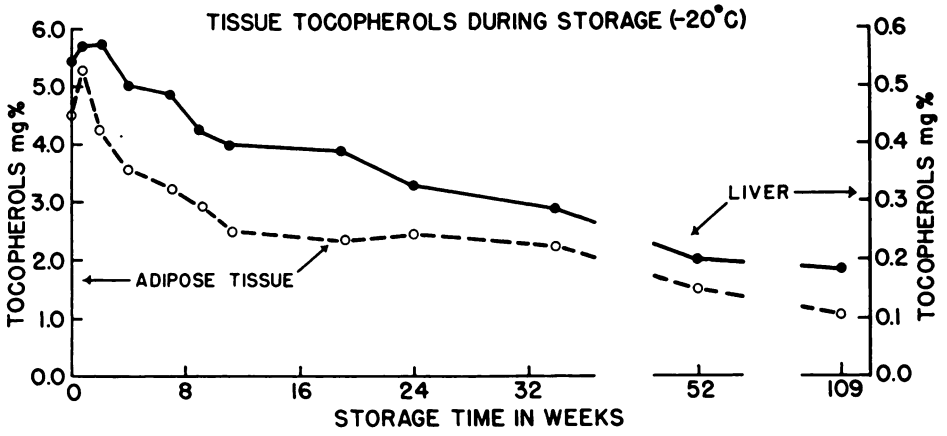

Fig. 1

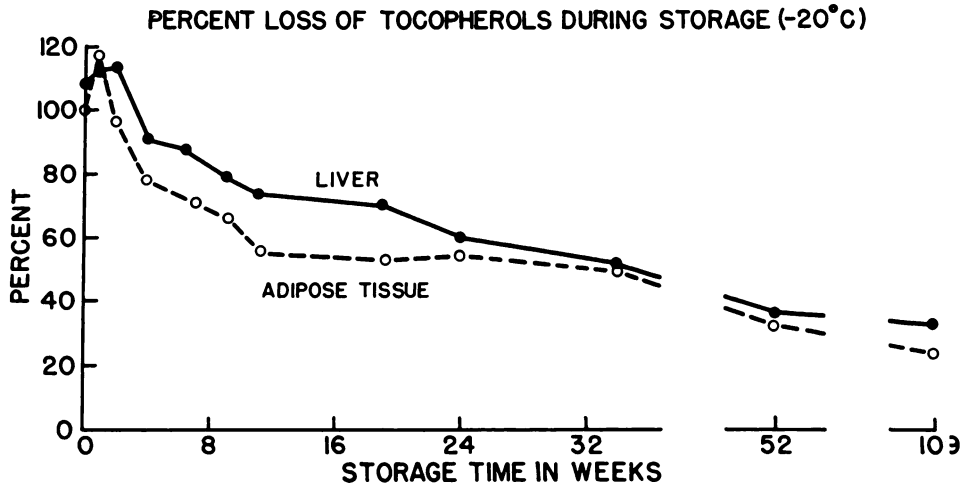

Fig. 2

± 1.3 and 69.7 ± 3.6 for adipose tissue fat, and 94.0 ± 2.0 and 94.7 ± 1.9 for liver fat, respectively; peroxide values of both fats were low throughout this period of storage, usually fluctuating within the range of 0 to 20–30 meq/kg of lipid. Thus neither storage nor molecular distillation appeared to produce any significant change in iodine number or peroxide content of the lipids in adipose tissue or liver.

DISCUSSION

Tocopherols are recognized as important antioxidants in both plant and animal tissues. Although ascorbic acid may have a similar function in animal fat[6] antioxidants other than tocopherols are not deposited to any appreciable extent in animal fat depots under the usual dietary conditions.[7] Even in animal fat, tocopherols are not so abundant but what stabilization can be improved by further addition of tocopherols, or other antioxidants such as ascorbic acid which can act synergistically with tocopherols.[8] In the lipids of human adipose tissue such synergistic antioxidants are probably absent or present in negligible amounts, and tocopherols per unit of fat are at a considerably lower concentration (usually one-half to one-fourth) than in other tissues and organs.[1,2] Such differences, combined with variations in the content of oxidative catalysts and lipoxidative enzymes capable of acting at rather low temperatures and the chain length and unsaturation of the lipids themselves, undoubtedly influence the rate and extent to which tocopherols diminish in various tissues and organs. Whether or not

this progressive loss on storage involves alpha-, gamma-, and delta-tocopherols to the same degree cannot be answered at the moment, since in the studies reported here separate determinations for γ- and δ-tocopherols, combined, were not carried out.

The observations recorded indicate that the loss of tocopherols in adipose tissue and liver during the first month or two of storage at deep-freeze or higher temperatures is of sufficient magnitude to justify some correction of analytical values obtained on tissue samples stored for variable periods of time prior to analyses. The rather limited data obtained for other tissues and organs suggest a lesser loss of total tocopherols on storage than in the case of the tissues mentioned.

SUMMARY

Total tocopherols in human tissues were determined chemically after various intervals of storage. During eight days storage at 4° C, tocopherol loss was greatest in adipose tissue, somewhat less in liver, brain and muscle, and least in lung. Removal of skin from an extremity prior to storage hastened tocopherol deterioration in underlying adipose tissue and muscle. After eight weeks storage at −20° C, tocopherol loss in adipose tissue and liver was somewhat less than after eight days at 4° C.

Prolonged storage at −20° C for 4, 9, 24, 34, 52, and 109 weeks resulted in a decline of tocopherol levels to 79, 66, 55, 50, 33, and 24 per cent of original values in adipose tissue, and to 93, 78, 60, 54, 37, and 34 per cent in liver. In neither tissue was there a significant change in lipid content, or in iodine number or peroxide value of the lipid, during the storage period of more than two years.

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