Effect of Tocopherol Feeding on the Composition of Turkey Tissues.* (18966)

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In vivo studies of the antioxidant value of the tocopherols have been relatively few. Prevention of peroxide accumulation in the fat of rats by feeding tocopherols has been shown(1,2) and the conclusion drawn that the normal source of tissue antioxidants is the tocopherol of the feed. Similar experiments on hog fat have indicated similar relationships between keeping quality of the fat and the presence of tocopherols and unsaturated fatty acid(3). However, an attempt to reduce rate of rancidification of pork fat by feeding or injecting tocopherols before slaughter proved largely unsuccessful(4). Major and Watts(5) obtained protection from rancidity in rabbit meat by feeding or injecting 1 or 11.1 mg per day of mixed tocopherols for an unspecified period. When 400 mg of mixed tocopherols or tocopherol phosphate was injected in 2 doses during the 3 weeks previous to sacrifice into rabbits fed a natural diet no protection against rancidity of the meat resulted. Carpenter and Lundberg(6) in contrast with Watts, Cunha, and Major(4) found the induction period for oxygen absorption much shorter in fat from hogs which had not received supplementary tocopherols than in those which had received a total of 31 g in 12 weeks.

Tocopherol content of tissues following feeding of tocopherols has been determined only in the rat(7) and hog(8). Bioassay used by Mason(7) indicated wide distribution of tocopherols in rat tissues with liver deposits most significantly changed by increases in intake. Only a small fraction of the excessive amounts ingested was found in the tissues, the total amount found after 2 to 4 months feeding being only 3 times the daily dose. This was confirmed by Hines and Mattill(9), who indicated large concentrations of tocopherols in the feces of rats given supplements of the vitamin. The muscle as well as the liver and other viscera of rats given graded doses of tocopherol was found to increase moderately in tocopherol content from 4.8 mg per kg on vit. E deficient diet to 7.5 and 11.9 with increasing levels of intake(7). Rabbits were found to store less tocopherol in the liver in comparison with the muscle than did rats(9).

No studies of tocopherol content and keeping qualities of poultry tissues appear to have been recorded. Since the development of rancidity in turkey fat and meat is usually rapid even under the best feeding and storage conditions, it appeared worth while to measure the effect of graded doses of tocopherols upon the production of peroxides and the development of rancidity in stored turkey carcasses. The tocopherol content of the tissues was determined simultaneously.

Experimental methods. Two lots of turkey hens of the Bronze strain were examined. The method of feeding, slaughtering and storing has been previously described(10). There

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2. Hanson, H. T., Barnes, R. H., Lundberg, W. O., and Burr, G. O., *J. Biol. Chem.*, 1944, v156, 673.

3. Chipault, J. R., Lundberg, W. O., and Burr, G. O., *Arch. Biochem.*, 1945, v8, 321.

4. Watts, B. M., Cunha, T. J., and Major, R., *Oil and Soap*, 1946, v23, 254.

5. Major, R., and Watts, B. M., *J. Nutrition*, 1948, v35, 103.

6. Carpenter, L. E., and Lundberg, W. O., *Ann. New York Acad. Sci.*, 1949, v52, 269.

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8. Bratzler, J. W., Loosli, J. K., Krukovsky, V. N., and Maynard, L. A., *J. Nutrition*, 1950, v42, 59.

9. Hines, L. R., and Mattill, H. A., *J. Biol. Chem.*, 1943, v149, 549.

were 12 birds in the first series, averaging 6 kg in weight when slaughtered at 34 weeks of age, and 16 birds in the second series averaging 6.5 kg when slaughtered at 36 weeks of age.

The mash fed contained alfalfa meal 7.5, wheat 32.5, wheat bran 10.0, corn 7.0, barley 25.0, fish meal 5.0, soybean meal 10.0, limestone and bone meal 2.0, salt 0.5, fish oil 0.5, and manganese sulfate 0.025. This ration was calculated to contain about 1.8 mg % tocopherols of which 0.9 mg % was α-tocopherol on the basis of the tocopherol analyses of Harris *et al.*(11). The maximum intake of α-tocopherol of the birds from the basal ration was not more than 1.5 mg per day and per kg per day not more than 0.25 mg, if this calculation is correct. No signs of vit. E deficiency were seen.

Tocopherols were added to this feed in the form of a concentrate containing per g 50 mg mixed tocopherols,† about one-half α and one-half mixed β and γ, chiefly γ. In the first series 4 groups of birds received for 5 weeks respectively per 100 g ration, 100 mg tocopherols, 10 mg tocopherols, none, and a vit. E-depleted feed. The latter was prepared by treatment with 1% ferric chloride solution. In the second series of 5 groups, 3 received the ration containing 20% of the tocopherol concentrate, or 1 g mixed tocopherols per 100 g ration, for 21, 7, and 2.5 days, respectively. The fourth group received 50% of the ration as the concentrate or 2 g mixed tocopherols per 100 g ration for 2.5 days, and the fifth group received the basal ration only.

At the end of various storage periods the carcasses were cut in half, one-half of each was roasted, judged for palatability and sampled for thiamine, riboflavin and niacin contents. The other half was used uncooked for determination of water, fat, tocopherols, peroxides, thiamine, riboflavin and niacin (10).

The tocopherol method used was a modification of the iron-α, α'-bipyridine color method of Hines and Mattill(9). Carotene was found to remain in the florisil treated extract of the livers but was removed successfully by treatment with concentrated (85%) sulfuric acid, (2 ml per 10 ml petroleum ether extract). The recovery of added tocopherols under these conditions when added cholesterol, carotene and vit. A were present in the tissue extracts, was 94 to 100%.

This method was unsatisfactory for the determination of tocopherols in fat. When only 30% of added tocopherol could be recovered from abdominal fat samples the analysis of fat for tocopherols was abandoned. The apparent loss of tocopherol occurred whether the tocopherol was added before or after extraction of the fat by the method of Chipault *et al.*(3).

The measure of fat rancidity was peroxide concentration measured by the method of French, Olcott, and Mattill(12). The Kreis test was also employed at first but since it was found that small amounts of tocopherol, 30 mg per g fat, reacted positively with the phloroglucinol reagent this test was eliminated.

The livers were analyzed for vit. A and carotene by the usual antimony trichloride and colorimetric procedures.

Bioassay of the liver and leg muscle extracts was carried out in order to estimate the character of the tocopherols measured since no such comparison had been previously made. Both depleted virgin and resorption female rats used as suggested by Mason(13) were found to respond similarly to tocopherol dosing. Only rats under 6½ months of age were used. The international standard for vit. E, racemic α-tocopherol acetate, was used at 3 levels, 1.2, 1.0 and 0.8 mg, to establish the mean fertility dose. Pooled liver and leg muscle samples were fed in 5 days after the mating in amounts calculated to provide 1.0 mg tocopherol as determined by the chemical method. The animals were allowed to deliver the litters instead of being sacrificed on the

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† This concentrate was supplied by Distillation Products, Inc., Rochester, N. Y.

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TABLE I. Tocopherol and Peroxide Content of Turkey Tissues As Affected by Tocopherol Feeding and Storage.

			Tocopherol feeding				Acceptability	Rancidity		Peroxides in mEq./kg		Tocopherols in mg per kg					
Series	Group	Storage, mo	Time, days	Total amt, g	Level in feed, %	Bird No.	Rating	Thigh	Fat	Skin fat	Abdominal fat	Liver	Heart	Gizzard	Leg	Breast	Liver vitamin A, μg/g
1	1	3	35	4.2	.1	1	Excel†	none	none	.2	.5	159					
	2		35	.4	.01	4	”	”	”	.1	.1	155					
	3		35	0	0	7	Fair	ran‡	ran‡	1.5	4.5	135					
	4		35*	0	0	10	Good	none	none	2.1	7.2	23					
	1	6	35	4.2	.1	2	Excel	”	”	.7	2.1	101			35	14	
	2		35	.4	.01	5	Good	ran	ran	1.5	5.4	67			22	9	
	3		35	0	0	8	Fair	none	none	4.3	13.4	42			12	9	
	4		35*	0	0	11	Poor	ran	ran	3.9	7.4	49			13	9	
	1	9	35	4.2	.1	3	Excel	none	none	1	4.8	80	107	45	24	11	4
	2		35	.4	.01	6	Poor	ran	ran	1.8	5.9	59	60	26	15	11	7
	3		35	0	0	9	”	none	”	2	6.7	52	52	19	13	10	4
	4		35*	0	0	12	”	ran	”	7.3	19.4	39	68	21	12	9	0
2	1	0	21	32.9	1	13	Excel	none	none	0	.7	218			50	25	
	2		7	11.3	1	17	”	”	”	0	1.2	119			43	10	
	4		2.5	8.7	2	25	Good	”	”	.3	1.9	153			13	9	
	3		2.5	3.4	1	21	”	ran	”	.4	.5	94			12	9	
	5		0	0	0	27	”	”	ran	.6	2	50			12	8	
	1	3	21	32.9	1	14	Excel	none	none	.9	2.2	210	80	26	27	11	50
	2		7	11.3	1	18	Good	”	”	.9	3.1	89	58	16	13	10	101
	4		2.5	8.7	2	26	”	”	”	2.7	5.4	87	28	16	11	9	37
	3		2.5	3.4	1	22	”	”	”	1.6	3.4	98	57	15	12	7	56
	5		0	0	0	28	Poor	ran	ran	1.1	4.6	40	63	18	11	8	72
	1	5	21	32.9	1	15	Excel	none	none	.7	5.5	275	133	28	22	10	44
	2		7	11.3	1	19	Good	”	”	1	1.6	108	74	18	10	9	21
	3		2.5	3.4	1	23	Poor	ran	ran	1	5.1	125	80	21	12	10	90
	1	5	21	32.9	1	16	Good	none	none	.6	1.5	227	145	33	17	9	4
	2		7	11.3	1	20	Poor	ran	ran	1	2.9	86	84	31	15	11	39
	3		2.5	3.4	1	24	”	”	”	1	7.4	85	86	21	10	9	29

* Fed a vit. E depleted diet for 35 days before slaughter.
† Excel = excellent; ‡ ran = rancid.

16th day, as recommended by Mason. Wide mesh screens were used in the delivery cages so that the young were dropped out of reach of the mother. Total implantation sites were counted and the number and weight of live and dead fetuses recorded. Fertility was judged by the per cent of litters containing live young and of implantations yielding live young(14). Occurrence of pseudopregnancies and first litter fertility was negligible.

Results. Tocopherol content of tissues. The tocopherol content of liver, heart, breast, gizzard and leg muscle as determined chemically is shown in Table I along with the peroxide numbers of skin and abdominal fat and the acceptability to a panel of judges of the meat and fat. The thigh muscle was also analyzed for tocopherols in a few birds, but since these values were found to be nearly the same as those for leg muscles they are not included in the table.

As shown by Mason(7) for the rat, the liver store was the best index of the tocopherol status of these birds since with increased intakes the liver tocopherol stores were in all cases increased. The maximum liver storage appeared to be limited, however, to about 225 mg per kg fresh tissue, no matter how great the intake. The minimum level was 23 to 52 as found in the livers of birds which received either no added tocopherols or a vit. E-depleted diet for 35 days before slaughter.

The heart tocopherol content, determined in only 15 birds, was usually somewhat smaller than that of the liver, but was less affected by the intake. The gizzards of these same 15 birds were found to contain about the same amount of tocopherols as the leg muscle with only small variations attributable to intake. Leg muscles showed definitely increased tocopherol content in the birds which had received the largest intakes but breast muscles had a fairly constant and minimum content of 8 to 11 mg per kg.

The thiamine, riboflavin and niacin values of the tissues of these birds as previously reported(10) show certain interesting correlations with the tocopherol concentrations. In all cases the tocopherol figures parallel roughly those of riboflavin and generally also of thiamine but not of niacin. Thus the heart had high concentrations of thiamine, riboflavin and tocopherol as compared with other tissues, but not of niacin. The liver was richest of all in riboflavin and tocopherol, the gizzard and leg muscles well endowed with riboflavin and tocopherol but poor in niacin, the breast muscle rich in niacin, poor in thiamine, riboflavin and tocopherol. Some parallelism in metabolic function of riboflavin and possibly also of thiamine with that of the tocopherols may be cautiously suggested.

Effect of storage on tocopherol of tissues. Except in the group in series 2 fed the largest amount of tocopherols the tocopherol content of the liver decreased with storage. This tendency is not evident in the limited number of cases in which the hearts and gizzards were examined, nor did leg and breast muscles show much change.

Rancidity judged organoleptically. A panel of 6 judged the cooked meat of breast, thigh and leg and skin and fat from the back of each of the birds. In no case was the breast meat found rancid. The thigh meat and fat were judged rancid in only 1 of 10 birds, which received any added tocopherols when examined at zero or 3 months storage, but 3 of 3 samples were judged rancid in those which had received no added tocopherols. After 5 or 6 months storage 3 of 6 birds which had received tocopherols and 1 of 2 which had not, exhibited some rancidity. After 9 months, only 1 of 4 birds, that which received the most tocopherol, had no rancidity. The general acceptability of the meat paralleled the amount of rancidity detected.

The peroxide number. The peroxide content of the skin and abdominal fat was generally greatest in the birds which received the least tocopherol. An exception was seen in the 2 birds of group 4, series 2, which received a total of 8.7 mg tocopherol in 2.5 days before slaughter. The peroxide numbers of the tissues were similar to those of the 2 birds in the same series which had no supplement and greater than those which had received half as much (group 3) tocopherol for 2.5

14. Kaunitz, H., and Beaver, J. J., *J. Biol. Chem.*, 1946, v166, 205.

TABLE II. Bioassay of Turkey Liver and Leg Muscle for Tocopherol Content.

Tocopherol fed		No. of rats	No. of litters containing live young	No. of implantation sites	Total No. of young	Live litter efficiency, %	Live implantation site efficiency, %
Form	Amt, mg						
α-tocopherol acetate	1.2	11	9	92	79	82	67
"	1	8	6	66	49	75	54
"	.8	7	3	80	51	43	39
Liver	1 *	15	12	100	79	80	63
Leg muscle	1 *	7	4	60	26	57	28
None		20	0		0	0	

* As determined chemically.

days. Perhaps the higher concentration of tocopherols in the feed promoted increased waste in absorption.

The peroxide content of fat under the skin was inversely related to the tocopherol content of the liver in both series. The same relationship held for the abdominal fat with minor differences.

Vitamin A of the liver as affected by tocopherol intake. As shown in Table I, there was no relationship between the amounts of tocopherol and of vit. A in the livers or between the intake of tocopherols and the liver vit. A. Since the tocopherol feeding periods were short compared with the age of the birds the liver storage prior to the period of tocopherol supplementation may have varied widely. Some loss in liver vit. A may have occurred in storage as indicated by the low levels found in the birds of series 1 examined after 9 months frozen storage. No data were collected, however, on birds of this series stored for shorter periods. The carotene or "yellow values" for the livers were fairly constant and bore no obvious relationship to the vit. A values or to the levels of tocopherol fed. These results differ from those of the better controlled experiments of Harris *et al.*(15) with rats.

Character of the tissue tocopherol. The mean fertility dose of the rats used was close to 0.9 mg and the turkey liver and leg muscle samples fed at the calculated 1.0 mg level produced litters to correspond with this expectation (Table II). Apparently the tocopherols measured in these 2 tissues were chiefly if not wholly α-tocopherol.

15. Harris, P. L., Kaley, M. W., and Hickman, K. C. D., *J. Biol. Chem.*, 1944, v152, 313.

Discussion. Total retention of the added tocopherols in all cases represented a very small fraction of the intake. In a representative of the group fed the most vitamin, the total retention in the organs analyzed and in the remainder of the edible carcass, assuming an average of leg and breast muscle tocopherol values for the whole carcass was only 76 mg out of the total intake of 32.9 g, of which approximately 16.5 g was α-tocopherol. This excludes any excess concentration of tocopherol in the fat and is therefore probably lower than the true value.

Importance of the time factor in securing tocopherol retention is seen in the superior content of the tissues of the birds fed relatively low levels over 35 days in series 1 as compared with the much larger amount for 7 days in series 2. Some increase in tocopherol of the tissues was secured nevertheless even when the feeding period was restricted to 2.5 days.

Relative increases in tocopherol content obtained by excess intake were 6 times for liver, 2-3 for heart, 2-4 for leg, 1-2 for breast and gizzard. This relatively large ability of the turkey to store vit. E in the liver as compared with the muscles resembles the behavior of the rat rather than the rabbit(9).

The response of vit. E-depleted female rats to administration of turkey liver or leg muscle indicated that the liver tocopherol was probably entirely α-tocopherol, since the calculated mean fertility dose corresponded with that found, but the turkey leg muscle had the value of 0.8 to 0.9 mg instead of the 1 mg obtained by chemical analysis. If the mean fertility dose of these rats was taken as 0.9 mg, the liver contained about 10% more and

the muscle about 10% less than the amount measured by the chemical method, which is probably within the limits of error of the methods.

There was little doubt that the rancidity as detected by the judges and indicated by the peroxide numbers was slowed by addition of tocopherols to the diet just previous to slaughter. The shortest period of supplementation, 2.5 days on feed containing either 1 or 2% tocopherols, appeared to afford little protection against rancidity although some increase in tissue tocopherol occurred. The longest period, 35 days at 0.1% addition was effective up to 9 months storage, but the same period of supplementation at the 0.01% level was ineffective. The 21-day feeding at 1% level was most effective of all and the same level maintained for only 7 days nearly as good. The lowest effective level used therefore was 0.1% of the feed for 35 days but at 1% nearly as effective protection was obtained by 7 days feeding.

Major and Watts(5) noted no protection of rabbit meat when 400 mg mixed tocopherols was fed with a natural feed 3 weeks before sacrifice of the animals. The amount and continuity of the dosage may have been insufficient. Our observations confirm the conclusions of Chipault *et al.*(3) and Carpenter and Lundberg(6) as to relationship of tocopherol content and keeping quality of hog fats.

Summary. Mixed tocopherols, about one-half α and the rest chiefly γ, fed to 28 female turkeys for 2.5 to 35 days before slaughter at levels 0.01, 0.1, 1 or 2% of the feed produced measurable increases in the tocopherol content of liver, heart, gizzard and muscle. The 0.1% level fed for 35 days produced the most efficient transfer of tocopherol, but the total amount of the supplement retained in this and the other groups was extremely small. The liver usually contained more tocopherol than any other tissue, 23 to 225 mg per kg fresh tissue, the heart contained 28 to 145, the gizzard 15 to 45, the leg muscle 11 to 50, and the breast muscle 8 to 25. Bioassay of liver and leg muscle indicated the presence of α-tocopherol in amounts within 10% of those obtained by the chemical procedure. No relationship was found between the tocopherol and the vit. A and carotene contents of the 15 livers examined. The peroxide numbers of the fat under the skin and the abdominal fat before and after frozen storage for 3, 5, 6, or 9 months were progressively greater with storage and with decreasing amounts of tissue tocopherol. The acceptability of meat and fat and the detection of rancidity to taste by a panel of judges fell in the same order as the peroxide numbers.

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A Non Adrenal-Mediated Action of ACTH on the Preputial Glands. (18967)

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Various steroids, particularly testoids, stimulate the growth of the preputial glands, both in male and in female rats(1). It has been shown, furthermore, that certain pituitary extracts, which in themselves are almost inactive, greatly augment the preputial gland stimulating effect of certain steroids(2,3). Although these pituitary extracts were rather rich in corticotrophic potency, some of the earlier investigators felt their effect upon the

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2. Noble, R. L., and Collip, J. B., *Endocrinology*, 1941, v29, 943.

3. Selye, H., and Clarke, E., *Rev. Canad. de Biol.*, 1943, v2, 319.