eration in renal tubules. Such pathologic changes were not observed in infected animals given this antibiotic orally in effective doses. The single LD_{50} on intramuscular administration was 600 mg per kilo in mice; on oral application, 1,500 mg per kilo.

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Tocopherol vs. Tocopherol Acetate as a "Sparer" of Vitamin A.

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The early observation[1] that materials containing vitamin E delayed the auto-oxidative destruction of carotene and vitamin A in fatty food mixtures was explained when the tocopherols were shown to be antioxidants.[2] That such protection may augment and conserve the vitamin A stores of an animal was first demonstrated by Moore[3] and has been confirmed repeatedly, as detailed in several reviews and some recent reports.[4]

The evidence suggests that inhibited oxidation in or near the alimentary tract[5] accounts for the survival of carotene and vitamin A. Recently,[6] this protective action appeared to be confined to preserving the store of vitamin A already deposited in the liver. The various forms of vitamin A behave differently. The results are modified by the form and method of administering the preparations of vitamin A[7] and vitamin E[8]. Other compounds having possible stabilizing effects[9] may be present and effective.

Several reports have stated or implied that the stabilizing action of the tocopherols is not limited to the alimentary tract but extends to the tissues of rats.[8,10] This was not demonstrable in rabbits.[11] Some information on this question might emerge from experiments with the esters or α-tocopherol, among them the acetate, which is also an oil, and is a dependable source of vitamin E, perhaps because it is not auto-oxidizable and has no antioxygenic action. Presumably, α-tocopherol acetate is hydrolyzed in the intestinal tract, and the free alcohol should therefore be available in the tissues in quantities undiminished by having provided prior stabilization in the food.

The only pertinent observation on the acetate appears to be that of Bacharach,[12] to the effect that only at high levels of feeding and for an extended period (60 days) was any conservation of vitamin A demonstrable. A comparison of α-tocopherol and its acetate was therefore undertaken, to assess the relative protective value of each by simple experiments patterned after the U.S.P. bio-

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assay for vitamin A.

Experimental. d,l,α-tocopherol acetate. Rats of the Sprague-Dawley strain were given a stock diet until they weighed 40-50 g. Half of them were then transferred to a diet deficient in both vitamins A and E and having the following percentual composition: Vitamin-free casein, 20%; glucose, 67.5%; salt mixture,[13] 2.5%; ground cellophane, 3%; lard, 2%; yeast, 5%; viosterol (in peanut oil), 40 drops per kg. The other half were given the same diet except that hydrogenated vegetable fat (Spry) replaced the lard and 12.5 μg of *d,l,α*-tocopherol acetate* were added per kg of diet. Based on food consumption each animal received 70-80 γ of α-tocopherol acetate and 4-5 γ of tocopherols daily. In a second series the amount of α-tocopherol acetate added to the diet was 100 mg per kg, giving a daily intake of about 600 γ.

At the end of the deprivation period of 3-4 weeks when xerophthalmia or decline in weight or both became evident, the animals were properly grouped and vitamin A containing oils were administered daily, orally, by syringe with bent blunt needle. The Standard U.S.P. Reference Oil No. 2 and the several shark liver oils† to be tested were diluted with cottonseed oil‡ to contain 2 I.U. of vitamin A per 0.1 cc (Reference Oil) or per 0.05 cc (liver oils). The oils were kept in the cold and new dilutions were made up fresh each week.

In Table I are the average figures obtained from these two series of animals. They indicate surprising and certainly acceptable uniformity in the times required for the development and cure of the symptoms of deficiency, whether α-tocopherol acetate was present in the diet or not. The weight gains on the reference oil were only half as great as those on the test oils, and the cure of xerophthalmia was also slower. This was doubtless due to the fact that this oil, as was reported later,[14] contained 30% less vitamin A than indicated. This figure was subsequently verified by the Carr-Price method against a standard vitamin A capsule kindly supplied by Distillation Products, Inc.

The vitamin A potency of the fish oils as indicated by the anti-xerophthalmic data agreed well with that of the chemical assays provided (Carr-Price, Rosenthal, Beckman). With the figures for gain in weight, the agreement was unsatisfactory, largely because of the unsuspected inferiority of the reference oil, and the consequent lack of a comparable standard dose-level.

d,l,α-tocopherol. The tests with the alcohol, made some time after those just described, differed from the earlier ones in several minor respects. Drisdol was used in place of viosterol as a source of vitamin D. Neither α-tocopherol nor vegetable oil was added to the basal diet of the experimental animals; α-tocopherol§ was given to them by mouth during the assay period only and in the same diluting olive oil which also contained the 2 I.U. of the vitamin A. The control animals received their vitamin A in olive oil. The sources of vitamin A were limited to the two reference oils U.S.P. Nos. 2 and 3 (the former after several years' storage in a dark ice-box). Finally, in lieu of a chemical determination of the liver stores of vitamin A, which at the moment could not be made on the necessary micro-scale, the survival time was determined after the close of the assay

[13] Hubbell, R. B., Mendel, L. B., and Wakeman, A. J., *J. Nutrition*, 1937, **14**, 273.

*Generously supplied by Hoffman-La Roche, Inc.

† We are indebted to Dr. J. Murray Luck for samples of these oils and the results of chemical assays on them, on the basis of which they were diluted for the bio-assays.

‡ Cottonseed oil was used designedly, if not wisely, to stabilize the vitamin A in the oils before absorption. During the assay period, therefore, the vitamin E deficient animals on the test and reference oils received daily 20 and 40 γ of tocopherols, respectively; those on the low level of dietary α-tocopherol acetate had a 20-50% increase in the potential vitamin E intake, those on the high level, 3-7% increase. The data indicate that cottonseed oil was inconsequential in its effect on the final results and that it may have accomplished its purpose.

[14] Collison, E. C., and Orent-Keiles, E., *Ind. Eng. Chem.*, Anal Ed., 1945, **17**, 378.

§ Kindly supplied by Merck and Company.

TABLE I.
Influence of α-Tocopherol Acetate on Bio-assay of Vit. A.
+E animals had 12.5 mg *d,l*,α-tocopherol acetate/kg of diet.

Source of curative Vit. A		Avg body wts at depletion, g	Avg gain in assay period, g	Xerophthalmia: Appeared in depletion period at avg days	Xerophthalmia: Cured in assay period at avg days
Refs. Oil 2	—E	84 (6)*	28 (1)†	23	22
Refs. Oil 2	+E	79 (6)	27 (1)	22	20
Oil 3	—E	74 (5)	58 (2)	23	21
Oil 3	+E	80 (8)	52	23	16
Oil 4	—E	80 (8)	46	24	18
Oil 4	+E	80 (7)	46	23	16
Oil 5	—E	80 (8)	47	23	19
Oil 5	+E	82 (7)	44 (1)	23	16
	+E animals had 100 mg *d,l*,α-Tocopherol acetate/kg of diet.				
Refs. Oil 2	—E	76 (6)	25 (1)	28	17
Refs. Oil 2	+E	78 (6)	33 (1)	22	18
Oil 7	—E	76 (8)	65	23	11
Oil 7	+E	74 (7)	54	22	15
Oil 11	—E	75 (5)	50 (3)	25	10
Oil 11	+E	76 (7)	54	23	11
Oil 14c	—E	72 (6)	57 (1)	25	10
Oil 14c	+E	72 (7)	53 (1)	22	12

* No. of animals.
† No. of animals which died during the period and not included in the average.

TABLE II.
Influence of α-Tocopherol on Bio-assay of Vitamin A.

Reference oil and amt. of tocopherol, (mg)	Avg body wt at depletion, g	Gain in assay period, g	Xerophthalmia: Appeared in depletion period at avg days	Xerophthalmia: Cured in assay period at avg days	Avg survival in post-assay depletion, days
2 .0	85 (8)*	3	29	13	11
2 .5	91 (6)	17 (2)†	31	12	19
2 1.5	91 (6)	11 (2)	29	13	19
3 .0	90 (5)	4 (4)	30	16	14
3 .5	80 (8)	25	28	15	19
3 1.5	87 (7)	13 (2)	34	10	19
2 .0	80 (7)	11 (1)	27	11	17
2 .3	85 (5)	24 (3)	28	6	24
3 .0	82 (8)	24	27	7	15
3 .7	78 (7)	38 (1)	27	8	28

* No. of animals.
† No. of animals that died during the assay period and not included in the average.

period when the administration of supplements ceased. Two series of observations were made with each of the reference oils; in the first, 0.5 and 1.5 mg of α-tocopherol were administered with each oil, in the second 0.3 mg with oil No. 2, and 0.7 mg with oil No. 3.

Despite the unexplained high mortality in some of the lots, the data in Table II show that (1) survival in the post-assay depletion period was prolonged by α-tocopherol given in the assay period; (2) there was an optimum α-tocopherol dosage, perhaps 0.5-0.7 mg daily, beyond which the effectiveness of the vitamin A dosage decreased, as indicated by gains in weight in the assay period. Both of these findings are confirmatory of earlier reports[5] and point to an antioxygenic rather than a biological function of α-tocopherol. The stabilization of fats by α-tocopherol ***in vitro*** also demonstrates an optimum concen-

tration; in the case of lard, this is between 0.05 and 0.10% of α-tocopherol.[15] Very recently, in similar experiments,[16] there is a suggestion that 0.5 mg α-tocopherol is an optimum dosage for the storage of vitamin A in the liver; the effect on vitamin A storage in the kidney was irregular. The liver stores of vitamin A from administered β-carotene were diminished by the larger doses of α-tocopherol and kidney storage was again irregular.

Unfortunately, no indication was secured as to the vitamin A stores of our animals on α-tocopherol acetate, following the assay period. The larger of the two dosages, 0.6 mg per day, even though it was within the optimum range and extended over both depletion and assay periods, may have been insufficient; it can hardly have been excessive. In any case, there was no response. The stabilization which the acetate can provide for vitamin A in the alimentary tract is limited to the amount of α-tocopherol available from it in the interval between hydrolysis and absorption. Perhaps, as with triglycerides, complete hydrolysis of the ester is not necessary for absorption, in which case any protective action in the tissues would depend upon the amount absorbed and the rate of subsequent hydrolysis. Information is needed on the rate of hydrolysis and absorption of tocopherol esters. The possible antioxygenic role of tocopherols in the tissues remains to be clarified.

Summary. 1. In bio-assays of vitamin A, α-tocopherol, but not α-tocopherol acetate, increased the rate of gain in the assay period. In a post-assay depletion period, animals that received α-tocopherol during the assay period survived 30-80% longer than controls.

2. As judged by gain in weight in the assay period, there is an optimum dosage of α-tocopherol; animals receiving 1.5 mg per day gained less than those receiving 0.5 mg. This conforms to the antioxygenic behavior of α-tocopherol.

3. Some of the possible causes for the failure of α-tocopherol acetate to conserve vitamin A are briefly discussed.

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On the Specificity and Differentiation of Cholinesterases.

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Cholinesterases (ChE) of different sources may be separated into two types, the "s" and the "e."[1-3] The behavior of the two enzymes toward noncholine esters has been supposed to be the major difference between them. It has been assumed that the e-type could catalyze only the hydrolysis of certain choline esters;[4] therefore it has been also called "specific" or "true" cholinesterase.

The venoms of the colubrid snakes can split acetylcholine[5] and some noncholine esters.[6] In a recent investigation, it was shown that

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