TABLE II. Mean TTC Reduction of Uterine Tissue from Female Rats at Various Stages of Estrous Cycle (20 Females with Duplicate Determination for Each of 4 Stages).

Stage of cycle	Estrus	Day 1 post-estrus	Day 2 post-estrus	Pro-estrus
μg formazan per mg uterine tissue				
Wet	.99 ± .021	.83 ± .040	.88 ± .045	1.14 ± .035
Dry	6.00 ± .20	4.83 ± .26	5.29 ± .29	6.96 ± .24

by uterine tissue was linear to the log-dose of estradiol administered. Variance among litter mates was statistically significant.

In normal cycling female rats, the level of TTC reduction by uterine tissue was lowest on day 1 post-estrus; during pro-estrus it was highest (Table II). When day 1 post-estrus level is taken as 100%, day 2 post-estrus averages 6% higher, pro-estrus 37% higher and during estrus about 20% higher. These data indicate that levels of estrogen increase at pro-estrus, decline during estrus, and reach a low level during diestrus. Analysis of these data shows that differences in TTC reduction during pro-estrus and estrus are significantly different from the levels during diestrus in the rat. Also differences in response between litters is significant. Comparing the mean values of TTC reduction obtained during the estrous cycle to the values obtained with spayed females injected with estradiol, it appears that at one-day post-estrus, the uterus is under the influence of an amount of estrogen equivalent to 0.40 μg estradiol administered daily. During pro-estrus the amount is approximately 1.6 μg estradiol daily. It is probable that the rat ovary secretes estradiol(6) and thus comparisons of TTC reduction levels in normal and estradiol treated spayed females may be valid.

Apparently the reduction rate of TTC by the uterine tissue of the rat represents a metabolic status that is primarily associated with degree of estrogenic stimulation. Thus it may serve as an estrogen status index. An estrogen status index may be of value in determining the optimal levels of estrogenic stimulation that are necessary for maximal reproductive performance.

Summary. An *in vitro* use of TTC reduction by uterine tissue in the female rat has been studied. Estrogen administration to spayed females results in levels of TTC reduction proportionate to the log-dose of estradiol. The level of TTC reduction fluctuates during the estrous cycle, being highest at pro-estrus and lowest on day 1 post-estrus.

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Biopotency of *l*-α-Tocopheryl Acetate for the Rat.* (29334)

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The recent availability(1,2) of *l*-α-tocopherol† (2 *l*, 4′ *d*, 8′ *d*-α-tocopherol /Distillation Products Industries/) has made possible the direct determination of the biopotency of this synthetic epimer of vitamin E. A report based on fetal resorption(3) indicated that the *l*

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† Dr. Stanley Ames of Distillation Products Industries.

epimer has only 21% of the activity of the natural *d* epimer while on the basis of erythrocyte hemolysis values of 34% and 41% have been reported(4,5). The accuracy of these reports is beyond question but it is felt that the results are not necessarily definitive. The *in vitro* erythrocyte hemolysis procedure becomes positive slightly below the lower end of the normal plasma tocopherol range(7) while Evans and Emerson(6) have pointed out that about 7 times as much α-tocopherol is needed to prevent fetal resorption in the rat as is needed to prevent nutritional muscular dystrophy. Both conditions depend on an early sign of minimal tocopherol deficiency(6,7), therefore the degree to which *l*-α-tocopherol can protect *d*-α-tocopherol in more severe deficiency states must be evaluated.

The present report considers the biopotency of *l*-α-tocopheryl acetate in terms of growth and time of onset of creatinuria as a sign of nutritional muscular dystrophy and endeavors to answer the question of synergism and sparing action. N,N′-diphenylphenylenediamine (DPPD) was used as a reference antioxidant.

Experimental. Male weanling rats of the Sprague-Dawley strain, generally in groups of 5 were fed a basal ration containing 21.8% of calories as casein and various levels of synthetic, tocopherol-free dietary fats with an iodine number(8) of 82 substituted isocalorically for carbohydrate as previously described(9). The synthetic fats were resynthesized from tocopherol-free fatty acids by transesterification of the methyl esters with glycerol triacetin under vacuum. The selenium content of the diet was varied by addition of sodium selenite and protein quality was improved by addition of 0.4% DL methionine.

Supplements of *d*-α-tocopheryl acetate in tocopherol-free resynthesized coconut oil were administered orally, by dropper, 3 times a week as was *l*-α-tocopheryl acetate or DPPD in tocopherol-free resynthesized triolein. Dosages were expressed as mg/kg rat body weight/week. Purity of the *l*-α-tocopheryl acetate was checked by gas-liquid chromatography and by the Emmerie-Engel reaction and was found to be about 90% with the remaining 10% as either the free alcohol or the quinone. Rats were weighed weekly and 24 hour urine collections were conducted every 2 weeks. Creatine and creatinine were determined by the procedure of Bonsnes and Taussky(10). Criteria of significant (p=.005) creatinuria have been described previously(9).

Results. Rat growth. As noted by Mason (11), rats on a tocopherol-deficient diet grow normally for a while, reach a plateau weight which may be maintained for several months and then lose weight with the appearance of obvious signs of muscle myopathy only after a protracted period. In Fig. 1A the growth curves for rats receiving the basal ration with 12.5% synthetic trienoic fat and various levels of *d*-α-tocopheryl acetate are shown in broken lines. Growth on the same ration supplemented with 0.10 ppm of selenium and 0.4% DL methionine is indicated by solid lines. In Fig. 1B the comparable data are given for groups supplemented with *l*-α-tocopheryl acetate. Fig. 1C and 1D are based on growth observed when the ration contained tocopherol-free resynthesized triolein rearranged with a portion of tocopherol-free fatty acids from cod liver oil. With added selenium and methionine, no growth depression below the control level was seen at any level of *d*-α-tocopheryl acetate used during the experimental period.

Note that in Fig. 1B the addition of 1.6 mg of the *l* epimer was only slightly more effective than the use of 0.2 mg of the *d* epimer in Fig. 1A, solid line; and that in Fig. 1C the use of 0.4 mg of the *d* epimer was only slightly more efficient than 1.6 mg of the *l* epimer in Fig. 1D, broken line.

Fig. 2A illustrates growth response to 2 levels of *d*-α-tocopheryl acetate on the ration supplemented with 0.10 ppm of selenium and 0.4% DL methionine and 12.5% of synthetic dienoic fat and Fig. 2B shows comparable data for 3 levels of *l*-α-tocopheryl acetate. The groups whose growth curves are shown in Fig. 2C received 0.2 mg *d*-α-tocopheryl acetate per kg rat body weight per week in addition to the indicated supplements of *l*-α tocopheryl acetate. Similarly in Fig. 2D the

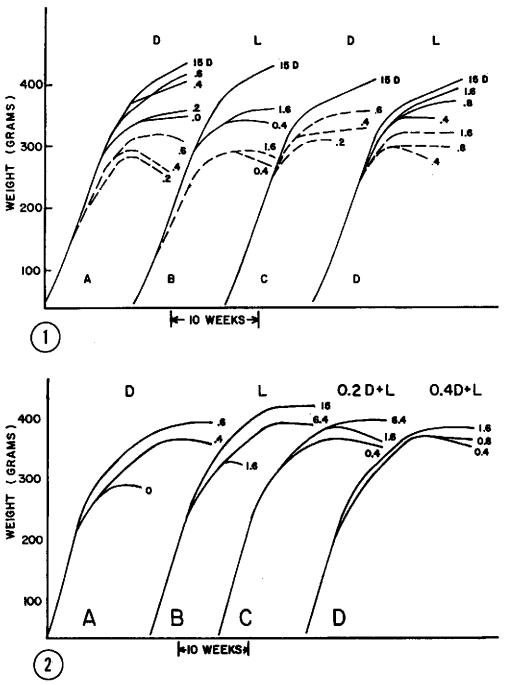

FIG. 1. Rat growth response at various levels of α-tocopherol supplementation. 1A *d* epimer, broken lines basal ration, and 12.5% synthetic trienoic fat; sclid lines basal ration plus 0.1 ppm selenium and 0.4% DL methionine. 1B same as 1A but *l* epimer. 1C and 1D corresponded to 1A and 1B, respectively, but contain 12.5% "mixed fat." Curves labeled 15D represent "optimal" growth on what was presumed to be an adequate level (15 mg/kg rat/week) for each diet used.

FIG. 2. Rat growth at various levels of α-tocopherol supplementation on 12.5% dienoic fat with 0.10 ppm selenium and 0.4% DL methionine added. 2A *d* epimer; 2B *l* epimer; 2C *l* epimer as indicated plus 0.2 mg per kg rat body weight per week *d* epimer; 2D same as 2C but 0.4 mg *d* epimer.

rats received 0.4 mg of *d*-α-tocopheryl acetate in addition to the *l*-α-tocopheryl acetate.

Creatinuria. The time of onset of significant creatinuria (p=.005)(9) in groups of rats receiving various levels of *d* and/or *l*-α-tocopheryl acetate is entered in Table I. On diets not supplemented with selenium and methionine, supplementation with low levels of either epimer had little or no effect in delaying onset of creatinuria.

With added selenium and methionine, however, as previously reported(9), the onset of creatinuria was delayed approximately 17.5 weeks per mg of *d*-α-tocopheryl acetate per kg rat body weight per week when either the dienoic or trienoic fat was fed. The "mixed" fat was specially formulated to be high in both monoenoic and penta and hexaenoic fatty acids. Such a combination generates a lower tocopherol requirement and creatinuria was delayed approximately 25 weeks per mg of *d*-α-tocopheryl acetate.

Low levels of *l*-α-tocopheryl acetate had little or no activity in delaying the onset of creatinuria while higher levels showed up to 20% of the biopotency of *d*-α-tocopheryl acetate. Similarly when fed in combination with low levels of the *d* epimer the *l* epimer

TABLE I. Time of Onset of Creatinuria in Rats Receiving Various Levels of *d*- and/or *l*-α-Tocopheryl Acetate.

Selenium & methionine addition	*d*-α-Tocopheryl acetate (mg/kg rat/wk)	*l*-α-Tocopheryl acetate (mg/kg rat/wk)	Onset of creatinuria (wk) 5 rat avg
	Trienoic fat		
—	.2	—	6
—	.4	—	7
—	.6	—	7
—	—	.4	6
—	—	1.6	6
+	0	—	9
+	.2	—	12
+	.4	—	15
+	.6	—	21
+	—	.4	9
+	—	1.6	10
	Mixed fat		
—	.2	—	7
—	.4	—	7
—	.6	—	7
—	—	.4	7
—	—	.8	7
—	—	1.6	7
+	.0	—	10
+	.4	—	20
+	—	.4	10
+	—	.8	10
+	—	1.6	12
	Dienoic fat		
+	.2	—	13
+	.4	—	17
+	.6	—	21
+	1.7	—	36
+	—	1.6	10
+	—	3.2	19
+	—	6.4	32
+	—	15	>36
+	.2	.4	8
+	.2	1.6	10
+	.2	6.4	36
+	.4	.4	16
+	.4	.8	19
+	.4	1.6	20
+	DPPD (5.0)	—	18
+	DPPD (10.0)	—	32
+	DPPD (30.0)	—	>36

showed little or no effect. Higher levels of the *l* epimer showed an additive response over and above the response to the *d* epimer. If sufficient *l* epimer is fed, complete protection is achieved in the complete absence of the *d* epimer. Synergism and sparing action were not demonstrated in this experiment.

The synthetic antioxidant, DPPD, appeared to have approximately 10% of the bioactivity noted for *d*-α-tocopheryl acetate. The toxicity of DPPD in the pregnant rat has been reported by several groups(12-14).

Discussion. By the criteria of growth and delay in onset of creatinuria *l*-α-tocopheryl acetate appears to have a biopotency for the rat of up to approximately 20% of that of *d*-α-tocopheryl acetate. This is in agreement with the work of Ames *et al*(3) but somewhat lower than the value obtained by Bruggemann *et al*(4) and Weiser *et al*(5). Since neither synergism nor sparing action could be demonstrated in the present study, this would appear to represent a true biological activity for the *l* epimer.

The requirement for both *d* and *l*-α-tocopherol is similarly affected by the level of dietary selenium and methionine, an interaction which has been discussed in detail for the *d* epimer elsewhere(9).‡

Summary. The *l* epimer of α-tocopheryl acetate, appears to have approximately 20% of the biological activity of the *d* epimer in the rat by the criteria of growth and delay in onset of creatinuria as a sign of nutritional muscular dystrophy. The *l* epimer alone can replace *d*-α-tocopherol but only if sufficient is supplied. No evidence of synergism or sparing action was observed.

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A Micro Method for Analysis of Plasma Salicylate. (29335)

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In recent years the use of dextran gel filtration for separation of large and small molecular weight compounds has come into widespread use(1-3). The cross linked dextran gel, (Sephadex) has also been applied to the study of protein-binding of drugs(4-6). The present report describes the application of this method in conjunction with fluorometric analysis of salicylates in plasma. Sephadex filtration eliminates the necessity

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