ovarian cyst induction in rats and is prevented by MER-25 as is the development of cystic ovaries. Whether the pituitary hypertrophy signals a contribution of this organ to ovarian cyst formation or is simply a reflection of ovarian cyst formation is not established. However, the pituitary is necessary for chorionic gonadotrophin to induce cystic ovaries in hypothyroid rats(12).

Summary. Chorionic gonadotrophin-treated hypothyroid rats develop cystic ovaries characterized by decreases in free and esterified cholesterol. Ethamoxytriphetol prevents cystic ovary formation and the associated cholesterol changes, blocks pituitary hypertrophy and partially protects against adrenal cholesterol depletion.

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The Biological Activity of Di-α-Tocopherone.* (28330)

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Di-α-tocopherone is the trivial name of a major metabolite of vitamin E which has been recently isolated from mammalian liver, synthesized by $K_3Fe(CN)_6$-oxidation of α-tocopherol, and assigned the structure shown in Fig. 1(1,2). In the studies reported here, the biological activity of this compound as a substitute for Vit. E in the nutrition of rats and rabbits has been investigated. Also, the hypothesis that di-α-tocopherone might represent an "active form" of the vitamin whose formation *in vivo* is genetically blocked in animals exhibiting hereditary *Muscularis dystrophia*, was tested using a strain of mice affected with this disease.

Methods. Rat fertility experiments. Fifty-eight weanling female rats of the Sprague-Dawley strain were maintained for 8 weeks on the following Vit. E-deficient diet (%): Glucose (Cerelose) 70.4, 'Labco' casein 20.0, salts 446(3) 4.0, methyl linoleate (60%)† 3.0, cod liver oil 2.0, vitamin mixture in Cerelose(4) 0.5, choline chloride 0.1. The females then were mated to normal males, divided into 4 treatment groups, and assigned to one of the following treatments: (a) none (negative controls) (b) 25 mg di-α-tocopherone (c) 100 mg di-α-tocopherone (d) 25 mg d-α-tocopheryl succinate. In each case the supplement was dissolved in 1.75 ml methyl linoleate and incorporated into the diet in 3 equally divided doses given on the 8th, 11th and 14th days after the start of the 5-day mating period. All the females were killed on the 21st day and the uteri were examined for the presence of live fetuses and for evidence of resorption.

Indications that the dimer was poorly absorbed from the lumen of the intestine prompted a second study in which the compound was administered parenterally. The

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† Nutritional Biochemicals Corp., Cleveland, Ohio.

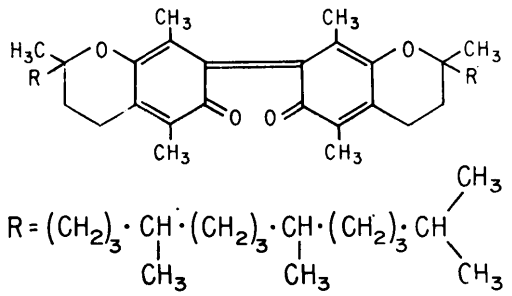

FIG. 1. Structural formula of di-α-tocopherone.

same procedure was followed except for minor dietary changes. The depletion diet contained 10% molecular-distilled lard instead of methyl linoleate and cod liver oil, a proportionate reduction being made in the concentration of Cerelose. Vitamins A and D were supplied at concentrations of 100 and 5 I.U. /g of diet, respectively. Four groups of female rats, totalling 50 animals, were given the following supplements: (a) none (negative controls) (b) 25 mg d-α-tocopheryl acetate (c) 50 mg di-α-tocopherone (d) 50 mg of $LiAlH_4$-reduced di-α-tocopherone(1). The supplements were injected intraperitoneally in 2 equal doses on the 8th and 11th days after the start of the mating period. As the dimers are insoluble in alcohol, they were first dissolved in 0.2 ml diethyl ether to which 0.2 ml of ethanol, 0.8 ml of glycerol and a drop of Tween 80 were added. The mixture was shaken vigorously for 10 minutes, during which time the ether evaporated and a fine emulsion was formed which was injected immediately. Nevertheless, in 3 cases part of the dose was recovered at the site of injection.

Rabbit experiments. The metabolite was also tested for curative activity with respect to muscular dystrophy in Vit. E-deficient rabbits. Eight New Zealand White rabbits weighing 1200-1400 g were maintained on a Vit. E-deficient diet(5) until the first clear sign of incoordination was detectable by the righting reaction (30-40 days). Two subsequently were treated with a single dose of 100 mg of d-α-tocopheryl succinate and 3 with 250 mg of di-α-tocopherone. The remaining 3 received 300 mg of di-α-tocopherone in 3 equal doses given on consecutive days. All supplements were administered by intraperitoneal injection in emulsified form.

Mouse experiments. Di-α-tocopherone was administered to 6 mice of the Bar Harbor dystrophic strain which were exhibiting symptoms of hereditary *Muscularis dystrophia.* Three were given a single 5 mg intraperitoneal dose as an emulsion in 0.5 ml of 20% ethanol containing 1% Tween 80. The other 3 were given 5 mg three times per week as a supplement to their commercial laboratory chow. The animals were observed for changes in body weight and in gross symptoms of dystrophy.

Absorption of di-α-tocopherone-C^{14}. The labelled compound was synthesized by ferricyanide oxidation of d-α-tocopherol-5-methyl-C^{14} and the reduced form was prepared by $LiAlH_4$ reduction(1). Four rats weighing about 250 g were fasted for 18 hours, then given either 0.66 mg of di-α-tocopherone-C^{14} or 0.52 mg of the ditocopheryl reduction product (both 1.5 μc/mg) in 5 g of diet. The animals were maintained on a commercial diet for 58 hours, whereupon they were killed and the feces, urine and liver were extracted(1) and analyzed for radioactivity.

Results. The results of the rat fertility experiments are tabulated in Table I. They indicated that, in relation to α-tocopherol, di-α-tocopherone has a low order of vitamin activity, if any. The only significant evidence of activity was observed following oral administration, in which case 2 healthy litters were produced at the 100 mg dosage level. Considering the limited extent to which the compound is absorbed (see below) a greater response might have been expected following parenteral dosage; however the response to intraperitoneal administration was uniformly negative.

Similar results were recorded with respect to muscular dystrophy in rabbits. The 3 animals which received a single intraperitoneal dose of di-α-tocopherone died after 3, 4 and 10 days, respectively, having exhibited a steady increase in the severity of symptoms. These survival times are typical of untreated animals. Tocopherol administration induced a complete remission of gross symptoms within this period. The 3 animals given 300 mg

TABLE I. Results of Rat Fertility Experiments.

Treatment	No. females	No. conceived	No. live litters	Avg No. fetuses Alive	Avg No. fetuses In resorption
Exp I—Oral administration					
None	15	8	0	0	8.8
α-tocopheryl succinate, 25 mg	15	7	7	8.3	0
di-α-tocopherone, 25 mg	15	9	1	0.2	8.0
di-α-tocopherone, 100 mg	13	7	2	2.7	7.2
Exp II—Intraperitoneal administration					
None	15	14	0	0	6.8
α-tocopheryl acetate, 25 mg	10	6	5	6.2	1.4
di-α-tocopherone, 50 mg	15	13	0	0	8.7
Reduced di-α-tocopherone, 50 mg	10	8	0	0	8.6

of di-α-tocopherone in divided doses survived for 27, 29 and 31 days, respectively. In the case of these individuals the treatment appeared to decrease the rate of weight loss and to sustain appetite to some extent, but no improvement in muscular incoordination was observed. The survival times for this group, however, are clearly longer than those observed for untreated animals and, together with the results of the rat experiments, suggest that di-α-tocopherone probably possesses some low order of Vit. E activity. If it is considered that an oral dose of 25 mg of α-tocopherol is sufficient to bring about a prompt remission in rabbits, the slight response to 300 mg of di-α-tocopherone would signify that the dimer possesses less than 5% as much activity by weight.

It is of interest that a dimer of α-tocopherol obtained via ferricyanide oxidation of α-tocopherol by Nelan and Robison(6), which possesses spectrophotometric characteristics similar to those of di-α-tocopherone but to which they have assigned a different structure, also was observed to have less than 5% of the activity of α-tocopherol with respect to resorption-gestation in rats. When di-α-tocopherone is treated with Emmerie-Engel reagent it undergoes a slow reaction which reaches a plateau after about 30 minutes. This indicates that in ethanol it is partially converted to a compound with reducing properties, a necessary requisite for Vit. E activity. The occurrence of a similar transformation *in vivo* seems doubtful, but there is a possibility that a limited conversion occurred by alcoholysis in the ethanolic emulsion before its administration.

Four of the genetically dystrophic mice given di-α-tocopherone succumbed within 30 days and the remaining 2, which had received a single dose, showed no evidence of remission. This mortality pattern was similar to that observed in untreated animals and indicates that the treatment was without effect.

Extraction of the livers taken from the 2 rats given an oral dose of di-α-tocopherone-C^{14} yielded only 0.15% and 0.13% of the administered activity. Eighty % of the dose was recovered from the feces and none was detected in the urine. The corresponding values for the reduced compound were 0.02% and 0.03% in the liver, 54% and 65% in the feces and zero activity in the urine. Paper chromatography(1) of the feces extracts indicated that the tocopherone was excreted largely unchanged, whereas the reduced form was partially converted to a second compound, probably an oxidation product.

Discussion. The foregoing results indicate that di-α-tocopherone is a terminal oxidation product of α-tocopherol *in vivo* rather than an active form of the vitamin. The dimer represents the second metabolite of Vit. E that has been isolated from animal tissues, the first being tocopheryl-p-quinone which has been detected by several workers and whose formation from C^{14}-d-α-tocopherol was recently confirmed(7). A degradation product which was isolated from human and rabbit urine by Simon *et al.*(8) apparently is absent from liver and muscle, since it cannot be detected in these tissues following administration of labeled α-tocopherol(7,9). The antioxidant view of Vit. E function presumes

that the active form is the free alcohol, and in support of this theory it is significant that the 2 metabolites whose formation *in vivo* has been established are also produced in a peroxidizing fatty acid medium *in vitro*(1).

Summary. Di-α-tocopherone, a recently characterized metabolite of α-tocopherol isolated from animal tissues, was tested for biological activity with respect to resorption-gestation in Vit. E-deficient rats, nutritional muscular dystrophy in rabbits and hereditary muscular dystrophy in mice. The results indicate that this metabolite has less than 5% of the Vit. E activity of α-tocopherol and that it possesses no therapeutic value in treatment of the congenital myopathy. Its probable role in metabolism is that of a terminal oxidation product rather than an active form of Vit. E.

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Lysozyme† Levels in the Kidneys of Cancer Patients. (28331)

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Lysozyme found to be present in many organs and secretions of humans has been described(1-5). Litwack has isolated chromatographically pure human lysozyme from the kidneys(6) and Jolles and Jolles recently have purified lysozyme from human milk(7). The authors have reported a 70-100 fold increase of lysozyme concentration in the kidneys of Sprague-Dawley rats bearing Jensen Sarcoma (8,9). Surgical removal or spontaneous regression of the tumor results in a return to its normal value in the kidney(10). Similar results have been published for other host-tumor systems by Cappuccino(11). Therefore, it was thought to be of interest to study the lysozyme levels in the kidneys of cancer patients.

Materials and methods. The kidney samples utilized for these experiments were obtained upon autopsy of cancer patients as soon as possible and kept at −20°C. They were homogenized by the technique already described(7,9). And the resulting extracts, after precipitation of most of the proteins with 1% acetic acid in 95% ethanol and centrifugation, were examined. Lysozyme activity was determined by a modification of Litwack method (12) by addition of supernatant's aliquots to a suspension of acetone dried *Micrococcus lysodeickticus* (*Rutgers strain 19*) in buffer (chloride-phosphate pH 6.2, OD. 0.600-0.700 mμ) and measurement of the change of OD. per minute. The results are expressed in mg equivalent of EGG WHITE LYSOZYME* per gram of wet tissue.

Results. The results of analysis performed on 29 kidney samples and reported in Table I show that the lysozyme values obtained vary over a wide range. If the normal value for lysozyme in human kidneys is considered to be 200 γ/g of wet tissue(13), the values found for the cancer-bearing human patient exam-

† New name recommended by the Commission on Enzymes of the International Union of Biochemistry: Muramidase (E.C.3.2.1.17).

* Worthington Lot #597.