studies have shown that the action of interferon is dependent on cellular RNA(3) and protein synthesis(11). A proposed mechanism of action for interferon which would account for most of the facts now known is that interferon acts as an inducer of a new cellular RNA which in turn causes the formation of a new protein(3). The antiviral action of this protein may be due to an inhibition of the accumulation of new viral RNA (3,12).

Summary. Treatment with interferon which markedly depressed vaccinia virus growth also inhibited the production of new virus DNA. This was indicated by the small number of cytoplasmic DNA grains due to tritiated thymidine found in radioautographic studies of vaccinia virus infected cells pretreated with interferon.

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Metabolism of *l*-Alpha-Tocopherol by the Vitamin E-Deficient Rabbit.* (30236)

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The cure of nutritional muscular dystrophy either by oral or by intravenous *l*-alpha-tocopheryl acetate is demonstrated by studies described in this report. The *l*-alpha-tocopheryl acetate was one-fifth as potent as the *d*-epimer and the lower potency was associated with a greater rate of loss of tocopherol from the body.

Methods. Young New Zealand rabbits of both sexes weighing approximately 500 g were fed a purified vitamin E-deficient diet (1) as modified by Diehl(2). All animals received 0.05% sodium sulfaquinoxaline in the drinking water 5 days of each week. The animals that served as controls were given an oral supplement of 8 mg of *dl*-alpha-tocopheryl acetate dissolved in 0.2 ml of corn oil 3 times weekly.

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Recovery experiments were begun when the unsupplemented rabbits showed severe muscular weakness as judged from the ability of the rabbit to right itself when placed on its back(3). The animals weighed between 600 and 900 g at this time, which was 3 to 4 weeks after the purified diet was begun. The oral dose for recovery was 50 mg either of *d*-alpha-tocopheryl acetate or of *l*-alpha-tocopheryl acetate dissolved in one ml of corn oil or olive oil. The intravenous dose was 50 mg of either epimer suspended in 4 ml of 0.15 M NaCl containing 1% polyoxyethylene sorbitan monooleate (Tween 80).

Total tocopherols of serum were measured by the method of Rindi(4) and liver tocopherols were measured by the method of Swick and Baumann(5). Urinary creatine and creatinine were measured by the method of Folin(6) with minor modifications. The ratio of urinary creatine to creatinine was obtained by dividing the μmoles of creatine by

the μmoles of creatinine present in a urine sample.

Results. After receiving a purified diet of the type used in these experiments for 3 to 4 weeks, young rabbits develop acute muscular dystrophy characterized by extreme muscular weakness and by unremitting urinary creatine to creatinine ratios of greater than one. With this acute syndrome, death occurs within 1 to 2 days after an animal is unable to regain an upright position when placed on its back. Weight gain is poor and the animals lose weight as the deficiency syndrome develops(1,7). By contrast, rabbits receiving *dl*-alpha-tocopheryl acetate supplements gain weight throughout the experimental period and their urinary creatine to creatinine ratios do not exceed 0.5. These observations were all confirmed in the present experiments.

Rabbits with severe muscular dystrophy responded to treatment with *l*-alpha-tocopheryl acetate given either orally or intravenously. In Fig. 1 examples of these responses are compared to the response of a rabbit treated with oral *d*-alpha-tocopheryl acetate. Qualitatively the response to *l*-alpha-tocopheryl acetate was similar to that evoked by *d*-alpha-tocopheryl acetate, but the duration of the response was much shorter. Treatment with either epimer caused creatinuria to cease, muscle strength to improve, weight loss to cease and weight gain to resume if the duration of response was sufficiently long.

Three severely dystrophic rabbits each received single 50 mg doses of *d*-alpha-tocopheryl acetate orally with survival after treatment ranging from 34 to 39 days. Five severely dystrophic rabbits each received a single 50 mg dose of *l*-alpha-tocopheryl acetate orally. Three of these had reached an irreversible stage of vit. E deficiency and died before a response could be observed, but the other 2 exhibited significant diminution of creatinuria and survived for 4 and 8 days.

After an intravenous dose of *d*-alpha-tocopheryl acetate the survivals of 2 rabbits were 35 and 63 days, whereas the survivals after intravenous *l*-alpha-tocopheryl acetate were 10 and 16 days for 2 rabbits. The response to intravenous administration of these compounds was as prompt and complete as the response to oral therapy. This is in contrast to the poor response to intramuscular or subcutaneous administration of *dl*-alpha-tocopheryl acetate observed by others(7-9).

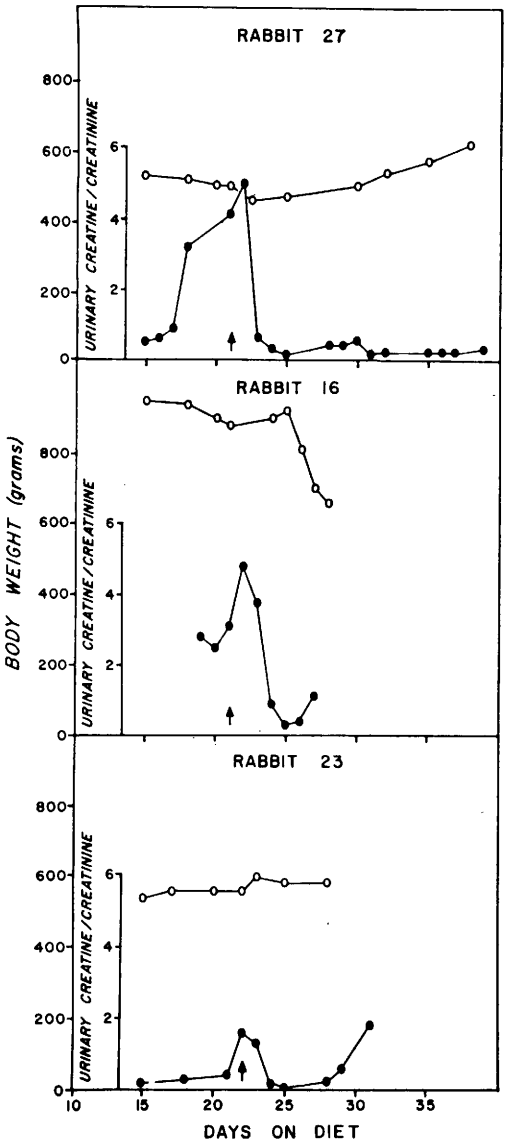

FIG. 1. Effects of *d*- and *l*-epimers of alpha-tocopherol on vitamin E-deficient rabbit. Weight is indicated by open circles and creatine to creatinine ratios by solid circles. The arrows mark the day that treatment was given. Rabbit 27 received 50 mg of *d*-alpha-tocopheryl acetate orally; rabbit 16 received 50 mg of *l*-alpha-tocopheryl acetate orally; and rabbit 23 received 50 mg of *l*-alpha-tocopheryl acetate intravenously. The times of death were day 55, day 29, and day 32 for rabbits 27, 16 and 23 respectively.

TABLE I. Tocopherol Concentrations in Liver.

Treatment	Days after treatment*	Liver tocopherol (μg/g)†
None		3.2 ± 1.2(9)
dl-alpha-tocopheryl acetate (control supplementation)		9.6 ± 2.7(10)
l-alpha-tocopheryl acetate	1	16.8; 28.7 (2)
Idem	4	7.1‡ (1)
Idem	8	1.6‡ (1)
d-alpha-tocopheryl acetate	3	31.1 (1)
Idem	9	12.2 (1)
Idem	20	7.9 (1)

* Oral treatment only.

† Mean ± standard deviation or range of values is shown. Number of rabbits in each group is given in parentheses.

‡ These animals died on days indicated and the livers were obtained within a few hours. The other animals were killed for liver tocopherol measurements.

Intravenous *dl*-alpha-tocopheryl acetate previously has been found to diminish the creatinuria of vit. E-deficient rats(10).

Based on body weight the average daily requirements estimated from the length of survival after *d*-alpha-tocopheryl acetate are 2.0 and 1.2 mg per kg body weight for oral and intravenous routes of administration, respectively. The estimated average daily requirements for oral and intravenous *l*-alpha-tocopheryl acetate are 9.5 and 8.0 mg per kg body weight, respectively. This method of comparing the effectiveness of the two epimers gives only a rough estimate(11) and the number of animals is small. Nevertheless, *l*-alpha-tocopheryl acetate whether given orally or intravenously, appears to be about one-fifth as effective as *d*-alpha-tocopheryl acetate in curing nutritional muscular dystrophy in the rabbit. This difference in effectiveness is of the same order of magnitude as has been observed in other biological tests (12-16).

It is evident from serum concentrations of tocopherol that significant gastrointestinal absorption of *l*-alpha-tocopheryl acetate occurs in the rabbit. Twenty-four hours after a single 50 mg oral dose of *l*-alpha-tocopheryl acetate the serum concentrations ranged from 15.0 to 48.0 μg per ml for 4 rabbits. After an equivalent oral dose of *d*-alpha-tocopheryl acetate the serum tocopherol concentrations ranged from 45.0 to 46.0 μg per ml for 3 rabbits. These values may be compared with 8.2 ± 4.0 (S.D.) μg per ml for 11 control rabbits and 3.0 ± 1.8 μg per ml for 18 severely dystrophic rabbits.

Data on the concentration of tocopherol in the liver after oral treatment are shown in Table I. Again, it is evident that significant amounts of *l*-alpha-tocopheryl acetate must have been absorbed from the gastrointestinal tract to account for the increase in liver tocopherol concentration. However, the liver tocopherol concentration after *l*-alpha-tocopheryl acetate decreased more rapidly in comparison to animals treated with *d*-alpha-tocopheryl acetate.

Serum tocopherol levels after intravenous injection of *l*- or *d*-alpha-tocopheryl acetate are shown in Fig. 2. Immediately after the injection, the serum tocopherol concentrations were higher in the rabbits that received *l*-alpha-tocopheryl acetate but the concentrations fell rapidly and reached much lower levels than those of the animals receiving *d*-alpha-tocopheryl acetate.

Discussion. Studies of the relative potencies of the pure *d*- and *l*-epimers of alpha-tocopherol in nutritional muscular dystrophy heretofore have relied entirely on prevention

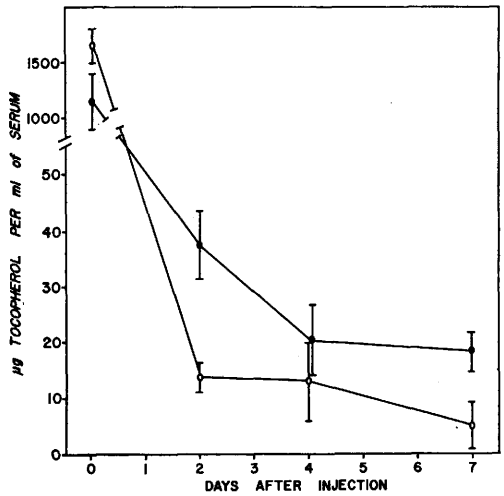

FIG. 2. Serum tocopherol after intravenous *d*- or *l*-alpha-tocopheryl acetate. Data were obtained from 2 rabbits given the *d*- and from 2 given the *l*-epimer. The curves connect average values and vertical bars show range. Open circles represent animals treated with *l*-alpha-tocopheryl acetate and solid circles represent those treated with *d*-alpha-tocopheryl acetate.

of deficiency signs and on the oral route of administration. Scott and Desai used prevention of nutritional muscular dystrophy in the chick(15), and Witting and Horwitt used prevention of creatinuria and prevention of growth retardation in rats receiving vit. E-deficient diets(16). In agreement with others who have used the fetal resorption test in pregnant vit. E-deficient rats(12) and the reversal of erythrocyte susceptibility to hemolysis by oxidizing agents(13,14), the *l*-epimer was observed to be considerably less potent than *d*-alpha-tocopherol.

To complete the evaluation of compounds exhibiting vit. E activity, such as *l*-alpha-tocopherol, curative experiments are essential. If conclusions are based entirely on preventive experiments, vit. E activity might be attributed to compounds which protect traces of *d*-alpha-tocopherol in the diet or in the animal's body. Earlier comparisons of the potency of *d*- to *dl*-alpha-tocopherol in curing nutritional muscular dystrophy produced variable results, but *dl*-alpha-tocopherol was somewhat inferior(11). Our data confirm the biological inferiority of *l*-alpha-tocopherol and provide information on its cause.

In considering the differences in biological activity between the *d*- and *l*-epimers of alpha-tocopherol, three questions may be asked: (a) Is the action of the *l*-epimer in metabolism qualitatively different from that of *d*-alpha-tocopherol? (b) If the activities of the 2 epimers are not qualitatively different, do they have different potencies in fulfilling the essential metabolic role(s) of alpha-tocopherol? or (c) Can the inferiority of *l*-alpha-tocopherol be explained by less effective gastrointestinal absorption, different tissue distribution, greater metabolic breakdown, or faster excretion? The present demonstration of the cure of nutritional muscular dystrophy in the rabbit strengthens the view that the *d*- and *l*-epimers of alpha-tocopherol are qualitatively exerting the same action(s) in metabolism. The alternative possibility that *l*-alpha-tocopherol acts by protecting or somehow facilitating the function of traces of *d*-alpha-tocopherol present in the diet or in the animal's body has not been conclusively eliminated, but there is no evidence in support of it.

If the activities of the *d*- and *l*-epimers are not qualitatively different, are they quantitatively different? Inasmuch as the metabolic role of alpha-tocopherol is unknown, this question cannot be answered with certainty. On the other hand, it has been observed that nutritional muscular dystrophy does not develop if serum concentrations of tocopherol are high in animals fed either the *d*- or *l*-epimers of alpha-tocopherol(15). Furthermore, equal amounts of the 2 epimers are not equally effective in maintaining serum and tissue concentrations (Table I, Fig. 2). Thus, the activities of the *d*- and *l*-epimers may be quantitatively similar with the biological inferiority of *l*-alpha-tocopherol being due to lower tissue concentrations.

The lower concentrations after *l*-alpha-tocopherol cannot be explained by inferior gastrointestinal absorption. Our data show that significant gastrointestinal absorption of *l*-alpha-tocopherol occurs in the rabbit, and Weber *et al* have found the initial absorption of the *l*-epimer by the rat to be faster than the absorption of *d*-alpha-tocopheryl acetate (17). In support of these observations, administration of *l*-alpha-tocopheryl acetate intravenously did not improve its relative effectiveness.

Preferential accumulation of *l*-alpha-tocopherol in one or more tissues could explain the lower concentrations in liver and serum (Table I, Fig. 2), but the observations of Weber *et al*(17) make this unlikely. These authors found no important differences in the pattern of distribution of the *d*- and *l*-epimers of alpha-tocopherol in liver, kidney, brain, skeletal muscle, depot fat, and adrenal glands of rats after oral administration of the radioactively labeled compounds. This observation, if it can be transposed to the rabbit, would indicate that the loss of tocopherol from serum and liver was not caused by an unusual redistribution of *l*-alpha-tocopherol in the body.

Therefore, we conclude that the lower biological activity of *l*-alpha-tocopherol is due, in part if not entirely, to faster loss from the organism. Whether the stereochemical difference between the two epimers results in

greater metabolic breakdown or in a faster excretion of the *l*-epimer remains to be clarified.

Summary. Nutritional muscular dystrophy in the rabbit can be cured by *l*-alpha-tocopheryl acetate administered either orally or intravenously. Based on duration of survival after a single 50 mg oral dose the average estimated daily requirement was 2.0 mg per kg of body weight for 3 rabbits that received *d*-alpha-tocopheryl acetate and 9.5 for 2 rabbits that received *l*-alpha-tocopheryl acetate. After intravenous treatment with the same dose of these compounds, using 2 rabbits in each group, the estimated requirements were 1.2 and 8.0 for the *d*- and *l*-epimers, respectively. Liver and serum concentrations of tocopherol decreased more rapidly after treatment with *l*-alpha-tocopheryl acetate than after treatment with the same amounts of *d*-alpha-tocopheryl acetate. These observations suggest that the biological inferiority of *l*-alpha-tocopherol is due, at least in part, to a faster rate of loss from the body.

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Autologous Skin Grafts in the Rat. Uptake of ^{35}S-sulfate.* (30237)

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In a previous report(1) the results of analyses on mucopolysaccharides and collagen in rat skin autografts during the healing process were described. Five days after surgery a reduction in content of acid mucopolysaccharides in the grafts was found. The following period was characterized by an increased content of acid mucopolysaccharides. Fifty-four and 103 days after surgery the content did not differ significantly from that of intact, symmetrical skin. The present study aimed at elucidating the incorporation of ^{35}S-sulfate into sulfomucopolysaccharides in full-thickness skin autografts of rats.

Material and methods. Among 67 young male Wistar rats of an inbred strain (State Serum Institute, Copenhagen) weighing 179 ± 3.2 g, seven served as controls, while 60 had full-thickness skin autografts. From the

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