Pronounced lag phase in utilization of substrates by GM1, *Bacillus macerans*, and *Micrococcus cerolyticus* might suggest that adaptation occurs during this period. However, the finding of esterase activity in cell free extracts of unadapted cells speaks against this possibility. In view of the apparent absence of wax esterase in the growth medium it also seems unlikely that the lag might have been caused by the time required for autolysis to liberate esterase. The results are compatible, however, with the view that saturation with endogenous substrate of the sites of esterase or other enzymes further along the chain of reactions might have been the cause of the observed lag.

Summary. 1) Various microorganisms were studied for ability to metabolize myricyl palmitate (beeswax), tubercle wax, and tributyrin. All cultures except one produced positive results varying only in rate and extent to which they attacked these substrates. A pronounced lag phase in utilization of substrate by 3 microorganisms tested was attributed to saturation of enzyme sites with endogenous substrates. 2) With the exception of *Candida albicans* intact cells did not show esterase activity. However, positive results were obtained with extracts which suggested that deesterification was an initial step in utilization of waxes as sources of energy for the various microorganisms tested.

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Action of N,N'-diphenyl-p-phenylenediamine in Tocopherol Deficiency Diseases.* (24485)

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The ability of certain synthetic antioxidants, notably N,N'-diphenyl-p-phenylenediamine (DPPD), to delay or prevent appearance of Vit. E deficiency symptoms, including muscular dystrophy and sterility, has been demonstrated. This activity generally has been attributed to conservation of traces of Vit. E present in the lipid portion of diet and in tissues of experimental animals. In conformity with this view, Shull and associates(1) found that DPPD and other synthetic antioxidants delayed but did not prevent eventual occurrence of muscular dystrophy in guinea pigs maintained on tocopherol-deficient diet. However, experiments with rats(2) showed that fertility could be partially restored in sterile females even when Vit. E was rigorously excluded from the regenerative diet. In the present study it has been found that DPPD has no conservational effect upon Vit.

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E present in liver and muscle of rabbits and rats maintained on tocopherol-deficient diet, and that the antioxidant brings about a rapid remission of symptoms of muscular dystrophy in rabbits fed a diet from which all lipoid materials have been removed.

Procedure. To determine the influence of DPPD on rate of depletion of tissue tocopherols, twenty-two 6-weeks-old rabbits were placed on tocopherol-low basal diet of the following composition: Labco casein 20, Cerelose 20.4, cornstarch 40, molecular-distilled lard 10, salts 446(3) 4, cellulose 5, Vit. premix(4) 0.5, choline chloride 0.1. Vit. A and D were provided at concentrations of 10,000 I.U. and 1000 I.U. kg of diet, respectively. During 2-week preliminary period the diet was supplemented with α-tocopheryl acetate at 1 g kg. At this time 4 animals were sacrificed and the remainder were divided into 2 groups of 9 each. One group was placed on basal tocopherol-low diet; the other received the same diet to which DPPD was added at 0.005%. Additional animals were sacrificed from each group at intervals of approximately 3 weeks. Livers and segments of thigh skeletal muscle were removed and frozen for Vit. E analysis. A similar experiment was carried out using 30 weanling rats. These animals were maintained for 2 weeks on tocopherol-low basal diet described elsewhere(4) plus a supplement of 30 mg α-tocopheryl acetate week. Then 6 rats were sacrificed and the remainder divided into 2 groups which were placed on basal diet with or without 0.001% DPPD. Additional animals were killed at intervals for liver and muscle analyses. Livers were ground with anhydrous sodium sulfate until dry. 10 g muscle samples were freed of extraneous connective tissue, ground with acid-washed sand and dried with sodium sulfate. The dried samples were placed in Erlenmeyer flasks and extracted with peroxide-free ether overnight in mechanical shaker. After filtration the ether was removed by warming in water bath under jet of nitrogen and saponification was carried out in the dark in the presence of pyrogallol according to slight modification of procedure of Eggitt and Ward(5). Sterols were removed from the non-saponifiable fraction of liver samples by overnight crystallization from ethanol in the cold. After evaporating the solvent under nitrogen, carotenoids and Vit. A were removed from liver extracts by dissolving the residue in benzene and passing through a column of Floridin XXS previously treated with stannous chloride in HCl as described by Beckmann(6). Finally, the benzene was evaporated in nitrogen atmosphere and the residue dissolved in ethanol for determination of Vit. E by the Emmerie-Engel reaction. 5 μg per sample could be determined readily by this procedure. In accord with the fact that α-tocopheryl acetate was the only dietary form used, chromatography on paper, using the systems of Green *et al.*(7), failed to detect any of the other natural isomers. The curative activity of DPPD was tested on dystrophic rabbits maintained on a lipid-free, tocopherol-free diet. Dystrophy was induced by feeding the diet of Young and Dinning(8) except that salts 446(3) and a different vitamin mixture were used(4). When distinct symptoms appeared, the dystrophic animals were placed on basal diet of the following composition: Labco casein 15, sucrose 43.9, cornstarch 36, salts 446(3) 4, choline chloride 0.1, vitamin premix in Cerelose(4) 1. The short recovery period required for regeneration of dystrophic rabbits with Vit. E permits removal of all lipids from the diet without risking a deficiency of essential fatty acids prior to recovery. The presence of 3% cod liver oil in the depletion diet also assured an adequate storage of Vit. A and D. The following supplements were administered daily to different groups of dystrophic animals: 10 mg α-tocopheryl acetate; 20 mg DPPD; none. The supplements were suspended in 1 ml glycerol containing 0.01 ml Tween 80. Negative controls received the carrier alone. Twenty-four hour urine collections were made at intervals for determination of total and preformed creatinine by modification of the method of Hare(9).

Results. The effect of feeding a Vit. E-deficient diet on tocopherol levels in tissues of rabbits and rats, and the influence of DPPD, are illustrated in Fig. 1. DPPD effected no conservation of Vit. E in tissues of either species, and such an effect does not,

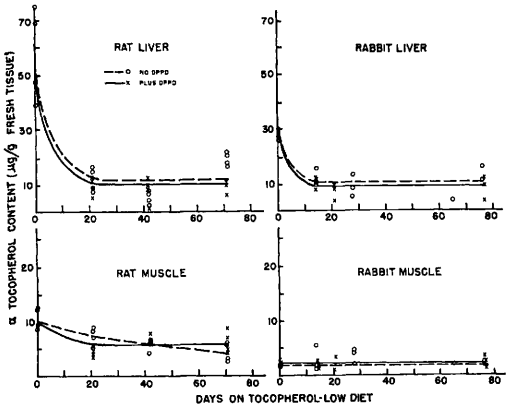

FIG. 1. Influence of DPPD on α-tocopherol concentration in tissues of rabbits and rats fed a Vit. E-deficient diet.

therefore, account for its ability to replace the vitamin in the diet. It is also noteworthy that the tocopherol level of skeletal muscle of rabbit is apparently unaffected by dietary depletion. This observation is in accord with that of Blaxter(10) who found that Vit. E content of muscle of dystrophic calves was at least as great as that of normal animals. The small decline noted in rat muscle tocopherol is of doubtful significance. It appears that the turnover of tocopherol in skeletal muscle of both species is exceedingly slow, and that after a period of relatively rapid depletion of "labile" tocopherol in the liver, the concentration in this organ also reaches a plateau which is maintained for extended periods. The rats made good growth during experiment while rabbits exhibited the customary slow gains observed with purified diets. Histological examination of skeletal muscle of rabbits maintained on basal diet for 10 weeks revealed evidence of mild dystrophy. Muscular degeneration on this diet requires a much longer period of depletion than is required when high levels of unsaturated fatty acids are ingested.

Five dystrophic rabbits maintained on lipid-free diet and treated with 20 mg DPPD daily showed symptomatic evidence of improvement in 2 to 3 days and a disappearance of symptoms in 5 to 6 days. These animals also underwent a remission of creatinuria, as indicated by creatine:creatinine ratios which declined from pretreatment range of 2.21-5.01 to 0.09-0.42 after treatment for 5 days. This group was continued on DPPD supplementation for 15 days, during which time growth was resumed and condition of the animals markedly improved. Terminal urine collections yielded creatine:creatinine ratios of 0.12-0.33. The condition of 3 control animals given Tween-glycerol carrier, deteriorated rapidly and they succumbed in 4 to 8 days. Three dystrophic animals treated with Vit. E. recovered in a manner similar to that described for the DPPD treatment.

These results demonstrate that DPPD possesses curative activity with respect to muscular dystrophy that is independent of the presence of Vit. E in the diet. Earlier observations(2) indicating that DPPD is also active in rehabilitation of sterile female rats have been confirmed in more recent experiments. Partial regeneration was obtained following administration of 0.005% DPPD in Vit. E-free diet in which a high-purity methyl linoleate-urea complex(11) served as the source of essential fatty acids and fat-soluble vitamins were provided in the crystalline state. Reproductive performance of rats fed DPPD is always inferior to that observed using Vit. E, however, owing to the high order of toxicity of the synthetic compound for pregnant females.

These findings support the view that biological activity of the tocopherols with respect to muscular dystrophy is associated with their antioxygenic potency, and are in accord with known activity of α-tocopherylhydroquinone and α-tocopherylquinone. The efficacy of DPPD with respect to rat sterility is, however, in contrast to the reported inactivity of these oxidation products of α-tocopherol, and implies that this syndrome is also a consequence of an inadequacy of suitable antioxygenic compounds. Moreover, the hydroquinone has been reported to have activity comparable to α-tocopheryl acetate in reversing testicular damage in Vit. E deficient hamsters (12). It is of interest that antioxygenic potencies of α- β- and γ-tocopherols, as determined in storage tests conducted at temperatures near the physiological range using carotene and ethyl oleate, are in the same order as their biological activities(13).

Tests of ability of DPPD to reactivate iso-

octane-extracted DPNH-cytochrome c reductase in the Keilin-Hartree heart muscle preparation have confirmed the finding of Nason and Lehman(14) that this antioxidant is ineffective in replacing α-tocopherol. However, serum albumin was found to be an unsatisfactory agent for suspending DPPD in this system and other materials are being investigated.

Curative activity of DPPD with respect to muscular dystrophy in the rabbit is in contrast to its reported inability to prevent hypercholesterolemia and muscular degeneration in guinea pigs fed a tocopherol-low diet (15). An explanation of this apparent discrepancy must await further investigation.

Summary. Administration of DPPD to growing rabbits and rats fed a Vit. E-low diet does not modify rate of depletion of vitamin from the liver. Tocopherol content of skeletal muscle was essentially unaffected by dietary restriction. DPPD was as effective as α-tocopherol, at level administered, in bringing about a remission of muscular dystrophy and creatinuria in rabbits maintained on diet devoid of tocopherols. Ability of DPPD to partially regenerate sterile female rats maintained on Vit. E-free diet was confirmed. These findings indicate that the effectiveness of DPPD as a substitute for Vit. E is not due to conservation of the vitamin in tissues or to the presence of residual tocopherols in the diet.

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Conversion of Serotonin (5-Hydroxytryptamine) to 5-Hydroxyindoleacetic Acid by Rabbit Blood. (24486)

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Serotonin (5-hydroxytryptamine) is oxidized to 5-hydroxyindoleacetic acid (5-HIAA) in animals and man(1). The latter compound is excreted in the urine. The catalyst for oxidation of serotonin is thought to be monamine oxidase. Although many tissues contain this enzyme, liver is usually the most active source. Werle and Roewer(2) have reported an amine oxidase present in dog blood. In other species, however, blood has been considered to be lacking in this enzyme activity(3). Tabor *et al.* (4) found an amine oxidase in beef plasma, but substrate and inhibition specificities differed markedly from those described for the monamine oxidase in liver. Reserpine causes a release of serotonin and histamine from platelets *in vivo*(5). *In vitro* studies have shown that incubation of reserpine with rabbit platelets suspended in plasma also liberates serotonin and histamine. However, after in-

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