Benedict's reagent was faster and perhaps more complete with the cornstarch-saliva mixture.

Summary. When 40% or more of anhydroglucose units of starch are oxidized to dialdehydes, the material is toxic to rats, as manifested by poor growth, increased mortality and testicular damage. No acute toxicity was noted. No toxicity occurred when 2% or less of the units were oxidized, even at levels of 20% of diet. Starch further oxidized to contain 5% of glucose units in the dicarboxyl form, produced no toxic symptoms with as much as 20% of diet consisting of this modified starch, but food intake was increased and utilization decreased, perhaps due to faulty digestion of the dicarboxyl starch.

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Biopotency of Non-Tocopherol Compounds in Vit. E Deficiency Diseases.* (25381)

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Recent isolation of several naturally occurring isoprenoid compounds bearing structural resemblances to tocopherols has raised the question of their possible Vit. E activity for animals. These include ubiquinone, a redox compound present in mitochondria necessary for succinoxidase activity(1), its hydroquinone, and solanachromene, a high molecular weight phenol isolated from tobacco(2) structurally related to α-tocopherol. In the present studies the biological activity of these substances, as well as that of acid reduction product of ubiquinone, has been evaluated in relation to muscular dystrophy in the rabbit. In addition, curative activity of the synthetic antioxidant N,N'-diphenyl-p-phenylenediamine (DPPD) with respect to hypercholesterolemia and impaired stability of red blood cells in Vit. E deficiency has been tested.

Procedure. New Zealand white rabbits weighing about 1200 g were depleted of Vit. E using the following diet (%): Labco casein 15, sucrose 37.9, corn starch 36.5, molecular-distilled lard 3, cod liver oil 3, salts 446(3) 4, choline chloride 0.1, vitamin premix in glucose(4) 0.5. All treatments were evaluated by curative assay when the first definite signs of muscular dystrophy were discernible by the righting reaction. At least 3 rabbits were used/treatment. The influence of DPPD on hypercholesterolemia associated with Vit. E deficiency(5) was studied in time series by sacrificing animals at 0, 8, 15 and 22 days after onset of treatment. Free plasma cholesterol was determined by the method of Nieft and Deuel(6). The unesterified cholesterol content of skeletal thigh muscle was also determined after grinding the samples with anhydrous sodium sulfate and acid-washed sand, and extracting for 48 hr with skelly-solve F in a Soxhlet apparatus. The hemolytic behavior in hydrogen peroxide of red blood cells from deficient and DPPD-treated animals was determined using modification of procedure of György *et al.*(7). DPPD was administered orally 20 mg daily as a suspension in 1 ml glycerin containing 0.1 ml Tween 80. One group received 35 mg dl-α-tocopheryl acetate in the same carrier 3 times weekly throughout experiment. Negative controls were treated with the carrier alone. Solanachromene was administered in 50 mg amounts daily, orally or intraperitoneally, as a suspension containing 20 parts ethanol and 80 parts 1% aqueous Tween 80. Ubiquinone, its hydroquinone and

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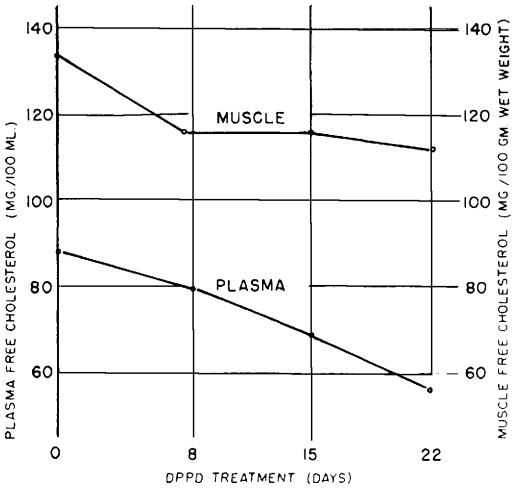

FIG. 1. Influence of DPPD administration on free cholesterol level in plasma and muscle of vitamin E deficient rabbits.

acid-reduction product were prepared immediately before use and formulated similarly. Acid-reduced ubiquinone was obtained by refluxing ubiquinone in ethanolic solution in the presence of stannous chloride and HCl until colorless, then extracting with peroxide-free ether after addition of water. The product was sensitive to Emmerie-Engel reagent, gave R_f values in accordance with literature values and exhibited an ultraviolet absorption maximum at 295 mμ. Hydroquinone was prepared by shaking ubiquinone in the presence of potassium borohydride in aqueous alcohol until the solution was colorless, then extracting with cyclohexane. Both reduced compounds were administered intraperitoneally. Solanachromene also was evaluated in a curative fertility assay using rats. Five female rats were depleted of Vit. E as described previously(8), were carried through a preliminary reproductive cycle to establish their sterility, then were remated to normal males. At 3 day intervals beginning one day prior to 5-day mating period, each female was given orally 18.4 mg solanachrome (a total of 128.8 mg) in an emulsion consisting of 10 parts ethanol and 90 parts 16% Tween 80. Control animals were given 5 mg dl-α-tocopheryl acetate daily.

Results. As found previously(9), gross symptoms of muscular dystrophy in rabbits responded rapidly and completely to treatment with DPPD. The data in Fig. 1 demonstrate that a gradual remission of hypercholesterolemia occurred during the regenerative period, as evidenced by decline in free plasma cholesterol from 88.5 to 55.7 mg % in 3 weeks. The latter value approaches the 47.6 mg % concentration for controls which received Vit. E throughout experiment, and indicates that a somewhat longer period is required for complete restoration of normal plasma levels. These results are not in conformity with these of Shull, Ershoff and Alfin-Slater(10) regarding the effect of DPPD on hypercholesterolemia of Vit. E deficiency. The cholesterol content of muscle lipid was greatly elevated in dystrophic animals and, although a decline during the period of DPPD treatment is evident, the final value of 112.5 mg/100 g is still in marked excess of 68.5 mg/100 g found for controls. A regenerative period of 3 weeks is obviously inadequate for restoration of normal cholesterol levels in skeletal muscle.

Erythrocytes taken from dystrophic rabbits were resistant to hemolysis by dialuric acid (11) but hemolyzed immediately in hydrogen peroxide. Following DPPD administration for 15 or 22 days, no hemolysis occurred for several hours. The cells were permeable to hydrogen peroxide, as indicated by oxidation of hemoglobin to methemoglobin, but this conversion did not appear to influence susceptibility to hemolysis and was not affected by DPPD.

No evidence of Vit. E activity was obtained from experiments with ubiquinone or acid-reduced ubiquinone. Up to 200 mg of either compound administered for 1 week, failed to influence the course of the disease or delay death. Similar results were obtained with hydroquinone, administered in 2 or 3 daily doses of 100 mg each. Fifty mg solanachromene daily, administered orally for 5 consecutive days or intraperitoneally for 4 days, also elicited no remission of symptoms or delay in mortality. Further, no offspring were obtained from female rats fed this compound as a supplement to Vit. E deficient diet.

Comparison of molecular structure of solanachromene with that of γ-tocopherol (substitution of a chromene for a chromane ring and 9 unsaturated isoprene units in the side

chain for 3 saturated isoprenes) is suggestive of similar functional activity. Based on a minimum effective dose of 0.4 mg α-tocopherol/kg/day for remission of dystrophy in rabbits(12) and a biopotency for γ-tocopherol in relation to α-tocopherol of 8%(13), it may be calculated that if solanachromene and γ-tocopherol were equivalent in activity on a molar basis, 15 mg of solanachromene/day should represent a curative dose for animals in this experiment. Since 50 mg/day failed to elicit any response, the activity of solanachromene, if any, is apparently considerably less than one-third of that of γ-tocopherol.

Our experiments with DPPD extend the number of Vit. E deficiency symptoms which already have been demonstrated to yield to this antioxidant. Previous studies(9) have shown that the preventive and curative effect of DPPD on muscular dystrophy in rabbits and sterility in rats is not due to conservation of Vit. E in tissues or in the diet. In only one respect has replacement been found to be quantitatively incomplete, *viz.*, in maintenance of reproduction in the female rat, which is highly susceptible to DPPD intoxication(8). These and other findings, including effectiveness of DPPD in reversing respiratory decline in necrotic liver degeneration when administered intraportally(14) and the reported absence of Vit. E in tissues of normal chicks fed low-fat diets deficient in this vitamin(15), strongly indicate that the tocopherols represent a class of natural antioxygenic compounds which play an important but non-specific role in metabolism.

Summary. Curative tests of Vit. E activity of ubiquinone, its hydroquinone and acid reduction product in relation to muscular dystrophy in the rabbit were negative. Similarly, solanachromene exhibited no biological activity with respect to dystrophy in rabbits or sterility in rats. Vit. E-deficient dystrophic rabbits underwent a remission of gross symptoms within approximately 5 days following oral treatment with N,N'-diphenyl-p-phenylenediamine. The elevated plasma concentration of unesterified cholesterol declined to near-normal levels within 3 weeks, while skeletal muscle concentration responded more slowly. The results confirm previous findings indicating that the Vit. E requirement of rabbits and rats represents a need for suitable antioxygenic compounds.

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