sensitization was probably due to a change in cells rather than alteration of absorbed neutral red.

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Utilization and Toxicity of Dialdehyde- and Dicarboxyl-Starches.* (25380)

ROBERT H. WILSON
Western Regional Research Laboratory,† Albany, Calif.

The modified physical and taste characteristics of oxidized starch have suggested the possibility of use in foods. A preliminary study of effects on the rat is presented here. Anhydroglucose units of starch can be oxidized by periodic acid to dialdehydes(1). The reaction is stoichiometric so that the number of glucose units thus oxidized can be regulated by amount of periodic acid used. An electrolytic procedure which regenerates the periodic acid as it is used up has recently been introduced(2). Further oxidation by chlorous acid quantitatively converts the aldehyde groups to carboxyl groups(3). The reactions are represented in Fig. 1.

Materials. Oxidized cornstarches were obtained from Northern Regional Research Lab., Peoria, Ill. Four dialdehyde starches had 0.05, 2, 40 and 100% of glucose units oxidized. These will be referred to as DAS-.05, -2, -40, and -100, respectively. The dicarboxyl starch had 5% of the glucose units oxidized (DCS-5). *Methods. Acute toxicity* tests were not performed with DCS, because some animals in the subacute study were ingesting daily 16-17 g/kg without apparent ill effects. Acute oral toxicity was studied with DAS-100. The material was dissolved with a minimum of NaOH, neutralized immediately with HCl and given to young adult rats (2 males and 5 females) at 1 g/kg. One male rat died after 11 days with multiple lung abscesses, presumably not due to the material. The others remained alive and healthy during 2 weeks observation. The basal diet for subacute toxicity experiments consisted of corn meal, 51.5; casein, 11.5; linseed oil cake meal, 10; alfalfa meal, 2; cod liver oil, 3; bone ash, 1.5; NaCl, 0.5; and cornstarch, 20%. Experimental diets were made by replacing an equal amount of corn starch with the desired amount of the appropriate oxidized starch. The animals were 30-day-old male rats, except for the DAS-0.05 test, where females were used. They were caged in groups of 4 to 6 animals, with water and food available *ad lib.* Feeding of diets continued for 90 to 100 days except when marked toxicity or limited supply forced earlier termination (lack of sufficient DAS-0.05 ended the feeding of 20% diet after 41 days). Concentrations of oxidized starches ranged from 1 to 20% of the diet for each of the DAS products, and from 0.2 to 20% for DCS-5.

* Histopathological examinations were done by Dr. Wm. E. Ribelin.

† Agric. Research Service, U. S. Dept. Agric.

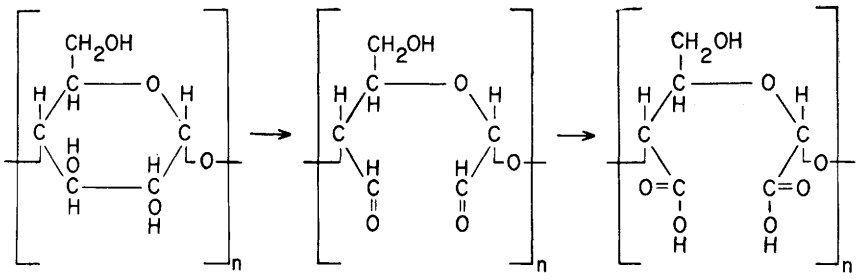

FIG. 1. Oxidation of anhydroglucose units of starch.

Results. Continued feeding of diets containing 10 and 20% of DAS-100 led to early death. Concentrations of 5 and 2%, and probably 1%, inhibited growth. DAS-40 permitted normal growth at dietary level of 1%, inhibited growth at 5%, very poor growth at 10%, and early death at 20%. Feeding of DAS-2 and -0.05 permitted normal growth at all levels including 20%. Low food intake accompanied poor growth; in no case was excessive food intake noted. Growth and appearance were normal with DCS-5, but food intake seemed excessive. This will be discussed below.

No gross abnormalities which could be attributed to the diets, were seen at autopsy. Histopathological examination of tissues showed testicular atrophy in rats receiving DAS-100 and -40. In spite of finding testicular atrophy in one control, it appears very probable that DAS-100 produces this atrophy in sexually mature rats in concentrations of 1% or more of the diet. There was an unusual multinucleated cell formation within testicular tubules. Diets containing DAS-40 produced various degrees of testicular atrophy in sexually mature rats in concentrations of 5% or more. Formation of multinucleated cells was not as conspicuous in this group. No significant microscopic abnormalities were detected in any of the DCS-5 animals.

Although growth rates were uniform between groups of DCS animals, there was from the start an apparently much greater food intake for rats receiving the 20% diet than for those on control diet (13.2 g vs 11.2 g/rat/day at the end of experiment), and possibly rats receiving the 2% diet ate more (11.7 g). To check on the possibility of food wastage, the following experiment was tried. Six male rats 90 days old were placed in individual false-bottom cages, with facilities for collection of feces and wasted food. Half received control diet and half the 20% DCS-5 diet. Food wastage was minimal, showing that rejection of objectionable food was not a factor in the results. Body weights, food intake, and weights of air-dried feces were obtained for individual animals at 3- or 4-day intervals for 2 weeks. Growth was fairly uniform. Food intakes and fecal outputs are shown in the Table. It seems clear that the animals were not utilizing the DCS diet as efficiently as that made with unmodified cornstarch. The greater food intake, however, furnished sufficient calories to maintain normal growth.

This poorer utilization evidenced by greater food intake and fecal output suggested that digestion of oxidized starch might be incomplete. This led to the following qualitative test. Boiled cornstarch and DCS solutions (1%) were mixed with saliva. Aliquots of the digestion mixtures were tested at intervals with dilute I-in-KI and with Benedict's qualitative sugar reagent. Both starches were digested to the point of no color with iodine, although rate of digestion seemed to be faster for cornstarch. Probably the reduction of

TABLE I. Effect of DCS-5 on Food Intake and Fecal Output.

Diet	Food intake, g/rat/day	Feces, g/rat/day	Feces × 100 / Food intake
Control	10.57	1.04	9.8
	11.21	1.06	9.5
	11.86	1.09	9.2
Avg	11.21	1.06	9.5
DCS-5	13.36	1.54	11.5
	11.29	1.26	11.2
	13.14	1.56	11.9
Avg	12.60	1.45	11.5

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)

H. H. DRAPER (Introduced by H. H. Mitchell)
Division of Animal Nutrition, University of Illinois, Urbana

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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