Toxicity Studies on Dibutyl- and Diamyl-Hydroquinone. (25715)

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Carlson and Brewer(1) reported on chronic toxicity of the antioxidant, hydroquinone. The derivatives of HQ, reported here, are even less toxic than the parent compound. High, Woods, and Wilson(2) gave mono-*tert*-butylhydroquinone (10 mg/day) to rats by mouth for 20 days to study effect on utilization of carotene. They reported no significant effect on growth and mentioned no adverse symptoms. Fassett and Roudabush(3) injected mono- and 2,5-di-*tert*-butylhydroquinone (DBH) intraperitoneally into growing rats 5 times weekly for 4 weeks, the highest daily dosage being 200 mg/kg or about half the LD_{50}. The highest level of mono compound interfered with growth, while there was no such response to 2,5-derivative. Oral administration of DBH in comparable dosage to dogs for 2 months, and to rats for an undisclosed time, produced no evidence of cumulative action. Effects of DBH and 2,5-di-*tert*-amylhydroquinone (DAH) to chickens has been reported(4). Acute oral administration of DBH produced toxic symptoms at levels of 2 g or more/kg, and 50% mortality when 10 g/kg was given. Continued administration of 0.25% in feed led to nervous symptoms, increased mortality, decreased growth, and reduced egg production and hatchability. On the other hand continued feeding of DAH produced no more than a slight impairment of growth. The present study deals with oral toxicities of DBH and DAH to rats, with skin toxicity and sensitization, and, briefly, with effects on hair pigmentation.

Methods. Chronic toxicity tests were made with weanling albino rats from our colony, incorporating test substances into diet and feeding *ad lib.* for considerable periods. DAH and DBH were mixed with 2 basal diets, because we(5) have found that chronic toxicity may be greatly affected by diet. The diets chosen were: (A), a ground, commercially available laboratory chow, and (B), our usual basal diet, containing in %: corn meal, 73; linseed oil cake meal, 10; alfalfa meal, 2; casein, 10; cod-liver oil, 3; bone ash, 1.5; and NaCl, 0.5. Substances under study were added with thorough mixing to basal diets to furnish following concentrations: 0, 0.0125, 0.025, 0.05, 0.1 and 0.2% of diet. One hundred fifteen male and 60 female rats were used, 5 animals to a cage. There were 10 males on the control and on each experimental diet except the one with 0.0125% in basal diet A. This group and all groups of females contained 5 animals each. Body weights, food intakes and general appearance were observed weekly for 200-216 days, then half of the males of each group were autopsied, and all females receiving Diet A. The remainder were continued with monthly weighings until approximately 500 days from start of feeding, and then autopsied. Gross appearance of tissues was noted, and organ weights were recorded for animals autopsied at 200 days. From all animals portions of heart, lungs, liver, kidney, intestine, stomach, thyroid, spleen, bladder and testis or ovary and uterus were fixed in formaldehyde. Paraffin sections stained with hemotoxyl and eosin were prepared for histological examination. Because our primary interest was on effects of continued ingestion, only a few animals were used in the negative study of acute oral toxicity. Each hydroquinone derivative was administered by stomach tube as 20% solution in ethyl alcohol to one rat at dosage levels of 500 and 1000 mg/kg, and as 30% solution in cottonseed oil to 5 rats at 1500 mg/kg level. Reactions were compared to those produced by hydroquinone as a 20% solution in ethyl alcohol. Rabbits and guinea pigs were studied for skin irritation. Ten per cent solutions of DAH and DBH in ethyl alcohol or cottonseed oil were prepared and 0.1 ml of each solution was placed on skin daily, except on week-end, for 2 weeks. Development of lesions and time of healing were noted. Studies of skin sensitization were made on guinea pigs by the

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method of Draize, Woodard and Calvery(6). For this test, suspensions of hydroquinones were made by dissolving 0.1 g of the crystals in 5 ml of ethyl alcohol, adding 0.1 ml of Tween 80 and diluting to 100 ml with 0.9% NaCl solution. Oettel(7) reported that daily oral administration of hydroquinone to black cats caused achromotrichia. To see if DAH and DBH had a similar action, each was mixed with ground rabbit chow and fed to black guinea pigs *ad lib.* 3 weeks. Concentration was 0.4% the first week, and 0.2% thereafter. No clearly discernible bleaching of hair occurred with either compound. Longer feeding was not possible because of unacceptability of food. A single black kitten was given DBH daily except week-ends for 2½ months. During final 6 weeks, the daily dose was 100 mg, in capsules. A partial browning (not graying) of hair occurred, probably attributable to aging, since the hair did not regain its former black color when DBH administration was discontinued.

Results. Body weights of experimental animals receiving DAH or DBH were, with one exception, similar to those of controls at end of 200 days. The exception was a significant decrease in rate of growth of female rats receiving 0.2% DBH in diet B. These animals weighed 221 ± 4.1 g at this time, compared to 250 ± 5.2 g for controls. The significance of this decreased growth is less apparent in view of the lack of effect on all other groups.

Red and white cell counts and hemoglobin concentrations of rats on diets containing 0.2% DAH or DBH did not differ significantly from values of control rats after 100 or 200 days on the diets. Normal hemoglobin values were found after 500 days.

Weights of organs (liver, spleen, kidneys, adrenals, heart, and ovaries or testes) were determined for animals autopsied after 200 days. No trend towards change in weight of any of these organs with increase in concentration was observed. The gross appearance of these tissues and those from animals autopsied after 500 days did not differ significantly from that of control animals. Microscopic examination of tissue sections revealed no lesions which could be attributed to the hydroquinone derivatives as fed in these concentrations to male and female rats.

DAH and DBH in amounts up to 1500 mg/kg by stomach tube produced no symptoms other than mild depression attributable to the ethyl alcohol or a loose stool produced by cottonseed oil. As opposed to this, 500 mg of hydroquinone/kg produced tremors lasting several hours, and 750 mg/kg killed 2 of 3 rats. The acute oral toxicity of derivatives was clearly less than that of unmodified hydroquinone, even when considered on an equimolar basis.

When DAH and DBH were dissolved in alcohol and applied to the skin, evaporation of alcohol left a visible, crystalline deposit which usually was not irritating. When dissolved in cottonseed oil, an erythema appeared in 24 hours, disappearing in the next 24 hours. Repeated applications of the oil solutions increased hyperemia, led to papular eruption followed by scab-formation over entire area. When treatment was stopped, lesions gradually disappeared, leaving a normal-looking skin after about a month.

Skin sensitization to these compounds was not found.

Discussion. If these compounds were to be used to stabilize a mixed feed ingredient such as alfalfa meal, it can be assumed that concentration of the compounds on the ingredient would never exceed 0.05% and maximum concentration in mixed feed would be 0.005%. Rats in this experiment receiving 40 times this concentration (0.2%) of hydroquinone derivatives for as long as 500 days were normal, as far as can be judged by available data. The only sign of toxicity noted was a reversible irritation of skin when oil solutions of derivatives were applied to this tissue. This would be a hazard to persons applying the material rather than to animals eating the alfalfa meal. Suitable protection of body and thorough washing with soap of exposed areas should decrease the chance of contact irritation.

In this study there was no indication that the basal diet modified toxicity.

Chickens appeared to be much more susceptible to the butyl compound than were rats. Neither species reacted adversely to the amyl compound.

Summary. Two hydroquinone derivatives, 2,5-di-*tert*-butylhydroquinone and 2,5-di-*tert*-amylhydroquinone, have been suggested as antioxidants to protect carotene of dehydrated alfalfa meal. Neither compound modified growth, appearance, organ weights or histological structure of rats eating diets containing as much as 0.2% of antioxidants for 500 days. No symptoms of intoxication developed in exploratory tests on rats when single doses of 1500 mg/kg of compounds were given by stomach tube. These materials did not sensitize the skin of guinea pigs but did produce a reversible lesion on rabbit and guinea pig skin when applied daily to the skin as 10% solutions in cottonseed oil.

Histopathological examination of tissues was made by Dr. Lelland J. Rather, Stanford Univ. School of Med.

1. Carlson, A. J., Brewer, N. R., PROC. SOC. EXP. BIOL. AND MED., 1953, v84, 684.
2. High, E. G., Woods, L. A., Jr., Wilson, S. S., *J. Biol. Chem.*, 1952, v195, 787.
3. Fassett, D. W., Roudabush, R. L., *Arch. Ind. Hyg. Occupational Med.*, 1952, v6, 525.
4. Heywang, B. W., Gassner, F. X., Thompson, C. R., *Poultry Sci.*, 1958, v37, 839.
5. Wilson, R. H., DeEds, F., *Arch. Ind. Hyg. Occupational Med.*, 1950, v1, 73.
6. Draize, J. H., Woodward, G., Calvery, H. O., *J. Pharmacol. Exp. Therap.*, 1944, v82, 377.
7. Oettel, H., *Arch. exp. Path. Pharmakol.*, 1936, v183, 319.

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Effect of Cortisone on Survival of Morphine Treated Guinea Pigs under Decompression Hypoxia.* (25716)

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It is well known clinically that morphine constitutes a hazard in respiratory impairment. It is generally stated that this is due to depression of the respiratory center. Morphine is known to inhibit release of ACTH following exposure to stressors(1,2) by actions on some site(s) in the central nervous system. Although the purpose served by increase in production of adrenal steroids following such exposure is not clear, it seems possible that this action of morphine may contribute to some of its adverse effects. The effect of simulated altitude upon mortality of guinea pigs with and without concurrent administration of morphine was investigated. Morphine increased mortality under these circumstances. Administration of cortisone and ACTH reduced mortality.

Methods. Guinea pigs weighing 300-350 g of either sex were employed. All animals received 5 ml of saline intraperitoneally at start of observation. Animals receiving morphine were injected with 2.5 mg (or 5 mg) of morphine sulphate/100 g body weight 10 minutes earlier. Those which received cortisone were injected subcutaneously with 60 mg/kg of cortisone acetate twice daily on 3 preceding days and ½ hour prior to injection of morphine. This seemingly large dose of cortisone acetate has been used routinely for experiments with guinea pigs in our laboratory since this species is relatively resistant to its anti-anabolic action(3). ACTH was administered in single dose of 8 U of corticotrophin gel (Organon) subcutaneously and 8 U of corticotrophin powder (Armour) intraperitoneally, 30 minutes prior to morphine injection. When administered in multiple doses, 8 U of gel was injected subcutaneously twice daily for 3 days prior to experiments. On day of experiment, gel and powder were administered 30 minutes prior to morphine injection as indicated above. Two types of evacuation chambers were used, one employing jars with a single animal and the other vacuum ovens with 4 animals at a

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