Lack of effect of butylated hydroxytoluene on dimethylhydrazine-induced colon carcinogenesis in rats[1]

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Summary. Rats were given 6600 ppm of butylated hydroxytoluene (BHT) in the diet along with 10 weekly oral doses of dimethylhydrazine (DMH, 30 mg/kg). The incidence and mean number of colonic tumors produced were similar to that of rats given DMH alone. Thus, BHT did not provide any protective effect against colon carcinogenesis.

Antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been shown to inhibit chemical carcinogenesis induced by polycyclic aromatic hydrocarbons[2-4] and N-2-fluorenylacetamide[5]. Disulfiram prevents the metabolic activation of a potent colon carcinogen, dimethylhydrazine (DMH), thereby inhibiting the induction of colonic tumors[6]. Recently, it has been reported that BHT too inhibits the formation of azoxymethane-induced colonic tumors[7]. The exact mechanism by which the phenolic antioxidants inhibit carcinogenesis is not fully understood, although it is assumed that BHT, by increasing the activity of hepatic microsomal enzymes, enhances the metabolism of carcinogens and the excretion of active metabolites[8,9].

Investigations of possible inhibitors of colon carcinogenesis are currently in progress in our laboratory. Using DMH-induced colonic tumors in rats as a model, we have recently reported the protective effect of ordinary wheat bran[10] as well as the role of macrophage lysosomes in various colonic tumors[11]. The present paper deals with the simultaneous administration of BHT and DMH to rats, and their effect on colon carcinogenesis.

Methods. Male Sprague-Dawley albino rats obtained from Charles River Breeding Laboratories, Inc., Massachusetts, were housed 2 per plastic box with hardwood chip bedding. Animals were allowed free access to a basal diet of ground Wayne® Lab-Blox® and tap water. Symmetrical 1,2-dimethylhydrazine dihydrochloride (DMH, 97% pure) obtained from Aldrich Chemical Co., Inc., Milwaukee, Wisconsin, was dissolved in physiologic saline at a concentration of 15 mg/ml. DMH (30 mg/kg) was given weekly by stomach tube for the first 9 weeks of the study (10 doses). BHT (6600 ppm) obtained from Sigma Chemical Co., St. Louis, Missouri, was mixed with the basal diet.

10 rats each were randomly assigned to 1 of 4 groups: group I: control, group II: DMH, group III: BHT, and group IV: BHT and DMH. DMH and BHT were given concurrently. Individual b.wts were recorded weekly and daily food consumption was calculated 3 times a week. Rats were killed at 23 weeks by an overdose of ether. The intestinal tract was opened longitudinally and carefully examined for polypoid or sessile tumors. Representative numbers of colonic and duodenal tumors were sampled and fixed in cold 70% ethanol:10% buffered formalin:glacial acetic acid (20:2:1) or in 10% neutral buffered formalin and embedded in paraffin. Sections, 5 µm thick, were stained with hematoxylin and eosin for histopathological examination. Statistical analyses were done using the Student's t-test.

Results. Rats from all groups were normal in appearance and behavior. Melena was seen around the anus of a few tumor-bearing animals toward the end of the test period. One rat from group IV was found dead at 20 weeks due to an intestinal obstruction by a large polypoid tumor. A tumor at the base of the external ear was observed at 22 weeks in another rat from group IV. The mean b.wts of rats from group II were depressed during weeks 5-10 (table 1). Following cessation of DMH treatment, the gain in mean b.wts exceeded those of controls. In group III, mean b.wts were depressed throughout the study, but recovered somewhat by 20 weeks. The effect of DMH and BHT individually in depressing mean b.wts was enhanced when both chemicals were given together. Thus, in group IV, mean b.wts remained depressed throughout the study. Food consumption data was similar in all groups.

Intestinal tumors were found only in rats from groups II and IV (table 2). The incidence of colonic tumors in group IV was not affected by BHT. The mean number of tumors/animal (6.4 and 6.1 tumors) was also similar in groups II and IV. Although there was a slight decrease in the incidence of duodenal tumors in group IV (56%) when compared to group II (80%), the mean number of tumors/animal was similar, or even slightly increased (2.0 tumors compared to 1.3 tumors for group II). The incidence and mean number of cecal tumors were slightly increased in group IV when compared to group II. Histopathologic

Table 1. Mean body weights (g) of rats given BHT and/or DMH

Group	Weeks on test					
	0	2	5	10	15	20
I	192±4	297±4	406±6	499±6	567±9	585±15
II	192±3	291±5	378±10*	451±13**	534±15	581±16
III	194±4	275±5**	378±7**	466±9**	529±10*	566±12
IV	201±3	272±5***	360±5***	432±7***	510±8***	536±10*

Values are represented as the mean±SE. Statistically different (*$p<0.05$, **$p<0.01$, ***$p<0.001$) from group I.

Table 2. Effect of BHT on dimethylhydrazine-induced intestinal tumors

Group	Colonic tumors		Duodenal tumors		Cecal tumors	
	Incidence	No./Rat*	Incidence	No./Rat*	Incidence	No./Rat*
II	10/10 (100%)	6.4±0.9 (3-12)	8/10 (80%)	1.3±0.3 (0-3)	1/10 (10%)	0.2±0.2 (0-2)
IV	9/9 (100%)	6.1±0.8 (2-10)	5/9 (56%)	2.0±0.8 (0-7)	4/9 (44%)	1.0±0.4 (0-3)

*Mean±SE for group (range).

examination of representative tumors from groups II and IV revealed the presence of polypoid adenomas, well-differentiated adenocarcinomas and undifferentiated mucinous adenocarcinomas, in keeping with observations made by Ward[12]. The ear tumor found in group IV was identified as a well-differentiated squamous cell carcinoma. The livers of animals given BHT were enlarged, but microscopically they were normal. Multiple metastatic nodules from an intestinal adenocarcinoma were observed throughout the liver in 1 rat from group IV.

Discussion. This study demonstrated that the simultaneous administration of a phenolic antioxidant (BHT) and a potent colon carcinogen (DMH) had no effect on the incidence and number of colon tumors, an observation in agreement with that of Wattenberg[6]. On the other hand, Weisburger et al.[7] claim that BHT inhibited intestinal carcinogenesis induced by a metabolite of DMH, azoxymethane (AOM). In their experiment, BHT was given to F344 rats 2 weeks prior to weekly s.c. injections of AOM. The observed differences between the 2 studies are perhaps due to the use of different strains of rats, the timing of the initiation of BHT feeding and the use of a metabolite of DMH.

BHT stimulates hepatic microsomal enzymes[8,9] and would therefore be expected to alter the metabolism of DMH by enhancing its oxidation to AOM and by increasing the rate of hydroxylation to form the proximate carcinogen, methylazoxymethanol (MAM). Increased formation of MAM-β-glucuronide could decrease the amount of active carcinogen available to react with cellular macromolecules[13]. From the morphologic results presented in this study, it appears that BHT does not alter the metabolism of DMH to decrease its carcinogenicity. Further, if BHT had any effect on the oxidative reactions of DMH metabolism, it would have been more apparent in the present study where DMH was used instead of AOM as in the work of Weisburger et al.[7], since AOM is already in an oxidized state[14]. The reason for the lack of inhibition of BHT on colon carcinogenesis in our study is not clear, but it seems likely that the rate limiting step may be the deconjugation of MAM-β-glucuronide, and this is not affected by BHT.

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Course of development of isolated rat embryonic ectoderm as renal homograft[1]

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Summary. When the isolated head-fold stage rat embryonic ectoderm is grafted under the kidney capsule, it gives rise to a new mesenchyme with the capacity to differentiate into mesodermal tissues.

The rat embryonic ectoderm can be cleanly separated from the mesoderm and tested for the capacity of differentiation in renal homografts[2,3]. We have previously shown that, in these experimental conditions, mesodermal tissues (cartilage, bone, muscle) developed even from isolated pieces of the head-fold stage ectoderm other than the primitive streak and the Hensen's node regions[4]. The possible explanations for this observation are: a) contamination of the ectoderm with adherent mesodermal cells, b) induction of mesodermal tissues in the connective tissue of the host renal capsule, c) neural crest origin of mesenchymal tissues, d) presence of undifferentiated stem cells in the ectoderm, and e) formation of a new ectodermal region of morphogenetic cell movements ('regeneration' of the primitive streak).

In this preliminary experiment, the histology of rat ectodermal grafts was studied at short time intervals in order to establish the course of events leading to the development of the final teratoma.

Material and methods. The head-fold stage rat embryos (9 days) of the inbred Fischer strain were used. The embryonic ectoderm was cleanly separated from the underlying mesoderm by the combined treatment with enzymes

Development of the isolated ectoderm as renal homograft

Time after grafting	Histological structure of grafts
2 days	Thickening of the ectoderm. Numerous mitoses. Differentiation and beginning invagination of the neuroepithelium (fig. 2, a). Immigration of loosely arranged cell groups (mesenchyme) beneath the ectoderm (figure 2, a, b).
5 days	Enlarged, vascularized graft. Predominantly immature neural tissue; massive cell necrosis (figure 2, c). 2-layered epidermis (cysts). Onset of myotube formation. The rest of the mesenchyme undifferentiated.
7 days	Advanced differentiation of the neural tissue (ependyma, choroid plexus) and epidermis (multilayered). Onset of chondrogenesis (figure 2, d).
9 days	Abundant neural tissue, muscle and cartilage. Osteogenesis. Epidermis: onset of keratinization, hair buds.
12 days	Onset of adipose tissue differentiation. Maturation of other tissues.