Effect of Feeding Butylated Hydroxyanisole to Dogs

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Groups of weanling Cocker Spaniel pups were fed BHA at levels of 0.0, 5.0, 50.0, and 250.0 mg. per kg. of body weight per day for a 15-month feeding period to see what effect, if any, these subacute levels of BHA might have on the dog. General health and weight gains were good throughout the period. Hemoglobin and blood cell counts were not appreciably affected by feeding BHA at the levels used here. Urine from dogs fed BHA contained higher levels of glucuronates and a higher ratio of total to inorganic sulfates, indicating that BHA excretion was by this route. Microscopic examination of tissue sections at the time of autopsy showed no changes beyond normal variation, except in three animals which had received the highest dosage, in which liver injury had occurred. The results indicate that dogs can ingest BHA for a long period without harm, at a level at least 220 times the maximum allowable level for this antioxidant in lard.

THE use of antioxidants to prevent the development of oxidative rancidity in animal fats used in food and feed has become very widespread. Permission for the use of butylated hydroxyanisole (a mixture of 2- and 3-*tert*-butyl-4-methoxyphenols, BHA) alone and in combination with other antioxidants and synergists in animal fats and shortenings containing animal fats at specified levels was granted in 1948 (*2*). Since that time, a large proportion of the edible animal fats produced have been treated with BHA as a preservative. The use of such materials in foods makes it desirable to find out more about the effects of the antioxidant when ingested at high levels over a long period of time. The present study was initiated to study the effects of feeding high levels of BHA to dogs.

Previous and concurrent work using rats to study the acute and chronic toxicity of BHA (*4*) indicated that levels of BHA up to 2% of the diet had no demonstrable effect over a 6-month period, and that the LD_{50} for rats was of the order of 4.1 to 5.0 grams per kg. of body weight. This information served as a guide in selecting levels of BHA to feed to dogs in a study of the chronic toxicity of the antioxidant in this species.

Procedure

Sixteen Cocker Spaniel pups were weaned at 6 weeks of age and within 1 to 2 months after weaning, litter mates were assigned to groups with four pups in each group as follows:

1. Control group, basal ration only.
2. Basal ration plus a low level of BHA which probably would produce no adverse effect. This group received 5 mg. of BHA per kg. of body weight per day.
3. Basal ration plus an intermediate level of BHA which might or might not produce injury. This group received 50 mg. of BHA per kg. of body weight per day.
4. Basal ration plus a high level of BHA which approached the highest level tolerated in the diet. This group received 250 mg. of BHA per kg. of body weight per day.

Each group consisted of one male and three females, except group 4 which had two males and two females.

Dosages were adjusted to changes in feed consumption and body weight to maintain the above levels. The basal ration was composed of toasted corn flakes, ground, 24.60%; toasted wheat flakes, ground, 24.56%; soybean oil meal (50% protein), 17.58%; meat and bone meal (50% protein), 13.80%; lard, 8.00%; fish meal (menhaden), 2.76%; wheat germ meal, 4.60%; dried skim milk powder, 2.30%; vitamins A and D oil (2250 U.S.P. units A and 300 I.C. units D per gram), 0.46%; iodized salt, 0.24%; dried brewer's yeast, 0.46%; and riboflavin supplement (BY-500), 0.74%. The BHA levels necessary to obtain the desired dosages were computed and the necessary amounts dissolved in the lard portion before mixing the diets. The dogs were housed in metal cages with expanded metal floors in a heated room and given their food allotment and fresh water twice daily. Body weight and food consumption records were kept for a 14- to 15-month period. Blood and urine analyses were made three times for each dog — twice during the test and again at the time the dogs were sacrificed for autopsy and histological examination of their tissues. The first two blood samples were drawn from a leg vein, and the third sample was collected by heart puncture at the time the dogs were sacrificed.

Results

Weight Gain and Feed Consumption. Weaning weight, final weight at 16 to 18 months of age, and average daily feed consumption are shown in Table I. No appreciable differences in weight gains in the different groups were noted except in the group 4 receiving the highest level of BHA (250 mg. BHA per kg. of body weight daily). The dogs in this group gained less weight and consumed less feed per day. The lowered feed consumption was due to the fact that BHA has a hot, peppery taste which the dogs do not like and it was difficult to disguise the taste of that amount of BHA in the feed. At lower levels (groups 2 and 3), the dogs consumed the feed without difficulty after building up gradually to those levels over an acclimatization period of a few days. During the course of the feeding period, the level of BHA in the diet had no influence on the age at which maximum weight was reached. Some dogs reached their peak weight at 7 months of age, while others continued to gain until they were sacrificed. In all dogs, minor fluctuations in body weight were noted, particularly after about 7 months of age. At the time of autopsy, dog number 11 in group 3 contained a large amount of adipose tissue in the body, and dog number 15 in group 4 was very thin. However, all dogs in the different groups appeared to be normal in general appearance.

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Table I. Effect of BHA Ingestion by Dogs

Group No.	Dog No.	Litter Source No.	Sex	Weaning Wt., G.	Final Wt., G.	Age at Sacrifice, Mo.	Av. Daily Feed Consumption, G.	Hb, %	Red Blood Cells, Blood 1000	Hemoglobin and Blood Cell Counts at Time of Sacrifice: White P.M.N., cells, no.	Differential White Cells[a]: P.M.N. no.	Lympho. no.	Mono. no.	P.M.E. no.	P.M.B. no.
1	1	IV	M	1500	10,000	16	225	17.5	5640	7,200	68	27	4	0	1
	2	II	F	1250	7,900	18	280	17.7	4960	4,500	81	19	0	0	0
	3	III	F	1150	10,800	18	280	17.3	6180	4,250	65	35	0	0	0
	4	I	F	1100	6,900	18	225	15.0	4870	7,000	75	23	2	0	0
							Av. 252	16.9	5412	5,737	72	26			
2	5	I	M	1450	9,000	18	240	17.0	5520	5,050	79	21	0	0	0
	6	II	F	1350	9,400	18	225	16.0	6200	8,900	69	30	1	0	0
	7	III	F	1200	10,300	18	270	16.7	4930	7,800	74	25	1	0	0
	8	IV	F	1200	6,450	16	255	16.5	6040	8,100	64	36	0	0	0
							Av. 245	16.6	5672	7,462	71.5	28			
3	9	II	M	1750	9,350	18	285	15.0	6760	3,250	83	23	4	0	0
	10	I	F	1450	6,150	18	240	15.2	5920	7,300	82	17	1	0	0
	11	III	F	1300	11,150	18	255	14.5	5530	10,900	72	26	2	0	0
	12	IV	F	1400	5,200	16	240	14.5	7070	13,200	79	18	3	0	0
							Av. 255	14.8	6213	8,681	79	21			
4	13	III	M	900	6,600	18	195	15.0	6670	5,450	78	18	3	1	0
	14	II	F	1400	6,500	18	210	15.0	6456	11,800	75	22	3	0	0
	15	I	F	1175	4,325	18	165	15.5	6310	10,900	79	20	1	0	0
	16	IV	M	1450	6,050	16	240	16.0	7510	7,450	62	33	3	2	0
							Av. 202	15.4	6736	8,900	73.5	25.7			

[a] Differential white cell counts. P.M.N. = polymorphonuclear neutrophils. Lympho. = lymphocytes. Mono. = monocytes. P.M.E. = polymorphonuclear eosinophils. P.M.B. = polymorphonuclear basophils.

Table II. Urinalyses of Dogs Fed BHA

(Mg./100 Ml.)

Group No.	Dog No.	Color	pH Reaction to Nitrazine Paper	Albumin	Sugar	Color Test for BHA in Urine	Glucuronic Acid in Urine[a]	Sulfates/100 Ml. Urine: Total sulfates	Inorganic sulfates	Ethereal sulfates	Ratio of Total to Inorganic Sulfates
1	1	Pale yellow	Alkaline, high	−	±		151.3	10.55	8.95	1.60	1.18:1
	2	Pale yellow	Alkaline	±	−		62.5	9.77	8.16	1.61	1.20:1
	3	Pale yellow	Alkaline, high	−	−		215.0	6.06	4.36	2.30	1.39:1
	4	Pale yellow	Alkaline, high	−	±						
							Av. 142.9	8.79	7.15	1.84	1.26:1
2	5	Yellow	Alkaline, high	±	−		240.0	9.41	7.31	2.10	1.29:1
	6	Pale yellow	Acid, slight	−	+		470.0	10.87	9.23	1.64	1.18:1
	7	Pale yellow	Alkaline	−	−	+	370.0	7.42	6.14	1.28	1.21:1
	8	Yellow	Alkaline, high	−	+	+					
							Av. 360.0	9.23	7.56	1.67	1.33:1
3	9	Pale yellow	Alkaline, high	−	−		117.5	4.45	3.19	1.26	1.40:1
	10	Pale yellow	Alkaline, high	−	±	+	437.5	7.04	4.11	2.93	1.71:1
	11	Pale yellow	Alkaline, high	−	−	+	187.5	6.75	4.34	2.41	1.56:1
	12	Pale yellow	Alkaline, high	−	±	+					
							Av. 247.5	6.08	3.88	2.20	1.56:1
4	13	Yellow amber	Alkaline, high	±	+	+	460.0	6.10	0.59	5.51	10.41:1
	14	Yellow amber	Alkaline, high	−	+		430.0	4.13	0.46	3.67	9.06:1
	15	Pale yellow	Neutral	−	+	+	427.5	5.91	2.22	3.69	2.66:1
	16	Yellow amber	Acid, slight	−	+	−					
							Av. 439.1	5.38	1.42	4.29	7.37:1

[a] Glucuronic acid in 1-day collection.
Microscopic appearance of urine was normal for all dogs except that urine of dog No. 16 contained white blood cells and bile.

Blood Studies. Results of blood studies are shown in Table I. Blood studies were made at intervals of approximately 5, 8, and 13 months after start of the feeding test. Hemoglobin, red cell counts, white cell counts, and differential white cells shown in Table I are values obtained at 13 months or when the dogs were sacrificed. While there was a tendency toward a slight increase in red cells and white cell counts as the level of BHA in the diet was increased, the values are within the normal range. The feeding of BHA at the levels used here appeared to have no appreciable effect upon hemoglobin level or upon cell counts.

Urinalysis. Routine urinalyses were run on each dog several times during the feeding test. Values obtained at the end of the feeding period when the dogs were sacrificed are shown in Table II. Positive sugar tests were obtained on some of the dogs that received both the control diets and the diets containing BHA, although there were only two dogs in groups 1, 2, and 3 whose urine showed only a faint positive sugar test while urine from all four dogs in group 4 was strongly positive for sugar. Average glucuronate levels were increased in the urine from dogs receiving BHA in their diet.

Samples of urine collected from the dogs at time of sacrifice were assayed qualitatively for BHA, using a color reaction based on the reagent *N*-2,6-trichloro-*p*-benzoquinoneimine. It was observed that all of the dog urine samples reacted with the reagent to give shades of blue and green, indicating that the urine contained small amounts of ma-

terials that react similarly to BHA to produce colored products. Absorbance curves were plotted, and indicated that BHA or other compounds giving a similar color reaction were present in the urine of dogs receiving BHA in their diet at the levels fed.

Quantitative assays of urine for total sulfates and inorganic sulfates were run on composite samples consisting of three or more urine samples from three dogs in each lot. Results show that dogs on the control diet, and on a low level of BHA (5 mg. of BHA per kg. of body weight) had normal urinary sulfate ratios. Dogs on the intermediate level (50 mg. of BHA per kg. of body weight) had a slight increase in organic sulfate, while dogs fed the higher level of BHA (250 mg. of BHA per kg. of body weight) had a considerably increased proportion of organic sulfates in the urine.

General Appearance of Dogs. All of the dogs were alert and active, had healthy hair coats and mild dispositions, and appeared to be healthy and contented throughout the course of the feeding period. The feeding of BHA at the levels here used appeared to have no effect upon the outward appearance or general health of the dogs.

Autopsy Findings and Histological Studies. Gross examination of the organs at autopsy revealed one animal (dog No. 3 in control group) with focal endocardial calcification of the left atrium without other lesions. The remainder of the animals showed no gross lesions except for one dog in the group receiving the highest dosage (dog No. 16, group 4) which had bile in the urine.

There was no obstruction of the common, cystic, or hepatic ducts in this dog, but the liver was bile stained and the capsular surface was finely granular.

Microscopic examination of representative sections from heart, liver, lung, pancreas, spleen, kidney, intestine, lymph node, stomach, thyroid, parathyroid, adrenal, gonad, and bone marrow revealed no changes beyond normal variation except in group 4 receiving the highest level of BHA in the diet. Abnormal variations in this group were all associated with the liver. Three of the four animals of group 4 had a liver cell degeneration and a diffuse granulocytic infiltration. The lobular structure of the liver was normal and there was no excessive connective tissue proliferation. There was diffuse parenchymal degeneration, with marked narrowing of the hepatic sinusoids. The Kupffer cells contained an increased amount of hemosiderin and there were focal accumulations of bile pigment in the periportal areas.

Functional and structural changes occurring during any hepatic disorder are usually more manifest in animals with insufficient dietary intake. The average daily feed intake of dogs 13, 14, and 15 in group 4 was lower than the average intake of dogs in other lots, but dog No. 15 with the lowest daily feed intake did not show signs of liver degeneration. However, during the course of the experiment it became apparent that this dog showed a definite dislike for feed containing the 250 mg. level of BHA. Because of this dog's dislike for BHA, it was decided to reduce the level of BHA in its feed, but to maintain as high a level as possible and yet allow proper feed intake for maintenance and growth. As a result, the average daily intake of BHA for dog No. 15 amounted to only 183 mg. of BHA per kg. of body weight, which may have accounted for the absence of liver degeneration in this dog.

Discussion and Conclusions

To all outward appearances, the dogs were not affected by the BHA in their diet except insofar as the BHA reduced palatability of the diet. There was a tendency toward reduced feed intake, particularly in group 4 receiving the highest level of BHA. Reduced feed intake was probably a contributing factor toward lower weight gains by the dogs in this group.

BHA in the concentrations here fed had no effect on the blood picture of the dog. Hemoglobin levels and cell counts did not seem to vary appreciably among the groups.

Results of urinalysis indicate increased levels of glucuronates in the dogs fed BHA, and a changed ratio of ethereal to inorganic sulfate. Positive sugar tests in urine from dogs fed BHA may have been due to the high levels of glucuronates in the urine. It would appear that the dog excretes BHA in the form of glucuronates and sulfate conjugates. Similar BHA excretion pathways have been reported for the rabbit (*1*).

At the time the dogs were sacrificed, they all appeared vigorous and well nourished, and of the treated animals, only the one animal in the group receiving the highest dosage of BHA appeared to have any gross pathological condition.

Liver injury was found by microscopic examination in three of the four animals in the group receiving the highest dosage of BHA. All other organs and tissues examined appeared to be normal.

It would appear from these studies that the dog is able to ingest BHA at the two levels here fed (5.0 mg. and 50.0 mg. per kg. of body weight per day) with no deleterious effects. However, when the dog is fed 250 mg. of BHA per kg. of body weight per day, the dog is unable to cope with this large amount without damage to the liver. Because three of the four dogs in group 4 weighed over 6000 grams at the end of the feeding period, these three dogs were each receiving more than 1.5 grams of BHA per day. The other dog in group 4, dog No. 15, did not consume as much food per day, did not gain as much weight, and refused to consume BHA at a level of 250 mg. per kg. of body weight. It did consume an average of 183 mg. of BHA per kg. per day, so that at the end of the test period this dog was consuming approximately 786 mg. of BHA per day, or roughly half of the amount consumed by the other three dogs in the group. This dog showed no sign of liver injury at the time of sacrifice.

Present regulations (*3*) allow the use of BHA in lard at a level not to exceed 0.01%. The lowest level of BHA fed here to dogs was 5 mg. per kg. of body weight per day, which amounted to approximately 0.22% of BHA in the lard of the diet or 22 times the maximum allowed in lard for human consumption. Group 3 received approximately 2.24% of BHA in the lard, or 220 times the maximum amount allowed in lard. At 22 and 220 times the maximum allowable levels of BHA in the lard, no evidence of injury was observed. It was only in group 4 where three of the dogs received 250 mg. of BHA per kg. of body weight per day, or approximately 11.0% of BHA in the lard of the diet and 1100 times the maximum level allowed in lard, that any liver injury was observed. The one dog in group 4 (dog No. 15) that actually consumed about half of this amount, or approximately 550 times the level allowed by Meat Inspection Division, Bureau of Animal Industry regulations, did not show liver injury.

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