Long-term Exposure to the Anti-inflammatory Agent Phenylbutazone Induces Kidney Tumors in Rats and Liver Tumors in Mice

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Long-term toxicity and carcinogenicity of phenylbutazone, a nonsteroidal anti-inflammatory drug, were evaluated in F344/N rats and B6C3F1 mice. In 2-year studies, phenylbutazone was given in corn oil by gavage 5 days per week to groups of 50 rats of each sex at doses of 0, 50, or 100 mg/kg body weight, and to groups of 50 mice at doses of 0, 150, or 300 mg/kg body weight. Body weights and survival were similar among groups. Major target organs are kidneys in rats and liver in mice. Kidney: inflammation, papillary necrosis, and mineralization in both sexes of rats, and hyperplasia and dilatation of the pelvis epithelium, and cysts in female rats. Uncommon tubular cell tumors of the kidney were found in 13 exposed rats: 5 in the 50 mg group and 4 in the 100 mg group of males; 4 in dosed female rats; none in controls. In female rats, dose-related increases in hyperplasia of the pelvis transitional epithelium, and 2 carcinomas were discovered. Urinary bladder: papillomas of the transitional epithelium were seen in 2 low-dose male and in 1 low-dose female rats. Forestomach: ulcers in rats, with acanthosis, hyperkeratosis, and basal cell hyperplasia in female rats; however, no neoplasms were associated with these lesions. Liver: primarily in male mice exposed to. phenylbutazone, hemorrhage, centrilobular cytomegaly and karyomegaly, fatty metamorphosis, cellular degeneration, and coagulative necrosis were seen; clear cell foci were observed in male mice. In summary, under the conditions of these 2-year oral intubation studies, phenylbutazone is associated with renal carcinogenicity in rats, as evidenced by increases in tubular cell neoplasms in both sexes. Evidence of carcinogenicity for male mice was shown by increased incidences and multiplicity of liver tumors. No carcinogenic activity was found for female mice.

Key words: F344 rat — B6C3F1 mouse — Hepatocellular tumor — Tubular cell tumor

Phenylbutazone (CAS #50-33-9; Butazolidin), first synthesized in 1914, was introduced in 1949 for the treatment of rheumatoid arthritis and related disorders for humans, and was introduced to veterinary medicine in 1952.[1] The antigout, antirheumatic, and antiphlogistic properties of phenylbutazone result from potent non-steroidal anti-inflammatory activity.[2] Of the pyrazolone derivatives, phenylbutazone is the most important, but serious side effects limit its suitability for long-term therapy (e.g., agranulocytosis). Extensive clinical experience with phenylbutazone revealed a number of toxicities. Mucosal ulceration is commonly observed in all species: rodents, dogs, horses, and humans.[3, 4] Signs of this, which have been most completely described in equines, include ulceration of mucosal surfaces in the oral cavity, glandular stomach, and colon.[5, 6] Focal hemorrhagic lesions induced by the administration of phenylbutazone to rats result from mucosal abrasion secondary to repeated vagal-stimulated peristaltic waves. Phenylbutazone-induced gastric acid synergizes, but is not essential for, the mucosal erosion.[7] In rats and mice, phenylbutazone (50–200 mg/kg) causes renal cell papillary necrosis, progressive proteinuria, and concomitant hyperplastic

changes in the collecting ducts.[8, 9] Similar lesions have been described in dogs and cats.[10]

Evidence for carcinogenicity to humans is inadequate[11]; while cases of leukemia have been reported in patients following phenylbutazone therapy, the significance of these observations is unclear given the widespread use of phenylbutazone. Lymphocytes from rheumatoid arthritis patients undergoing phenylbutazone therapy have shown significant increases in chromosomal aberrations following treatment with 300 mg/day for 3–7 months[12] or 600 mg/day by infusion for 10 days.[13]

Phenylbutazone is not mutagenic to bacteria.[1, 14–18] Similarly, two metabolites of phenylbutazone, oxyphenbutazone and γ-ketophenylbutazone, did not induce gene mutations in bacteria.[14, 19, 20] However, phenylbutazone is genotoxic and clastogenic in some mammalian assay systems; chromosomal aberrations are induced by phenylbutazone in cultured hamster lung fibroblasts,[16] in Chinese hamster ovary cells,[1, 21] and in human lymphocyte cultures.[22]

The cytotoxic and genotoxic activities of phenylbutazone toward mammalian cells combined with some evidence of carcinogenicity in Donryu rats[23] suggested that this chemical should be further evaluated as a possible carcinogenic hazard to humans. We describe herein

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the results of long-term toxicity and carcinogenicity experiments on phenylbutazone in rats and mice.

MATERIALS AND METHODS

Animals Weanling inbred Fischer 344/N rats and B6C3F1 hybrid mice obtained from Harlan Industries (Indianapolis, IN; 13-week studies) or Charles River Breeding Laboratories (Kingston, NY; two-year studies) were held in quarantine for up to 4 weeks for acclimatization and to document the absence of parasites or diseases. Animals of the same species and sex were housed 5 per cage on hardwood bedding in polycarbonate cages in rooms controlled for temperature (20–27°C), relative humidity (50–79%), and light cycle (12 h fluorescent light, 12 h dark). NIH-07 open formula rodent ration (Ziegler Bros., Gardner, PA), and tap-water were freely available.

Chemical Phenylbutazone, as a single lot (#62642) from Ciba Geigy Corporation (Summit, NJ), was checked by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. Its purity was determined by elemental analysis. Karl Fischer water analysis, a USP titration method using tetrabutylammonium hydroxide as the titrant, USP chloride and sulfate assays, thin-layer chromatography, and high-performance liquid chromatography (HPLC). Cumulative data (not shown) indicated that the phenylbutazone used was more than 99% pure. Stability of the bulk chemical was monitored periodically by HPLC and no deterioration was seen over the course of these studies.

The phenylbutazone/corn oil suspensions were stable for at least 14 days when stored in the dark at room temperature or at 5°C. During the studies, dosing mixtures were stored for no longer than 2 weeks at 0°C. For quality control, suspensions were analyzed at approximately 8-week intervals and composite data (not shown) indicate that the formulations were within 10% of target concentrations 98% of the time.

Experimental designs Thirteen-week studies: These 90-day studies were conducted to evaluate cumulative toxic effects, to identify target organs of repeated administration of phenylbutazone, and to help select doses to be used in the 2-year studies. Groups of 10 rats (6 weeks old) were administered 0, 25, 50, 100, 200, or 300 mg phenylbutazone/kg body weight in corn oil by gavage, 5 days per week for 13 weeks. Groups of 10 mice (7–8 weeks old) of each sex were administered 0, 40, 80, 150, 300, or 600 mg/kg phenylbutazone on the same schedule. Animals were observed twice daily and individual animal weights were recorded initially and once per week thereafter. At the end of the 13-week studies, survivors were killed, and necropsies were performed on all animals. Histologic examinations were performed on all vehicle control animals, on rats in the 100 and 200 mg/kg groups, on rats in the 300 mg/kg groups that died before the end of the studies, and on mice in the 300 and 600 mg/kg groups. Approximately 40 tissues/organs were examined.

Two-year studies: For the 2-year studies, groups of 50 rats of each sex were administered 0, 50, or 100 mg phenylbutazone/kg in corn oil by gavage 5 days per week for up to 103 weeks. Groups of 50 mice of each sex were administered 0, 150, or 300 mg/kg on the same schedule.

Complete necropsy was performed on all animals in the 2-year studies. All organs/tissues were examined for visible lesions and preserved in 10% formalin. Organs/tissues selected for histopathologic examination were trimmed, embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically, using an "inverse pyramid design."[24, 25] That is, tissues/organs in all high-dose and control rats and mice, and in all low-dose rats and mice dying before the end of the study were examined, as were any gross lesions observed at necropsy. Because the kidney was a prime target organ, three or four additional sections per kidney per animal were evaluated.

Statistical analyses Tumor incidences were analyzed for dose-related trends and for pair-wise comparisons of exposed groups with controls by life-table analysis (for "fatal" tumors), logistic regression tests (for "non-fatal" tumors observed at necropsy), and Fisher's exact and Cochran-Armitage trend tests (based on proportions of tumor-bearing animals).[26–28] Survival probabilities in the 2-year studies were estimated by a product-limit procedure,[29] and analyzed for chemical effects by the methods of Cox[30] and Tarone.[31] Complete details of these studies can be obtained from the 205-page technical report.[1]

RESULTS

Rats

Thirteen-week studies Final mean body weights of rats that received 200 or 300 mg/kg were 8–13% lower than that of vehicle controls for males and 12–13% lower for females. Relative liver weights were increased in the 200 and 300 mg/kg groups. Clinical signs at 200 or 300 mg/kg included diarrhea, unkempt fur, and decreased activity. Renal papillary edema and necrosis, and multifocal mineralization (1 control male) were observed in dosed rats (Table I); severity of mineralization increased with increasing dose. Testicular degeneration occurred in 4/6 males that received 300 mg/kg, 2/10 males that received 200 mg/kg, and 1/10 males that received 100 mg/kg. Lymphoid depletion of the thymus, spleen, mesenteric lymph node, or mandibular lymph node was seen in 6/7 males and 6/8 females that received 300 mg/kg and 1/10 males and 1/10 females that received 200 mg/kg; none in

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Table I. Number of Rats with Selected Renal Lesions in the Thirteen-week Gavage Studies of Phenylbutazone

Lesion	Male					Female					
	Vehicle control	50 mg/kg	100 mg/kg	200 mg/kg	300 mg/kg	Vehicle control	25 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	300 mg/kg
Number examined	10	10	10	10	7	10	10	10	10	10	8
Papillary edema	0	0	5*	0	0	0	0	5*	3	2	1
Multifocal mineralization	1	1	2	9**	4*	0	0	4*	7**	10**	6**
Papillary necrosis	0	0	1	3	3	0	0	0	2	3	3

* $P<0.05$ vs. controls (Fisher's exact test).
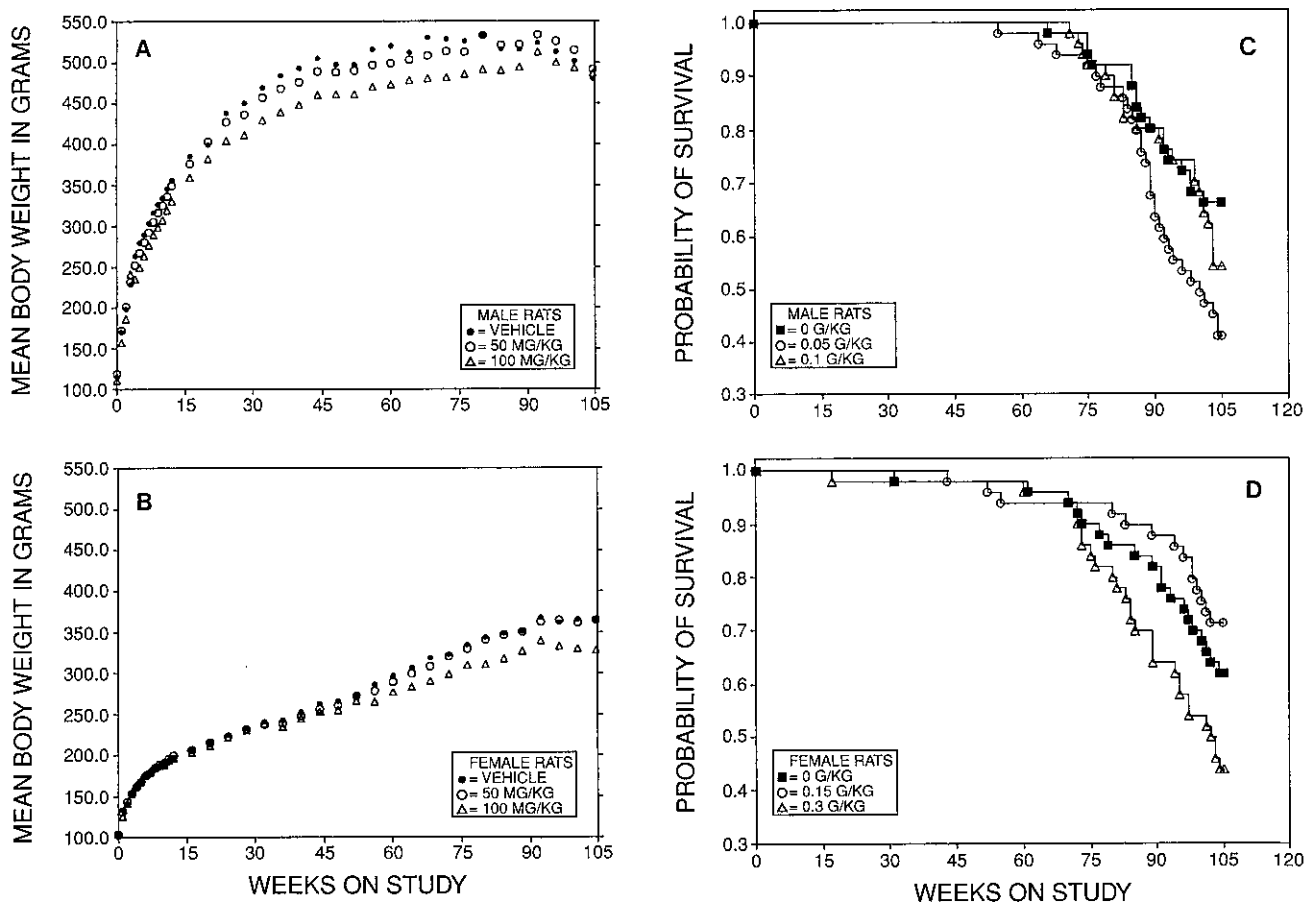** $P<0.01$ vs. controls (Fisher's exact test).

Fig. 1. Growth curves for male (A) and female (B) rats and Kaplan-Meier survival curves for male (C) and female (D) rats exposed to 0, 50, or 100 mg phenylbutazone/kg body weight by gavage.

vehicle controls. Lymphoid depletion was observed only in rats that died. Seven on 10 males and 8/10 females that received 300 mg/kg and 1/10 male and 2/10 female rats that received 200 mg/kg died before the end of the studies. Based on the relative severity of degenerative and necrotic papillary changes, body weight differences, and early deaths, doses selected for the 2-year studies were 50 and 100 mg/kg phenylbutazone.

Two-year studies No compound-related clinical signs were seen. Mean body weights of the top-dose group of male rats were generally 7%–11% lower than those of vehicle controls between week 4 and week 80 (Fig. 1A).

Table II. Incidences and Severity of Nephropathy in Rats in the Two-year Gavage Studies

Nephropathy	Male			Female		
	Vehicle control	50 mg/kg	100 mg/kg	Vehicle control	50 mg/kg	100 mg/kg
Incidence of nephropathy	49/50	49/49	50/50	38/50	46/50	47/50
Severity[a]						
None	1	0	0	12	4	3
Minimal	11	6	5	22	11	1
Mild	25	19	11	14	19	6
Moderate	9	12	15	2	16	17
Marked	4	12	19	0	0	23
Mean severity $\pm$ SE[b]	2.1$\pm$0.1	2.6$\pm$0.1**	3.0$\pm$0.1**	1.1$\pm$0.1	1.9$\pm$0.1**	3.1$\pm$0.2**

a) Number of animals with indicated severity.
b) Mean severity of animals with lesion; 1=minimal; 2=mild; 3=moderate; 4=marked.
** $P < 0.01$ vs. vehicle controls (Mann-Whitney U test).

Table III. Urinary Sysyem Lesions in the Two-year Gavage Studies of Phenylbutazone[a]

Site/Lesion	Male			Female		
	Vehicle control	50 mg/kg	100 mg/kg	Vehicle control	50 mg/kg	100 mg/kg
Kidney						
Number examined	50	49	50	50	50	50
Kidney						
Pelvis dilation	1	1	1	1	0	22**
Papilla necrosis	1	10**	25**	0	18**	41**
Tubule						
Inflammation	6	13	27	2	4	26
Mineralization	5	10	35**	3	31**	46**
Cyst	2	0	2	1	2	16**
Hyperplasia	3	1	4	1	1	2
Adenoma	0	0	3	0	0	0
Carcinoma	0	1	0	0	0	0
Pelvis epithelium						
Hyperplasia	14	7	12	7	20**	37**
Carcinoma	0	0	0	0	0	2
Kidney						
Carcinoma	0	0	0	0	1	0
Urinary bladder						
Number examined	45	43	48	50	49	49
Transitional epithelium						
Hyperplasia	0	0	0	1	0	3
Papilloma	0	2	0	0	1	0

a) Observed at original single-section histopathologic review.
** $P < 0.01$ vs. vehicle contols.

Mean body weights of the top-dose group of female rats were 6%–10% lower than those of vehicle controls, starting at week 56 and continuing until the end of the study (Fig. 1B). Estimates of the probabilities of survival for male and female rats administered phenylbutazone at the doses used in these studies and for vehicle controls are shown in Fig. 1, C and D. For male rats, survival at 2 years was 33/50 in the control group, 20/50 in the low-dose group (P=0.025), and 27/50 in the top-dose group. In female rats, respective survival rates were 31/50, 35/50, and 22/50 (P=0.091).

Kidney A spectrum of nonneoplastic lesions occurred in the kidney of male and female rats given phenylbutazone, including a dose-related increase in the severity of aging-related nephropathy (Table II), necrosis of the papilla, mineralization of the collecting tubules in the papilla

Table IV. Incidence of Selected Tubule Lesions Observed upon Step-section Review of Rat Kidneys[a]

Lesion	Male			Female		
	Vehicle control	50 mg/kg	100 mg/kg	Vehicle control	50 mg/kg	100 mg/kg
Number examined	50	49[d]	50	50	49[d]	49[d]
Cystic hyperplasia	2[b]	4	3[b]	0	0	0
Hyperplasia	2	6	1	1	0	2
Adenoma	0	4	2[c]	0	3	1
Oncocytic hyperplasia	0	1	0	0	0	0
Oncocytoma	0	0	0	0	0	1

a) Diagnoses from original sections are not included.
b) One animal had a diagnosis of renal tubule hyperplasia on the original section.
c) One animal had a diagnosis of renal tubule adenoma on the original section.
d) There was insufficient tissue from one low-dose (50 mg/kg) male and female and one top-dose (100 mg/kg) female rat for step-sectioning. Consequently, the denominators for these groups do not equal 50.

(Table III), and acute inflammation of the proximal convoluted tubules. Nephropathy consisted of degeneration of renal tubular epithelium in the cortex, with atrophy and dilatation of the tubules, formation of hyaline casts, regeneration of the tubular epithelium, interstitial fibrosis, and glomerulosclerosis. The less severe stages of papillary necrosis were characterized by loss of cellular detail in the mid or distal papilla due to degeneration and loss of epithelial cells, necrosis of interstitial cells, and hyalinization of the vasa recta. In more advanced lesions, distal papillae were completely necrotic with dystrophic mineralization of the devitalized tissue, or necrotic tissue had sloughed completely, causing deformity of the papilla. In addition, there was mild inflammation consisting of accumulations of neutrophils and cellular debris in the lumina of individual tubules in the cortex of dosed rats. Hyperplasia of the renal pelvis epithelium, dilatation of the pelvic lumen, and cortical cysts were also increased in dosed female rats relative to vehicle controls.

In the routine single sections of kidney tissue, renal tubular cell adenomas (Table III) occurred in 3/50 high-dose male rats, and a tubular cell carcinoma occurred in one low-dose male rat. The historical incidence of renal tubular cell neoplasms in corn oil vehicle control male F344/N rats in National Toxicology Program (NTP) studies is 11/2,092 (0.5%), making these uncommon in control animals. Tubular cell hyperplasia was not increased. An anaplastic carcinoma of uncertain histogenesis was seen in the kidney of a low-dose female rat; because of the undifferentiated nature of the neoplasm, it could not be determined if it was of tubular or of transitional cell origin. Transitional cell carcinomas of the renal pelvis occurred in two high-dose females. No transitional cell neoplasms of the kidney have been observed in 2,094 corn oil vehicle control female F344/N rats in the contemporary NTP data base.

Table V. Number of Rats with Renal Tubular Cell Adenomas or Carcinomas (Combined) Determined by Single-section and Multiple-section Sampling in the Two-year Gavage Studies of Phenylbutazone

	Vehicle control	50 mg/kg	100 mg/kg
Male			
Single section	0	1	3
Multiple sections	0	4	2[a]
Composite values (combined)	0	5	4
Female			
Single section	0	0	0[b]
Multiple sections	0	3	1
Composite values (combined)	0	3[c]	1[d]

a) One of these adenomas was also diagnosed in the single-section sampling.
b) Carcinomas of the transitional epithelium were also observed in two high-dose female rats.
c) An additional neoplasm of uncertain classification was observed with both sectioning techniques in one low-dose female rat.
d) An oncocytoma was also observed in one high-dose female rat.

In the current studies, the conventional single-section histopathologic evaluation of the kidney identified a tubular cell carcinoma in a low-dose male and tubular cell adenomas in three high-dose male rats. Renal tubular cell hyperplasia was observed in three vehicle control, one low-dose, and four high-dose male rats. Although the number of male rats with renal tubular cell neoplasms is low, renal tubular cell tumors are particularly uncommon in control F344/N rats, and even more so in females.[32, 33] The highest number of tubular cell neoplasms observed in a corn oil vehicle control group in the current NTP historical data base is 1/48. However three tubular cell

adenomas have been reported in each of two recent untreated control groups. Thus, the four renal tubular cell neoplasms identified by conventional histologic techniques in male rats given phenylbutazone is difficult to interpret fully. For these reasons and because the majority of renal tubular cell neoplasms in these and other studies are microscopic,[34] additional tissue sections for extended histopathologic review of proliferative or neoplastic lesions were taken from kidneys of male and female rats.

Microscopic examination of the additional sections revealed no tubular cell neoplasms in vehicle control rats (Table IV), although cystic hyperplasia, hyperplasia, or adenomas were found in dosed groups of both sexes. No additional neoplasms of the pelvic transitional epithelium were located. Lesions classified as cystic hyperplasia consisted of greatly dilated tubules with focal stratification of the tubule epithelium, whereas hyperplasias were characterized by tubules partially or completely filled with epithelial cells. Adenomas were larger, usually

greater in diameter than five tubules, and consisted of solid clusters of tubule epithelial cells. The oncocytoma was similar to the adenomas but consisted of cells with eosinophilic cytoplasmic granules.

Results of the two histological reviews are compiled in Table V. In male rats the composite low-dose incidence was significantly increased and the top-dose incidence was marginally increased ($P = 0.058$) compared with the vehicle control incidence. The composite incidence in either dosed group of female rats was not statistically different from that in vehicle controls. Nonetheless, the combined observations from the original evaluation and the additional sections showed neoplasms in all four dosed groups. Because these tumors are rare in this strain of rats, it seems probable that the lesions were chemically related, especially since they occurred in both sexes and only in exposed animals.

Other tissues Medullary hyperplasia of the adrenal gland was increased ($P < 0.01$) only in high-dose female rats (male: control, 11/50; low dose, 15/49; high dose,
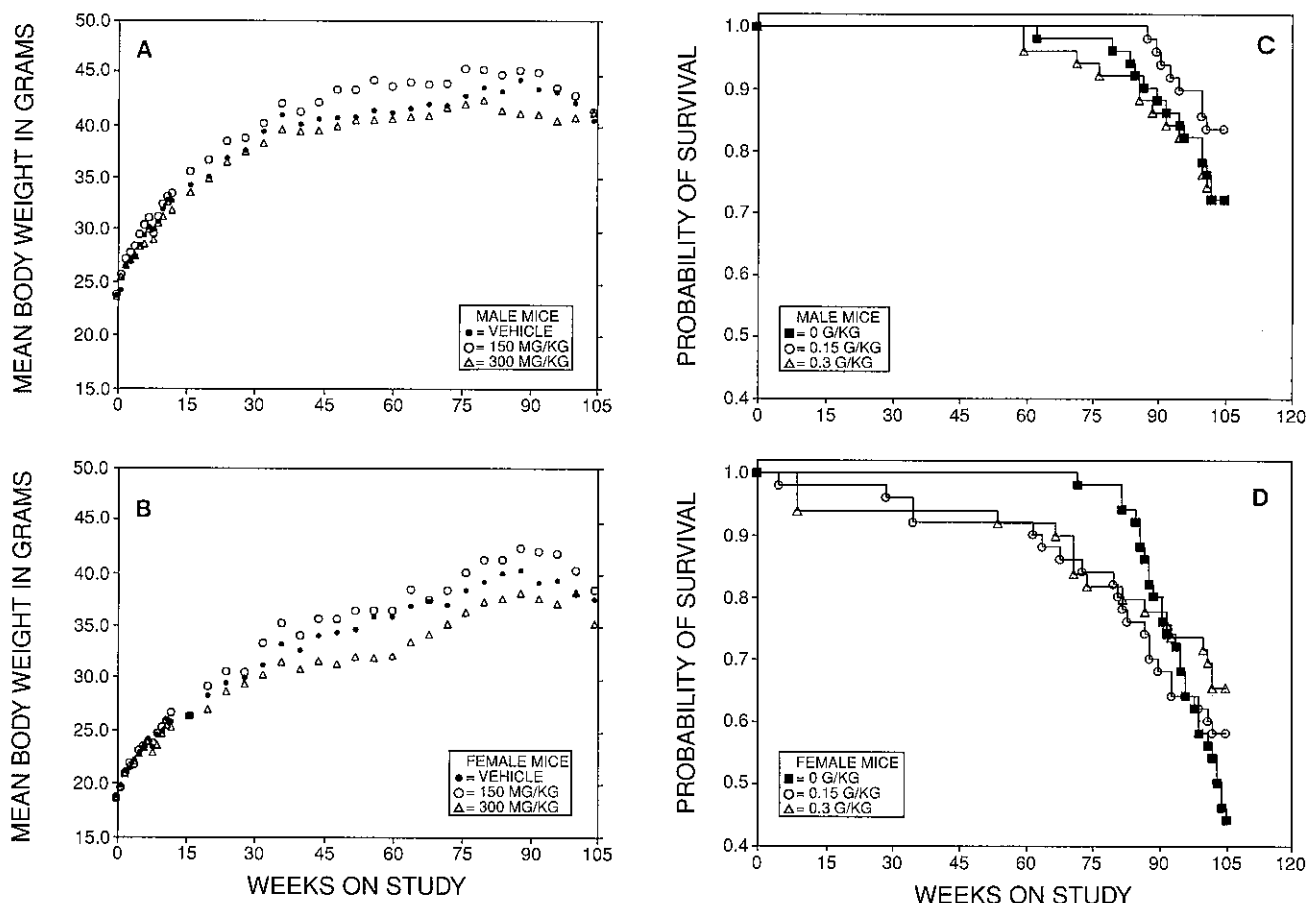

Fig. 2. Growth curves for male (A) and female (B) mice and Kaplan-Meier survival curves for male (C) and female (D) mice exposed to 0, 50, or 100 mg phenylbutazone/kg body weight by gavage.

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Table VI. Incidence of Mice with Selected Liver Lesions in the Two-year Studies With Phenylbutazone

Lesion	Male			Female		
	Vehicle control	150 mg/kg	300 mg/kg	Vehicle control	150 mg/kg	300 mg/kg
Number examined	50	50	50	50	49	50
Clear cell focus	0	0	5*	0	0	1
Hemorrhage (peliosis hepatis)	1	16**	27**	0	6*	1
Pigmentation	1	1	26**	0	2	0
Fatty change	11	7	20**	5	6	0
Centrilobular cytomegaly	0	34**	45**	0	0	0
Centrilobular karyomegaly	0	34**	44**	0	0	0
Degeneration	0	22**	38**	0	1	0
Coagulative necrosis	4	11*	36**	8	14	12
Adenoma	8	12	24**	4	5	7
Carcinoma	8	4	11	1	0	0
Adenoma or carcinoma[a]	16	14	31**	5	5	7

a) Historical incidence at study laboratory (mean±SD): 111/348 (32%±8%); historical incidence in NTP studies, 688/2,084 (33%±9%).
* $P<0.05$ vs. vehicle controls. ** $P<0.01$ vs. vehicle controls.

12/49; female: 3/50; 6/50; 19/50). Pheochromocytomas in female rats were not increased (3/50; 1/50; and 3/50). Ulcers of the forestomach were increased ($P<0.05$) in male (0/50; low dose, 5/50; high dose, 6/50) and high-dose female rats (2/49; 1/49; 12/49), as were acanthosis (4/49; 0/49; 12/49), hyperkeratosis (3/49; 0/49; 12/49), and basal cell hyperplasia (4/49; 1/49; 12/49). Interestingly, no tumors of the forestomach were seen, and fibroadenomas of the mammary gland in female rats were dose-relatedly decreased (22/50; 15/50; 7/50).

Mice

Thirteen-week studies No compound-related clinical signs were observed. Mean body weights were similar among groups. Liver-to-body-weight ratios were increased for mice that received 300 and 600 mg/kg (data not shown). No compound-related histopathologic effects were found. Five of 10 male mice and 4/10 female mice that received 600 mg/kg died early. No other chemical-related deaths occurred. Because of an absence of effects on body weight or other adverse effects at 300 mg/kg and the 9/20 deaths at 600 mg/kg, doses of phenylbutazone selected for the 2-year studies were 150 and 300 mg/kg.

Two-year studies No compound-related clinical signs were observed in mice over the course of the 104-week studies. Mean body weights of low-dose mice were 98%–108% of those of vehicle controls throughout the study (Fig. 2). Mean body weights of high-dose male mice were within 4% of vehicle controls until week 84 and within 7% thereafter, while those of high-dose female mice were generally 4%–11% lower than controls after

week 36. Estimates of the probabilities of survival are depicted in Fig. 2, C and D. Two-year survivals of male mice were 35/50, 40/50, and 36/50, but somewhat lower in female mice (22/50, 29/50, and 32/50).

Liver In male mice, phenylbutazone caused a variety of toxic lesions in the liver, including cytomegaly, karyomegaly, hepatocellular degeneration and necrosis, fatty change, hemorrhage, and pigment accumulation (Table VI). Cytomegaly was primarily centrilobular in distribution and consisted of enlarged hepatocytes with homogeneous eosinophilic cytoplasm, and usually with enlarged nuclei (karyomegaly). Multiple foci of hepatocellular degeneration were characterized by shrunken, deeply basophilic cells with pyknotic nuclei or individual vacuolated cells. Individual necrotic hepatocytes with or without minimal inflammatory response often accompanied this change. Lesions diagnosed as hemorrhage consisted of widely dilated sinusoids filled with blood (angiectasis or peliosis hepatis). A granular yellow-green pigment (presumably bile pigment) was present in widely scattered macrophages in the liver sinosoids. Clear cell foci (discrete areas of hepatocytes with pale cytoplasm) occurred in five high-dose male mice, but not in controls or females.

Hepatocellular adenomas and adenomas or carcinomas in male mice occurred with positive trends, and the incidences in the high-dose group were greater than that in the vehicle controls (Table VI). Multiple tumors were dose-related in 2 controls, 4 low-dose, and 9 high-dose males. Metastases were found in the heart and lung. No increases were seen in female mice.

DISCUSSION

The observations of phenylbutazone-associated renal toxicity in all dosed groups and decreased body weights in high-dose rats, and the fact that 90% of rats survived for at least 20 months, indicate that the doses used were adequate for assessing the long-term toxicity and carcinogenicity of phenylbutazone; somewhat higher doses might have been tolerated but were considered inappropriate. The decrease in survival in the low-dose group of male rats began at about week 85 and is unexplained. Mortality of high-dose female rats was increased, and was probably due to renal failure, since a large number of high-dose female rats exhibited marked nephropathy (46%) and/or renal papillary necrosis (82%).

The kidney was confirmed, as anticipated, to be the major target organ for phenylbutazone-induced toxicity in male and female rats,[8, 9, 35] as has been the case in other studies involving nonsteroidal analgesics.[36] In the 13-week studies, renal papillary necrosis was observed in male and female rats given phenylbutazone at doses as low as 100 mg/kg. In the 2-year studies, papillary necrosis was seen in over 80% of females and 50% of males given the top dose of 100 mg/kg; lesions were often more severe and extensive in females than in males, though they are typically more common in males.

Although the mechanism of papillary necrosis caused by nonsteroidal analgesics has not been clarified, the lesion is characteristic of an ischemic-induced infarct probably arising from impairment of blood supply to regions of the papilla.[37] Within the affected region of the papilla, necrosis of the cells forming the interstitial tissue, of interstitial capillaries, and of the thin loops of Henle was present. Electron microscopy of kidney lesions induced by various analgesics has shown that the earliest lesions appear in interstitial cells, the vasa recta supplying blood to the medulla and the papilla, and the loop of Henle.[38]

A major effect of nonsteroidal analgesics is inhibition of prostaglandin synthesis, and it has been proposed that decreases in prostaglandin production in the renal papilla may play a key role in the pathogenesis of papillary necrosis, possibly by decreasing blood flow, thereby leading to ischemia. Prostaglandin E2 is produced in the renal medulla and may be involved in the autoregulation of medullary blood flow[39]; an increase in prostaglandin synthesis appears to occur in response to a variety of stimuli that reduce renal blood flow. Many analgesics, including phenylbutazone, lower medullary blood flow. Because of the countercurrent exchange mechanism in the kidney, the concentration of diffusible analgesic metabolites will be highest in the papilla. In studies of the experimental induction of papillary necrosis with 2-bromoethylamine hydrobromide, increasing urine flow and decreasing papillary solute concentrations protected against development of this lesion.[40, 41] Conversely, dehydration enhanced the severity of papillary necrosis induced by aspirin or combinations of aspirin, phenacetin, and caffeine.[39]

Although no microscopic evidence of injury to the renal tubules in the cortex was found in our 13-week studies, others have reported cortical tubular necrosis after administration of a single dose of oxyphenbutazone, the major metabolite of phenylbutazone.[8] Not surprisingly, therefore, there were dose-related increased severities of nephropathy in male and female rats in our 2-year studies of phenylbutazone. Age-related nephropathy primarily involves the convoluted tubules of the renal cortex and the glomeruli; some degree of nephropathy is seen in nearly all 2-year-old rats, more so in males. This "spontaneous" renal disease of rats is not what has been described as analgesic nephropathy in humans.[39] The pathogenesis of nephropathy in rats has not been fully elucidated, but high protein diets are known to exacerbate the lesions.[42] Because of the complex lesions involving the nephron in this disease and the limited manner in which individual cells are able to respond to injury, chemical-induced damage to the tubular epithelium is often manifested as a dose-related increase in severity of nephropathy.

Interpretation of neoplastic responses in male rats exposed to phenylbutazone is based in these considerations: 1) routine histopathologic evaluation revealed a low, albeit noteworthy, incidence of tubular cell neoplasia (0 vs. 2 and 6%). 2) Additional sections indicated the presence of more tubular cell adenomas in the dosed groups, bringing the composite observations to 0 vs. 10 and 8%. Statistically, these show a marginal trend, and the pairwise comparisons between control and dosed groups fluctuated around significance. 3) Comparisons of these data with historical control incidence rates for multiple sectioning revealed that the number of adenomas/carcinomas observed in the low-dose phenylbutazone group has been equalled in control male rats of another NTP study.[43] 4) The nine tumors in dosed males, together with four tumors in females, versus none in the 100 concurrent controls, lends support to the judgment that these are chemically related. 5) Data reported here also indicate clearly that the kidney is a target organ of phenylbutazone-induced nonneoplastic lesions, as evidenced by increased severity of nephropathy, dose-related increases in papillary necrosis, and mineralization of the collecting tubules. Considered collectively, these observations provide evidence, albeit equivocal, of phenylbutazone-induced carcinogenicity in male F344/N rats. Further supporting evidence comes from promotion studies whereby N-ethyl-N-nitrosourea (ENU) and N-propyl-N-nitrosourea (PNU) initiation followed by 2-year dietary exposure to phenylbutazone (PBZ) showed

enhanced occurrence of tubular cell tumors of the kidney for both nitrosoureas: ENU=6.7% vs. ENU+PBZ= 33% and PNU=5% vs. PNU+PBZ=16%.

Other evidence to support the view that phenylbutazone is associated with kidney neoplasia in the tubular cells of dosed rats is speculative. Although not mutagenic in bacterial cells, phenylbutazone is genotoxic and clastogenic in some mammalian assay systems.[1, 16, 44, 45] Investigators have demonstrated that phenylbutazone has a promoting effect on kidney neoplasms initiated by N-ethyl-N-nitrosourea or N-propyl-N-nitrosourea.[23] There was no evidence of hyaline droplet formation or other aspects of the α 2u-globulin nephrotoxic syndrome[46, 47] in the studies reported here.

In female rats given phenylbutazone, hyperplasia of the renal pelvis transitional epithelium was increased (14% in controls vs. 40 and 74%), and occurred typically near the fornices of the renal pelvis. Dose-related increased severity of nephropathy was seen in female rats and the transitional cell hyperplasia might be considered as an adaptive response in female rats with marked nephropathy. However, this is untenable because more animals exhibited hyperplasia than those with marked or moderate nephropathy. Further, Maekawa et al.[23] reported an increase in hyperplasia of the pelvis mucosa in female rats (2% vs. 12 and 21%), while finding no increase in male rats. Two high-dose female rats had transitional cell carcinomas, a lesion never seen in our controls. Hyperplasia of the transitional epithelium occurred in the urinary bladder of one vehicle control and three top-dose female rats, and a transitional cell papilloma occurred in one low-dose female rat. The low incidences of these uncommon effects, however, preclude unequivocal association of these latter lesions with the administration of phenylbutazone, despite the commonality of tissue types seen in both the kidney and urinary bladder.

Two transitional cell carcinomas in the renal pelvis in the top-dose group of female rats were not enhanced by the 3 or 4 additional sections. These neoplasms have not been observed in nearly 2,100 contemporary control (corn oil vehicle) female F344/N rats or in over 1,600 historical untreated controls in the NTP data base. A statistical comparison between the two observed in these studies versus the historical data base is highly significant ($P<0.001$) and thereby unlikely to be due to chance. Although it was not possible to determine precisely the tissue of origin of the carcinoma found in the low-dose female rat, the tumor displayed characteristics of transitional cell morphology and was clearly malignant. The rarity of these neoplasms in the renal pelvic epithelium of female F344/N rats, combined with the substantial numbers of transitional cell hyperplasias and the fact that this topography is a target for phenylbutazone-induced toxicity, supports the conclusion that the three carcinomas

observed are related to the administration of phenylbutazone. It is noteworthy that transitional cell carcinomas have been associated with nonsteroidal analgesic-induced nephropathy in humans.[48–51]

Results of the additional sections identified the presence of an oncocytoma in the top-dose group of female rats. Although the pathogenesis of this rare lesion is not known with certainty, some investigators[52, 53] consider an oncocytoma to be a benign tumor that develops in the distal parts of the nephron.[54] This contrasts with tubular cell adenomas, which apparently originate in the proximal renal tubules and often progress to adenocarcinomas.[55–57]

Although phenylbutazone has been widely reported to cause acute ulceration of the glandular stomach[5, 6] these lesions were not observed in rats in either the 13-week or 2-year studies. However, Maekawa et al.[23] reported phenylbutazone-induced erosions of the glandular stomach in female Donryu rats, and ulcers in the small intestine in both sexes. In our experiments, ulcers and associated regenerative epithelial hyperplasia of the forestomach were observed in male and female rats in the 2-year studies. Similar lesions have been observed in other gavage studies, and some authors have associated these with irritant effects of chemicals, although the evidence is weak and inconsistent.[58, 59] Direct trauma to the forestomach associated with gavage procedures may also have contributed to the development of the ulcers, although this too seems unlikely. For this particular chemical, the dietary studies of Maekawa et al.[23] do not support either of these causal hypotheses; there was little irritant effect and the gavage route of exposure was not used.

Regarding the experiments with mice, because body weights were somewhat decreased only in the top-dose groups and because survival of each sex of the dosed mice in the 2-year studies was similar to that of vehicle controls, the doses chosen for mice could have been higher, but are considered to have been adequate to detect a carcinogenic effect.

Results of the 2-year studies in mice showed that phenylbutazone induced liver tumors (primarily adenomas) in male mice. The incidence of hepatocellular neoplasms observed in this group (62%) is nearly twice the mean incidence observed in corn oil vehicle controls at the study laboratory or in NTP cross-laboratory studies (33%) and exceeds the highest incidence observed in historical controls (50%). Although there is no statistically significant increase in the combined incidences of adenomas and carcinomas in the low-dose male mice, there is evidence of a dose-dependent increase in preneoplastic lesions in the liver. The high-dose male mice had an increased incidence of clear cell foci, which are believed to be preneoplastic changes.[60, 61]

Hepatotoxicity and hepatocarcinogenicity of phenylbutazone have not previously been reported. Another 2-year study with rats suggested a slight positive effect of phenylbutazone administration on the occurrence of neoplastic nodules in the liver (2% vs. 6 and 9.5%), but the incidences were not statistically significant.[23] No nonneoplastic lesions of the liver were reported by these authors. Clinically, however, overt hepatic injury has been reported in about 2–3 persons per 1,000 given phenylbutazone therapeutically.[62]

Conversely, phenylbutazone was associated with decreases or negative trends for two site-specific neoplasms. Mammary gland fibroadenomas in female rats were reduced in both exposed groups compared to concurrent controls (23/50 vs. 15/50 and 7/50); hyperplasia was not greatly different (41/46 vs. 32/40), yet strengthens the evidence when combined with fibroadenomas. Although such trends have been seen in animals with substantially decreased body weights,[63] the decreases demonstrated in this study occurred in the absence of marked dose-dependent decreases in body weight, and despite reduced survival.

Hematopoietic system tumors (lymphomas) were decreased in top-dose male mice: 7/50 vs. 8/50 and 1/50. Previous findings in mice[64] and in rats[65] indicate an inverse relationship between increases in liver tumors and decreases in leukemia. Analogous findings were observed in this study in the mice. No mechanistic explanation for these empirical observations is available; nonetheless, there seems to be some feedback message from the liver rather than a forward message from the bone marrow.

In summary, under the conditions of these 2-year oral intubation studies, phenylbutazone was associated with renal carcinogenicity in rats, as evidenced by increases in tubular cell neoplasms in both sexes. The case for carcinogenic activity is supported by the occurrence of transitional cell carcinomas of the kidney in two female rats in the top-dose group; none has been seen in nearly 20,000 control female rats. Evidence of carcinogenicity for male mice is provided by increased incidences and multiplicity of liver tumors. No evidence of carcinogenic activity was found in female mice.

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