

Factors Modulating Benzidine Carcinogenicity Bioassay¹

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SUMMARY

An integrated series of studies was presented in which several factors were assessed as to their capability to influence the outcome of carcinogenicity of benzidine dihydrochloride in mice. In all studies C57BL/6J × C3HeB/FeJ F₁ mice of both sexes were utilized. Animals were either 6 or 1 week of age at the beginning of carcinogenic treatment. Six-week-old mice were exposed to p.o. administration of carcinogen delivered either in food (50 or 100 ppm daily) or by stomach intubation at equivalent dose levels at twice-weekly intervals. In addition, a 150-ppm dose level in food was administered for 39, 54, or 84 weeks. A limited 3-week, daily intubation of benzidine (30 or 100 μg/mouse) was also explored in 1- and 6-week-old mice. Animals were killed in all studies at 90 weeks of age, at which time their tumor incidence was evaluated.

Depending upon experimental conditions, benzidine treatment effected development of liver tumors, lung adenomas, Harderian gland cystadenomas, and lymphoreticular neoplasms. Continuous feeding of adult mice for 84 weeks at three dose levels of benzidine resulted in development of liver tumors with a positive dose-response relationship in both sexes. The analysis of data revealed a greater susceptibility of females than of males (94% versus 44% at 150 ppm). Twice-weekly administration of benzidine by stomach intubation was shown to be less hepatocarcinogenic than continuous feeding of equivalent amounts. In the series in which male mice were fed food containing 150 ppm of benzidine for only 34 or 54 weeks, in contrast to the above 84-week schedule, a negative relationship was observed between the incidence of liver tumors and the duration of treatment.

Daily administration of 30 μg of benzidine to infants by stomach intubation for a 3-week period significantly enhanced development of liver tumors only in males (66%). Introduction of 150 ppm of benzidine into food offered to mother and offspring from delivery to weaning led to development of liver tumors in 95% of male mice and in 5% of females. No liver tumors developed following similar 3-week treatment of 6-week-old adults.

INTRODUCTION

For the last 10 years our laboratory has been engaged in studies aimed at defining the role of age, strain, and sex in modifying the outcome of carcinogenic processes in various tissues of mice in order to assess their influence upon carcinogenicity bioassay. In this attempt, representatives of chemical classes of carcinogens, such as *N*-nitroso agents, polycyclic hydrocarbons, and aromatic amines, have been utilized (8, 10, 18, 21). This presentation deals with factors modulating carcinogenesis in C57BL/6J × C3HeB/FeJ F₁ (hereafter called B6C3F₁) mice following p.o. administration of BZ·2HCl,² an acknowledged human carcinogen (2, 16, 22). It is obvious that such information would be of great significance in the further use of the mouse as a laboratory animal for evaluation of carcinogenicity of various classes of chemical agents. Because there have been comparatively few studies performed on mice to date and these were limited to s.c. administration of benzidine (1, 12), it was deemed necessary to conduct a series of subacute toxicity investigations following p.o. administration of this agent prior to embarking upon long-term carcinogenicity studies (13). The overview of the current results of both series is presented herein. A review of benzidine carcinogenesis in other species by various routes of administration has been detailed recently (4).

MATERIALS AND METHODS

Mice. The 1st generation of B6C3F₁ mice was used. The parent strains were purchased from The Jackson Laboratories, Bar Harbor, Maine. Animals were housed in plastic cages in groups of 10 and were kept in temperature-controlled laboratories. Sanicel was used as bedding, and powdered Purina laboratory chow and water were given *ad libitum*. At 2-week intervals, the animals were weighed and inspected for the presence of any external neoplasms or other symptoms indicating the development of internal tumors and/or nonspecific pathological changes affecting their health.

Treatment. BZ·2HCl (certified A.C.S. grade; Fisher Scientific Co., Fair Lawn, N. J.) was mixed with powdered Purina laboratory chow in a mechanical blender in the amounts to contain specified ppm of carcinogen in diet. The concentrations of BZ·2HCl were verified by a colorimetric method using *p*-dimethylaminobenzaldehyde reagent. The

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²The abbreviation used is: BZ·2HCl, benzidine dihydrochloride.

analyses were performed twice weekly throughout the experiment. Food containing BZ·2HCl was prepared at weekly intervals because analyses carried out during preliminary subacute toxicity studies indicated no deterioration of the agent over that period of time. Fixed amounts of fresh food were introduced in clean containers into experimental cages daily, while the soiled feeders containing 24-hr residual food were removed. Periodic weighing of residual food gave indications of average daily consumption of the carcinogen.

In order to arrive at optimally tolerated doses of BZ·2HCl, which would not interfere adversely with the animals' weight gain and their survival to permit tumor expression, a subacute dose tolerance test was performed before embarking on the long-term carcinogenesis studies (13). In these preliminary studies, mice were fed BZ·2HCl in concentrations of 100, 200, 400, 600, and 800 ppm in powdered Purina laboratory chow for a period of 6 weeks. During the experimental phase, food consumption, growth rate, and clinical signs of toxic manifestations were recorded. At the end of 6 weeks, all animals were killed, carcasses and the major internal organs were weighed, gross morphological changes were recorded, and tissue specimens were taken for histopathological examination.

In carcinogenicity studies, BZ·2HCl was administered in food daily or by stomach intubation delivered daily to infants or twice to adults. In the short-term treatments (3 weeks) animals were given 30 μ g of BZ·2HCl every day, while in the long-term treatment series (from 6th to 90th week of age) estimated weekly consumption of specified benzidine dose delivered in food (50 or 100 ppm) was administered by stomach intubation in 2 weekly treatments (Table 2).

Duration of the Experiment. Regardless of mode of treatment (in food or by intubation) or its duration, as indicated within the text in reference to individual experimental series, the animals were kept under observation to their 90th week of age at which time they were killed. Animals tolerated treatment well and very few died during the experiment. Complete autopsies were performed on all of the animals and specimens from grossly visible tumors and other tissues were taken for histological evaluation. The specimens were fixed in buffered formalin, processed, and stained with hematoxylin and eosin (8).

Tumor incidence was scored on the basis of histological evaluations. The χ^2 method of analysis was applied in assessing the significance of differences in specific tumor incidences between various experimental groups of a given series.

RESULTS

Subchronic Toxicity

The reduction in body weight in relation to benzidine concentration was observed as early as 2 weeks following inception of feeding. By the end of the 6th week, a significant dose-dependent inhibition of growth was ob-

served in male groups exposed to 400, 600, and 800 ppm of BZ·2HCl. These groups lost 5, 10, and 18%, respectively, of their initial body weight. Although less pronounced, a similar pattern of body weight changes were observed in the female mice. Thus, at the highest dose level their weight loss was only 7.5%. At the end of the BZ·2HCl feeding period, both sexes showed negative linear correlation between the doses of benzidine fed and the body weights, the males showing a significantly steeper slope than females.

Carcinogenicity Studies

Dose-Response Effect. Based on the subchronic investigations, dose levels of 50, 100, and 150 ppm of BZ·2HCl were used to assess the dose-response relationship regarding hepatocarcinogenesis by this carcinogen. Chart 1 shows a linear dose-response relationship in both sexes between dose of benzidine and development of liver tumors. Thus, in males the incidence rose from 6% at 50 ppm to 44% at 150 ppm, while in females these values were 26 and 94%, respectively ($p < 0.01$). On the average, BZ·2HCl was 2.5-fold more effective in inducing liver tumors in females than in males ($p < 0.01$).

Depending upon the dose, up to 70% of liver tumors were hepatocellular carcinomas of trabecular or acinar pattern with a tendency to metastasize to the lungs. Their morphology and behavior were similar to those induced by diethylnitrosamine in the same hybrid strain (8). Histologically, the primary hepatocellular carcinomas appeared as multifocal,

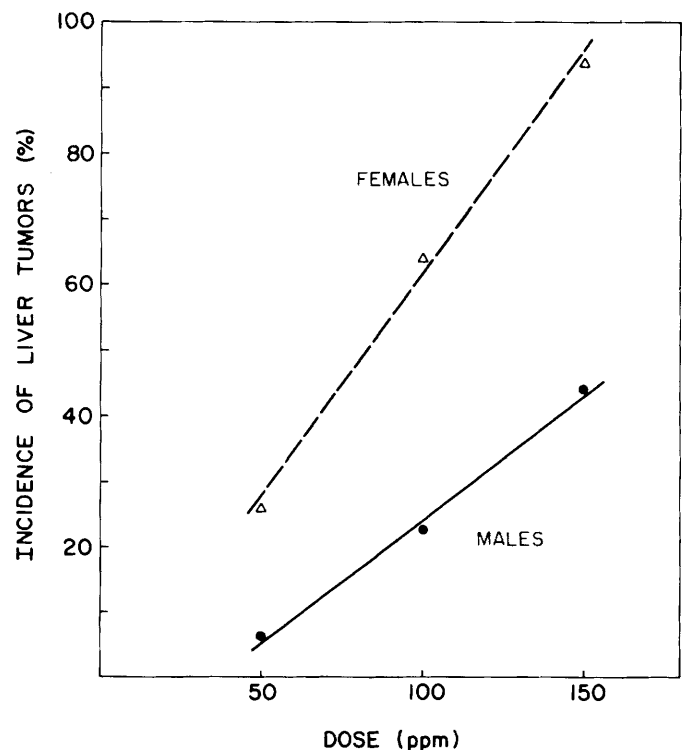


Chart 1. Incidence of liver tumors in B6C3F₁ male and female mice fed diet containing 50, 100, or 150 ppm of BZ·2HCl in powdered Purina laboratory chow from the 6th to the 90th week of age.

solitary, or confluent growths. They were composed of small rounded or large polygonal cells arranged occasionally in cords, but primarily in trabecular or acinar formations. In many areas, blood-filled sinusoid-like spaces were separating cords and trabeculae of tumor cells. Occasionally, blood spaces were discrete. The tumor cells demonstrated a moderate to severe degree of anisocytosis and pleomorphism. Their cytoplasm was basophilic and, in certain places, eosinophilic. Nuclear atypia of varying degrees, mitotic activity, and intranuclear and intracytoplasmic inclusion bodies were observed. In some places, neoplastic cells formed solid tumor masses.

These tumors metastasized to the lungs mainly by the hematogenous route. Metastatic foci were usually multifocal and were composed of small groups of cells or of larger tumor masses showing a close morphological resemblance to the primary tumor cells. They occupied mainly the alveolar capillaries and the small and medium-sized branches of the pulmonary artery adjacent to the small bronchi and bronchioles. The metastatic foci were growing concentrically. Frank infiltration of the lung parenchyma was observed in certain areas, but the most frequent feature was the formation of larger tumor masses by the confluence of adjacent metastatic foci. Thirty-eight % of primary tumors transplanted into isologous hosts grew successfully and metastasized to various sites (S. D. Vesselinovitch and N. Mihailovich, unpublished data).

Modifying Role of Mode of Benzidine Administration in Tumor Development. Concurrently with the above study, animals were given BZ·2HCl intermittently by stomach tube, twice weekly, in the amounts 0.5 or 1.0 mg/mouse at each intubation corresponding to weekly consumption of benzidine by mice fed 50 or 100 ppm. Table 1 shows that intermittent treatment by intubation was a significantly less efficient method of inducing liver tumors, which had been manifested only in females at both dose levels [5 versus 26% ($p < 0.01$) and 23 versus 64% ($p < 0.01$)]. Differences between males and females in the incidence of liver tumors were not observed in the intermittent feeding series.

In addition to liver tumors, benzidine-treated mice also developed Harderian gland tumors, lung adenomas, and

lymphoreticular tumors. Harderian gland tumors (Table 1) were observed exclusively in male animals as in the case of liver tumors. Continuous feeding was once again shown to be a more effective type of treatment, with the dose having a significant effect on the incidence (36 versus 15%; $p < 0.01$).

Table 1 also shows that, in contrast to liver and Harderian gland, tumors of the lung developed more frequently following intermittent benzidine treatment, especially in the males.

Besides liver, Harderian gland, and lung tumors, benzidine had a marginal effect upon development of lymphoreticular tumors. Because of their small number and in order to demonstrate this effect, this incidence has been calculated for each mode of benzidine administration after combining groups regardless of sex and dosage. Table 2 shows the combined data indicating a small but significant increase of these tumors in the intermittent benzidine-treated series (2 versus 9.5%; $p < 0.01$).

Effect of Duration of Treatment on Tumor Response. Parallel to studies mentioned above, groups of 6-week-old male mice were fed food containing 150 ppm of BZ·2HCl for periods of 39, 54, or 84 weeks, respectively. All animals

Table 2
Incidence of lymphoreticular tumors in B6C3F₁ mice following exposure to BZ·2HCl delivered in food or by intubation

Mode of exposure to BZ·2HCl ^a	Ratio ^b	%
Continuous ^c	19/200	9.5
Intermittent ^d	17/300	5.7
Controls	4/194	2.0

^a Animals were exposed to carcinogen from the 6th through the 90th week of age regardless of mode of exposure.

^b Number of mice bearing lymphoreticular tumors/number of carcinogen-treated animals; combined data for both dose levels and sex.

^c Food containing 50 to 100 ppm of BZ·2HCl was offered *ad libitum*.

^d Mice received 0.5 or 1.0 mg of BZ·2HCl per mouse by stomach intubation twice weekly, being comparable in amounts to those consumed in continuous series; total amounts delivered by intubation were 84 or 168 mg/mouse.

Table 1
Comparison of continuous versus intermittent administration of BZ·2HCl on development of liver, Harderian gland, and lung tumors

Dose (ppm)	Sex	Liver tumors				Harderian gland tumors				Lung adenomas			
		Continuous ^a		Intermittent ^b		Continuous		Intermittent		Continuous		Intermittent	
		Ratio ^c	%	Ratio	%	Ratio	%	Ratio	%	Ratio	%	Ratio	%
50	M	3/50	6	3/75	4	9/50	18	8/75	11	2/50	4	17/75	23
	F	13/50	26	4/75	5	3/50	6	2/75	3	2/50	4	5/75	7
100	M	11/50	22	12/75	16	18/50	36	11/75	15	3/50	6	19/75	25
	F	32/50	64	17/75	23	3/50	6	5/75	7	2/50	4	4/75	5

^a Animals were given food containing specified amounts of BZ·2HCl.

^b Mice received BZ·2HCl by stomach intubation in amounts equivalent to specified dosages; mice were intubated twice weekly. The amounts at each treatment were 0.5 mg (corresponding to 50-ppm series) or 1.0 mg (corresponding to 100-ppm series) per animal at treatment.

^c Number of mice bearing specified tumors/number of BZ·2HCl-treated animals.

were killed at 90 weeks of age and the incidence of liver tumors was evaluated. Table 3 shows that groups of mice exposed to shorter feeding periods developed liver tumors with higher incidence than those exposed to the longest period of time. Thus, while 84 weeks of benzidine feeding resulted in an incidence of 44% liver tumors, 39 weeks of feeding led to development of these tumors in 70% of the treated animals ($p < 0.01$). The average weight of mice in the group fed benzidine for only 39 weeks was significantly higher than that of the mice given benzidine for 84 weeks (35 versus 25 g).

Table 4 illustrates emergence of liver tumors following removal of BZ·2HCl from food. In this series, animals were fed 150 ppm of benzidine from the 6th to the 45th week of age and sets of 50 animals were killed at 45, 60, 75, and 90 weeks of age to evaluate liver tumor incidence. At the time of termination of treatment (45 weeks), only 4% of animals bore hepatocellular carcinomas. However, their incidence rose to 10, 28, and 48% in animals killed at 60, 75, and 90 weeks, respectively. This demonstrates that, once carcinogenesis has been initiated, the neoplastically committed cells expressed their malignant character once a sufficient observational period has been allowed.

Role of Age at the Time of Benzidine Administration in the Incidence of Tumors. Because the age of animals at the time of carcinogenic exposure can influence outcome of carcinogenesis in various tissue in general but in liver in particular, an experimental study was undertaken in which animals were exposed daily to benzidine (30 $\mu\text{g}/\text{infant}$ and 100 $\mu\text{g}/\text{adult}$) by stomach tube for a period of only 33 weeks. Table 5 shows that liver tumors developed with high frequency only in males treated during infancy. In another series, when food containing 150 ppm of benzidine was offered to mother and offspring from delivery to weaning, 95% of the male mice and 5% of females developed liver tumors.

Table 6 gives comparative evaluation of the role of age and sex in benzidine hepatocarcinogenesis. Thus, in males, administration of a total dose of 0.63 mg of BZ·2HCl delivered between the 7th and 27th day of life or 4.20 mg delivered between the 1st and 27th day of age resulted in development of liver tumors in 66 and 95%, respectively. In contrast, a total dose of 188 mg consumed by the adults resulted in only 44% liver tumor incidence. In the case of females, the age-associated variation in hepatocarcinogenesis was reversed; mice treated during infancy developed liver tumors with 0 and 5%, respectively, while 94% of the females treated during adulthood bore the same type of tumors.

Table 3

Effect of duration of BZ·2HCl treatment (150 ppm) on the incidence of liver tumors in B6C3F₁ males

Duration of treatment ^a (wk)	Estimated consumption of BZ·2HCl (mg/mouse)	Incidence of liver tumors	
		Ratio ^b	%
39	117	35/50	70
54	162	25/50	50
84	188	22/50	44

^a Animals were 6 weeks old at start of treatment and were killed at 90 weeks of age.

^b Number of tumor-bearing animals/number of carcinogen-treated animals.

Table 4

Incidence of liver tumors in B6C3F₁ male mice at various time intervals following removal of BZ·2HCl

Animals in all groups were fed food containing 150 ppm of BZ·2HCl from 6th to 45th week of age.

Age animals killed (wk)	Incidence of mice bearing liver tumors					
	Benign		Malignant		Total	
	No.	%	No.	%	No.	%
45	6	12	2	4	8	16
60	15	30	5	10	20	40
75	17	34	14	28	31	62
90	11	22	24	48	35	70

Table 5

Effect of age and sex on BZ·2HCl hepatocarcinogenesis in B6C3F₁ mice

Group	Effective no. of mice	Sex	Treatment ^a	Age at treatment (days)	Liver tumors	
					No.	%
1	98	M	None		1	1
2	89		Yes ^b	7-27	59	66
3	50		Yes ^c	42-62	2	4
4	100	F	None		0	0
5	82		Yes	7-27	0	0
6	50		Yes	42-62	0	0

^a Animals received 5 μg of BZ·2HCl per g of body weight estimated at 7 and 42 days, respectively.

^b BZ·2HCl, 30 μg , delivered daily in 0.05 ml of distilled water by stomach intubation.

^c BZ·2HCl, 100 μg , delivered daily in 0.05 ml of distilled water by stomach intubation.

genesis was reversed; mice treated during infancy developed liver tumors with 0 and 5%, respectively, while 94% of the females treated during adulthood bore the same type of tumors.

DISCUSSION

Using dose levels of BZ·2HCl that appeared nontoxic in the short-term investigations, several protocols were developed to assess its carcinogenicity in the mouse and to explore various factors capable of modifying neoplastic expression. The significance of these integrated studies lies in bringing forth the importance of experimental design in disclosing carcinogenic potential of a given chemical agent. These studies showed, under specific experimental conditions, benzidine treatment effected development of liver tumors, lung adenomas, Harderian gland tumors, and lymphoreticular neoplasms.

Continuous feeding of adult mice for 84 weeks at 3 dose levels of BZ·2HCl (50, 100, or 150 ppm) resulted in development of liver tumors with a positive dose-response relationship in both sexes, the females being more susceptible. Similarly, higher susceptibility of adult female mice to hepatocarcinogenesis by dimethylaminoabenzene (9) and 2,7-diacetamidofluorene (7) has been observed when these carcinogens were administered in food continuously.

Table 6
Comparative evaluation of role of age and sex in BZ·2HCl hepatocarcinogenesis

Age period at treatment (days)	Estimated total exposure to BZ·2HCl (mg/animal)		Incidence of liver tumors			
	Males	Females	Males		Females	
			Ratio	%	Ratio	%
7-27 ^a	0.63	0.63	59/89	66	0/82	0
1-27 ^b	4.20	3.36	62/65	95	2/43	5
42-630 ^c	187.87	150.29	22/50	44	47/50	94

^a Thirty μ g of BZ·2HCl delivered daily in 0.05 ml of distilled water by stomach intubation.

^b Food containing 150 ppm of BZ·2HCl was offered to mother and offspring from delivery to weaning.

^c Food containing 150 ppm of BZ·2HCl was fed to animals *ad libitum* from the 42nd through the 630th day of age.

Twice-weekly administration of benzidine by stomach intubation for 84 weeks in the amounts equivalent to continuous feeding was shown to be less carcinogenic to liver and Harderian gland but more to the lung. In another series, in which male mice were fed food containing 150 ppm of BZ·2HCl for only 34 or 59 weeks, in contrast to the above 84-week schedule, a negative relationship was observed between duration of treatment and development of liver tumors. These findings suggest that variation in tumor response with duration of treatments might be due to differential toxicity, overall consumption of BZ·2HCl, and/or indirect "inhibiting" effect of protracted lower food consumption upon neoplastic expression. Regardless of the mechanism, the generally accepted belief that longer exposure to carcinogen would lead to higher tumor response does not hold true, especially if the test agent might possess toxic and/or carcinolytic activity due to its chemical nature and/or excessive dose load. These concepts should be kept in mind when contemplating so-called lifelong treatment studies regarding carcinogenesis.

Daily administration of 30 μ g of benzidine to infant mice by stomach intubation for 3 weeks induced liver tumors only in males, which is consistent with the observation of other investigators administering aromatic amines during infancy (3, 5, 6, 11, 15). This observation is in keeping with our studies showing greater susceptibility of infant males than of females to hepatocarcinogenesis by different chemical carcinogens (14, 17, 18, 20, 21). Such sex difference in hepatocarcinogenesis has been abolished by gonadectomy performed 3 weeks following carcinogenic treatment (19). Besides the modifying effect of hormonal environment on neoplastic expression, one should not exclude the possibility of variation in the degree of activation of procarcinogens due to a possible difference in the enzymatic competence between the sexes, which may vary with age of the animals (14). The latter consideration as a possible mechanism of observed sex difference is in keeping with the fact that during adulthood the females became responsive to benzidine hepatocarcinogenesis.

These integrated studies on BZ·2HCl carcinogenesis showed (a) the necessity of protracted treatment of adults with benzidine to reveal its carcinogenicity potential, (b) a positive dose-response relationship regarding hepatocar-

cinogenesis, (c) greater susceptibility of adult females for development of liver tumors, and (d) high sensitivity of the liver of preweaning males to carcinogenesis. Therefore, the data indicate the necessity of taking into consideration the modifying role of age, sex, duration of treatment, dose of carcinogen, and nonspecific toxicity of the agent when selecting a bioassay system for evaluation of carcinogenicity.

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REFERENCES

1. Bonser, G. M., Clayson, D. B., and Jull, J. W. The Induction of Tumours of the Subcutaneous Tissues, Liver and Intestine in the Mouse by Certain Dyestuff and Their Intermediates. *Brit. J. Cancer*, 10: 653-667, 1956.
2. Goldwater, L. J., Rosso, A. J., and Kleinfeld, M. Bladder Tumors in a Coal Tar Dye Plant. *Arch. Environ. Health*, 11: 814-817, 1965.
3. Gorrod, J. W., Carter, R. L., and Roe, F. J. C. Induction of Hepatomas by 4-Aminobiphenyl and Three of Its Hydroxylated Derivatives Administered to Newborn Mice. *J. Natl. Cancer Inst.*, 41: 403-410, 1968.
4. International Agency for Research on Cancer. The Evaluation of Carcinogenic Risk of Chemicals to Man, Vol. 1, pp. 80-86. Lyon, France: International Agency for Research on Cancer, 1972.
5. Klein, M. Influence of Low Dose of 2-Acetylaminofluorene on Liver Tumorigenesis in Mice. *Proc. Soc. Exptl. Biol. Med.*, 101: 637-638, 1959.
6. Klein, M., and Weisburger, E. K. Carcinogenic Effect of *N*-Hydroxy-*N*-2-fluorenylacetylacetamide, 2',4'-Dimethylacetanilide, and 2',4',6'-Trimethylacetanilide on Liver in Suckling Mice. *Proc. Soc. Exptl. Biol. Med.*, 122: 111-114, 1966.
7. Kozuka, S. Inhibiting Effect of Reserpine and Female Sensitivity in Hepatic Tumor Induction with 2,7-Diacetylaminofluorene in SMA/MA Strain Mice. *Cancer Res.*, 30: 1384-1386, 1970.
8. Kyriazis, A. P., Koka, M., and Vesselinovitch, S. D. Metastatic Rate of Liver Tumors Induced by Diethylnitrosamine in Mice. *Cancer Res.*, 34: 2881-2886, 1974.

9. Leatham, J. H., and Harding, H. R. Hepatic Tumor Induction in Swiss and C3H Mice. *Proc. Am. Assoc. Cancer Res.*, 2: 319, 1953.
10. Lombard, L. S., Rice, J. M., and Vesselinovitch, S. D. Renal Tumors in Mice: Light Microscopic Observations of Epithelial Tumors Induced by Ethylnitrosourea. *J. Natl. Cancer Inst.*, 53: 1677-1685, 1974.
11. Nishizuka, Y., Ho, K., and Nakakuki, K. Liver Tumor Induction by a Single Injection of *o*-Aminoazotoluene to Newborn Mice. *Gann*, 56: 135-142, 1965.
12. Prokofjeva, O. G. Induction of Hepatic Tumours in Mice by Benzidine. *Vopr. Onkol.*, 17: 61-64, 1971.
13. Rao, K. V. N., Rust, J. H., Mihailovich, N., Vesselinovitch, S. D., and Rice, J. M. Subacute Toxicity of Benzidine in the Young Adult Mice. *Federation Proc.* 33: 44, 1971.
14. Rao, K. V. N., and Vesselinovitch, S. D. Age- and Sex-associated Diethylnitrosamine Dealkylation Activity of the Mouse Liver and Hepatocarcinogenesis. *Cancer Res.*, 33: 1625-1627, 1973.
15. Roe, F. J. C., Carter, R. L., and Adamthwaite, S. Induction of Liver and Lung Tumours in Mice by 6-Aminochrysene Administered during the First Three Days of Life. *Nature*, 221: 1063-1064, 1969.
16. Scott, T. S. Carcinogenic and Chronic Toxic Hazards of Aromatic Amines. New York: Elsevier Publishing Co., 1962.
17. Vesselinovitch, S. D. The Sex-dependent Difference in the Development of Liver Tumors in Mice Administered Dimethylnitrosamine. *Cancer Res.*, 29: 1024-1027, 1969.
18. Vesselinovitch, S. D. Transplacental and Neonatal Carcinogenesis: Experimental Approach. *In* W. Davis and C. Maltoni (eds.), *Characterization of Human Tumors*, Vol. 1, pp. 135-150. Amsterdam: Excerpta Medica, 1974.
19. Vesselinovitch, S. D., and Mihailovich, N. The Effect of Gonadectomy of the Development of Hepatomas Induced by Urethan. *Cancer Res.*, 27: 1788-1791, 1967.
20. Vesselinovitch, S. D., Mihailovich, N., Wogan, G. N., Lombard, L. S., and Rao, K. V. N. Aflatoxin B₁, a Hepatocarcinogen in the Infant Mouse. *Cancer Res.*, 32: 2298-2291, 1972.
21. Vesselinovitch, S. D., Rao, K. V. N., Mihailovich, N., Rice, J. M., and Lombard, L. S. Development of Broad Spectrum of Tumors by Ethylnitrosourea in Mice and the Modifying Role of Age, Sex and Strain. *Cancer Res.*, 34: 2530-2538, 1974.
22. Wendel, R. G., Hoegg, U. R., and Zavon, M. R. Benzidine: A Bladder Carcinogen. *J. Urol.*, 111: 607-610, 1974.