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Conditions Modifying Development of Tumors in Mice at Various Sites by Benzo(*a*)pyrene[1]

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SUMMARY

The modifying roles of age, sex, and strain of mice on the incidence, multiplicity, and spectrum of tumors induced by benzo(*a*)pyrene have been investigated. The first-generation (F_1) hybrids of C57BL/6J × C3HeB/FeJ and C3HeB/FeJ × A/J mice of both sexes were given single i.p. injections (75 or 150 μg/g) of benzo(*a*)pyrene at 1, 15, or 42 days of age. Experimental animals were allowed to live their life-spans, while animals in control groups were killed at 52, 90, 142, or 170 weeks of age.

Animals treated with benzo(*a*)pyrene died, in general, by the 100th week of age due to development of liver, lung, stomach, and lymphoreticular tumors. Few of the control animals died during that same observational period.

The age of mice at the time of exposure to the carcinogen modified development of tumors at all the sites. The sex of animals influenced the development of liver and lymphoreticular tumors. The C3HeB/FeJ × A/J F_1 hybrids developed lung tumors more readily than did the C57BL/6J × C3HeB/FeJ F_1 mice, which had significantly more liver tumors and neoplasms of the lymphoreticular system than the former strain. No strain difference was observed in regard to tumors at other sites. Higher doses of benzo(*a*)pyrene were more effective in inducing lung, liver, and stomach tumors. In addition, 5 cases of pancreatic ductal adenoma and adenocarcinoma were observed in carcinogen-treated mice.

INTRODUCTION

A series of integrated studies was initiated a few years ago in an attempt to assess the sensitivity of 2 hybrid strains of mice to carcinogenic stimuli at specific age periods. Animals of both sexes were exposed to either a single or a limited number of administrations of the *N*-nitroso compounds ENU[3] (19), *N*-nitroso-*N*,*N*-diethylamine (10), or of the polycyclic aromatic hydrocarbon, BP. While ENU under physiological conditions undergoes spontaneous, rapid, heterolytic decomposition generating a short-acting carcinogenic moiety in all tissues (2, 5), *N*-nitroso-*N*,*N*-diethylamine and BP (4, 6, 8, 14) require the presence of specific enzymes for their activation to a carcinogenic moiety. The latter capability varies from tissue to tissue, thus restricting the carcinogenicity of the agent to the tissue(s) possessing enzymatic activity and susceptibility at the time procarcinogen is administered. This report deals with the roles of age, sex, and strain on carcinogenesis in several tissues following single administration of BP.

MATERIALS AND METHODS

Mice. The 1st generation of C57BL/6J × C3HeB/FeJ and C3HeB/FeJ × A/J hybrids, hereafter called B6C3F_1 and C3AF_1, respectively, were used. The parent strains had been purchased from The Jackson Laboratory, 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 Rockland mouse diet 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 signs indicating the development of internal tumors and/or nonspecific pathological changes.

Treatment. BP, after being checked for purity, was dissolved (0.75 and 1.50%) in trioctanoin (Eastman Kodak Co., Rochester, N. Y.) at the time of treatment and was administered as specified in Table 1. Four nontreated and 1 solvent-treated group served as the source of information on the spontaneous tumor development in each of the hybrids (19).

Duration of Experiment. Groups of nontreated control mice were killed at 52, 90, and 142 weeks of age, whereas survivors of a 4th set of nontreated controls and a set of trioctanoin-treated groups were killed at 170 weeks. Animals given BP were observed throughout their life-spans. 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, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

Statistical Analysis of Data. The incidence of tumors of a given organ was calculated from the number of animals still alive in each experimental group at the time that a tumor of that organ was first observed at autopsy. The χ^2 method of analysis was applied in the assessment of difference in tumor incidence between groups, while the differences in the average age at which tumors were detected at autopsy were

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[3] The abbreviations used are: ENU, *N*-ethyl-*N*-nitrosourea; BP, benzo(*a*)pyrene.

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assessed by Student's *t* test. The variation in the multiplicity of lung tumors was evaluated by the Poisson method.

RESULTS

The animals tolerated BP administration well, since practically none died due to its toxic effect within 4 weeks following treatment with the carcinogen. The effects of the carcinogen were manifested late in life in the variation of the survival rates of the animals, their body weights, and the incidence and spectrum of tumors that developed.

In general, animals treated at 42 days of age had lower weight loss and lived 10 to 20 weeks longer than mice given BP at birth or in infancy. The females of both strains lived 10 to 20 weeks longer than the males of comparable groups. Of the 2 hybrid strains, the $B6C3F_1$ strain showed better survival. The mortality of the animals, in general, was associated with development of tumors, mainly of the liver, the stomach, and the lymphoreticular system.

Development of Tumors in the Controls. Detailed information on the incidence of tumors in the nontreated controls has been presented in a paper reporting on the multicarcinogenicity of ENU which was evaluated concurrently with this study and used the same biological system (19). For proper evaluation of the presented data and at the same time to avoid repetition *in toto*, Table 2 presents only the incidences of tumors observed in animals sacrificed at 90 and 142 weeks. By the 90th week of life, tumors had developed at a low incidence with the exception of lung adenomas observed in the $C3AF_1$ males (49%) and females (26%) ($p < 0.01$). At 142 weeks of age, however, various primary tumors were observed in both strains, each type of neoplasm not exceeding an incidence of 10%, except lung tumors, which developed in 60% of male and 40% of female $C3AF_1$ mice, in contrast with only 13 and 9% observed in the $B6C3F_1$ male and female mice, respectively.

Development of Tumors in BP-treated Animals. The primary tumors developed in 9 organs (Table 3) but principally in the liver, lung, stomach, and lymphoreticular system.

Liver Tumors. Table 4 presents the incidence of liver tumors and the average age of mice at the time of tumor detection at autopsy. In general, hepatomas developed with significantly higher incidence ($p < 0.01$) and at an earlier age in mice that were treated with BP within 24 hr of birth or at 15 days of age than they did in animals receiving similar treatment at 42 days of age. In the latter case, the incidence of hepatomas was essentially the same as that observed in the nontreated controls (Table 2).

Females of both strains developed hepatomas with significantly lower incidence than did corresponding male mice ($p < 0.01$). This incidence did not differ significantly from that observed in the nontreated controls with the exception of $B6C3F_1$ females treated at birth.

Table 1

Experimental design

Age at treatment (days)	No. of animals in experimental groups			
	BP, 75 μg/g[a]		BP, 150 μg/g[a]	
	M	F	M	F
	$B6C3F_1$			
1	47	45	63	45
15	60	55	55	45
42	55	47	47	46
	$C3AF_1$			
1	62	45	52	56
15	56	49	53	57
42	30	32	32	40

[a] Given i.p. in 0.01 ml of trioctanoin per g of body weight.

Table 2

Incidence of tumors in various organs of nontreated control mice at 90 and 142 weeks of age

Sex	Age[a] (wk)	No. of mice	% of mice bearing primary tumors in							
			Lung	Liver	Harderian glands	Stomach	Kidney	Lympho-reticular system	Nervous system	Mam-mary gland
			$B6C3F_1$							
M	90	98	7	1	5	0	0	2	0	0
	142	100	13	7	4	0	0	8	0	1
F	90	96	2	0	1	0	0	2	0	1
	142	100	9	1	4	1	1	8	0	4
			$C3AF_1$							
M	90	97	49	3	3	0	0	0	0	0
	142	100	60	8	4	3	2	3	0	2
F	90	100	26	0	1	0	1	2	0	2
	142	100	50	1	1	2	3	13	0	1

[a] Age at which survivors of the respective groups were killed.

Table 3
Tumors diagnosed in various organs, tissues, and systems of BP-treated mice

Liver
Adenoma
Hepatocellular carcinoma
Lung
Adenoma
Adenocarcinoma
Stomach
Papilloma
Squamous cell carcinoma
Reticuloendothelial system
Lymphocytic
Granulocytic
Reticulum cell
Kidney
Solid granular-clear-cell adenocarcinoma
Papillary cystadenoma
Ovaries
Tubular adenoma
Granulosa cell tumor
Uterus
Sarcoma
Leiomyosarcoma
Central nervous system
Oligodendroglioma
Ependymoma
Pancreas
Ductal papillary adenoma
Ductal adenocarcinoma

Higher doses of BP significantly increased tumor incidence and shortened the time of their emergence in males given carcinogen at birth ($p < 0.05$). In the 15-day-old animals, the higher dose resulted in reduced longevity (Table 4) so that a significant difference in tumor incidence was observed between groups of mice treated at 1 and 15 days of age, respectively ($B6C3F_1$, 81% *versus* 58%, $p < 0.01$; $C3AF_1$, 46% *versus* 23%, $p < 0.05$).

Of the 2 strains, the $B6C3F_1$ hybrids developed hepatomas at a significantly higher incidence. Thus, all 4 experimental conditions, *i.e.*, age, sex, dose, and strain, modulated hepatocarcinogenesis by BP.

Lung Tumors. Data on the incidence of multiplicity of lung tumors are presented in Table 4. Of the 2 strains, the $C3AF_1$ mice developed significantly more tumors than did the $B6C3F_1$ hybrids ($p < 0.001$); multiplicity of lung tumors was also higher in the former hybrid strain.

The lower susceptibility of $B6C3F_1$ enabled demonstration of variation in tumor incidence in relation to age and dose. Thus, both sexes developed lung tumors with higher incidence when treated with BP at birth than at 15 or 42 days of age ($p < 0.05$). Higher doses of BP, however, significantly enhanced lung carcinogenesis in newborn $B6C3F_1$ female mice ($p < 0.05$).

Stomach Tumors. Table 5 lists data on the incidence of stomach tumors, equally represented by squamous cell papillomas and carcinomas. Regardless of age and sex, 97 of 583 (17%) mice exposed to the low dose of BP developed stomach tumors. With the high dose, this incidence was increased to 30%, mainly due to the consistently high response of animals treated at 15 or 42 days of age. Animals treated with the higher dose at birth were consistently less responsive (62 of 415; 15%) than were mice given the carcinogen in infancy (119 of 430; 28%) or in young adulthood (103 of 329; 31%). In contrast to tumor development in liver and lungs, no strain and sex differences were observed with regard to development of tumors in the gastric epithelium.

Lymphoreticular Tumors. Both "dose" groups for each strain were combined because no statistically significant difference between the 2 dose levels was observed. Table 6, therefore, presents the incidence of lymphoreticular tumors in relation to age, sex, and strain. Data show that the age at which mice received BP had a modifying effect upon tissue response. Thus, when the treatment was initiated within the 1st 24 hr after birth, 23% of males and 43% of females developed lymphoreticular tumors. When BP was given at 6 weeks of age, however, 45% of males and 61% of females developed tumors. This positive age-associated difference in the incidence of lymphoreticular tumors was statistically significant in both sexes ($p < 0.001$ for males; $p < 0.05$ for females).

The overall incidence of lymphoreticular tumors in the $B6C3F_1$ hybrids was 32% (104 of 327) and 52% (148 of 283) for males and females, respectively ($p < 0.001$). This sex-associated difference was primarily due to development of reticulum cell sarcomas, as reported elsewhere (18).

The $C3AF_1$ strain was significantly less responsive to BP treatment than were $B6C3F_1$ mice [76 of 564 (13%) *versus* 252 of 610 (41%); $p < 0.001$]. The increase in the incidence of lymphoreticular tumors with the age of the animals at exposure to BP was observed only in females, which were also more responsive than similarly treated males [50 of 274 (18%) *versus* 26 of 290 (9%); $p < 0.001$].

Miscellaneous Tumors. In addition to the above-mentioned tumors which were observed with sufficiently high frequencies to make possible the assessment of the role of various experimental conditions on their development, various tumors were observed at other sites at levels too low to permit any such evaluation. For this reason, percentages for all tumors that appeared in a particular tissue were combined for males and females of each of the strains, except for those tumors developing in sex organs, and results are presented in Table 7. Corresponding values for the nontreated controls are also included. Statistical analyses of the data revealed a BP effect only in 3 tissues, namely, kidneys ($p < 0.10$ in $B6C3F_1$; $p < 0.01$ in $C3AF_1$), ovaries ($p < 0.01$ in both hybrid strains), and uteri ($p < 0.05$ in both strains). Kidney tumors encompassed papillary adenomas and adenocarcinomas. Ovarian tumors included tubular adenomas and granulosa cell tumors, while uterine tumors were mostly mesenchymal sarcomas. The latter type of tumor was also seen arising occasionally from the soft

Table 4
Incidence of liver and lung tumors

Sex	Age[a] (days)	Liver tumors: Dose: 75 μg/g body wt: % [b]	Liver tumors: Dose: 75 μg/g body wt: Av. age[c] (wk)	Liver tumors: Dose: 150 μg/g body wt: %	Liver tumors: Dose: 150 μg/g body wt: Av. age (wk)	Lung tumors: Dose: 75 μg/g body wt: %	Lung tumors: Dose: 75 μg/g body wt: Av. age (wk)	Lung tumors: Dose: 75 μg/g body wt: Multiplicity[d]	Lung tumors: Dose: 150 μg/g body wt: %	Lung tumors: Dose: 150 μg/g body wt: Av. age (wk)	Lung tumors: Dose: 150 μg/g body wt: Multiplicity
					B6C3F$_1$						
M	1	55	86	81	81	43	103	3	59	84	4
	15	60	93	58	81	25	103	2	36	82	2
	42	13	108	9	87	36	119	2	38	95	2
F	1	7	129	18	121	49	126	3	62	112	4
	15	7	116	7	90	33	122	2	40	101	3
	42	0		0		26	131	2	17	118	3
					C3AF$_1$						
M	1	34	80	46	69	93	78	6	92	70	8
	15	27	90	23	77	93	87	5	94	75	6
	42	0		3	79	93	91	5	87	85	6
F	1	2	91	2	70	93	82	7	93	73	7
	15	2	102	2	62	94	98	5	91	79	6
	42	0		0		87	93	5	90	83	6

[a] Age at which animals received i.p. injections of BP, dissolved in trioctanoin, and delivered in the amounts specified.
[b] Ratio of number of mice bearing liver or lung tumors to effective number expressed as a percentage.
[c] Average age at which tumors were observed.
[d] Average number of grossly visible lung tumors per whole lung.

Table 5
Incidence of stomach tumors

Sex	Age[a] (days)	BP, 75 μg/g: %[b]	BP, 75 μg/g: Age[c] (wk)	BP, 150 μg/g: %	BP, 150 μg/g: Age (wk)
			B6C3F$_1$		
M	1	23	98	22	75
	15	25	97	45	77
	42	25	113	51	94
F	1	11	118	11	97
	15	11	107	58	99
	42	23	129	26	101
			C3AF$_1$		
M	1	6	79	17	68
	15	9	81	34	72
	42	20	87	41	84
F	1	13	69	14	69
	15	18	99	26	82
	42	12	101	20	81

[a] Age at which animals were given BP.
[b] Ratio of number of mice bearing stomach tumors to effective number expressed as a percentage.
[c] Average age at which tumors were observed.

retroperitoneal tissues. In addition, between the 2 strains there were 5 cases of pancreatic ductal papillary adenomas and adenocarcinomas (Table 3).

DISCUSSION

The main objective of this series of experiments was to assess the roles of age, sex, strain, and dose of BP on carcinogenesis in various tissues so that one could define the optimal biological conditions favoring carcinogenesis by polycyclic aromatic hydrocarbons and compare them with conditions favoring carcinogenesis by representatives of other classes of carcinogens in the same biological system. The summary of the results presented in Table 8 illustrates trends in factors influencing carcinogenesis similar to those recently reported for ENU (19).

In both strains, the age of the animals at the time of exposure to BP has been the most effective modulator of carcinogenesis in liver, lung, stomach, and lymphoreticular system. Newborn and infant mice developed liver and lung carcinogenesis more readily than did young adults. In contrast, infant and young adult mice were more prone to stomach and lymphoreticular system tumorigenesis. This is consistent with the observed high incidence of pulmonary tumors in newborns (9, 12, 16, 17) and gastric tumors in adult mice (1, 3, 11, 13) following single exposures to BP.

The sex of the animals affected the development of liver

Table 6

Incidence of lymphoreticular tumors in mice following administration of BP[a] at 1, 15, or 42 days of age

Sex	Age at treatment (days)	$B6C3F_1$ Effective no.	$B6C3F_1$ No.	$B6C3F_1$ %	$B6C3F_1$ Age at death (wk)	$C3AF_1$ Effective no.	$C3AF_1$ No.	$C3AF_1$ %	$C3AF_1$ Age at death (wk)
M	1	112	26	23	85	114	7	6	76
	15	112	32	29	85	109	15	14	78
	42	102	46	45	98	62	4	6	87
F	1	89	38	43	117	101	8	8	78
	15	100	54	54	92	105	22	21	89
	42	92	56	61	97	72	20	28	89

[a] BP, dissolved in trioctanoin, was injected i.p. once at specified ages; each animal received 75 or 150 μg of carcinogen per g of body weight.

Table 7

Development of the miscellaneous tumors in various tissues and organs of nontreated controls and BP-exposed mice

Organ	% incidence of tumors in given organs: $B6C3F_1$ Controls	$B6C3F_1$ BP	$C3AF_1$ Controls	$C3AF_1$ BP
Brain	0.0	0.3	0.0	0.9
Harderian glands	3.5	3.3	2.2	0.9
Salivary glands	0.0	0.2	0.0	0.0
Esophagus	0.0	0.3	0.0	0.0
Intestines	0.0	1.1	0.0	1.4
Pancreas	0.0	0.3	0.0	0.1
Kidneys	0.2	6.8	1.3	6.2[a]
Adrenals	0.0	0.6	0.0	0.0
Ovaries	2.2	10.6[a]	1.0	6.8[a]
Breast	2.5	0.7	1.5	0.0
Uterus	0.5	4.6[a]	0.5	4.3[a]
Blood vessels	0.0	1.3	0.0	0.9
Skin	0.0	0.3	0.0	0.1

[a] Statistically significant different in the specified incidence of tumors between the untreated controls and BP-treated mice.

Table 8

Experimental conditions modulating carcinogenesis by BP in various tissues

Carcinogenesis in	Conditions: Age Newborn[a]	Age Infant	Age Adult	Sex M	Sex F	Strain $B6C3F_1$	Strain $C3AF_1$	Dose Low	Dose High
Lung	+						+		+
Liver	+	+		+		+			+
Stomach		+	+						+
Lymphoreticular system		+	+		+	+			

[a] Newborn, <24 hr; infant, 15 days; adult, 42 days; +, more favorable condition.

and lymphoreticular tumors. Males were consistently more responsive to hepatocarcinogenesis (12), while females developed malignant lymphomas with greater frequency. Of the 2 hybrids, the $C3AF_1$ mice were more susceptible to lung and kidney carcinogenesis, whereas $B6C3F_1$ mice developed hepatomas and lymphoreticular tumors more frequently. The higher dose of BP significantly enhanced development of hepatomas (newborn males), lung tumors (newborns), and stomach tumors (both sexes).

The data show that the spectrum of tumors induced by

BP (Table 3) has been strikingly narrower than that reported for the ENU series (19). In the latter case, 59 primary tumors were observed in 22 tissues; whereas in the current study, only 19 primary tumors developed in 9 organs. This difference may be attributed to physicochemical properties of the agents, mechanisms of their activation to ultimate carcinogenic moieties and catabolic removal, and the administered molar doses. Thus, while ENU is water soluble and spontaneously undergoes rapid heterolytic decomposition, BP has low solubility in aqueous media and requires, for its activation, specific aryl hydrocarbon hydroxylases, the levels of which may vary between tissues and strains (4, 7). Comparison of tumor responses to approximately isomolar doses of these 2 agents (150 μg of BP per g *versus* 60 μg of ENU per g) showed lower carcinogenicity of BP to lungs, liver and kidneys. Thus, differences in the solubility and the activation of these 2 agents appear to be directly related to variations in their carcinogenicity.

Although the high susceptibility of the newborn animal to chemical carcinogens had been repeatedly demonstrated, few studies were designed to evaluate concurrently the age-associated differences in response to specific carcinogens (15, 20). An overall view of the present data indicates that the preweaning animals are more sensitive to carcinogenic response than the adults are. The common features of tissues during the infant age period, namely, relative cell immaturity, high rate of macromolecular synthesis, and a consequently high rate of cell replication, as well as low immunological competence, are probably causally related to the high susceptibility of tissues to the inception of carcinogenesis and earlier expression of tumor development.

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