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Benzene Inhalation Effects Upon Tetanus Antitoxin Responses and Leukemogenesis in Mice[1,2,3]

Richard D. Stoner, Robert T. Drew and David M. Bernstein

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[2]From the Medical Research Center, Brookhaven National Laboratory, Upton, N.Y. 11973. Telephone No. (516)345-3601, FTS (516)666-3601.

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ABSTRACT

This report describes the effects of inhaled benzene on primary and secondary antibody responses and the incidence of leukemia in mice. Young adult mice (15/group) were given 5, 12 or 22 exposures to 400 ppm benzene for 6 hours/day 5 days/week. After the exposure periods, the mice were immunized with absorbed tetanus toxoid (APTT) and/or fluid tetanus toxid (FTT). Exposure to benzene increasingly suppressed primary antitoxin responses to both antigens. Secondary antibody responses to FTT were nearly normal in animals similarly given 10, 15 or 20 exposures to 400 ppm benzene. Other groups of mice were similarly exposed to either 200 ppm or 50 ppm benzene. Primary antitoxin responses elicited with FTT and/or APTT were nearly normal in all mice exposed to 50 ppm benzene and in mice exposed to 200 ppm benzene for 5 days. However, 10 and 20 exposures to 200 ppm benzene inhibited antibody production.

An experiment was carried out to test the effects of inhaled 300 ppm benzene on the time of onset and incidence of leukemia in 400 female HRS/J mice. Two genotypes were used; the (hr/hr) hairless mice are leukemia-prone, whereas the (hr/+) haired mice are more resistant to leukemia. Chronic exposure to benzene and/or air-control conditions were started when the mice were 7 months of age. The exposures continued for a period of 6 months. Lymphoid, myeloid and mixed (lymphoid and myeloid) leukemias were observed. Ninety percent of the (hr/hr) mice exposed to benzene died from leukemia as compared with 91 percent for the (hr/hr) air control group. Eighty-five percent of the (hr/+) mice exposed to benzene died from leukemia as compared with 81 percent for the (hr/+) air control group. Exposures to 300 ppm benzene did not alter the time of onset or the incidence of leukemia commonly expected in HRS/J mice.

INTRODUCTION

There are no reports in the literature concerning the effects of inhaled benzene upon antibody responses to specific antigens. The early studies by Rusk (1914), Hektoen (1915, 1916) and Simonds and Jones (1915) demonstrated that subcutaneous injection of rabbits with benzene in olive oil and/or corn oil mixtures depressed antibody production to sheep red cells and inhibited the formation of agglutinins for killed typhoid bacilli. A more recent report by Lange, Smolik, Zatonski and Szymanska (1973) indicated that serum complement, IgG and IgA, globulin levels were decreased in workers occupationally exposed to benzene. IgM globulin levels were slightly elevated in the group exposed to benzene vapors; based upon clinical records, these individuals were more susceptible to bacterial infections.

In this paper we present the results of studies designed to test the inhibitory effects of inhaled benzene on primary and secondary (booster) tetanus antitoxin responses in mice. Mice were exposed to 50, 200 and 400 ppm benzene in these experiments. All animals were immunized at the termination of exposure; similar air chamber control groups also were maintained. In general, primary antibody responses to tetanus toxoid were suppressed after exposures to inhaled benzene. Nearly normal secondary antitoxin responses were observed in mice exposed to inhaled benzene. Heiniger, Meier, Kaliss, Cherry, Chen and Stoner (1974) observed a functional immunodeficiency in the capacity of hairless (hr/hr) mutants of the HRS/J strain of mice to produce primary tetanus antitoxin responses. Meier, Myers and Huebner (1969) reported that 70 percent of the homozygous (hr/hr) mice, as compared with 20 percent of the heterozygous (hr/+) haired mice, developed lymphoid leukemia after 8 months of age. Both genotypes later developed myeloid leukemia after 18 months of age. Chronic injection of

an immunosuppressant (azathioprine) increased the incidence of leukemias up to 90 percent in both genotypes (Meier and Myers, 1974). All HRS/J mice of both genotypes carry equivalent amounts of endogenous murine C-type RNA virus (MuLV) in their tissues. The (hr) locus on chromosome 14 (Linkage Group III) is believed to influence susceptibility to leukemia (Deringer, 1951). The observation of benzene induced suppression of primary immune responses together with the possible role of the mutant (hr) gene, or a closely linked gene in immunosurveillance against leukemogenesis, led us to investigate the immunosuppressant effects of inhaled 300 ppm benzene upon the incidence of leukemia in HRS/J mice.

MATERIALS AND METHODS

Animals

The animals used for the antibody response experiments were pathogen-free female Swiss albino mice (Hale-Stoner strain). All animals were raised in the Brookhaven colony and weaned when 4 weeks of age. Breeders and weanlings were maintained on HCl acidified drinking water, pH 2.5, to prevent carrier infections with *Pseudomonas aeruginosa*. The animals were 10 to 12 weeks of age at the time of exposure to inhaled benzene.

The two genotypes (hr/hr) and (hr/+) of the HRS/J strain of mice were obtained as weanlings from the Jackson Laboratory, Bar Harbor, Maine. The two genotypes are congenic and differ only at the mutant gene locus, or in a closely linked region to the mutant gene locus.

Antigens and Immunization

Primary antibody responses were elicited with either a single subcutaneous injection of 0.2 ml fluid tetanus toxoid (FTT) or a single injection of 0.05 ml aluminum phosphate-adsorbed tetanus toxoid (APTT). Secondary (booster) responses were elicited with a single subcutaneous injection of 0.1 ml FTT. The toxoids were obtained commercially from Lederle Laboratories, Pearl River, New York.

Serology and Titration of Antitoxin

Mice were bled from the caudal artery (0.2 ml) and the pooled sera from each group was titrated as a single specimen. Serum antitoxin titers were determined in test mice by the Ehrlich method (neutralization of potent tetanus toxin). The toxin contained 2×10^5 mouse MLD/ml. Minimal paralysis at the end of 4 days was used as the end point. Each sample of serum was titrated several

times. A more detailed description of titration procedures may be found in an earlier report (Hale and Stoner, 1956).

Pathological and Hematological Examinations

All animals were clinically observed daily and killed when moribund under deep ether anesthesia. Where possible, a total white count, hematocrit and white cell differential count was performed. The blood smears were stained with myeloperoxidase stain using benzidine dihyrochloride and recounterstained with fresh Giemsa stain for 10 minutes (Kaplow, 1965). The carcesses were fixed in 10% buffered formalin and sections stained with hematoxylon and eosin.

The classification of the observed leukemias was based on the pathological descriptions of Meier, Myers and Huebner (1969). Lymphoid leukemia was grossly characterized by thymoma, lymphadenopthy and hepatosplenomagly. The predominant cell type was lymphocytic. The myeloid leukemic animals exhibited splenomegaly rather than lymphadenopathy and thymoma. Spleens were enlarged 5 to 10 times their normal size. The lymph nodes were normal in size and khaki-colored. These animals showed high peripheral white blood cell counts (predominantly immature granulocytes). Mixed lymphoid and myeloid leukemias were also observed.

Exposure Methodology for Antibody Response Experiments

Animals were exposed to benzene in a double barrier chamber system (Laskin, Kuschner and Drew, 1970). The stainless steel and plexiglass system consisted of five interconnected compartments, two of which were used for housing the animals. One was used for the controls and the other for the exposed animals. When in these compartments, food and water were available ad libitum. Air entered through the compartment housing control mice and was exhausted by way of the compartment housing the exposed mice. The entire system was kept at a negative pressure of approximately 1 of inch water (-1"wg). The third compart-

ment, also maintained at -1" wg, contained a small (128 liter) chamber into which the animals were placed for exposure to benzene. This exposure chamber was operated at approximately -2" wg relative to the chamber room. Animals were transferred from the living quarters to the exposure chamber through the use of glove ports. An interlocking system was employed such that if negative pressure was lost in the system the benzene supply would be terminated.

The benzene vapor was generated in a compartment located above the internal exposure chamber, also kept at one inch negative of water. A regulated supply of filtered dry compressed air was passed through a bubbler containing benzene. The output of the bubbler led into the main air supply for the exposure chamber (perpendicular to the direction of airflow). The chamber air was exhausted through charcoal filters to remove the benzene. The benzene concentration was monitored downstream from the charcoal filters with a Wilks Miran infrared spectrophotometer to insure that no benzene breakthrough occurred. A parallel alternate charcoal system was available for switchover in case a benzene breakthrough was detected. The benzene concentration in the exposure chamber was monitored on a routine basis with a gas chromatograph (GC) (Packard Model 417). A continuous flow sampling system from the exposure chamber to the GC was employed using an automatic sampling valve to inject a sample into the column. In addition, a wet chemical method was used daily to insure that the GC was operating correctly. This method consists of adsorbing benzene in ethanol in two adsorbers in series and then subsequently detecting the benzene with a UV spectrophotometer.

Exposure System for the Large Scale, Long-Term Leukemia Benzene Experiment

A 0.38 cubic meter stainless steel and glass chamber, similar to that described by Hinners, Burkhart and Punte (1968) except that the upper pyramid

was at an angle of 67° with the horizontal, was used in these studies. The benzene was generated as described above in an enclosed glove box system. The benzene vapor was fed perpendicularly into the intake air to the chamber. The feed line was wrapped in heating tape and kept at 50°C to prevent any condensation. The exhaust was passed through large charcoal filters in parallel to allow a switchover in case a breakthrough occurred. The chamber was modified to insure the safety of the personnel operating the system. An electronic solonoid valve in the supply line was used with an interlocking system arranged so that the flow of vapor was terminated immediately from either a loss of negative pressure in the chamber or by releasing one of the latches securing the chamber door. The animals were maintained during exposure without food or water. One hour after the benzene generator was shut off, the animals were transferred to separate housing facilities. During housing and exposure, the animals were maintained at approximately 22°C + 2°C and 50% relative humidity.

RESULTS

Effects of Inhaled Benzene on Primary Antibody Responses to Fluid Tetanus Toxoid

The experiment shown in Table 1 demonstrated the inhibitory effects of 400 ppm benzene upon primary tetanus antitoxin responses to FTT in mice. Three groups of BNL mice (15 per group) were exposed to inhaled benzene for 6 hours/day for periods of 5, 12 and 22 days. Control groups were exposed to air under similar temperature and air flow conditions. After the various periods of exposures to benzene, the animals were removed from the chambers and given a primary immunization of 0.2 ml FTT by subcutaneous injection. Pooled serum samples from each group were obtained 21 days later. Antibody titers are expressed as the number of minimum lethal doses (MLD) of tetanus toxoid neutralized per cm^3 of pooled serum and/or as International Units (IU) of tetanus antitoxin per cm^3 of serum. Primary antibody responses to FTT were greatly repressed (75 to 88 percent) in mice exposed to 400 ppm benzene for all time periods. Primary responses to FTT were also slightly repressed (6 to 22 percent) in mice maintained under similar air chamber conditions as compared with animals not transported to the inhalation chambers. It should be emphasized that animals were only exposed to 400 ppm benzene prior to primary immunization and then returned to our mouse facility; thus, recovery from the inhibitory effects of exposure to inhaled benzene could occur during the period of 21 days before serum was obtained for antitoxin titration.

Effects of Inhaled Benzene on Primary Antibody Responses to Adsorbed Tetanus Toxoid

The data summarized in Table 2 describes the repressive effects of 400 ppm benzene upon primary responses to APTT in mice. Animals were exposed to benzene

and/or air control conditions for 6 hours/day for periods of 5, 12 and 22 days. Primary immunization with 0.05 ml APTT was given by subcutaneous injection at the end of the various exposure periods. The animals were then removed from the exposure chambers and housed in our mouse facility. Pooled sera from the various groups were obtained 21 days later. Exposure to 400 ppm benzene for periods of 5, 12 and 22 days increasingly suppressed primary antitoxin responses with duration of exposure. Primary responses to APTT were not altered in mice exposed to air control conditions for 5 and 12 days; however, antibody responses appeared to be inhibited (26 percent) in air control mice exposed for 22 days.

Effects of Inhaled Benzene Upon Secondary Antibody Responses.

The results of an experiment to test the effects of 400 ppm benzene on secondary antitoxin responses are presented in Table 3. All animals were given a primary immunization with 0.05 ml APTT by subcutaneous injection when 4 weeks of age. Two months later, the animals were randomly distributed into the various groups and exposed to benzene and/or air control conditions for periods of 10, 15 and 20 days. A second (booster) injection of 0.1 ml FTT was given subcutaneously at the end of the periods of exposure. Pooled samples of serum were obtained from each group 7 days later. The results of the antitoxin titrations are presented as MLD of toxin neutralized and as IU of antitoxin per cm^3. It is apparent that exposure to 400 ppm benzene or to air control condition did not significantly alter secondary antibody responses. Similar results were observed in several repeat experiments. These findings with benzene are rather similar to earlier studies concerning the radiosensitivity of antibody responses wherein primary responses were inhibited by doses of ionizing radiation whereas secondary antibody responses were radioresistant.

Effects of 200 ppm Benzene on Primary Responses to Fluid Tetanus Toxoid

The experiment plotted in Figure 1 shows the effects of 200 ppm benzene upon primary responses to FTT. Seven groups of 15 mice each were exposed either to benzene or to air control conditions. As in the previous experiments, the animals were immunized at the end of the exposure periods with 0.2 ml FTT. Serum was obtained 21 days later. Antibody titers are expressed as the number of MLD of tetanus toxin neutralized per cm^3 of serum. Primary antibody responses were increasingly repressed as the exposure period to 200 ppm benzene was increased through 5, 10 and 20 days. Although primary antitoxin responses were greatly inhibited in these benzene-exposed animals, all groups of mice responded with excellent secondary antitoxin responses when a booster dose of 0.1 ml FTT was given on day 28 after primary immunization (day 7 after the 21-day serum samples were obtained).

Effects of 200 ppm Benzene on Primary Antibody Responses to Adsorbed Tetanus Toxoid

The data shown in Figure 2 illustrates the effects of 200 ppm upon primary antitoxin responses to APTT. Seven groups of 15 mice each were exposed to benzene and/or air control conditions for periods of 5, 10 and 20 days. The animals were given primary immunizations of 0.05 ml APTT at the end of their exposure periods. Pooled serum samples were obtained for titration of antitoxin 21 days later. Antibody titers are expressed in MLD toxin neutralized per cm^3 of serum. Primary responses were not inhibited in animals exposed to 200 ppm benzene for 5 days; however, exposure periods of 10 and 20 days increasingly repressed antibody formation. As in the preceding experiment, (200 ppm benzene and primary immunization with FTT) all groups of animals responded with excel-

lent secondary antitoxin responses elicited with 0.1 ml FTT on day 28 after the time of primary immunization.

Effects of 50 ppm Benzene on Primary Antibody Responses

The data from two experiments are plotted in Figure 3. Fourteen groups of mice (15 mice/group) were exposed to 50 ppm benzene and/or air control conditions for periods of 5, 10 and 20 days. Seven groups of mice were immunized with 0.05 ml APTT and 7 groups were immunized with 0.2 ml FTT by subcutaneous injections. Nearly normal primary antibody responses were obtained to both forms of tetanus toxoid in mice exposed to 50 ppm benzene. In the preceding comparable experiments, benzene exposure dose levels of 400 ppm and/or 200 ppm severly repressed primary antitoxin responses elicited with FTT and APTT. It is apparent from these data that the threshold dose level required for repression of primary antitoxin responses may be found between 50 ppm and 200 ppm of inhaled benzene.

Effects of 300 ppm Inhaled Benzene Upon Leukemogenesis in HRS/J Mice

The foregoing experiments clearly demonstrated the repressive effects of 200 to 400 ppm benzene upon primary antibody responses in BNL mice. The genetic control of susceptibility and resistance to viral leukemogenesis has been reported in HRS/J mice (Meier, Myers and Huebner (1969). They studied two genotypes hairless (hr/hr) and haired (hr/+) mice and observed leukemia in 72 percent of the (hr/hr) mice at 18 months and 20 percent leukemia in the heterozygous (hr/+) mice at 18 months of age. The mutant gene (hr) appeared to enhance susceptibility and the wild-type allele (+) induced resistance to leukemogenesis. Both genotypes contained similar amounts of murine C-type RNA virus in their adult and fetal tissues. A severe thymic cortical atrophy was observed in (hr/hr) mice together with a reduced capacity to respond to primary

immunization with APTT and/or FTT (Heiniger, Meier, Kaliss, Cherry, Chen and Stoner, 1974). Since chronic injection of an immunosuppressant (aziothioprine) increased the incidence of leukemia up to 90 percent in both genotypes (Meier and Myers, 1973), this led us to investigate the possible role of benzene-induced immunosuppression upon the time of onset and incidence of leukemia in HRS/J mice.

Four hundred female HRS/J mice were used in this experiment. One hundred (hr/hr) mutants and 100 heterozygous (hr/+) mice were exposed to 300 ppm benzene for 6 hours/day, 5 days/week for 6 months. The same numbers of (hr/hr) and (hr/+) mice were exposed to air under similar chamber conditions. Because of a delay in the construction of the inhalation facility, the exposures were not started until the animals were 7 months of age. The HRS/J mice begin to develop leukemia when 7 to 10 months of age. It was deemed worthwhile to expose them to benzene vapor at this rather late age to determine if a late exposure period to 300 ppm benzene of 6 months duration would alter the expected course of leukemogenesis.

The cumulative percent leukemic mortality observed is plotted in Figure 4. These data cover the exposure period of 6 months, along with an additional observation period of 5 months until the animals were 18 months of age. All mice dying from lymphoid, myeloid and mixed (lymphoid and myeloid) leukemia are plotted together. It is evident from the leukemic mortality curves shown in Figure 4 that exposure to 300 ppm benzene over a period of 6 months failed to hasten the time of onset of leukemia in both (hr/hr) and (hr/+) mice. On the contrary, the appearance of leukemia in (hr/hr) mice exposed to benzene was delayed during their exposure to benzene; this was followed by an increased incidence in leukemia so that by 18 months of age there was no difference observed

in the total percent leukemia (74 to 85 percent) between the four groups exposed to benzene and/or air chamber control conditions.

The gross pathological results and types of leukemia observed in (hr/hr) and (hr/+) animals are summarized in Table 4. Exposure of (hr/hr) and (hr/+) mice to 300 ppm benzene resulted in an increased number of myeloid leukemias; this was counterbalanced by an increase in the number of mixed (myeloid and lymphoid) leukemias observed in (hr/hr) and (hr/+) mice maintained under air chamber control conditions. There was no significant difference in percent leukemic mortality in the four test groups of HRS/J mice. Although it may be assumed that exposure to 300 ppm benzene repressed immune responsiveness in (hr/hr) and (hr/+) animals, this apparently did not affect any genetic mechanisms of immunosurveillance which may operate against leukemogenesis.

A total of 24 of the 400 test animals demonstrated hemorrhagic lungs when moribund. Heart blood samples from 15 of these animals were cultured for anaerobic bacteria at the time of autopsy. There was no evidence for a generalized bacteremia in these animals.

DISCUSSION

The results of the present series of experiments clearly demonstrate that inhaled benzene in doses of 200 to 400 ppm suppress primary tetanus antitoxin responses when fluid toxoid and adsorbed toxoid are administered after 5 to 20 days of exposure. These findings are in keeping with the earlier reports of Rusk (1914), Hektoen (1915, 1916) and Simonds and Jones (1915) in which inhibition of antibody production to sheep red cells and agglutinins for killed typhoid bacilli were demonstrated in rabbits after subcutaneous injection of benzene in olive oil and/or corn oil mixtures. These pioneer investigators were primarily interested in the use of chemical agents to abolish antibody formation so as to determine the identity of the various lymphoid organs involved in the production of specific antibodies.

No attempt was made in the present study to determine the cytological effects of inhaled benzene upon lymphoreticular tissues; this topic will be covered in a subsequent report. The observed suppression of primary antibody responses after exposure to inhaled benzene resembles previous results wherein sub-lethal doses of ionizing radiations greatly suppressed primary antibody responses (Stoner, Terres, Cottier and Hess, 1976). It should also be noted that exposure to 400 ppm benzene failed to inhibit secondary responses which is comparable to the well-known radioresistant nature of secondary antibody responses (Stoner and Hale, 1962). If the mechanisms for suppression of primary antibody formation are similar for inhaled benzene and ionizing radiation, it could be assumed that exposure to benzene interferes with the proliferation of plasmocytoid cells involved in the formation of antibodies. It should also be emphasized that in the present series of experiments animals were exposed to benzene only prior to the time of primary and/or booster immunizations and then

removed from the exposure chambers for the duration of their primary and secondary antitoxin responses. Possibly, antibody responses would have been reduced to even lower levels if the animals had been returned to the benzene-exposure chambers for the duration of their antibody responses. Even though primary antitoxin responses were greatly reduced by exposures of 200 to 400 ppm benzene, all groups of mice responded with nearly normal secondary antibody responses.

Chronic exposure of man to benzene has long been closely related to depression of the bone marrow with subsequent development of aplastic anemia. The appearance of leukemia in man has long been thought to be related to chronic exposure to benzene. The actual exposure doses experienced by man have been very difficult to determine. The estimated doses vary from about 10 ppm to 500 ppm over a period of many years (Askoy, Erdem and Dincol, 1976, and Infante, Rinsky, Wagover and Young, 1979). Direct evidence for benzene as a leukemic agent has been equivocal in laboratory animals. Ward, Weisburger, (1975) tested the effects of a subcutaneous injection of a 30% solution of benzene in corn oil in C57BL/6N mice and found no increase in thymic lymphomas. An increase in thymic lymphomas was observed in a positive control group injected with nitrosobutylurea. Maltoni and Scarmato (1979) administered benzene in olive oil by stomach tube to rats and reported a dose-correlated increase in lymphoreticular neoplasia and carcinoma of the Zymbal gland. Snyder, Goldstein, Sellakurnar Bromberg, Laskin and Albert (1980) gave lifetime exposures of inhaled 300 ppm benzene to C57BL/6J male mice and observed a significant increase in hematopoietic neoplasms which included 6 cases (15 percent) of thymic lymphomas.

The present study was designed to test the effects of 300 ppm benzene upon the time of onset and incidence of lymphoid and myeloid leukemia in HRS/J mice.

Two principal genotypes (hr/hr) hairless and (hr/+) haired mice were exposed to benzene and to similar air chamber control conditions. The results shown in Figure 4 illustrate that exposure to benzene failed to hasten the time of onset of leukemia in (hr/hr) mice and did not increase the incidence of leukemia in (hr/+) mice. These findings are in contrast with the earlier report of Meier, Myers and Huebner (1969) wherein they observed a greater incidence of leukemia in (hr/hr) mice (72 percent) than in (hr/+) mice (20 percent) at 18 months of age. Their observations were based upon retired breeders from their animal colony (337 (hr/hr) mutants and 279 (hr/+) mice). The animals were handled only at weekly intervals when changed to fresh cages. In the present study, we used virgin female animals and all animals were exposed either to benzene or to air chamber control conditions and were handled twice daily, 5 days each week. We have no idea if a handling effect plays a role in our observed greater incidence in leukemia in our control animals. Perhaps a genetic shift has occurred in the HRS/J mice during the past 10 years so that the (hr) gene locus no longer influences the previously observed greater susceptibility to leukemia in homozygous (hr/hr) animals. Meier, Myers and Huebner (1969) observed mostly lymphoid leukemias in both genotypes up to 12 to 15 months of age; whereas animals older than 15 months succumbed to myeloid (granulocytic) leukemia. We were unable to detect a distinct type of leukemia at any specific time in our benzene-exposed or control animals. Lymphoid, myeloid and mixed (lymphoid-myeloid) leukemias appeared at random.

It is obvious that under the experimental conditions employed in our study that exposure of HRS/J mice to 300 ppm benzene did not hasten the time of onset or influence the incidence of leukemia. Perhaps the animals were too old (7 months of age) when first exposed to 300 ppm benzene for a period of 6 months.

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TABLE 1.

Primary Antitoxin Responses to Fluid Tetanus Toxoid

In Mice Exposed to Benzene

Group No.*	Days Exposed To Benzene (400 ppm)	Tetanus Antitoxin Titer Day 21 Response		Percent Repression of Controls
		MLD Toxin Neutralized/cm^3	$IU/cm^3 \times 10^{-3}$	
1.	5	35	0.88	80.0
2.	12	45	1.12	74.3
3.	22	20	0.5	88.6
4.	Air Chamber Controls 5	150	3.78	14.3
5.	Air Chamber Controls 12	165	4.13	5.8
6.	Air Chamber Controls 22	135	3.38	22.9
7.	Room Temp. Controls	175	4.38	-

* 15 female BNL mice/group.

TABLE 2.

Primary Antitoxin Responses To Adsorbed Tetanus Toxoid

In Mice Exposed To Benzene

Group No.*	Days Exposed To Benzene (400 ppm)	Tetanus Antitoxin Titer Day 21 Response		Percent Repression of Controls
		MLD Toxin Neutralized/cm^3	IU/cm^3 x 10^{-3}	
1.	5	150	3.8	8.0
2.	12	105	2.6	35.6
3.	22	25	0.63	84.7
4.	Air Chamber Controls 5	160	4.0	1.9
5.	Air Chamber Controls 12	168	4.2	+3.0
6.	Air Chamber Controls 22	120	3.0	26.4
7.	Room Temp. Controls	163	4.1	--

* 15 female BNL mice/group.

TABLE 3.

The Effects of Inhaled Benzene (400 ppm) Upon

Secondary Tetanus Antitoxin Responses

Group No.*	Days Exposed To Benzene (400 ppm)	Day of Booster FTT (s.c.)	MLD of Toxin Neutralized /cm^3**	International Units of Tetanus Antitoxin /cm^3
1.	10	10	144	3.6
2.	15	15	316	8.9
3.	20	20	300	7.5
4.	Air Chamber Controls 10	10	225	5.6
5.	Air Chamber Controls 15	15	215	5.4
6.	Air Chamber Controls	20	246	6.1
7.	Room Temp. Controls	20	262	7.1
8.	Primary Only Controls	None	17.5	0.44

* 15 female BNL mice/group.

** Sera obtained 7 days after the booster injection of fluid toxoid.

TABLE 4

Type of Leukemia and Mortality

Observed in HRS/J Mice

Pathological Findings	Benzene (300 ppm)	Air Control	Benzene (300 ppm)	Air Control
	(hr/hr)	(hr/hr)	(hr/+)	(hr/+)
Lymphoid	9	13	20	20
Myeloid	50	29	47	32
Lymphoid and Myeloid (mixed)	17	32	18	25
Ovarian Tumor	0	1	2	1
Hemorrhagic Lungs	4	1	10	9
Eye Abscess	1	0	2	0
Total Leukemia Mortality	76/84	74/81	85/99	77/95
Percent Leukemia Mortality	90.4	91.3	85.8	81.1

LEGENDS FOR FIGURES

Figure 1. Showing primary antitoxin responses in seven groups of mice (15 mice/group). Primary immunizations were given on days 0, 5, 10 and 20 after exposure to benzene.

Figure 2. Showing primary antitoxin responses in seven groups of mice (15 mice/group). Primary immunizations were given on days 0, 5, 10 and 20 after exposure to benzene.

Figure 3. Showing primary antitoxin responses obtained in fourteen groups of mice (15 mice/group). Primary immunizations with either APTT or FTT were given on days 0, 5, 10 and 20 after exposure to benzene.

Figure 4. Showing the cumulative percent mortality of mice dying from lymphoid, myeloid and mixed leukemia. Each group consisted of 100 mice of (hr/hr) or (hr/+) genotypes. Exposure to 300 ppm benzene and/or air chamber control conditions was terminated after 6 months when the animals were 13 months of age.

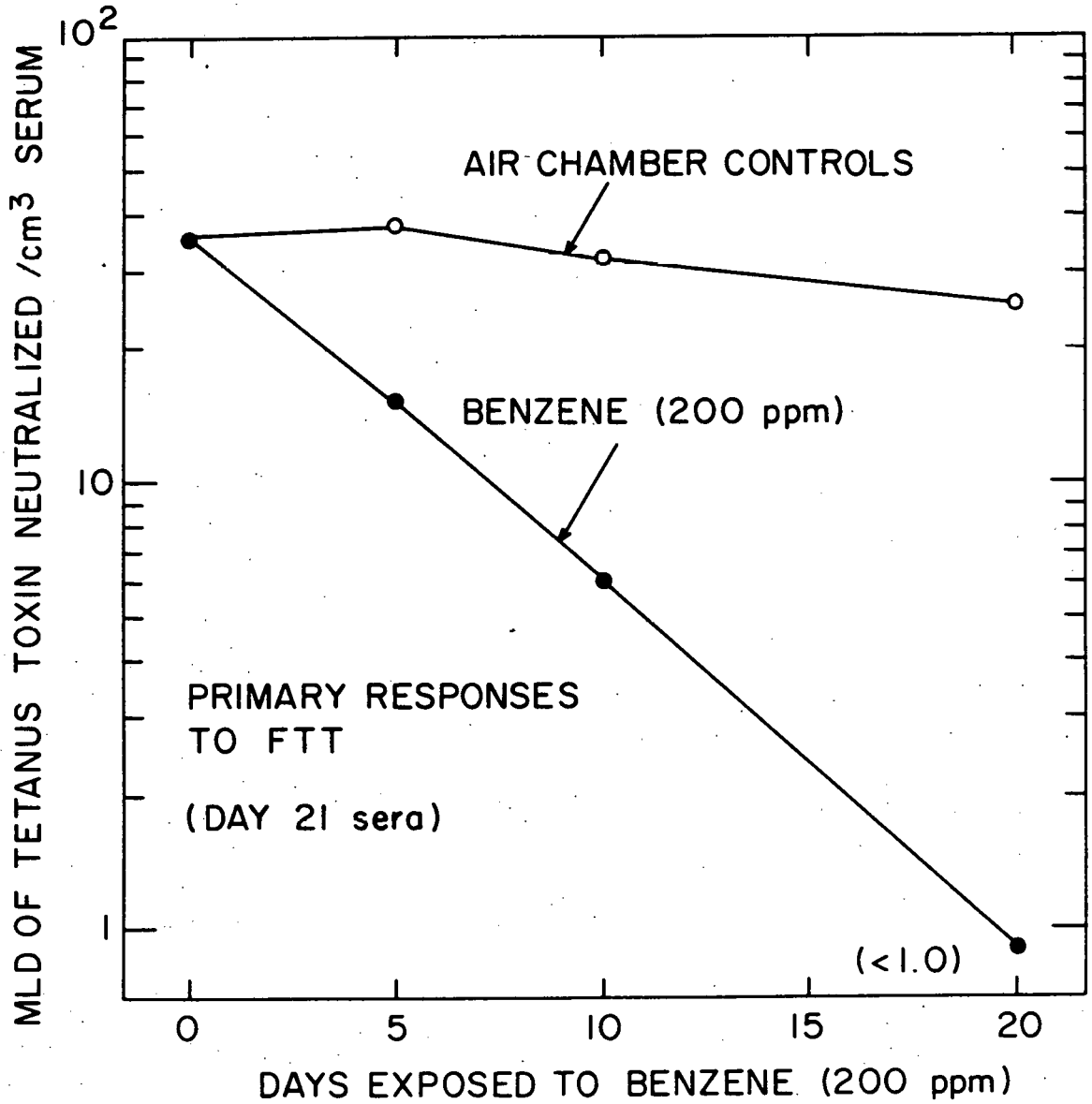
MLD OF TETANUS TOXIN NEUTRALIZED /cm3 SERUM
10^2
10
1
AIR CHAMBER CONTROLS
BENZENE (200 ppm)
PRIMARY RESPONSES
TO FTT
(DAY 21 sera)
(<1.0)
0
5
10
15
20
DAYS EXPOSED TO BENZENE (200 ppm)

FIGURE 1.

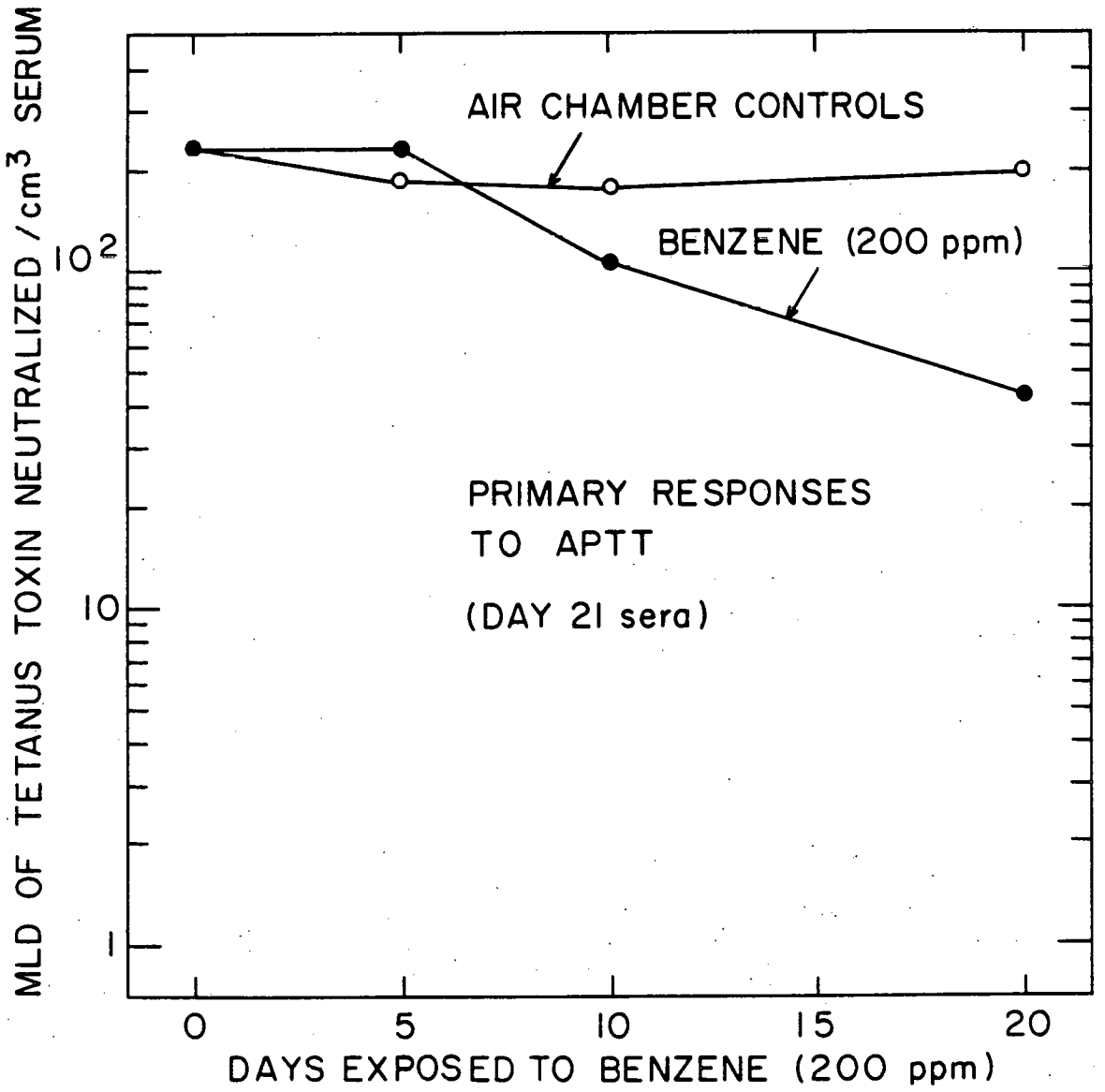
MLD OF TETANUS TOXIN NEUTRALIZED / cm3 SERUM
10 2
10
1
AIR CHAMBER CONTROLS
BENZENE (200 ppm)
PRIMARY RESPONSES
TO APTT
(DAY 21 sera)
0
5
10
15
20
DAYS EXPOSED TO BENZENE (200 ppm)

FIGURE 2.

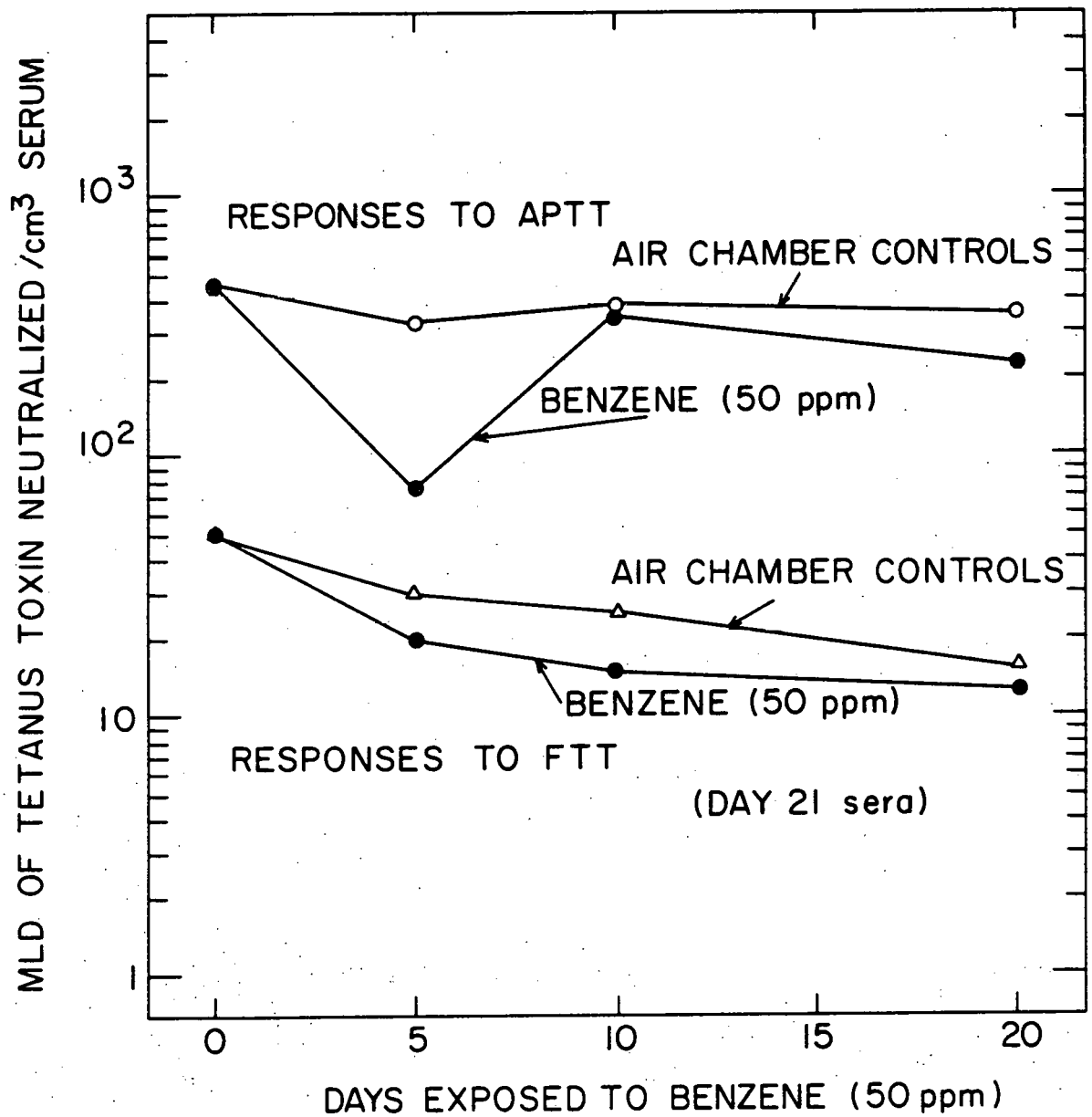
MLD OF TETANUS TOXIN NEUTRALIZED /cm3 SERUM
10^3
10^2
10
1
RESPONSES TO APTT
AIR CHAMBER CONTROLS
BENZENE (50 ppm)
AIR CHAMBER CONTROLS
BENZENE (50 ppm)
RESPONSES TO FTT
(DAY 21 sera)
0
5
10
15
20
DAYS EXPOSED TO BENZENE (50 ppm)

FIGURE 3.

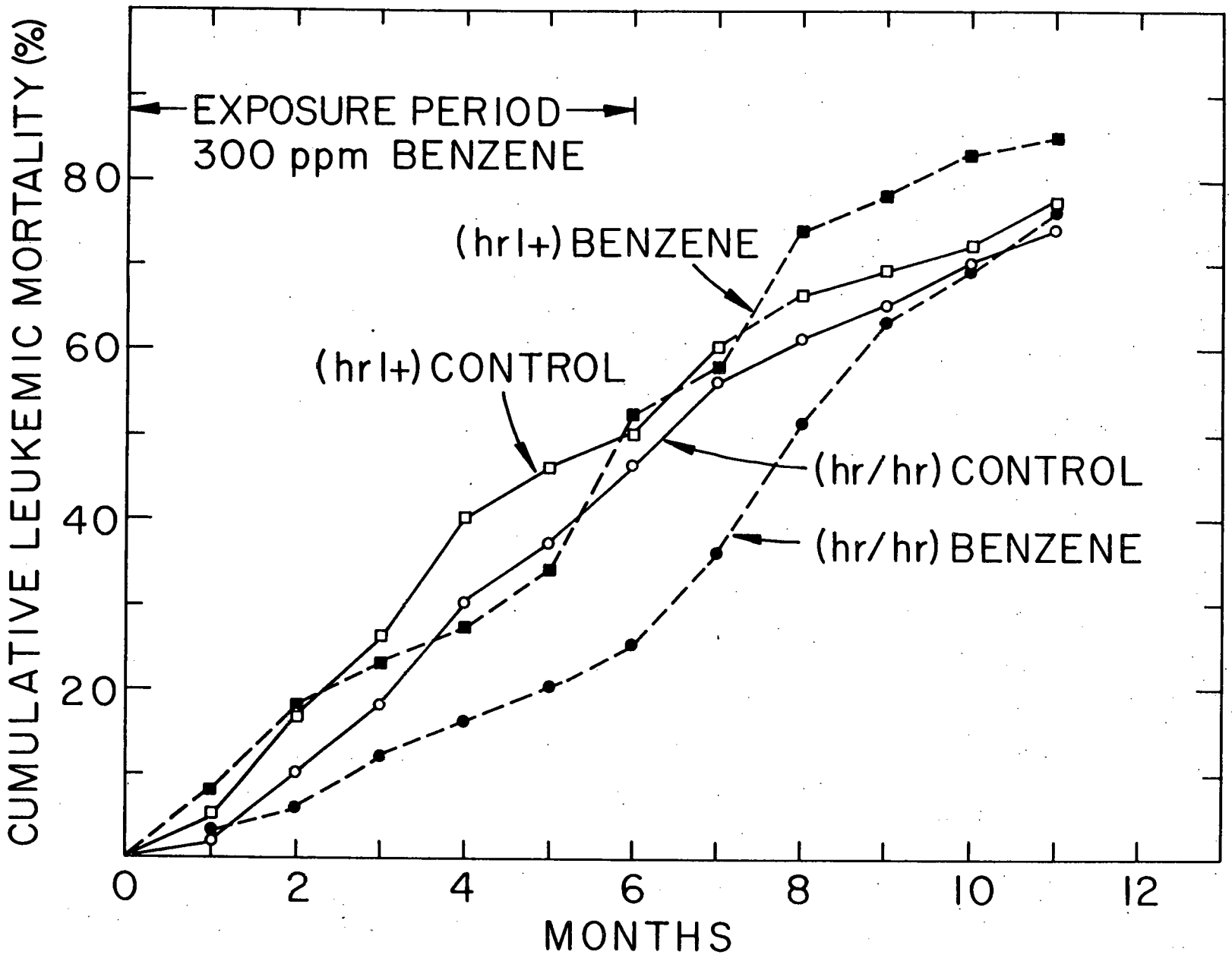
EXPOSURE PERIOD
300 ppm BENZENE
(hr l+) BENZENE
(hr l+) CONTROL
(hr/hr) CONTROL
(hr/hr) BENZENE
CUMULATIVE LEUKEMIC MORTALITY (%)
20
40
60
80
0
2
4
6
8
10
12
MONTHS

FIGURE 4.