National Institute of
Environmental Health Sciences

NIEHS Technical Report on the Subchronic Toxicity Study of 3'-Azido-3'-deoxythymidine (AZT) and Pyrazinamide Combinations (CAS Nos. 30516-87-1 and 98-96-4) Administered by Gavage to $B6C3F_1$ Mice

AIDS 05

October 1999

NIEHS AIDS Therapeutics
Toxicity Report
Number 5

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NIH Publication 00-3949
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United States Department of Health and Human Services
Public Health Service
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FOREWORD

Infection with human immunodeficiency virus (HTV) causes immunosuppression and leads to acquired immunodeficiency syndrome (AIDS) with a broad spectrum of opportunistic infections. Prophylaxis and treatment of AIDS are generally combination therapies of antiretroviral agents with antimicrobial drugs specific for the opportunistic infections. The National Institute of Environmental Health Sciences (NIEHS), under the AIDS research program, is evaluating AIDS therapeutics for reproductive, developmental, and general toxicity in rodents. These evaluations may include single therapeutic agents or combination therapies when the toxic potential of these agents in animal models is not available or is incomplete.

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CONTRIBUTORS

This report on the subchronic toxicity study of 3'-azido-3'-deoxythymidine (AZT) and pyrazinamide combinations is based primarily on studies that began in August 1993 and ended in December 1993 at Southern Research Institute, Birmingham, AL.

National Institute of Environmental Health Sciences

Evaluated experiment, interpreted results, and reported findings

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PEER REVIEW

The report on the subchronic toxicity study of 3'-azido-3'-deoxythymidine (AZT) and pyrazinamide combinations was evaluated by the reviewers listed below. These reviewers serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, reviewers determine if the design and conditions of these studies are appropriate and ensure that this general toxicity study report presents the experimental results and conclusions fully and clearly. The comments of the reviewers were reviewed prior to the finalization of this document. Changes were made such that the concerns of the reviewers were addressed to the extent possible.

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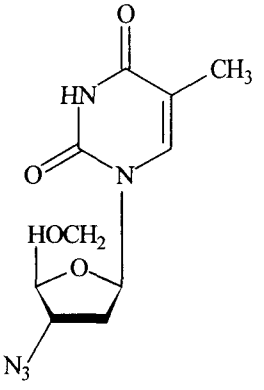
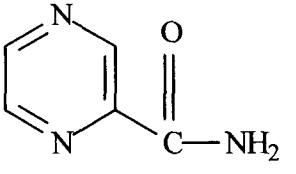
ABSTRACT

3′-Azido-3′-deoxythymidine (AZT) and Pyrazinamide Combinations



AZT
Molecular Formula: $C_{10}H_{13}N_5O_4$
Molecular Weight: 267.24
CAS No.: 30516-87-1



Pyrazinamide
Molecular Formula: $C_5H_5N_3O$
Molecular Weight: 123.12
CAS No.: 98-96-4

The toxicity of combinations of AZT (200 or 400 mg) and pyrazinamide (1,000 or 1,500 mg) was evaluated in B6C3F$_1$ mice treated by gavage for up to 94 days. The primary toxic effect of AZT was bone marrow suppression manifested by macrocytic anemia, thrombocytosis, and reticulocytopenia followed by reticulocytosis. Cellular depletion of bone marrow was observed microscopically. Administration of pyrazinamide alone caused mild hepatotoxicity, as evidenced by increased liver weights and by glycogen depletion of hepatocytes in a zonal pattern. AZT and pyrazinamide administered together resulted in a significant exacerbation of the hematopoietic toxicity induced by AZT alone. The hepatotoxicity of pyrazinamide was slightly augmented by AZT.

INTRODUCTION

AIDS is a lethal multi-system disease that has become a major public health problem since its recognition in 1981 (Gottlieb *et al.*, 1981; Masur *et al.*, 1981; Siegle *et al.*, 1981). The etiological agent of AIDS is a retrovirus now referred to as HIV (Coffin, 1986). To date, the most effective single agent in the treatment of HIV has been the first dideoxynucleoside analogue used in clinical trials, zidovudine (3′-azido-3′-deoxythymidine, AZT, Retrovir, azidothymidine, compound S, BW A509U, CAS No. 30516-87-1), commonly referred to as AZT (Vince *et al.*, 1988; Amin, 1989).

AZT therapy produces numerous beneficial effects in AIDS patients, including decreases in morbidity and increases in lifespan (Amin, 1989; Jeffries, 1989). The most important adverse effects of AZT are anemia and granulocytopenia, which are believed to reflect bone marrow toxicity (Richman, 1988; Amin, 1989). Two types of anemia may occur with AZT therapy: macrocytic megaloblastic anemia and normocytic normochromic anemia.

Several subacute and subchronic rodent toxicity studies have demonstrated that the primary toxicity of AZT is myelosuppression. Male Swiss (CD-1®) mice were administered 100, 250, 500, or 1,000 mg/kg body weight by gavage for 30 days (Mansuri *et al.*, 1990). No mortality or body weight effects were evident from AZT treatment. Erythropenia and increased mean cell volume were observed at all doses, and anemia was observed at the 1,000 mg/kg dose. Pathologic findings in the AZT-treated mice were consistent with the hematologic results and included lymphoid depletion, reticuloendothelial hyperplasia in spleen and thymus, and bone marrow hypocellularity.

In a 14-week subchronic study (NTP, 1999), B6C3F$_1$ mice were treated with 0, 25, 50, 100, 400, or 1,000 mg AZT/kg body weight in 0.5% methylcellulose by gavage. All doses were administered twice daily as one half of the total daily dose. On day 5, statistically significant dose-related decreases were observed in reticulocyte counts in both males and females. Dose-related anemia was evident on days 23 and 93. To evaluate the ability of treated animals to reverse any compound-related effects when treatment is stopped, additional groups of mice received AZT in 0.5% methylcellulose at doses of 0, 50, 400, or 1,000 mg/kg twice daily by gavage for 14 weeks and then were held without additional treatment for 4 weeks. Improvement of hematology parameters indicated recovery of the bone marrow after treatment stopped. An apparently nontoxic, treatment-related clinical finding that affected AZT-treated B6C3F$_1$ mice was a darkening of the skin on the tail, feet, and muzzle.

AZT alone, when administered by gavage to B6C3F$_1$ mice at 100, 200, or 300 mg/kg per day for 13 weeks, caused hematopoietic toxicity with dose-related bone marrow suppression, macrocytic anemia, and thrombocytosis (Rao *et al.*, 1998).

Oral bioavailability of AZT was determined in female B6C3F$_1$ mice by comparison of the area under the curve obtained from an oral dose to that of an intravenous dose (Trang *et al.*, 1993). Bioavailability was found to be 0.86, 0.78, and 0.97 for the 15, 30, and 60 mg/kg oral doses. The mean elimination half-life values ranged from 17.3 to 19.9 minutes for the three intravenous doses and from 16.5 to 21.9 minutes for the three oral doses. Based on these results, the internal dose of AZT was linear and dose proportional over the oral-dose concentration range administered.

Standard teratology studies of AZT have been performed in rats and rabbits (Ayers, 1988). Rats were dosed orally with 125 to 500 mg/kg on gestation days 6 to 15. No fetal toxicity or teratogenicity was found. Fetal AZT concentrations 30 minutes post-dosing were 61 μg/g, or 76 times the antiviral ID_{50} (inhibitory dose for 50% of the viral population being tested). Rabbits were orally dosed at 75 to 500 mg/kg on gestation days 6 to 18. The high dose caused an increased incidence of late resorptions with no evidence of teratogenicity, and no fetal toxicity was found. Fetal AZT concentrations 30 minutes post-dosing were 40.2 μg/g, or 50 times the human antiviral ID_{50}.

Female Wistar rats were dosed three times orally with 100 mg/kg AZT at 5-hour intervals on gestation day 10 for a total dose of 300 mg/kg (Greene *et al.*, 1990). No adverse effects on maternal weight gain, feed consumption, fertility, hematologic parameters or growth or survival of offspring were observed. AZT concentration measurements 30 minutes after the last dose were 62.6 μg/mL in maternal plasma and 21.1 μg/g in fetal tissue.

Studies in C_3H/He mice concluded that AZT has a direct toxic effect on the developing mouse embryo (Toltzis *et al.*, 1991). Female mice were exposed to 0, 0.25, 0.5, or 2.5 mg AZT/mL in the drinking water for 8 weeks during mating and throughout gestation. All AZT groups had fewer pregnant mice per group, fewer pups per litter, and increased resorptions per mouse. Dose-related embryolethality was observed.

Because AIDS is a disease of immune suppression, the majority of AIDS patients actually die from characteristic opportunistic infections (Hardy, 1991; Harkins and Herriot, 1992), and the treatment of AIDS is increasingly one of combination therapy of anti-HIV drugs and antimicrobial drugs (Goldschmidt and Dong, 1992). Tuberculosis (TB) is one of the opportunistic diseases leading to mortality in AIDS patients (Nolan, 1992).

AIDS patients with TB receive combination therapy with AZT and antituberculosis drugs. Treatment for TB involves combination therapy with multiple antibacterial agents in order to eliminate the strains of organisms inducing TB, including those resistant to isoniazid, the primary drug used in treating TB.

The standard treatment regimen is isoniazid (300 mg/day), rifampin (600 mg/day or 450 mg/day for persons weighing less than 50 kg), and pyrazinamide (20 to 30 mg/kg per day) for the first 2 months of treatment. Isoniazid and rifampin are continued for another 7 months, for a total therapy duration of 9 months (CDC, 1987; Barnes *et al.*, 1991). The inclusion of pyrazinamide in treatment regimens has shortened the length of therapy to 6 months (East and Central African/British Medical Research Council, 1986). Pyrazinamide is the synthetic pyrazine analogue of nicotinamide. The daily dose for adults is 20 to 35 mg/kg orally, given in three or four equally spaced doses. The maximum quantity to be administered is 3 g per day, regardless of body weight.

Pyrazinamide is well absorbed from the gastrointestinal tract and is widely distributed throughout the body. The oral administration of 1 g produces plasma concentrations of about 45 μg/mL at 2 hours and 10 μg/mL at 15 hours. The drug is excreted primarily by renal glomerular filtration; urinary concentrations are 50 to 100 μg/mL for several hours after a single dose. Pyrazinamide is hydrolyzed to pyrazinoic acid and subsequently hydroxylated to 5-hydroxypyrazinoic acid, the major excretory product (Weiner and Tinker, 1972). Grossett *et al.* (1992) reported that 150 mg/kg pyrazinamide (considered to be a therapeutic dose) administered by gavage to female Swiss (CD-1®) mice resulted in serum concentrations similar to those of humans receiving pyrazinamide therapy.

Injury to the liver is the most common and serious side effect of pyrazinamide. When a dose of 3 g per day (40 to 50 mg/kg body weight) is orally administered, signs and symptoms of hepatic disease appear in about 15% of patients, with jaundice in 2% to 3% of patients, and death due to hepatic necrosis occurring in rare instances (Mandell and Sande, 1990). Mild and transient elevations of the plasma alanine and aspartate aminotransferases are the earliest abnormalities produced by the drug and occur approximately 2 weeks after initiation of therapy (Ramakrishnan *et al.*, 1968). Baron and Bell (1974) have noted that such transient asymptomatic increases in the serum hepatic enzyme concentrations are also common during the early weeks of antituberculosis chemotherapy with other drugs, and these usually return to normal without interrupting or altering the regimen and are not clinically important.

Hepatotoxicity and nephrotoxicity occurred in male Wistar rats following the administration of 2.5 g/kg of pyrazinamide by gavage in 10% gum arabic (Zitkova *et al.*, 1983). Bederka *et al.* (1975) have reported an LD_{25} for pyrazinamide in young adult female Swiss albino and Charles River mice (20 to 32 g) of 705 mg/kg following intraperitoneal administration in dimethyl sulfoxide. The LD_{50} data for pyrazinamide reported in the Registry of Toxic Effects of Chemical Substances are as follows: mouse intraperitoneal, 1,680 mg/kg; mouse subcutaneous, 2,793 mg/kg (RTECS, 1983).

Roman and Georgian (1977) studied the comparative cytogenetic effects of para-aminosalicylic acid sodium salt, pyrazinamide, and rifampin in human peripheral blood cultures. The structural chromosomal lesions were randomly distributed between the different chromosome groups of the human karyotype; nearly 20% to 30% of affected cells had more than one lesion per metaphase. In the treatments with sodium para-aminosalicylate and pyrazinamide, the analysis of the frequencies of the cells carrying chromosomal aberrations and of the chromosomal lesion types indicated a dose-response correlation. The *in vitro* studies indicate a potential genetic hazard in the use of these drugs.

STUDY RATIONALE

The objective of this study was to obtain controlled laboratory data on the toxicity of AZT and pyrazinamide combination therapy with subchronic exposure. Male and female $B6C3F_1$ mice were dosed orally with AZT (200 or 400 mg/kg per day) alone, pyrazinamide (1,000 or 1,500 mg/kg per day) alone, or with combinations of AZT and pyrazinamide for up to 94 days. Mice were evaluated for clinical findings, mean body weights, sperm function, vaginal cytology, and clinical pathology parameters. Selected animals were necropsied and subjected to histopathologic evaluations.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF CHEMICALS

3′-Azido-3′-deoxythymidine (AZT; lot 1401-R-8) was manufactured by Raylo Chemicals (Edmonton, Alberta) and supplied as a powder. Pyrazinamide (lot 98-6-4) was manufactured by Aldrich Chemical Co. (Milwaukee, WI) and supplied as a powder. Methylcellulose (lot 876672) was obtained as powder from Fisher Scientific Company (Pittsburgh, PA).

The relative purity of AZT was determined by high-performance liquid chromatography to be 100%. The purity of the pyrazinamide was estimated to be 99.4% by high-performance liquid chromatography. Lot 876672 of methylcellulose met the USP specifications for all analyses and was considered to be suitable for use as a dosing vehicle. The control vehicle used in this study was an aqueous solution containing 0.5% methylcellulose.

DOSE FORMULATIONS

The dosing vehicle was prepared by mixing methylcellulose with heated deionized water and then diluting with water to form a 0.5% solution, which was allowed to cool. AZT, pyrazinamide, or combination of the two drugs was then mixed with the dosing vehicle until a homogeneous suspension was obtained to give the required concentrations (Table 1).

Stability studies indicated that the dose formulations were stable for 30 days when stored refrigerated or at room temperature. Dose formulations for this study were stored refrigerated, protected from light, and used within 22 days. Samples at each dose concentration from the initial, mid point, and final dose formulations were analyzed by high-performance liquid chromatography for concentration. The concentrations of AZT ranged from 93.5% to 102% of the target concentrations; concentrations of pyrazinamide were 101% to 108% of the target concentrations. Residual dosing formulations taken from the animal room after dosing with the initial and final mixes were also analyzed. Concentrations of the residual formulations were within 10% of the target concentration, except one AZT sample (70.7%) and four pyrazinamide samples (51.2% to 121%).

STUDY DESIGN

Mice were obtained from an NIEHS/NTP colony at Taconic Laboratory Animals and Services (Germantown, NY) and were approximately 6 weeks old when placed on study. The mice were housed five males or five females per cage during quarantine before randomization. After randomization, males were individually housed, and females continued to be housed five per cage.

The mice were housed in polycarbonate cages with solid bottoms and sides. Average temperature in the animal rooms was 71.0° F, with a standard deviation of 0.9° F; average relative humidity was 51.4%, with a standard deviation of 7.4%.

At terminal sacrifice, blood samples were collected from five male and five female sentinel animals as part of the animal disease screening program. Results indicated all animals were free of viral antibodies.

AIDS patients frequently receive combination therapy with pyrazinamide and AZT to treat tuberculosis, which is one of the complicating opportunistic infections in these immunologically suppressed individuals. At the present time, no acceptable alternatives to animal models provide adequate toxicity information regarding this combination therapy. Grossett *et al.* (1992) reported eradication of tuberculosis in Swiss (CD-1®) mice with 150 mg pyrazinamide/kg body weight per day. In a previous reproductive toxicity study of pyrazinamide, no toxicity was observed at doses up to 1,200 mg pyrazinamide/kg body weight per day (eight times the therapeutic dose) for 30 days (NIEHS, 1997). Therefore, doses below (1,000 mg/kg) and above (1,500 mg/kg) the nontoxic dose of pyrazinamide were selected for this study. The AZT dose concentrations for this study were based on results of previous toxicity studies conducted in mice, which demonstrate that measurable evidence of toxicity will be obtained at 200 and 400 mg AZT/kg body weight per day. Even though the high dose of pyrazinamide selected for this study was 10 times the therapeutic dose, based on the previous study (NIEHS, 1997) it may not cause measurable toxicity; therefore, doses of AZT less than 200 mg/kg, which may not cause consistently measurable toxicity, were not included to evaluate combination toxicity with pyrazinamide.

A brief summary of the study design is provided in Table 1. The study was conducted in B6C3F_1 mice because this strain is routinely utilized for toxicity evaluations of this type by the NIEHS/NTP. The oral route of administration was selected because this is the clinically relevant route used in humans. Combinations of AZT and pyrazinamide were administered by gavage as a single dose formulation. Total daily dose volumes of 20 mL/kg were divided into two equal doses of 10 mL/kg administered approximately 6 hours apart. There were 20 male and 20 female mice per dose group. Core study animals (10 per group) were dosed until the day prior to sacrifice (91 to 94 days). Clinical pathology study animals (10 per group) were dosed until day 59. All mice were observed twice daily (morning and afternoon) for mortality/moribundity.

On study days 4, 30, and 60 (clinical pathology study animals) or prior to terminal sacrifice on study days 92 to 95 (core study animals), blood was drawn for hematology determinations. Vaginal cytology evaluations were conducted on all core study females on days 76 to 87, and sperm count, function, and motility were assessed on core study males on days 92 to 95. All surviving clinical pathology study animals were euthanatized on day 60 using 100% CO_2 and discarded; all surviving core study animals were sacrificed on days 92 to 95 using 100% CO_2. A complete gross necropsy was conducted on all core study animals and early death clinical pathology study animals.

Clinical Pathology

All blood samples were collected from the retroorbital sinus under CO_2/O_2 anesthesia into tubes containing EDTA. Animals were selected in random order for blood collection, and samples were analyzed in the order collected.

Erythrocyte, platelet, and leukocyte counts; hematocrit; hemoglobin concentration; mean cell hemoglobin; mean cell volume; mean cell hemoglobin concentration; and leukocyte differentials were determined on whole blood using a Technicon H•1™ automated hematology analyzer. Reticulocyte counts were conducted using a Coulter Model Elite Flow Cytometer™. Blood smears were prepared to manually verify leukocyte differentials and platelet morphology if necessary.

Vaginal Cytology and Sperm Function Evaluations

Samples of vaginal fluid were collected from females using a medicine dropper moistened with 0.9% saline. Samples from 12 consecutive days per group were placed on two slides per animal, which were evaluated for relative frequency of estrous phases and the estrous cycle length.

Sperm motility was evaluated at necropsy in the following manner. The left epididymis was isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis). Modified Tyrode's buffer was applied to slides, and a small incision was made at the border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers.

Following completion of sperm motility estimates, each left cauda epididymis was placed in buffered neutral saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution and then heat fixed at 65°C. Sperm density was then determined microscopically with the aid of a hemocytometer. To quantify spermatogenesis, a testicular spermatid head count was determined by removing the tunica albuginea

and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemocytometer.

Histopathology

Necropsy was performed on all core study animals and early death clinical pathology study animals. The heart, right kidney, liver, lungs, right testis, and thymus were weighed. Histopathology was conducted on the tissues listed in Table 1. The tissues were preserved in the appropriate fixative, trimmed to a maximum thickness of 0.3 cm for processing, embedded in paraffin, sectioned at 4 to 6 μm, stained with hematoxylin and eosin (testes were stained with PAS), and examined by light microscopy.

TABLE 1
Experimental Design and Materials and Methods for the 13-Week Toxicity Study of AZT and Pyrazinamide Combinations

Study Laboratory	Southern Research Institute, Birmingham, AL
Strain and Species	$B6C3F_1$ mice
Animal Source	Taconic Laboratory Animals and Services, Germantown, NY
Time Held Before Study	11 or 12 days
Average Age When Study Began	42 or 43 days
Date of First Dose	August 30, 1993
Duration of Dosing	Day 1 through day prior to sacrifice
Date of Last Dose	December 1, 1993
Necropsy Dates	November 29 to December 3, 1993
Average Age at Necropsy	133 days
Size of Study Groups	10 males and 10 females
Method of Animal Distribution	Animals were assigned to groups using a stratified weight method and then assigned to study groups in random order
Animals per Cage	Five males or five females per cage during quarantine, then one male per cage and five females per cage after randomization
Method of Animal Identification	Microchip implant
Diet	NIH-07 pelleted feed, available *ad libitum*
Water	Tap water (Birmingham, AL), available *ad libitum*
Cages	Polycarbonate cages with solid bottoms and sides
Bedding	Heat-treated hardwood chips (Sani-Chips®, P.J. Murphy Forest Products Corporation, Montville, NJ)

TABLE 1
Experimental Design and Materials and Methods for the 13-Week Toxicity Study of AZT and Pyrazinamide Combinations

Cage Filters
Reemay® spun-bonded polyester (Andico, Birmingham, AL)

Racks
Stainless steel (Lab Products, Maywood, NJ)

Animal Room Environment
Temperature: 71.0° ± 0.9° F
Relative humidity: 51.4% ± 7.4%
Fluorescent light: 12 hours fluorescent light/day
Room air: minimum of 10 changes/hour

Doses
Daily doses in an aqueous solution of 0.5% methylcellulose by gavage:
0 mg AZT + 0 mg pyrazinamide per kg body weight per day
200 mg AZT + 0 mg pyrazinamide per kg body weight per day
400 mg AZT + 0 mg pyrazinamide per kg body weight per day
0 mg AZT + 1,000 mg pyrazinamide per kg body weight per day
200 mg AZT + 1,000 mg pyrazinamide per kg body weight per day
400 mg AZT + 1,000 mg pyrazinamide per kg body weight per day
0 mg AZT + 1,500 mg pyrazinamide per kg body weight per day
200 mg AZT +1,500 mg pyrazinamide per kg body weight per day
400 mg AZT + 1,500 mg pyrazinamide per kg body weight per day
All doses were administered daily (7 days per week) in two equal doses approximately 6 hours apart.

Type and Frequency of Observations
Mortality/moribundity: twice daily
Clinical findings: once weekly
Body weights: once weekly

Method of Sacrifice
CO_2 asphyxiation

Necropsy
Complete necropsies were performed on all core study animals and early death clinical pathology study animals. The heart, right kidney, liver, lungs, right testis, and thymus were weighed.

Histopathology
The following tissues were examined: brain, femur, gross lesions, heart and aorta, kidneys, liver, lungs and bronchi, mandibular and mesenteric lymph nodes, ovaries, spleen, testes with epididymis, thymus, tissue masses.

Clinical Pathology
Hematology evaluations were conducted on all clinical pathology study animals on days 4, 30, and 60 and on all core study animals at terminal sacrifice. The parameters evaluated included erythrocyte, reticulocyte, and platelet counts; hematocrit; hemoglobin concentration; mean cell hemoglobin; mean cell volume; mean cell hemoglobin concentration; leukocyte counts and differentials; and platelet morphology (if necessary).

Sperm Function Evaluation
Sperm samples were collected from all core study males at terminal sacrifice. The left epididymis and cauda epididymis were weighed. The parameters evaluated included spermatid heads, spermatid counts, and motility.

Vaginal Cytology Evaluation
Vaginal fluid samples were collected from all core study females on days 76 to 87. The parameters evaluated included relative frequency of estrous phases and the estrous cycle length.

STATISTICAL METHODS

Group means and standard deviations were calculated for hematology parameters and for final mean body and organ weights. Organ-weight-to-body-weight ratios were also calculated. Final mean body weights, mean organ weights, and organ-weight-to-body-weight ratios for each dosed group were compared to those of the control group by a two-tailed Student's *t*-test. The standard deviations used in the *t*-tests were obtained by pooling the individual values for the control and dosed groups. Hematology data were evaluated using Dunnett's (1955) test.

Severity grades for lesions in liver, spleen, and bone marrow were analyzed for interaction using two-way analysis of variance. If a significant interaction between AZT and pyrazinamide was observed, mean values were plotted to graphically display the interaction between the two chemicals. In the absence of interaction, vehicle control and dosed group means were compared using either Williams' (1971, 1972) or Dunnett's (1955) multiple comparison procedures. The choice between the two tests was based on evidence of a dose-related trend in the data as determined by Jonckheere's test (Jonckheere, 1954). Williams' and Dunnett's tests were also used to assess the significance of differences between vehicle control and dosed group responses for male final mean body weights and reproductive parameters. Jonckheere's test was used to assess the significance of dose-response trends. Trend sensitive tests were used when Jonckheere's test was significant at $P \leq 0.01$. If the P value from Jonckheere's test for a dose-related trend was $P \geq 0.10$, Dunn's (1964) test was used. If the P value was less than 0.10, Shirley's (1977) test was used. The outlier test of Dixon and Massey (1951) was employed to detect extreme values. Implausible values, extreme values from animals that were suspected of being sick due to causes other than treatment, and values that were indicated to be inadequate due to measurement problems were eliminated from analysis.

Treatment effects on vaginal cytology data were investigated by applying a multivariate analysis of variance (using Wilke's Criterion [Stevens, 1986] as the test statistic) to test for the simultaneous equality of measurements across dose concentrations. Because the data are proportions (the proportion of the observation period that an animal was in a given estrous phase), an arcsine transformation was used to bring the data into closer conformance with the normality assumptions required for the multivariate analysis of variance.

RESULTS

SURVIVAL AND CLINICAL FINDINGS

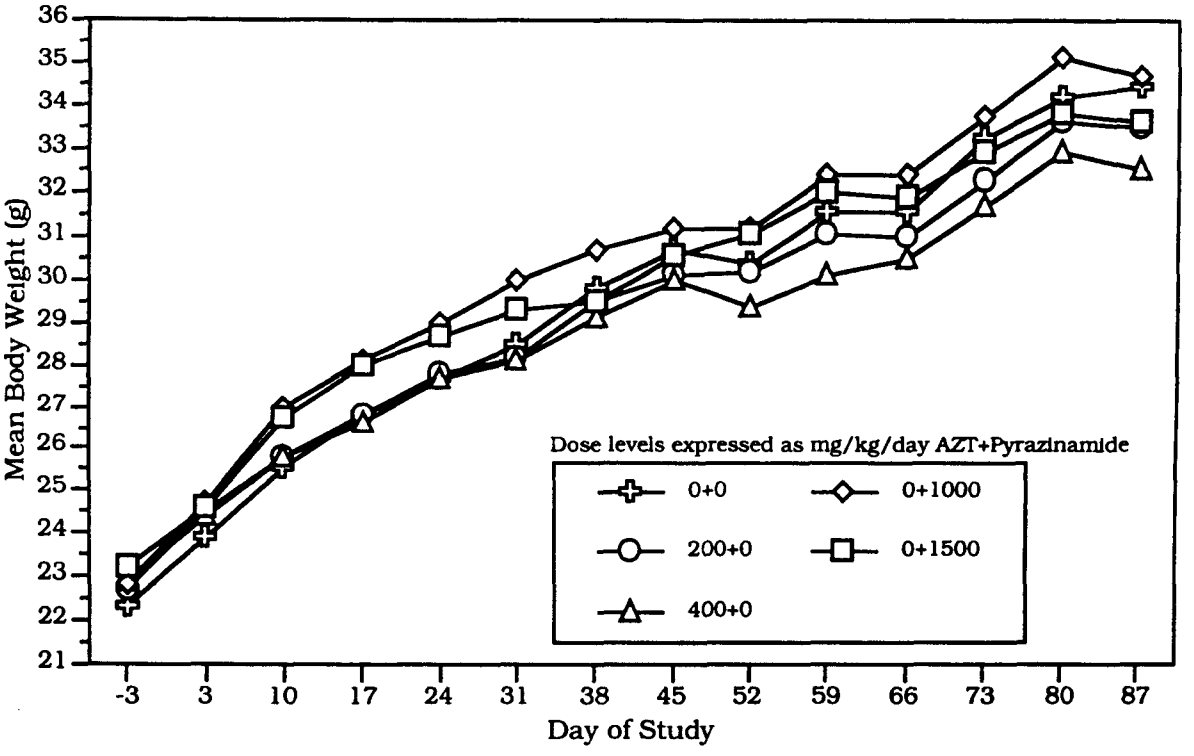
Four mice from the clinical pathology groups died before the end of the study. One female mouse from the 1,000 mg/kg pyrazinamide group died on day 9 from an apparent dosing accident. One male from the 400 + 1,000 mg/kg AZT + pyrazinamide group, one female from the 400 mg/kg AZT group, and one female from the 400 + 1,500 mg/kg AZT + pyrazinamide group either died or were sacrificed in moribund condition on day 30 or 31. These three deaths were thought to be the result of the bleeding procedure being performed on animals that had an underlying anemia. None of the deaths were thought to have been directly caused by the administration of the test article. There were no significant clinical findings in mice treated with AZT alone, pyrazinamide alone, or the AZT and pyrazinamide combinations. However, all mice treated with AZT or AZT with pyrazinamide had darkening of the skin on the tail, feet, and muzzle.

BODY AND ORGAN WEIGHTS

Male Mice

The administration of AZT alone, pyrazinamide alone, and AZT and pyrazinamide combined did not result in any significant changes in mean body weight gain in male B6C3F$_1$ mice during the course of this study (Figure 1). For male mice treated with 400 mg/kg AZT alone, a slight decrease in the final mean body weights occurred. The final mean body weight (33.8 g) was approximately 6% less than that of the vehicle control group (36.1 g; Table B1). There were no significant decreases in final mean body weights in any of the groups treated with AZT alone, pyrazinamide alone, or combinations of AZT and pyrazinamide.

Slight treatment-related increases were evident in the liver weights of male mice treated with pyrazinamide alone or combinations of pyrazinamide and AZT (Figure 2 and Table B1). There were no significant changes in absolute kidney weights of male mice treated with AZT alone, pyrazinamide alone, or combinations of AZT and pyrazinamide. Increases in thymus weights occurred in male mice administered 1,000 mg/kg pyrazinamide and male mice administered 200 + 1,500 mg/kg (Table B1). These increases were not considered to be biologically significant because dose-related patterns were not evident. Slight increases in heart weights were observed in males treated with 400 + 1,000 mg/kg or 400 + 1,500 mg/kg (Table B1). The increased relative



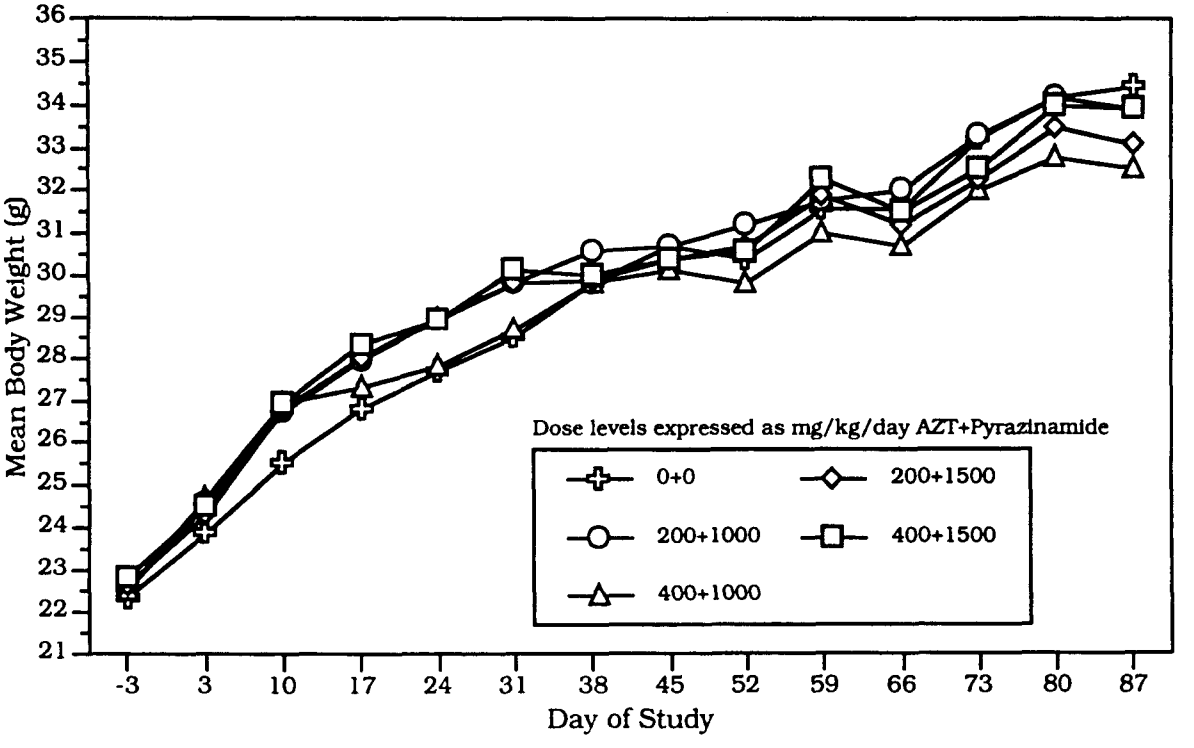


FIGURE 1
Mean Body Weights of Male Mice in the 13-Week Study of AZT and Pyrazinamide Combinations

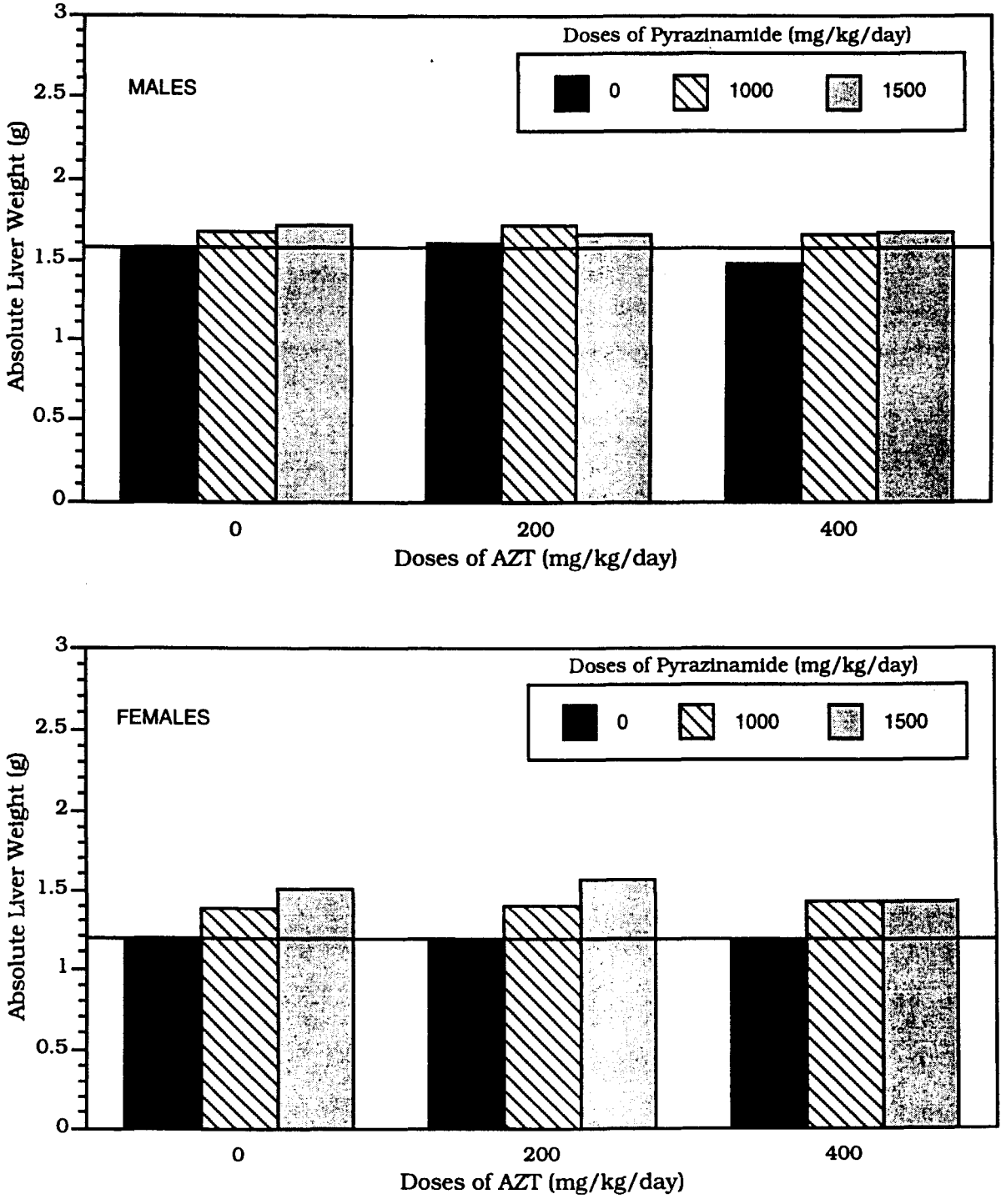


FIGURE 2
Mean Absolute Liver Weights for Male and Female Mice in the 13-Week Toxicity Study of AZT and Pyrazinamide Combinations

heart weights may represent a compensatory myocardial hypertrophy in anemic animals subsequent to increased heart rate and/or force of contraction in an attempt to maintain adequate tissue oxygenation. Significant changes were not observed in lung weights of male mice treated with AZT alone, pyrazinamide alone, or combinations of AZT and pyrazinamide. Absolute right testis weights were lower in the group treated with 400 mg/kg AZT and in all groups treated with pyrazinamide alone or in combination with AZT (Table B1). Changes of greatest magnitude were observed in the group treated with 400 + 1,500 mg/kg, in which the absolute testis weight was approximately 20% less than that of the vehicle control group.

Female Mice

Administration of AZT alone, pyrazinamide alone, and combinations of AZT and pyrazinamide to female $B6C3F_1$ mice did not result in any significant alterations in mean body weight gain during the course of the study (Figure 3). For female mice treated with 1,500 mg/kg pyrazinamide, a slight increase in final mean body weight occurred (Table B1). The final mean body weight (29.8 g) was approximately 1.05 times that of the vehicle control group (28.4 g). Statistically significant decreases in final mean body weights were not observed in any of the groups treated with AZT alone, pyrazinamide alone, or combinations of AZT and pyrazinamide.

Treatment-related increases were evident in the liver weights of female mice treated with pyrazinamide alone or combinations of pyrazinamide and AZT (Figure 2 and Table B1). The liver weights of female mice treated with 1,000 or 1,500 mg/kg pyrazinamide alone were approximately 1.16 times (1.381 g; $P \leq 0.05$) and 1.26 times (1.509 g; $P \leq 0.05$) the weight of the corresponding vehicle control group (1.195 g). Liver weights were increased in all dosed groups that received combinations of AZT and pyrazinamide. For female mice receiving combination therapy, the highest liver weights were observed in the group administered 200 + 1,500 mg/kg: the absolute liver weight was approximately 1.31 times (1.563 g; $P \leq 0.05$) that of the vehicle control group (1.195 g). The absolute kidney weights of female mice administered 1,500 mg/kg and of all female groups treated with combination therapy (Table B1) were increased. The significance of these increased kidney weights was not known. There were no significant changes in thymus weights of female mice. Increases in heart weights were observed in all female groups administered AZT and pyrazinamide in combination (Table B1). Increased heart weights of the combination groups were approximately 1.10 to 1.14 times (0.125 to 0.129 g) the weight of the vehicle control group (0.113 g). These increases in heart weights may represent a compensatory myocardial hypertrophy in anemic animals subsequent to increased heart rate and/or force of contraction in an attempt to maintain adequate tissue oxygenation. The increased relative heart weight of female mice administered 1,000 mg/kg pyrazinamide was thought to be an incidental finding,

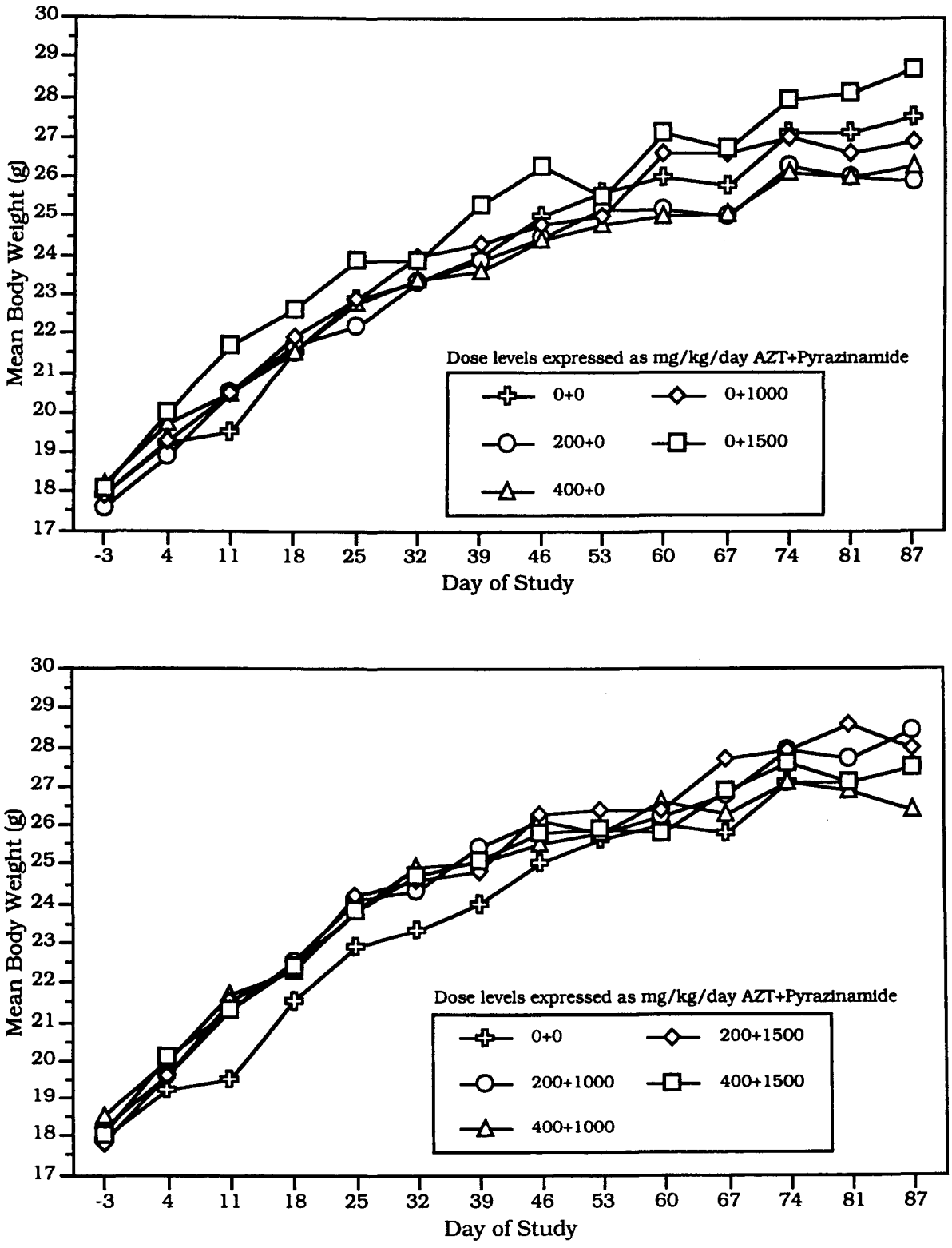


FIGURE 3
Mean Body Weights of Female Mice in the 13-Week Toxicity Study of AZT and Pyrazinamide Combinations

as a dose-related pattern was not evident. Significant changes were not detected in lung weights of female mice treated with AZT alone, pyrazinamide alone, or combinations of AZT and pyrazinamide (Table B1).

HEMATOLOGY

AZT Alone

Administration of 200 or 400 mg/kg AZT alone produced anemia, reticulocytopenia followed by reticulocytosis, thrombocytosis, and leukopenia. In general, alterations were more prevalent in females than in males. The anemic process was mild, usually dose related, and accompanied by elevations in mean cell volume (MCV) and mean cell hemoglobin (MCH) values and, as such, could be classified as macrocytic. On day 4, slight decreases were detected in erythrocyte (RBC) counts of male mice treated with AZT alone. Respective RBC counts of male mice administered 200 or 400 mg/kg AZT were approximately 5% ($9.02 \times 10^6/mm^3$; $P \leq 0.05$) and 6% ($8.90 \times 10^6/mm^3$; $P \leq 0.01$) less than that of the vehicle control group ($9.45 \times 10^6/mm^3$) (Figure 4 and Table A1). Minor decreases in hemoglobin (Hgb) and hematocrit (Hct) values accompanied the treatment-related decreases in RBC counts. Significant changes were not detected in any of the RBC parameters evaluated on day 4 in female mice administered AZT alone (Figure 5 and Table A2).

On day 30, significant ($P \leq 0.01$) dose-related decreases in RBC counts were detected in male and female mice administered AZT alone. Respective RBC counts of male mice administered 200 or 400 mg/kg AZT were approximately 17% ($8.47 \times 10^6/mm^3$; $P \leq 0.01$) and 23% ($7.89 \times 10^6/mm^3$; $P \leq 0.01$) less than that of the vehicle control group ($10.22 \times 10^6/mm^3$) (Figure 4 and Table A1). Declines in Hgb and Hct values paralleled the decreased RBC counts and were accompanied by significant increases in MCVs and MCH values. For female mice on day 30, respective RBC counts for groups administered 200 or 400 mg/kg AZT were approximately 16% ($7.92 \times 10^6/mm^3$; $P \leq 0.01$) and 21% ($7.46 \times 10^6/mm^3$; $P \leq 0.01$) less than that of the vehicle control group ($9.45 \times 10^6/mm^3$) (Figure 5 and Table A2). Minor decreases in Hgb and Hct values and significant increases in MCV and MCH values ($P \leq 0.01$) accompanied the dose-related decreases in RBC counts. Minor significant decreases in mean cell hemoglobin concentration (MCHC) values of the female mice were not considered to be biologically significant.

On day 60, changes in RBC parameters of male and female mice administered AZT alone were similar to those observed on day 30. Respective RBC counts of male mice administered 200 or 400 mg/kg AZT were approximately 21% ($8.38 \times 10^6/mm^3$; $P \leq 0.01$) and 27% ($7.81 \times 10^6/mm^3$; $P \leq 0.01$) less than that of the vehicle control group ($10.63 \times 10^6/mm^3$) (Figure 4 and Table A1). Hgb and Hct values paralleled the

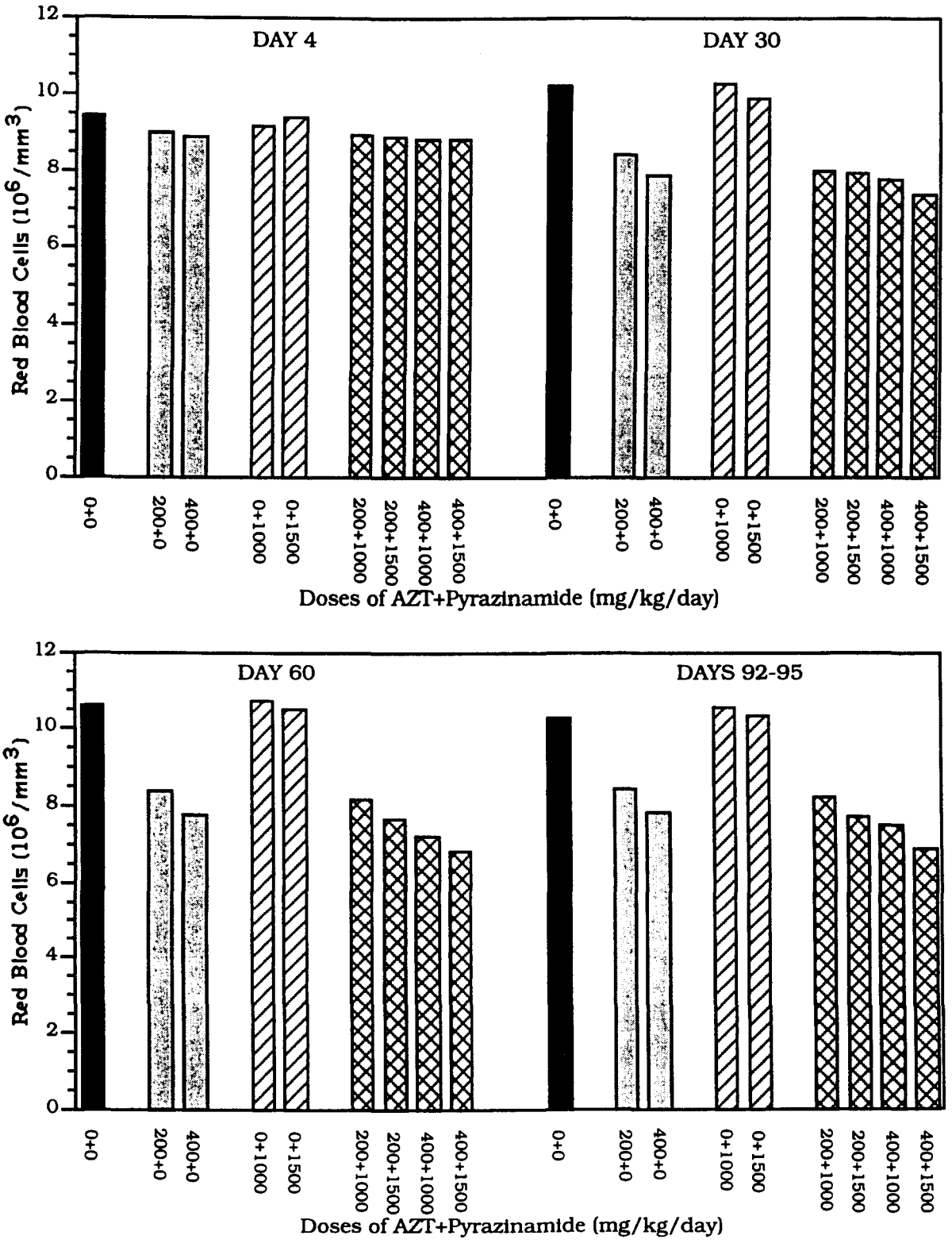


FIGURE 4
Mean Red Blood Cell Values for Male Mice in the 13-Week Toxicity Study of AZT and Pyrazinamide Combinations

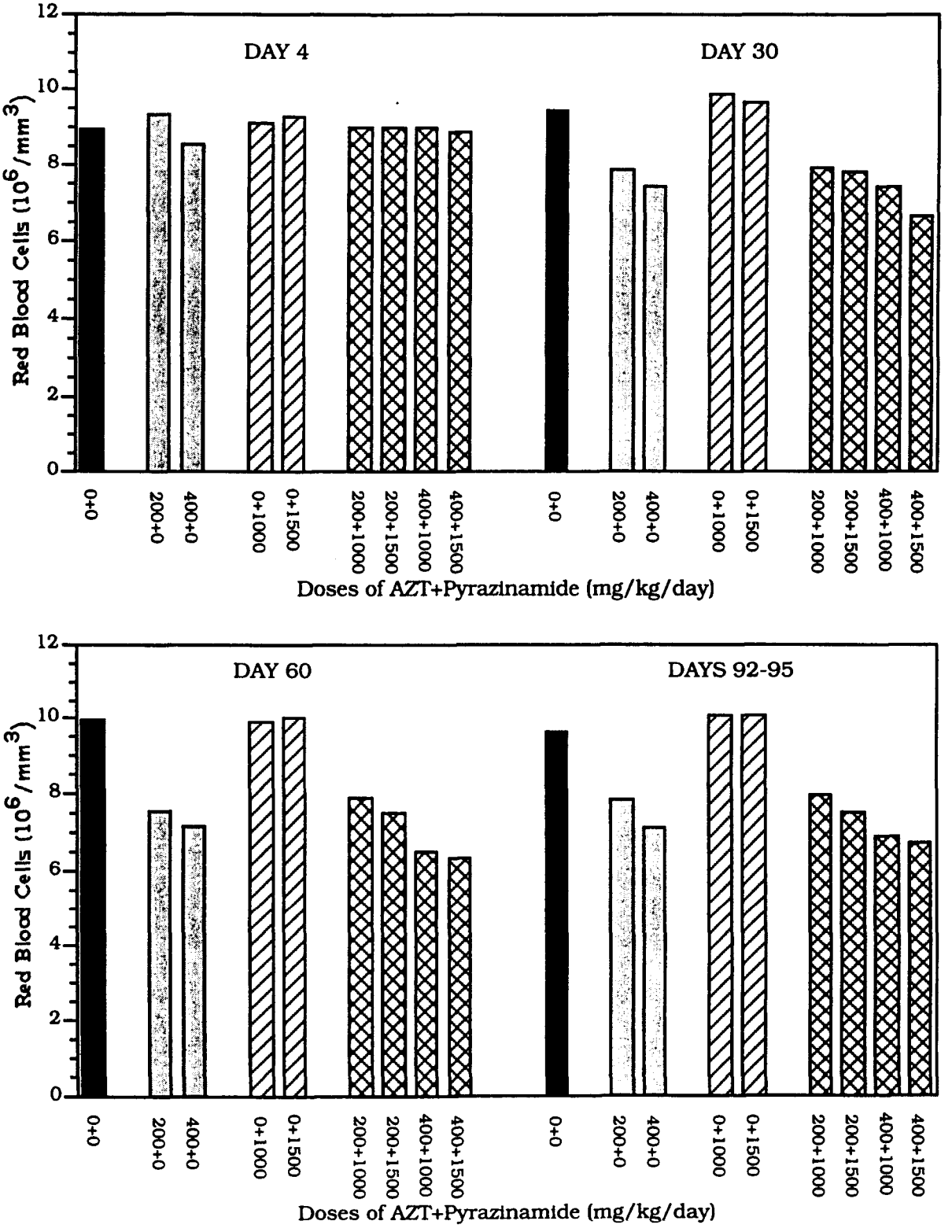


FIGURE 5
Mean Red Blood Cell Values for Female Mice in the 13-Week Toxicity Study of AZT and Pyrazinamide Combinations

decreases in RBC counts, and significant increases ($P \leq 0.01$) in MCV and MCH values also accompanied the anemia. Respective MCV values of male mice administered 200 or 400 mg/kg AZT were approximately 16% (56.3 fL; $P \leq 0.01$) and 23% (59.6 fL; $P \leq 0.Q1$) greater than that of the vehicle control group (48.6 fL).

Similar changes were observed in female mice on day 60. Respective mean RBC counts of female mice treated with 200 or 400 mg/kg AZT were approximately 24% ($7.55 \times 10^6/mm^3$; $P \leq 0.01$) and 28% ($7.19 \times 10^6/mm^3$; $P \leq 0.01$) less than that of the vehicle control group ($9.98 \times 10^6/mm^3$) (Figure 5 and Table A2). Decreased Hgb and Hct values accompanied the dose-related decreases in RBC counts. MCV values of female mice administered 200 or 400 mg/kg AZT were approximately 19% (58.5 fL; $P \leq 0.01$) and 27% (62.4 fL; $P \leq 0.01$) greater than that of the vehicle control group (49.3 fL). Minor significant decreases in MCHC values of males and females were not believed to be biologically significant.

An anemia of similar magnitude was observed in male and female mice at the time of terminal sacrifice on days 92 to 95. Respective RBC counts of male mice treated with 200 or 400 mg/kg AZT were approximately 18% ($8.44 \times 10^6/mm^3$; $P \leq 0.01$) and 24% ($7.83 \times 10^6/mm^3$; $P \leq 0.01$) less than that of the vehicle control group ($10.28 \times 10^6/mm^3$) (Figure 4 and Table A1). Decreased Hgb and Hct values paralleled the dose-related decreases in RBC counts. Increased MCV and MCH values ($P \leq 0.01$) also accompanied the compound-related anemia. MCV values observed at the time of terminal sacrifice in male mice treated with 200 or 400 mg/kg AZT were approximately 16% (54.6 fL; $P \leq 0.01$) and 22% (57.5 fL; $P \leq 0.01$) greater than that of the vehicle control group (47.1 fL).

Changes observed in the RBC parameters of female mice at the end of the study were similar to those described in males. Respective RBC counts of female mice administered 200 or 400 mg/kg AZT alone were approximately 19% ($7.85 \times 10^6/mm^3$; $P \leq 0.01$) and 26% ($7.13 \times 10^6/mm^3$; $P \leq 0.01$) less than that of the vehicle control group ($9.65 \times 10^6/mm^3$) (Figure 5 and Table A2). Decreases in Hgb and Hct values paralleled the dose-related decreases in RBC counts. Increases in MCV and MCH values ($P \leq 0.01$) also accompanied the dose-related anemia. Respective MCV values of female mice administered 200 or 400 mg/kg AZT alone were approximately 19% (55.7 fL; $P \leq 0.01$) and 26% (59.3 fL; $P \leq 0.01$) greater than that of the vehicle control group (46.9). No other significant changes were observed in the RBC parameters evaluated in male or female mice administered AZT alone.

Administration of AZT alone to male and female mice also produced statistically and biologically significant alterations in reticulocyte counts. Initially, a reticulocytopenia developed, which was followed by a reticulocytosis later in the study. Respective reticulocyte counts on day 4 of male mice treated with 200 or

400 mg/kg AZT alone were approximately 28% ($3.4 \times 10^5/mm^3$; $P \leq 0.01$) and 32% ($3.2 \times 10^5/mm^3$; $P \leq 0.01$) less than that of the vehicle control group ($4.7 \times 10^5/mm^3$) (Table A1). Respective reticulocyte counts on day 4 of female mice treated with identical doses were approximately 20% ($3.3 \times 10^5/mm^3$; $P \leq 0.05$) and 27% ($3.0 \times 10^5/mm^3$; $P \leq 0.01$) less than that of the vehicle control females ($4.1 \times 10^5/mm^3$) (Table A2). Reticulocyte counts rebounded rapidly, as a dose-related reticulocytosis was evident in male and female mice on day 30. Respective reticulocyte counts on day 30 of male mice administered 200 or 400 mg/kg AZT were approximately 1.1 times ($4.3 \times 10^5/mm^3$) and 1.3 times ($5.1 \times 10^5/mm^3$; $P \leq 0.01$) the reticulocyte count of the vehicle controls ($4.0 \times 10^5/mm^3$). Respective reticulocyte counts of the female groups treated with identical doses were approximately 1.3 times ($4.1 \times 10^5/mm^3$; $P \leq 0.05$) and 1.5 times ($4.7 \times 10^5/mm^3$) the reticulocyte count of the vehicle controls ($3.1 \times 10^5/mm^3$).

A dose-related reticulocytosis was also evident in male and female mice on day 60. Respective reticulocyte counts on day 60 of male mice treated with 200 or 400 mg/kg AZT alone were approximately 1.2 times ($4.0 \times 10^5/mm^3$; $P \leq 0.01$) and 1.6 times ($5.1 \times 10^5/mm^3$; $P \leq 0.01$) the reticulocyte count of the vehicle controls ($3.3 \times 10^5/mm^3$) (Table A1). Respective reticulocyte counts on day 60 of female mice treated with identical doses were approximately 1.4 times ($4.9 \times 10^5/mm^3$; $P \leq 0.01$) and 1.7 times ($5.9 \times 10^5/mm^3$; $P \leq 0.01$) greater than that of the vehicle controls ($3.5 \times 10^5/mm^3$) (Table A2).

A dose-related reticulocytosis similar to that described above was observed in male and female mice at the end of the study. Respective reticulocyte counts at terminal sacrifice of male mice treated with 200 or 400 mg/kg AZT alone were approximately 1.3 times ($5.4 \times 10^5/mm^3$; $P \leq 0.01$) and 1.4 times ($6.0 \times 10^5/mm^3$; $P \leq 0.01$) that of the vehicle control group ($4.3 \times 10^5/mm^3$) (Table A1). Respective reticulocyte counts at the end of the study of female mice treated with identical doses were approximately 1.1 times ($4.9 \times 10^5/mm^3$) and 1.4 times ($6.0 \times 10^5/mm^3$; $P \leq 0.01$) that of the reticulocyte count of the vehicle control group ($4.3 \times 10^5/mm^3$) (Table A2).

A compound-related increase in platelets, although not always statistically significant, was observed in male and female mice treated with AZT alone. Respective platelet counts observed on day 30 of male mice administered 200 or 400 mg/kg AZT alone were approximately 1.1 times ($1,275 \times 10^3/mm^3$) and 1.1 times ($1,293 \times 10^3/mm^3$) that of the platelet count of the vehicle controls ($1,150 \times 10^3/mm^3$) (Table A1). Respective platelet counts on day 30 of female mice administered doses identical to the male doses were approximately 1.1 times ($1,171 \times 10^3/mm^3$) and 1.2 times ($1,209 \times 10^3/mm^3$; $P \leq 0.05$) that of the platelet count of the vehicle controls ($1,044 \times 10^3/mm^3$) (Table A2).

A compound-related thrombocytosis was evident on day 60. Respective platelet counts on day 60 of male mice administered 200 or 400 mg/kg AZT alone were approximately 1.3 times (1,259 $\times$ 10^3/mm^3; P$\leq$0.01) and 1.4 times (1,305 $\times$ 10^3/mm^3; P$\leq$0.01) that of the platelet count of the vehicle control group (949 $\times$ 10^3/mm^3) (Table A1). Respective platelet counts obtained on day 60 of female mice treated with identical doses were approximately 1.3 times (1,276 $\times$ 10^3/mm^3; P$\leq$0.01) and 1.3 times (1,292 $\times$ 10^3/mm^3; P$\leq$0.01) that of the vehicle control group (1,010 $\times$$10^3$/mm^3) (Table A2).

Although not statistically significant, platelet counts of male and female mice treated with AZT alone were slightly increased at the end of the study (days 92 to 95). Respective platelet counts at the end of the study of male mice administered 200 or 400 mg/kg AZT alone were approximately 1.1 times (1,520 $\times$ 10^3/mm^3) and 1.1 times (1,502 $\times$ 10^3/mm^3) that of the vehicle controls (1,347 $\times$ 10^3/mm^3) (Table A1). Respective platelet counts of female mice treated with identical doses were approximately 1.1 times (1,275 $\times$ 10^3/mm^3) and 1.1 times (1,319 $\times$ 10^3/mm^3) that of the vehicle controls (1,176 $\times$ 10^3/mm^3) (Table A2).

Administration of AZT alone also resulted in a leukopenia that appeared to be more pronounced in female mice than in males. On day 4, statistically significant changes in leukocyte (WBC) values were not observed in male mice treated with AZT alone (Figure 6 and Table A1). For the female mice, respective WBC counts of the groups treated with 200 or 400 mg/kg AZT alone were approximately 29% (5.77 $\times$ 10^3/mm^3; P$\leq$0.01) and 30% (5.64 $\times$ 10^3/mm^3; P$\leq$0.01) less than that of the vehicle controls (8.11 $\times$ 10 /mm)^3 (Figure 7 and Table A2). Evaluation of the corresponding differential data revealed declines in both granulocytes and mononuclear cell counts.

On day 30, similar findings were evident in the WBC parameters of male and female mice, as biologically significant alterations were not observed in male mice treated with AZT alone (Table A1). WBC counts on day 30 of female mice administered 200 or 400 mg/kg AZT alone were approximately 15% (4.47 $\times$ 10^3/mm^3) and 32% (3.58 $\times$ 10^3/mm^3; P$\leq$0.01) less than that of the vehicle controls (5.24 $\times$10 /mm)^3 (Table A2). Evaluation of the corresponding differential data revealed dose-related significant declines in segmented neutrophil, lymphocyte, and monocyte counts.

On day 60, only minor alterations were evident in some of the WBC parameters evaluated in male and female mice treated with AZT alone (Tables A1 and A2). Although significant decreases were not detected in the WBC counts of male or female mice, minor decreases were evident in segmented neutrophil and monocyte counts of males and females. At the end of the study (days 92 to 95), although not statistically significant, slight declines were evident in WBC, segmented neutrophil, lymphocyte, and monocyte counts. The only statistically

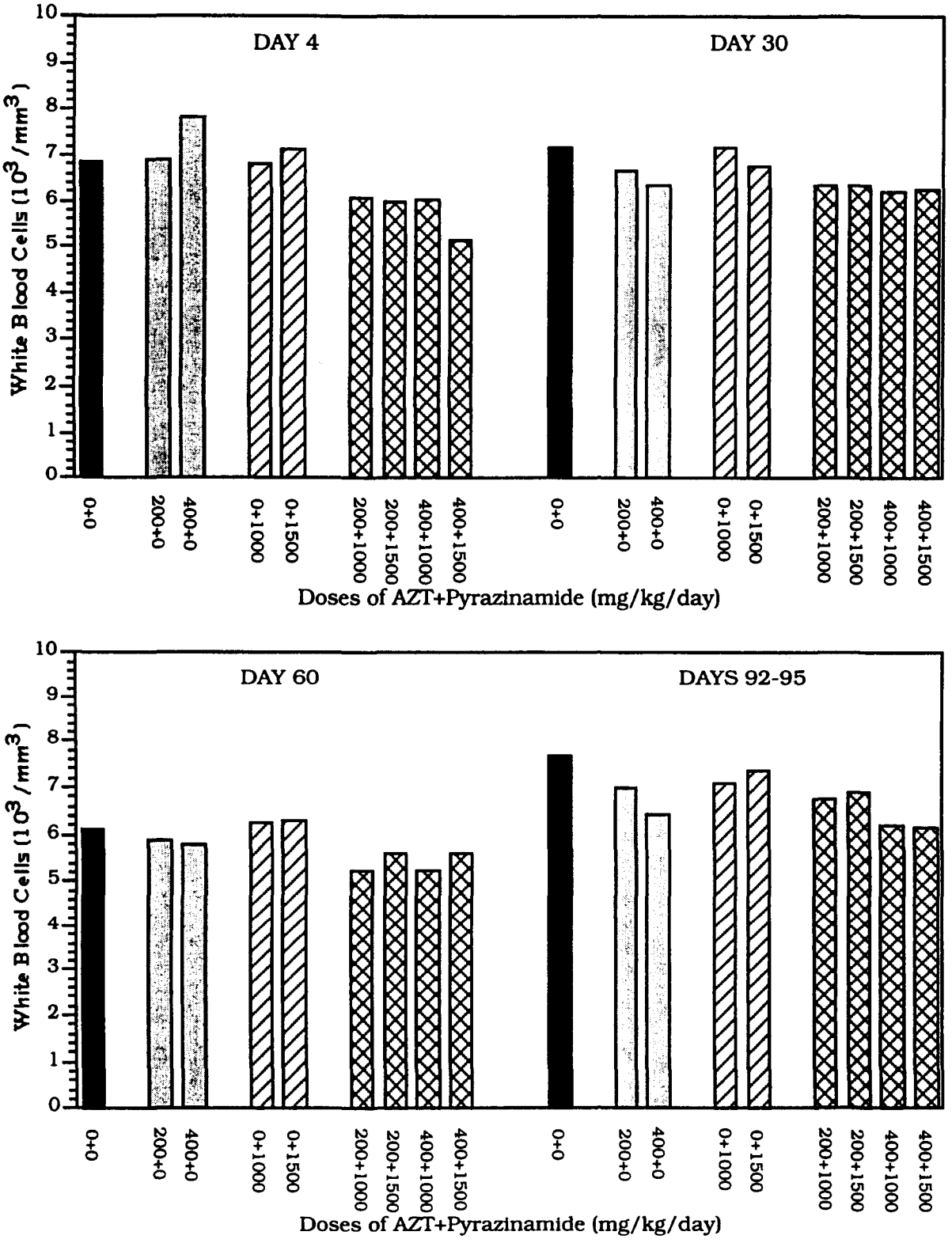


FIGURE 6
Mean Leukocyte Values for Male Mice in the 13-Week Toxicity Study of AZT and Pyrazinamide Combinations

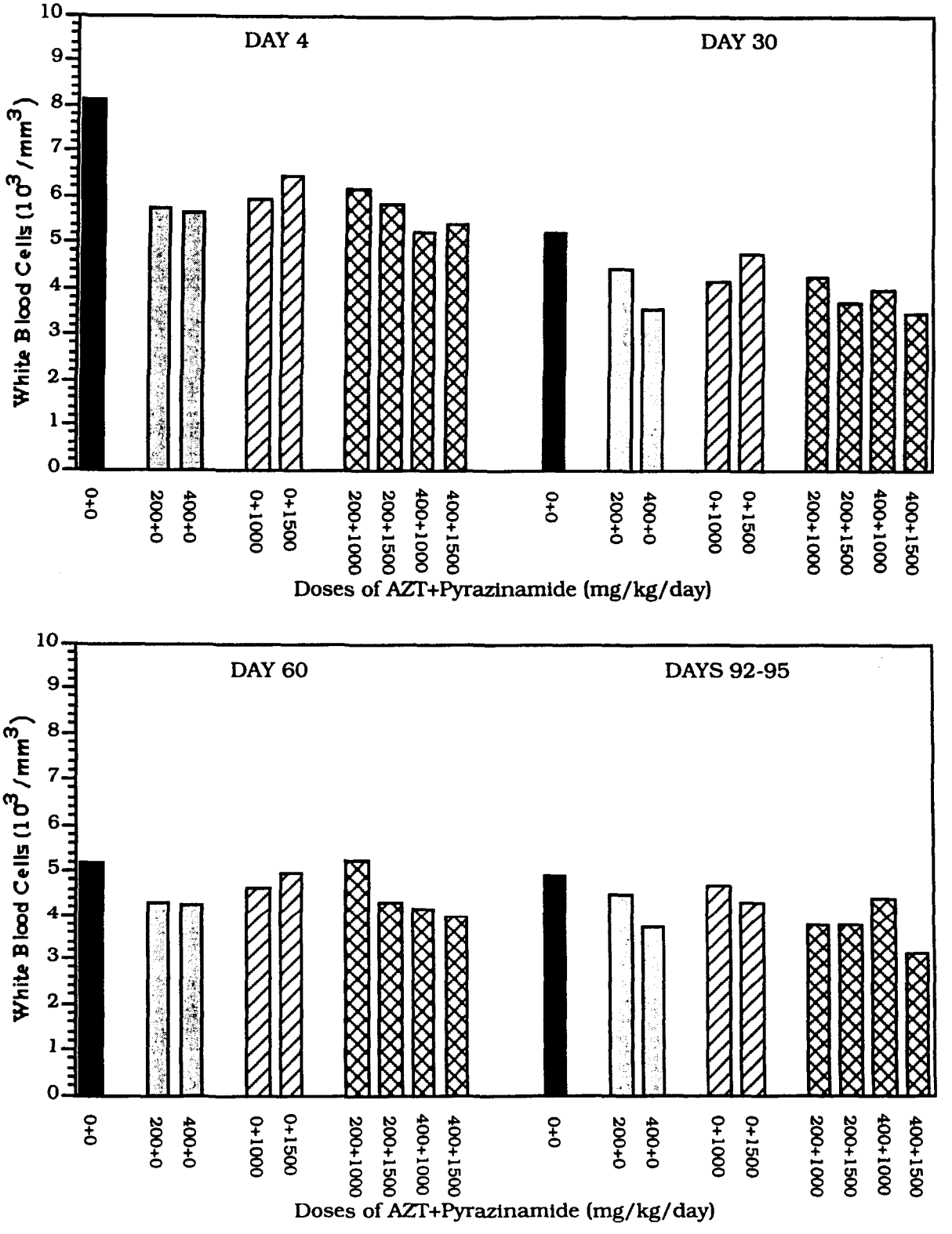


FIGURE 7
Mean Leukocyte Values for Female Mice in the 13-Week Toxicity Study of AZT and Pyrazinamide Combinations

significant change observed at the end of the study was a decreased eosinophil count of male mice administered 400 mg/kg AZT.

Pyrazinamide Alone

Administration of pyrazinamide at concentrations of 1,000 or 1,500 mg/kg to male and female mice did not result in any biologically significant alterations in any of the hematology parameters evaluated during this study. Occasional significant changes in some parameters were observed with a random or sporadic distribution. On day 4, a minor significant increase in monocyte counts was observed in male mice administered 1,500 mg/kg. This change was not considered to be biologically significant, as an overall leukocytosis was not observed. Also on day 4, minor statistically significant decreases were observed in WBC, lymphocyte, and eosinophil counts of female mice treated with 1,000 mg/kg. These decreased values were not considered to be biologically significant, as a dose-related response was not evident, and female mice administered 1,500 mg/kg had no significant changes in any of the parameters evaluated. On day 30, minor significant increases were observed in segmented neutrophil and monocyte counts of the male groups treated with 1,000 or 1,500 mg/kg. A significant decrease was also observed in the eosinophil count of the male group treated with 1,500 mg/kg. Those minor changes in differential parameters were not considered to be biologically significant, as WBC counts were not altered. A significant increase in MCH values of males administered 1,500 mg/kg was within normal limits. Significant changes were not observed in female groups on day 30. On day 60, significant increases in MCH values of male and female mice of the 1,500 mg/kg groups and a minor increase in MCHC values of female mice administered 1,500 mg/kg were considered to be within normal limits.

At the end of the study for mice administered pyrazinamide alone (days 92 to 95), increases in Hgb values of male and female mice in both dose groups and increases in MCH values of female mice in the 1,500 mg/kg group were within normal limits. Significant decreases in eosinophil counts of male mice in the 1,000 and 1,500 mg/kg groups were not considered to be biologically significant, as alterations in WBC counts were not observed.

AZT/Pyrazinamide Combinations

In general, administration of combinations of AZT and pyrazinamide to male and female mice resulted in hematologic alterations of far greater magnitude than those observed subsequent to the administration of AZT alone. Although overt anemia was not evident in male or female mice on day 4, statistically significant declines in RBC counts were observed in male mice treated with combinations of AZT and pyrazinamide. These slight decreases were similar in magnitude among the different combination groups (Figure 4 and Table A1). Slight

declines in Hgb and Hct values accompanied the decreased RBC counts. Significant changes were not observed in the RBC parameters evaluated on day 4 of female mice administered combinations of AZT and pyrazinamide.

On day 30, the severity of the anemia had increased, and male mice administered combinations of AZT and pyrazinamide were more anemic than those treated with AZT alone. On day 30, the RBC count of male mice administered 400 + 1,500 mg/kg was approximately 28% ($7.40 \times 10^6/mm^3$; $P \leq 0.01$) less than that of the vehicle controls ($10.22 \times 10^6/mm^3$) (Figure 4 and Table A1). Decreased Hgb and Hct values paralleled the decline in RBC count, and significant increases ($P \leq 0.01$) in MCV and MCH values also accompanied the anemia. The peak MCV value of male mice administered 400 + 1500 mg/kg was approximately 18% (61.4 fL; $P \leq 0.01$) greater than that of the vehicle controls (52.0 fL). An anemia of similar magnitude was observed in female mice on day 30, except that the only difference between the effect of AZT alone versus combination treatment was in the 400 + 1,500 mg/kg group. The RBC count of the female group receiving 400 mg/kg AZT alone was 23% less than that of the vehicle control group, and the RBC count of the 400 + 1,500 mg/kg group was 29% less than that of the vehicle control group. The RBC count of the high-dose combination group was $6.70 \times 10^6/mm^3$ ($P \leq 0.01$) versus $9.45 \times 10^6/mm^3$ for the female vehicle control group. Dose-related declines in Hgb and Hct values paralleled the treatment-related decrease in the RBC counts. Increased MCV and MCH values ($P \leq 0.01$) also accompanied the anemia. The MCV value on day 30 of the female group administered 400 + 1,500 mg/kg was approximately 16% (56.5 fL; $P \leq 0.01$) greater than that (48.9 fL) of the vehicle control group. Minor significant decreases in MCHC values observed in male and female mice administered combinations of AZT and pyrazinamide were considered to be within normal limits.

On day 60, the magnitude of the changes in RBC parameters was slightly greater in both males and females than those described for day 30, and the changes were dose related. On day 60, the RBC count of male mice administered 400 + 1,500 mg/kg was approximately 36% ($6.84 \times 10^6/mm^3$; $P \leq 0.01$) less than that of the vehicle controls ($10.63 \times 10^6/mm^3$) (Figure 4 and Table A1). Hgb and Hct values paralleled the decreases in RBC counts. Marked compound-related increases in MCV and MCH values ($P \leq 0.01$) accompanied the anemia. The peak MCV value observed on day 60 of male mice administered 400 + 1,500 mg/kg was approximately 34% (65.3 fL; $P \leq 0.01$) greater than that of the vehicle controls (48.6 fL). An anemia of similar magnitude was evident on day 60 in female mice administered combinations of AZT and pyrazinamide. The RBC count (Figure 5) on day 60 of female mice administered 400 + 1,500 mg/kg was approximately 36% ($6.34 \times 10^6/mm^3$; $P \leq 0.01$) less than that of the vehicle controls ($9.98 \times 10^6/mm^3$). Hgb and Hct values paralleled the decrease in RBC counts, and marked increases in MCV and MCH values ($P \leq 0.01$) also accompanied the dose-related anemia. The peak MCV value observed on day 60 of female mice administered 400 + 1,500 mg/kg was approximately 30% (64.3 fL; $P \leq 0.01$) greater than that (49.3 fL) of the vehicle

controls. Minor changes in MCHC values observed in both males and females were considered to be within normal limits.

At the end of the study (days 92 to 95), the anemia in male and female mice treated with combinations of AZT and pyrazinamide was similar to that described for day 60. At the end of the study, the RBC count of male mice administered 400 + 1,500 mg/kg was approximately 33% ($6.89 \times 10^6/mm^3$; $P \leq 0.01$) less than that of the vehicle controls ($10.28 \times 10^6/mm^3$) (Figure 4 and Table A1). Decreased Hgb and Hct values paralleled the compound-related decrease in RBC counts. Marked increases in MCV and MCH values ($P \leq 0.01$) also accompanied the anemia. The peak MCV value of male mice administered 400 + 1,500 mg/kg was approximately 34% (63.1 fL; $P \leq 0.01$) greater than that of the vehicle controls (47.1 fL). A treatment-related anemia of similar severity was observed at the end of the study in female mice treated with combinations of AZT and pyrazinamide. The RBC count (Figure 5 and Table A2) of female mice administered 400 + 1,500 mg/kg was approximately 30% ($6.76 \times 10^6/mm^3$; $P \leq 0.01$) less than that of the vehicle controls ($9.65 \times 10^6/mm^3$). Decreased Hgb and Hct values paralleled the decreases in reticulocyte counts, and marked increases in MCV and MCH values ($P \leq 0.01$) also accompanied the anemia. The peak MCV value observed in female mice administered 400 + 1,500 mg/kg was approximately 36% (63.6 fL; $P \leq 0.01$) greater than that of the vehicle controls (46.9 fL). Minor changes in MCHC values observed in male and female mice administered combinations of AZT and pyrazinamide were considered to be within normal limits.

Male and female mice administered combinations of AZT and 1,500 mg/kg pyrazinamide developed a prominent reticulocytopenia early (day 4) in the study, and the severity of this change was greater than that observed subsequent to the administration of AZT alone. Combinations of AZT and 1,000 mg/kg pyrazinamide had little or no effect on the reticulocytopenia compared with the administration of AZT alone. The reticulocyte count on day 4 of the male group treated with 400 + 1,500 mg/kg was approximately 43% ($2.7 \times 10^5/mm^3$; $P \leq 0.01$) less than that of the vehicle controls ($4.7 \times 10^5/mm^3$) (Table A1). The reticulocyte count on day 4 of female mice administered the identical combination was approximately 37% ($2.6 \times 10^5/mm^3$; $P \leq 0.01$) less than that of the vehicle controls ($4.1 \times 10^5/mm^3$) (Table A2).

Reticulocyte counts rebounded rapidly, and a significant reticulocytosis was evident by day 30. All combinations of AZT and pyrazinamide in males caused an increase in reticulocytosis in comparison to males administered AZT alone. The reticulocyte count of male mice administered 400 + 1,500 mg/kg was approximately 1.4 times ($5.6 \times 10^5/mm^3$; $P \leq 0.01$) that of the vehicle controls ($4.0 \times 10^5/mm^3$) (Table A1). The magnitude of reticulocytosis was also increased in females receiving combinations of AZT and pyrazinamide; however, the effect at 400 + 1,000 mg/kg was greater than that at 400 + 1,500 mg/kg

(Table A2). The reticulocyte count of female mice administered 400 + 1,000 mg/kg was approximately 1.7 times ($5.4 \times 10^5/mm^3$; $P \leq 0.01$) that of the vehicle controls ($3.1 \times 10^5/mm^3$).

The reticulocytosis on day 60 in male and female mice administered combinations of AZT and pyrazinamide was similar to that described for day 30. The reticulocyte count observed on day 60 of male mice administered 400 + 1,500 mg/kg was approximately 1.9 times ($6.1 \times 10^5/mm^3$; $P \leq 0.01$) that of the vehicle controls ($3.3 \times 10^5/mm^3$) (Table A1). In females, as on day 30, the magnitude of reticulocytosis in the group administered 400 + 1,000 mg/kg was greater than that in the group given 400 + 1,500 mg/kg (Table A2). The reticulocyte count on day 60 of female mice administered 400 + 1,000 mg/kg was approximately 1.7 times ($6.1 \times 10^5/mm^3$; $P \leq 0.01$) that of the vehicle controls ($3.5 \times 10^5/mm^3$).

At the end of the study (days 92 to 95), the reticulocytosis in male and female mice was dose related, and the magnitude of this change was greater in groups administered the highest combinations of AZT and pyrazinamide when compared to groups treated with AZT alone. The reticulocyte count at the end of the study for male mice administered 400 + 1,500 mg/kg was approximately 1.8 times ($7.8 \times 10^5/mm^3$; $P \leq 0.01$) that of the vehicle controls ($4.3 \times 10^5/mm^3$) (Table A1). The reticulocyte count of the female group administered identical combinations was approximately 1.9 times ($8.0 \times 10^5/mm^3$; $P \leq 0.01$) that of the vehicle controls ($4.3 \times 10^5/mm^3$) (Table A2).

Administration of combinations of AZT and pyrazinamide to male and female mice caused biologically and statistically significant increases in platelet counts of far greater magnitude than those observed subsequent to the administration of AZT alone. On day 4, significant changes in platelet counts were not observed in male mice administered combinations of AZT and pyrazinamide (Table A1). However, a significant thrombocytosis was observed on day 4 in some of the female groups receiving combination therapy. The platelet count of the female group treated with 200 + 1,500 mg/kg was approximately 1.1 times ($1,275 \times 10^3/mm^3$; $P \leq 0.05$) that of the vehicle controls ($1,144 \times 10^3/mm^3$) (Table A2). On day 30, the treatment-related thrombocytosis was distinctly dose related, with peak platelet counts in male and female mice administered the highest combination of AZT and pyrazinamide. The platelet count on day 30 of male mice administered 400 + 1,500 mg/kg was approximately 1.4 times ($1,637 \times 10^3/mm^3$; $P \leq 0.01$) that of the vehicle controls ($1,150 \times 10^3/mm^3$). The platelet count of female mice administered identical combinations was approximately 1.5 times ($1,561 \times 10^3/mm^3$; $P \leq 0.01$) that of the vehicle controls ($1,044 \times 10^3/mm^3$).

A dose-related thrombocytosis similar in magnitude to that described for day 30 was observed on day 60 in both male and female mice administered combinations of AZT and pyrazinamide. The platelet count on day 60 of male mice administered 400 + 1,500 mg/kg was approximately 1.6 times ($1,510 \times 10^3/mm^3$; $P \leq 0.01$) that of the vehicle controls ($949 \times 10^3/mm^3$) (Table A1). The platelet count of female mice administered an identical combination was approximately 1.5 times ($1,515 \times 10^3/mm^3$; $P \leq 0.01$) that of the vehicle controls ($1,010 \times 10^3/mm^3$) (Table A2).

The compound-related thrombocytosis persisted until the end of the study (days 92 to 95), with platelet counts of male and female mice administered combinations of AZT and pyrazinamide being greater than the platelet counts observed in mice treated with AZT alone. The platelet count of male mice administered 400 + 1,500 mg/kg was approximately 1.3 times ($1,770 \times 10^3/mm^3$; $P \leq 0.01$) greater than that of the vehicle controls ($1,347 \times 10^3/mm^3$) (Table A1). The platelet count of the female group administered identical doses was approximately 1.4 times ($1,609 \times 10^3/mm^3$; $P \leq 0.01$) that of the vehicle controls ($1,176 \times 10^3/mm^3$) (Table A2).

A slight leukopenia developed in male and female mice treated with AZT and pyrazinamide. In general, the magnitude of this leukopenia was slightly greater than that observed subsequent to the administration of AZT alone (Figures 6 and 7, Tables A1 and A2). The lowest mean WBC count on day 4 of male mice was observed in the group administered 400 + 1,500 mg/kg and was approximately 25% ($5.15 \times 10^3/mm^3$; $P \leq 0.01$) less than that of the vehicle controls ($6.87 \times 10^3/mm^3$). Evaluations of the corresponding differential data revealed slight declines in granulocytes as well as mononuclear cells. Similar changes were observed in female mice administered AZT and pyrazinamide combinations. The lowest WBC count on day 4 of female mice was observed in the group administered 400 + 1,000 mg/kg and was approximately 36% ($5.22 \times 10^3/mm^3$; $P \leq 0.01$) less than that of the vehicle controls ($8.11 \times 10^3/mm^3$). Decreases were observed in granulocytes as well as in mononuclear cell counts.

Alterations in WBC parameters were also observed on day 30. The lowest mean WBC value on day 30 of male mice was observed in the group administered 400 + 1,000 mg/kg and was approximately 13% ($6.22 \times 10^3/mm^3$; $P \leq 0.05$) less than that of vehicle controls ($7.18 \times 10^3/mm^3$) (Figure 6 and Table A1). Evaluation of the differential data revealed slight decreases in granulocytes and mononuclear cells. The leukopenia observed on day 30 in the female mice was distinctly dose related. The lowest WBC count of female mice was observed in the group administered 400 + 1,500 mg/kg and was approximately 34% ($3.48 \times 10^3/mm^3$; $P \leq 0.01$) less than that of vehicle controls ($5.24 \times 10^3/mm^3$) (Figure 7 and Table A2). Slight decreases were observed in both granulocyte and mononuclear cell counts.

The mild compound-related leukopenia was also observed on day 60 in male and female mice. The lowest WBC count in male mice on day 60 was observed in the group administered 200 + 1,000 mg/kg and was approximately 14% ($5.23 \times 10^3/mm^3$; $P \leq 0.05$) less than that of the vehicle controls ($6.11 \times 10^3/mm^3$) (Table A1). Slight decreases in granulocyte and mononuclear cell counts accompanied the decreased leukocyte values. Although not significant, the lowest WBC count on day 60 of female mice was observed in the group administered 400 + 1,500 mg/kg and was approximately 23% ($4.00 \times 10^3/mm^3$) less than that of vehicle controls ($5.18 \times 10^3/mm^3$) (Table A2). Slight declines in granulocyte and mononuclear cell counts were also observed.

The mild leukopenia continued through the end of the study (days 92 to 95), although none of the values were significant. The lowest WBC count observed at the end of the study in male mice was in the group administered 400 + 1,500 mg/kg and was approximately 20% ($6.19 \times 10^3/mm^3$) less than that of the vehicle controls ($7.70 \times 10^3/mm^3$) (Figure 6 and Table A1). The lowest WBC count of females was in the same high-dose combination group and was approximately 36% ($3.16 \times 10^3/mm^3$) less than that of vehicle controls ($4.90 \times 10^3/mm^3$) (Figure 7 and Table A2). Evaluation of the differential data revealed slight declines in granulocyte and/or mononuclear cell counts in both male and female mice.

NECROPSY OBSERVATIONS

Of 180 total mice in the core study groups, less than 10% had gross lesions of any type. None of the gross lesions were attributed to administration of AZT alone, pyrazinamide alone, or the combination of AZT and pyrazinamide. Four mice from the clinical pathology study groups died early and were the only mice in those groups necropsied. One of these four mice had a pale carcass, probably caused by anemia associated with cellular depletion of bone marrow resulting from administration of AZT and pyrazinamide at the highest dose concentrations. None of the other three mice had gross lesions attributed to administration of AZT and/or pyrazinamide.

HISTOPATHOLOGIC OBSERVATIONS

Suspected or actual target organs for microscopic lesions caused by the test chemicals included bone marrow, liver, and spleen (Table 2). Photomicrographs of representative compound-related lesions are shown in Plates 1 through 9 and summarized in Table 3.

TABLE 2
Incidence and Mean Severity of Histopathologic Alterations in Mice in the 13-Week Toxicity Study of AZT and Pyrazinamide Combinations[a]

	Vehicle Control	200 + 0	400 + 0	0 + 1,000	200 + 1,000	400 + 1,000	0 + 1,500	200 + 1,500	400 + 1,500
n	10	10	10	10	10	10	10	10	10
Male									
Bone marrow									
Cellular depletion	0	9 (1.1)	9 (1.4)	0	8 (1.3)	9 (1.4)	0	10 (1.5)	9 (1.3)
Liver hepatocyte									
Centrilobular glycogen depletion	5 (2.2)	6 (1.5)	10 (2.0)	10 (2.9)	10 (2.9)	10 (3.0)	10 (2.4)	10 (2.8)	10 (2.7)
Spleen									
Hematopoietic cell proliferation	10 (1.4)	10 (2.3)	10 (2.4)	10 (1.3)	10 (2.2)	10 (2.5)	10 (1.6)	10 (2.5)	10 (2.4)
Female									
Bone marrow									
Cellular depletion	0	6 (1.0)	8 (1.4)	0	10 (1.7)	9 (1.7)	0	10 (1.2)	8 (1.6)
Liver hepatocyte									
Centrilobular glycogen depletion	2 (1.0)	5 (1.2)	7 (1.4)	10 (2.7)	10 (2.9)	10 (2.6)	10 (2.8)	10 (2.4)	9 (3.0)
Spleen									
Hematopoietic cell proliferation	10 (1.9)	10 (2.9)	10 (2.8)	10 (1.9)	10 (2.7)	10 (3.0)	10 (2.5)	10 (2.6)	10 (3.1)

[a] Daily gavage doses are given as AZT + pyrazinamide (mg/kg per day). Data are given as incidence (number of animals with lesion) followed by the mean severity (which is based on the numeric scale of 1 = minimal, 2 = mild, 3 = moderate, 4 = marked)

TABLE 3
Summary of Microscopic Examinations of Compound-Related Lesions in the 13-Week Toxicity Study of AZT and Pyrazinamide Combinations

Dose[a]	Tissue	Magnification	Plate Number	Treatment-Related Lesions
0 + 0	Liver	400×	1	None
0 + 1,500	Liver	400×	2	Depletion, glycogen, hepatocyte, centrilobular (moderate)
0 + 0	Spleen	100×	3	None
400 + 0	Spleen	100×	4	Hematopoietic cell proliferation (moderate)
0 + 1,500	Spleen	100×	5	Hematopoietic cell proliferation (mild)
0 + 0	Bone marrow	100×	6	None
		200×	7	None
400 + 0	Bone marrow	100×	8	Cellular depletion (mild)
		200×	9	Cellular depletion (mild)

[a] AZT + pyrazinamide (mg/kg per day)

Bone Marrow Lesions

Cellular depletion (depletion of hematopoietic cells) of bone marrow was observed in all male and female groups treated with AZT alone or AZT in combination with pyrazinamide. Criteria for severity grades of bone marrow lesions are as follows:

Minimal - Depletion of approximately 5% or less of the hematopoietic cells of the bone marrow, based on examination of the metaphyseal region where available

Mild - Depletion of approximately 6% to 20% of the hematopoietic cells of the bone marrow, based on examination of the metaphyseal region where available

Moderate - Depletion of approximately 21% to 50% of the hematopoietic cells of the bone marrow, based on examination of the metaphyseal region where available

Marked - Depletion of more than 50% of the hematopoietic cells of the bone marrow, based on examination of the metaphyseal region where available

None of the mice in the vehicle control groups and none of the mice treated with pyrazinamide alone developed cellular depletion of bone marrow. The mean severity of cellular depletion of bone marrow was significant ($P \leq 0.01$ and $P \leq 0.05$) in female groups treated with 200 + 1,000 mg/kg or 200 + 1,500 mg/kg. Mean severity grades in all other groups treated with combinations of AZT and pyrazinamide were similar to grades in their respective vehicle control groups. In general, the degree of severity of cellular depletion of the bone marrow corresponded with diminished erythrocyte counts observed in the peripheral circulation.

Spleen Lesions

The spleen was examined routinely as a possible target organ, and hematopoietic cell proliferation was diagnosed in all spleens. Hematopoiesis occurs normally in the spleen of mice; thus, comparison of the splenic lesions among groups was, by necessity, a comparison only of mean severity, as the incidence was 100% in every group. Criteria for severity grades for hematopoietic cell proliferation follow:

Minimal - approximately 15% or less of the red pulp occupied by hematopoietic cells of the types normally found in bone marrow

Mild - approximately 16% to 50% of the red pulp occupied by hematopoietic cells of the types normally found in bone marrow

Moderate - approximately 51% to 90% of the red pulp occupied by hematopoietic cells of the types normally found in bone marrow

Marked - approximately 91% to 100% of the red pulp occupied by hematopoietic cells of the types normally found in bone marrow

Although slight increases in the mean severity were evident in all groups treated with AZT alone or in combination with pyrazinamide (Table 2), none of the severity scores were statistically significant when compared to the vehicle controls.

Liver Lesions

Administration of AZT and pyrazinamide resulted in an increased incidence and/or severity of a cytoplasmic alteration in hepatocytes diagnosed as glycogen depletion. As glycogen does not stain with routine hematoxylin and eosin stains, this diagnosis represents a cytoplasmic alteration in hepatocytes manifested by diminished vacuolization. Criteria for severity grades of glycogen depletion of hepatocytes are as follows:

Minimal - slight reduction of the irregularly shaped cytoplasmic vacuoles that are characteristic of the presence of glycogen in hepatocytes in areas surrounding the central veins, such areas usually extending not more than 1/3 to 1/2 the distance through the liver lobule from the central to portal veins

Mild - almost complete reduction of the irregularly shaped cytoplasmic vacuoles that are characteristic of the presence of glycogen in hepatocytes in areas surrounding the central veins, such areas usually extending not more than 1/3 to 1/2 the distance through the liver lobule from the central to portal veins

Moderate - almost complete reduction of the irregularly shaped cytoplasmic vacuoles that are characteristic of the presence of glycogen in hepatocytes in areas surrounding the central veins, such areas usually extending 1/2 or more of the distance through the liver lobule from the central to portal veins

A statistical analysis was performed on the mean severity grades of glycogen depletion in hepatocytes (Table 4). In male and female mice, the administration of 200 or 400 mg/kg AZT alone resulted in increased incidences of glycogen depletion but did not affect the severity of the lesion. The administration of 1,000 or 1,500 mg/kg pyrazinamide resulted in increases in both the incidences and severities ($P \leq 0.01$) of glycogen depletion in male and female mice; these effects were not enhanced by the coadministration of AZT. The degree of severity appeared to peak with 1,000 mg/kg, as severity grades in male and female groups treated with 1,000 or 1,500 mg/kg pyrazinamide alone or in combination with AZT were all similar.

SPERM MOTILITY AND VAGINAL CYTOLOGY EVALUATIONS

Pyrazinamide alone had a significant effect on left caudal and epididymal weights and spermatid heads per testis (Appendix C). Left caudal weights ($P \leq 0.01$) and spermatid heads per testis ($P \leq 0.01$) were significantly decreased at 1,500 mg/kg, while left epididymal weights were significantly decreased ($P \leq 0.05$) at 1,000 and 1,500 mg/kg. Left testicular weights were affected by AZT alone and pyrazinamide alone. Left testicular weights were significantly decreased ($P \leq 0.01$) in groups administered 400 + 1,000 mg/kg or 400 + 1,500 mg/kg. Sperm motility was significantly decreased ($P = 0.048$) by an interaction of AZT and pyrazinamide.

The average estrous cycle length and estrual cycle were not affected by AZT alone, pyrazinamide alone, or combinations of AZT and pyrazinamide.

TABLE 4
Statistical Analysis of Mean Severity of Glycogen Depletion of Hepatocytes in Male and Female Mice in the 13-Week Toxicity Study of AZT and Pyrazinamide Combinations

Dose[a]	Mean Severity[b]	Standard Error	Ratio[c]
Male			
0 + 0	1.10	0.41	—
0 + 1,000	2.90**	0.10	2.64
0 + 1,500	2.40	0.16	2.18
200 + 0	0.90	0.28	—
200 + 1,000	2.90**	0.10	3.22
200 + 1,500	2.80**	0.13	3.11
400 + 0	2.00	0.15	—
400 + 1,000	3.00**	0.00	1.50
400 + 1,500	2.70**	0.15	1.35
Female			
0 + 0	0.20	0.13	—
0 + 1,000	2.70**	0.15	13.50
0 + 1,500	2.80**	0.13	14.00
200 + 0	0.60	0.22	—
200 + 1,000	2.90**	0.10	4.83
200 + 1,500	2.40**	0.22	4.00
400 + 0	1.00	0.26	—
400 + 1,000	2.60**	0.22	2.60
400 + 1,500	2.70**	0.30	2.70

** Significantly different from the vehicle control group ($P \leq 0.01$) by Jonckheere's test
[a] Daily gavage doses of AZT + pyrazinamide (mg/kg per day); n = 10
[b] Mean severity is based on numerical scale of 1 = minimal, 2 = mild, 3 = moderate, 4 = marked
[c] Dosed group mean/vehicle control group mean

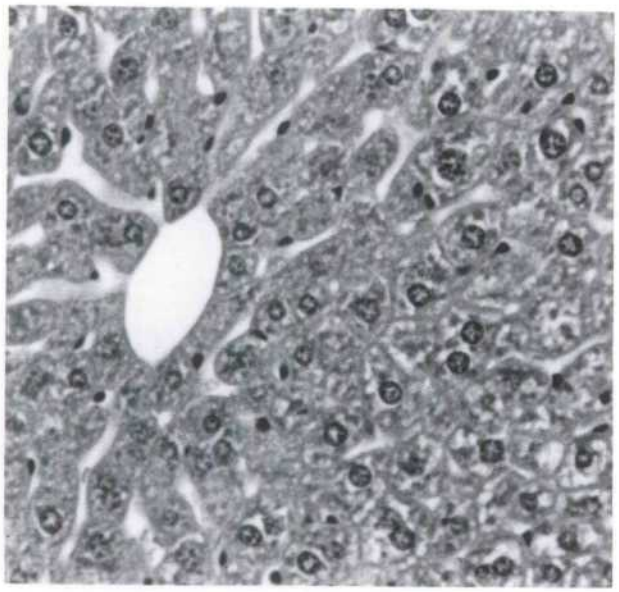


PLATE 1
Liver of a vehicle control female B6C3F$_1$ mouse showing no lesions. H&E; 400×

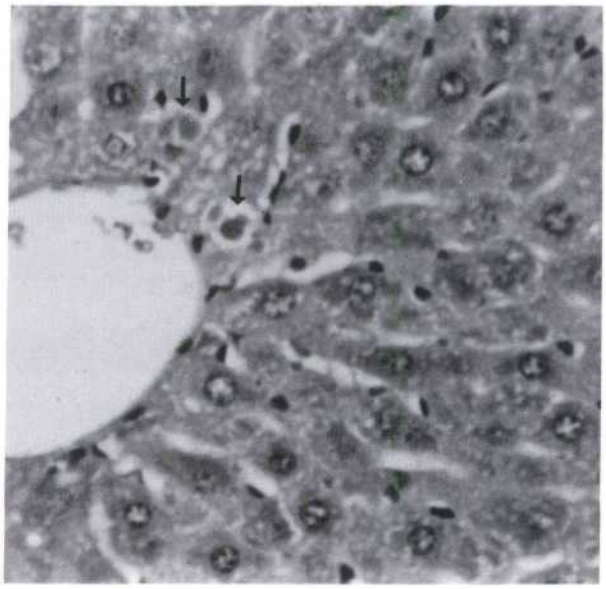


PLATE 2
Liver of a female B6C3F$_1$ mouse given 1,500 mg pyrazinamide per kg body weight per day by gavage for 13 weeks showing moderate depletion of glycogen in hepatocytes in the centrilobular zone. Hepatocyte remnants, apoptotic bodies (arrows), are visible near the central vein. H&E; 400 x

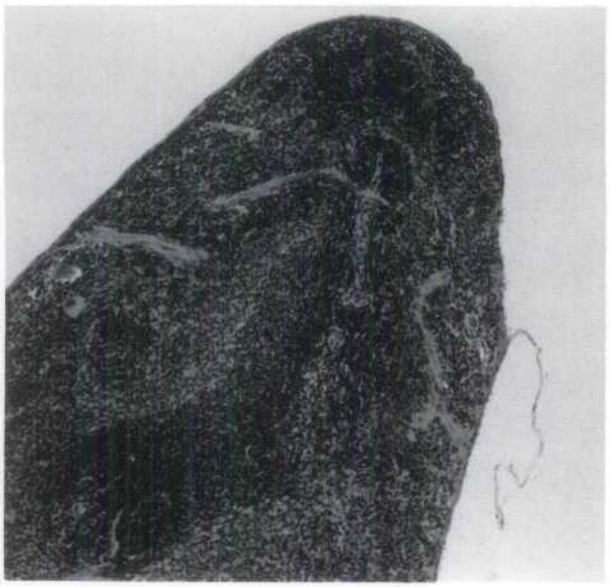


PLATE 3
Spleen of a vehicle control male B6C3F$_1$ mouse showing no lesions H&E-100X

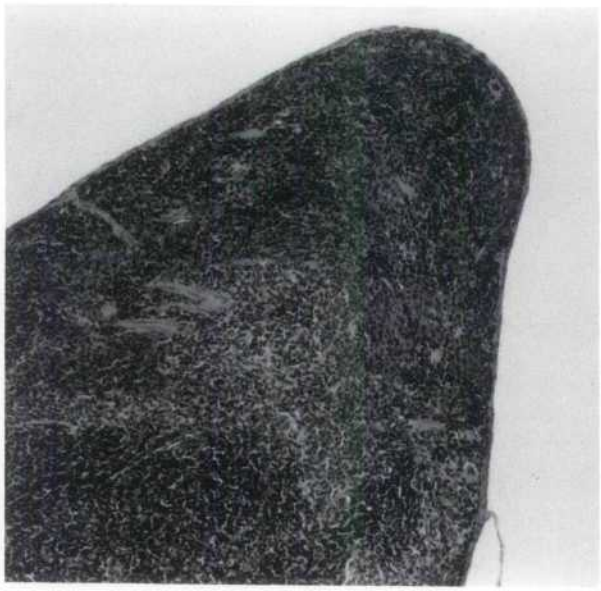


PLATE 4
Spleen of a male B6C3F$_1$ mouse given 400 mg AZT per kg body weight per day by gavage for 13 weeks showing moderate cell proliferation. H&E; 100 X

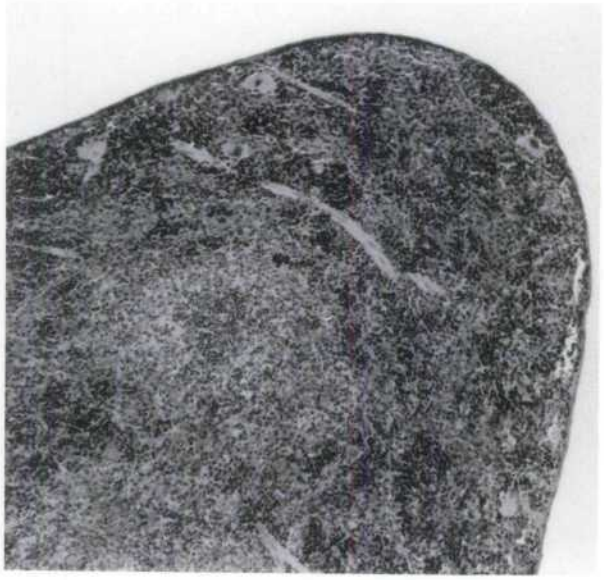


PLATE 5
Spleen of a female B6C3F$_1$ mouse given 1,500 mg pyrazinamide per kg body weight per day by gavage for 13 weeks showing mild hematopoietic cell proliferation. H&E; 100 X

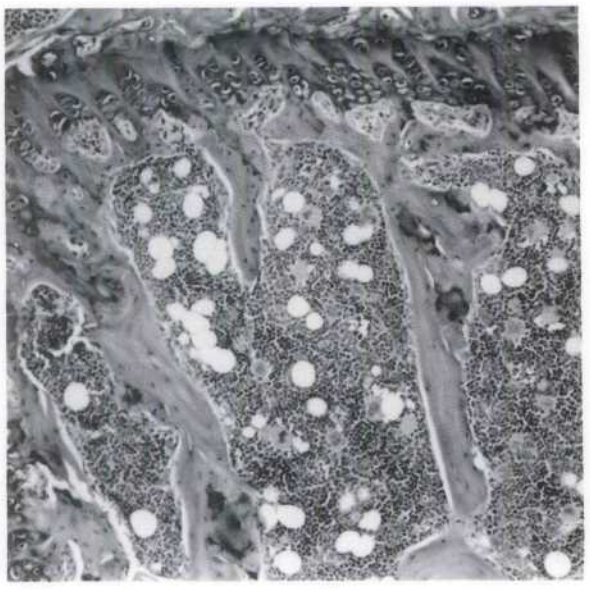


PLATE 6
Bone marrow (femur) of a vehicle control female B6C3F_1 mouse showing no lesions. H&E; 100 x

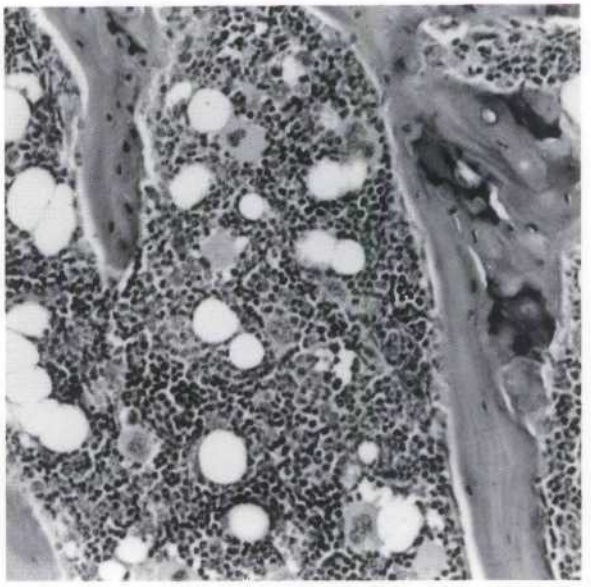


PLATE 7
Bone marrow (femur) of a vehicle control female B6C3F_1 mouse showing no lesions. H&E; 200 x

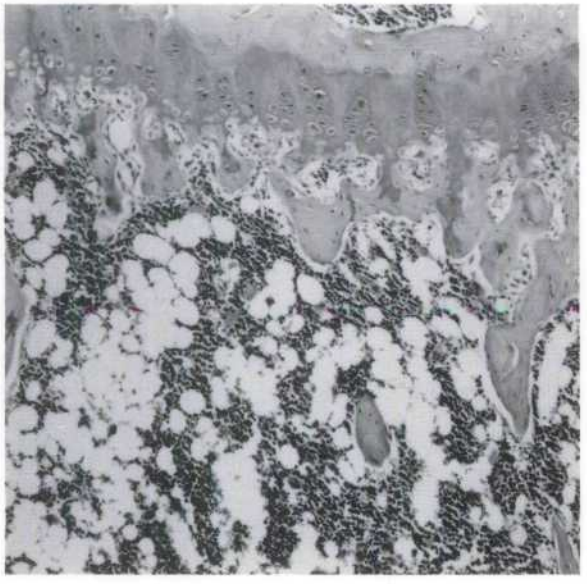


PLATE 8
Bone marrow (femur) of a female B6C3F_1 mouse given 400 mg AZT per kg body weight per day by gavage for 13 weeks showing mild cellular depletion. H&E; 100 x

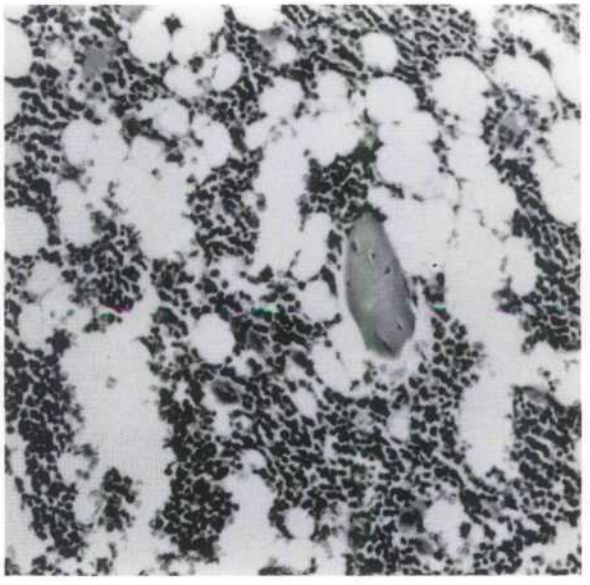


PLATE 9
Bone marrow (femur) of a female B6C3F_1 mouse given 400 mg AZT per kg body weight per day by gavage for 13 weeks showing mild cellular depletion. H&E; 200 X

DISCUSSION AND CONCLUSIONS

Tuberculosis is one of the most common opportunistic infections in AIDS patients, and these patients frequently receive combination therapy with pyrazinamide and AZT. In this study, administration of pyrazinamide at 1,000 or 1,500 mg/kg did not cause any biologically significant changes in hematologic parameters; however, minor statistically significant increases in hemoglobin concentration at the end of the study (days 92 to 95) were observed in male and female mice (Tables A1 and A2). AZT at 200 or 400 mg/kg caused a dose-related increase in severity of hematologic toxicity. Pyrazinamide at 1,000 mg/kg did not markedly increase the toxicity of AZT at doses up to 400 mg/kg. Pyrazinamide at 1,500 mg/kg, when coadministered with AZT at 400 mg/kg, caused more severe hematologic changes than those caused by AZT alone (Tables A1 and A2). These changes included significant decreases in erythrocyte counts and significant increases in platelet counts (Tables A1 and A2). An initial reticulocytopenia was observed on day 4 followed by reticulocytosis on days 30 and 60 and at the end of the study (days 92 to 95), indicating a regenerative response of the hematopoietic system. Pyrazinamide did not markedly influence the cellular depletion of bone marrow caused by AZT alone. AZT at 400 mg/kg caused a significant increase in the incidence but not the severity of glycogen depletion in the liver. Pyrazinamide, at the doses tested, caused increases in liver weights and increased the incidence and severity of liver glycogen depletion. These effects of pyrazinamide were not enhanced by coadministration of AZT. Pyrazinamide alone decreased the testicular, caudal and epididymal weights and epididymal sperm motility. AZT alone caused deceases in testicular weights and epididymal sperm motility. There appears to be some interaction between AZT and pyrazinamide on epididymal sperm motility.

CONCLUSIONS

AZT caused a dose-related increase in the severity of hematological toxicity. Pyrazinamide up to 10 times the therapeutic dose (1,500 mg/kg) did not cause general or hematologic toxicity. However, 10 times the therapeutic dose of pyrazinamide increased the hematologic toxicity of AZT, indicating that pyrazinamide at high doses may have potentiated the hematologic toxicity of AZT.

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APPENDIX A
HEMATOLOGY RESULTS

TABLE A1
Hematology Data for Male B6C3F$_1$ Mice in the 13-Week Toxicity Study of AZT and Pyrazinamide[a]

	Vehicle Control	200 + 0	400 + 0	0 + 1,000	200 + 1,000
n	10	10	10	10	10
Hematocrit (%)					
Day 4	45.7 ± 1.8	44.0 ± 1.6	43.5 ± 3.3**	44.9 ± 1.7	43.9 ± 1.2
Day 30	53.1 ± 2.1	48.6 ± 1.3**	47.0 ± 1.8**	53.8 ± 2.2	47.5 ± 1.8**
Day 60	51.7 ± 1.6	47.2 ± 1.6**	46.5 ± 2.1**	53.0 ± 1.9	49.0 ± 2.4*
Week 13	48.4 ± 1.8	46.1 ± 1.3	45.0 ± 2.4**	50.5 ± 2.4	47.3 ± 2.5
Hemoglobin (g/dL)					
Day 4	14.9 ± 0.5	14.4 ± 0.4	14.3 ± 1.0*	14.7 ± 0.4	14.4 ± 0.5
Day 30	16.7 ± 0.6	15.2 ± 0.4**	14.7 ± 0.5**	16.9 ± 0.6	14.8 ± 0.6**
Day 60	17.7 ± 0.4	15.8 ± 0.4**	15.4 ± 0.5**	18.2 ± 0.7	16.2 ± 0.7**
Week 13	16.3 ± 0.5	15.4 ± 0.5*	14.7 ± 0.8**	17.1 ± 0.9*	15.7 ± 0.9
Erythrocytes ($10^6/\mu L$)					
Day 4	9.45 ± 0.32	9.02 ± 0.30*	8.90 ± 0.60**	9.17 ± 0.32	8.98 ± 0.25*
Day 30	10.22 ± 0.42	8.47 ± 0.24**	7.89 ± 0.34**	10.30 ± 0.42	8.03 ± 0.34**
Day 60	10.63 ± 0.29	8.38 ± 0.23**	7.81 ± 0.31**	10.76 ± 0.43	8.20 ± 0.46**
Week 13	10.28 ± 0.31	8.44 ± 0.31**	7.83 ± 0.26**	10.58 ± 0.39	8.26 ± 0.33**
Reticulocytes ($10^5/\mu L$)					
Day 4	4.7 ± 0.35	3.4 ± 0.77**[b]	3.2 ± 0.32**	5.0 ± 0.54	3.6 ± 1.18**
Day 30	4.0 ± 0.18	4.3 ± 0.40	5.1 ± 0.46**	4.0 ± 0.36	5.1 ± 0.55**
Day 60	3.3 ± 0.19	4.0 ± 0.21**	5.1 ± 0.19**	3.1 ± 0.22	5.1 ± 0.40**
Week 13	4.3 ± 0.47	5.4 ± 0.46**	6.0 ± 0.56**	4.1 ± 0.43	6.2 ± 0.24**
Mean cell volume (fL)					
Day 4	48.4 ± 0.8	48.8 ± 0.8	48.9 ± 0.8	49.0 ± 0.8	48.9 ± 0.9
Day 30	52.0 ± 0.4	57.4 ± 0.6**	59.5 ± 1.5**	52.3 ± 0.7	59.2 ± 0.8**
Day 60	48.6 ± 1.1	56.3 ± 1.0**	59.6 ± 0.7**	49.3 ± 0.6	59.8 ± 1.3**
Week 13	47.1 ± 1.6	54.6 ± 1.8**	57.5 ± 1.8**	47.7 ± 1.2	57.2 ± 1.6**
Mean cell hemoglobin (pg)					
Day 4	15.8 ± 0.2	16.0 ± 0.2	16.0 ± 0.2	16.0 ± 0.2	16.0 ± 0.3
Day 30	16.4 ± 0.2	17.9 ± 0.3**	18.6 ± 0.3**	16.4 ± 0.3	18.4 ± 0.1**
Day 60	16.7 ± 0.2	18.9 ± 0.2**	19.7 ± 0.4**	16.8 ± 0.1	19.8 ± 0.3**
Week 13	15.8 ± 0.5	18.2 ± 0.4**	18.8 ± 0.5**	16.1 ± 0.4	19.0 ± 0.5**
Mean cell hemoglobin concentration (g/dL)					
Day 4	32.7 ± 0.6	32.7 ± 0.7	32.8 ± 0.6	32.7 ± 0.7	32.8 ± 0.8
Day 30	31.4 ± 0.4	31.2 ± 0.4	31.3 ± 0.4	31.4 ± 0.4	31.1 ± 0.4
Day 60	34.3 ± 0.9	33.4 ± 0.4**	33.2 ± 0.6**	34.2 ± 0.5	33.1 ± 0.5**
Week 13	33.7 ± 0.8	33.3 ± 0.8	32.8 ± 0.8	33.9 ± 1.0	33.2 ± 1.0
Platelets ($10^3/\mu L$)					
Day 4	1,271 ± 166.1	1,447 ± 129.8	1,411 ± 196.3	1,406 ± 207.3	1,294 ± 172.5
Day 30	1,150 ± 207.1	1,275 ± 113.0	1,293 ± 156.2	1,216 ± 236.0	1,337 ± 119.0
Day 60	949 ± 190.2	1,259 ± 109.5**	1,305 ± 171.8**	1,019 ± 137.0	1,291 ± 170.2**
Week 13	1,347 ± 119.6	1,520 ± 161.0	1,502 ± 116.2	1,347 ± 226.4	1,577 ± 151.7
Leukocytes ($10^3/\mu L$)[c]					
Day 4	6.87 ± 0.98	6.90 ± 0.80	7.82 ± 2.29	6.81 ± 0.86	6.10 ± 0.99
Day 30	7.18 ± 0.76	6.67 ± 0.81	6.36 ± 0.46	7.17 ± 0.77	6.35 ± 0.83
Day 60	6.11 ± 0.42	5.88 ± 0.61	5.81 ± 0.66	6.26 ± 0.79	5.23 ± 0.72*
Week 13	7.70 ± 1.03	7.01 ± 1.49	6.46 ± 1.13	7.08 ± 0.94	6.76 ± 1.00
Segmented neutrophils ($10^3/\mu L$)					
Day 4	0.83 ± 0.20	0.78 ± 0.12	1.09 ± 1.10	0.94 ± 0.19	0.77 ± 0.22
Day 30	0.67 ± 0.09	0.71 ± 0.11	0.62 ± 0.09	0.91 ± 0.21**	0.71 ± 0.12
Day 60	0.65 ± 0.10	0.60 ± 0.11	0.47 ± 0.07*	0.71 ± 0.19	0.52 ± 0.21
Week 13	0.95 ± 0.19	0.85 ± 0.17	0.79 ± 0.30	1.04 ± 0.34	0.86 ± 0.18

TABLE A1
Hematology Data for Male B6C3F$_1$ Mice in the 13-Week Toxicity Study of AZT and Pyrazinamide

	400 + 1,000	0 + 1,500	200 + 1,500	400 + 1,500
n	10	10	10	10
Hematocrit (%)				
Day 4	43.1 ± 1.1**	45.8 ± 0.6	43.5 ± 1.3*	43.2 ± 1.6*
Day 30	47.3 ± 1.2**	52.4 ± 2.1	47.9 ± 1.4**	45.4 ± 2.2**
Day 60	45.8 ± 1.6**[b]	52.4 ± 1.9	46.6 ± 1.2**	44.6 ± 2.1**
Week 13	45.5 ± 1.9**	50.3 ± 1.9	46.4 ± 1.3	43.4 ± 1.2**
Hemoglobin (g/dL)				
Day 4	14.2 ± 0.4**	14.9 ± 0.3	14.3 ± 0.4*	14.2 ± 0.4**
Day 30	14.7 ± 0.4**	16.6 ± 0.5	14.8 ± 0.5**	13.9 ± 0.6**
Day 60	15.2 ± 0.4**[b]	18.1 ± 0.6	15.7 ± 0.5**	14.7 ± 0.7**
Week 13	14.9 ± 0.5**	16.9 ± 0.6	15.2 ± 0.6**	14.0 ± 0.2**
Erythrocytes ($10^6/\mu L$)				
Day 4	8.86 ± 0.18**	9.39 ± 0.23	8.89 ± 0.32**	8.85 ± 0.33**
Day 30	7.78 ± 0.25**	9.89 ± 0.37	7.95 ± 0.26**	7.40 ± 0.34**
Day 60	7.25 ± 0.26**[b]	10.53 ± 0.41	7.66 ± 0.21**	6.84 ± 0.36**
Week 13	7.53 ± 0.41**	10.36 ± 0.31	7.76 ± 0.27**	6.89 ± 0.24**
Reticulocytes ($10^5/\mu L$)				
Day 4	3.0 ± 0.70**	5.3 ± 0.36	3.0 ± 0.26**	2.7 ± 0.47**
Day 30	5.4 ± 0.35**	3.9 ± 0.28	5.3 ± 0.48**	5.6 ± 0.38**
Day 60	5.8 ± 0.28**[b]	3.3 ± 0.15	5.6 ± 0.19**	6.1 ± 0.31**
Week 13	7.0 ± 0.54**	4.5 ± 0.32	6.6 ± 0.29**	7.8 ± 0.91**
Mean cell volume (fL)				
Day 4	48.7 ± 0.9	48.8 ± 0.9	48.9 ± 1.0	48.8 ± 0.4
Day 30	60.8 ± 0.9	53.0 ± 0.4	60.3 ± 1.1*	61.4 ± 0.7**
Day 60	63.2 ± 1.1**[b]	49.7 ± 0.3	60.8 ± 0.8**	65.3 ± 1.2**
Week 13	60.5 ± 2.5**	48.6 ± 1.2	59.9 ± 2.0**	63.1 ± 2.4**
Mean cell hemoglobin (pg)				
Day 4	16.0 ± 0.2	15.9 ± 0.2	16.1 ± 0.2	16.0 ± 0.2
Day 30	18.8 ± 0.2**	16.8 ± 0.2**	18.7 ± 0.2**	18.8 ± 0.2**
Day 60	21.0 ± 0.2**[b]	17.1 ± 0.1**	20.5 ± 0.2**	21.5 ± 0.4**
Week 13	19.8 ± 0.6**	16.4 ± 0.5	19.6 ± 0.6**	20.4 ± 0.6**
Mean cell hemoglobin concentration (g/dL)				
Day 4	32.9 ± 0.7	32.6 ± 0.6	32.9 ± 0.7	32.8 ± 0.5
Day 30	31.0 ± 0.3	31.7 ± 0.5	31.0 ± 0.5	30.7 ± 0.3**
Day 60	33.2 ± 0.6**[b]	34.5 ± 0.3	33.7 ± 0.5	32.9 ± 0.4**
Week 13	32.8 ± 0.6	33.7 ± 0.8	32.7 ± 1.1	32.3 ± 0.9**
Platelets ($10^3/\mu L$)				
Day 4	1,374 ± 215.3	1,311 ± 100.0	1,380 ± 116.0	1,401 ± 183.2
Day 30	1,430 ± 145.4**	1,212 ± 173.6	1,418 ± 123.4**	1,637 ± 100.3**
Day 60	1,485 ± 179.0**[b]	1,006 ± 164.3	1,379 ± 135.3**	1,510 ± 234.9**
Week 13	1,730 ± 251.3	1,346 ± 124.8	1,645 ± 164.6**	1,770 ± 203.7**
Leukocytes ($10^3/\mu L$)				
Day 4	6.01 ± 1.27	7.13 ± 0.92	5.97 ± 0.66	5.15 ± 0.64**
Day 30	6.22 ± 0.49	6.79 ± 0.93	6.34 ± 0.79	6.28 ± 0.75
Day 60	5.24 ± 0.64**[b]	6.32 ± 0.44	5.60 ± 0.60	5.60 ± 0.48
Week 13	6.21 ± 1.41	7.37 ± 1.16	6.89 ± 1.21	6.19 ± 0.64
Segmented neutrophils ($10^3/\mu L$)				
Day 4	0.66 ± 0.14	0.95 ± 0.14	0.67 ± 0.15	0.65 ± 0.12
Day 30	0.60 ± 0.09	0.93 ± 0.22**	0.75 ± 0.13	0.59 ± 0.07
Day 60	0.51 ± 0.12 [b]	0.78 ± 0.14	0.63 ± 0.13	0.53 ± 0.13
Week 13	0.83 ± 0.13	1.08 ± 0.16	0.88 ± 0.19	0.85 ± 0.15

TABLE A1
Hematology Data for Male B6C3F$_1$ Mice in the 13-Week Toxicity Study of AZT and Pyrazinamide

	Vehicle Control	200 + 0	400 + 0	0 + 1,000	200 + 1,000
n	10	10	10	10	10
Lymphocytes ($10^3/\mu L$)					
Day 4	5.71 ± 0.82	5.81 ± 0.78	6.37 ± 1.21	5.49 ± 0.75	4.97 ± 1.06
Day 30	6.21 ± 0.72	5.71 ± 0.77	5.53 ± 0.46	5.94 ± 0.56	5.42 ± 0.78
Day 60	5.16 ± 0.37	5.03 ± 0.51	5.07 ± 0.59	5.25 ± 0.63	4.50 ± 0.64*
Week 13	6.30 ± 0.75	5.79 ± 1.33	5.37 ± 0.85	5.64 ± 0.88	5.54 ± 0.84
Monocytes ($10^3/\mu L$)					
Day 4	0.17 ± 0.04	0.15 ± 0.02	0.18 ± 0.06	0.21 ± 0.05	0.20 ± 0.05
Day 30	0.11 ± 0.02	0.09 ± 0.02	0.07 ± 0.02**	0.17 ± 0.05**	0.10 ± 0.05
Day 60	0.12 ± 0.03	0.08 ± 0.02**	0.07 ± 0.03**	0.12 ± 0.02	0.08 ± 0.03**
Week 13	0.17 ± 0.04	0.16 ± 0.03	0.13 ± 0.05	0.21 ± 0.11	0.17 ± 0.04
Basophils ($10^3/\mu L$)					
Day 4	0.02 ± 0.007	0.02 ± 0.006	0.03 ± 0.014	0.02 ± 0.007	0.02 ± 0.008
Day 30	0.02 ± 0.008	0.02 ± 0.009	0.02 ± 0.010	0.03 ± 0.012	0.02 ± 0.011
Day 60	0.02 ± 0.007	0.02 ± 0.022	0.02 ± 0.007	0.02 ± 0.009	0.02 ± 0.013
Week 13	0.02 ± 0.010	0.02 ± 0.012	0.01 ± 0.004	0.02 ± 0.008	0.02 ± 0.009
Eosinophils ($10^3/\mu L$)					
Day 4	0.13 ± 0.04	0.11 ± 0.03	0.13 ± 0.01	0.13 ± 0.03	0.11 ± 0.04
Day 30	0.15 ± 0.06	0.13 ± 0.03	0.11 ± 0.05	0.11 ± 0.05	0.09 ± 0.04*
Day 60	0.14 ± 0.05	0.13 ± 0.07	0.18 ± 0.11	0.13 ± 0.04	0.10 ± 0.05
Week 13	0.24 ± 0.16	0.16 ± 0.05	0.14 ± 0.08*	0.14 ± 0.03*	0.15 ± 0.05
Large unstained cells ($10^3/\mu L$)					
Day 4	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01
Day 30	0.01 ± 0.01	0.02 ± 0.00	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01[b]
Day 60	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01[b]
Week 13	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01

TABLE A1
Hematology Data for Male B6C3F$_1$ Mice in the 13-Week Toxicity Study of AZT and Pyrazinamide

	400 + 1,000	0 + 1,500	200 + 1,500	400 + 1,500
n	10	10	10	10
Lymphocytes ($10^3/\mu L$)				
Day 4	5.04 ± 1.09	5.76 ± 0.79	4.98 ± 0.51	4.20 ± 0.60**
Day 30	5.38 ± 0.45	5.56 ± 0.82	5.34 ± 0.71	5.50 ± 0.66
Day 60	4.49 ± 0.60*[b]	5.23 ± 0.36	4.72 ± 0.53	4.83 ± 0.35
Week 13	5.08 ± 1.27	5.93 ± 1.05	5.64 ± 1.06	4.96 ± 0.64
Monocytes ($10^3/\mu L$)				
Day 4	0.18 ± 0.05	0.23 ± 0.04**	0.17 ± 0.03	0.15 ± 0.03
Day 30	0.11 ± 0.02	0.17 ± 0.01**	0.11 ± 0.01	0.08 ± 0.02
Day 60	0.08 ± 0.02**[b]	0.15 ± 0.03	0.10 ± 0.03	0.10 ± 0.02
Week 13	0.15 ± 0.04	0.20 ± 0.04	0.18 ± 0.05	0.19 ± 0.03
Basophils ($10^3/\mu L$)				
Day 4	0.02 ± 0.006	0.02 ± 0.010	0.02 ± 0.008	0.02 ± 0.007
Day 30	0.02 ± 0.007	0.02 ± 0.006	0.02 ± 0.007	0.01 ± 0.007
Day 60	0.02 ± 0.009[b]	0.02 ± 0.009	0.02 ± 0.007	0.01 ± 0.008
Week 13	0.01 ± 0.007	0.02 ± 0.012	0.02 ± 0.006	0.02 ± 0.007
Eosinophils ($10^3/\mu L$)				
Day 4	0.10 ± 0.03	0.14 ± 0.05	0.12 ± 0.05	0.11 ± 0.03
Day 30	0.10 ± 0.03	0.10 ± 0.02*	0.11 ± 0.03	0.08 ± 0.03**
Day 60	0.12 ± 0.04[b]	0.14 ± 0.05	0.11 ± 0.03	0.12 ± 0.05
Week 13	0.12 ± 0.05**	0.12 ± 0.04**	0.14 ± 0.08*	0.14 ± 0.07*
Large unstained cells ($10^3/\mu L$)				
Day 4	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
Day 30	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01
Day 60	0.02 ± 0.01[b]	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01
Week 13	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunnett's test
** $P \leq 0.01$
[a] Daily gavage doses of AZT + pyrazinamide (mg/kg per day). Data are given as mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=9
[c] Leukocyte counts corrected for nucleated erythrocyte counts greater than 10 per 100 leukocytes

TABLE A2
Hematology Data for Female B6C3F$_1$ Mice in the 13-Week Toxicity Study of AZT and Pyrazinamide[a]

	Vehicle Control	200 + 0	400 + 0	0 + 1,000	200 + 1,000
n	10	10	10	10	10
Hematocrit (%)					
Day 4	46.2 ± 1.5	47.5 ± 1.6	44.4 ± 6.1	46.6 ± 1.4	46.2 ± 1.6
Day 30	46.1 ± 1.2	44.0 ± 1.2	43.0 ± 0.7	48.4 ± 2.4	44.8 ± 1.5
Day 60	49.2 ± 2.7	44.2 ± 1.8**	44.8 ± 3.0**[b]	49.0 ± 0.8[b]	45.8 ± 1.3**
Week 13	45.2 ± 1.4	43.7 ± 2.2	42.2 ± 3.1	47.8 ± 3.1	46.1 ± 3.4
Hemoglobin (g/dL)					
Day 4	15.1 ± 0.4	15.6 ± 0.7	14.4 ± 2.0	15.3 ± 0.4	15.1 ± 0.4
Day 30	15.4 ± 0.3	14.2 ± 0.4	13.8 ± 0.2*	16.1 ± 0.7	14.6 ± 0.4
Day 60	16.0 ± 0.6	14.0 ± 0.5**	14.0 ± 1.0**[b]	16.2 ± 0.3[b]	14.7 ± 0.4**
Week 13	15.5 ± 0.5	14.7 ± 0.5	14.0 ± 0.8**	16.6 ± 0.9*	15.5 ± 0.8
Erythrocytes ($10^6/\mu L$)					
Day 4	8.99 ± 0.26	9.34 ± 0.42	8.55 ± 1.25	9.15 ± 0.34	9.02 ± 0.26
Day 30	9.45 ± 0.26	7.92 ± 0.21**	7.46 ± 0.16**	9.89 ± 0.50	7.97 ± 0.24**
Day 60	9.98 ± 0.51	7.55 ± 0.34**	7.19 ± 0.56**[b]	9.92 ± 0.16[b]	7.89 ± 0.31**
Week 13	9.65 ± 0.40	7.85 ± 0.32**	7.13 ± 0.44**	10.08 ± 0.38	7.98 ± 0.46**
Reticulocytes ($10^5/\mu L$)					
Day 4	4.1 ± 0.61	3.3 ± 0.79*	3.0 ± 0.65**	4.4 ± 0.47	3.3 ± 0.61*
Day 30	3.1 ± 0.50	4.1 ± 0.44*	4.7 ± 0.36**	3.3 ± 0.69	4.5 ± 0.85**
Day 60	3.5 ± 0.51	4.9 ± 0.54**	5.9 ± 0.82**[b]	3.3 ± 0.60[b]	4.7 ± 0.98*
Week 13	4.3 ± 1.17	4.9 ± 0.82	6.0 ± 0.91**	4.0 ± 0.91[b]	6.1 ± 0.91**
Mean cell volume (fL)					
Day 4	51.4 ± 1.0	50.9 ± 0.9	52.1 ± 1.2	51.0 ± 1.0	51.2 ± 0.9
Day 30	48.9 ± 0.8	55.6 ± 0.5**	57.7 ± 0.9**	48.9 ± 0.4	56.2 ± 0.7**
Day 60	49.3 ± 0.6	58.5 ± 1.1**	62.4 ± 0.8**[b]	49.3 ± 0.5[b]	58.1 ± 1.0**
Week 13	46.9 ± 1.4	55.7 ± 2.2**	59.3 ± 2.8**	47.4 ± 1.6	57.8 ± 1.9**
Mean cell hemoglobin (pg)					
Day 4	16.8 ± 0.1	16.7 ± 0.2	16.9 ± 0.5	16.7 ± 0.4	16.8 ± 0.4
Day 30	16.3 ± 0.2	18.0 ± 0.1**	18.5 ± 0.2**	16.3 ± 0.3	18.3 ± 0.2**
Day 60	16.0 ± 0.2	18.6 ± 0.2**	19.5 ± 0.3**[b]	16.3 ± 0.2[b]	18.6 ± 0.3**
Week 13	16.1 ± 0.4	18.7 ± 0.5**	19.7 ± 0.5**	16.5 ± 0.4	19.5 ± 0.5**
Mean cell hemoglobin concentration (g/dL)					
Day 4	32.7 ± 0.6	32.7 ± 0.6	32.5 ± 0.7	32.8 ± 0.8	32.8 ± 0.9
Day 30	33.3 ± 0.5	32.3 ± 0.3**	32.1 ± 0.5**	33.3 ± 0.4	32.5 ± 0.3**
Day 60	32.5 ± 0.7	31.7 ± 0.6**	31.3 ± 0.4**[b]	32.9 ± 0.5[b]	32.1 ± 0.3
Week 13	34.3 ± 0.7	33.6 ± 0.8	33.2 ± 1.0	34.8 ± 1.0	33.7 ± 0.9
Platelets ($10^3/\mu L$)					
Day 4	1,144 ± 75.3	1,066 ± 118.9	1,245 ± 139.3	1,124 ± 61.6	1,233 ± 63.4
Day 30	1,044 ± 82.9	1,171 ± 154.3	1,209 ± 111.1*	988 ± 153.8	1,228 ± 134.1*
Day 60	1,010 ± 203.8	1,276 ± 206.3**	1,292 ± 116.5**[b]	1,179 ± 165.5[b]	1,403 ± 106.2**
Week 13	1,176 ± 94.9	1,275 ± 168.0	1,319 ± 197.7	1,197 ± 162.2	1,455 ± 252.0*
Leukocytes ($10^3/\mu L$)[c]					
Day 4	8.11 ± 1.33	5.77 ± 2.18**	5.64 ± 1.66**	5.92 ± 0.71*	6.18 ± 2.28*
Day 30	5.24 ± 0.98	4.47 ± 1.66	3.58 ± 0.53**	4.15 ± 0.71	4.27 ± 1.07
Day 60	5.18 ± 0.90	4.30 ± 1.03	4.28 ± 1.06[b]	4.62 ± 0.84[b]	5.23 ± 0.94
Week 13	4.90 ± 1.15	4.50 ± 1.74	3.77 ± 0.99	4.67 ± 1.37	3.82 ± 1.00
Segmented neutrophils ($10^3/\mu L$)					
Day 4	0.90 ± 0.24	0.66 ± 0.23	0.73 ± 0.26	0.72 ± 0.22	0.61 ± 0.31*
Day 30	0.62 ± 0.22	0.48 ± 0.28	0.30 ± 0.09*	0.47 ± 0.16	0.42 ± 0.17
Day 60	0.52 ± 0.17	0.38 ± 0.10	0.32 ± 0.10*[b]	0.45 ± 0.14[b]	0.47 ± 0.09
Week 13	0.59 ± 0.18	0.57 ± 0.27	0.38 ± 0.19	0.61 ± 0.19[b]	0.47 ± 0.21

TABLE A2
Hematology Data for Female B6C3F$_1$ Mice in the 13-Week Toxicity Study of AZT and Pyrazinamide

	400 + 1,000	0 + 1,500	200 + 1,500	400 + 1,500
n	10	10	10	10
Hematocrit (%)				
Day 4	46.0 ± 2.0	47.5 ± 0.8	45.8 ± 1.3	45.9 ± 1.2
Day 30	43.8 ± 2.8	47.8 ± 1.1	44.4 ± 1.5	38.4 ± 10.1**
Day 60	42.6 ± 1.8**	49.6 ± 2.1	45.5 ± 1.9**	40.8 ± 2.3**[b]
Week 13	42.6 ± 2.7	48.7 ± 5.1	44.3 ± 3.1	43.0 ± 1.9[d]
Hemoglobin (g/dL)				
Day 4	15.1 ± 0.5	15.6 ± 0.4	15.1 ± 0.4	15.0 ± 0.4
Day 30	14.1 ± 0.8	15.9 ± 0.4	14.4 ± 0.4	12.4 ± 3.2**
Day 60	13.3 ± 0.6**	16.5 ± 0.7	14.7 ± 0.5**	12.8 ± 0.7**[b]
Week 13	13.9 ± 0.6**	16.8 ± 1.5**	14.9 ± 0.9	14.1 ± 0.4**[d]
Erythrocytes ($10^6/\mu L$)				
Day 4	9.02 ± 0.30	9.31 ± 0.25	9.00 ± 0.23	8.90 ± 0.21
Day 30	7.47 ± 0.47**	9.71 ± 0.23	7.87 ± 0.30**	6.70 ± 1.60**
Day 60	6.50 ± 0.29**	10.03 ± 0.41	7.53 ± 0.31**	6.34 ± 0.33**[b]
Week 13	6.92 ± 0.40**	10.06 ± 0.86	7.51 ± 0.44**	6.76 ± 0.39**[d]
Reticulocytes ($10^5/\mu L$)				
Day 4	2.5 ± 0.41**	4.6 ± 0.39	3.4 ± 0.63*	2.6 ± 0.52**
Day 30	5.4 ± 0.69**	3.4 ± 0.66	5.2 ± 0.76**	4.6 ± 1.40**
Day 60	6.1 ± 1.06**	3.2 ± 0.82	5.6 ± 0.77**	5.6 ± 1.34**[b]
Week 13	6.9 ± 1.08**	4.4 ± 1.26	5.6 ± 1.08*	8.0 ± 0.89**[d]
Mean cell volume (fL)				
Day 4	51.0 ± 0.7	51.1 ± 0.7	50.9 ± 0.7	51.6 ± 1.5
Day 30	58.6 ± 1.4**	49.2 ± 0.9	56.4 ± 1.1**	56.5 ± 4.4**
Day 60	65.5 ± 1.1**	49.5 ± 0.5	60.5 ± 1.4**	64.3 ± 1.8**[b]
Week 13	61.5 ± 2.6**	48.4 ± 1.8	59.0 ± 1.6**	63.6 ± 3.3**[d]
Mean cell hemoglobin (pg)				
Day 4	16.7 ± 0.2	16.7 ± 0.3	16.7 ± 0.2	16.8 ± 0.2
Day 30	18.9 ± 0.3**	16.4 ± 0.4	18.3 ± 0.3**	18.3 ± 1.1**
Day 60	20.5 ± 0.1**	16.4 ± 0.2**	19.5 ± 0.2**	20.3 ± 0.4**[b]
Week 13	20.1 ± 0.6**	16.7 ± 0.4*	19.8 ± 0.2**	20.9 ± 0.9**[d]
Mean cell hemoglobin concentration (g/dL)				
Day 4	32.7 ± 0.6	32.8 ± 0.6	33.0 ± 0.6	32.6 ± 0.8
Day 30	32.2 ± 0.5**	33.2 ± 0.4	32.5 ± 0.4**	32.4 ± 0.7**
Day 60	31.3 ± 0.5**	33.3 ± 0.4**	32.3 ± 0.5	31.6 ± 0.4**[b]
Week 13	32.7 ± 1.0**	34.5 ± 1.0	33.6 ± 0.9	32.8 ± 0.9**[c]
Platelets ($10^3/\mu L$)				
Day 4	1,263 ± 70.6*	1,239 ± 108.2	1,275 ± 109.4*	1,187 ± 75.4
Day 30	1,422 ± 154.6**	1,020 ± 109.4	1,219 ± 109.1*	1,561 ± 186.1**
Day 60	1,493 ± 198.8**	1,133 ± 209.8	1,402 ± 134.0**	1,515 ± 233.7**[b]
Week 13	1,529 ± 187.1**	1,170 ± 234.1	1,428 ± 197.1*	1,609 ± 325.4**[d]
Leukocytes ($10^3/\mu L$)				
Day 4	5.22 ± 1.40**	6.47 ± 1.46	5.84 ± 1.24**	5.43 ± 1.00**
Day 30	4.00 ± 0.51*	4.79 ± 1.43	3.71 ± 0.65**	3.48 ± 0.51**
Day 60	4.18 ± 0.75	4.98 ± 1.37	4.31 ± 1.09	4.00 ± 0.79[b]
Week 13	4.41 ± 1.41	4.33 ± 1.25	3.80 ± 1.18	3.16 ± 0.65[d]
Segmented neutrophils ($10^3/\mu L$)				
Day 4	0.65 ± 0.19	0.89 ± 0.30	0.67 ± 0.12	0.61 ± 0.20
Day 30	0.43 ± 0.07	0.80 ± 0.56	0.38 ± 0.11	0.42 ± 0.08
Day 60	0.41 ± 0.11	0.67 ± 0.30	0.47 ± 0.12	0.43 ± 0.13[b]
Week 13	0.47 ± 0.09	0.50 ± 0.15	0.45 ± 0.14	0.39 ± 0.15[d]

TABLE A2
Hematology Data for Female B6C3F$_1$ Mice in the 13-Week Toxicity Study of AZT and Pyrazinamide

	Vehicle Control	200 + 0	400 + 0	0 + 1,000	200 + 1,000
n	10	10	10	10	10
Lymphocytes ($10^3/\mu L$)					
Day 4	6.78 ± 1.19	4.77 ± 1.91**	4.66 ± 1.47**	4.89 ± 0.71*	5.28 ± 1.96
Day 30	4.35 ± 0.78	3.81 ± 1.43	3.12 ± 0.47**	3.48 ± 0.57	3.63 ± 0.89
Day 60	4.44 ± 0.81	3.74 ± 0.98	3.79 ± 0.96[b]	3.96 ± 0.75[b]	4.54 ± 0.85
Week 13	4.09 ± 0.97	3.73 ± 1.45	3.20 ± 0.76	3.82 ± 1.16[b]	3.09 ± 0.69
Monocytes ($10^3/\mu L$)					
Day 4	0.20 ± 0.05	0.14 ± 0.07*	0.13 ± 0.04*	0.17 ± 0.05	0.14 ± 0.06*
Day 30	0.10 ± 0.03	0.06 ± 0.02	0.06 ± 0.02*	0.09 ± 0.03	0.09 ± 0.03
Day 60	0.09 ± 0.02	0.06 ± 0.03	0.05 ± 0.03*[b]	0.08 ± 0.02[b]	0.08 ± 0.03
Week 13	0.11 ± 0.04	0.08 ± 0.03	0.07 ± 0.03	0.12 ± 0.04[b]	0.11 ± 0.04
Basophils ($10^3/\mu L$)					
Day 4	0.03 ± 0.012	0.02 ± 0.009	0.02 ± 0.007*	0.02 ± 0.005	0.02 ± 0.012*
Day 30	0.01 ± 0.008	0.01 ± 0.005	0.01 ± 0.004	0.01 ± 0.006	0.01 ± 0.004
Day 60	0.01 ± 0.007	0.01 ± 0.003	0.01 ± 0.007[b]	0.01 ± 0.006[b]	0.02 ± 0.007
Week 13	0.01 ± 0.007	0.01 ± 0.006	0.01 ± 0.007	0.01 ± 0.006[b]	0.01 ± 0.006
Eosinophils ($10^3/\mu L$)					
Day 4	0.17 ± 0.05	0.16 ± 0.05	0.08 ± 0.04*	0.09 ± 0.05*	0.12 ± 0.04
Day 30	0.15 ± 0.06	0.10 ± 0.05	0.10 ± 0.03	0.10 ± 0.04	0.11 ± 0.04
Day 60	0.09 ± 0.05	0.10 ± 0.03	0.10 ± 0.05[b]	0.11 ± 0.07[b]	0.11 ± 0.05
Week 13	0.10 ± 0.03	0.10 ± 0.03	0.10 ± 0.06	0.10 ± 0.07[b]	0.14 ± 0.10
Large unstained cells ($10^3/\mu L$)					
Day 4	0.03 ± 0.02	0.02 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
Day 30	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
Day 60	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01[d]	0.01 ± 0.00[b]	0.02 ± 0.01
Week 13	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01[b]	0.01 ± 0.01

TABLE A2
Hematology Data for Female B6C3F$_1$ Mice in the 13-Week Toxicity Study of AZT and Pyrazinamide

	400 + 1,000	0 + 1,500	200 + 1,500	400 + 1,500
n	10	10	10	10
Lymphocytes ($10^3/\mu L$)				
Day 4	4.31 ± 1.29**	5.18 ± 1.26	4.83 ± 1.08*	4.56 ± 0.80**
Day 30	3.38 ± 0.48*	3.74 ± 0.99	3.13 ± 0.53**	2.91 ± 0.44**
Day 60	3.61 ± 0.67	4.08 ± 1.06	3.64 ± 0.97	3.37 ± 0.73[b]
Week 13	3.72 ± 1.35	3.58 ± 1.03	3.13 ± 1.08	2.59 ± 0.55[d]
Monocytes ($10^3/\mu L$)				
Day 4	0.14 ± 0.04*	0.20 ± 0.06	0.16 ± 0.05	0.13 ± 0.03*
Day 30	0.07 ± 0.03	0.13 ± 0.08	0.07 ± 0.02	0.06 ± 0.03
Day 60	0.07 ± 0.03	0.10 ± 0.04	0.09 ± 0.03	0.08 ± 0.02[b]
Week 13	0.10 ± 0.04	0.11 ± 0.04	0.10 ± 0.04	0.09 ± 0.04[d]
Basophils ($10^3/\mu L$)				
Day 4	0.02 ± 0.005*	0.02 ± 0.007	0.02 ± 0.011*	0.01 ± 0.005**
Day 30	0.01 ± 0.005	0.01 ± 0.005	0.01 ± 0.005	0.01 ± 0.008
Day 60	0.01 ± 0.008	0.01 ± 0.005	0.01 ± 0.006	0.01 ± 0.004[b]
Week 13	0.01 ± 0.007	0.01 ± 0.011	0.01 ± 0.009	0.01 ± 0.005[d]
Eosinophils ($10^3/\mu L$)				
Day 4	0.09 ± 0.05*	0.17 ± 0.12	0.14 ± 0.08	0.09 ± 0.03*
Day 30	0.09 ± 0.04*	0.11 ± 0.05	0.12 ± 0.06	0.07 ± 0.03**
Day 60	0.08 ± 0.03	0.11 ± 0.07	0.11 ± 0.05	0.09 ± 0.03[b]
Week 13	0.10 ± 0.03	0.11 ± 0.08	0.10 ± 0.06	0.07 ± 0.03[d]
Large unstained cells ($10^3/\mu L$)				
Day 4	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
Day 30	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.00
Day 60	0.01 ± 0.00[b]	0.01 ± 0.01	0.01 ± 0.01[b]	0.01 ± 0.01[b]
Week 13	0.01 ± 0.01[b]	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01[d]

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunnett's test
** $P \leq 0.01$
[a] Daily gavage doses of AZT + pyrazinamide (mg/kg per day). Data are given as mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=9
[c] Leukocyte counts corrected for nucleated erythrocyte counts greater than 10 per 100 leukocytes
[d] n=8

APPENDIX B
ORGAN WEIGHTS AND ORGAN-WEIGHT-TO-BODY-WEIGHT RATIOS

TABLE B1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 13-Week Toxicity Study of AZT and Pyrazinamide[a]

	Vehicle Control	200 + 0	400 + 0	0 + 1,000	200 + 1,000
Male					
n	10	10	10	10	10
Necropsy body wt	36.1	35.3	33.8*	35.9	35.2
Heart					
Absolute	0.147 ± 0.0116	0.139 ± 0.0120	0.140 ± 0.0105	0.147 ± 0.0067	0.146 ± 0.0084
Relative	4.1 ± 0.3	4.0 ± 0.5	4.1 ± 0.2	4.1 ± 0.3	4.2 ± 0.3
R. Kidney					
Absolute	0.286 ± 0.0276	0.285 ± 0.0246	0.269 ± 0.0191	0.281 ± 0.0185	0.283 ± 0.0177
Relative	7.9 ± 0.4	8.1 ± 0.9	8.0 ± 0.5	7.8 ± 0.5	8.1 ± 0.5
Liver					
Absolute	1.563 ± 0.1702	1.603 ± 0.1251	1.474 ± 0.0952	1.673 ± 0.1517[b]	1.720 ± 0.1883*
Relative	43.4 ± 2.8	45.6 ± 4.2	43.6 ± 2.8	46.5 ± 2.6*	48.8 ± 2.8*
Lung					
Absolute	0.183 ± 0.0255[b]	0.192 ± 0.0319	0.165 ± 0.0151	0.197 ± 0.0411	0.189 ± 0.0335
Relative	5.1 ± 0.5[b]	5.5 ± 0.9	4.9 ± 0.5	5.5 ± 1.1	5.4 ± 0.9
R. Testis					
Absolute	0.118 ± 0.0102	0.113 ± 0.0045	0.109 ± 0.0081*	0.109 ± 0.0080*	0.109 ± 0.0068*
Relative	3.3 ± 0.2	3.2 ± 0.1	3.2 ± 0.2	3.0 ± 0.3*	3.1 ± 0.2
Thymus					
Absolute	0.031 ± 0.0041[b]	0.033 ± 0.0042[b]	0.030 ± 0.0037	0.038 ± 0.0044*	0.035 ± 0.0039
Relative	0.9 ± 0.1[b]	0.9 ± 0.1[b]	0.9 ± 0.1	1.1 ± 0.1*	1.0 ± 0.1*
Female					
n	10	10	10	10	10
Necropsy body wt	28.4	26.9	27.1	27.5	28.3
Heart					
Absolute	0.113 ± 0.0082	0.116 ± 0.0070	0.116 ± 0.0107	0.120 ± 0.0050[b]	0.129 ± 0.0088*
Relative	4.0 ± 0.5	4.3 ± 0.3*	4.3 ± 0.4	4.4 ± 0.4*[b]	4.6 ± 0.3*
R. Kidney					
Absolute	0.174 ± 0.0135	0.174 ± 0.0126	0.173 ± 0.0142	0.184 ± 0.0178	0.190 ± 0.0149*
Relative	6.1 ± 0.5	6.5 ± 0.5	6.5 ± 0.9	6.7 ±0.5	6.7 ± 0.5*
Liver					
Absolute	1.195 ± 0.1407	1.164 ± 0.1096	1.181 ± 0.1392	1.381 ± 0.1695*	1.402 ± 0.1122*
Relative	42.0 ± 3.4	43.3 ± 3.0	43.7 ± 2.9	50.2 ± 4.7*	49.6 ± 3.0*
Lung					
Absolute	0.177 ± 0.0275	0.180 ± 0.0408	0.190 ± 0.0333	0.192 ± 0.0342[b]	0.208 ± 0.0444[b]
Relative	6.3 ± 1.0	6.7 ± 1.4	7.0 ± 1.0	7.0 ± 1.1[b]	7.3 ± 1.6[b]
Thymus					
Absolute	0.042 ± 0.0073	0.041 ± 0.0048	0.042 ± 0.0046[b]	0.039 ± 0.0068	0.040 ± 0.0078
Relative	1.5 ± 0.2	1.5 ± 0.2	1.5 ± 0.2[b]	1.4 ± 0.2	1.4 ± 0.3

TABLE B1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 13-Week Toxicity Study of AZT and Pyrazinamide

	400 + 1,000	0 + 1,500	200 + 1,500	400 + 1,500
Male				
n	10	10	10	10
Necropsy body wt	34.0*	35.3	34.6	35.0
Heart				
Absolute	0.150 ± 0.0094	0.151 ± 0.0110	0.146 ± 0.0117	0.154 ± 0.0135
Relative	4.4 ± 0.3*	4.3 ± 0.3	4.2 ± 0.2	4.4 ± 0.3*
R. Kidney				
Absolute	0.284 ± 0.0207	0.292 ± 0.0239	0.278 ± 0.0155	0.293 ± 0.0258
Relative	8.4 ± 0.5	8.3 ± 0.5	8.0 ± 0.5	8.4 ± 0.7
Liver				
Absolute	1.660 ± 0.1327	1.716 ± 0.1365*	1.666 ± 0.1188	1.681 ± 0.1314
Relative	48.9 ± 2.5*	48.7 ± 2.2*	48.2 ± 3.2*	48.1 ± 2.6*
Lung				
Absolute	0.188 ± 0.0210	0.194 ± 0.0276	0.181 ± 0.0137	0.179 ± 0.0136[b]
Relative	5.6 ± 0.6	5.5 ± 0.7	5.2 ± 0.4	5.1 ± 0.5[b]
R. Testis				
Absolute	0.101 ± 0.0080*[b]	0.100 ± 0.0102*	0.098 ± 0.0096*	0.094 ± 0.0081*
Relative	3.0 ± 0.2*[b]	2.8 ± 0.2*	2.8 ± 0.2*	2.7 ± 0.2*
Thymus				
Absolute	0.032 ± 0.0036	0.035 ± 0.0048[b]	0.037 ± 0.0055*	0.032 ± 0.0035
Relative	0.9 ± 0.1	1.0 ± 0.1*[b]	1.1 ± 0.2*	0.9 ± 0.1
Female				
n	10	10	10	9
Necropsy body wt	27.6	29.8	28.9	28.0
Heart				
Absolute	0.128 ± 0.0114*	0.122 ± 0.0155	0.125 ± 0.0118*	0.125 ± 0.0093*[c]
Relative	4.6 ± 0.4*	4.1 ± 0.4	4.3 ± 0.3*	4.5 ± 0.3*[c]
R. Kidney				
Absolute	0.196 ± 0.0217*	0.196 ± 0.0165*	0.203 ± 0.0231*	0.199 ± 0.0125*[c]
Relative	7.1 ± 0.8*	6.6 ± 0.4	7.1 ± 0.9*	7.1 ± 0.4*[c]
Liver				
Absolute	1.432 ± 0.1750*	1.509 ± 0.2121*	1.563 ± 0.1152*[b]	1.426 ± 0.1211*[c]
Relative	51.9 ± 5.5*	50.6 ± 3.4*	55.1 ± 5.6*[b]	50.9 ± 2.7*[c]
Lung				
Absolute	0.180 ± 0.0262	0.191 ± 0.0411[b]	0.200 ± 0.0422	0.181 ± 0.0135[d]
Relative	6.5 ± 1.0	6.4 ± 1.5[b]	6.9 ± 1.4	6.6 ± 0.6[d]
Thymus				
Absolute	0.042 ± 0.0065	0.041 ± 0.0054	0.042 ± 0.0065	0.045 ± 0.0085[d]
Relative	1.5 ± 0.2	1.4 ± 0.2	1.5 ± 0.3	1.6 ± 0.3[d]

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or Dunnett's test
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
[b] n=9
[c] n=8
[d] n=7

APPENDIX C
REPRODUCTIVE TISSUE EVALUATIONS

TABLE C1
Summary of Reproductive Tissue Evaluations for Male B6C3F$_1$ Mice in the 13-Week Toxicity Study of AZT and Pyrazinamide

Left Caudal Weight (g)

Pyrazinamide (mg/kg/day)	AZT (mg/kg/day)		
	0	200	400
0	0.0171 ± 0.0003 (10)[a]	0.0176 ± 0.0007 (10)	0.0168 ± 0.0007 (10)
1,000	0.0170 ± 0.0003 (10)	0.0171 ± 0.0004 (10)	0.0167 ± 0.0004 (10)
15,000	0.0155 ± 0.0006 (10)	0.0167 ± 0.0005 (10)	0.0159 ± 0.0004 (10)

Note: A two-way analysis of variance indicates a significant pyrazinamide effect at 1,500 mg/kg.

Left Epididymal Weight (g)

Pyrazinamide (mg/kg/day)	AZT (mg/kg/day)		
	0	200	400
0	0.0429 ± 0.0006 (10)[a]	0.0420 ± 0.0007 (10)	0.0417 ± 0.0010 (10)
1,000	0.0414 ± 0.0005 (10)	0.0415 ± 0.0007 (10)	0.0393 ± 0.0008 (10)
15,000	0.0387 ± 0.0010 (10)	0.0390 ± 0.0009 (10)	0.0379 ± 0.0008 (10)

Note: A two-way analysis of variance indicates a significant pyrazinamide effect at 1,000 and 1,500 mg/kg.

Left Testicular Weight (g)

Pyrazinamide (mg/kg/day)	AZT (mg/kg/day)		
	0	200	400
0	0.1169 ± 0.0020 (10)[a]	0.1129 ± 0.0021 (10)	0.1077 ± 0.0022 (10)
1,000	0.1073 ± 0.0013 (10)	0.1054 ± 0.0030 (10)	0.0964 ± 0.0026 (10)
15,000	0.0977 ± 0.0027 (10)	0.0963 ± 0.0024 (10)	0.0916 ± 0.0023 (10)

Note: A two-way analysis of variance indicates a significant AZT effect at 400 mg/kg and a significant pyrazinamide effect at 1,000 and 1,500 mg. There was no AZT/pyrazinamide interaction effect.

Epididymal Sperm Motility (%)

Pyrazinamide (mg/kg/day)	AZT (mg/kg/day)		
	0	200	400
0	80.26 ± 2.41 (10)[a]	66.04 ± 2.74 (9)[b]	56.61 ± 6.00 (10)
1,000	73.15 ± 2.50 (10)	49.40 ± 8.98 (10)	69.80 ± 5.95 (10)
15,000	73.81 ± 6.31 (10)	66.63 ± 4.27 (10)	52.82 ± 9.31 (10)

Note: Results of a two-way analysis of variance indicate that the dose response to AZT differs across doses of pyrazinamide (P=0.048), including a significant pyrazinamide interaction.

[a] Mean ± SE; number of animals providing the data is indicated in parentheses.
[b] For 1 out of 10 animals in the 200 + 0 mg/kg group, sperm motility counts were performed approximately 1.5 hours after death.

TABLE C1
Summary of Reproductive Tissue Evaluations for Male B6C3F$_1$ Mice in the 13-Week Toxicity Study of AZT and Pyrazinamide

Epididymal Sperm Density ($\times 10^6$)

Pyrazinamide (mg/kg/day)	AZT (mg/kg/day)		
	0	200	400
0	1,213.02 ± 61.70 (10)[a]	1,337.90 ± 101.54 (10)	1,331.52 ± 103.40 (10)
1,000	1,248.23 ± 119.50 (10)	1,380.79 ± 102.12 (10)	1,328.51 ± 107.12 (10)
15,000	1,336.62 ± 103.68 (10)	1,293.78 ± 124.50 (10)	1,265.77 ± 55.73 (10)

Spermatid Heads per Testis ($\times 10^7$)[b]

Pyrazinamide (mg/kg/day)	AZT (mg/kg/day)		
	0	200	400
0	1.67 ± 0.05 (10)[a]	1.51 ± 0.05 (10)	1.62 ± 0.04 (10)
1,000	1.56 ± 0.05 (10)	1.58 ± 0.05 (10)	1.40 ± 0.08 (10)
15,000	1.42 ± 0.06 (10)	1.46 ± 0.06 (10)	1.36 ± 0.08 (10)

Note: A two-way analysis of variance indicates a significant pyrazinamide effect at 1,500 mg/kg.

Spermatid Heads per Gram of Testis ($\times 10^7$)[c]

Pyrazinamide (mg/kg/day)	AZT (mg/kg/day)		
	0	200	400
0	14.30 ± 0.53 (10)[a]	13.37 ± 0.46 (10)	15.09 ± 0.54 (10)
1,000	14.50 ± 0.38 (10)	14.99 ± 0.45 (10)	14.45 ± 0.52 (10)
15,000	14.46 ± 0.34 (10)	15.16 ± 0.43 (10)	14.91 ± 0.82 (10)

[a] Mean ± SE; number of animals providing the data is indicated in parentheses.
[b] X(10,000)Y; (X=average number of spermatid heads per tertiary square; Y=dilution factor)
[c] Total spermatid heads per testis/testicular weight in grams

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