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EFFECTS OF EXPOSURE TO MONOMETHYLHYDRAZINE AND 1,1-DIMETHYLHYDRAZINE ON THE IMMUNOLOGICAL RESPONSIVENESS OF GUINEA PIGS

AEROSPACE MEDICAL RESEARCH LABORATORY

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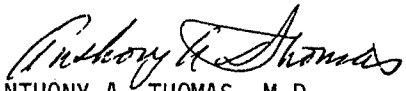

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The purpose of this project was to establish methods for evaluating the immunological effects of various toxic compounds of Air Force interest. The humoral immune response of guinea pigs was measured by determining serum antibody titers to bovine serum albumin. Cell mediated immunity was tested in vivo by evaluating the delayed hypersensitivity to tuberculin. Three compounds were tested. One, 6-mercaptopurine (6MP), was a known immunosuppressive drug. Its administration resulted in significantly depressed humoral and cellular

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responses, demonstrating that these methods could be used to evaluate immunosuppression in routine toxicity testing. Exposure to the two toxic rocket propellants monomethylhydrazine and 1,1-dimethylhydrazine resulted in a similar but less pronounced decrease in immune responsiveness.

PREFACE

This research was performed from June 1974 to September 1975 by the Pathology Branch, Toxic Hazards Division of the Aerospace Medical Research Laboratory, Aerospace Medical Division, Wright-Patterson Air Force Base, Ohio, under Project 6302, "Toxic Hazards of Propellants and Materials," Task 63020620, "Pathological Evaluation of Toxic Injury." Dr. Michael K. Pangburn was the principal investigator and MSgt Peter Veno provided valuable technical assistance.

INTRODUCTION

The purpose of this study was to evaluate the effects of monomethylhydrazine (MMH) and 1,1-dimethylhydrazine (UDMH) on the immune system of animals. MMH and UDMH are fuels used in rocket propulsion systems and consequently represent an exposure hazard to fuel manufacturing and handling personnel. Since the immune system is an important part of an animal's defense against disease, determination of the immunosuppressive potential of substances to which humans will be exposed is of the utmost toxicological concern.

Several investigators have reported on the toxicologic and pharmacologic properties of simple alkylhydrazines (Rothberg and Cope, 1956; Rinehart et al., 1960; Back and Thomas, 1963; Reynolds and Back, 1966; Clark et al., 1968). Others have examined aspects of immunosuppression by MMH (Bollag, 1963; Floersheim, 1966). The object of the present report is to examine the chemical suppression of two distinct types of immune responses which occur upon challenge by a foreign cell or antigen. These are: (1) the humoral immunity characterized by synthesis of antibodies which are released into the blood and lymphatic fluids and (2) cell-mediated immunity in which sensitized lymphocytes are produced that possess the ability to identify and destroy specific foreign cells.

MATERIALS AND METHODS

Forty male guinea pigs (300-400 g) were used in groups of 10. All the animals were bled (0.5-1.0 cc) by heart puncture using a 22 gauge, 1 inch needle previously wetted with a heparin solution. They were bled on the first day of each week during the experiment (see schedule, Table I). They received food and water ad lib. The test groups received an intraperitoneal injection of a solution of MMH (1.0 mg/kg), UDMH (10 mg/kg) both from Matheson, Coleman and Bell, 6-mercaptopurine (5 mg/kg) from Aldrich Chemical Company, or saline. The known immunosuppressive drug, 6-mercaptopurine (6MP), was used as an additional control to evaluate the methodology. All of the compounds were made up fresh each day in 0.9% saline at a concentration such that injection of 1 ml/kg yielded the correct final dosage. The 6MP solution contained 50% dimethyl sulfoxide because of the insolubility of 6MP in 0.9% saline alone. These injections were given each day for 5 consecutive days during the first and fourth week of the experiment.

Antigenic sensitization was accomplished by the intraperitoneal injection of 0.2 ml of sterile bovine serum albumin (BSA, crystallized, Miles Laboratories) in saline (50 mg/ml) on the second day of the first week. A similar injection was given on the second day of the fourth week as a booster. Sensitization to tuberculin was accomplished by a single intraperitoneal injection of 0.2 ml of Freund's Complete

TABLE I

TREATMENT SCHEDULE

Week	Day	Event
1	1	Bled, Test compounds injected.
	2	Test cmpds injected, BSA injected, Freund's Adjuvant injected.
	3	Test cmpds injected.
	4	" " "
	5	" " "
2	1	Bled.
3	1	Bled.
4	1	Bled, Test cmpds injected.
	2	Test cmpds injected, BSA booster injected, Tuberculin Skin Test Inoculation.
	3	Test cmpds injected, 24 hr Skin Test read.
	4	Test cmpds injected, 48 hr Skin Test read.
	5	Test cmpds injected.
5	1	Bled.
6	1	Bled.

TABLE II

AVERAGE SERUM ANTIBODY (ANTI-BSA) TITERS AT WEEK SIX

Treatment	Titer ± S.E.	No. of Animals
Controls	910 ± 350	8
MMH	550 ± 410	6
UDMH	740 ± 390	8
6MP	190 ± 90[a]	7

[a] $p<0.10$

Adjuvant (Calbiochem). On the second day of the fourth week, the tuberculin sensitivity was measured using a multiple puncture scarifier with Old Tuberculin (Mono-vacc Test, Lincoln Laboratories, Inc.). The skin test was administered in duplicate on the shaved hind flank of each animal. The erythema and palpable induration were measured after 24 and 48 hours.

Antibody titers to BSA were measured in serum samples taken weekly for six weeks. Serum was prepared from heparinized blood by addition of thrombin followed by clot removal. Titers were determined by passive hemagglutination using sheep red blood cells as described by Campbell et al., 1964.

RESULTS

A comparison of the treatment schedule (Table I) and weight gain graph (Figure 1) shows that both control and treated animals experienced little weight gain during the weeks of exposure (the first and fourth week). There was never, however, any significant difference between the weights of the control animals and those given the test compounds.

Serum antibody titers to bovine serum albumin (BSA) rose rapidly following the booster injection (Table II). Only the known immunosuppressive agent 6MP showed significant suppression ($p<0.1$) although all of the exposed animals showed depressed titers relative to saline controls. The large booster injection of BSA at 22 days resulted in the death of seven guinea pigs due to anaphylactic shock. Since this procedure had been tested previously

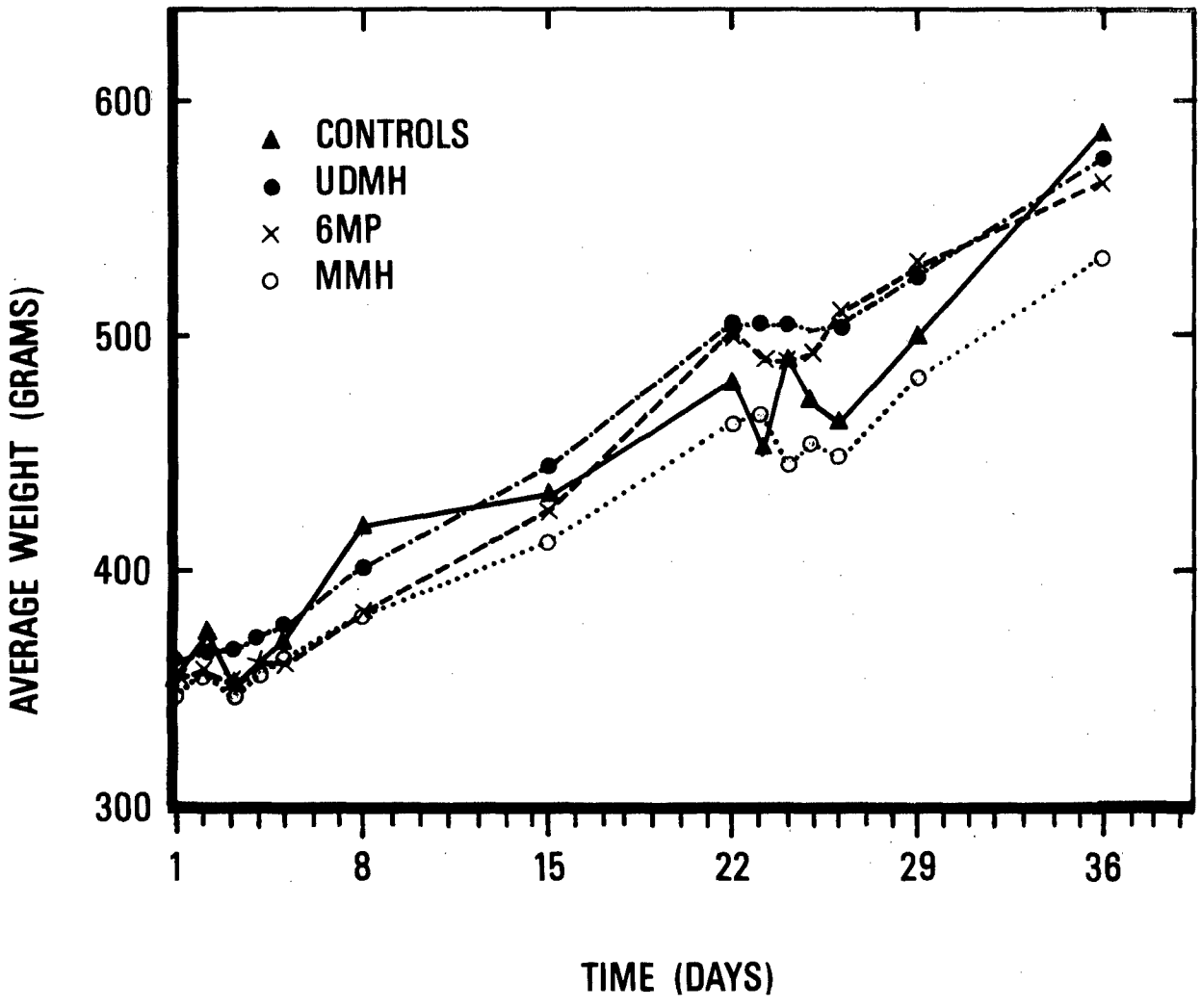

FIGURE 1. Average weights of guinea pigs vs time. The animals were weighed daily during the administration of toxic compounds and weekly between exposures.

without loss and since none of the deaths occurred in the control group, the losses might have been due to the weakened condition of the exposed animals.

Of the animals exposed to MMH, UDMH and 6MP, all showed some decrease in delayed hypersensitivity to tuberculin as judged by 24 and 48 hr skin reactions. After 24 hrs, significantly reduced induration was found only in the 6MP treated animals (Table III). However, a reduction of erythema was apparent in all of the exposed animals at this time. By 48 hrs, only the animals exposed to UDMH and 6MP showed significantly decreased induration with respect to the control animals.

TABLE III

EFFECT OF TOXIN ADMINISTRATION ON THE TUBERCULIN SKIN REACTION[a]

	After 24 hrs		After 48 hrs		
Treatment	Induration	Erythema	Induration	Erythema	No. of Animals
Controls	1.75 ± 0.40	4.55 ± 0.57	1.85 ± 0.35	3.15 ± 0.53	10
MMH	1.67 ± 0.63	2.92 ± 1.03[d]	1.83 ± 0.58	3.66 ± 0.74	6
UDMH	1.25 ± 0.38	3.06 ± 0.44[d]	0.63 ± 0.28[d]	4.12 ± 0.41	8
6MP	0.50 ± 0.20[c]	1.07 ± 0.37[b]	0.75 ± 0.25[d]	2.62 ± 0.75	7

[a]Reported as mm diameter ± standard error.

[b]$p < 0.01$ with respect to controls.

[c]$p < 0.05$ with respect to controls.

[d]$p < 0.2$ with respect to controls.

DISCUSSION

The results show that this methodology may prove useful in detecting immunosuppressive effects of toxic agents. The animals given 6-mercaptopurine, the known immunosuppressant, exhibited significantly decreased ($p < 0.05$) cellular responses to tuberculin as well as depressed antibody titers. The test compounds decreased these immune responses also, but to a lesser degree. The data suggest that both MMH and UDMH decrease immune responsiveness.

Generally it has been found that the most effective way to administer immunosuppressive drugs is concurrent with or 24 hrs prior to the antigenic challenge (Gabrielsen and Good, 1967). The initial dose is followed by regimes which vary with the drug. The procedure used here was 5 days of daily administration with the antigen given on the second day. The LD_{50}'s of MMH and UDMH have not been reported for guinea pigs but preliminary experiments indicated that the daily doses given were approximately one-tenth of the LD_{50}. The animals showed no adverse effects at this level and no deaths could be attributed directly to the toxicity of the compounds tested. However, a large number of deaths did occur following the booster injection as described in the Results section. This resulted in a decrease in the statistical reliability of the data. To correct this in future experiments the size of the booster injection should be much smaller (10% of that given here).

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