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A STUDY OF THE MECHANISM OF ACUTE TOXIC EFFECTS OF HYDRAZINE, UDMH, MMH, and SDMH

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FOREWORD

This study was initiated by Project TORES (Toxicology Research) under the sponsorship of The Advanced Research Projects Agency, DOD, and procured by the U.S. Army Chemical Center, Maryland. Upon termination of Project TORES, procurement and administrative functions were transferred to the Aerospace Medical Division, AFSC, Brooks Air Force Base, Texas. Technical monitorship was delegated to the Toxic Hazards Branch, Physiology Division, Biomedical Laboratory, 6570th Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio. The research was performed in support of Project 6302, "Toxic Hazards of Propellants and Materials," Task 630202,"Pharmacology and Biochemistry," from September 1962 to September 1963. Mr. F. W. Weir was the principal investigator and Dr. C. H. Hine technical consultant for The Hine Laboratories, Inc., San Francisco, California, and Drs. A. A. Thomas and K. C. Back were contract monitors for the 6570th Aerospace Medical Research Laboratories.

The authors are grateful to Drs. W. F. Ganong and B. L. Wise, who provided consultation and instruction on surgical procedures and experimental techniques during this investigation and to Miss M. L. Dewey, under whose administrative supervision these experiments were conducted.

The experiments reported herein were conducted according to the "Principles of Laboratory Animal Care," established by the National Society for Medical Research.

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ABSTRACT

The mechanism and site of toxic activity of hydrazine, UDMH (1,1-dimethylhydrazine), MMH (methylhydrazine), and SDMH (1,2-dimethylhydrazine) were investigated by acute toxicity studies in mice, by studying cardiovascular and autonomic effects in dogs, by noting the effect on convulsions of transection of the central nervous system at several levels in dogs, and by evaluating selected protective agents in mice. Four separate mechanisms of action are suggested by differences in pharmacologic activity. SDMH has a markedly delayed toxicity, in mice, as compared to the other hydrazines. MMH manifests its MAO inhibitory activity by intensifying the response to tyramine. Hydrazine convulsions are cortical in origin, while UDMH, SDMH and MMH convulsions originate in a pre-pontine area. Protection from convulsions and death due to hydrazine is afforded, in mice, by arginine or ornithine. Protection is provided from the effects of both UDMH and MMH, in mice, by pyridoxine and amino-oxyacetic acid. p-chlorobenzaldehyde and p-nitrobenzaldehyde protect against the effects of UDMH, but not MMH; p-dimethylaminobenzaldehyde protects against the effects of MMH but not UDMH.

PUBLICATION REVIEW

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A STUDY OF THE MECHANISM OF ACUTE TOXIC EFFECTS OF HYDRAZINE, UDMH, MMH, AND SDMH

I. INTRODUCTION

At the onset of this study a considerable body of information was available concerning the toxicology and descriptive pharmacology of hydrazine; of 1,1-dimethylhydrazine (UDMH); of 1,2 dimethylhydrazine (SDMH) and of monomethylhydrazine (MMH) (refs. 1, 2,3,4). It was possible, therefore, to adopt more specific goals for the present study. These were to relate, if possible, the hydrazines to more familiar and more thoroughly studied drugs; to establish any similarities or differences among them; to find an index of their activity more convenient for analysis than convulsions and death; to begin an analysis of the mechanism of their central nervous system toxicity; and to attempt to extend our data to the development of therapeutic agents. To accomplish this our investigation was carried out in three general areas:

Evaluation of therapeutic agents in mice;

Cardiovascular studies in dogs;

Localization of the site of convulsant action in dogs.

In addition, some effort was expended to develop a more rapid and specific method for monitoring UDMH vapor in the atmosphere.

II. ACUTE TOXICITIES OF THE HYDRAZINES IN MICE

Introduction

The acute toxicities of the hydrazines under study were re-evaluated to provide standard values for the strain selected and laboratory conditions existing and for use in further testing of the compounds. At the same time, observations were made as to the pattern of toxic response. For descriptive and classification purposes the hydrazines were compared with the reference convulsant drugs: procaine, pentylenetetrazol (Metrazol®) and strychnine.

Methods

Groups of ten male Swiss-Webster mice, 20-25 grams, were administered IP graded doses of hydrazine, MMH, UDMH and SDMH to determine the occurrence and progression of toxic signs. The final mortality was determined after 24 and 168 hours. All solutions were adjusted to pH7 and the concentration adjusted so that the appropriate dose would not exceed 1 ml per mouse.

Results

Tables 1 and 2 summarize the quantitative toxicity data deter-

mined and the typical response seen at lethal doses of the reference compounds in mice. Differences in manifestation of CNS stimulation between Metrazol, strychnine and procaine were clearly discernible. None of the hydrazines resembled the reference CNS stimulants in which convulsions were the principal toxic manifestation prior to death. Those mice which received hydrazine, MMH or UDMH would frequently not convulse so long as they were isolated singly in cages but would convulse when returned to a larger cage in groups.

SDMH was distinctive in that the 24-hour LD_{50} value was very high (940 mg/kg) but delayed deaths reduced the LD_{50} to 47 mg/kg at 168 hours. Doses are reported as the dihydrochloride salt (45.5% of SDMH dihydrochloride is base). Except at very high doses, deaths were delayed, usually about 48 hours. Some animals appeared hyperactive at lower doses, but convulsions were observed only at doses of 750 mg/kg and above. Convulsions from the high doses usually appeared between 90 and 170 minutes after administration of the compound. Violent aggression was usually exhibited during this period. Mice that suffered the delayed death showed decreased responsiveness and decreased spontaneous movement prior to death.

Table 1: LD_{50} and LD_{90} Values in Mice for the Hydrazines and Reference Compounds.

Compound	24 hours			168 hours
	LD_{50}* mg/kg	95% Confidence Limits	LD_{90}* mg/kg	LD_{50} mg/kg
Metrazol®	76	72.7 - 79.5	83	
Strychnine[1]	1.39	1.29 - 1.51	1.55	
Procaine	218	200 - 238	500	
Hydrazine	75	69 - 81	85	75
UDMH	122	111 - 134	150	122
MMH[2]	82		100	82
SDMH[3]	940	800 - 1110		47 (42-53)

1. Dosage of strychnine is reported as the sulfate salt of which approximately 78% is free base.

2. Dosage of MMH is reported as the sulfate salt (approximately 32% of MMH sulfate is free base).

3. Dosage of SDMH is reported as the dihydrochloride salt (45.5% of SDMH is free base).

* Method of Litchfield and Wilcoxon.

Table 2: Summary of the Typical Responses to Administration of
Effective Doses of the Reference Compounds and the
Hydrazines in Mice.

Compound	Dose mg/kg	Average Time to First Response	Response
Metrazol®	83	3.2 min.	Intermittent clonus, brief tonic extension. Immediate death.
Strychnine	1.55	2.9 min.	Tremor, brief clonus, tonic flexion, prolonged extension. Immediate death.
Procaine	500	2.9 min.	Incoordination, spasmodic jerking, clonus, occasion-ally tonus. Immediate death.
Hydrazine	85	16 min.	Depression, excitation, tremor, violent intermit-tent clonus, slow tonic flexion, extension - - - death.
UDMH	150	104 min.	Depression, excitation, sudden clonus, tonus - - - death.
MMH	100	129 min.	Depression, agitation, violent clonus, tonus - - - death.
SDMH	60	48-72 hrs.	Depression - - - death.

Discussion

The several hydrazines appear qualitatively different from the
primary stimulants with which they were compared. The suscepti-
bility of the "grouped" animals to convulsions is similar to that
seen after amphetamine in that convulsions could be precipitated
in non-convulsing animals by grouping. The hyperactivity of a
single animal would precipitate convulsions among all animals.
This process also increased mortality.

Only SDMH showed a marked difference between immediate and
delayed toxicities. It is possible that two different toxic
mechanisms are acting.

III. EVALUATION OF POSSIBLE PROTECTIVE AGENTS IN MICE

Introduction

The purpose of this series of experiments has been two-fold:

to screen for drugs with a protective action that might be useful therapeutically, and, by a rational selection of the compounds to be screened, to further the physiological analysis of the effects of the hydrazines.

Methods

The LD_{90} values determined above were used in all further testing. Compounds to be tested were dissolved in or diluted with water (or other suitable solvent when necessary), and the pH adjusted to 7. Concentrations of solutions were administered so that the mice received the appropriate dose in a volume of 0.01 ml/gm body weight. Unless otherwise noted below, the therapeutic agent to be tested was administered 1 hour prior to administration of the convulsant compound. In the series of aldehydes, the protective compound was administered either once, one hour before the challenge by the agent, or twice, one hour before and one hour after the challenge. Control animals were run with each protection experiment and received solvent in the same volume and frequency as "protected" animals.

The animals were observed for a period of about 3 hours to record convulsions or death, and mortalities were recorded at 24, 48 and 168 hours.

Results and Discussion

Eighteen compounds were tested against hydrazine, UDMH and MMH. Results from these tests are summarized in Table 3.

Phenobarbital

Phenobarbital (30 and 45 mg/kg) partially protected against convulsions and death from hydrazine, UDMH and MMH. When convulsions did occur, death did not necessarily follow.

Paraldehyde

The reported protective effects of paraldehyde against thiosemicarbazide (ref. 5) and its familiar hypnotic properties suggested that some protection against hydrazine, UDMH and MMH would be seen. No protection was demonstrated even though paraldehyde was given in a number of doses (200, 400 and 800 mg/kg) and at various time intervals before the challenge dose of hydrazine.

Diphenylhydantoin (Dilantin®)

In the highest dose levels used (50 mg/kg), partial protection was demonstrated against the effects of MMH. Whereas 9/10 or 10/10 of the unprotected animals died, only 3/10 or 4/10 of the Dilantin-pretreated animals died. No protection was noted from the effects of UDMH or hydrazine.

Chlorpromazine

Chlorpromazine (25, 50 and 100 mg/kg) was chosen as a representative phenothiazine tranquilizer. No protective activity was

Table 3: Summary of Evaluation of Possible Protective   Agents
           in Mice.

Protective Agent	Hydrazine	UDMH	MMH
Phenobarbital	+ +	+ +	+ +
Paraldehyde	O	O	O
Dilantin	O	O	+
Chlorpromazine	O	O	O
Mephenesin	O	O	O
Pyridoxine	O	+ +	+ +
Arginine	+ +	O	O
Ornithine	+ +	O	O
AOAA	O	+ +	+ +
Reserpine	O	O	O
DOPA	O	O	O
p-Nitrobenzaldehyde	O	+ +	O
p-Chlorobenzaldehyde	O	+ +	O
p-Dimethylaminobenzaldehyde	O	O	+ +
Benzaldehyde	O	O	O
Salicylaldehyde		O	
Piperonal	O	O	O
trans-Cinnamaldehyde	O	O	O

Legend

+ +   Indicates protection. While 8/10 to 10/10 of the animals in
      the control groups died, either 0/10 or possibly 1/10 of the
      animals died in the treated groups.

+   Indicates partial protection. While 8/10 to 10/10 of the
      control group animals died, only 3/10 or 4/10 of the treated
      animals died.

O   Indicates no protection. 8/10, 9/10 or 10/10 of the animals
      in both control and treated groups died.

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demonstrated against the effects of hydrazine, UDMH or MMH.

Mephenesin

Mephenesin (60, 120 and 240 mg/kg) afforded no protective activity. Mephenesin is a depressant of polysynaptic neural transmission within the spinal cord, and failure to protect against convulsions or death from the hydrazines suggests that the action of the hydrazines is at a level above the spinal cord. Demonstration of protection against the convulsant and lethal effects of strychnine established the effectiveness of these doses of mephenesin as a cord depressant.

Reserpine and DOPA (Dihydroxyphenylalanine)

Reserpine pretreatment was used to deplete the animal of its intrinsic catecholamines. Reserpine (0.5 mg/kg) was administered daily to three groups for one, two, and three days, respectively, before the challenge. Control animals received water in the same volume and with the same frequency. All injections were IP. The rationale for this procedure is based on analogy to cocaine effects in dogs. The convulsions and increased activity induced by cocaine are prevented by reserpine pretreatment (refs. 10,11). However, reserpine did not protect against Metrazol, hydrazine, MMH or UDMH. Reserpine itself is a convulsant, and questionable potentiation was seen.

Following the observation that some of the hydrazines block the liberation of norepinephrine by tyramine, DOPA was screened to see whether its ability to elevate catecholamine levels would be protective. DOPA (500 mg/kg) was given IP, but no protective effect was seen.

Arginine and Ornithine

Hydrazine elevates blood and CSF ammonia levels (ref. 7). Arginine and the related ornithine afford effective protection against ammonium chloride toxicity and lower blood ammonia levels (ref. 8). Arginine administered at a dose of 4 mM/kg (728 mg/kg) demonstrated almost complete protection against hydrazine, but none against UDMH or MMH. Likewise, ornithine, when administered at 8 mM/kg, demonstrated protection against hydrazine, but not UDMH or MMH.

These data suggest that hydrazine is distinctive when compared with the other hydrazines because its action may be in part at least, secondary to ammonia or ammonium toxicity.

Pyridoxine

Pyridoxine has been shown by other investigators to be effective in preventing convulsions and death against UDMH (ref. 6). In our experiments doses of 50 mg/kg afforded no protection against hydrazine but did afford complete protection from both convulsions and death due to UDMH and MMH.

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Amino-oxyacetic Acid (AOAA)

As a result of the interest in the possible role of GABA in convulsions a large number of GABA analogues were screened for their ability to elevate brain GABA levels and protect against thiosemicarbazide-induced convulsions (ref. 9).

AOAA (50 mg/kg), administered 1 hour prior to and immediately before the challenge dose, gave clear protection against UDMH and MMH but not against hydrazine.

The effectiveness of AOAA is of interest, but it must be cautioned that its originators now offer objections to interpreting its action in terms of elevation of GABA levels, a change that occurs later than the time when effectiveness begins (ref. 5).

Aldehydes

Pyridoxine has been shown to be effective in preventing the acute and chronic toxicity of isoniazid and the convulsant effect of semicarbazide, UDMH and similar compounds bearing an amine function. It was assumed that pyridoxine acts after conversion to pyridoxal because the latter is the form in which it functions as a cofactor for a variety of enzymes. The known reactivity of amines or hydrazines with aldehydes was further assumed to explain the depletion of pyridoxal. No one of the postulated specific mechanisms, for example the GABA level hypothesis, remains tenable. It is, however, still possible that pyridoxine acts because it is converted to pyridoxal.

This series of experiments asks whether pyridoxine acts because it is converted to a specific, biochemically important aldehyde or whether other aldehydes might have comparable activity. The demonstrated reactivity of UDMH with another carbonyl-bearing compound, glucose (ref. 12), further suggested that perhaps other carbonyl compounds that could react with the hydrazines to form a stable Schiff base in vivo would be of therapeutic value.

If this concept of antagonism of the hydrazines by combination with an aldehyde is valid, then those aldehydes that form the most stable Schiff bases should be most effective. Aldehydes for our tests were selected from a group which Kumler (ref. 13) has indicated would give the best yield of hydrazones when reacted with guanylhydrazine.

Seven substituted benzaldehydes were evaluated as possible therapeutic agents against UDMH, MMH and hydrazine. Results of tests with the three effective aldehydes are summarized in Table 4.

p-Nitrobenzaldehyde and p-Chlorobenzaldehyde: Neither a single administration of 300 mg/kg nor 600 mg/kg administered in two doses of 300 mg/kg each provided any significant protection against the toxic effects of either MMH or hydrazine.

Against UDMH, protection from both convulsions and death was afforded by 600 mg/kg of either aldehyde when administered in two

Table 4: The Effect of Three Aldehydes on Convulsions and Mortalities Due to Hydrazines

Compound* vs. Toxicant (mg/kg)	Group	Number Convulsed	Number of Deaths	Mean Time to Convulsions
p-Nitrobenzalde-hyde (600) vs. UDMH (175)	Control	9/10	8/10	2.8 hrs.
	Treated	0/10	0/10	-
p-Nitrobenzalde-hyde (600) vs. MMH (100)	Control	10/10	8/10	2.6 hrs.
	Treated	9/10	8/10	2.7 hrs.
p-Nitrobenzalde-hyde (600) vs. hydrazine (90)	Control	9/10	9/10	0.33 hrs.
	Treated	10/10	10/10	0.52 hrs.
p-Chlorobenzalde-hyde (600) vs. UDMH (175)	Control	10/10	9/10	3.6 hrs.
	Treated	0/10	0/10	-
p-Chlorobenzalde-hyde (600) vs. MMH (100)	Control	10/10	8/10	2.6 hrs.
	Treated	9/10	8/10	2.6 hrs.
p-Chlorobenzalde-hyde (600) vs. hydrazine (90)	Control	9/10	9/10	0.33 hrs.
	Treated	9/10	9/10	0.41 hrs.
p-Dimethylamino-benzaldehyde (600) vs. UDMH (175)	Control	10/10	9/10	1.7 hrs.
	Treated	8/10	8/10	2.4 hrs.
p-Dimethylamino-benzaldehyde (600) vs. MMH (100)	Control	7/10	5/10	1.5 hrs.
	Treated	1/10	0/10	1.2 hrs.
p-Dimethylamino-benzaldehyde (600) vs. hydrazine (90)	Control	9/10	9/10	0.4 hrs.
	Treated	10/10	10/10	0.33 hrs.

*All aldehydes given in two divided doses of 300 mg/kg each, one hour before and one hour after administration of the challenging hydrazine. Thus, the total amount of aldehyde given was 600 mg/kg.

doses of 300 mg/kg each. In each case, either 8/10 or 9/10 of the control animals convulsed and died while none of the aldehyde-treated animals either convulsed or died.

Single administration or lower doses of these aldehydes did not provide consistent protective effects; higher doses of these aldehydes produce death. We found the median lethal dose for p-nitrobenzaldehyde to be 690 mg/kg with 95% confidence limits of 657 to 725 mg/kg.

p-Dimethylaminobenzaldehyde: Neither single administration of 300 mg/kg nor double administration, 600 mg/kg total dose, of p-dimethylaminobenzaldehyde provided protection against the toxic effects of UDMH or hydrazine. When this aldehyde was tested against the effects of MMH and given in two doses of 300 mg/kg each, 0/10 mice died and 1/10 convulsed. In untreated control mice administered MMH, 7/10 convulsed and 5/10 died.

Lower doses or single administration did not provide consistent protection against MMH. The compound is toxic at higher doses. We found the median lethal dose in mice to be 575 mg/kg, with 95% confidence limits of 523 to 632 mg/kg.

When the above aldehydes were administered with an interval of 2 hours between doses, no cumulative toxic effects were noted and 600 mg/kg total dose could be administered. The animals did appear to be sedated for a period following injection, but sedation was probably not a factor in the protective action. Whereas phenobarbital sodium will protect mice against all three hydrazine compounds, p-nitrobenzaldehyde and p-chlorobenzaldehyde will protect only against UDMH and p-dimethylaminobenzaldehyde will protect only against the effects of MMH. Further evidence of specificity is the observation that the aldehydes do not show protection against Metrazol (120 mg/kg) or procaine hydrochloride (250 mg/kg).

Each of the above aldehydes was further tested using sub-cutaneous administration techniques to minimize any chance of direct interaction of the aldehyde with the toxicant in the peritoneal cavity. In each case, the results were similar to those reported above.

Other aldehydes: Benzaldehyde itself, tested at several dose levels and time intervals, afforded no protection from death due to UDMH, MMH, or hydrazine. There was some decrease in the number and frequency of convulsions, as compared to the control animals, when tested against UDMH, but there was no difference in the final mortality between the control animals and the benzaldehyde-treated ones.

Salicylaldehyde, piperonal, and trans-cinnamaldehyde were also tested against the effects of the hydrazines, at several doses and time intervals. None of these aldehydes provided any significant protection from the toxic effects of the hydrazines tested.

We wish to emphasize that the aim of these studies is not alone to suggest agents which might be applied in a therapeutic context but primarily to find protective activity to gain insight as to

mechanism of action of the convulsant agents.

That different aldehydes protect preferentially against UDMH or MMH cannot be explained at this time. The aldehydes were chosen, in part, to provide para substituents that were electropositive (nitro and chloro) and uncharged (dimethylamino). The difference in response could as easily be due to a difference in chemical or biological reactivity of the hydrazines.

IV. CARDIOVASCULAR EFFECTS OF THE HYDRAZINES ON ANESTHETIZED AND UNANESTHETIZED DOGS

Introduction

Since information regarding the toxicology and general descriptive pharmacology of the four hydrazines was available at the outset (refs. 1,2,3,4), the present study could adopt more specific goals. These were to use cardiovascular and autonomic nervous system effects of the hydrazines to relate, if possible, the hydrazines to more familiar and more thoroughly studied drugs, to establish any similarities or differences among them, and most important, to find some peripheral index of their activity more convenient for analysis than convulsions.

Methods

Anesthetized dogs: Male, mongrel dogs were anesthetized with sodium pentobarbital, 30 mg/kg. Blood pressure was recorded from a femoral artery through a polyethylene catheter connected to a Statham transducer. The electroencephalogram was recorded between needle electrodes placed in the frontal and occipital regions of the scalp. Standard electrocardiographic leads were used, and respiration was recorded with a pneumotachygraph attached to an endotracheal tube. All measurements were recorded on a polygraph (Grass). Drug injections were made into an indwelling cannula in the femoral vein.

Conscious dogs: Male, mongrel dogs were anesthetized with a minimal dose (25 mg/kg IV) of sodium thiopental. Cannulae were placed in the femoral artery and vein for the recording of blood pressure and the injection of drugs. The area around the incision was infiltrated with procaine and the animal allowed to awaken. An animal handler remained with the animal throughout the experiment.

Results

A. Effect of the Hydrazines on the Response to Tyramine

Tyramine is the classic substrate for the assay of monamine oxidase (MAO), and the prolongation and intensification of the tyramine effect is an accepted evidence of MAO inhibition. Many hydrazines and hydrazides are MAO inhibitors, and an early goal of our experiments was to demonstrate or exclude such an effect.

Moreover, tyramine exerts its sympathomimetic effect entirely by the liberation of norepinephrine, which almost certainly has a role within the brain as well as its established peripheral effect. Challenge doses of tyramine 25 or 50 mcg/kg IV were therefore given at intervals after the injection of the hydrazines and the amount and duration of the blood pressure increase measured.

MMH, 50 mg/kg, given to two anesthetized dogs increased the pressor effect of tyramine 35% and doubled the duration of the response. The effect appeared in 30 minutes and persisted for 2 hours. This result is consistent with the demonstration by biochemical studies that MMH is a monamine oxidase inhibitor (ref. 14). In this experiment, as in those described below, the response to epinephrine was unaltered.

Hydrazine, 50 mg/kg IV, given to one anesthetized dog blocked the response to tyramine for a period lasting from 30 to 300 minutes after injection. At 360 minutes the response had returned almost to normal.

SDMH, 100 mg/kg, given to one conscious dog reduced the tyramine response by 50% for a similar period.

UDMH, 50 and 75 mg/kg, given in three trials reduced or blocked the response to tyramine.

Cocaine is the only other drug known to us that is able, in certain doses, to block the tyramine release of norepinephrine. Since this is a central stimulant with effects grossly similar to the hydrazines and since norepinephrine has been suggested as an inhibitory factor within the central nervous system, the area of amine function deserves further study.

B. Effect of the Hydrazines on Blood Pressure and Pulse Rate

A further separation of the hydrazines is based on their effects on blood pressure and heart rate. These changes were studied not only in intact, conscious and anesthetized dogs but also after decortication, decerebration and spinal cord section, and after efferent autonomic pathways had been blocked by hexamethonium. A separation of centrally and peripherally originated influences is therefore possible.

In describing changes in blood pressure and pulse, the following distinctions or definitions of phases in the sequence of effects are made:

(a) The first or immediate effect is transient and outlasts the actual injection time only a few minutes. This phase is of minor interest.

(b) Following the immediate phase and persisting until convulsions appear is the period presumed to be of greatest

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interest because the vascular effects develop concurrently with the acute toxic effects. This period is described below.

(c) During convulsions the central stimulation, and, even more important, the asphyxia lead to signs of epinephrine release.

(d) Following the convulsive phase is the terminal period characterized by vasomotor collapse or "post-convulsive depression".

MMH. MMH sulfate was administered to four anesthetized animals in the following milligram per kilogram doses: 25 (1 trial), 50 (2 trials), and 100 (1 trial).

In all cases, administration of the drug caused a minor fall in blood pressure accompanied by cardiac slowing. Systolic pressure decreased first (approximately 20 minutes after injection), and then diastolic pressure (after about 90 minutes). Recovery was still incomplete after 4 hours. Two conscious dogs were given 25 and 50 mg/kg with comparable effects.

After autonomic blockade with hexamethonium in two animals, the fall in blood pressure usually seen after 50 mg/kg of MMH is replaced by a pronounced (25-30 mm) rise in systolic and diastolic pressure. Rate is not changed by MMH after hexamethonium. Hexamethonium was given in an initial dose of 2 mg/kg supplemented hourly by 1 mg/kg.

SDMH. SDMH-dihydrochloride given to three anesthetized dogs in doses of 75, 100 and 200 mg/kg decreased systolic and diastolic pressures as much as 40 mm Hg.

The decrease in blood pressure was accompanied by marked cardiac slowing. At 50 mg/kg, pulse pressure decreased but systolic pressure was maintained and the rate slowed. In one conscious dog given 100 mg/kg a similar effect on blood pressure was seen, but pulse rate increased.

The appearance and recovery of the cardiovascular effects parallel the change in tyramine reactivity described above.

After hexamethonium, SDMH (1 trial at 100 mg/kg) was given without cardiovascular effect.

Hydrazine. Hydrazine, given to two anesthetized dogs in doses of 50 mg/kg and one dog in a dose of 100 mg/kg, caused a rise in diastolic and, later, systolic pressure accompanied by cardiac slowing.

In a single conscious animal given successive doses of 5, 10 and 15 mg/kg at time 0, 30 and 45 minutes, the blood pressure also increased, but the rise was accompanied by an increase in pulse

rate. The blood pressure change persisted after hexamethonium but the cardiac slowing was not seen in one dog given 50 mg/kg of hydrazine.

UDMH. UDMH given to anesthetized dogs in doses of 75 mg/kg (2 trials) caused an increase in systolic pressure and an increase in cardiac rate. We have not described the immediate, transient change in blood pressure that accompanies the injection.

Two animals were given UDMH (50 and 75 mg/kg) after autonomic efferent pathways were blocked with hexamethonium. In these animals, systolic pressure did not change but diastolic pressure rose slightly (15-20 mm) even though pulse rate now slowed from 150 to 120.

The many trials on decorticate, decerebrate, and cord sectioned animals detailed below also included measurements of pulse and blood pressure. These dogs provide corroboration of the above observations in that the effects seen on the intact dogs described above were seen in an exaggerated degree.

Discussion

The four hydrazines are apparently not a homogeneous group. MMH is an amine oxidase inhibitor whereas the others can block the release of norepinephrine by tyramine.

The pressor effect of hydrazine is peripheral in origin whereas the blood pressure effect of the others (pressor in the case of UDMH; depressor after MMH and SDMH) is central in origin.

The cardiac slowing induced by MMH, SDMH, and hydrazine may safely be ascribed to medullary (vagal) stimulation since changes in rate are not seen after hexamethonium.

V. LOCALIZATION OF THE SITE OF CONVULSANT ACTION

Introduction

The intent of these experiments was to determine the lowest portion of the central nervous system initiating the convulsions caused by the various hydrazines. It has been our plan to test response to the hydrazines of animals that have had transection of the neuraxis at various levels.

Methods

Male mongrel dogs were anesthetized with sodium thiopental and prepared for the recording of blood pressure, using a Statham transducer attached to a cannula in the femoral artery; of respiration, via an endotracheal tube and pneumotachygraph; of the electrocardiogram, using standard leads; and of body temperature, using a thermister probe in the rectum. All drugs and solutions were administered via an indwelling catheter in the femoral vein. Whole blood and, less regularly, saline were infused during the

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operation to replace lost body fluids, and, if needed, after section to maintain blood pressure.

In the animals that were decorticated or decerebrated, a midline skin incision was made in the scalp with an electrosurgical knife, the temporalis muscles were stripped from the bone, using rongeurs, and an extensive craniotomy was performed.

In the animals that were decorticated, each cerebral hemisphere was rapidly removed by blunt dissection and gentle suction. The plane of deep dissection passed immediately superior to the corpus callosum and rostral to the basal ganglia. Blood vessels were occluded with clips and electrocautery. The mid-brain and diencephalon, optic nerves and chiasm, and the pituitary were left intact.

In the animals that were decerebrated, the midbrain was transected and the diencephalon and pituitary were removed. In some animals, the plane of section passed dorsally between the superior colliculi and the thalmus, and ventrally just superior to the red nuclei (high sections). In others (low sections), the plane of section passed through the inferior colliculus and superior to the border of the pons.

In each case the vacated space in the cranial cavity was packed with Gelfoam and cotton sponges soaked in saline, and the muscles and skin closed with clips and sutures.

At the end of each experiment, the remaining portion of the brain was carefully removed, fixed in formalin and examined after hardening. The exact level of transection, as well as the symmetry of section, presence or absence of hemorrhage and any areas of possible local damage were recorded for each animal. These results of the post mortem examination were then correlated with the results of the experiments, i.e. the presence, absence, or significant modification of convulsive activity.

In cord sectioned animals, incision was made in the midline of the dorsal aspect of the neck. Muscles were retracted, exposing the dorsal and lateral processes. Rongeurs were used to remove bone and expose the cord between last cervical and first thoracic vertebrae. The cord was ligated (extra-durally) at two points and cut between the ties. The level of the spinal cord section was verified at post-mortem examination.

In all cases, the animals were permitted to recover for four hours after the last administration of sodium thiopental. Convulsant doses of the compounds to be tested which had been adjusted to pH7 were then injected IV and the animals were observed until death. Blood pressure, respiration, electrocardiogram and body temperature were monitored continuously, and body temperature was maintained with external heat when necessary.

Results

The results of these studies are summarized in Table 5. For purposes of clarity these results are presented separately for each

Table 5:   Effect of Transection of the Neuraxis on the Convul-
sant Activity of the Hydrazines

Convulsant Agent	Level of Transection	Number of Dogs Tested	Effects
UDMH	Decortication	6	5 dogs-convulsions typ- ical for UDMH 1 dog -no convulsions
	High-level Decerebration	2	Convulsions typical for UDMH
	Standard Decerebration	10	6 dogs-typical convul- sions 4 dogs-no convulsions
	Low-level Decerebration	2	No convulsions
	Cervical Cord Preparation	6	No convulsions caudal to the shoulder region
Hydrazine	Decortication	7	No convulsions
MMH	Decortication	5	Convulsions typical for MMH
	Decerebration	7	No convulsions
SDMH	Decortication	5	Convulsions typical for SDMH
	High-level Decerebration	5	4 dogs-somewhat subdued and modified convulsant activity 1 dog- no convulsions
	Low-level Decerebration	4	No convulsions
	Cervical Cord Preparation	4	No convulsions caudal to the shoulder region

compound and for each level of transection. Since the primary
interest of this portion of the investigation was to study the
convulsant action of the various hydrazines, these results are
presented from that point of view.

UDMH - Decortication

Administration of 100 mg/kg UDMH to four decorticate dogs
and 150 mg/kg to two decorticate dogs produced convulsant activity
like that seen in both anesthetized and conscious control dogs in-
jected with the same dose. In several of these animals, however,
the time to death was somewhat longer than in the control animals.

In the four animals injected with 100 mg/kg UDMH, tremors
developed between 90 and 120 minutes, generally followed by eme-
sis, then convulsions, first tonic, then clonic, within 2 to 3
hours. One of the animals died shortly thereafter, one was sac-
rificed, but two survived in their cages for up to 24 hours.

One of two animals injected with 150 mg/kg developed tremors
within 45 minutes, then tonic and clonic convulsions after 60
minutes, continuing to 120 minutes. Immediately after this time
respiration became increar ngly more irregular and shallow until
death occurred after 150 minutes. The second animal injected
with 150 mg/kg was atypical. This dog failed to show significant
change in nervous system activity during a 5-hour observation
except for a period of restlessness about 2.5 hours after injec-
tion of the UDMH. The animal died overnight. This animal, how-
ever, had inadvertently become somewhat hypothermic during the
observation period.

UDMH - Decerebration, High and Low Level

Ten animals were decerebrated and injected with 150 mg/kg
UDMH. In every case, blood pressure, cardiac rate and respira-
tion were within the normal range before injection. Body temper-
ature was supported when necessary with a heating pad.

Six of the ten animals demonstrated basically unchanged toxic
responses; they went through periods of tonic, then clonic convul-
sions, and died, usually within 4 hours. However, four of the ten
animals did not convulse. There was some evidence of central
stimulation (random movements, twitching of limbs, mild tonic
jerks, and slight tremors) in three of the four animals $1\frac{1}{2}$ to 2
hours after injection of the UDMH, but one animal demonstrated no
observable CNS changes, was returned to its cage and died over-
night.

Generally the apparent cause of death in the animals which
did not convulse was respiratory arrest, or, if the respiration
was supported artificially, progressive hypotension.

When the preserved brain stems of the ten decerebrated ani-
mals were sectioned, it was found that the animals which did con-
vulse had more midbrain tissue remaining, i.e. the level of
transection was slightly higher.

On the basis of this finding, two animals were intentionally

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decerebrated at a low level, and two at a high level. The two animals with the high level transections convulsed normally, while the two animals transected at a low level did not. As in the other animals, the blood pressure, respiration and temperature were within normal limits before the administration of the convulsant compound. There was some activity, such as random movements and twitching of limbs, between 90 and 180 minutes after administration of the UDMH, but no convulsions. One animal died 180 minutes after injection of the UDMH, but the other was returned to his cage after four hours of observation, and died overnight.

Thus, there was a total of 8 dogs with a "high decerebration", all of which convulsed, and 6 dogs with a "low decerebration", none of which convulsed.

UDMH - Cervical Cord Section

The spinal cords of six dogs were transected at the level of C_7-T_1, as described above. After the usual 4-hour recovery period, the animals were injected with 150 mg/kg UDMH. One animal demonstrated no CNS stimulation at all, and died about 85 minutes after administration of the UDMH. In five of the six dogs, essentially normal convulsant activity was observed in the head and shoulder region, starting 60 to 120 minutes after administration of the UDMH. Four of these animals showed no spontaneous activity caudal to the shoulder region except for a few isolated tremors in the hind legs. In one dog, about 100 minutes after administration of the UDMH, there was a series of subdued tonic extensions in the hindquarters. Four of the dogs died between 80 and 185 minutes. One animal was observed for 4 hours, returned to his cage and died overnight.

All animals exhibited an essentially normal patellar reflex when tested 30 minutes after transection, and about every 15 minutes thereafter until the end of the experiment.

Hydrazine - Decortication

Seven dogs were decorticated in the manner described and after the 4-hour recovery period, were injected IV with 100 mg/kg hydrazine.

In every case, prior to injection, blood pressure, cardiac rate, respiration, and body temperature were relatively normal. Immediately after administration, in all animals, there was a period of hyperpnea which lasted for 15 to 30 minutes. There were no other observable CNS changes seen in any of the animals, and they died between 60 and 240 minutes after injection of the hydrazine. Immediate cause of death appeared to be cardiovascular failure.

Two sham-operated control animals subjected to a craniotomy and prepared in the same way except for the actual removal of the cerebral cortex demonstrated tonic-clonic convulsions starting about 45 minutes after administration of 100 mg/kg hydrazine and died within 100 minutes.

MMH - Decortication

Five animals decorticated in the manner described, and injected with a solution of MMH-sulfate, 100 mg/kg, demonstrated toxic effects similar to animals which had received the same dose but had not been decorticated. Typical effects included moderate hyperpnea immediately after injection of the drugs, followed by tremors, tonic extension and tonic, then clonic convulsions within 60 minutes. Death occurred between 60 and 150 minutes after administration of the compound. The immediate cause of death in these animals appeared to be severe hypotension.

MMH - Decerebration

Seven animals were subjected to low decerebration and injected intravenously with a solution of MMH-sulfate, 100 mg/kg. In all of the animals there was a short period of hyperpnea following injection of the drug. One dog showed no other central stimulation and died after 190 minutes. In the other six animals, there was some evidence of central stimulation starting 30 to 60 minutes after administration of the MMH. Typical effects included emesis, some body tremors, fasciculation of muscle groups in the hindquarters, and with two dogs, a short period of tonic extension. There were no convulsions in any of these animals. These animals died between 75 and 325 minutes after administration of the MMH. The apparent cause of death was respiratory failure, or if the respiration was supported artificially, progressive hypotension.

SDMH - Decortication

Administration of 500 mg/kg SDMH-dihydrochloride to five decorticate dogs produced convulsant activity similar to that seen in conscious control dogs injected with the same dose.

The effects noted in these animals fell into three distinct categories:

(a) Quiet period.

Except for a very transient decrease in blood pressure immediately after injection of the solution of SDMH, there were no remarkable changes in blood pressure, heart rate, respiration or spontaneous activity during a period of between 75 and 150 minutes after administration.

(b) Excited period.

The end of the quiet period was marked by the development of fine tremors in the legs followed by coarse tremors, body movements, and tonic, then clonic convulsions interrupted by short rest intervals. This increased CNS activity lasted anywhere from 20 to 150 minutes.

(c) Depressed period.

The increased CNS activity stopped abruptly and was replaced by a period of depression during which spontaneous activity ceased, blood pressure began to decrease, respira-

tion became labored, and in 3/5 of the animals voluntary respiration stopped. Artificial respiration was applied in these cases.

The blood pressure continued to fall in four of the five dogs, and they died between 180 and 360 minutes after administration of the SDMH.

The fifth animal remained quiet, with a decreased but stable blood pressure, for 270 minutes, at which time he was returned to his cage. He subsequently died overnight.

SDMH - High-level Decerebration

Five animals were decerebrated with the plane of section passing dorsally between the superior colliculi and the thalmus, and ventrally just superior to the red nuclei. In every case blood pressure, heart rate and respiration were within normal limits before injection of the SDMH.

As with decorticate animals, there was a quiet period after administration of the SDMH which lasted approximately 120 minutes.

In 4/5 of the animals, at the end of the quiet period tremors developed, followed by mild tonic extension with opisthotonos. In three animals there were several short intervals of subdued tonic-clonic convulsions. Generalized depression followed in these animals. Voluntary respiration stopped,and breathing was supported with a respirator. The blood pressure decreased to zero between 150 and 175 minutes after injection of the SDMH.

The fourth animal was returned to his cage 430 minutes after injection and died overnight.

One dog of this series demonstrated no increased CNS activity during a 240-minute observation period and was returned to his cage where he died overnight.

SDMH - Low-level Decerebration

Four dogs were decerebrated with the plane of section passing through the inferior colliculus and superior to the border of the pons.

In every case, blood pressure, heart rate and respiration were within normal limits before injection. After injection, in each of the four dogs, there was a transient decrease in blood pressure and increase in respiration which lasted about 5 minutes. There were no other remarkable changes during the period between 60 and 90 minutes after injection. After this, there developed a marked increase in respiratory activity lasting for 10 to 30 minutes. This was followed by complete cessation of voluntary respiratory activity. Respiration was then supported with a pump. All four of these animals died between 120 and 240 minutes after administration of the SDMH. The apparent immediate cause of death was a decrease in the blood pressure to zero. There were no convulsions noted in any of these dogs as a result of the injection of the SDMH.

SDMH - C7-T1 Cord Section

The cervical cords of four dogs were transected at the anatomical level of C7-T1. The exact level of transection was verified at post-mortem examination.

After injection all of these animals demonstrated convulsant activity, similar to that described for the decorticate animals, in the head and shoulder region. This activity started between 90 and 150 minutes after injection.

None of these animals showed any effects of increased CNS activity caudal to the shoulder region except for a few isolated tremors in the hind legs.

One of the four dogs died 140 minutes after injection. The other animals were returned to their cages and died between 18 and 24 hours after administration of the SDMH.

Each of these dogs exhibited a patellar reflex approximately equal to the control reflex when tested 30 minutes after transection and during the observation period.

Discussion

These results indicate that a small area in the midbrain is essential for the occurrence of convulsions in dogs poisoned with UDMH, MMH and SDMH. Other investigators have reported that convulsions occur in the midbrain animal, but are absent in the low decerebrate animal when picrotoxin or Metrazol is injected (ref. 15). For various reasons, we thought that the structure in the midbrain that is involved is the reticular formation, and that the convulsions spread from this structure to the cortex and the spinal cord.

Hydrazine, on the other hand, fails to cause convulsions in the absence of the cerebral cortex alone. It is, therefore, unique among the hydrazines, and one of only two or three compounds the convulsant action of which is known to be abolished by decortication.

VI. COMPARISON AND SUMMARY OF THE PHARMACOLOGICAL ACTION OF THE HYDRAZINES

At the beginning of our study of the hydrazines, it seemed likely that these four hydrazines (hydrazine, UDMH, MMH and SDMH) would be somewhat alike in their activity and furthermore could be related, in some ways at least, to the more widely studied hydrazine derivatives such as phenylhydrazine or semicarbazide. As data accumulated, however, it became evident that there were at least three and perhaps even four separate problems, and none of them showed direct correlation to the more widely studied compounds. The evidence that separates the activities of the members of this related series of hydrazines has been presented above, but the distinctions are summarized in Table 6, and are discussed below. For purposes of clarity each compound is discussed in its order.

Table 6: Comparison of Pharmacological Action of the Hydrazines

Test	Hydrazine	UDMH	SDMH	MMH
MAO Inhibition	O	O	O	+
Tyramine Block	+	+	+	O
Arginine Protection	+	O		O
AOAA Protection	O	+		+
Pyridoxine Protection	O	+		+
p-Nitrobenzaldehyde Protection	O	+		O
p-Chlorobenzaldehyde Protection	O	+		O
p-Dimethylamino-benzaldehyde Protection	O	O		+
Convulsions Blocked Decortication	+	O	O	O
Decerebration (Low-level)		+	+	+
Cord Section		+	+	
Time to Death	Immediate*	Immediate*	Delayed**	Immediate*

Legend:
+   Positive
O   Negative
*   Within 24 hours
** Not within 24 hours

Hydrazine. On the basis of our experiments, the administration of hydrazine definitely blocks the normal tyramine response for a considerable time-interval after administration of the hydrazine. We use this evidence to suggest that hydrazine produces little or no MAO inhibition.

Significant differences can be seen between the activity of hydrazine and at least two of its derivatives as a result of the protection studies in mice. Arginine, and the closely related ornithine, produce clear-cut protection from death and convulsions against hydrazine, but have absolutely no protective activity against UDMH and MMH.

The transection experiments demonstrate another area of dif-

ference in that decortication clearly blocks convulsions due to hydrazine but not its derivatives.

UDMH. Although the administration of UDMH does not reduce the response to tyramine to the degree demonstrated by hydrazine and SDMH, the fact that UDMH does not potentiate the effect of tyramine suggests lack of inhibition of MAO in vivo.

Amino-oxyacetic acid and Pyridoxine demonstrate complete protection from the effects of UDMH in mice. These compounds did not exhibit any protective activity against the effects of hydrazine. Certain substituted benzaldehydes further differentiate the activity of UDMH from that of MMH. p-Nitrobenzaldehyde and p-chlorobenzaldehyde provide protection from convulsions and death due to UDMH in mice. These compounds are not effective against the toxic effects of MMH.

Decortication or "high-level" decerebration do not block the convulsive activity of UDMH; "low-level" decerebration and transection of the cervical cord do block the activity of UDMH.

SDMH. As in the case of hydrazine, the normal response to tyramine is reduced or blocked for several hours after the administration of SDMH, indicating little or no MAO inhibition.

Unlike the other hydrazines, SDMH produces a delayed pattern of deaths, at least in rodents. When administered in high doses, central stimulation is evident, but depression is the predominant toxic sign in the case of the dose levels that do not produce death for several days.

"Low-level" decerebration and transection of the cervical cord in dogs block the convulsant activity of high doses of SDMH.

MMH. MMH shows a marked potentiation of tyramine response. This information and the results of in vitro studies of Horita and McGrath (ref. 14) indicate that MMH definitely produces MAO inhibition.

As in the case of UDMH, complete protection against convulsions and death from MMH was demonstrated in mice with amino-oxyacetic acid and Pyridoxine. Unlike UDMH, however, the toxic activity of MMH in mice was blocked with effective doses of p-dimethylaminobenzaldehyde.

"Low-level" decerebration effectively blocks the convulsions due to MMH in dogs.

Discussion

The evidence presented in this section clearly suggests that these compounds cannot be investigated as a group. It is reasonable to assume that we are dealing with at least two separate groups of compounds, and probably four completely separate mechanisms of activity.

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VII: DETERMINATION OF UDMH BY GAS LIQUID CHROMATOGRAPHY

Introduction

Although we had assured ourselves that the published methods for analysis of the hydrazines could be duplicated and that existing chemical methods were satisfactory insofar as their accuracy in the ranges of necessary applications was concerned, the methods for the hydrazines were all non-specific and time-consuming. It seemed desirable, therefore, to develop more rapid and specific methods for monitoring exposure chamber air. Our immediate goal was for a method for UDMH.

Because of the specificity and sensitivity of GLC methods and based on our previous experience with other organic compounds, this seemed to offer considerable advantage over other procedures.

Instrumentation

After some preliminary experiments with different columns and using available equipment in the laboratory, we developed the following procedure: The GLC apparatus was a Hi-Fi gas chromatograph, made by Wilkens Instrument & Research, Inc., Walnut Creek, California, equipped with a hydrogen flame ionization detector. The particular model was A-600 B. To this was attached a Leeds & Northrup Speedomax H Recorder with 0-1mV capacity. The column was 1/8" outside diameter, .093" inside diameter, 5' in length, made of stainless steel tubing and packed with chromosorb W, 60/80 mesh solid support coated with Carbowax 400 to 20% by weight. Oven temperatures were established at 110°C, plus or minus 1°, and the injector temperatures at 160°, plus or minus 10°. The inlet pressure was 14 lbs. per square inch of nitrogen and 12 lbs. per square inch of hydrogen. The machine settings were at output sensitivity of 1, input impedance 10^{9} ohms, and attenuation of X 32, i.e. 1/32 of the full recorder scale of 1 millivolt. The recorder chart speed was set at 0.5 inches per minute.

Preparation of the Standard Curve

UDMH air standards, of 500, 1000 and 1500 ppm, were prepared in gas bottles by delivering 5, 10 and 15 μl of UDMH through a micropipette and allowing about 1 hour for equilibration. Glass beads in the bottles provided mixing action as the bottle was periodically shaken. The UDMH peak height or area resulting from each injection of UDMH air standard was measured with a 10 cm rule graduated to mm. The calibration curve was prepared by plotting micrograms injected against UDMH peak responses. On repeated trials variations in peak height was less than the equivalent of 0.1 μg UDMH or 40 ppm at the 1000 ppm concentration for an injection of 1 ml UDMH air standard sample.

UDMH was dissolved in water or carbon disulfide for use as a second standard. This practice was discontinued when interactions with the solvent were noted. Reaction with water was evidenced by the diminution of the UDMH peak and appearance of new peaks with time. The effect of carbon disulfide was evidenced by the formation of a yellow reaction product, presumably sulfur from oxidation of carbon disulfide.

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Application of Procedure to Air Analysis

No special sample preparation or handling is required for application of the GLC procedure to an analysis. A sample of the air to be analyzed is collected in a 10 ml syringe equipped with a two-way stopcock. A Hamilton syringe is loaded from this sampling syringe by slight positive pressure on the collecting syringe and negative pressure on the plunger of the Hamilton gas-tight syringe, 5 ml capacity (#1005 Hamilton Company, Inc., Whittier, California), the needle of the former being inserted into the barrel of the sampling syringe. One ml of the air sample is injected through the rubber septum of the inlet system of the gas chromatograph. The amount of UDMH present is determined by measuring height or area at the appropriate elution time, comparison being made with the previously established standard curve. The mcg/ml of sample may be converted to parts per million at 25°C and 760 mm of mercury barometric pressure according to standard conversion tables. For UDMH, which has a molecular weight of 60.10, 1 mg/L would equal 408 parts per million, and 1 part per million would equal 0.0025554 mg/L. Therefore, 2.55 mcg/ml would equal 1000 parts per million of UDMH.

Comparison of GLC and Chemical Method

In order to check on the accuracy of the method, a comparison was made with the results of simultaneously performed chemical analyses of an air sample prepared dynamically in a 215 liter exposure chamber. Quantitation by the chemical method was made by Feinsilver's modification of the Jamieson method (ref. 16), which consists of sample collection of air passing through a scrubber containing 25 ml of HCl:HOH(25:1). The UDMH is quantitated by titration with potassium iodate and the oxidation reduction stoichiometric calculation. The potassium iodate is standardized against 0.100 N potassium dichromate. The chemical method for a single determination of a sample collected from 17.5 liters of air during the interval of repeated sampling gave a result of 983 parts per million on a nominal concentration of 1000 parts per million. The values obtained in six samples drawn during this time and monitored by the GLC technique described above ranged from 980 to 1020. The results are depicted in Table 7. Additional checks between the two methods on three different occasions show that the chemical method gives results which average slightly less than those obtained by the GLC method and less than the nominal concentration (Table 8). The high degree of accuracy and sensitivity of the GLC method, in addition to the ease of determination and the rapidity with which results can be obtained all commend this procedure over the chemical methods for monitoring UDMH concentrations in the air, especially as it relates to animal exposure chambers. The sensitivity of the method may be increased to quantitate 5 ppm of UDMH by simple adjustment of the attenuation on the gas chromatograph and increase in the size of sample injected. This has not been attempted in this laboratory with UDMH, but increase of the method's sensitivity by these means is standard GLC technique.

Table 7: Determination of the Concentration[a] of UDMH in
Ambient Air at Various Time Periods

Sample Number	Time (minutes)	Concentration (ppm)
1	0	983
2	12	1020
3	20	1000
4	35	990
5	45	980
6	60	1020

[a]Nominal Concentration 1000 ppm. Concentration
as Indicated by Chemical Method 983 ppm.

Table 8: Comparison of UDMH Concentrations in Ambient Air
by GLC and Chemical Methods

Nominal Concentration	Value by[a] GLC	Value by[b] Chemical Det.	Difference[c] ppm	%
980	960 980 940	973	-13	-1.3
490	450 470 470	412	+51	+12.3
450	435 432 432	404	+29	+7.2

[a]Three spot samples.

[b]Sample collected over 15 minutes.

[c]Based on the value as determined by the chemical method.

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