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FURTHER STUDY OF THE MECHANISM OF ACUTE TOXIC EFFECTS OF 1,1-DIMETHYLHYDRAZINE, METHYLHYDRAZINE, AND 1,2-DIMETHYLHYDRAZINE

FRANCIS W. WEIR
FREDERICK H. MEYERS
ROBERT H. ARBUCKLE
JESUS H. NEMENZO

THE HINE LABORATORIES, INC.

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BIOMEDICAL LABORATORY
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FOREWORD

This research was performed under Contract AF33(657)-11756 in support of Project 6302, "Toxic Hazards of Propellants and Materials," Task 630202, "Pharmacology and Biochemistry," from September 1963 to September 1964 for the Toxic Hazards Branch, Physiology Division, Biomedical Laboratory, Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio.

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WAYNE H. McCANDLESS
Technical Director
Biomedical Laboratory

ABSTRACT

Investigations were designed to explore mechanisms of toxic action of SDMH, UDMH, and MMH. SDMH is more toxic when administered in strongly acid (less than pH 1.0) or alkaline (pH 11.0) solutions than when administered in buffered solutions. The acute toxicity to mice of unbuffered SDMH-dihydrochloride is not different from hydrochloric acid. The tenfold difference between the acute toxicity of SDMH at 24 and 168 hours for mice is not seen in rats or dogs. The degree and time course of liver damage in mice is such that it is probably responsible for the delayed deaths seen in this species. The previously reported selective protective action of various substituted benzaldehydes against UDMH and MMH in mice was not observed in rats. Prophylactic treatment with amino-oxyacetic acid provided protection to rats against the lethal effects of UDMH, but not against the effects of MMH. The mechanism and site of action of UDMH (1,1-dimethylhydrazine), MMH (methylhydrazine), and SDMH (1,2-dimethylhydrazine) were investigated. Discrete localized lesions produced in specific areas of the otherwise intact brain stem by suction or electrolytic destruction modify or abolish UDMH-induced convulsions in dogs. The area from which these convulsions arise has been localized to a ventral mid-collicular site.

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

SECTION I

INTRODUCTION

Subsequent to the first report on our investigations of mechanisms of toxic effects of hydrazine, UDMH, MMH, and SDMH (Ref. 1), further work was undertaken to clarify results of acute poisoning with SDMH, evaluate protective agents against UDMH and MMH, and localize site of action of the hydrazines. The results of these extended experiments are contained in this report together with recommendations for intensive effort on site of action studies. The techniques described offer promise of fruitful research.

SECTION II

ACUTE TOXICITY OF SDMH

The previously reported LD_{50} of SDMH-dihydrochloride (1,2-dimethylhydrazine) for mice was 940.0 mg/kg at 24 hours, but only 47.0 mg/kg at 168 hours (Ref. 1). Since other laboratories have obtained discrepant results (Ref. 2), the toxicity of SDMH was reevaluated by systematic variation of the pH of injected solutions of SDMH-dihydrochloride, and by comparison of liver damage produced by this compound. The governing role of hepatotoxicity was suggested by the widely divergent dose-response data obtained at 24 and 168 hours in mice. The possibility of species specificity indicated the need to extend the study of SDMH toxicity to rats and dogs.

1. Methods.

a. Influence of the pH of Injected Solutions on Acute Toxicity of SDMH in Mice:

(1) Groups of 10 male Swiss Webster mice (20.0-30.0 gms) were injected intraperitoneally with graded doses of SDMH-dihydrochloride unadjusted for pH, adjusted to pH 3.0, pH 7.0, or pH 11.0 with sodium hydroxide.

(2) Comparable groups of mice were injected intraperitoneally with graded doses of hydrochloric acid, unadjusted for pH.

Concentrations of all solutions were calculated so that mice received appropriate doses in a volume of 0.01 ml solution/gm body weight. LD_{50} values were computed according to the method of Litchfield and Wilcoxon (Ref. 3) for both the dihydrochloride salt and the free base.

b. Comparative Toxicity of SDMH to Rats, Mice, and Dogs:

(1) Groups of five male Long Evans rats (150.0-225.0 gms) were injected intraperitoneally with graded doses of SDMH-dihydrochloride. The compound was dissolved in distilled water and resultant solutions adjusted to pH 7.0 with sodium hydroxide. Concentrations of SDMH were calculated to provide appropriate doses in a volume of 0.01 ml solution/gm body weight.

(2) Groups of 10 male Swiss Webster mice (20.0-30.0 gms) were injected intraperitoneally with solutions of SDMH in distilled water finally adjusted to pH 7.0. The mice received injections at 0.01 ml solution/gm body weight.

(3) Male mongrel dogs (5.0-8.0 kg) were similarly treated except that they received the appropriate doses in a volume of 1.0 ml solution/kg body weight.

Groups of control animals for each species received intraperitoneal injections of distilled water in suitable volume for body weight. Deaths were recorded each 24 hours for 168 hours after injections. LD_{50} values were obtained from these death records after the method of Litchfield and Wilcoxon (Ref. 3).

c. Hepatotoxicity of SDMH:

(1) A group of 10 male Long Evans rats (150.0-225.0 gms) was injected intraperitoneally with 75% of the 168 hour LD_{50} dose of SDMH-dihydrochloride. The compound was administered in a volume of 0.01 ml/gm, adjusted to pH 7.0.

(2) Groups of 10 male Swiss Webster mice (20.0-30.0 gms) were injected with 75% of the 168 hour LD_{50} dose of SDMH adjusted to 7.0, or unadjusted for pH.

(3) A group of 10 male Swiss Webster mice was injected with hydrochloric acid.

(4) Control groups of rats and mice were injected with distilled water.

At 24, 48, 72, and 96 hours after injection, two animals from each of the six groups were killed; liver slices were removed, fixed in 10% formalin and prepared for histologic study with hematoxylin eosin.

2. Results and Discussion.

a. Influence of the pH of Injected Solutions on Acute Toxicity of SDMH in Mice: Since the dihydrochloride of SDMH liberates free hydrochloric acid upon solution, these experiments may be viewed as controlling the total influence of the acid because the available acid depends on the pH of the solution. The results were as follows:

Solution	24 Hour LD_{50} I.P. mg/kg	168 Hour LD_{50} I.P. mg/kg
Concentrated HCl* (pH ≦ 1.0)	240.0 (196.0- 295.0)	110.0 (100.0-121.0)
SDMH** Unadjusted (pH ≦ 1.0)	185.0 (174.0- 197.0)	72.0 (57.0- 90.0)
SDMH Adjusted to pH 3.0	1,380.0 (1,180.0-1,615.0)	120.0 (95.0-151.0)
SDMH Adjusted to pH 7.0	1,025.0 (933.0-1,130.0)	103.0 (81.0-131.0)
SDMH Adjusted to pH 11.0	545.0 (419.0- 709.0)	73.0 (50.0- 88.0)

* Approximately 37% HCl.
**Free base approximately 45% of doses listed.

If the above results are recalculated on the basis of the active portion, i.e. 37% of the HCl and 45% of the SDMH, the results appear as follows:

Solution	24 Hour LD_{50} I.P. mg/kg	168 Hour LD_{50} I.P. mg/kg
Concentrated HCl	89.0 (72.0-109.0)	41.0 (37.0-45.0)
SDMH Unadjusted	83.0 (78.0- 89.0)	32.0 (26.0-43.0)
SDMH Adjusted to pH 3.0	621.0 (532.0-683.0)	54.0 (43.0-68.0)
SDMH Adjusted to pH 7.0	462.0 (420.0-509.0)	46.0 (36.0-59.0)
SDMH Adjusted to pH 11.0	245.0 (185.0-390.0)	38.0 (27.0-40.0)

Detailed data appear in Tables I, II, III, and IV.

Interpretation of the above data requires consideration of the form in which SDMH is available. Whether the results are expressed as mg/kg of the base or the dihydrochloride salt, the experiment begins with the dihydrochloride which does not exist as such in solution. At least one mole of hydrochloric acid per mole of SDMH is liberated upon contact of the compound with distilled water. The pK of SDMH is not found in the literature, but reasoning from knowledge of the properties of hydrazine, we may assume that the second mole of hydrochloric acid is also dissociated. Thus, unneutralized solutions of SDMH-dihydrochloride could exhibit toxic effects due to the action of the acid. The above data suggest that such is the case. Inspection of the recalculated mortality data for HCl and unadjusted SDMH-dihydrochloride shows that there is little or no difference in the lethal effects at either the 24 hour or 168 hour intervals. When the acid level is controlled by addition of sodium hydroxide to change the pH to 3.0, 7.0, or 11.0, the toxicity of SDMH at the 24 hour interval is remarkably reduced. However, the toxicity at the 168 hour interval is not affected much by adjustment of the pH, indicating perhaps a different mechanism of action in the delayed deaths. Therefore, the 24 hour toxicity of unbuffered solution of SDMH does appear to be largely due to the free hydrochloric acid which it contains.

b. Comparative Toxicity of SDMH to Rats, Mice, and Dogs: The immediate and delayed toxic action of SDMH was determined in three species using SDMH-dihydrochloride adjusted to pH 7.0. The following LD_{50}s for intraperitoneal injections were obtained:

	24 Hour LD_{50} I.P. mg/kg	168 Hour LD_{50} I.P. mg/kg
Mouse	1,025.0 (933.0-1,130.0)	103.0 (81.0-131.0)
Rat	660.0 (565.0- 770.0)	610.0 (535.0-695.0)
Dog	140.0 (96.0- 203.0)	118.0 (87.0-159.0)

Detailed data are presented in Tables V, VI, and VII.

From the above data it is seen that delayed toxicity, i.e., deaths occurring at about 48 hours after injection, is found in mice but not in the other two species.

c. Hepatotoxicity of SDMH:

(1) Results of Intraperitoneal Injections of Buffered Solutions of SDMH:

The livers from rats injected with 495.0 mg/kg SDMH solutions buffered to pH 7.0 showed moderate focal necrosis and infiltration by polymorphonuclear leucocytes; the degree of the changes appeared similar in all rats sacrificed at any of the time intervals. The presence of a chronologically uniform pattern of liver damage is consistent with the absence of a significant number of delayed deaths, suggesting that the liver damage is not a major causative factor in the deaths of rats.

Livers of mice sacrificed at 24 hours after injection of SDMH solutions adjusted to pH 7.0 showed marked vacuolization and granularity of the cytoplasm of the hepatic cells. Some areas of regeneration and mitotic division were noted. The greatest degree of change in mice livers was seen 48 hours after injection of the pH 7.0 buffered SDMH. In addition to the pathology seen at 24 hours, widespread areas of focal necrosis were noted. The necrosis showed no architectural pattern, and involved different sections of the central, mid-zonal, and peripheral areas of the lobules. Liver tissues of mice killed at 72 and 96 hours showed areas of regeneration and vacuolization but no necrosis. When the extent of damage to livers of mice given SDMH at pH 7.0 is compared with mortality data, correlation is found between times of death and times of greatest obvious impairment.

(2) Results of Intraperitoneal Injections of Unbuffered Solutions of SDMH:

After the injection of strongly acid SDMH solutions, mice showed more intense and persistent liver damage. Even 96 hours after injection of 77.0 mg/kg SDMH, areas of necrosis were seen throughout the lobules with predominance in the peripheries.

Rats and mice injected with distilled water showed no lesions of the livers at any time intervals. Mice injected with hydrochloric acid at 75% of the LD_{50} exhibited no liver damage.

Table I

The Toxicity of SUMH Dihydrochloride in Mice of a Solution with pH Unadjusted

Doses in mg/kg*	24 Hour Mortality	168 Hour Mortality
40.0	0/10	0/10
60.0	0/10	4/10
80.0	0/10	6/10
120.0	0/10	9/10
140.0	1/10	10/10
160.0	1/10	10/10
180.0	3/10	10/10
200.0	6/10	10/10
200.0	8/10	10/10
400.0	10/10	10/10
LD_{50}	185.0 mg/kg (174.0-197.0)**	72.0 mg/kg (57.5-90.0)**

* Free Base 45% of doses listed
** 95% Confidence Limits: Method of Litchfield and Wilcoxon

Table II

The Toxicity of SDMH Dihydrochloride in Mice of a Solution Adjusted to pH 3.0

Doses in mg/kg*	24 Hour Mortality	168 Hour Mortality
50.0	0/10	0/10
70.0	0/10	1/10
90.0	0/10	0/10
100.0	0/10	5/10
110.0	0/10	4/10
300.0	0/10	9/10
500.0	0/10	10/10
700.0	0/10	10/10
700.0	0/10	10/10
800.0	0/10	8/10
900.0	1/10	10/10
1,000.0	0/10	10/10
1,100.0	1/10	10/10
1,400.0	6/10	10/10
1,700.0	7/10	10/10
2,000.0	8/10	10/10
LD_{50}	1,380.0 mg/kg	120.0 mg/kg

* Free Base 45% of doses listed

Table III

The Toxicity of SD[illegible]H Dihydrochloride in Mice of a Solution Adjusted to pH 11.0

Doses in mg/kg*	24 Hour Mortality	168 Hour Mortality
20.0	0/10	0/10
50.0	0/10	2/10
70.0	0/10	3/10
90.0	0/10	8/10
100.0	0/10	8/10
110.0	0/10	10/10
300.0	2/10	10/10
500.0	3/10	10/10
700.0	3/10	10/10
700.0	9/10	10/10
800.0	8/10	10/10
900.0	8/10	10/10
1,000.0	8/10	10/10
1,100.0	10/10	10/10
LD_{50}	545.0 mg/kg (419.0-709.0)**	83.0 mg/kg (60.8-87.5)**

* Free Base 45% of doses listed

** 95% Confidence Limits: Method of Litchfield and Wilcoxon

Table IV

The Toxicity of Concentrated HCl* in Mice

Doses in mg/kg**	24 Hour Mortality	168 Hour Mortality
50.0	0/10	0/10
100.0	0/10	0/10
110.0	1/10	5/10
120.0	1/10	8/10
130.0	1/10	8/10
140.0	1/10	8/10
150.0	3/10	10/10
200.0	3/10	10/10
250.0	2/10	10/10
300.0	5/10	10/10
350.0	8/10	10/10
400.0	9/10	10/10
450.0	9/10	10/10

* Approximately 37% of doses listed are free HCl

Table V

The Toxicity of SDMH Dihydrochloride in Mice of a Solution Adjusted to pH 7.0

Doses in mg/kg*	24 Hour Mortality	168 Hour Mortality
10.0	0/10	0/10
30.0	0/10	0/10
50.0	0/10	1/10
90.0	0/10	2/10
110.0	0/10	4/10
800.0	3/10	10/10
900.0	1/10	10/10
1,000.0	4/10	10/10
1,100.0	6/10	10/10
1,300.0	9/10	10/10
LD_{50}	1,025.0 mg/kg (933.0-1,130.0)**	103.0 mg/kg (81.0-131.0)**

* Free Base 45% of doses listed
** 95% Confidence Limits: Method of Litchfield and Wilcoxon

Table VI

The Toxicity of SDMH Dihydrochloride in Rats of a Solution Adjusted to pH 7.0

Doses in mg/kg*	24 Hour Mortality	168 Hour Mortality
400.0	0/5	0/5
500.0	2/5	2/5
600.0	1/5	1/5
700.0	2/5	4/5
800.0	4/5	5/5
900.0	5/5	5/5
LD_{50}	660.0 mg/kg (565.0-770.0)**	610.0 mg/kg (535.0-696.0)**

* Free Base 45% of doses listed
** 95% Confidence Limits: Method of Litchfield and Wilcoxon

Table VII

The Toxicity of SDMH Dihydrochloride in Dogs of a Solution Adjusted to pH 7.0

Doses in mg/kg*	24 Hour Mortality	168 Hour Mortality
60.0	0/1	0/1
125.0	1/3	2/3
250.0	2/2	2/2
500.0	3/3	3/3
LD_{50}	140.0 mg/kg (96.5-203.0)**	118.0 mg/kg (87.5-159.0)**

* Free Base 45% of doses listed
** 95% Confidence Limits: Method of Litchfield and Wilcoxon

SECTION III

EVALUATION OF AGENTS PROTECTING AGAINST THE TOXIC EFFECTS OF UDMH AND MMH IN RATS

1. Introduction.

The purpose of this series of experiments has been twofold: To screen drugs of a protective nature which might be useful therapeutically and to further the physiological analysis of the effects of hydrazines through rational selection of the compounds to be screened.

In a previous report from this laboratory (Ref. 1) protection afforded by several agents against the toxicities of various hydrazines to mice was reported. For example, amino-oxyacetic acid administered to mice one hour prior to and immediately before the challenge dose gave clear protection against UDMH (1,1-dimethylhydrazine) and MMH (methylhydrazine), but offered no protection against hydrazine. A series of substituted benzaldehydes were evaluated as possible therapeutic agents against UDMH, MMH, and hydrazine in mice. p-Nitrobenzaldehyde and p-chlorabenzaldehyde provided protection against the toxic effects of UDMH but not against those of MMH or hydrazine. p-Dimethylaminobenzaldehyde offered protection against MMH but not against either hydrazine or UDMH.

During this contracting period the study of protective agents was extended to rats. Incidental to this work was the necessity of determining the toxicities of the hydrazines and aldehydes to our strain of rats.

2. Methods.

Groups of five male Long Evans rats (150.0-225.0 gms) were used in all experiments. Intraperitoneal injections at appropriate dose levels were given in a volume of 0.01 ml/gm body weight.

The two hydrazines were dissolved in or diluted with distilled water. The resultant solutions were adjusted to pH 7.0 with saturated sodium hydroxide and administered at LD_{90} doses (Ref. 1).

The amino-oxacetic acid (AOAA) was dissolved in distilled water and adjusted to pH 7.0. The substituted benzaldehydes were dissolved in corn oil. The candidate protective agents were administered at levels found to be effective in protecting mice (Ref. 1). AOAA was injected one hour before or immediately prior to challenge with the hydrazine.

Control animals received appropriate solvents for the protective agent in the same volume and number of doses as the treated rats.

3. Results.

Rat mortality data were analysed after the method of Litchfield and Wilcoxon. The following results show 95% confidence limits in parentheses:

	24 Hour LD_{50} I.P. mg/kg	24 Hour LD_{90} I.P. mg/kg
UDMH	101.0 (93.5-109.0)	130.0
MMH	85.0 (68.2- 82.5)	100.0
p-Nitrobenzaldehyde	545.0 (462.0-643.0)	
p-Dimethylaminobenzaldehyde	620.0 (548.0-701.0)	

The administration of AOAA either one hour pretreatment or immediately pretreatment provided protection against UDMH poisoning. None of these treated rats convulsed or died. AOAA administered either 30 or 60 minutes after UDMH injection provided no protection.

AOAA did not provide protection against MMH poisoning, as was also true in previous experiments with mice (Ref. 1). None of the aldehydes tested afforded protection against either UDMH or MMH. In every experiment all animals convulsed and died. The results are presented below in tabular form.

	Mortality (No. Dead/No. Tested)	
	UDMH	MMH**
Protective Agent	130.0 mg/kg	100.0 mg/kg
AOAA 60 minute pretreatment 50 mg/kg	0/5	4/5
AOAA immediately pretreatment 50 mg/kg	0/5	5/5
AOAA 30 minute posttreatment	5/5	-
AOAA 60 minute posttreatment	5/5	-
p-Dimethylaminobenzaldehyde 300 mg/kg*	5/5	5/5
p-Chlorobenzaldehyde 300 mg/kg*	5/5	5/5
p-Nitrobenzaldehyde*	5/5	5/5

* The animals were intended to receive 600 mg/kg of aldehyde in two divided doses of 300 mg/kg each, one hour before and one hour after the administration of the hydrazine; however, the second dose of aldehyde was never administered because the animals died within one hour of the hydrazine challenge.

**Dose of MMH is of the sulfate salt, of which 32% is free base.

SECTION IV

LOCALIZATION OF SITE OF CONVULSANT ACTION OF THE HYDRAZINES

1. Introduction.

A previous report from this laboratory described diminution or abolition of convulsions by transection of the neuraxis at various levels in dogs administered one of the hydrazines (Ref. 1). Using these classical neurophysiological techniques it was demonstrated that only the convulsions precipitated by hydrazine are blocked by decortication.

The convulsions caused by UDMH, SDMH, and MMH persist after pre-collicular decerebration but are eliminated by a lower decerebration where the plane of section is ventrally just prepontine.

In order to precisely define the site of convulsant activity, a series of experiments were designed to produce discrete, localized lesions in various portions of the otherwise intact brain stem. To accomplish this end, techniques were developed for the stereotaxic placement of electrodes in dogs for the creation of electrolytic lesions with direct current.

2. Methods.

a. Creation of Electrolytic Lesions Using Direct Current:

The equipment utilized for this work was a modification of the Hume-Ganong stereotaxic equipment (Ref. 4). The modified apparatus incorporated a spindle carrier which was adjustable in all directions by means of worm gears and was calibrated with vernier scales in each plane. The experimental animal was rigidly positioned by stainless steel plugs fitted into each external auditory canal, by a support which held the upper jaw, and by an adjustable bar which held the lower jaw. The top of the frame was lifted away, permitting complete exposure of the head.

In use, the top framework was removed, the animal positioned, and a midline incision developed down to the skull. The top framework was then replaced, and a pointer attached to a collet was positioned over the saggital suture. An x-ray cassette was placed in a holder attached to the framework a known distance from the midline, and film exposed from the accurately positioned X-ray tube.

The bony landmarks seen in the x-ray permitted selection of a point which was generally in the center of the third ventricle. The distance to position the pointer above this site was measured from the developed x-ray. After repositioning the pointer, a drill was attached, and a hole to accept a 20 gauge needle was drilled through the skull at the midline.

A 20 gauge needle was inserted to the predetermined depth, and 0.15 cc sodium acetrizoate (Urokon^{R}) injected into the third ventricle. A second X-ray was taken immediately thereafter and this film, showing the third ventricle filled, provided an accurate reference point for all further stereotaxic implantations in the subject.

Destructive electrodes were prepared from 27 gauge stainless wire insulated with teflon, except for a 3.0 mm portion at the tip. The destructive electrode was stereotaxically placed into the brain; an indifferent electrode of about 3.0 cm^2 was attached to the tongue. Further procedures were similar to those described by Rowland et al. (Ref. 5). Twenty-two dogs were prepared with lesions of varying size and configuration, using the direct current technique.

The electrical source was a 6 volt dry cell. The current was regulated by rheostats placed in series with the cathodal destructive electrode. Current and voltage were monitored using ammeters and voltmeters in the circuit.

b. Lesions Created by Suction Techniques:

Supplementary to the experiments in which brain stem lesions were produced electrolytically, a series of experiments were performed in which discrete lesions of the midbrain were created by removing tissue with controlled suction.

Male mongrel dogs, anesthetized with sodium thiopental, were prepared as described in a previous report (Ref. 1). The scalp was incised on the midline down to the calvarium. An opening approximately 2.5 cm x 5.0 cm was made in the calvarium. After ligation of blood vessels as necessary, the dura was retracted to allow access to the brain. A 2.5 mm suction tube was passed down between the cerebral hemispheres into the midbrain at the level of the superior colliculi. Gentle suction was applied and by manipulation of the apparatus, discrete areas of the midbrain were removed. The area was then packed with Gelfoam and the incisions of the dura and scalp closed with sutures. A four hour recovery period elapsed before the dog received an injection of hydrazine.

3. Results.

a. Electrolytic Lesions Created with Direct Current:

Macroscopic examination of sectioned brain tissue indicates that individual lesions created with the apparatus and techniques described are approximately 3.0 mm in diameter. To destroy an area of sufficient size to block convulsions in dogs, a rectangular pattern of lesions was created along a plane transecting the anterior colliculi and passing immediately anterior to the pons. All lesions were confined to the area ventral to the cerebral aqueduct. The only differences planned for this series of experiments were the variation in total number and position of the individual lesions.

In six of the 22 animals, there were no convulsions after UDMH. These dogs died between 45 minutes and three and one-half hours after UDMH. The apparent cause of death in these animals was respiratory arrest. When respiration was supported artificially, progressive hypotension caused death.

Nine dogs showed convulsant activity after UDMH which was markedly subdued and modified from the usual pattern of convulsions in UDMH-poisoned animals (Ref. 1). These animals showed only mild tonic extension accompanied by periods of tremor and muscle fasciculation. Death in all cases was a result of respiratory failure at one to three and one-half hours after UDMH.

Intact dogs given 150.0 mg/kg UDMH (both anesthetized and conscious) generally demonstrated predictable patterns of activity beginning with body tremors followed by emesis within one hour, then clonic convulsions, then a continuing series of tonic-clonic convulsions and death within two to three hours (Ref. 1).

Four dogs administered UDMH after receiving destructive lesions demonstrated a pattern of effects, including convulsions, similar to that described above for intact animals. The operated animals showed tremors followed by continuing tonic-clonic convulsions, then respiratory failure, and death at one to two hours 35 minutes.

Three dogs died from unexplained causes before injection of UDMH although these animals were prepared and handled in the same way as all other dogs. Death was apparently the result of progressive hypotension.

b. Lesions Created by Suction Techniques:

Twelve dogs were prepared in the manner described and injected with a convulsant dose of UDMH (150.0 mg/kg). Six dogs were prepared with suction-created lesions which completely transected the brain stem at mid-collicular level. In each of these six dogs, there were no remarkable changes 30-60 minutes after administration of the compound. At the end of this time some tremors usually developed and a five minute period of emesis followed. Subsequent to emesis, there was a gradual decrease in blood pressure, as well as rate and depth of respiration. In five of the six dogs, respiratory arrest caused death. This usually occurred two to three hours after administration of the compound. The sixth dog did not die spontaneously but was killed three and one-half hours after receiving UDMH.

Six dogs were prepared as above except that the lesions incompletely transected the brain stem, leaving structures ventral to the aqueduct intact. These subjects demonstrated a pattern of effects similar to that seen in unoperated animals injected with toxic doses of UDMH. The usual pattern of convulsant activity, including tonic-clonic convulsions preceded by emesis and tremors, was apparent in each of these animals. In all cases convulsions occurred 45-90 minutes after administration of UDMH and death followed within two hours.

4. Discussion.

This series of experiments sought to determine whether convulsions due to the hydrazines could be blocked by a less traumatic procedure than surgical ablation. We have demonstrated, at least for UDMH, that the suction technique permits blockage by transection of the brain stem without removal of rostral structures. Further, it is apparent that the area necessary to effect convul-

sions is located in the ventral structures of the midbrain. On the basis of these experiments we felt certain that convulsions due to UDMH could be blocked with even more discrete lesions produced by direct current as described above.

The data obtained from this series of experiments using electrolytic lesions do not yet permit precise localization of site of convulsant action. However, production of exact electrolytic lesions would appear to be the single most useful technique for investigation of the site of convulsant effects. Since convulsions are commonly accepted end points in so many toxicological studies, and the pattern of UDMH effects appear similar to other common convulsants, identification of the nuclei or tracts essential for the appearance of convulsions after UDMH may well have sweeping significance.

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