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Biochemical Response to 1,1-Dimethylhydrazine (UDMH)

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Owing to their present use as rocket fuels and their pharmacologic activity as central nervous system stimulants, substituted hydrazines and hydrazine derivatives have come into increasing use in recent years. The parent compound hydrazine and the methyl-substituted hydrazines are of potential hazard because of their toxicity, volatility, and explosive nature. Exposure to hydrazine vapor brings about central nervous system (CNS) effects resulting in clonic-tonic convulsions and death if the exposure is severe; in addition, hydrazine at lower levels of exposure will severely damage the liver, resulting in necrosis and fatty infiltration (Comstock *et al.*, 1954). Inhalation of 1,1-dimethylhydrazine (unsymmetrical dimethylhydrazine, UDMH), while producing similar CNS effects, is apparently lacking the severe hepatotoxic effects of hydrazine (Rinehart *et al.*, 1960).

The differences in hepatotoxic action and the exact mechanisms by which these compounds exert their CNS effects are not clear, although several recent reports have demonstrated that UDMH inhibits glutamic acid decarboxylase and γ-aminobutyric-α-ketoglutaric transaminase activities in brain tissue. This inhibition can be reversed and CNS symptoms alleviated by pyridoxine hydrochloride (Medina *et al.*, 1962; Reeves, 1961).

The present study was undertaken with two objectives in mind: to study the cytotoxic effect of UDMH on tissues and to evaluate several techniques that might be of value as sensitive indexes of tissue damage due to UDMH exposure. Serum glutamic-oxalacetic transaminase (GOT) was determined since it has been shown to be a relatively sensitive index of liver damage. Urinary amino acid excretion was measured since it may reflect liver or kidney damage or altered protein and amino acid metabolism. Brain and liver sulfhydryl levels were also studied, as they may be affected by the chemical reactivity of UDMH or by its effect on the oxidation-reduction state of the tissue. Histopathologic examination of the various tissues was also included in this study.

MATERIALS AND METHODS

UDMH used in these studies was Eastman Kodak practical grade. For intraperitoneal injection, UDMH was dissolved in 27 m*M* bicarbonate in 0.9% saline solution (Reeves, 1961).

All animals were male rats of the Sprague-Dawley strain (Rawley Farms), approximately 225–275 g in weight. The animals utilized for enzyme studies and sulfhydryl levels were maintained on an ad libitum diet of Rockland Chow. The rats used for amino acid excretion studies were fed a normal protein test diet from Nutritional

Biochemical Corporation. These rats were housed in individual metabolism cages designed for the collection of urine uncontaminated by feces. Twenty-four-hour urine samples were collected, diluted to 20 ml with distilled water, and filtered. Three drops of toluene were added to each filtrate and all samples were kept in a refrigerator for subsequent paper chromatographic studies. Urinary amino acids were determined in triplicate according to the method of Khachadurian *et al.* (1960). Each rat served as its own control, and 24-hour urine samples were collected for a 5-day period before and after UDMH injection. The urines were analyzed for their creatinine content by the Jaffe reaction. The results are also expressed as the ratio of α-amino acid nitrogen to creatinine nitrogen.

Ascending paper chromatography was carried out on 18 × 22-cm sheets of Whatman no. 1 paper. The solvent systems used were: methanol, *n*-butanol, benzene, water (2:1:1:1) and phenol saturated with buffer (6.3% sodium citrate, 3.7% sodium dihydrogen phosphate). For the detection of amino acids, the dried chromatograms were sprayed with 0.2% ninhydrin in 95% ethanol. Serum glutamic-oxalacetic transaminase (GOT) activity was determined by the method of Steinberg *et al.* (1956). These enzyme studies were done on serum samples obtained from rats 18 hours and 5–7 days after the injection of UDMH. The 18-hour time interval was chosen on the basis of previous studies which indicate that in acute liver intoxication from CCl_4 poisoning, the serum enzyme levels reach a maximum 18–24 hours after administration of the toxic compound (Cornish and Block, 1960). The 5–7 day studies were included to check for any latent toxicity that might develop.

Animals to be used for sulfhydryl determinations were stunned by a blow on the head and killed by decapitation. Tissues to be analyzed were removed at once, weighed, and homogenized immediately with 9 parts of cold 2.5% sulfosalicylic acid in an all-glass tissue grinder. Liver samples were cut into small pieces, disintegrated with 4–6 strokes, and finally given 20 strokes to complete the process. Brain tissue was given 20 strokes to disintegrate the whole organ. The homogenates were centrifuged for 15 minutes at 8000 rpm in a cold room. The supernatant fluid was analyzed according to the amperometric titration method of Benesch and Benesch (1950). The titrations were done in 4.8 *M* methanol using a supporting electrolyte 1.5 *M* in NH_4NO_3 and 7.5 *M* in NH_4OH. One milliliter of standard glutathione (0.001 *N*), 2 ml liver homogenate, or 3 ml brain homogenate was added to a mixture of 1 ml supporting electrolyte and enough aqueous methanol to give a total of 31 ml. Nitrogen was bubbled through this solution for 10 minutes, the rotating platinum electrode acting as a stirrer. The titration was carried out with 1×10^{-3} normal $AgNO_3$, using a syringe microburette. The end point was determined from a plot of current versus milliliters of $AgNO_3$ used in the titration.

RESULTS

Acute Intraperitoneal Toxicity

In order to find an appropriate working level where relatively large amounts of UDMH could be routinely injected, an acute toxicity study was carried out with the following results (Table 1). Eighty milligrams of UDMH per kilogram was the maximum intraperitoneal dose which allowed all animals to survive. At 100 mg/kg, 2 of 4 rats died and at 120 mg/kg, all rats died. The approximate intraperitoneal LD_{50}

value, calculated on the basis of these preliminary studies, is 100 mg/kg. Mild to severe convulsions were evident in the 80–120 mg/kg dose range at the times noted in Table 1.

TABLE 1

MORTALITY IN RATS FOLLOWING THE INTRAPERITONEAL INJECTION OF UDMH

Time after injection (hours)	Dosage			
	60 mg/kg	80 mg/kg	100 mg/kg	120 mg/kg
1½	NS[a] 0/4	NS 0/4	SC[b] 0/4	SC 0/4
2	NS 0/4	MC[c] 0/4	SC 0/4	SC 0/4
4	NS 0/4	MC 0/4	SC 2/4	SC 1/4
6	NS 0/4	MC 0/4	SC 2/4	SC 4/4
8	NS 0/4	MC 0/4	SC 2/4	—
Total deaths	0/4	0/4	2/4	4/4

[a] NS, no symptoms.
[b] SC, severe convulsions.
[c] MC, mild convulsions.

The growth curves of rats given a single intraperitoneal injection of 40, 60, or 80 mg/kg of UDMH on day 5 are shown in Fig. 1. Only at the 80 mg/kg dose level was an actual weight loss noticeable. This occurred only on the day following the injection. Food consumption was also decreased by approximately 30% during the 24-hour

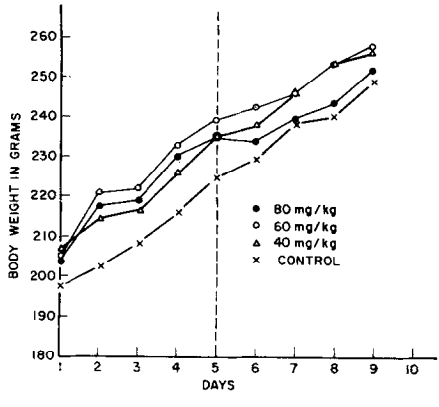

FIG. 1. Growth curves of rats receiving a single intraperitoneal dose of UDMH.

period following the injection of 80 mg/kg of UDMH. Both food consumption and weight gain were normal on subsequent days.

Serum GOT Levels

The results of these studies are shown in Table 2. Normal serum GOT levels averaged 188 ± 54 units. No significant changes occurred in serum GOT levels measured 18 hours and 5–7 days after the injection of UDMH.

Tissue Sulfhydryl Levels.

The free sulfhydryl levels in liver and brain of a number of normal rats are presented in Table 3. Normal free sulfhydryl levels averaged 217 ± 25 mg per 100 g

TABLE 2

SERUM GLUTAMIC OXALACETIC TRANSAMINASE LEVELS IN NORMAL AND UDMH-TREATED RATS (80 MG/KG)

	UDMH treated	
Control (units/ml)	After 18 hours (units/ml)	After 5/7 days (units/ml)
165	205	220
110	190	160
150	350	240
145	180	175
290	230	188
248	150	183
200	200	170
160	185	—
222	250	—
	182	—
188 ± 54.4[a]	212 ± 52.9	191 ± 24.2

[a] Average ± standard deviation.

TABLE 3

SULFHYDRYL LEVELS OF NORMAL AND UDMH-INJECTED RATS

Normal		UDMH Injected		
Glutathione (mg%)		Post Injection	Glutathione (mg%)	
Liver	Brain		Liver	Brain
215	31	2 hours	239	39
241	34		211	40
220	41		232	40
230	44		232	42
230	52		217	42
255	46		204	40
			222[a]	**40**
209	48			
251	42	18 hours	203	44
227	53		194	46
206	46		158	48
162	37		204	38
190	44		196	37
210	36		169	43
			187	**43**
182	46			
232	37			
217 ± 25[b]	41 ± 9	5–7 days	201	51
			160	38
			211	44
			210	41
			208	33
			202	42
			199	**42**

[a] Averages for UDMH-injected rats indicated by boldface figures.

[b] Average ± standard deviation.

of liver. These results are quite comparable with several values reported by Benesch and Benesch (1950). Normal brain sulfhydryl levels were considerably less than liver values and averaged 41 ± 9 mg per 100 g of brain. The sulfhydryl levels in rat tissues after the injection of UDMH are also shown in Table 3. Animals sacrificed at 2 hours, 18 hours, and 5–7 days after the injection averaged 222, 187, and 199 mg% in liver and 40, 43, and 42 mg% respectively in brain. These values are not significantly different from control levels.

Amino Acid Excretion

The analytical data on amino acid excretion are shown in Table 4. Daily creatinine nitrogen values were quite constant within each group of animals and were not affected

TABLE 4

CREATININE N AND α-AMINO ACID N EXCRETION IN RATS INJECTED WITH UDMH[a]

Doses (mg/kg)	Days	Creatinine N		α-Amino acid N		αAAN:Creatinine N	
		Control	UDMH	Control	UDMH	Control	UDMH
80	1	2.6	2.7	0.360	0.510	0.136	0.187
	2	2.6	2.4	0.300	0.240	0.114	0.100
	3	2.9	2.8	0.274	0.240	0.094	0.085
	4	3.1	3.1	0.353	0.200	0.112	0.065
	5	3.0	3.0	0.300	0.218	0.100	0.074
						0.111 ± 0.011	
60	1	2.6	2.8	0.310	0.450	0.121	0.163
	2	2.6	2.5	0.246	0.296	0.095	0.119
	3	3.0	2.7	0.242	0.248	0.081	0.092
	4	3.2	3.1	0.320	0.215	0.102	0.070
	5	3.1	3.0	0.307	0.220	0.100	0.076
						0.100 ± 0.009	
40	1	2.6	3.1	0.360	0.490	0.138	0.158
	2	2.6	2.4	0.284	0.316	0.107	0.131
	3	3.0	3.0	0.240	0.306	0.082	0.104
	4	2.7	3.0	0.344	0.233	0.129	0.078
	5	3.0	3.1	0.353	0.274	0.114	0.088
						0.114 ± 0.016	

[a] Each value in the table is the average obtained on four animals. Standard deviation is shown for nitrogen ratio of controls.

by UDMH injection. α-Amino acid nitrogen excretion, however, showed a marked increase during the first 24 hours after the injection of 80, 60, or 40 mg of UDMH per kilogram. Since creatinine nitrogen values remain relatively constant for any given animal, the α-amino acid nitrogen to creatinine nitrogen ratio represents more clearly the change in amino acid excretion over a 24-hour period. At all levels of UDMH injection, this ratio is considerably elevated during the first 24 hours after UDMH injection. This is followed by a period of decreased amino acid excretion so that 5 days after the injection of UDMH the α-amino acid N to creatinine N ratios are well below the mean for the control period. A similar group of control animals, injected only with the bicarbonate-saline solution, showed no alteration in daily amino acid excretion.

Paper chromatographic studies of the urinary amino acids did not visually reveal any major abnormalities in amino acid distribution. There appears to be an overall increase in amino acid excretion which could readily account for the elevations found by the analytical procedures.

Tissue Pathology

Eight animals were injected with 80 mg of UDMH per kilogram. Four animals were sacrificed 1 day, and four animals sacrificed 4 days, after the injection. The tissues appeared normal. Histopathologic studies of kidney, pancreas, cerebellum, cerebral cortex, choroid plexus, lung, liver, spleen, heart, and pancreas were essentially negative and comparable to the control group.

DISCUSSION

The approximate acute LD_{50} of 100 mg/kg found in this study is similar to that of 104 mg/kg reported by Back and Thomas (1963). Tremors and mild convulsions began approximately 2 hours after the injection of 80 mg/kg of UDMH. With doses of 100 mg/kg, the convulsions began approximately 1½ hours after injection and rapidly progressed to severe tonic-clonic convulsions. Two of the four animals injected with 100 mg/kg died in a convulsive state approximately 4 hours after the injection. The other two rats convulsed periodically but gradually recovered and survived this dose. At a dose level of 120 mg/kg, all four of the injected animals died in a convulsive state 4–6 hours after the injection.

As illustrated in Fig. 1, body weight changes in rats injected with 40, 60, or 80 mg UDMH per kilogram were not great. Only the rats injected with 80 mg/kg showed a slight loss of weight on the first day after injection with subsequent normal weight gains.

The normal serum GOT levels found in rats injected with relatively large single doses of UDMH suggest that liver cell permeability has not been markedly altered and that sudden extensive cell death has not occurred in the liver. This is in contrast with the findings in CCl_4 studies where serum GOT levels may be 50 times normal levels 24 hours after an acute exposure (Cornish and Block, 1960). Histopathologic examination of liver and other tissues 24 hours after the UDMH injection showed no tissue pathology. This finding is consistent with the serum enzyme studies. The inability to demonstrate direct tissue damage and the normal enzyme levels found in these studies would lead one to suggest that the acute toxic effects of UDMH are related primarily to its biochemical activity.

The free sulfhydryl levels in liver and brain of UDMH-treated rats were not significantly different from control levels. Animals sacrificed at 2 hours, 18 hours, and 5–7 days after UDMH injection maintained normal free sulfhydryl levels in brain and liver during this period. Thus it would appear that alterations in the availability of free sulfhydryl groups does not contribute to the pharmacologic action of UDMH.

Free amino acid excretion in urine was definitely increased 24 hours after rats were injected with UDMH at the 40, 60, or 80 mg/kg dose level. There appears to be a direct correlation of increasing amino acid excretion with increasing dose level of UDMH. UDMH when added to 24-hour control urines in amounts equal to the entire dose given to a single rat, does not interfere with this colorimetric method

for the determination of α-amino acids. The chromatographic studies also indicate that there is a rather general increase in overall amino acid excretion, and abnormalities related to specific amino acids are not apparent. Increased amino acid excretion, as found in these studies, could result from a number of physiological or biochemical disturbances. Tissue damage could release free amino acids into the bloodstream. However, the apparent lack of tissue damage histopathologically as well as the failure of serum GOT activity to detect liver damage suggests that the increased free amino acid excretion may be due to other factors. Massien *et al.* (1962) have recently shown that mice treated with L-glutamic acid-δ-hydrazide tend to accumulate alanine and δ-aminobutyric acid in brain, presumably due to the inhibition of enzymes involved in amino acid metabolism. Thus it is quite possible that UDMH may also interfere with amino acid metabolism not only in brain, but in other tissues as well. Interference with protein synthesis or gluconeogenesis could also result in the excretion of increased amounts of amino acids. A toxic effect on kidney resulting in partial failure to reabsorb amino acids may also be a factor. However, with the lack of evidence for structural tissue damage by histopathologic methods, it is more likely that the amino acid findings are associated with an effect of UDMH upon some more basic physiologic function or biochemical reaction.

SUMMARY

The effect of the intraperitoneal injection of UDMH on rat tissue sulfhydryl levels, serum GOT, and urinary amino acid excretion has been studied. The approximate LD_{50} in rats was found to be 100 mg/kg.

Rats given a single injection of 80 mg/kg of UDMH show no changes in liver or brain sulfhydryl levels, nor are serum GOT levels altered by this dose.

Analyses of urine samples show that rats injected with UDMH at levels of 40, 60, or 80 mg/kg excrete correspondingly increased amounts of amino acids in the urine during the subsequent 24-hour period. It appears that this is a general increase in amino acid excretion, and abnormalities associated with specific amino acids are not apparent.

Histopathologic studies do not reveal any tissue pathology as a result of this single injection of UDMH. Since neurologic symptoms are present in these animals, the present findings tend to support the evidence for a biochemical mechanism mediating the acute toxic effects of UDMH.

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