Acknowledgement—This work was supported by the Cancer Chemotherapy National Service Center, National Cancer Institute, under National Institutes of Health Contracts SA-43-ph-3784, SA-43-ph-1740, PH43-65-594, and PH43-64-51.

Medicinal Chemistry Section; Y. FULMER SHEALY
JOE D. CLAYTON
Virus Section; GLEN J. DIXON
ELIZABETH A. DULMADGE
Microbiology Section; ROBERT F. PITTILLO
Kettering-Meyer Laboratory, DALE E. HUNT
Southern Research Institute,
Birmingham, Ala., U.S.A.

REFERENCES

1. L. L. BAMBAS, in *The Chemistry of Heterocyclic Compounds*, (Ed. A. WEISSBURGER), pp. 205–11, Interscience, New York (1952).
2. A. CARR, E. SAWICKI and F. E. RAY, *J. org. Chem.* **23**, 1940 (1958).
3. V. G. PESIN, A. M. KHALETSKII and L. V. ZOLOTOVA-ZOLOTUKHINA, *Zhur. Obshchei Khim.* **31**, 3000 (1961); see English trans., *J. gen. Chem. USSR* **31**, 2798 (1961), Consultants Bureau, Inc., New York, N.Y.
4. M. CARMACK and D. SHEW have prepared 1,2,5-selenadiazole-3,4-dicarbonitrile and the corresponding diacid by a method different from that reported here; D. Shew, *Dissertation Abstracts* **20**, 1593 (1959).
5. Y. F. SHEALY and J. D. CLAYTON, *J. org. Chem.* **28**, 1491 (1963).
6. Y. F. SHEALY and J. D. CLAYTON, *J. org. Chem.* **29**, 2141 (1964).
7. H. EAGLE, *Proc. Soc. exp. Biol. Med.* **89**, 362 (1955).
8. H. EAGLE and G. E. FOLEY, *Am. J. Med.* **21**, 739 (1956).
9. Cancer Chemotherapy National Service Center, *Cancer Chemother. Rep.* **25**, 57 (1962).
10. V. D. OYAMA and H. EAGLE, *Proc. Soc. exp. Biol. Med.* **91**, 305 (1956).
11. H. EAGLE, *J. exp. Med.* **102**, 595 (1955).
12. R. F. PITTILLO, C. MONCRIEF, R. W. BROCKMAN and P. CHAMBERS, in *Antimicrobial Agents and Chemotherapy*—1964 (Ed. J. C. SYLVESTER), pp. 474–84, American Society for Microbiology, Ann Arbor, Mich.

Biochemical Pharmacology, 1966, Vol. 15, pp. 1614–1618. Pergamon Press Ltd., Printed in Great Britain.

Vitamin B_6 and the toxicity of 1,1-dimethylhydrazine*

(*Received* 23 *February* 1966; *accepted* 10 *June* 1966)

IT IS well known that 1,1-dimethylhydrazine (UDMH) inhibits certain enzymatic reactions requiring vitamin B_6 as a cofactor,[1, 2] apparently through the formation of a UDMH-pyridoxal or pyridoxal phosphate hydrazone which depletes the tissues of vitamin B_6. Other workers have demonstrated that intraperitoneally administered pyridoxine (PY) protects rats from UDMH toxicity, while pyridoxal (PAL) and pyridoxal phosphate (PALP) do not.[2, 3] When PAL and PALP were injected immediately after UDMH, convulsions and death occurred much sooner than with UDMH alone. Similar findings with PAL[4] and PALP[2] have been reported for hydrazine and a number of substituted hydrazines.

In the present study, the B_6 vitamers were injected intracerebrally into UDMH-treated rats to circumvent the blood–brain barrier. On the basis of these findings, the protective effects of intraperitoneally injected vitamers then were re-examined.

* This study was sponsored by the Commission on Environmental Hygiene of the Armed Forces Epidemiological Board, and was supported in part by the Medical Research and Development Command, Office of the Surgeon General, Department of the Army of the United States.

MATERIALS AND METHODS

Adult (250–350 g) male rats of the Sprague-Dawley (Rawley Farms) strain were used. The animals received Rockland rat and mouse chow and water *ad libitum*.

UDMH-treated animals were given an LD_{100} i.p. dose of 120 mg/kg. UDMH (Matheson, Coleman & Bell) was dissolved in 27 mM sodium bicarbonate in 0·9% saline such that the final solution contained 50 mg UDMH/ml. The three vitamers used for intracerebral injections were dissolved in 0·9% saline. It was necessary to add a few drops of dilute NaOH to achieve complete solution of high concentrations of PALP.

Intracerebral injections were carried out as follows. A small hole was drilled through the skull of anesthetized rats at a point 2 mm posterior to the coronal suture and 2 mm dorsal to the sagittal suture. A microliter syringe with a 26-gauge needle inserted to a depth of 3 mm from the skull surface was used for the injections. The complete procedure required about 10 min, and recovery was rapid. Although the largest dose of B_6 was injected in a volume of 30 μl, most volumes were 15 μl or smaller. For each of the three B_6 vitamers, a wide range of dosages was administered intracerebrally to normal rats to determine the response to the compound, the minimum lethal dose, and the dose level below which no response occurred. The work was repeated in rats previously injected with UDMH. B_6 vitamers were given intracerebrally just after the first convulsion, which occurred approximately 90 min after UDMH injection. The rats were observed for 6 hr after UDMH administration. Previous studies have shown that most deaths in UDMH-treated rats occur within this period.[5]

A wide range of intraperitoneal dosages of PAL and PALP was then examined for their ability to protect rats from UDMH-induced convulsions and death.

RESULTS

Intracerebral studies. Intracerebral doses of 3–6 mg PY or 4 mg PAL produced convulsions and death in nearly all animals, whereas smaller doses were less toxic (Table 1). PALP appeared more

TABLE 1. EFFECT OF INTRACEREBRAL INJECTION OF PYRIDOXINE (PY), PYRIDOXAL (PAL), AND PYRIDOXAL PHOSPHATE (PALP) ON NORMAL AND UDMH-TREATED* RATS

Dose of B_6 vitamer (mg)	PY		PAL		PALP	
	C†	M‡	C	M	C	M
Control rats						
6	2/2	2/2				
4	2/2	2/2	3/4	3/4		
3	2/3	2/3				
2	1/4	1/4	1/5	0/5	3/3	3/3
1	1/4	0/4	1/5	0/5	3/3	3/3
0·17					3/3	0/3
0·08					2/5	0/5
UDMH-treated rats						
6	5/5	4/5				
4	5/8	2/8	3/5	3/5		
3	4/6	1/6				
2			3/3	0/3		
1	0/2	0/2	0/3	0/3	5/6	5/6
0·50	0/4	0/4	0/11	0/11		
0·35	1/3	0/3				
0·25	2/6	1/6	0/3	0/3		
0·17					7/7	2/7
0·10	2/2	0/2			2/3	2/3
0·07					2/2	0/2
0·05	3/6	0/6	4/6	1/6	4/5	1/5
0·03					3/6	1/6
0·02					3/6	5/6

* UDMH (120 mg/kg; i.p.) 90 min before B_6 administration.
† Convulsions: number convulsing/number treated.
‡ Mortality: number dead/number treated.

toxic in normal rats than the other B_6 vitamer, since 1 mg given intracerebrally resulted in death of all animals tested. Doses ranging from 0·05 to 1 mg PY prevented death in 22 of 23 rats which had received an ordinarily lethal dose of UDMH ($P < 0·001$). Convulsions were observed, however, in UDMH-treated rats receiving more than 1 mg or less than 0·5 mg of PY.

PAL in doses ranging from 0·25 to 1·00 mg per rat prevented convulsions and fatalities in all 17 rats receiving UDMH ($P < 0·001$). Dosages of PAL greater or less than these amounts were not completely effective in preventing UDMH toxicity.

Doses of PALP ranging from 0·03 to 0·17 mg protected 18 of 23 rats from the lethal effects of UDMH ($P < 0·001$), but no dose was found to be completely effective in preventing convulsions.

Intraperitoneal studies. Doses of 100 mg/PY, PAL, or PALP per kg had no apparent effect on normal rats (Table 2). Ten of twelve rats given 25–100 mg PAL/kg 90 min after UDMH convulsed, but only three died. Rats given 5 or 10 mg PAL/kg appeared to be completely protected from the toxic effects of UDMH.

Although 5–10 mg PALP/kg partially protected against UDMH toxicity, no dosage of PALP

TABLE 2. EFFECT OF INTRAPERITONEAL INJECTION OF PYRIDOXAL (PAL), PYRIDOXAL PHOSPHATE (PALP), OR PYRIDOXINE (PY) ON NORMAL AND UDMH-TREATED RATS*

B_6 Vitamer injected	Dose (mg/kg)	Convulsions	(%)	Mortality	(%)
Control rats					
PAL	100	0/4	(0)	0/4	(0)
PALP	100	0/4	(0)	0/4	(0)
PY	100	0/4	(0)	0/4	(0)
UDMH-Treated rats					
PAL	100	4/4	(100)	1/4	(25)
	50	3/4	(75)	1/4	(25)
	25	3/4	(75)	1/4	(25)
	10	0/11	(0)	0/11	(0)
	5	0/3	(0)	0/3	(0)
	2	2/6	(33)	2/6	(33)
PALP	60	2/2	(100)	1/2	(50)
	30	6/6	(100)	2/6	(33)
	10	2/6	(33)	0/6	(0)
	5	1/6	(17)	1/6	(17)
	0·6	2/2	(100)	2/2	(100)
	30 × 2†	4/4	(100)	4/4	(100)
	5 × 2†	5/10	(50)	0/10	(0)
	2 × 2†	3/6	(50)	2/6	(33)
PY	50	0/6	(0)	0/6	(0)
	25	0/6	(0)	0/6	(0)
	10	0/6	(0)	0/6	(0)
	5	3/6	(50)	0/6	(0)
UDMH only		90/90	(100)	85/90	(94)

* See footnotes to Table 1.

† First dose of PALP given 90 min after UDMH and second dose given after an additional 45 min.

was found that would completely prevent convulsions in UDMH-treated animals. Attempts to protect UDMH-injected animals by giving two doses of 2 mg PALP/kg 45 min apart were only partially effective, and two 5 mg/kg doses prevented fatalities, but some animals convulsed.

UDMH-treated animals were completely protected by 10–50 mg PY/kg (Table 2). Three of six rats treated with 5 mg PY/kg had single convulsions.

Since appropriate doses of PY, PAL, or PALP protected against UDMH toxicity when given 1·5 hr after UDMH, the effect of various doses of each vitamer when given simultaneously with UDMH was examined (Table 3). Animals injected with UDMH and PAL (30–100 mg/kg) or PALP (50–60 mg/kg) convulsed and died within 1 hr. All rats given 10 mg PAL/kg convulsed, but there were no deaths. Of six rats treated with 10 mg PALP/kg, five convulsed and one died. Ten to 50 mg PY/kg protected against UDMH toxicity, although one rat convulsed. All UDMH rats treated with 5 mg PY/kg convulsed and two of six died.

TABLE 3. EFFECT OF SIMULTANEOUS INTRAPERITONEAL INJECTION OF PYRIDOXAL (PAL), PYRIDOXAL PHOSPHATE (PALP), OR PYRIDOXINE (PY) WITH UDMH*

B_6 Vitamer injected	Dose (mg/kg)	Convulsions†	(%)	Mortality‡	(%)
PAL	100	4/4	(100)	4/4	(100)
	50	4/4	(100)	4/4	(100)
	30	6/6	(100)	6/6	(100)
	10	6/6	(100)	0/6	(0)
	5	6/6	(100)	3/6	(50)
PALP	60	3/3	(100)	3/3	(100)
	50	4/4	(100)	4/4	(100)
	10	5/6	(83)	1/6	(17)
	5	5/6	(83)	1/6	(17)
PY	50	0/6	(0)	0/6	(0)
	25	0/6	(0)	0/6	(0)
	10	1/6	(17)	0/6	(0)
	5	6/6	(100)	2/6	(33)

* See footnotes to Table 1.

DISCUSSION AND CONCLUSIONS

This study demonstrates that appropriate intracerebral or intraperitoneal doses of PAL, PALP, and PY are effective antidotes against UDMH. The inability of previous workers[2, 4] to demonstrate a protective effect of PAL and PALP appears to be related to the dosage used. Doses above or below rather closely defined limits were not effective in protecting against UDMH toxicity. In addition, treatment was more effective when delayed until the onset of severe symptoms. Several factors may account for this finding. The excretion of UDMH begins rather rapidly,[6, 7] thus a smaller amount of UDMH would be available for the formation of toxic hydrazones[2] with injected B_6. Injection of the vitamer nearer to the time of tissue depletion of B_6 would make high levels of the coenzyme available to the cell at the most critical time. Injected PAL or PALP may be converted to UDMH-hydrazones *in situ*, leaving little coenzyme available for metabolic functions at distant sites. In contrast, the injection of PY provides a B_6 reservoir which does not form a UDMH hydrazone, thereby providing for its conversion to active coenzyme at the site of enzymatic activity.

It becomes apparent that successful treatment of UDMH toxicity with PAL or PALP presents a rather difficult problem of balance among the effects of the B_6 vitamer, the UDMH-hydrazone, and UDMH. Thus pyridoxine, which is effective over a wide dosage range, remains the vitamer of choice in treating UDMH toxicity.

Department of Industrial Health,
School of Public Health and
Institute of Industrial Health,
The University of Michigan,
Ann Arbor, Mich., U.S.A.

CAROL L. GEAKE
MARY L. BARTH
HERBERT H. CORNISH

REFERENCES

1. M. A. MEDINA, H. D. BRAYMER and J. L. REEVES, *J. Neurochem.* **9**, 307 (1962).
2. M. A. MEDINA, *J. Pharmac. exp. Ther.* **140**, 133 (1963).

3. R. D. O'Brien, M. Kirkpatrick and P. S. Miller, *Toxicol. appl. Pharmac.* **6**, 371 (1964).
4. B. Dubnick, G. A. Leeson and C. C. Scott, *Toxicol. appl. Pharmac.* **2**, 403 (1960).
5. H. H. Cornish and M. L. Barth, *Toxicol. appl. Pharmac.* **6**, 569 (1964).
6. K. C. Back, M. K. Pinkerton, A. B. Cooper and A. A. Thomas, *Toxicol. appl. Pharmac.* **5**, 401 (1963).
7. F. N. Dost, D. J. Reed and C. H. Wang, Aerospace Med. Res. Labs. Rep. No. AMRL-TR-64-111, Wright Patterson AFB (Dec. 1964).

Biochemical Pharmacology, 1966, Vol. 15, pp. 1618–1621. Pergamon Press Ltd., Printed in Great Britain.

Studies of the inhibition of lipolytic enzymes—III. The inhibition of a Tween hydrolase from rat adipose tissue *in vitro* and *in vivo* by *n*-butyl carbamic acid methyl ester, U-14641

(*Received* 5 *May* 1966; *accepted* 13 *June* 1966)

n-Butyl carbamic acid methyl ester (BCME) has been demonstrated to be a potent species-specific inhibitor of canine liver and kidney lipase.[1] The isolation and extensive purification of a Tween hydrolase from rat adipose tissue has also been reported.[2] The experiments reported herein demonstrate that BCME is an inhibitor, both *in vitro* and *in vivo*, of Tween hydrolase from rat adipose tissue.

METHODS

For the kinetic experiments, Tween hydrolase was highly purified by the procedure hitherto reported.[2] Enzyme activity *in vivo* was determined in male Sprague-Dawley rats weighing 250–300 g, and fed Purina lab chow *ad libitum*. BCME was made up in water at a concentration of 0·1 M and was administered i.v. into the tail veins. The rats were killed by decapitation, and the epididymal and perirenal fat pads were quickly excised and chilled in cold water. The water was then decanted and the pads, blotted dry and weighed, were homogenized in a chilled Waring Blendor for 1 min at top speed in 2 volumes (v/w) of cold water. The resulting homogenate was centrifuged at 5000 *g* for 10 min at 0°. The supernatant fluid was filtered through cheesecloth to remove particles of fat, and the Tween hydrolase activity was assayed as follows. To 15-ml conical centrifuge tubes was added 500 μmoles ammonium chloride buffer, pH 8·35; 0·25 ml adipose tissue extract prepared as described; 25 μmoles Tween 20 (Atlas Chemical Industries), and water to a final volume of 2 ml. Incubations were for 10 min at 38°.

In the kinetic experiments, because small volumes of highly purified enzyme (30 units*/tube) were needed, the final volume of the incubation mixture was reduced to 1 ml and 250 μmoles of buffer was used, along with varying amounts of substrate. All components were added except the substrate, and a 3-min preincubation period was employed to ensure establishment of steady-state conditions between enzyme and inhibitor. The tubes were then removed from the water bath, chilled, and the Tween substrate added, following which the tubes were again incubated for 10 min at 38°. At this time the tubes were again removed from the bath, chilled in ice slush, and the reaction stopped by addition of 7·5 ml of a solvent mixture of 4:1:0·1 isopropanol:heptane:1 N H_2SO_4 per ml incubation medium. Fatty acids were extracted and titrated by the method of Ko and Royer.† Appropriate enzyme and substrate blanks were carried in every experiment to correct for endogenous fatty acid.

RESULTS AND DISCUSSION

Under the described conditions for kinetic experiments, with 10 μmoles Tween substrate, BCME was found to inhibit the enzyme 93 per cent at a concentration of 5×10^{-3} M, 85 per cent at 5×10^{-4} M, and 56 per cent at 5×10^{-5} M.

* According to international usage, 1 unit of enzyme is that amount of enzyme required to liberate 1 μmole fatty acid/hr under specified conditions of assay.

† Unpublished.