ment on rubella virus antibodies and herpes simplex antibodies appear to differ, in that antibodies produced long after immunization are not enhanced in the herpes simplex system but are enhanced by complement in the rubella virus system. Further studies will be necessary to determine the action of complement in the enhancement of rubella virus antibody titer.

Summary. The hemadsorption-negative plaque assay technique has provided a means of quantitatively studying rubella virus neutralization. A 3-day assay for rubella antibodies has been developed which is sensitive and reproducible. The addition of fresh guinea pig serum enhanced rubella antibody titers up to 16 times those obtained in heated sera. The factor or factors responsible for the enhancement of antibody titers have the properties of complement.

The authors gratefully acknowledge the able assistance provided by Miss Marian Moore and Mrs. Marjorie Burkhardt. The helpful advice and consultation of Dr. Gordon R. Dreesman is deeply appreciated.

1. Parkman, P. D., Buescher, E. L., Artenstein, M. S., Proc. Soc. Exp. Biol. and Med., 1962, v111, 225.

2. Parkman, P. D., Buescher, E. L., Artenstein, M. S., McCown, J. M., Mundon, F. D., Druzd, A. D., J. Immunol., 1964, v93, 595.

3. Weller, T. H., Neva, F. A., Proc. Soc. Exp. Biol. and Med., 1962, v111, 215.

4. McCarthy, K., Taylor-Robinson, C. H., Pillinger, S. E., Lancet, 1963, v2, 593.

5. Leerhoy, J., Acta Path. et Microbiol. Scandinav., 1966, v67, 158.

6. Hull, R. N., Butorac, G., Am. J. Epid., 1966, v83, 509.

7. Parkman, P. D., Mundon, F. K., McCown, J. M., Buescher, E. L., J. Immunol., 1964, v93, 608.

8. Schiff, G. M., Sever, J. L., Huebner, R. J., Science, 1963, v142, 58.

9. Sever, J. L., Huebner, R. J., Fabiyi, A., Monif, G. R., Castellano, G., Cusumano, C. L., Traub, R. G., Ley, A., Gilkeson, M. R., Roberts, J. M., Proc. Soc. Exp. Biol. and Med., 1966, v122, 513.

10. Neva, F. A., Weller, T. H., J. Immunol., 1964, v93, 466.

11. Dudgeon, J. A., Butler, N. R., Plotkin, S. A., Brit. Med. J., 1964, v2, 155.

12. Plotkin, S. A., Arch. Virusforsch., 1965, v16, 423.

13. Marcus, P. L., Carver, D. H., Science, 1965, v149, 983.

14. Melnick, J. L., Ann. N. Y. Acad. Sci., 1955, v61, 754.

15. Hopps, H. E., Bernheim, B. C., Nisalak, A., Tjio, J. H., Smadel, J. E., J. Immunol., 1963, v91, 416.

16. Stoker, M., MacPherson, I., Nature, 1964, v203, 1355.

17. Rawls, W. E., Melnick, J. L., Rosenberg, H. S., Bayatpour, M., Proc. Soc. Exp. Biol. and Med., 1965, v120, 623.

18. Rawls, W. E., Melnick, J. L., J. Exp. Med., 1966, v123, 795.

19. Lennette, E. H., in Diagnostic Procedures for Viral and Rickettsial Diseases, 3rd Ed., Am. Pub. Health Assn., Inc., New York, N.Y. 1964, pp51-54.

20. Dulbecco, R., Vogt, M., Strickland, A. G. R., Virology, 1956, v2, 162.

21. Sabin, A. B., Bact. Rev., 1950, v14, 225.

22. Westaway, E. G., Virology, 1965, v26, 517.

23. Yoshino, K., Taniguchi, S., ibid., 1964, v22, 193.

24. ———, J. Immunol., 1966, v96, 196.

Received October 6, 1966. P.S.E.B.M., 1967, v124.

A Comparison of Alkylhydrazines and Their B_6-Hydrazones as Convulsant Agents.* (31693)

ARTHUR FURST AND WALDEMAR R. GUSTAVSON (Introduced by J. H. Gast)
Institute of Chemical Biology, University of San Francisco, San Francisco, Calif.

The rocket propellants 1,1-dimethylhydrazine (UDMH) and monomethylhydrazine (MMH) are powerful convulsant agents(1, 2,3,4). Data are available on the acute

* This study was supported by U. S. Air Force Contract AF 33(615)-2332. Further reproduction of this material is authorized to satisfy the needs of the United States Government.

TABLE I. Physical Constants of Hydrazones.

B_6	Hydrazine	Recrystallizing solvent	m.p.	R_f*
Pyridoxal	UDMH	EtOH-H_2O	122° dec.	.42
"	MMH	Benzene-pet ether	170° dec.	.39
Pyridoxal-5-phosphate	UDMH	H_2O	243° dec.	.26
" " "	MMH	Benzene-pet ether	Decomp only	.17

* Solvent system used: butanol-pyridine-water (2:1 saturated).

toxicity of these compounds, and on some of their pharmacological(5), biochemical(6), and pathological(7) effects.

A comparison of the convulsigenic action of these hydrazines can be made on a quantitative basis by plotting the log of dose against the time lapse between administration of the agent and a) onset of the first convulsion, or b) time of death of experimental animal. An estimate of the LD_{50} (10 day) is another criterion for comparison of activity.

Studies along these lines are presented in this report. Mice were used in this work as they exhibited a more consistent response to the convulsant agents than did rats.

The respective pyridoxal and pyridoxal-5-phosphate hydrazones† of UDMH and MMH were made and also evaluated. The inclusion of these hydrazones was suggested because some congeners of vitamin B_6, namely pyridoxol and pyridoxamine, can be used as antidotes for UDMH and MMH toxicity(2,8), as well as for other substituted hydrazines such as thiosemicarbazide (TSC)(9). The third congener, pyridoxal, seemed to exacerbate rather than alleviate the convulsigenic activity of both UDMH and MMH(4).

Materials and methods. Synthesis of hydrazones. Hydrazones were prepared by dissolving the calculated amount of pyridoxal or pyridoxal-5-phosphate in water and heating the solution to 50°C. An equimolar amount of the alkylhydrazines: UDMH (K&K Laboratories or Eastman), or MMH (Aldrich Chemical Co.) dissolved in 5 ml of water was added and the temperature kept at 50°C for an additional 1 to 2 hours. The solutions were cooled to 0°C, whereupon crystals appeared. These were separated by filtration and purified by recrystallization. Melting points are similar to those reported by Wiley (10), who prepared these compounds by a different method.

Convulsion experiments.‡ Various doses of convulsant agents were injected intraperitoneally into Swiss albino mice (average weight 22 ± 1 g). The convulsigens were dissolved or suspended in normal saline without buffering. Doses were administered in a volume of 0.01 ml per gram of body weight. Dose levels ranged from 2.5-1000 mg/kg, depending upon the convulsant agent. The agent was administered to each animal at one-minute intervals.

The animals were housed individually(3) in cylindrical cages (dimensions: 8″ diameter × 7″ height). Experiments were conducted in a quiet laboratory with subdued lighting. Care was taken to prevent a convulsing mouse or external stimuli from inducing another mouse into premature convulsions.

Five animals were tested at each dose level. The animals were under constant observation for 3 to 5 hours. The time of onset of first and subsequent convulsions and time of death were recorded to the nearest minute. Some mice, however, remained in a state of continuous seizure from the onset of the first convulsion to the time of death. After 24 hours, mortality checks were made twice daily for 10 days. For each series of convulsion experiments, 5 control mice were injected with saline.

Results. The physical constants for the synthetic hydrazones are given in Table I.

Table II presents the time lapse (lag-time) between administration of the convulsigen,

† The hydrazones will be designated at B_6-Al-UDMH, B_6-Al-5PO_4-UDMH, B_6-Al-MMH, and B_6-A1-5PO_4-MMH, respectively.

‡ In all animal work we have complied with the principles of "Laboratory Animal Care" as established by the National Society for Medical Research (N.S.M R.).

TABLE II. Effective Lag-Times of Hydrazines and Their B_6-Hydrazones.

Compound	Dose (mg/kg)	Time (min)* First convulsion	Time (min)* Death
UDMH	85	sub-convulsive	non-lethal
	125	113 ± 40	156 ± 42
	150	76 ± 19	104 ± 28
	166	63 ± 10	91 ± 16
	220	48 ± 8	77 ± 18
	420	27 ± 4	35 ± 13
	500	26 ± 3	28 ± 3
	840	18 ± 1	24 ± 12
MMH	5	sub-convulsive	non-lethal
	8	158 ± 8	non-lethal
	10	37 ± 19	175 ± 10
	20	16 ± 4	92 ± 20
	50	7 ± 2	12 ± 6
	100	5 ± 1	6 ± 2
B_6-Al-UDMH	5	29 ± 5	2 days
	10	33 ± 9	49 ± 6
	25	27 ± 6	43 ± 15
	50	23 ± 4	41 ± 5
	100	12 ± 4	27 ± 13
B_6-Al-MMH	3	sub-convulsive	non-lethal
	13	40 ± 7	62 ± 18
	25	28 ± 2	37 ± 8
	50	25 ± 3	37 ± 9
	100	15 ± 3	23 ± 6
B_6-Al-5PO_4-UDMH	6	35 ± 12	30 ± 17
	12	25 ± 10	40 ± 24
	25	24 ± 10	35 ± 12
	50	27 ± 13	38 ± 11
	100	25 ± 14	34 ± 12
B_6-Al-5PO_4-MMH	10	36 ± 9	50 ± 5
	13	28 ± 12	48 ± 18
	25	29 ± 2	39 ± 7
	50	26 ± 3	36 ± 5
	100	21 ± 4	32 ± 5

* All times are given in min ± S.D.

and onset of the first seizure, and the time of death. Table III presents the 50% mortality doses with 95% confidence intervals calculated by the Litchfield-Wilcoxon method (14). No convulsions or deaths occurred in the control animals which were injected with saline only.

Discussion. Characteristic behavior was noted after administration of either the alkylhydrazines or their hydrazones. During the preconvulsant stages the mice were hyperactive, exhibiting continuous movement about the cage (appeared to be seeking food). Just prior to onset of the seizure, the mice appeared to drift occasionally into sleep and remained in a state of mild depression, easily aroused by the smallest noise. Typically, the first grand mal seizure was preceded by smaller lighter seizures. Grand mal seizures sometimes occurred without complex prodromal. In this event, the animal which was lying or walking about the cage suddenly fell unconscious and immediately progressed into a violent clonic or tonic seizure. The usual major seizure, however, seemed to be merely an extension and intensification of the petit mal. Clonic pattern occasionally persisted, became progressively more violent, then subsided (clonic twitching). The clonic pattern frequently phased into a strong tonic form, which proceeded back through clonic seizure or progressively decreased in severity (semicomatose between seizures). Death usually came with opisthotonos and general body rigidity. Other syndromes noted were: paralysis of extremities (especially in hydrazone convulsion), a drift from mydriasis into miosis and of exophthalmos into enophthalmos, hyperventilation, piloerection, and apparent Straub tail. A few mice at the lower level recovered from the seizures.

MMH-induced convulsions were more rapid in onset and much more severe in nature than those of UDMH.

The hydrazones as a rule were more toxic than the hydrazines themselves. The differences were much greater for UDMH than for MMH. At 100 mg/kg UDMH was no longer convulsant, but the B_6-Al-UDMH and B_6-Al-5PO_4-UDMH were active at 5 mg/kg. The B_6-Al-5PO_4-MMH was only slightly more toxic than B_6-Al-MMH itself, but both hydrazones were convulsant at 5 mg/kg also. It was of interest that the lag-time prior to convulsions after administration of B_6-Al-UDMH or B_6-Al-5PO_4-UDMH did not

TABLE III. 50% Lethal Doses.

Compound	LD_{50} (mg/kg) Experimental	95% Confidence interval*
UDMH	113	103–124
MMH	15	10– 23
B_6-Al-UDMH	60	40– 90
B_6-Al-MMH	11	9– 13
B_6-Al-5PO_4-UDMH	90	45–180
B_6-Al-5PO_4-MMH	7	5– 8

* The 95% confidence interval was computed by the Litchfield-Wilcoxon method(14).

change throughout the entire dose range investigated.

These results are similar to those of O'Brien(4) who found that when UDMH and B_6-Al were administered simultaneously in rats, the LD_{50} of UDMH decreased from 102 mg/kg to 87 mg/kg. A similar experiment with MMH and B_6-Al showed practically no change in the range of LD_{50}; *i.e.*, 28 mg/kg and 27 mg/kg. It is possible that the hydrazones were formed *in vivo*.

Two of the vit. B_6 congeners, namely pyridoxol and pyridoxamine, have been shown to be somewhat effective as antidotes for seizures induced by TSC(9) as well as UDMH and MMH(2,8). The TSC inhibited some of the vit. B_6 enzymes(11), and logically it would seem that the addition of pyridoxal would overcome this inhibition.

One conclusion drawn from this work is that *pyridoxal* must not be tried as an antidote for the alkylhydrazine-induced convulsions. The administration of pyridoxal apparently aided the formation of the hydrazones, B_6-Al-UDMH, or B_6-Al-MMH, which are, as noted, more toxic. A similar finding by Dubnick(12) showed that pyridoxal exacerbated the toxicity of phenylethylhydrazine.

Our data give indirect evidence that the mechanism of action of UDMH and MMH parallels the suggestion of McCormick and Snell(13), who postulated that the toxicity of TSC was due to hydrazone formation with pyridoxal.

Summary. A comparative study was made of the toxicity and convulsion activity of 1,1-dimethylhydrazine (UDMH), monomethylhydrazine (MMH), and their pyridoxal and pyridoxal-5-phosphate hydrazones. The hydrazones of UDMH are more toxic than UDMH itself when 3 criteria are compared: a) lag-time before convulsions at various dose levels, or b) time of death, or c) LD_{50}. In the case of MMH, the convulsion times of hydrazones and free hydrazine are about equal; death appears earlier with the hydrazones. This work implies that pyridoxal exacerbates rather than alleviates the convulsant activity of UDMH and MMH, and thus should not be considered an antidote.

The authors wish to thank Mr. George Ledin, Jr. for his help in statistical analysis of the data, and Mr. Eric Gustavson for excellent laboratory assistance.

1. Back, K. C., Pinkerton, M. K., Cooper, A. B., Thomas, A. A., Toxic. Appl. Pharm., 1963, v5, 410.
2. Back, K. C., Pinkerton, M. K., Thomas, A. A., Technical Document Rep. AMRL-TDR 63-44, Wright-Patterson Air Force Base, Ohio, 1963, 1.
3. Jenney, E. H., Pfeiffer, C. C., J. Pharm. & Exp. Therap., 1958, v122, 110.
4. O'Brien, R. D., Kirpatrick, M., Miller, S. P., Toxic. Appl. Pharm., 1964, v6, 371.
5. Back, K. C., Thomas, A. A., J. Am. Ind. Hyg. Assn., 1963, v24, 23.
6. Cornish, H. H., Barth, M. L., Toxic. Appl. Pharm., 1964, v6, 568.
7. Dominguez, A. M., Amenta, J. S., Hill, C. S., Domanski, T. J., Aerospace Med., 1962, v33, 1094.
8. Reeves, J. L., Report 62-31, Brooks Air Force Base, Texas, 1961.
9. Parks, R. E., Jr., Kidder, G. W., Dewey, V. C., Proc. Soc. Exp. Biol. and Med., 1952, v79, 287.
10. Wiley, R. H., Irik, G., J. Pharm. and Med. Chem., 1962, v5, 49.
11. Killam, K. F., Bain, J. A., J. Pharm. & Exp. Therap., 1957, v119, 255.
12. Dubnick, B., Leeson, G. A., Scott, C. C., Toxic. Appl. Pharm., 1960, v2, 403.
13. McCormick, D. B., Snell, E. E., Proc. Nat. Acad. Sci., 1959, v45, 1371.
14. Litchfield, J. T., Jr., Wilcoxon, F., J. Pharm. & Exp. Therap., 1949, v96, 99.

Received July 25, 1966. P.S.E.B.M., 1967, v124.