THE ROLE OF VITAMIN B_6 IN THE TOXICITY OF HYDRAZINES*

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Within recent years hydrazine and the methyl substituted hydrazines have come into considerable use as rocket fuels. As a result of this increased production of hydrazines, the possibility of accidental human exposure is of considerable interest. Obvious concern results from the fact that hydrazine had been used as an experimental means of producing fatty liver in experimental animals for many years. On an acute toxicity basis, O'Brien and coworkers (1964) reported comparative lethal $dose_{50}$ (LD_{50}) values for hydrazine 64 mg/kg (2 mmoles/kg), monomethylhydrazine (MMH) 28 mg/kg (0.61 mmoles/kg), 1,1-dimethylhydrazine (unsymmetrical dimethylhydrazine, UDMH) 102 mg/kg (1.7 mmoles/kg), and 1,2-dimethylhydrazine (symmetrical dimethylhydrazine, SDMH) essentially nontoxic even at dosages of 500 mg/kg. The structures of these four compounds are shown in FIGURE 1, and it appears that a free amino group is essential for biological activity in this group of compounds. Barth and associates (1967) have previously reported the diuretic effect induced by UDMH, and it is interesting to note that of the four hydrazines shown in FIGURE 1, only SDMH is without diuretic effect, again emphasizing the biological activity of the free amino groups in this series of compounds. Killam and Bain reported in 1957 that thiosemicarbazide, furoyl hydrazide, and isonicotinic acid hydrazide would inhibit, *in vitro*, enzyme systems catalyzed by vitamin B_6. Thus it was suggested that the epileptiform convulsions produced by these compounds could be due to an inhibition of one or more of the enzyme systems requiring pyridoxal phosphate as a cofactor. In related findings, Reeves (1961) and Back and colleagues (1963) reported the use of pyridoxine and pyridoxamine as effective antidotes for UDMH and MMH toxicity. Similar findings on the effectiveness of pyridoxine in alleviating the convulsive symptoms of hydrazine toxicity have not been consistent. Medina (1963) reported that convulsions induced by hydrazine could be blocked by pyridoxine, but not by pyridoxal.

O'Brien and associates (1964) reported that although hydrazine can be a convulsant at high doses, at doses near the LD_{50} it is not primarily a convulsant but is a depressant. On this basis, they suggest that hydrazine probably has a mode of action different from UDMH and MMH. It is interesting to note that this suggestion correlates with the early reports that brain levels of γ-amino-butyric acid (GABA) are depressed after UDMH treatment but apparently elevated after hydrazine treatment.

Reversal of Hydrazine Toxicity by Vitamin B_6

A number of studies from this laboratory have investigated the relationship of the various hydrazines to the B_6 vitamers both *in vivo* and *in vitro*.

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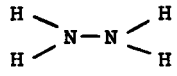
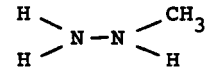
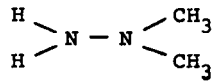


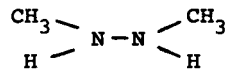
Hydrazine



Monomethylhydrazine (MMH)



1,1-Dimethylhydrazine (UDMH)



1,2-Dimethylhydrazine (SDMH)

FIGURE 1. Structure of hydrazine and methyl substituted hydrazines.

Due to the time lag between administration of the hydrazines and the appearance of convulsions, it appeared possible that the formation of metabolites might be responsible for convulsive activity of these compounds. The direct injection of 3.5 to 4.0 mg of UDMH intracerebrally brought about severe convulsive seizures in rats similar to those seen following intraperitoneal injections of UDMH. Considerably higher quantities, however, must be injected intraperitoneally (40 to 50 mg/kg) before consistent convulsive seizures appear in rats. Thus it appears that the convulsive seizures are mediated centrally. In a related series of

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.
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studies, it has been shown that intracerebral injections of the various B_6 vitamers are effective antidotes to intraperitoneal injections of UDMH (TABLE 1). This dose of UDMH, (120 mg/kg I.P.) produces convulsions in all animals and has a mortality rate of 94%. Intracerebral injections of 3 to 6 mg of pyridoxine (PY), 4 mg of pyridoxal (PAL), or 1 mg of pyridoxal phosphate (PALP) without hydrazine produced convulsions and death in nearly all rats. In animals receiving 120 mg/kg of UDMH I.P. and 3 to 4 mg of PY intracerebrally (I.C.), 11 of 14 animals survived; thus the two compounds were mutually protective. At lower doses of PY ranging from 0.05 to 3 mg, nearly all rats survived the normally lethal effect of this dose of UDMH. PAL was also an effective antidote to UDMH over a somewhat similar dosage range. PALP, however, was effective only within a relatively limited range of values. A related study of the effect of intraperitoneal injections of the B_6 vitamers on UDMH toxicity is shown in TABLE 2. Ten mg or more of PY was effective in preventing both convulsive seizures and mortality in rats receiving 120 mg/kg of UDMH I.P. PAL was effective over the relatively narrow range of 5 to 10 mg/kg. Dosages of PAL either above or below these amounts were only partially effective or ineffective. In a similar way, intra-

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)

* UDMH (120 mg/kg, I.P.) 90 minutes before B_6 administration.

† First dose of PALP given 90 minutes after UDMH; second dose 45 minutes later.

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TABLE 3

EFFECT OF B_6 VITAMERS ON HYDRAZINE-TREATED* RATS

Vitamer	Dose (mg/kg)	B_6 Route	Mortality 1 hr	Mortality 6 hr	Mortality 24 hr	% Survived
Pyridoxine	20	I.P.	5/6	6/6	6/6	0
	15	I.P.	2/6	2/6	2/6	67
	15	I.V.	0/18	0/18	3/18	83
	100	S.C.	4/6	4/6	4/6	33
	0.5 mg/rat	I.C.	0/4	0/4	0/4	100
	1.0 mg/rat	I.C.	0/4	0/4	0/4	100
Pyridoxal	10	I.P.	5/6	6/6	6/6	0
	10	I.V.	1/6	1/6	2/6	67
Pyridoxal phosphate	5	I.P.	3/6	5/6	5/6	17
	5	I.V.	3/6	3/6	5/6	17
Hydrazine	100 mg/kg	—	21/41	37/41	41/41	0

* All rats received 100 mg/kg of hydrazine, I.P.

peritoneally administered PALP prevented all deaths only when administered at one dose level (10 mg/kg). No dosage of PALP was completely effective in preventing convulsions.

Since studies have revealed that PY, PAL, and PALP are all successful antidotes for UDMH, these B_6 vitamers were studied for their effectiveness in hydrazine toxicity. The doses, routes of injection, and results are given in TABLE 3. PY proved to be the most effective B_6 vitamer for therapy of hydrazine toxicity. PY was most successful at a dose of 15 mg/kg given I.V. or at a dose of 0.5 or 1.0 mg/rat given I.C. By both routes, PY was given 15 to 20 minutes after hydrazine. Five rats which are not recorded in the Table were given 15 mg of PY/kg I.V. immediately prior to impending death from 100 mg of hydrazine/kg. These rats survived, indicating that PY is a quite good antidote for hydrazine toxicity, even when injected rather late. Intravenous PAL at 10 mg/kg protected four of six rats, but PALP was ineffective, with only one of six rats surviving. Surviving rats always appeared lethargic the day after the experiment but were quite normal on the third day. Eleven of 18 rats receiving 400 to 500 mg/kg of PY orally also survived a dose of 100 mg/kg of hydrazine. In this case, PY was given 30 minutes before the hydrazine injection.

Two hydrazine-treated rats that received 0.5 mg PY (I.C.) had moderate to severe hyaline droplet change in the renal tubular epithelium and in the hepatic cord cells. The cord cells also showed small necrotic foci with neutrophil accumulation. There was severe periportal lipid infiltration. Two rats that received 1 mg PY I.C. were sacrificed 48 hours after the hydrazine injection. These rats had renal tubular necrosis, and there were numerous areas of necrosis in the liver as well as severe periportal lipid infiltration. Intracerebral B_6 apparently prevents convulsions by action within the brain, but perhaps because of the small quantities used, the severe histopathologic effects of hydrazine on other organs was not diminished.

Tryptophan Metabolism

Since vitamin B_6 is involved in tryptophan metabolism, it was of interest to determine whether UDMH would alter the amounts of xanthurenic acid or kynu-

renic acid excreted in the urine. Such a finding would also be of value in assessing human exposures to UDMH. Six-hour post-UDMH urine samples were collected under toluene from rats kept in individual metabolic cages. Six or more animals were included in each group. Fluorometric determination of xanthurenic and kynurenic acids was carried out by the method described by Satoh and Price (1958). Normal animals excreted an average of 3.5×10^{-4} mg of kynurenic acid and 5.0×10^{-4} mg of xanthurenic acid over the six-hour period. The values obtained for UDMH-treated rats (80 mg/kg I.P.) were 2.8×10^{-4} mg and 5.2×10^{-4} mg respectively, not significantly different from the control animals. Unfortunately, short-term changes during the six-hour period would not have been detected by this procedure.

Electrolyte Distribution

Studies on electrolyte distribution in the urine of rats treated with UDMH have been previously reported (Barth *et al.*, 1967). The effect of UDMH on brain electrolytes has also been investigated. Homogenates of whole brain were made in distilled deionized water and the electrolyte concentration measured with a Beckman flame photometer. The results are shown in TABLE 4. Total electrolyte levels of Na, K, and Ca in treated animals were not significantly different from the controls. The values were rather consistent except for one group of UDMH rats where the mean calcium level appears depressed. Due to considerable animal variation within groups, this is not a statistically significant difference. Mitochondrial preparations from brains of normal rats and rats given 100 mg/kg of UDMH were also analyzed for potassium and calcium levels. Animals were sacrificed two hours after treatment and mitochondria prepared according to the method of Lovtrup and Zelander (1962). The results of these measurements are shown in TABLE 5. No differences were noted in mitochondrial Ca or K levels in these two groups of rats. It is realized that these are rather gross measurements in that the whole brain was utilized, thus electrolyte changes within a small localized area would perhaps go undetected.

TABLE 4

ELECTROLYTE LEVELS IN WHOLE BRAIN HOMOGENATES FROM NORMAL AND UDMH-TREATED RATS

UDMH mg/kg	Sacrifice Time (hr)	No. of Rats	mEq/kg Tissue*		
			Na	K	Ca
Controls	–	12	49.4 ± 0.4	90 ± 1	3.0 ± 0.7
80	2	13	50.9 ± 0.6	88 ± 1	1.7 ± 0.2
100	2	12	50.5 ± 0.5	91 ± 1	3.1 ± 0.4

* Average ± SE.

TABLE 5

ELECTROLYTE LEVELS IN BRAIN MITOCHONDRIAL PREPARATIONS FROM NORMAL AND UDMH (100 mg/kg)-TREATED RATS

	Sacrifice Time (hr)	No. of Rats	K μg/mg N*	Ca μg/mg N*
Normal	–	10	161 ± 11	72 ± 13
UDMH-Treated	2	10	161 ± 4	74 ± 17

* Average ± SE.

Amino Acid Metabolism

As early as 1926, Lewis and Izume suggested a hydrazine block of amino acid utilization. Subsequent findings with *in vitro* systems (Amenta & Johnston, 1963; Killam & Bain, 1957, McCormick & Snell, 1961) have demonstrated that hydrazine and some of its derivatives do produce a block in amino acid metabolism which can be reversed by pyridoxal phosphate. Simonsen and Roberts, (1967) have shown increased levels of certain amino acids in tissues of hydrazine-treated rats. However, whether amino acid imbalance bears any causal relationship to the convulsive seizures in hydrazine-treated rats is not clear. A recent paper from our laboratory reported plasma and tissue amino acid levels, as well as the effect of amino acid loading on hydrazine treated rats. FIGURE 2 illustrates the effect of tyrosine loading on brain and plasma tyrosine levels in control and hydrazine-treated rats. Plasma tyrosine levels in tyrosine-loaded rats are two to three times greater than control levels one to two hours after dosing. Brain levels show a continuous rise, and at three hours they are approximately four times control levels. Plasma and brain levels show tremendous increases in tyrosine levels when tyrosine-loaded rats are given hydrazine. Plasma levels are approximately 15 times control values, while brain tryosine levels rapidly increase five- or sixfold within three hours after treatment. When a non-tryosine-loaded group of animals were injected with hydrazine, it was found that plasma levels at three hours were five times those of the control group receiving only the tyrosine load. At the same time, brain tyrosine levels in hydrazine-treated rats were quite comparable with those of rats that were tyrosine-loaded. The elevated tyrosine levels in plasma and brain of tyrosine-loaded hydrazine-treated rats demonstrates either a remarkable increase in the rate of absorption or a severe inhibition of metabolism or excretion of tyrosine. Several earlier studies when both hydrazine and tyrosine were given intraperitoneally showed similar results. To obviate peritoneal irritation, and perhaps a direct effect on the rate of tyrosine absorption, hydrazine was given subcutaneously and tyrosine intraperitoneally in the present studies. Under similar experimental conditions, total plasma amino acids were measured (FIGURE 3). Tyrosine loading was not a factor in these studies, since it did not materially influence total α-amino nitrogen levels of the control group. The three hydrazine treated groups showed a continuous rise in plasma α-amino nitrogen levels over the three-hour period following injection with 60 mg/kg hydrazine subcutaneously. Eighteen hours after hydrazine injection, plasma α-amino nitrogen levels were 15.2 ± 1.7 mg/100 ml, liver 0.89 ± 0.02, and brain 0.62 ± 0.01 mg/g, compared to control levels of 6.6 ± 0.43, 0.45 ± 0.02, 0.57 ± 0.01 respectively. Thus although plasma and liver levels were still elevated, brain levels were near normal at this time. Since these studies were carried out on 18-hour fasted rats, the amino acid elevations in plasma and tissue must arise from endogenous metabolism. These findings are consistent with the hypothesis that amino acid metabolism is inhibited in hydrazine rats, apparently due to an acute vitamin B_6 deficiency. Several pieces of information, however, suggest that factors other than PALP deficiency may play a role in the effect of hydrazine on amino acid levels. These will be considered in the general discussion.

Chemical Reactivity

In vivo studies of the reaction rates between the various hydrazines and PALP (Cornish & Ling) show that the reaction between hydrazine and PALP is almost immediate, while UDMH and MMH are considerably slower. SMDH,

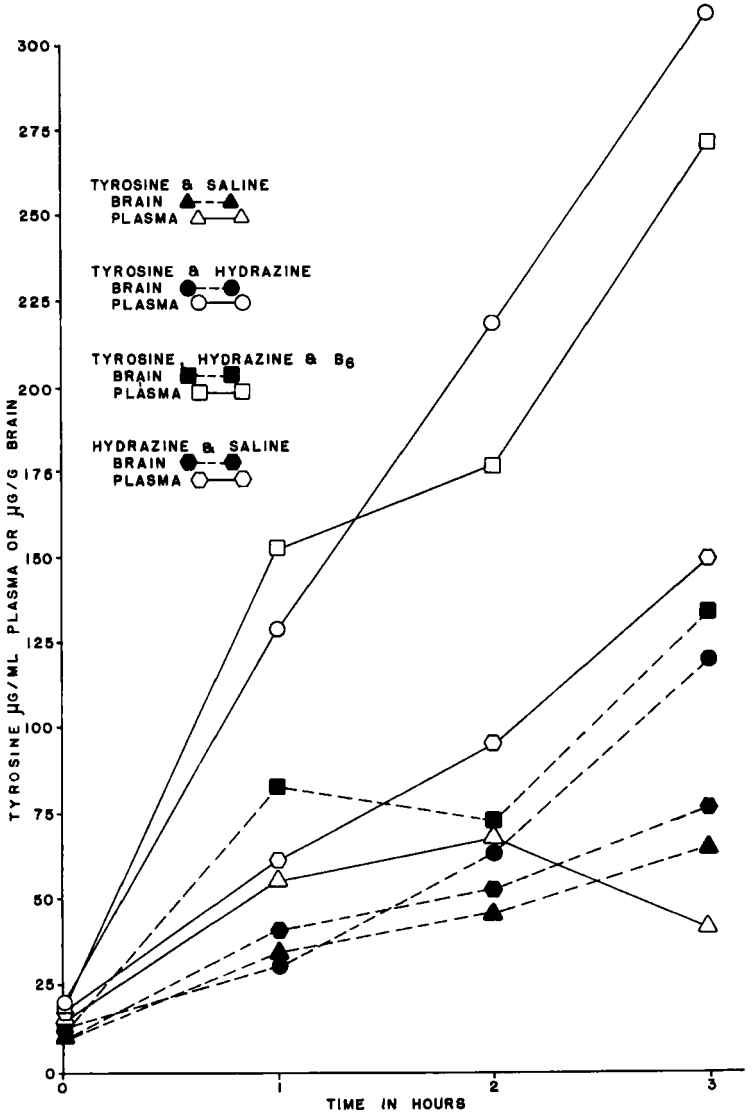

FIGURE 2. Tyrosine levels in plasma and brain. Rats were dosed as follows: tyrosine, 100 mg/rat I.P.; hydrazine, 60 mg/kg S.C.; vitamin B_6 (pyridoxine HCl), 15 mg/kg I.P. (Copyrighted figure reproduced by permission of *Toxicology and Applied Pharmacology* (Cornish & Wilson, 1968).)

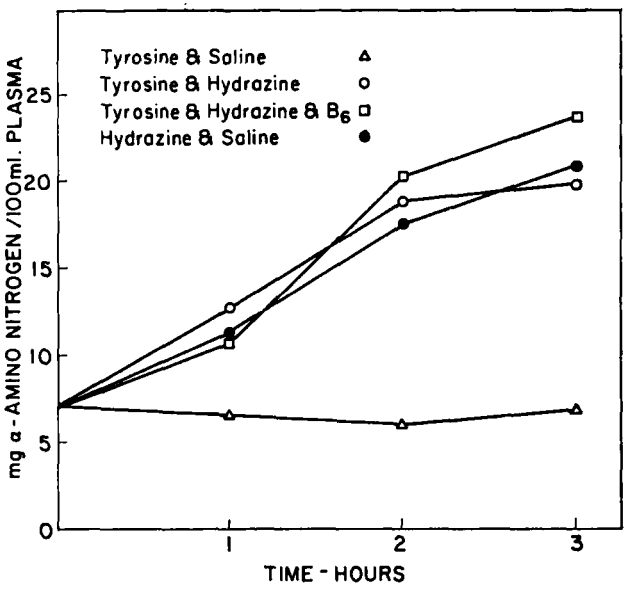

FIGURE 3. α-Amino nitrogen levels in plasma. Rats were dosed as follows: tyrosine, 100 mg/rat I.P.; hydrazine, 60 mg/kg S.C.; vitamin B_6 (pyridoxine HCl), 15 mg/kg I.P. (Copyrighted figure reproduced by permission of *Toxicology and Applied Pharmacology* (Cornish & Wilson, 1968).)

without a free amine, does not react with PALP. This is consistent with *in vitro* findings that with relatively high doses (LD_{100}), animals dosed with hydrazine die within a few minutes, whereas animals receiving UDMH or MMH may survive for a considerable time. Hydrazine, being a reactive compound, may of course react with other biological components at an equally rapid rate.

General Discussion

The earliest reports of the ability of pyridoxine to reverse the toxic effects of UDMH made the simple hypothesis of an acute vitamin B_6 deficiency most attractive. This was particularly true since it can be demonstrated, both *in vitro* and *in vivo*, that UDMH forms a hydrazone with PALP. Unfortunately the hydrazones themselves appear to have considerable toxicity and may play a role in the *in vivo* response to various hydrazines. A recent report by Furst and Gustavson (1967) compared the toxicity of UDMH, MMH, and the corresponding hydrazones of pyridoxal and pyridoxal-5-phosphate. In mice, the characteristic convulsive seizures were brought on by both the hydrazines and the hydrazones. In general, the hydrazones were more toxic than the hydrazines themselves. For example, at 100 mg/kg I.P., UDMH was not a convulsant in the mouse, while the corresponding hydrazones of PAL and PALP were convulsants at dosages of 5 mg/kg. One must keep in mind, however, that the intraperitoneal injection of a reactive hydrazine may not be comparable to the injection of a hydrazone in which the chemically reactive groups of both compounds have been combined. Thus, certainly absorption and distribution of these two materials will vary greatly. For comparison on a mechanistic basis, it would be necessary to know

the concentrations of each reaching the pharmacologically active site, presumably in the brain. Such findings, however, are consistent with the report of Uchida and O'Brien (1964), which showed that total PALP levels in the brain of rats was not reduced at the time of seizures when rats were given UDMH. However, intraperitoneal injections of PY increased brain levels of PALP in control and SDMH-treated rats somewhat more than in UDMH-treated animals. The authors conclude that the short-term effects of these hydrazines are not due to a reduction of the prevailing levels of total brain PALP. Several alternative hy-hypotheses are suggested, including metabolic compartmentalization of PALP, or perhaps a direct action of the hydrazines on brain glutamic decarboxylase.

The data shown in TABLES 1, 2 & 3, which indicate that all forms of the B_6 vitamer are at least partially effective against the convulsive effects of the hydrazines, clarifies to some extent the conflicting data on the ability of PY but not PAL or PALP to prevent convulsive seizures in UDMH-treated rats. It illustrates the relatively narrow range of effective dosages of PAL and PALP, amounts above or below these ranges not being effective. Thus, findings will vary, depending upon the dosage used.

With respect to specific enzymatic activity that might be affected, early studies obviously concerned themselves with brain glutamic acid decarboxylase (GAD) and γ-aminobutyric acid (GABA) levels. Medina reported (1963) rat brain glutamic acid decarboxylase activity severely inhibited by both hydrazine and UDMH. γ-aminobutyric acid transaminase activity was markedly inhibited by hydrazine but only slightly affected by UDMH. GABA levels were elevated in hydrazine-treated rats and depressed in UDMH-treated animals. Pyridoxine, at the single dosage used, did not alter the activity of GAD or GABA-transaminase in hydrazine-treated animals, but did return GAD and GABA to essentially normal values in UDMH-treated animals. The authors conclude that a direct relationship between the metabolism of GABA and the convulsive action of hydrazines could not be shown.

Any overall hypothesis of the mode of action of the convulsigenic hydrazines must take into account the initial problems of varying rates of absorption and distribution, passage through the blood-brain barrier, the formation of PAL or PALP hydrazones, which in themselves are convulsants, the inhibition of a number of B_6-requiring enzymes by the hydrazines or their hydrazones, and the possible localized B_6 deficiency within critical brain areas. Although PALP deficiency within the brain has not been demonstrated, the ability of appropriate small intracerebral and intraperitoneal doses of the B_6 vitamers to completely or partially protect animals against the toxic effects of UDMH, MMH, and hydrazine most simply implies a deficiency state at some critical metabolic point.

Acknowledgment

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