# Plasma Pyridoxal Phosphate Depletion by the Carcinostatic Procarbazine (34378)

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(Introduced by D. P. Rall)

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In 1956, Weigand (1) demonstrated a reaction in aqueous solution between one of several hydrazides and pyridoxal, resulting in the formation of a pyridoxal hydrazone (Fig. 1). Subsequent investigations revealed that pyridoxal hydrazones were potent inhibitors of pyridoxal kinase, the enzyme which phosphorylates pyridoxine in its conversion to the biologically active pyridoxal-5′-phosphate (PALP) (2). Pyridoxal hydrazone formation and pyridoxine depletion are thought to be responsible for signs of pyridoxine deficiency which occur in the clinical use of such drugs as isoniazid (3) and hydralazine (4). Lowered brain levels of PALP in animals (5) and other indirect evidence of PALP depletion in man (6) after administration of these drugs have been reported in support of this interaction.

Recently, procarbazine (*N*-isopropyl-α-(2-methylhydrazino)-*p*-toluamide, hydrochloride, MIH) a derivative of methylhydrazine, has proved effective in treating advanced Hodgkin's disease (7). The metabolism of this drug is incompletely understood, but rapid transformation to an azo derivative occurs in aqueous solution (8). Further metabolism to methylhydrazine and *N*-isopropylterephthalamic acid has been proposed by Oliverio *et al.* (9) on the basis of the isolation of the latter from urine after drug administration (Fig. 2). Further support for this hypothesis has come from the demonstration of the *in vivo* formation of $CO_2$ and methane from the hydrazine methyl group of MIH (10). Since methylhydrazine is a proposed metabolic intermediate of MIH, it was considered that MIH might be implicated in pyridoxine metabolism by virtue of pyridoxal methylhydrazone formation.

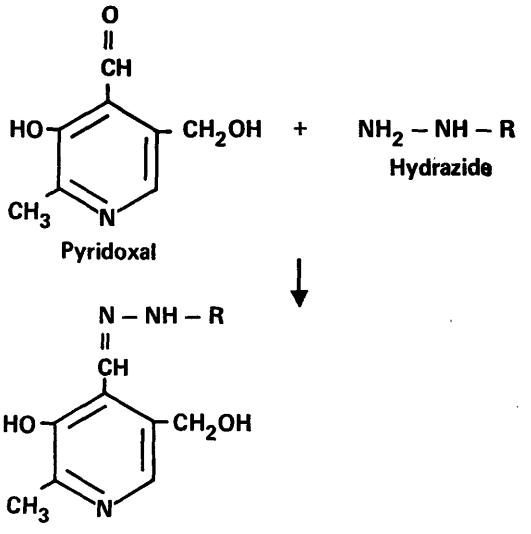


FIG. 1. Interaction of pyridoxal and a hydrazide with formation of a Schiff base, a pyridoxal hydrazone derivative. R can be any of a number of substituents yielding such compounds as semicarbazide, isonicotinylhydrazide, thiosemicarbazide, methylhydrazine, etc.

In succeeding experiments, procarbazine, monomethylhydrazine (MMH), and pyridoxal methylhydrazone (PMH), all caused a rapid fall in plasma PALP levels after intraperitoneal injection. In light of these findings, the proposed metabolic pathway for procarbazine and general aspects of hydrazine-pyridoxal interaction are reevaluated.

*Materials and Methods.* Procarbazine (Lot PP-5) was obtained from Hoffmann-LaRoche. Monomethylhydrazine (MMH) was obtained from Matheson, Coleman, and Bell. Pyridoxal and pyridoxal-5′-phosphate (PALP) were purchased from Calbiochem.

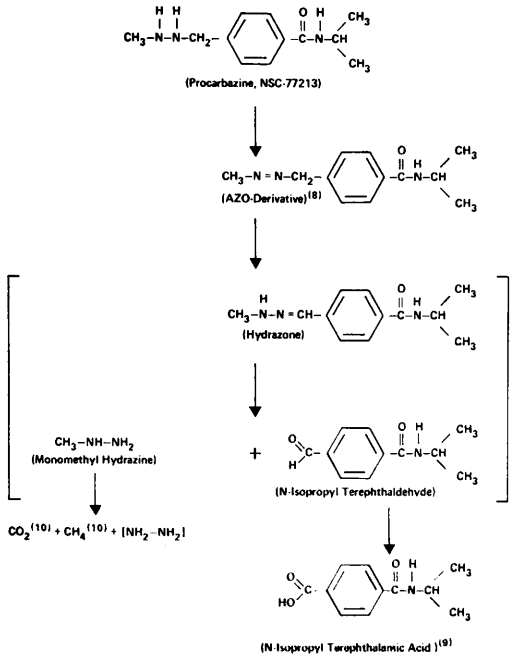


FIG. 2. Proposed degradative route of MIH *in vivo*. The metabolites not included in brackets have been identified.

Pyridoxal methylhydrazone (PMH) was synthesized from monomethylhydrazine and pyridoxal hydrochloride according to the method of Wiley and Irick (11), and was injected as a fine suspension in 2% sodium carboxymethylcellulose. Procarbazine and monomethylhydrazine were administered in physiological saline.

Male $CDF_1$ mice weighing 22–27 g were given single $LD_{10}$ doses of procarbazine, MMH, or PMH. In one experiment, however, mice were given a single intraperitoneal injection of procarbazine at 450 mg/kg, which is an effective antitumor dose against murine leukemia L1210 and other experimental rodent tumors (9). Animals were given water, but no food during the experiments. At designated time intervals, four animals were sacrificed by decapitation. Blood was collected in 0.1 ml of 0.1 *M* NaEDTA, pH 7.4, and was centrifuged at 700*g* for 20 min to obtain the plasma which was stored at −70° until assayed. Plasma levels were also determined in control animals injected with 2% sodium carboxymethylcellulose or physiological saline.

At the time of assay, plasma was deproteinized by addition of 0.1 ml of 50% TCA to 0.9 ml plasma. The supernatant fraction was neutralized with 0.025–0.035 ml of 4.5 *M* NaOH and a 0.025-ml aliquot was assayed for PALP content.

Plasma PALP levels were determined by modification of the method of Umbreit *et al.* (12). Tyrosine decarboxylase from *S. faecalis* was partially purified and fully resolved of cofactor by ammonium sulfate precipitation and Sephadex G-200 gel filtration. In the presence of L-tyrosine-1-$^{14}C$ (New England Nuclear Corp.) and apoenzyme, $^{14}CO_2$ production is a linear function of added PALP. The PALP content of plasma can thus be calculated by comparing the reaction velocity generated by a plasma aliquot to a standard curve of reaction velocity vs. PALP concentration (13).

*Results.* After intraperitoneal administration of MIH, there is a rapid fall in plasma PALP such that less than 10% of baseline PALP remains after 1 hr. Low plasma levels persist for at least 6 hr, and rise slowly thereafter toward normal (Fig. 3). A similar fall in plasma PALP occurs after intraperitoneal administration of monomethyl hydrazine of PMH (Fig. 4). However, there is a more rapid return toward normal with PMH as plasma PALP levels return to 80% of baseline values within 1 hr of PMH injection.

Plasma PALP fell gradually to 55% of baseline values in mice injected with physiologic saline and fasted for 24 hr (Fig. 3). Mice injected with 2% sodium carboxymethylcellulose, the vehicle for PMH administration, showed a gradual fall in plasma PALP comparable to that observed in the saline-injected controls.

It is possible that the low plasma PALP determinations resulted from the action of a direct inhibitor of the enzymatic assay reaction, rather than an actual decline in plasma PALP. To demonstrate that such an inhibitor was not present, a known amount of PALP ($10^{-9}$ g) was added to a 0.025-ml aliquot of plasma from an animal treated with PMH. The $^{14}CO_2$ production generated by plasma with added PALP equalled the sum of activities of the same plasma and an aqueous solu-

tion of PALP $10^{-9}$ g assayed separately.

The administration of pyridoxal, 300 mg/kg, intraperitoneally, 1 hr prior to giving MIH subcutaneously resulted in a sharp drop in the $LD_{50}$ in mice from 800 to 400 mg/kg.

*Discussion.* The fall in plasma PALP after intraperitoneal injection of MIH suggested that a hydrazine is formed during metabolism of the parent compound. The proposed pathway for MIH metabolism postulates the formation of MMH as an intermediate, and the ability of this compound to lower plasma PALP has also been shown in these experiments.

*In vitro* evidence suggests that PALP depletion after hydrazine administration could result from direct combination of the hydrazine and pyridoxal phosphate, and/or inhibition of the pyridoxal kinase reaction by a pyridoxal hydrazone. The present experiments demonstrate the effectiveness of the latter mechanism, in that small doses of PMH rapidly lowered plasma PALP.[1]

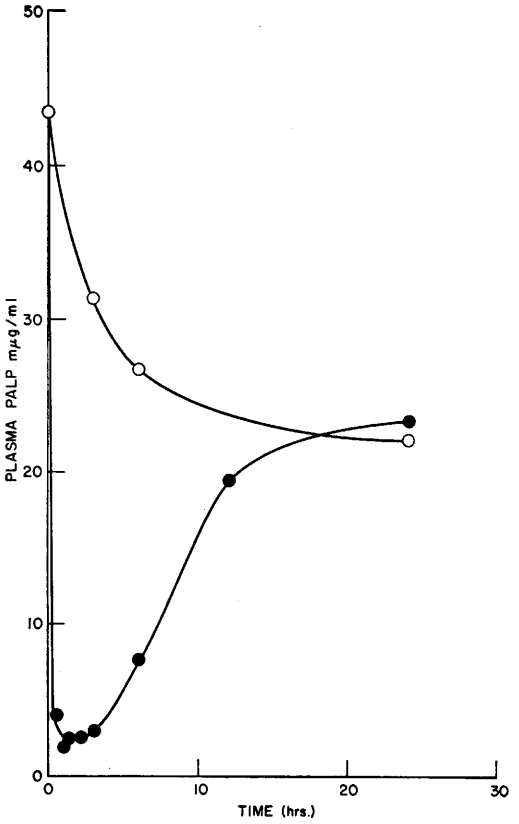


FIG. 3. Depression of plasma PALP in mice given a single intraperitoneal injection of 450 mg/kg of MIH (●); control mice injected with physiological saline (○). Each point represents an average of four mice.

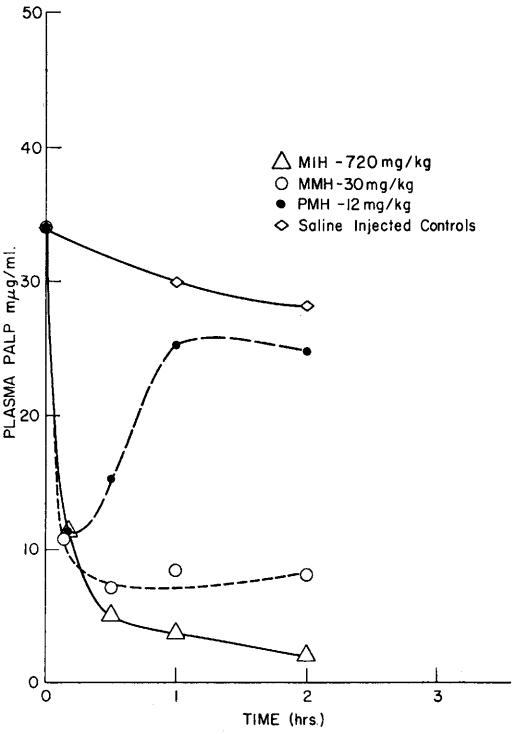


FIG. 4. Depression of plasma PALP in mice given single intraperitoneal injections of $LD_{10}$ doses of procarbazine (MIH), monomethylhydrazine (MMH), and pyridoxal methylhydrazone (PMH). Each point represents an average of four mice.

We have isolated and identified pyridoxal methylhydrazone (PMH) from chloroform extracts of mice given a single subcutaneous injection of 800 mg/kg of MIH followed by an intraperitoneal injection of 300 mg/kg of pyridoxal 30 min later (unpublished data). Bain and Williams (5) have likewise identified the pyridoxal and pyridoxal-5′-phosphate hydrazones in the brains of animals given isoniazid and observed a fall in the brain levels of PALP that coincided with appearance of the hydrazones.

[1] The marked enhancement of MIH toxicity by prior administration of pyridoxal suggests that PMH formation may be of critical importance in the toxicity of this drug.

The rapid fall of plasma PALP after injection of PMH indicates a turnover rate of less than 15 min for plasma PALP.[2] Many of the acute effects of hydrazine administration, such as hyperaminoacidemia (14), hypoglycemia (15), and convulsions (16), can be explained by the inactivation of PALP-dependent enzymes such as transaminases (gluconeogenesis) and glutamic acid decarboxylase ($\gamma$-aminobutyric acid synthesis) (17).

Procarbazine has caused a wide range of toxic effects, aside from bone marrow suppression. The nausea and vomiting seen with high oral or intravenous doses is thought to be of central nervous system origin (18). Other neurologic side effects include somnolence, confusion, and cerebellar ataxia, which are particularly prominent after intravenous administration (19), and mimic the acute effects of the drug in mice. In mice these neurological changes temporally parallel the fall in plasma PALP and disappear with the return of plasma PALP to normal. A peripheral neuropathy similar to the pyridoxine-responsive neuropathy produced by isoniazid has also been described in 17% of patients treated with MIH (18). Monoamine oxidase inhibition by MIH has been demonstrated (20) and may also account for some central nervous system effects of the drug. However, liver monamine oxidase is not a PALP-dependent enzyme and its inactivation is probably not related to PALP depletion.

It is hoped that further investigation of the interaction of hydrazine derivatives and pyridoxine will provide a better understanding of the toxicity and potential uses of this group of drugs.

*Summary.* Intraperitoneal injection of the antitumor agent, procarbazine (MIH), produced a rapid and prolonged fall in plasma levels of pyridoxal phosphate in mice. A similar fall in plasma PALP followed administration of monomethylhydrazine, a postulated metabolite of MIH, and pyridoxal methylhydrazone. The effectiveness of the latter compound, a probable inhibitor of the pyridoxal kinase reaction, in depressing plasma PALP levels indicates a rapid turnover rate of plasma PALP. It appears likely that PALP depletion plays a role in the neurotoxic effects of this new chemotherapeutic agent.

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[2] Thus, an acute interruption of PALP synthesis would be expected to produce immediate effects on enzymatic reactions requiring PALP.

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