

DISCERNING THE MAUVE FACTOR, PART 2

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“Mauve Factor” was once mistaken for kryptopyrrole but is the hydroxylactam of hemopyrrole, hydroxyhemopyrrolin-2-one (HPL). Treatment with nutrients—particularly vitamin B₆ and zinc—reduces urinary excretion of HPL and improves diverse neurobehavioral symptoms in subjects with elevated urinary HPL. Heightened HPL excretion classically associates with emotional stress, which in turn is known to associate with oxidative stress. For this review, markers for nutritional status and for oxidative stress were examined in relationship to urinary HPL.

In cohorts with mixed diagnoses, 24-hour urinary HPL correlated negatively with vitamin B₆ activity and zinc concentration in red cells ($P < .0001$). Above-normal HPL excretion corresponded to subnormal vitamin B₆ activity and subnormal zinc with remarkable consistency. HPL correlated inversely with plasma GSH and red-cell catalase, and correlated directly with plasma

nitric oxide ($P < .0001$). Thus, besides implying proportionate needs for vitamin B₆ and zinc, HPL is a promising biomarker for oxidative stress. HPL is known to cause non-erythroid heme depression, which lowers zinc, increases nitric oxide, and increases oxidative stress.

Administration of prednisone reportedly provoked HPL excretion in animals. Since adrenocorticoid (and catecholamine) stress hormones mediate intestinal permeability, urinary HPL was examined in relationship to urinary indicans, presumptive marker for intestinal permeability. Urinary HPL associated with higher levels of indicans ($P < .0001$). Antibiotics reportedly reduce HPL in urine, suggesting an enterobic role in production. Potentially, gut is reservoir for HPL or its precursor, and stress-related changes in intestinal permeability mediate systemic and urinary concentrations. (*Altern Ther Health Med.* 2008;14(3):##.)

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Disclosure

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The biological origin of HPL (Mauve Factor) is unknown. Over decades, serious consideration has been given to derivation from (1) dietary sources, (2) heme, (3) porphobilinogen (PBG), or (4) porphyrins. A known polymorphism for coproporphyrinogen oxidase (CPOX), as yet unexamined in relation to Mauve, presents a potential genetic basis for increased HPL excretion, invites modulation by specific environmental toxins, and invokes an enterobial role in production of HPL.

Ingestion of large amounts of dietary sources of the pyrrole moiety—coffee, tea, cola drinks, chlorophyll, tobacco, tryptophan, vitamin B₁₂—did not increase HPL excretion in hospitalized psychiatric patients.¹ Another potential source of pyrrole, D-lysergic diethylamide (LSD), did increase HPL excretion in 20% of subjects.^{2,3}

Pfeiffer suggested that HPL results from breakdown of heme,^{4,6} and Irvine identified a distinctive triad of urobilinoids in the stool of high-Mauve subjects which were possibly consistent with derivation of HPL from microbial degradation of bile pigment.^{7,8} Irvine was unable to produce HPL from heme, bilirubin, or bile pigments under mild laboratory conditions⁹ and found

that a large dose of hemoglobin (1.6 kg of blood sausage over 48 hours) produced no effect on HPL excretion.¹⁰

Irvine hypothesized that HPL is a metabolite of PBG or porphyrins from the heme biosynthetic pathway, citing the structural similarity of these compounds to HPL, the porphyrinogenicity of HPL, and very high levels of HPL in acute intermittent porphyria (AIP).⁹ The side-chains of PBG correspond exactly to HPL once they are decarboxylated and deaminated, and an endogenous enzyme is known to convert PBG to the corresponding hydroxylactam. Irvine included as precursor candidates all porphyrins with a methyl, vinyl, or ethyl group found in hemo-configuration on ring I.⁹ Response to a large oral dose of aminolevulinic acid (ALA) (1.5 g) did double urinary HPL over baseline in one subject for several days.¹¹ Irvine failed to produce HPL from porphyrins under mild laboratory conditions.⁹

Of all the porphyrins, isocoproporphyrin is most homologous to HPL.^{9,12} Isocoproporphyrins are an abnormal series of porphyrins from altered human heme biosynthesis.^{13,14} A polymorphism for CPOX increases isocoproporphyrins, as do toxins such as mercury,¹⁵ diazinon,¹⁶ and hexachlorobenzene.¹⁴ Suggestively, urinary coproporphyrin concentrations were greater in high-Mauve schizophrenics than other schizophrenics,¹⁷ and intraperitoneal injection of rats with 0.65 $\mu\text{mol/kg}$ of HPL (Cutler's low dose, as discussed in part 1 of this article) quintupled urinary coproporphyrins.¹⁸

The formation of isocoproporphyrin from altered human heme biosynthesis requires participation of gut flora. Altered host CPOX produces dehydroisocoproporphyrinogen, which is degraded by gut flora to produce isocoproporphyrin in stool.^{13,14,16} If, as Irvine suggested, isocoproporphyrin is a precursor to HPL, then either host or microbe¹⁹ could effect the final conversion from isocoproporphyrin. But the preceding step—dehydroisocoproporphyrinogen to isocoproporphyrin—is microbial.

Preliminary evidence does suggest bacterial involvement in the formation of Mauve. Oral tetracycline reversibly abolished urinary HPL excretion in 4 previously Mauve-positive subjects. Oral dosing with kanamycin, a non-absorbable antibiotic, reversibly abolished or sharply reduced urinary HPL in 9 subjects.^{1,20}

MAUVE AND THE GUT

There has been no quantification of HPL in stool or direct measurement of intestinal permeability of high-Mauve subjects, but multiple observations suggest that intestinal permeability modulates HPL excretion in urine. As these facts are considered, it should be kept in mind that Mauve not only is associated with neurobehavioral symptoms, but abdominal signs and symptoms exist in many high-Mauve subjects. Abdominal tenderness was reported in a large percentage of high-Mauve subjects,²¹ and Pfeiffer associated Mauve with sharp abdominal pains, as "stitch-in-side."^{22,23} Schizophrenics²⁴ and autistics²⁵⁻²⁹ have more abdominal symptoms, and abdominal pain is characteristic of AIP.

It is known that zinc deficiency results in intestinal epithelial damage and increased permeability mediated by greater intestinal NO.³⁰ Since urinary HPL associates with zinc deficiency,

HPL might be expected to associate with intestinal permeability. Suppression of urinary HPL with zinc (admittedly, in combination with B_6 ; there is no record of attempts to suppress HPL with zinc alone)⁶ comports with evidence that zinc lessens bowel permeability in animals^{31,33} and in humans³⁴⁻³⁷ and reduces bacterial adherence to enterocytes.³⁸

Irvine observed that laxatives and enemas increase urinary HPL.¹ The types of enemas and laxatives were not specified, but the range of possibilities would appear to increase intestinal permeability. Magnesium sulfate³⁹ and biscodyl⁴⁰ significantly increase intestinal permeability. Soap-suds or tap-water enemas result in epithelial loss,⁴¹ which would be expected to increase intestinal permeability. The effect of laxatives and enemas suggests that intestinal permeability affects urinary HPL.

To explain the clinical observation that HPL excretion and stress are associated, Sohler specifically proposed that urinary excretion of HPL relates to a "stress-induced anomaly of intestinal permeability which permits these pyrroles to get into the systemic circulation."^{5(p278)} It is well established that stress increases intestinal permeability. One hour of water-avoidance stress⁴² or 4 hours of restraint stress⁴³ significantly increased intestinal permeability in rats. Psychosocial stress results in intestinal inflammation and greater intestinal permeability in humans.⁴⁴⁻⁴⁶ More specifically, emotional stress increases urinary excretion of compounds normally retained in the bowel, including bilirubin metabolites^{47,48} and indoles⁴⁹⁻⁵¹ from bacterial degradation of tryptophan.

The permeabilized intestinal epithelium of stressed rodents is characterized by greater numbers of bacteria adhering to or penetrating bowel epithelium, infiltration by mononuclear cells,^{52,53} mast cell activation, and depletion of mucous.^{44,54} Catecholamines and glucocorticoids—the so-called "stress hormones"—increase many-fold during stress. Experimentally, administration of stress hormones duplicates the effects of experimental stress on intestine.

Application of norepinephrine to sheets of large bowel increased bacterial adherence within 30 minutes.⁵⁵ Intestinal epithelial permeability is increased by glucocorticoid injection and mediated by glucocorticoid receptors.⁵⁶ Central or peripheral injection of corticotropin releasing factor (CRF) mimics stress-induced degranulation of mast cells and increased permeability in colon.^{42,57} Dexamethasone injections of rats decreased IgA and increased bacterial adherence to epithelium within 24 hours.⁵⁸

Irvine's attempts to provoke HPL in animals with a number of treatments were futile, with one notable exception: urinary HPL increased significantly in Sprague-Dawley rats and female hyperproliferative (PRO/Re) mice treated with prednisone.^{59,60} Other mechanisms—including porphyrinogenic—are possible, but the response to prednisone is consistent with a permeability effect on HPL excretion.

Roman Lietha presented data that suggested an association between urinary HPL and indicans in 154 patients at a Princeton BioCenter conference in 1988. The indicans test is a qualitative assay for urinary indoles, which result from enterobial degradation of tryptophan. It is known that intestinal permeability associates with increased accumulation of tryptophan in intestinal mucous.⁶¹

Indicans correlate poorly with dietary protein and small bowel flora but do associate with enteric protein loss,⁶² which in turn associates with intestinal permeability.⁶³ Urinary indicans, while lacking specificity, would be expected to associate with intestinal permeability. Data from a mixed cohort of 2726 subjects from the Biocenter Laboratory in Wichita, Kansas, were examined to determine if a relationship exists between indicans and HPL in urine. Indicans positively correlated with HPL by colorimetric assay ($P < .0001$) (Figure 1). Mauve appears to relate to gut (Figure 2).

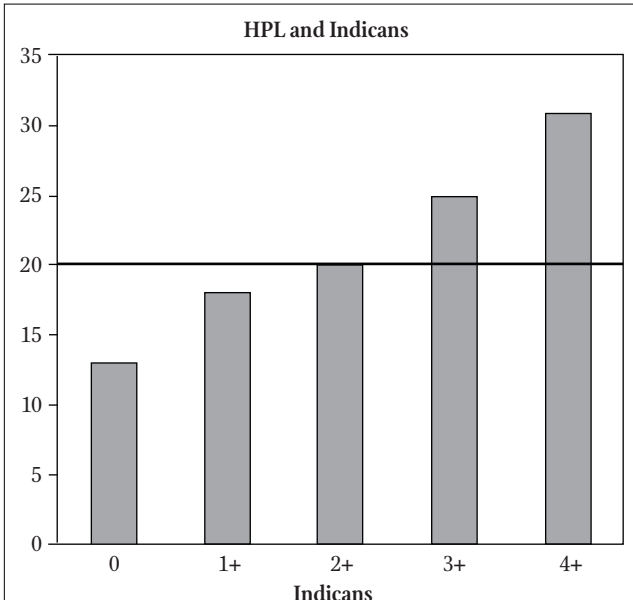


FIGURE 1 Median Values for Colorimetric HPL Equivalents* Correlated With Indicans in Single-void Urine From Mixed Cohort of 2726 Subjects

*Normal $< 20 \mu\text{g/dL}$.
HPL values for the 4+ indicans group were skewed, necessitating use of median values for significance testing.
 $P < .0001$ for difference in medians of all groups.

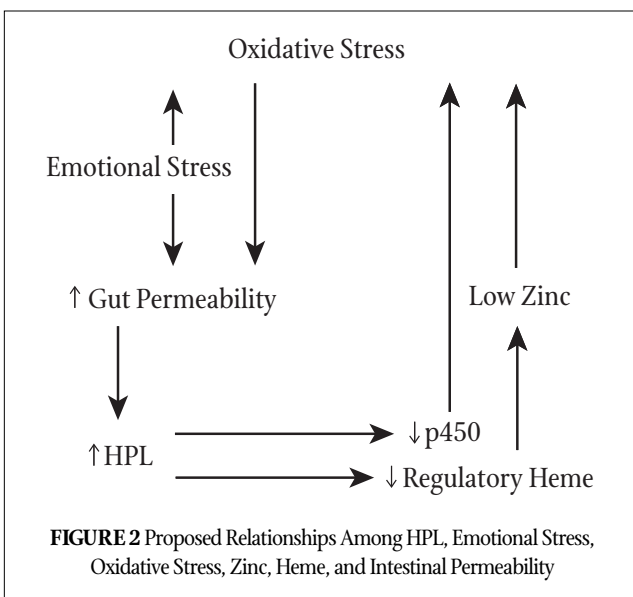


FIGURE 2 Proposed Relationships Among HPL, Emotional Stress, Oxidative Stress, Zinc, Heme, and Intestinal Permeability

MISLEADING LITERATURE AND OTHER OBSTACLES

After a period of initial activity, no basic research on Mauve has graced the peer-reviewed literature for many years. This inactivity contrasts rather sharply with ongoing enthusiasm for Mauve among a subgroup of nutritional practitioners and families. The disparity probably stems in part from continuing misidentification of the compound as KP. Also, HPL is a highly labile, technically challenging compound to study. What's more, only recently did the prevailing psychoanalytic paradigm open to underlying physical causation of "psychiatric" disease and the facilitative concept of oxidative stress. Finally, unwarranted negative conclusions about Mauve in the peer-reviewed literature discourage potential investigators.

An article in the *American Journal of Psychiatry* disparagingly entitled "Pyroluria: a Poor Marker in Chronic Schizophrenia"⁶⁴ found no relationship between qualitative urinary Mauve and a presumptive list of signs and symptoms for zinc and vitamin B₆ deficiency. Remarkably, the cohort had only 2 subjects with positive urine.

An article in *Clinical Science* stated unequivocally in title and abstract that Mauve, "is not causally related to schizophrenia."^{65(p469)} The text of the article softened the conclusion by stating that Mauve was "unlikely" to be causal, because no difference was found between schizophrenics and controls. The technical handling of specimens is suspect because Mauve was undetected in half of samples, at a purported detection limit of 0.25 $\mu\text{g/dL}$. The use of subjects with active somatic illness as controls was unfortunate, because Mauve is elevated in somatic illness.^{21,66-70}

As discussed in part 1 of this article, Cutler minimized potential neurotoxicity of HPL in humans in *Pharmacology and Toxicology* on the basis of hypothetical estimates of HPL in human blood contradicted by prior published values.⁷¹ In addition, the article indicated that thesis work by Graham failed to demonstrate a positive correlation between HPL levels and symptom severity in schizophrenia. In fact, only 1 of 7 schizophrenics in the thesis data had above-normal urinary HPL.⁷²

Kershner reported in *Journal of Nutrition*⁷³ the results of a randomized trial that sought to evaluate HPL as a screening test for response to nutrients. Twenty children with learning disability/hyperactivity received a low carbohydrate diet for 6 months, and 18 of 20 improved. Then the cohort was divided into groups of 10. One group received daily vitamin C, nicotinamide, vitamin B₅, and vitamin B₆ (500-750 mg); the other group received placebo. After 6 months, neither group showed additional improvement. Kershner concluded that, "Kryptopyrrole [colorimetric HPL] proved invalid as a screening test for vitamin-dependent learning disorders,"^{73(p819)} because pre-treatment levels did not demonstrate statistical relationship with improvement on vitamins.

The mean HPL values for the 2 groups were well within normal limits, and only 6 subjects (presumably 3 in either cohort, but not specified) had elevated HPL prior to nutrients. To compensate for the inadequate number of subjects with abnormal HPL, Kershner arbitrarily adjusted the normal range for HPL prior to statistical analysis. The choice of nutrients for the

Kershner study would be considered suboptimal by current standards and at the time of the study. Hoffer embraced vitamin B₆ and zinc as superior to multi-gram doses of B₃ years earlier, yet Kershner used no zinc. Pfeiffer^{4,22} and McCabe²¹ reported that without zinc, as much as 3 g of B₆ were required to suppress HPL, but Kershner used far less.

From the Kershner study is extracted a useful clinical point. Behavioral deterioration in some subjects after vitamins was relieved by the addition of magnesium. The late Bernard Rimland, PhD, founder and former Director of Autism Research Institute, San Diego, California, affirmed in oral communications from 1997 to 2006 that optimal response to higher doses of B₆ often is achieved with concurrent magnesium.

CONTEMPORARY THERAPEUTIC APPROACHES

Vitamin B₆ (200-800 mg daily) in combination with zinc (25-100 mg daily) usually is sufficient to suppress HPL and achieve optimal symptomatic response. Generally, higher urinary HPL suggests the need for proportionately higher dosing of zinc and B₆, and repeated measurements of HPL influence dosing decisions.

Clinical symptoms and HPL suppression are the primary determinants of B₆ dosing. Poor dream recall or morning nausea/anorexia reportedly are useful signs of insufficient B₆.²² Blood tests for EGOT⁷⁴ or P5P²³ may be used to confirm functional status of B₆, but pyridoxine blood levels are not considered useful. Long-term treatment of 3000 high-Mauve patients with high-dose vitamin B₆ resulted in no cases of peripheral neuropathy,⁷⁴ but reversible median nerve paresthesia has been reported.²³ P5P is unassociated with neuropathy.^{22,23} As orally communicated by William Walsh, PhD, in 2005, P5P may be effective in combination with B₆ or instead of B₆ in some high-Mauve subjects, including "slender malabsorbers."

Zinc requirement may be quite high in subjects with elevated HPL. Subnormal and low-normal levels of cellular or plasma zinc indicate the need for more zinc. For assessment with plasma zinc, avoidance of zinc supplementation for 24 hours excludes artifact. Long-term zinc requirements may be less than optimal doses in initial months of treatment, during accelerated growth, or during extended periods of psychosocial stress. Recurrence of leukonychia or striae is a relatively sure sign of lagging zinc.

Excessive zinc supplementation, which results in copper depression and depressed immunity, can be avoided with periodic blood testing. Especially in adults receiving more than 50 mg of elemental zinc daily, cellular or plasma zinc should not exceed upper limits of normal, and cellular or serum copper should not be below the normal range. Supplemental copper is rarely indicated in high-Mauve subjects and can aggravate symptoms.

Suppression of manganese may result from aggressive zinc supplementation. Small dosages (approximately 5 mg manganese for each 30 mg of supplemental zinc) reportedly improve symptoms in some high-Mauve subjects. Serum or red-cell levels may be monitored during supplementation with manganese, which in excess is pro-oxidant.

FUTURE RESEARCH

As a high priority, randomized clinical trials are needed to examine symptom improvement in high-Mauve subjects after treatment with B₆ and zinc. There are many corollary questions to answer: Does P5P, which protects intestinal GSHPx,⁷⁵ warrant an expanded role, and if so, in which patients? Would zinc, which blocks intestinal lipoxidation and permeability,⁷⁶ be useful in high boluses or perhaps in poorly absorbable forms? If the relationship to oxidative stress is fundamental, would antioxidants such as GSH or coenzyme Q₁₀ be useful treatments?

Suggested novel applications of Mauve in behavioral disorders include prevention of suicide,⁷⁷ prevention of psychiatric illness,²¹ and treatment and prevention of criminal behavior.^{78,79} Hoffer found that sudden, unexpected deviant behavior in previously well-adapted adults associated with Mauve.⁸⁰

The status and use of fatty acids in high-Mauve subjects needs elucidation. Owing to the vulnerability of double bonds to oxidative stress, both omega-3 and omega-6 fatty acid depletion might be expected in high-Mauve subjects. Preliminary data on 23 schizophrenics suggests this is the case, at least in schizophrenia. Plasma from the schizophrenics contained significantly less docosahexanoic acid (DHA, omega-3), as a percentage of total lipids, than controls. Only the 6 high-Mauve subjects from this cohort also had lower arachidonic acid (AA, omega-6) ($P < .01$).⁸¹

A number of studies have found lower concentrations of omega-3 and omega-6 fatty acids in blood from schizophrenics,⁸²⁻⁸⁵ including lower red-cell membrane DHA and AA.⁸³ Multiple trials report improvement in groups of schizophrenics receiving omega-3 supplementation.⁸⁶ It is possible that Mauve identifies a subgroup which would benefit from omega-6 supplementation in combination with omega-3 or as a higher priority.

Evening primrose oil (EPO), a rich source of gamma linolenic acid (GLA), is a precursor for both AA and dihomo-gammalinolenic acid (DGLA), immediate precursor for prostaglandin E1 (PGE-1). PGE-1 rapidly lowers intestinal permeability, including stress-induced intestinal permeability.⁸⁷⁻⁸⁹ EPO also improves zinc absorption.⁹⁰⁻⁹² EPO and misoprostol, a commercial PGE-1 analogue, are logical possibilities for Mauve research.

HPL is a potentially useful screen for other biochemical abnormalities, including dysregulation of homocysteine, a neurotoxic⁹³ metabolite. Cystathionine beta-synthase, which metabolizes homocysteine, is uniquely dependent on both vitamin B₆ and heme as cofactors.^{94,95} In written communication, Allen Lewis, MD, Medical Director of Pfeiffer Treatment Center, Warrenville, Illinois, reported in 2005 that serum HCY and colorimetric HPL (single-void, unadjusted) from unsupplemented autistic children seen at the Pfeiffer Treatment Center correlated significantly ($N=114$; $P=.002$). Plasma HCY associates with greater risk of cardiovascular and neurodegenerative⁹⁶ disease.

Mauve assay may be useful in the care of subjects with strictly somatic diagnoses or in the optimization of health in subjects without diagnoses. Mauve elevation in somatic illness^{21,66-68,70} presumably reflects unrecognized nutritional deficits and greater oxidative stress. In allergy, which is associated with zinc deficiency,⁹⁷ Mauve

testing might enhance nutritional awareness. (Pfeiffer suggested allergic diathesis in association with mauve, citing the zinc requirement for histamine storage in mast cells.¹¹) Similarly, cancer associates with zinc deficiency⁹⁸ and B₆ deficiency.^{99,102}

Corroboration of HPL as marker for biotin deficiency should be a high priority. The finding is plausible. Biotinidase, necessary for maintenance of biotin levels, is sensitive to oxidative modification, and oxidative stress is suspected to lower biotinidase in plasma.^{103,104} Biotin deficiency causes brain dysfunction, and subjects with partial biotinidase deficiency remain asymptomatic until stressed.¹⁰³

HPL testing is potentially useful in pregnancy, when nutritional demands are enormous and when nutritional deficits can result in fetal disease or malformation. Biotin deficiency, for example, is relatively common in pregnancy,¹⁰⁴ and marginal biotin deficiency is teratogenic in mice.¹⁰⁴ Deficiencies of biotin,^{105,106} zinc,¹⁰⁷ and B₆¹⁰⁸⁻¹¹⁰ independently result in cleft palate in animals. Presumably, combined deficiency of these nutrients increases teratogenic risk. Hyperhomocysteinuria associates with fetal disease and malformation,¹¹¹ and heme modulates neurogenesis.¹¹² Pfeiffer associated Mauve with miscarriage.⁷⁷

Predisposition to Mauve may be heritable. Some clinicians consider elevated Mauve in one family member an indication for family-wide testing. Polymorphisms favoring HPL production are considered most likely in genes for altered expression of CPOX, porphyrins, metabolism of stress hormones,^{113,114} or production of endogenous antioxidants such as metallothionein.¹¹⁵ Defective enzymes may associate with HPL, especially those that are sensitive to oxidative impairment¹¹⁶ and also are either B₆-dependent (eg, glutamic acid decarboxylase) or zinc-dependent (eg, pyridoxal kinase).

Porphyrin profiles in high-Mauve subjects might confirm origin in the heme biosynthetic pathway, any defect of which can be magnified by factors that increase activity of ALA synthase (ALA-S). Treatment of animals with HPL significantly increased ALA-S activity,^{12,117} presenting the intriguing possibility that an aberrant product of porphyrin metabolism may exert positive feedback on its own production. A number of toxins—especially heavy metals and alcohol—increase ALA-S activity.¹¹⁷

Research is needed to confirm the relationship between HPL excretion and stress, and to more completely characterize HPL as a biomarker for oxidative stress. H₂O₂ should be measured in high-Mauve subjects. Besides catalase, depression of other heme-dependent enzymes could potentiate greater oxidative stress in high-Mauve subjects. For instance, lower cytochrome p450 might confer greater sensitivity to medications or toxins.

Association of HPL excretion with stress hormones and oxidatively modified biomolecules such as 8-OHdG and isoprostane seems likely. The perspective for research in this area is provided by the understanding that emotional stress causes increased oxidation of biomolecules, including brain, as surely as do toxins or deficiency of antioxidant nutrients (as reviewed by McGinnis in 2007).¹¹⁸

The biological effects of HPL are mostly unexplored, and interpretation of such studies will be abetted by accurate quantification of HPL in blood and stool. Specific microbes may associ-

ate with HPL. Downstream effects of heme suppression by HPL may be detectable in animals and high-Mauve subjects. HPL may exert significant effects on gut or brain, including hallucinosis. Associated EEG abnormality¹¹⁹ may normalize with Mauve suppression, but modern brain scans have eluded Mauve.

CONCLUSION

At the very least, this review should clarify the identity and history of Mauve (HPL). Hopefully, it will strengthen commitment to careful handling and refined laboratory approaches to urinary assay of HPL, including normalization to specific gravity or creatinine. And for the first time, herein were presented organized data that examined—and confirmed—urinary HPL as a yardstick for functional B₆ and zinc deficiency. The findings are congruous with clinical observations over the decades and should stimulate independent corroboration and further research.

In its search for clinically relevant biomarkers for oxidative stress, modern medicine would do well to consider urinary Mauve as a means to quantify oxidative stress and to guide the use of specific antioxidant therapies. Besides practical potential, Mauve provides an exquisite conceptual model for the interplay of oxidative stress, emotional stress, nutrients, and gut as they pertain to disease of brain and body.

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