

## REVIEW ARTICLE

## DISCERNING THE MAUVE FACTOR, PART 1

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"Maue Factor" was once mistaken for kryptopyrrole but is the hydroxylactam of hemopyrrole, hydroxyhemopyrrolin-2-one (HPL). Treatment with nutrients—particularly vitamin B<sub>6</sub> 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 B<sub>6</sub> activity and zinc concentration in red cells ( $P < .0001$ ). Above-normal HPL excretion corresponded to subnormal vitamin B<sub>6</sub> activity and subnormal zinc with remarkable consistency. HPL correlated inversely with plasma glutathione and red-cell catalase, and correlated directly

with plasma nitric oxide ( $P < .0001$ ). Thus, besides implying proportionate needs for vitamin B<sub>6</sub> 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 a reservoir for HPL or its precursor, and stress-related changes in intestinal permeability mediate systemic and urinary concentrations. (*Altern Ther Health Med*. 2008;14(2):40-50.)

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## Disclosure

The following authors affiliate with commercial laboratories that perform HPL assay: Audhya (Vitamin Diagnostics, Inc, Cliffwood Beach, New Jersey); Jackson (Bio-Center Laboratory, Wichita, Kansas); and McLaren-Howard (Biomed Medical Unit, London).

*Editor's note: The following is part 1 of a 2-part article. Part 2 will appear in the May/June 2008 issue of Alternative Therapies in Health and Medicine.*

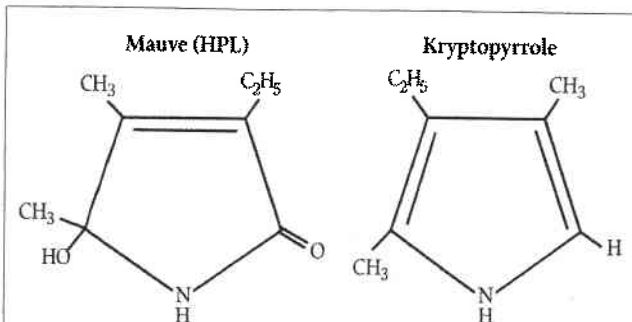
"Maue Factor," or "Maue" (mōv) for brevity, first was detected in the urine of psychiatric patients by the Hoffer group in 1958<sup>1-4</sup> and named for its appearance on paper chromatograms. Irvine extracted the compound from urine,<sup>1,5</sup> correctly assigned the structure to the pyrrole family,<sup>1,6</sup> and conferred the common name.<sup>1</sup> Early technology permitted only qualitative assay.<sup>2,5-8</sup>

Hoffer observed that recovery from acute schizophrenia associated with disappearance of Maue from the urine, regression with reappearance.<sup>2,4,7</sup> Large doses of vitamin B<sub>3</sub> suppressed Maue in schizophrenics.<sup>6,7,9</sup> Pfeiffer reported superior clinical results with combined vitamin B<sub>6</sub> and zinc, which suppressed Maue and improved symptoms in many neurobehavioral disorders.<sup>10-17</sup>

The Pfeiffer group introduced a colorimetric quantitative assay for Maue,<sup>18</sup> which utilizes kryptopyrrole (KP) as standard. Structural similarity affords the use of KP as standard for HPL assay, but the 2 molecules are distinct (Figure 1). Maue was identified mistakenly as KP by Irvine in a high-profile scientific journal in 1969<sup>19</sup> and again by Sohler in 1970.<sup>20</sup> A flurry of research on the experimental effects of KP eventuated.<sup>3,10,21-33</sup> Improved technology demonstrated that KP is not found in human urine,<sup>34,35</sup> and Maue was identified indisputably by synthesis as HPL.<sup>36-41</sup>

"HPL" and "Maue" are used synonymously in this article and

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**FIGURE 1** Mauve Factor (HPL) Is Distinct From Kryptopyrrole (KP)  
Structural similarity of the compounds affords the use of KP as standard in the colorimetric assay for HPL.

for clarity may be substituted for erroneous use of "kryptopyrrole" in older documents. "High-Mauve" denotes subjects or groups with elevated HPL or with a tendency to excrete excess HPL. "Pyrroluria" lacks specificity, as many pyrroles appear in urine.

HPL is unstable outside the body, readily interconverting with other structures.<sup>39,39,42-45</sup> Exposure to light or to seemingly mild chemical treatments reduces detectable HPL,<sup>8,16,39</sup> which also is acid labile<sup>44</sup> (a study that unadvisedly used hydrochloric acid to preserve urine failed to detect HPL in schizophrenia,<sup>46</sup> a condition well known for HPL elevation). Graham reported the half-life of HPL in urine at room temperature to be 10 to 12 hours, although the extent of light exposure was unspecified.<sup>39</sup>

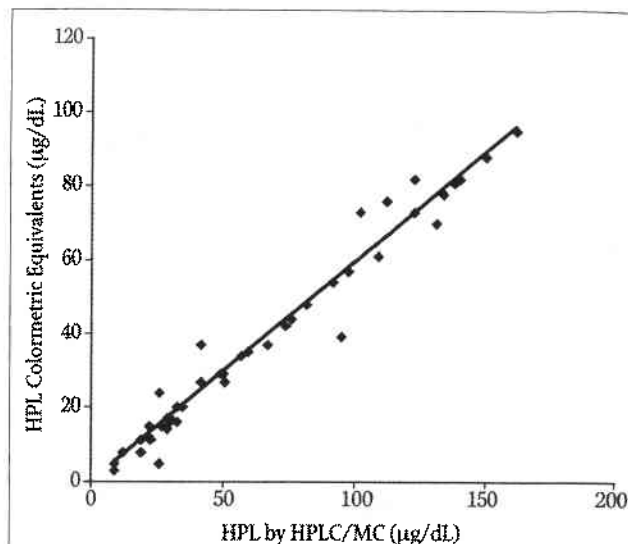
Addition of ascorbate preservative and protection from light and heat maximize detection of HPL. Besides light-shielding transport tubes, one laboratory (Vitamin Diagnostics, Cliffwood Beach, New Jersey) recommends urine collection under dim light and employs darkroom assay conditions. If assay for HPL cannot be performed immediately, overnight shipment and/or freezing of the urine sample are required by all North American laboratories surveyed for this review. Gorchein found that freezing to -8° C stabilized HPL in urine for up to 4 months.<sup>47</sup> Re-freezing of thawed specimens diminishes detectable HPL (Ellen Hanson, Laboratory Supervisor, Direct Health Care Access II Laboratory, Inc, Mount Prospect, Illinois; oral communication, September 2006).

KP is readily oxidized,<sup>39,48</sup> so laboratories take special precautions to maintain purity of KP used for colorimetric HPL assay. Occasionally, the colorimetric assay is invalidated by the presence of other Ehrlich-reactive compounds which produce spectrophotometric interference at 540 nm. Urobilinogen is the most common offender.<sup>18,49</sup> Others reportedly include hemoglobin, bilirubin, and mendeamine (oral communication, September 2006, from Irwin Sommerfeld, Laboratory Director of Direct Health Care Access II Laboratory).

#### VALIDATION OF THE COLORIMETRIC ASSAY FOR URINARY HPL

HPL assay utilizing high-pressure liquid chromatography/mass spectroscopy (HPLC/MS) and synthetic HPL standard is

highly sensitive and specific. In a comparison of split-urine samples by Vitamin Diagnostics Laboratory, the simpler colorimetric assay for HPL correlated very highly with HPLC/MS ( $r=0.98$ ;  $P<0.0001$ ) (Figure 2). It should be noted that absolute HPL values varied on the 2 assays. The normal range for colorimetric assay was  $<15 \mu\text{g/dL}$ , but for MS/HPLC, normal was  $<25 \mu\text{g/dL}$ . The latter compares favorably with Graham's normal range of  $<26 \mu\text{g/dL}$  utilizing gas-liquid chromatography and synthetic HPL standard.<sup>39</sup>



**FIGURE 2** HPL by HPLC/MS and Colorimetric Assay

Validation of colorimetric assay for urinary HPL. Same as Figure 1 cohort, but excludes extremely high ( $>100 \mu\text{g/dL}$  on colorimetric) values.  $N=44$ ;  $r=0.98$ ;  $P<0.0001$ .

#### EFFECTS OF VARIABLE HYDRATION ON HPL CONCENTRATION

Normalization of values to urinary specific gravity (SG)<sup>50</sup> or creatinine corrects for variable hydration. Pfeiffer encouraged normalization of the colorimetric assay to SG in his later years, according to Tapan Audhya, PhD (oral communication, June 2006). Examination of results from 600 colorimetric assays from the BioCenter Laboratory in Wichita, Kansas, revealed that 20% of HPL values moved into or out of the normal range after adjustment to SG by refractometry. Examination of data from the Biocenter Laboratory and from the Direct Health Care Access II Laboratory revealed that normalization affects reported HPL values up to 4-fold.

Normalization was found to improve correlation with other laboratory parameters. Before normalization to SG, HPL in single-void specimens from subjects with mixed diagnoses failed to correlate significantly with plasma zinc ( $N=87$ ;  $r=-0.15$ ;  $P=.18$ ). In written communication from July 2006, William Walsh, PhD, reported that significant correlations were achieved after normalization of colorimetric HPL to SG ( $r=-0.28$ ,  $P=.009$ ) and to creatinine ( $r=-0.30$ ,  $P=.004$ ). Graham's peer-reviewed publications adjusted HPL to creatinine.<sup>31,52</sup>

Addition of ascorbate to urine collections protects HPL from

degradation. However, typical quantities of ascorbate employed (250-500 mg) alter SG of urine in the usual 10 mL transport tube. Laboratories have found ways to overcome this difficulty. Vitamin Diagnostics Laboratory divides the urine at time of collection, yielding a second, unpreserved specimen for determination of SG. Vitamin Diagnostics Laboratory director Tapan Audhya, PhD, reports that for 24-hour urine collection, conservation of HPL with negligible effects on SG are achieved by addition of 500 mg of ascorbate to the large, refrigerated container, from which a small aliquot is examined for HPL and SG.

### MAUVE IN BIOLOGICAL FLUIDS

All humans apparently excrete small quantities of HPL in urine. As assayed by HPLC/MS under strict darkroom conditions, Vitamin Diagnostics Laboratory finds that the normal concentration of HPL in urine is 2 to 25 µg/dL. In our survey of labs in North America, Europe, and Australia, the upper limit of normal for HPL by colorimetric assay varies between 8 and 20 µg/dL.

As an approximate yardstick, clinicians consider urinary HPL levels over twice the upper limit of normal as highly elevated. Very high HPL measurements—hundreds of micrograms per deciliter—are reported and not strictly limited by primary diagnosis. HPL is detectable in human blood<sup>3,39,45,53,54</sup> and cerebrospinal fluid.<sup>45</sup>

In schizophrenics with elevated urinary HPL, Durko reported that whole blood levels for HPL (2-dimensional thin-layer chromatography, synthetic HPL standard) ranged between 4 and 10 µg/dL. Dialysis cleared HPL from both blood and urine.<sup>54</sup> Interfering substances have frustrated efforts to develop a practical blood test for HPL.

### Mauve Excretion Patterns

In most cases, day-to-day deviations around a baseline mean do not preclude identification of subjects prone to HPL elevation. Sporadic spikes in HPL well above baseline associate with stress, as will be discussed later. There is evidence that HPL excretion can increase very rapidly. In 1992, a study for the US Navy measured urinary HPL (colorimetric, normalized to SG) after male volunteers were subjected to brief cold-water immersion stress. In an oral summary of the study, Tapan Audhya, PhD, reported in 2002 the observation of significant increases in HPL excretion at 30 minutes, with peaks (as high as 80 µg/dL) at 1 hour and reversion to baseline at 24 hours.

HPL excretion appears to be greater during waking hours than during sleep. According to William Walsh, PhD, Pfeiffer suggested second-void spot urine specimens for HPL because he considered first-void measurement misleading (oral communication, July 2006). At Vitamin Diagnostics Laboratory, HPL in urine collected from subjects over 24 hours was higher from noon until midnight than from midnight until noon. It is noted that specific biomarkers for oxidative stress—8 hydroxydeoxyguanosine (8-OHdG), malondialdehyde (MDA), and 8-isoprostane—peak in early evening.<sup>55</sup>

While 24-hour urine collection circumvents intra-day variations in HPL excretion, as a practical matter, most laboratories

accept single-void urines, randomly timed. Hoffer favored same-time collection of specimens to improve comparability (written communication, August 2006).

### HPL IN NEUROBEHAVIORAL DISORDERS

The discovery of HPL grew out of Hoffer's interest in the possible biochemical etiology of schizophrenia. In 1961 he reported for the first time that certain urinary "unknown substances" on chromatograms were detectable in most schizophrenics hospitalized for active symptoms or relapses but not detectable after symptoms improved or abated ( $P < .001$ ).<sup>7,8,56,57</sup> It is now understood that these substances were HPL and its interconverting isomers. Hoffer applied the qualitative urinary test as an indicator for treatment with vitamin B<sub>3</sub>, which reduced schizophrenic symptoms and excretion of HPL.<sup>4</sup>

In an article that accompanied Hoffer's initial report, Irvine first used the term *Mauve factor*.<sup>1</sup> The compound associated significantly with psychometric scores for abnormal perception, paranoia, depression, and other symptoms in schizophrenics.<sup>6,45,58</sup> Electroencephalographic (EEG) abnormality associated with the compound in psychiatric patients.<sup>5</sup>

It became clear that Mauve is not confined to schizophrenia. In 1965, O'Reilly reported Mauve elevations in affective psychosis, alcoholism, psychoneurosis, and "disturbed children."<sup>59</sup> According to Joan Mathews Larson, executive director of the Health Recovery Center, Minneapolis, Minnesota, Mauve is elevated in approximately 75% of subjects seeking treatment for substance abuse (oral communication, July 2002). Mauve elevation is documented in many cognitive, affective, and neurobehavioral disorders (Table 1).<sup>4,8,22,23,30,32,52,58-69</sup>

### HPL AND STRESS

O'Reilly hypothesized that Mauve excretion increases during

**TABLE 1** Neurobehavioral Disorders Associated With Elevated HPL\*

Diagnosis	Percentage High-Mauve
AIP <sup>22,32</sup>	100
Latent AIP <sup>52</sup>	70
Down syndrome <sup>60</sup>	71
Schizophrenia, acute <sup>8,58,61,62</sup>	59-80
Schizophrenia, chronic <sup>23,30,63,64</sup>	40-50
Criminal behavior	
Adults, sudden deviance <sup>65</sup>	71
Youths, violent offenders <sup>66</sup>	33
Manic depression <sup>30,59</sup>	47-50
Depression, non-schizophrenic <sup>8,64,67</sup>	12-46
Autism <sup>67,68</sup>	46-48
Epilepsy <sup>67</sup>	44
Learning disability/ADHD <sup>64,67</sup>	40-47
Neuroses <sup>69</sup>	20
Alcoholism <sup>4,8,39,63,64,69</sup>	20-84

\*AIP indicates acute intermittent porphyria; ADHD, attention deficit hyperactivity disorder.

physical or psychosocial ("emotional") stress.<sup>59</sup> Over decades, clinicians formed the strong opinion that, irrespective of behavioral diagnosis, stress increases associated symptoms and excretion of Mauve.<sup>11,16,30</sup> Pfeiffer came to state unequivocally that Mauve is "a stress-induced factor."<sup>14(p775)</sup> Sohler reportedly induced HPL with experimental stress.<sup>45</sup> The effect of cold-water stress in the unpublished US Navy study was described earlier.

McCabe advocated short-term increases in B<sub>6</sub> dosing to blunt symptomatic deterioration in high-Mauve subjects during physical or emotional stress.<sup>48</sup> Clinicians give higher short-term "stress doses" of both B<sub>6</sub> and zinc.<sup>16,70</sup>

## VITAMIN B<sub>6</sub> AND ZINC

Pfeiffer discovered the clinical response of high-Mauve subjects to B<sub>6</sub> and zinc in 1971 and saw remarkable improvements in a series of 1000 high-Mauve patients.<sup>16,71</sup>

Treatment with B<sub>6</sub> and zinc reportedly reduced mean urinary HPL in 99 patients from 60 µg/dL to 30 µg/dL in 1 month.<sup>30</sup> Although randomized trials have not been performed, combined B<sub>6</sub> and zinc are now entrenched as core treatment for high-Mauve subjects. According to William Walsh, PhD, neurobehavioral symptoms associated with elevated HPL may improve after only a few days of therapy with B<sub>6</sub> and zinc (oral communication, 2006). Discontinuation may result in severe deterioration within 48 hours.<sup>11</sup>

Clinicians report proportionality between Mauve excretion and symptom severity<sup>30</sup> and according to the late Hugh Riordan, MD, former director of the Center for the Improvement of Human Functioning International, Wichita, Kansas (oral communication, 2000), higher Mauve excretion usually requires higher dosages of B<sub>6</sub> and zinc for suppression. HPL in urine decreased progressively with higher B<sub>6</sub> dosing,<sup>16</sup> and progressive B<sub>6</sub> dosing associates with normalization of erythrocyte glutamate oxaloacetate transaminase (EGOT).<sup>72</sup>

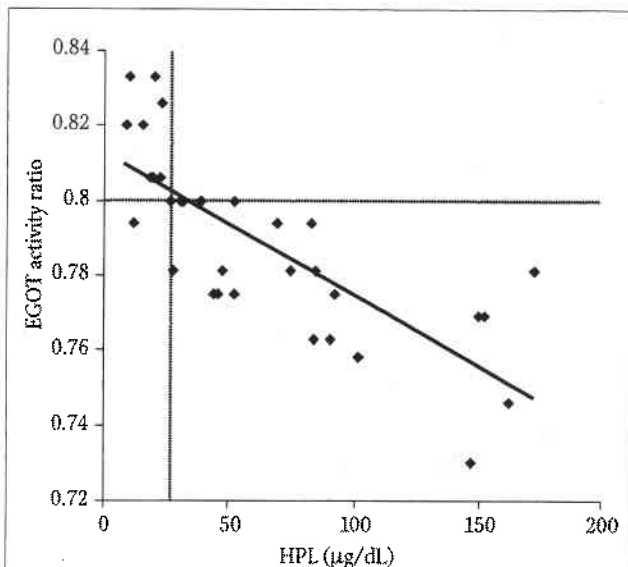
Initially, Pfeiffer tended to use high doses of vitamin B<sub>6</sub> (400-3000 mg daily) and relatively modest ("dietary") doses of zinc. Later, some patients were noted to respond optimally to B<sub>6</sub> and as much as 160 mg daily of elemental zinc.<sup>11</sup> In the collective experience of the authors, long-term treatment with B<sub>6</sub> and zinc usually is needed for ongoing HPL suppression and symptom management. Optimal initial dosages may be higher than maintenance dosages. Zinc requirements in high-Mauve subjects are noted to increase during growth spurts then decline abruptly. Pfeiffer reported that on occasion, previously high-Mauve subjects no longer may require high doses of B<sub>6</sub> and zinc<sup>16</sup>; the phenomenon was confirmed in oral communication in 2003 with Mark Vonnegut, MD, a former high-Mauve Pfeiffer patient and now a practicing pediatrician in Quincy, Massachusetts.

Pfeiffer's claims of a "double deficiency" of B<sub>6</sub> and zinc in association with abnormal Mauve excretion<sup>30</sup> were based on the clinical response to supplementation and a pattern of lower blood levels of zinc and functional B<sub>6</sub> status (pyridoxal-5-phosphate [P5P] and EGOT) among his high-Mauve patients.<sup>11,72,74</sup> Numerical data were not published.

Pfeiffer and Sohler proposed that functional B<sub>6</sub> deficiency and zinc deficiency in high-Mauve subjects results from increased urinary loss of P5P and zinc due to complexation with Mauve, and they cited 20 µg/dL higher zinc content in spot urines of Mauve-positive subjects.<sup>11</sup> The finding would extrapolate to relatively insubstantial total zinc loss, unless the effect extended to other routes of excretion. Pfeiffer published evidence of binding between P5P and KP<sup>12</sup> and between zinc and KP<sup>18</sup> but did not study HPL.

## Validation of HPL as a Marker for B<sub>6</sub> Status

The original data presented in this review were retrieved retroactively and anonymously from laboratory records, without regard to primary diagnosis or other criteria. In samples collected at the Biolab Medical Unit, London, colorimetric urinary HPL (single-void, unadjusted to SG or creatinine), correlated moderately with EGOT (n=58; r=-0.42; P=.001). In samples collected at the Vitamin Diagnostic Laboratory, HPL by HPLC/MS in 24-hour urines, normalized to SG, correlated strongly with EGOT (n=32; r=-0.77; P<.0001); all 24 subjects with abnormal HPL had below-normal or borderline-low EGOT (Figure 3).



**FIGURE 3 HPL and B<sub>6</sub> Activity\***

HPL by high-pressure liquid chromatography/mass spectroscopy in 24-hour urine correlates strongly with EGOT in mixed cohort. EGOT indicates erythrocyte glutamate oxaloacetate transaminase. Normal ranges: urinary HPL <25 µg/dL; EGOT activity ratio >0.8. N=32; r=-0.77; P<.0001.

The data affirm HPL as biomarker for functional B<sub>6</sub> deficiency and rationalize treatment with B<sub>6</sub>. Clinical response of high-Mauve subjects to B<sub>6</sub> may relate to known mechanisms by which B<sub>6</sub> subserves neuronal function. Numerous signs, symptoms, and traits have been observed in association with Mauve (Table 2). Poor dream recall and mild morning nausea/breakfast anorexia may relate especially to B<sub>6</sub> deficiency.<sup>15,16,73,75</sup> Pfeiffer suggested that stretch marks result from a combined deficiency of B<sub>6</sub> and zinc.<sup>13</sup>

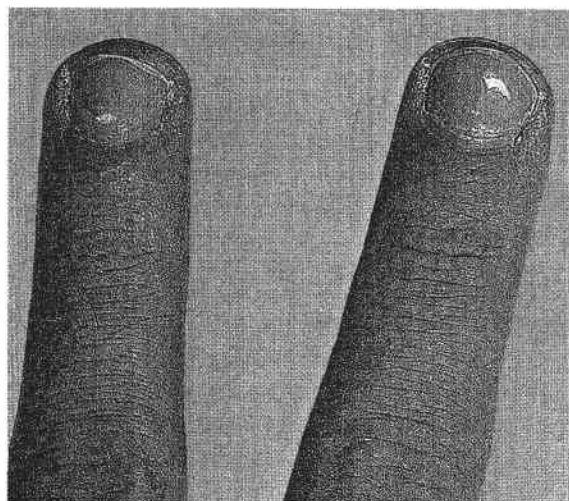
**TABLE 2** Signs, Symptoms, and Traits Clinicians Report as More Prevalent in High-Mauve Patients\* 6,10,11,13,16,30,38,54,11,13

Poor dream recall	Impotence
Nail spots	Eosinophilia
Stretch marks (striae)	B <sub>6</sub> responsive anemia
Pale skin/poor tanning	Attention deficit/hyperactivity
Coarse eyebrows	Crime and delinquency
Knee and joint pain	Substance abuse
Acne	Alcoholism
Allergy	Stress intolerance
Cold hands or feet	Emotional lability
Abdominal tenderness	Explosive anger
Stitch in side	Anxiety
Constipation	Pessimism
Morning nausea	Dyslexia
Light/sound/odor intolerance	Familial or social withdrawal
Tremor/shaking/spasms	Depression
Hypoglycemia/glucose intolerance	Paranoia
Obesity	Hallucinations
Migraine	Disordered perception
Delayed puberty	Bipolar disorder
Amenorrhea/irregular periods	Autism

\*The frequency of these features and their relationship to biochemical abnormalities associated with HPL are not well-studied.

#### Validation of HPL as a Marker for Zinc Status

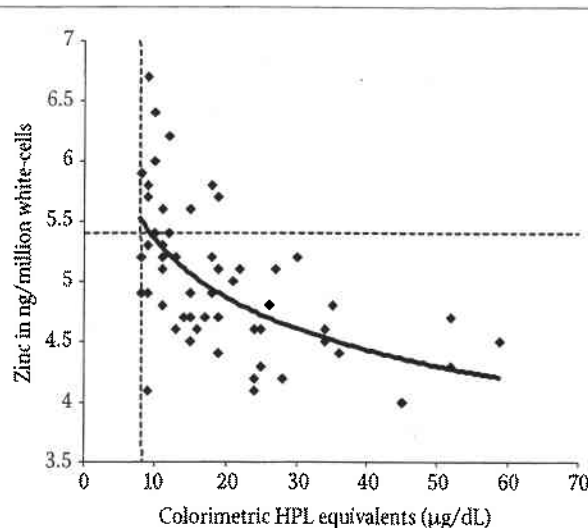
White flecks in the nails (Figure 4) are responsive to zinc<sup>16,71,76</sup> and reportedly detectable in 60% of high-Mauve subjects.<sup>64</sup> HPL was examined in relationship to 3 different measurements for zinc. As discussed earlier, Walsh reported that plasma zinc and single-void colorimetric HPL correlated significantly once normalized to SG ( $r=-0.28$ ;  $P=.009$ ) or to creatinine ( $r=-0.30$ ;  $P=.004$ ).



**FIGURE 4** Leukodysplasia Implies Zinc Deficit

In this high-Mauve subject, white flecks in nails resolved after institution of 100 mg of elemental zinc daily, reoccurred after dosage was lowered to 40 mg, and again abated on higher dosage.

Cellular zinc levels correlated more strongly with urinary HPL. In samples at the BioLab Medical Unit, single-void colorimetric HPL (unadjusted to SG) from a mixed cohort correlated substantially with white-cell zinc ( $N=58$ ;  $r=-0.60$ ;  $P<.0001$ ). Abnormal HPL corresponded to below-normal white-cell zinc in 42 of 58 patients (Figure 5). In samples at Vitamin Diagnostic Laboratory, stronger association existed between red-cell zinc and 24-hour urinary HPL (HPLC/MS, adjusted to SG) in a mixed cohort ( $N=37$ ;  $r=-0.88$ ;  $P<.0001$ ). Twenty-four of 24 subjects with elevated HPL had below-normal red-cell zinc (Figure 6).



**FIGURE 5** Single-void HPL and White-cell Zinc

Colorimetric HPL equivalents in single-void urines, unadjusted to specific gravity, correlates with white-cell zinc in mixed cohort. Normal values: HPL < 8 µg/dL; white-cell zinc > 5.4 ng/106 leukocytes.  $N=58$ ;  $r=-0.60$ ;  $P<.0001$ .

#### HPL AND OTHER NUTRITIONAL PARAMETERS

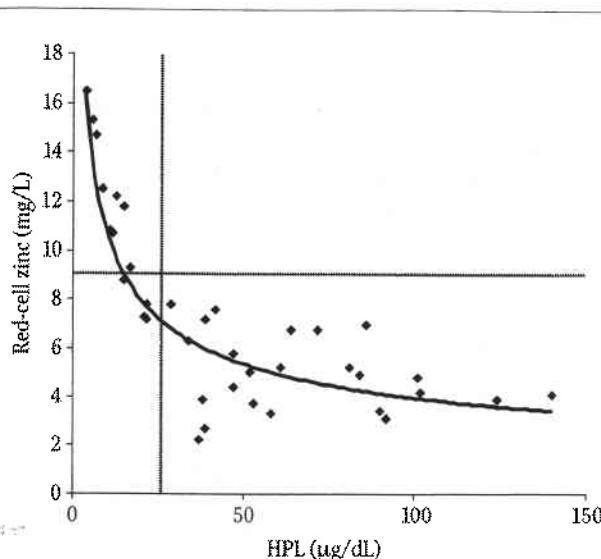
Oscar Kruesi, MD, former academic dean for Integrative Medicine, Capitol University, Washington, District of Columbia, reported a pattern of low plasma biotin levels in high-Mauve patients (oral communication, 2005). At the Vitamin Diagnostics Laboratory, 24-hour urinary HPL (HPLC/MS, adjusted to SG) and plasma biotin concentrations from a small, mixed cohort strongly correlated ( $N=24$ ;  $r=-0.88$ ;  $P<.0001$ ). Elevated HPL predicted below-normal plasma biotin in 16 of 16 subjects (Figure 7). These data are the first to suggest biotin deficiency in association with HPL. Biotin deficiency causes neurological disease in animals and humans<sup>77,78</sup> and is more common than thought.<sup>79</sup>

Examination of laboratory records found no association between HPL and markers for vitamin B<sub>3</sub> (urinary n-methyl nicotinamide), vitamin B<sub>12</sub> (urinary methylmalonic acid), folate (urinary formiminoglutamic acid, FIGLU), or thiamine (red cell transketolase).

#### POSSIBLE NEUROTOXICITY OF HPL

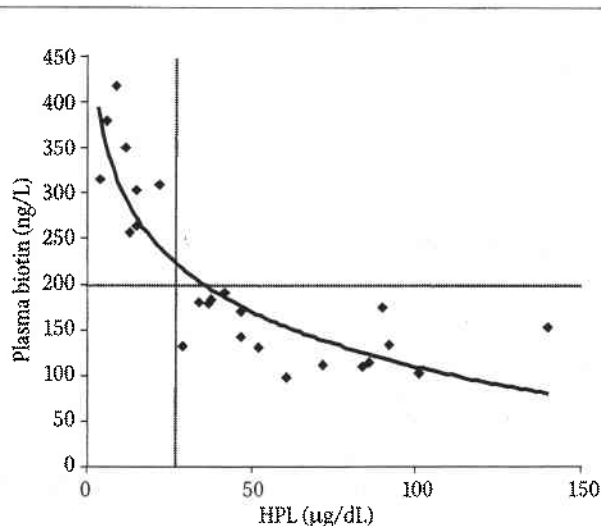
Several findings suggest that HPL is neurotoxic in humans: (1) structural homology to known neurotoxin; (2) acute behavioral effects in animals; (3) porphyrinogenicity in animals; (4)





**FIGURE 6 HPL and Red-cell Zinc**

HPL by mass spectroscopy/high-pressure liquid chromatography in 24-hour urine correlates with red-cell zinc in mixed cohort. Normal values: HPL <25 µg/dL; red-cell zinc >9 mg/L. N=37;  $r=-0.88$ ;  $P<.0001$ .



**FIGURE 7 HPL and Plasma Biotin**

HPL by high-pressure liquid chromatography/mass spectroscopy in 24-hour urine correlates with plasma biotin in mixed cohort. Normal range: urinary HPL <25 µg/dL; biotin >200 ng/L. N=24;  $r=-0.88$ ;  $P<.0001$ .

association in humans with porphobilinogen (PBG) and aminolevulinic acid (ALA), potential neurotoxins; (5) acute depression of non-erythroid heme in animals.

As a class, pyrroles have been called "nerve poisons."<sup>780</sup> HPL is from the subclass of monopyrroles, well known for biotoxicity.<sup>3,45</sup> Batrachotoxin (poison-dart frog)<sup>81</sup> and PBG<sup>82</sup> are monopyrroles which exert potent effects on the nervous system. KP<sup>3,10,22,24,25,28,31-33,45,83</sup> and the hydroxylactam of kryptopyrrole (KPL),<sup>84,85</sup> highly homolo-

gous to HPL, cause acute neurobehavioral effects in animals. Structural similarity of HPL to pyroglutamate and kainic acid<sup>41</sup> suggests possible direct effects on neurotransmission.

Irvine produced ptosis, locomotor abnormalities, and hypothermia in rats with unspecified doses of HPL.<sup>86</sup> Cutler found that intraperitoneal injection of HPL 0.65 µmol/kg produced relatively mild acute effects: decreased gross activity, increased preference for light areas of the cage, and a trend toward more aggressive behavior. A higher dose of 1.95 µmol/kg increased head-twitch and backward locomotion,<sup>41</sup> behaviors seen in rats treated with hallucinogens.<sup>87</sup>

Strictly by estimation, Cutler discounted significant behavioral effects in humans from HPL, because the plasma concentration of 0.3 µmol/kg (equivalent to 4.6 µg/dL) achieved in rats with the higher dose of HPL was adjudged "many-fold" greater than plausible HPL blood levels in humans.<sup>41</sup> The estimation overlooked published data from Semmelweis Medical University, which reported a whole-blood range for HPL of 4 to 10 µg/dL in schizophrenics.<sup>84</sup> Cutler's higher dose of HPL marginally achieved this range.

HPL definitely is porphyrinogenic in animals. Cutler's lower dose of HPL significantly increased total urinary porphyrin excretion in rats.<sup>82,85,88</sup> Graham documented peak urinary HPL immediately prior to a severe attack of acute intermittent porphyria (AIP),<sup>39</sup> but alteration of porphyrin metabolism by HPL has not been proven in humans. Nevertheless, elevation of HPL in the porphyrias is well documented.<sup>22,32,89-93</sup> In AIP, HPL is elevated consistently<sup>22,32</sup> and during AIP neurovisceral crisis may reach urinary concentration as high as 946 µg/dL.<sup>39</sup> In AIP—including the latent state—HPL consistently associates with urinary PBG and ALA.<sup>92,93</sup>

The association of HPL with ALA is not limited to AIP. In a mixed group of psychiatric patients (N=128), urinary HPL and ALA correlated positively.<sup>94</sup> ALA is a potent oxidant and neurotoxin<sup>95</sup> with known effects on neuronal energy production<sup>96</sup> and neurotransmission.<sup>97,98</sup> ALA binds P5P and produces free radicals by autooxidation.<sup>99</sup> Animal studies that failed to increase ALA after injection with HPL<sup>88</sup> used the Cutler doses.

Ex vivo, guinea-pig ileal contractions were inhibited by HPL at seemingly high concentrations of 8.5 µmol/kg (132 µg/dL),<sup>100</sup> but HPL in human bowel or stool has not been quantified for reference.

#### HPL DEPRESSES HEME

Heme is tightly coupled to neuronal metabolic activity.<sup>101</sup> Depression of heme leads to metabolic crisis, with mitochondrial<sup>102</sup> and neuronal<sup>103</sup> decay. Injection of rats with Cutler's lower dose (0.65 µmol/kg) of HPL at 0 and 24 hours reduced hepatic microsomal heme (by 42%) and heme-containing cytochrome P-450 (by 55%) at 48 hours.<sup>88</sup> Equivalent reduction of heme in cultured neurons with N-methylprotoporphyrin IX (NMP) reduces mitochondrial complex IV, upregulates nitric oxide synthase (NOS), and reduces intracellular zinc by half.<sup>101</sup> NMP inhibits heme synthesis, the proposed mechanism for HPL.<sup>92,88</sup> It is possible that HPL directly binds heme, as does KPL in vitro.<sup>104</sup>

Non-erythroid heme in high-Mauve subjects has not been measured, but depressed levels are predictable. Besides potential depression by HPL, deficiencies of zinc, B<sub>6</sub>, and biotin (all cofactors for heme synthesis) independently decrease non-erythroid heme.<sup>99,102</sup> And heme is degraded by stress.<sup>102</sup> It should be mentioned as well that heavy metals, which have not been examined in relation to Mauve, are renowned dysregulators of porphyrin metabolism and increase heme degradation.<sup>102</sup>

Heme plays a central role in energy production and is required by a family of biomolecules needed for detoxification and antioxidant defense: catalase, cystathionine synthase, cytochrome, guanylate cyclase, heme-hemopexin (for production of metallothionein), NOS, pyrrolase, sulfite reductase. Ultimately, heme depression increases oxidant leak from mitochondria and oxidative damage to cells.<sup>99,102</sup>

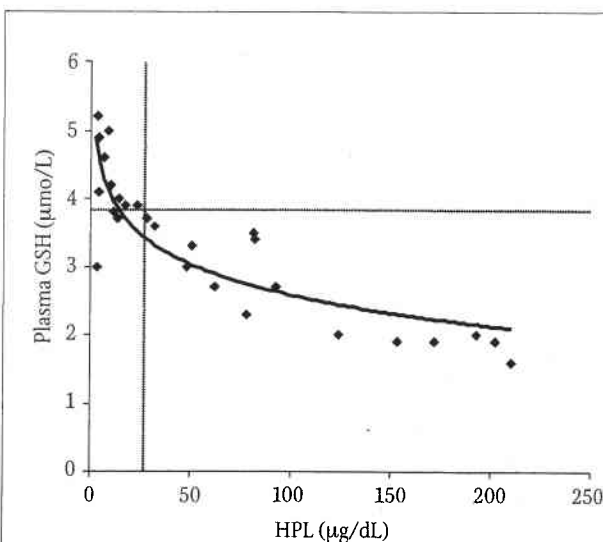
### HPL AND OXIDATIVE STRESS

Oxidative stress clearly results from deficiency of zinc or B<sub>6</sub>, as reviewed by McGinnis.<sup>105</sup> For example, even marginal B<sub>6</sub> deficiency is associated with lower glutathione peroxidase (GSHPx), lower glutathione (GSH) reductase, lower reduced/oxidized glutathione ratios, higher lipid peroxide levels, and mitochondrial decay.<sup>106-108</sup> The B<sub>6</sub> vitamins are themselves highly vulnerable to damage by oxidative species.<sup>109-111</sup> P5P protects neurons from oxidative stress, apparently by increasing energy production and lowering excitotoxicity,<sup>112,113</sup> and zinc supplementation decreases oxidized biomolecules.<sup>114,115</sup> Since HPL is a marker for B<sub>6</sub> and zinc deficiency, HPL is a potential biomarker for oxidative stress.

Biomarkers for oxidative stress are known to be higher in high-Mauve disorders such as schizophrenia,<sup>116,117</sup> autism,<sup>118-120</sup> ADHD,<sup>121,122</sup> Down syndrome,<sup>123-125</sup> and alcoholism.<sup>126-128</sup> In schizophrenia, lower blood levels of glutathione and response to intravenous glutathione were reported nearly 50 years ago.<sup>129</sup>

Plasma levels of reduced GSH, the ubiquitous intracellular antioxidant, are decreased in diseases associated with greater oxidative stress,<sup>130</sup> including Down syndrome.<sup>131</sup> In Alzheimer's disease, in which oxidative modification of brain precedes appearance of neurofibrillary tangles and plaque,<sup>132,133</sup> plasma GSH correlates inversely with brain levels of oxidatively-modified biomolecules.<sup>134</sup> It is reasonable to view plasma GSH as a biomarker for pathological effects of oxidative stress.

Initial data from a small cohort of Austrian patients with mixed diagnoses suggested an association between urinary HPL and plasma GSH. Peter Lauda, MD, reported that single-void colorimetric HPL, adjusted to creatinine, correlated modestly with red-cell GSH ( $r=-0.41$ ) in a group of patients in whom HPL was elevated only in 1 of 13 subjects (written communication, 2005). In samples from the Vitamin Diagnostics Laboratory, 24-hour urinary HPL (HPLC/MS, normalized to SG) from a mixed cohort strongly correlated with plasma GSH ( $N=30$ ;  $r=-0.85$ ;  $P<.0001$ ), and abnormal HPL associated with below-normal plasma GSH in 17 of 17 subjects (Figure 8). Very strong correlation with plasma GSH substantiates urinary HPL as biomarker for oxidative stress.



**FIGURE 8** HPL and Plasma-reduced Glutathione

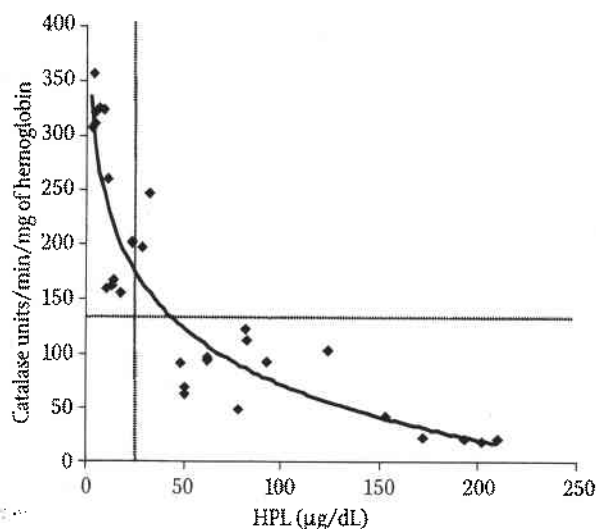
Urinary HPL by high-pressure liquid chromatography/mass spectroscopy in 24-hour urine correlates with plasma GSH in mixed cohort. Normal values: urinary HPL <25 μg/dl; plasma GSH >3.8 μmol/L.  $N=30$ ;  $r=-0.88$ ;  $P<.0001$ .

### HPL AND CATALASE

Catalase is an endogenous antioxidant that prevents excess cellular hydrogen peroxide ( $H_2O_2$ ), a freely-diffusible and potent oxidant. Catalase consists of 4 protein subunits, each requiring a heme group. Since catalase requires heme and HPL suppresses heme, it follows that HPL may associate with lower catalase. Lower catalase in blood is reported in schizophrenia<sup>116,135</sup> and autism.<sup>136</sup>

In samples collected at the Vitamin Diagnostics Laboratory, red-cell catalase activity in a mixed cohort was found to correlate inversely with 24-hour urinary HPL by HPLC/MS, normalized to SG ( $N=30$ ;  $r=-0.92$ ,  $P<.0001$ ). Abnormal HPL corresponded to subnormal catalase in 15 of 17 subjects (Figure 9). In addition to proposed direct effects of HPL on heme synthesis, depression of catalase may result from greater oxidative stress in high-Mauve subjects, because catalase is sensitive to oxidative degradation<sup>137</sup> (as is GSHPx,<sup>138</sup> which also can remove  $H_2O_2$  in a reaction using GSH as substrate).<sup>139</sup>

Depressed catalase hypothetically predisposes high-Mauve subjects to excess  $H_2O_2$  and presents a possible explanation for hypopigmentation of skin associated with Mauve—including, in the extreme, classic “china-doll” complexion.<sup>10,11</sup> The pathogenesis of vitiligo illuminates the effect of abnormal catalase and  $H_2O_2$  on pigmentation. A genetic polymorphism for catalase apparently predisposes patients to vitiligo.<sup>140</sup> All patients with vitiligo exhibit decreased catalase and increased  $H_2O_2$  in epidermis.<sup>141</sup> In the presence of excess  $H_2O_2$ , melanocytes<sup>142</sup> and melanin (which normally functions to bind redox-active metals and thereby reduce oxidative stress) are damaged, resulting in lesser pigment production.<sup>130</sup> If destruction of melanocytes by excess  $H_2O_2$  is not complete, treatment with pseudo-catalase restores skin pigmentation by reducing  $H_2O_2$ .<sup>143,144</sup>



**FIGURE 9 HPL and Red-cell Catalase**

HPL by high-pressure liquid chromatography/mass spectroscopy in 24-hour urine correlates with red-cell catalase in mixed cohort. Normal values: HPL < 25 µg/dL; red-cell catalase > 130 units/min/mg of hemoglobin. N=30,  $r=-0.92$ ;  $P<.0001$ .

As stress classically associates with Mauve, so do stressful life events associate with the onset of vitiligo.<sup>145,146</sup> Catecholamines, which increase as a consequence of stress, are increased in vitiligo patients, particularly during the active phase.<sup>147</sup> Both the synthesis of catecholamines and their auto-oxidation produce  $H_2O_2$ .<sup>139,148,149</sup>

Catecholamine excess is cytotoxic in diverse tissues, and the toxicity is oxidatively mediated by  $H_2O_2$ . Excess is implicated clearly in human heart disease, and cardiomyocyte apoptosis produced by catecholamine infusion is prevented by antioxidant vitamins.<sup>150</sup> In cultured neurons, toxicity of epinephrine and norepinephrine is reproduced by addition of equimolar  $H_2O_2$  or blocked completely by addition of catalase.<sup>151</sup> Catecholamine excess in neurobehavior was anticipated by Abram Hoffer in the Adrenochrome Hypothesis of Schizophrenia in 1954.<sup>152,153</sup>

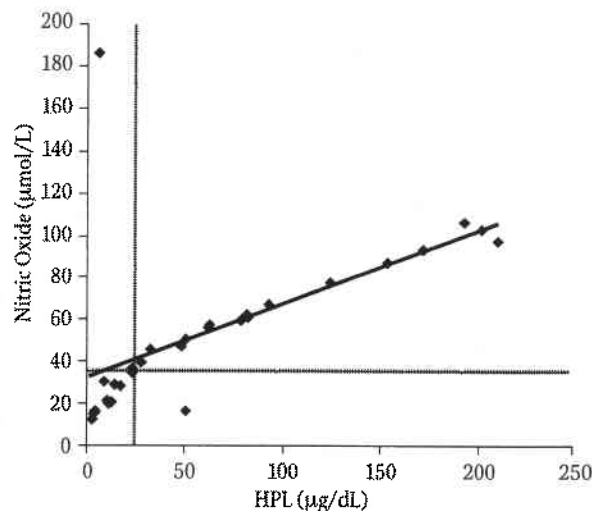
Besides lighter skin, lighter hair coloration than siblings and earlier gray is reported in high-Mauve subjects. Excess  $H_2O_2$  is known to increase proportions of oxymelanin in hair, with lightening analogous to the effect achieved by topical application of bleach for cosmetic purposes.<sup>154</sup> Excess  $H_2O_2$  remains hypothetical until levels are measured in the high-Mauve population. Zinc deficiency alone may explain hypopigmentation associated with Mauve. Melanin is rich in zinc and requires zinc for synthesis and maintenance.<sup>155,156</sup> Zinc protects melanocytes from oxidation,<sup>154,156</sup> and zinc deficiency grays the coats of rats.<sup>157</sup> Oxidants, including  $H_2O_2$ ,<sup>158</sup> displace zinc from binding proteins, and it has been suggested that clinical zinc depletion results inherently from greater oxidative stress.<sup>165</sup>

## HPL AND NITRIC OXIDE

Heme depression results in excess nitric oxide (NO),<sup>161</sup>

which is injurious to the brain<sup>159,160</sup> and is suspected to play a role in such high-Mauve disorders as schizophrenia,<sup>161</sup> autism,<sup>162</sup> and Down syndrome.<sup>163</sup> In schizophrenia and autism, stable metabolites of NO are elevated in conjunction with greater thiobarbituric acid-reactive substances in plasma.<sup>163</sup>

In samples from a mixed cohort at Vitamin Diagnostics Laboratory, plasma NO, measured directly, and 24-hour urinary HPL by HPLC/MS, normalized to SG, correlated positively (N=30;  $r=0.60$ ;  $P<.0001$ ). The statistical relationship strengthens substantially ( $r=0.96$ ) if an extreme outlier is excluded on the presumption of poor sample preservation (Figure 10). The strong association with NO enhances Mauve as a biomarker for oxidative stress.



**FIGURE 10 HPL and Plasma Nitric Oxide**

HPL by mass spectroscopy/high-pressure liquid chromatography in 24-hour urine correlates with plasma nitric oxide in mixed cohort. Normal values: HPL < 25 µg/dL; plasma nitric oxide 18-36 µmol/L. N=30;  $r=0.60$ ; with exclusion of an extreme outlier (6.4, 186),  $r=0.97$ ,  $P<.0001$ .

It should be noted that while altered functional  $B_6$ , zinc, biotin, GSH, catalase, and NO all point toward increased oxidative stress in association with urinary HPL, the data presented are from non-congruent cohorts. Proof that these parameters move together would require same-subject measurement of each.

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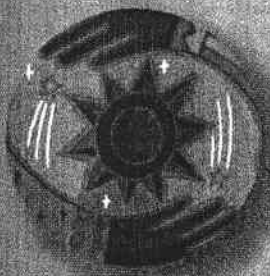
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