Embedded Sentinels of Toxicity

Scientists are beginning to identify when, where and how certain toxic substances affect the body by studying aberrations in body levels of chemicals known as porphyrins

By JANET RALOFF

Two people, both carrying the same relatively high body burden of a toxic substance like inorganic lead, will not necessarily suffer equally. For years scientists have not only wondered why, but also sought some kind of indicator to show who will suffer most. It now appears that, at least for certain classes of chemicals and metals, porphyrins may be the answer.

This research is already suggesting practical strategies for limiting toxicity. One study indicates that the susceptibility to lead in those at highest risk — inner-city children in low-income families — can be dramatically reduced by ensuring their diet has sufficient iron.

Porphyrins are small, ring-shaped chemicals involved in the body's multistep synthesis of heme. The heme molecule is best known as the the iron-carrying component of hemoglobin in red blood cells. Heme synthesis, however, occurs in almost all tissues. Bound to a protein, heme plays a role in a range of functions, including cellular respiration, energy generation and chemical oxidation.

However, enzymes that convert one porphyrin to the next during the synthesis of heme "are quite sensitive, as a group, to chemically induced disturbances," explains toxicologist Bruce Fowler of the National Institute of Environmental Health Sciences in Research Triangle Park, N.C. As soon as the action of one enzyme in the process is blocked, the porphyrin on which it was to have acted begins accumulating in the urine or blood.

Inorganic lead, for example, appears somehow capable of inactivating three different heme-cycle enzymes, according to Michael R. Moore of the University of Glasgow's Gardiner Institute in Scotland. Identifying a relative excess of one or more porphyrins and a shortage of succeeding ones in the heme-building process "sort of points the finger at which enzymes are being inhibited by a toxic chemical," Fowler says. In this way, abnormal ratios of porphyrins in the body not only signal a chemical's enzymatic site of action, but also might serve as an early-warning flag of ongoing low-dose toxicity.

Old, leaded paint is a major source of lead toxicity in children.

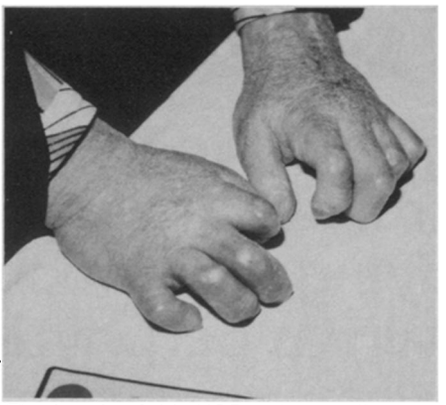
Moreover, Fowler's preliminary animal research indicates that porphyrin ratios may offer a specific and very sensitive signature of toxic exposure. In other words, the porphyrin "fingerprint" of lead's effects is different from that of arsenic's effects, which is different from that of the two metals combined. Add cadmium to the pair of metals, and a fourth fingerprint appears.

This raises the possibility, Fowler says, that it may someday be possible to diagnose the source of a person's toxicity by matching his or her body levels of various porphyrins to a map of ratios that typify exposures to a specific chemical or mix of chemicals. It's even possible, he says, that by measuring the strength of that fingerprint, scientists might be able to estimate the dose.

Porphyrins are already routinely used as a biological marker of lead toxicity. In fact, blood screening for an abnormal ratio of porphyrins — a condition known as porphyrinopathy — has replaced blood lead measurements as the routine test for detecting elevated body levels of lead, especially in children, according to Ellen Silbergeld, a lead toxicologist with the Environmental Defense Fund in Washington, D.C. A finding

of a lead-mediated porphyrinopathy rules out the chance that any excess lead in the blood results from accidental contamination of the sample, she notes. Moreover, the porphyrin test is easier and less expensive than actual lead-in-blood measurements, she says.

Only in the past 10 years or so, however, has there been much interest in using abnormal porphyrin ratios as potential indicators of other toxic exposures. It was largely to identify progress in this area that Fowler and Silbergeld several months ago convened an international conference in Rye, N.Y., under the aegis of the New York Academy of Sciences. What the meeting established, Fowler says, is that for some toxicants, a porphyrinopathy may eventually prove more useful as an index of exposure – and perhaps even of developing toxicity – than any gauge that now exists. The conference also identified several potential near-term applications of porphyrin-biomarker research.



Henry Peters

The hands of this HCB-poisoned Turkish woman, at age 30, show evidence of chemically induced porphyria, including severe arthritis and a foreshortening of finger joints. What's not obvious are symptoms of residual nerve damage, including a loss of feeling in her arms that worsens toward the hands.

One of the earliest examples of porphyrins' use as a biomarker for substances other than lead is the study of porphyrinopathies among Turks who, during the mid to late 1950s, ate wheat that had been treated with hexachlorobenzene (HCB). The chemical had been used in Turkey as a fungicide on grain that was intended for use as seed. But during a famine, 3,000 to 4,000 people ate bread made from the HCB-poisoned grain. (There was little confusion about which grain had been treated, since it had been marked with a dye. But the hungry had assumed that when washing removed the dye, it also removed the HCB.)

Roughly 10 percent of those who ate the contaminated grain died. Many of the rest, particularly children, developed porphyrias – diseases causing neurological damage and disfiguring skin sensitivity to sunlight. Previously, all porphyrias were believed to be genetic in origin.

Over the past 30 years, an international team of scientists has been studying changes in body porphyrin ratios among many of these HCB victims. Headed by Henry Peters and Derek Cripps at the University of Wisconsin in Madison, and by Ayhan Gõcmen from Hacettepe University Medical School in Ankara, Turkey, the group is acquiring clues as to how some of the porphyrias were initiated by the HCB and why they persist for decades.

Since the Turkish incident, many other polyhalogenated aromatic hydrocarbons (PAHs) – including the dioxins and polychlorobenzenes – have been found capable of affecting porphyrin ratios in animals and cultured cells. For example, George H. Elder and his co-workers at the University of Wales College of Medicine in Cardiff have shown that some PAHs seem to irreversibly inactivate or modify a porphyrin-converting enzyme known as UROD through a process that involves highly reactive oxygen species.

Working with the most toxic dioxin, TCDD – now generally considered the most porphyrin-disrupting compound as well – Lavinia Cantoni and her colleagues at the Institute of Pharmacological Research Mario Negri in Milan, Italy, have found that the liver, and secondarily the kidney, appear to be the main sites of UROD impairment by liver-damaging PAHs. Cantoni's work with HCB indicates that other porphyrin enzymes can be affected as well. One of these other affected enzymes, she says, may even be a source of the reactive oxygen species the Welsh group suspects of threatening UROD.

The Welsh and Italian programs, like the Turkish studies, suggest porphyrinopathies may hold promise as biomarkers of human PAH exposures. At the Centers for Disease Control in Atlanta, Renate Kimbrough has already made several attempts to use them in this way.

In one study, Kimbrough looked at urinary porphyrin levels of the 200 people believed to have been most heavily exposed to polybrominated biphenyls (PBBs) during a 1974 Michigan feed-grain accident, comparing their levels with those of people who had not been exposed to the chemical. Though animal data suggest that PBBs can upset porphyrin ratios in the body, Kimbrough says, "we have not been able to establish a difference between the two groups." She sees this as indicating that porphyrinopathies may not be useful human biomarkers of PAH exposures.

Pharmacologist Gerald Marks of Queens University in Kingston, Ontario, sees it somewhat differently. Noting that "even negative results may be useful," he says the apparent absence of porphyrinopathies in the Michigan residents exposed to PBBs might signal that this population survived the chemical exposures without much harm.

Moreover, Marks points out, Kimbrough didn't look for porphyrin changes in the Michigan residents until six years after their exposure. It's possible, he says, that by then any temporary porphyrin changes had self-corrected. Or, he says, "looking in the urine may not be the way to go" – it may turn out that blood levels are more sensitive monitors of initial adverse effects.

In addition to the work with PAHs, there is growing evidence that porphyrinopathies might serve as biomarkers of toxic exposure to many metals. Alfred M. Bernard and his colleagues at the University of Louvain in Brussels, Belgium, have shown in animals and cultured cells that copper, mercury, silver, zinc and cadmium can all reduce or block the action of the porphyrin-building enzyme known as ALAD.

Most recently, Bernard has found that aluminum also inhibits ALAD activity in people undergoing blood dialysis. (Aluminum hydroxide is a common antacid preparation given to dialysis patients to bind with, and thereby control, their body phosphate levels.) Bernard now suspects that the porphyrinopathy he's uncovered in these patients may be responsible for – and a potential biomarker of – the developing anemia so often observed in dialysis patients.

Work by researchers at the Battelle Seattle Research Center indicates that early signs of developing mercury toxicity might be detected by monitoring human urinary porphyrin levels. Other potential toxicants recently shown to induce porphyrinopathies include gallium arsenide and the anesthetizing gas nitrous oxide.

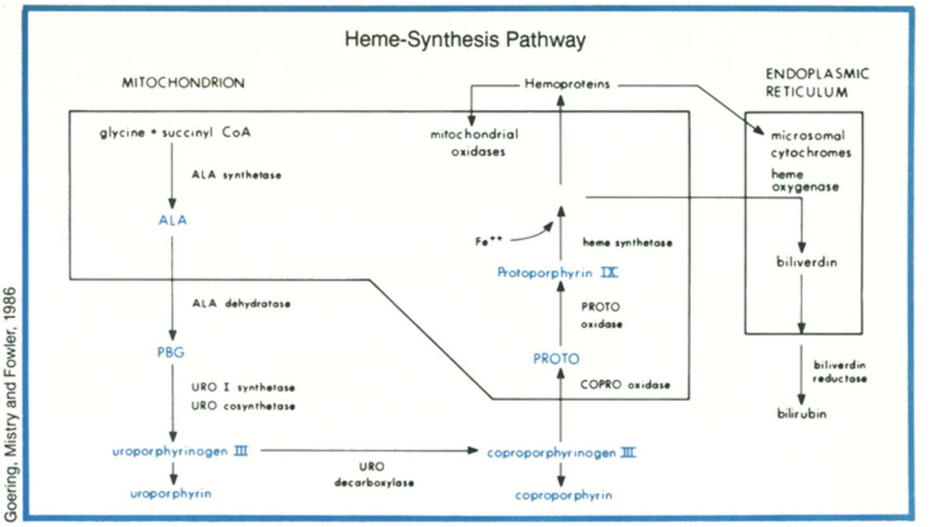
Already some of this research into the development of porphyrinopathies is suggesting practical benefits. A case in point is the work by George S. Drummond at Rockefeller University in New York City. He has been examining factors that alter the effectiveness of the enzyme that breaks down heme. That's important because there are basically two ways to affect the rate of heme synthesis – by interfering with heme production or by slowing its degradation. Drummond and his colleagues have found that several non-iron-based metal porphyrins – most notably tin protoporphyrin – can suppress the activity of the heme-breakdown enzyme.

An unexpected dividend suggested by this work is a potential new treatment for infants suffering from a toxic buildup of bilirubin, a heme-breakdown product, in

the blood. The condition, which can cause brain damage, occurs most commonly in premature infants.

Another potential by-product of porphyrin research is the preliminary identification of a genetic factor in humans that appears to increase an individual's susceptibility to lead toxicity. New work by Robert J. Desnick at the Mt. Sinai School of Medicine in New York City has identified what appears to be a specific "structural gene mutation." Desnick says this mutation, coding for ALAD synthesis, may make that ALAD enzyme – and hence, the porphyrin pathway – more sensitive to lead.

"It's intriguing to conceive that there might be a genetic predisposition to lead poisoning," Desnick says. In fact, he says, his preliminary data hold open the prospect of one day being able to prevent many lead poisoning episodes in the workplace by screening out those persons at highest risk. If developed, such a test would be the first specific toxic-susceptibility screen for an environmental chemical, he says.



Porphyrins and porphyrin precursors are shown in blue. Chemicals ending in "-ase" alongside the arrowed route are the enzymes used to transform porphyrins, heme and its breakdown products.

Sergio Piomelli and his colleagues at Columbia University in New York City have identified yet another factor that can make individuals, especially children, more susceptible to porphyrin-system upset and lead toxicity. That factor is iron deficiency.

In 1972, Piomelli's group developed what has become the standard porphyrin-based fingerprick test to screen children for high lead levels. Ten years later, they demonstrated that, in children, lead's adverse effects on levels of one porphyrin start at blood lead levels as low as 14 to 17 micrograms per deciliter – well below what is currently considered to constitute lead toxicity (SN: 2/16/85, p.103). "Thus," Piomelli says, "well before clinical symptoms become obvious, profound alterations of the heme-biosynthetic pathway occur."

His new research indicates that one way lead alters the heme system is by blocking the final enzyme in the heme-building pathway. Iron and lead apparently compete for a binding site on the enzyme, Piomelli says, and when the binding site is filled with iron, as it should be, the enzyme is less susceptible to inactivation by lead. But when it is lead, rather than iron, that binds to the enzyme – as may occur in iron-deficient children – the enzyme's ability to complete the heme-building process is impaired.

Piomelli's study of blood porphyrin levels in several thousand New York City children now confirms that porphyrin changes – indicating initial adverse lead effects – occur at lower blood lead levels in those children who are most iron-deficient.

"Both lead poisoning and iron deficiency occur at greatest frequency in the youngest and most socially deprived [poorest] children," Piomelli says. Inner-city children from low-income families are the population group at highest risk for lead toxicity, being exposed both to peeling lead-based paint and to high levels of exhaust from cars fueled with leaded gasoline. Supplementing the diet with iron, Piomelli suggests, could limit lead toxicity in such populations.

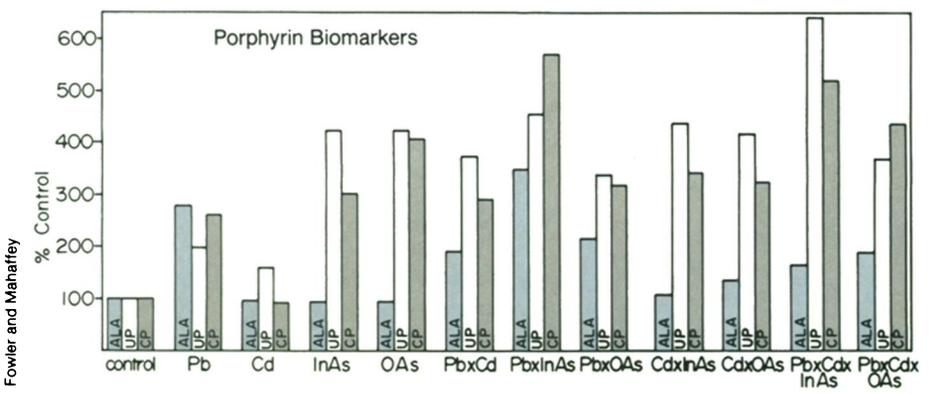
Today the study of porphyrins as toxic sentinels "is basically a small and relatively esoteric field," says Fowler. But it has the potential for revolutionizing human toxicology, he believes.

"In the past we've had to be content with monitoring whether an individual has been exposed to a substance or carries it in his or her body," he says. "Now, by studying porphyrinopathies, we can detect how an animal or person is responding to a chemical. We can actually detect initial biochemical disturbances in their metabolism."

Moreover, he says, these biomarkers offer the advantage of being "specific, very sensitive and something that can usually be studied relatively noninvasively – with a blood or urine sample."

By offering a way to detect early adverse changes – as well as inherent, individual susceptibility – porphyrinopathies may ultimately prove useful to regulatory agencies. The public generally wants limits on exposure to toxic chemicals, and wants to have those limits set at levels below which serious toxicity occurs, Fowler notes.

However, he adds, before imposing limits on chemical exposures, regulators like to have clear-cut evidence of the exposure level at which demonstrable toxicity occurs. "What these porphyrins are finally allowing us to do," says Fowler, "is detect precisely when and where adverse changes begin." □



Varying "fingerprints" of biomarkers in urine, following a rat's 10-week exposure to various mixes of metals/metalloids. ALA is the pre-porphyrin aminolevulinic acid; UP is uroporphyrin; and CP is coproporphyrin. Pb is lead; Cd is cadmium; InAs is inorganic arsenic; and OAs is arsanillic acid. These data show that detectable elevations in excreted porphyrin-related compounds occur after exposure to toxic substances, and that the excretion pattern for each combination of metals and metalloids is unique.