



pediatric and
maternal-fetal

The Monitor

Volume 29 Number 2 July, 2010

The newsletter of the Pediatric and Maternal-Fetal Division of the AACC

Division Officers

Chair-

Nathalie Lepage, PhD

Chair Elect-

Sharon Geaghan, MD

Secretary-

David Grenache, PhD

Treasurer-

Sihe Wang, PhD

Nominating Committee-

Carol Lee, Chair
David Carpentieri

Members at Large-

Dean Carlow, MD, PhD
Uttam Garg, PhD
Angela Ferguson, PhD
Linda Rogers, PhD

Newsletter Editor-

Dennis J. Dietzen, PhD

Newsletter Editorial Board-

Uttam C. Garg, PhD
Stanley F. Lo, PhD
Michael P Metz, MD

In this Issue:

L is for Lead.....2

Interview with an organic
acid.....8

Excerpts from the
Literature.....9

Other News.....12

Annual Meeting
Highlights.....13

PMF Awards.....14

From the Mind of..... The Chair

Dear colleagues and friends in the Pediatric and Maternal Fetal Division, the AACC Annual meeting in July 2010 promises to be full of exciting educational and networking opportunities for all of us. The Pediatric and Maternal-Fetal Division collaborated in the preparation of several educational activities (one brown bag and four symposia). Please make sure you attend the Annual meeting in Anaheim!

In addition, the division will host a meeting on July 27 on "Pediatric Reference Range Initiative Forum and Update". This meeting has been open to all AACC attendees for the past 5 years, and brings the latest developments in the establishment of pediatric reference intervals in the United States and across the world. You will also not want to miss the Division Mixer and Awards Ceremony on July 27 at 18:00 in the Carmel room of the Anaheim Hilton. You will be able to meet colleagues from our division. Light food and refreshment will be served.

The Pediatric and Maternal Fetal Division is proud to provide the members with a listserv that is relevant to our discipline.

Personally, I appreciated all the comments posted from our members in the past six months. It is extremely useful to compare practices between institutions to improve current status.

Members of the Pediatric and Maternal-Fetal Division should also take note that many of our own are candidates for important national AACC offices in the upcoming elections. These include candidates for the Board of Directors including Dennis Dietzen, David Grenache, and Patti Jones, as well as a candidate for President, Mike Bennett.

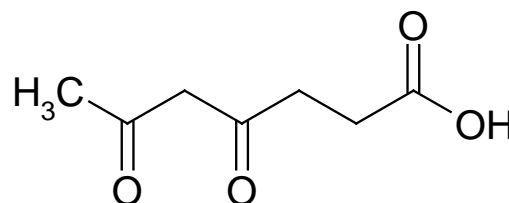
The feature article of *The Monitor* is always selected based on alphabet. In the June version, you will enjoy reading an article on Lead. Please feel free to forward your comments and current practice to the newsletter editor. We would like to develop a section on "comments to the Editor" based on your contributions.

Thank you all for your support to our Division.

Looking forward to seeing you in Anaheim,
Nathalie Lepage, PhD

Interview with an organic acid: Succinylacetone

Once again we bring you in depth coverage of the world of organic acids. This edition's contribution is our first from outside the United States. I guess scientists from other parts of the world also like to converse with our organic acid friends. From the streets of Izmir, Turkey, we listen to the story of the reclusive marker of type I tyrosinemia, succinylacetone. This rare look into the world of this organic acid is sure to be one of the highlights of this issue.



-ed.

The ABC's of Pediatric Laboratory Medicine – L is for Lead

Boris Calderon, MD
-edited by DJD

Clinical Chemistry Fellow
Department of Pathology and Immunology
Washington University School of Medicine



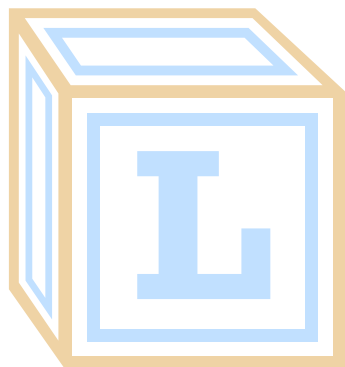
Pb Lead
Atomic Number: 82
Atomic Mass: 207.20

Background

Lead makes up only 0.0013% of the earth's crust but is easily mined and refined. As a result, lead has been redistributed in the environment in multiple forms. It has been used in construction, decoration, and even as a food additive. Even though complications from lead poisoning were first acknowledged in the United States in the early 20th century, lead continued to be utilized in gasoline. Lead was not banned in gasoline until 1996 by the Clean Air Act.¹ A second important contributor to environmental lead was paint, in which lead was used as a pigment, as a curing agent, and to preserve its appearance. Lead-based paint was banned in 1977 by the Consumer Product Safety

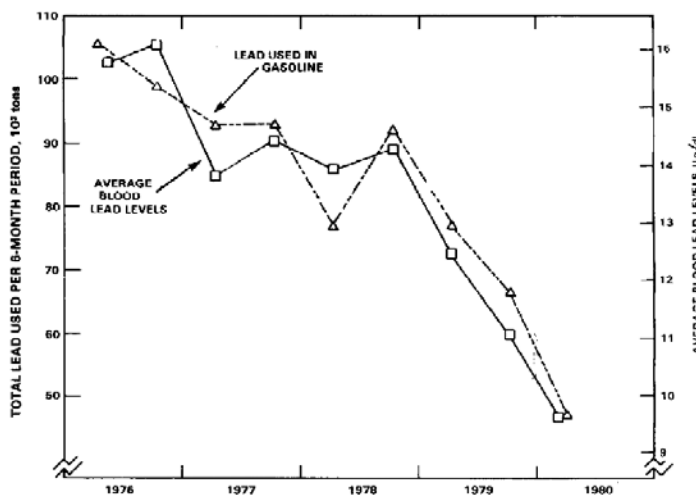
Commission to reduce the risk of lead poisoning in children who may ingest paint chips or peelings.² After these actions, the average blood lead concentration in children decreased by about 80% (Figure 1).^{3,4}

Residual lead remains a problem in the environment because lead cannot be degraded. In many part of the world, lead continues to be used in many products including pigments, gasoline, jet fuel, pottery, solder, cooking vessels, and medications. Table 1 shows a list of common sources of lead exposure. These products, which are occasionally imported into the United States, are potential sources of lead exposure.



Lead makes up only 0.0013% of the earth's crust but is easily mined and refined. As a result, lead has been redistributed in the environment in multiple forms

Figure 1: Parallel decreases in blood lead values observed in the NHANES II study and amounts of lead used in gasoline during 1976-1980. Needleman H. Annu Rev Med 2004. 55:209-22.



Epidemiology

The Centers for Disease Control and Prevention (CDC) currently designates a blood lead level of 10 µg/dl (0.48 µmol/L) or higher as toxic.⁵ The prevalence of lead toxicity has decreased substantially since the 1970s due to environmental control, screening programs, and public awareness. While the incidence of lead poisoning has decreased, an estimated 310,000 children in the United States younger than five years still have elevated blood lead levels. Children in this age group are more susceptible to the toxic effects of lead than

adults.⁶ This susceptibility is due to an immature blood-brain barrier in the developing nervous system and also due to the higher prevalence of iron deficiency, which leads to increased absorption of lead and other metals from the gastrointestinal tract. Other important exposures are from lead dust and hand to mouth contamination. There is a higher prevalence of elevated lead levels in urban rather than rural areas, particularly in inner-city children who live in houses built before the 1970s.⁷

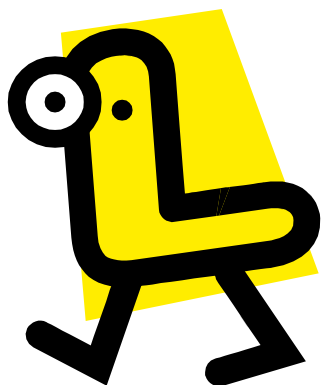


Table 1. Sources of Lead Exposure

Dust containing lead from renovations
Traditional medicine remedies
Imported candy
Imported cosmetics
Imported toys
Paint chips from lead-base paint
Pottery and ceramics
Soil contaminated with lead
Contaminated water by lead from pipes, solder, valves and fixtures
Occupational (construction workers, plumbers, lead miners, steel welders)
Take-home exposures (based on occupational exposure)
Vinyl mini blinds (imported before 1997)
Tobacco smoking

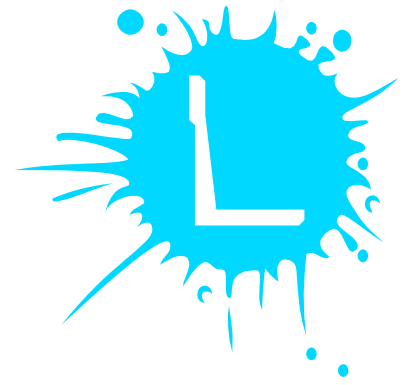
Pathology and Toxicology

The amount of lead absorbed depends on the form of lead presented, the route of exposure, and other factors such as nutritional status and age.⁸ The exposure routes that can cause a significant toxicity are ingestion, inhalation, and dermal contact. Inhaled lead is completely absorbed regardless of the age. Absorption by gastrointestinal exposure differs by age; children absorb a greater proportion than adults (70% versus 20%, respectively).⁸⁻¹⁰ Other factors or deficiencies may increase lead absorption in the gastrointestinal tract, such as fasting and iron or calcium deficiency.^{8,11,12} Dermal contact has become an uncommon exposure route, as leaded gasoline additives are no longer used and lead absorption through the skin is slow.

Once absorbed, 99% of circulating lead is found in erythrocytes. Erythrocyte lead is dispersed into the soft tissues (liver, renal cortex, medulla, pancreas, ovary, spleen, prostate, adrenal gland, brain, fat, testis, heart and lungs) and mineralized tissues (bone and teeth) over the following 4 to 6 weeks (Figure 2). Lipid-dense tissues, such as the central nervous system (CNS), are sensitive to organic forms of lead. Due to the short half-life of lead in blood (28 to 36 days), blood lead levels cannot be used to diagnose or rule out evidence of exposure that occurred more than six weeks prior to testing.¹³

A major problem with lead excretion is its tissue dependent half-life. As mentioned previously, blood lead half-life ranges from 28 to 36 days, while in soft tissue the half-life may be 40 days. Mineralizing tissues have the longest half-life (greater than 25 years). Lead storage varies between children and adults. In adults, approximately 80 to 95% of retained lead is stored in bones, while in children about 70% is stored in bones with the remainder preferentially distributing to soft tissues. Due to slow turnover, lead concentration in bone increases significantly with age.

Lead is distributed in two different compartments within mineralizing tissue: a labile compartment that can easily exchange lead with the blood, and the second compartment referred to as the inert pool. This inert compartment is the one that has the longest half-life. The inert pool of lead in the mineralizing tissues accumulates over lifetime and is the main source of elevated lead level in blood even after the exogenous exposure source has been removed.⁸ Conditions resulting in increased bone turnover such as pregnancy, lactation, postmenopausal osteoporosis, hyperthyroidism, and cisplatin chemotherapy have been shown to increase blood lead concentration through mobilization of bone stores.¹⁴⁻¹⁷ Lead deposition in bone can also be transferred to the fetal skeleton during pregnancy.



A major problem with lead excretion is its tissue dependent half-life. Blood lead half-life ranges from 28-36 days. Mineralizing tissues have the longest half-life (> 25 years).



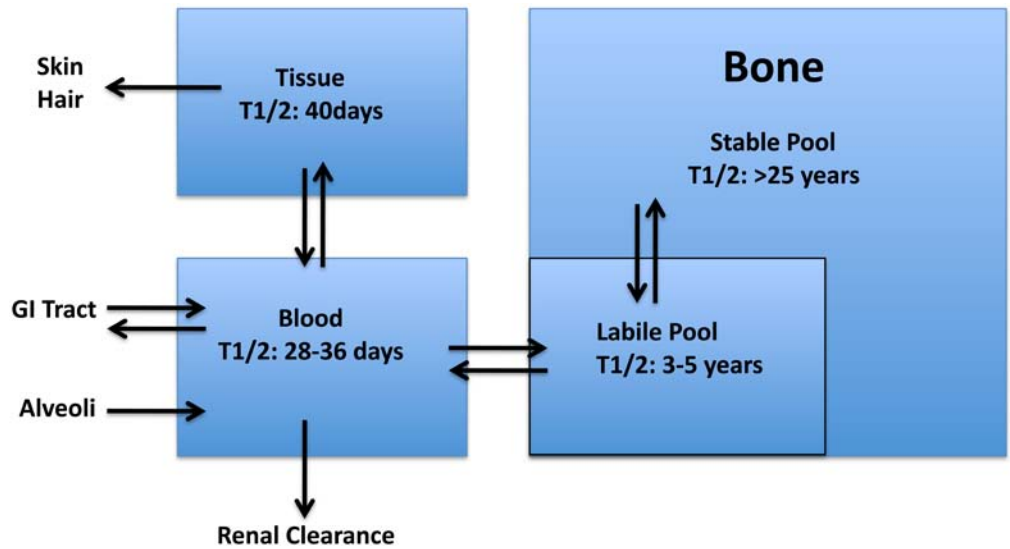
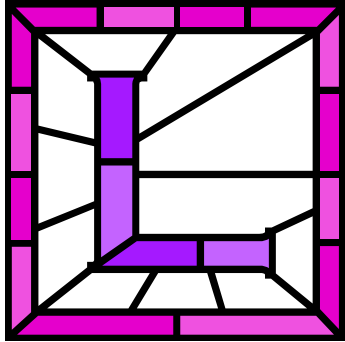


Figure 2: Absorption, distribution, elimination and half-life of lead in blood, soft tissues, and bones.

Lead toxicity can affect several systems but mainly hematological, nervous, and renal.

Pathology and Toxicology, continued

Inorganic lead is not metabolized in the body and is excreted unchanged primarily in the urine. Other pathways for excretion that account for one-third of the total include bile, gastric fluid, and saliva. Alkylated forms of lead undergo oxidative dealkylation to potent neurotoxins, triethyl and trimethyl lead. Transformation is catalyzed by hepatic cytochrome p450-monooxygenase systems.

The basis of lead's toxicity is its interference with a variety of enzymes and structural proteins due to strong binding affinity for sulfhydryl groups. Lead toxicity can affect several systems, but mainly, hematological, nervous, and renal. Since there is no safe lead concentration, any amount of lead in blood is now considered to have some degree of toxicity (Table 2).



Table 2: Effects of Lead Levels at Which Clinical Effects are Observed in Children.

Blood lead concentration (µg/dl)	Effect:
All	Lead deposition in bones
Less than 10	Production of red blood cells slows down
15-20	Abnormally high amount of erythrocyte protoporphyrin blood
Below 25	Subtle behavioral and learning changes
50-60	Loss of nerve signals from brain to muscle
80-100	Brain damage (lead encephalopathy)



Hematologic Toxicity of Lead

The best known of lead's toxic effects involves interference with the heme synthetic pathway by direct inhibition of delta-aminolevulinic acid synthetase (ALAS) and delta-aminolevulinic acid dehydratase (ALAD) (Figure 3). Whole blood lead concentrations greater than 20 µg/dl, inhibit ALAD by about 50%. The two major consequences are decreased hemoglobin synthesis and hemolysis. Decreased hemoglobin has been observed with blood lead levels around 40 µg/dl and prolonged exposure to high levels of lead has been shown to reduce erythrocyte survival. Acute lead exposure (with lead concentrations greater than 70 µg/dl) has been associated with hemolytic anemia. Since ALAD can also be affected by other states of disease such as porphyria, liver cirrhosis, and alcoholism, ALAD activity cannot be used to diagnose lead toxicity. The use of urinary excretion of aminolevulinic acid (ALA), which is the substrate that accumulates as a result of decrease ALAD, has been used in the past as a marker for lead toxicity. However, it is not a useful marker in low-level toxicity, since it can only be detected when blood

lead concentrations exceed 35 µg/dl in adults and 25 µg/dl in children.

Human ALAD is a polymorphic enzyme with two common alleles, ALAD1 and ALAD2.¹⁸ Expression of the ALAD2 allele appears to increase susceptibility to lead toxicity. It has also been reported that a common cause of hemolytic anemia, glucose-6-phosphate dehydrogenase (G6PD) deficiency may also increase the susceptibility to lead toxicity.²⁰ Lead can also inhibit ferrochelatase, an enzyme that catalyzes the insertion of iron into protoporphyrin IX (Figure 3). This inhibition, results in an increase of the erythrocyte protoporphyrin (EP), typically assayed as zinc protoporphyrin (ZPP). However, ZPP may also be found elevated in iron deficiency. EP levels usually are not elevated until lead levels are greater than 30 µg/dl, making it a poor screening test for mild lead toxicity, but helpful in monitoring response to therapeutic interventions. Impairment of pyrimidine 5'-nucleotidase is another effect of lead toxicity. The inhibition of this enzyme causes the accumulation of pyrimidine nucleotides inside erythrocytes leading to a lack of maturation and eventually anemia.



The most well known of lead's toxic effects involves interference with the heme synthetic pathway by direct inhibition of delta-aminolevulinic acid synthetase and delta-aminolevulinic acid dehydratase.

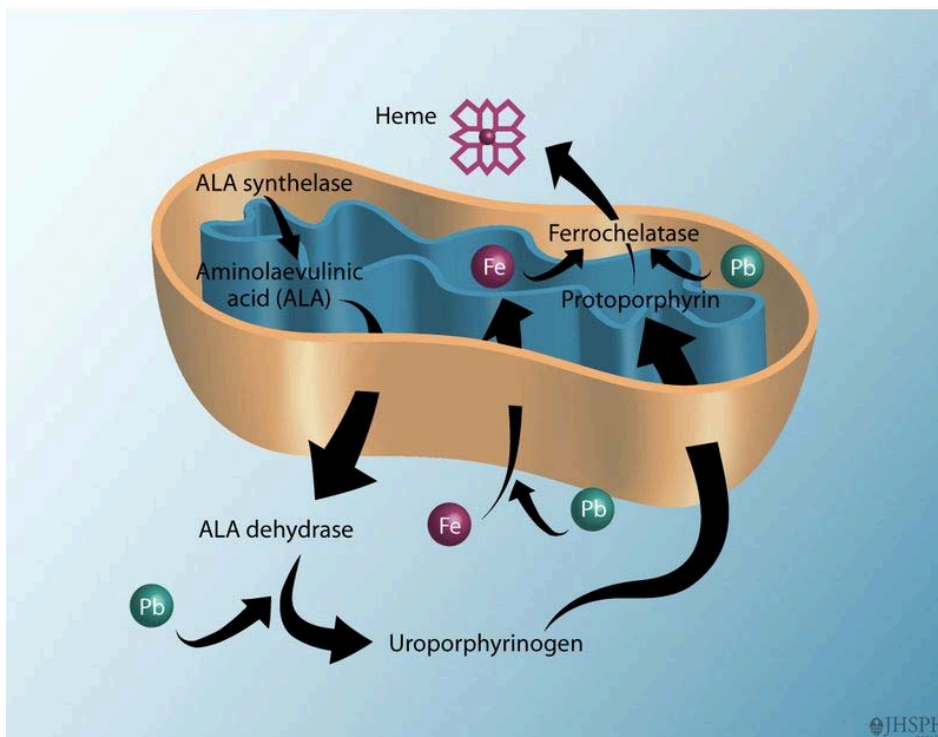


Figure 3. Effects of Lead on Heme Synthesis in the Mammalian Erythrocyte from *Public Health Toxicology*. Copyright © Johns Hopkins Bloomberg School of Public Health. Creative Commons BY-NC-SA.

Nervous System Toxicity

Even though the pathophysiology of lead in the nervous system has not been entirely clarified, it is thought that lead inhibits energy metabolism by uncoupling mitochondrial oxidative phosphorylation and altering phosphocreatine (a surrogate for ATP) availability. The manifestations of lead neurotoxicity in adults and children include irritability, headache, attention deficit, memory loss, and cognitive impairment (Table 2). In adults, the most common documented neurological symptom of lead exposure is peripheral

neuropathy, typically involving extensor muscles. It is known that lead competes with or mimics the action of calcium. Lead competes for calcium binding sites in the cerebellum on Protein Kinase C. This process affects calcium entry into cells altering neuronal function, mitochondrial structure, cellular respiration, and neuronal signaling.²⁰ Lead is particularly damaging to the developing nervous system of the fetus that lacks the lead-binding proteins found in mature astroglia, which normally sequester and eliminate lead.

Renal Toxicity

Lead nephropathy manifests as proximal tubular damage, glomerular sclerosis and interstitial fibrosis. Renal insufficiency occurs by acute lead exposure, however, there is evidence that renal damage occurs

at much lower exposure levels. Multiple studies have defined a strong connection between blood lead levels and a decline in renal function.²¹⁻²⁴

Analytic Considerations for Lead Determination

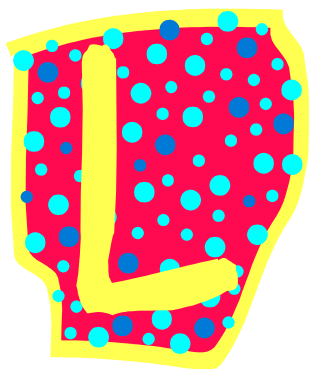
As circulating lead is primarily localized to erythrocytes, the sample of choice for determination of lead is whole blood. Urine may also be employed when evaluating excretion of lead in chelation therapy. For evaluation of chronic exposure, lead determination in nails or hair has also been used. Capillary blood sampling is used primarily in community based screening programs but is subject to interference if not performed properly. Capillary samples may be subject to contamination with exogenous lead and may give a false-positive result. The most common causes of falsely elevated lead levels from capillary sampling include the inadequate use of gloves by phlebotomist, use of alcohol wipes contaminated with lead-base ink, inadequate cleaning of finger, and failure to wipe off the first drop of blood.²⁵ Venous blood testing may also run into contamination problems that may also give false positive results. Such contaminations have been reported from lots of heparin containing lead that are used as an anticoagulant. Lead-free heparinized vacutainers or EDTA as an anticoagulant is recommended. No differences in blood lead concentrations have been observed between properly collected venous and capillary specimens. Heparinized, refrigerated blood samples are stable for 2 weeks, but EDTA blood samples are stable for several months if frozen at -20°C.

Urine collection must be performed in lead-free borosilicate or polyethylene bottles. Urine is stable in preserved urine for 1 week at 4°C. High concentrations of gadolinium and iodine are known to interfere with most metal analyses. If gadolinium or iodine-containing contrast media has been administered, a specimen should not be collected for 96 hours.²⁵

The definitive method for lead determination is inductively coupled plasma mass spectrometry (ICP/MS). This method has been employed by the Center for Disease Control and Prevention (CDC) for establishing lead concentrations in samples for licensure and proficiency testing. In this technique the sample is mixed with argon plasma at 10,000°C which accomplishes desolvation and ionization. The three most abundant, naturally occurring lead isotopes measured are at m/z 206, 207, and 208. Anodic stripping voltammetry is a fast, relatively simple technique employed in hand-held instruments (e.g., LeadCare®, ESA, Chelmsford, MA) that is used in community screening programs. In this technique lead from the sample is first reduced to its ground state then reoxidized by increasing electrochemical potential. The number of electrons released as lead is ionized is proportional to the amount of lead in the sample. Elevated results derived from these screening programs are typically confirmed by ICP/MS.



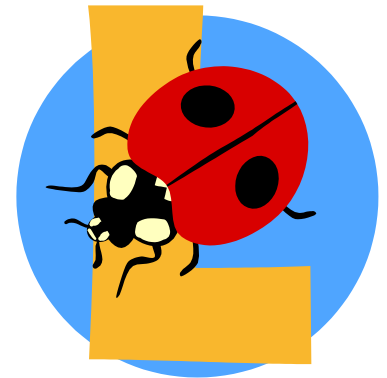
The specimen of choice for blood lead determination is anticoagulated whole blood. The definitive method for blood lead determination is inductively coupled plasma-mass spectrometry (ICP/MS).



Summary and Conclusion

In conclusion, removal of contributors to environmental lead has drastically reduced blood lead levels over the past 30 years. Secondary prevention programs that employ screening continue to identify children with significant exposure to residual environmental lead. Determination

of blood lead concentration is the preferred screening tool. Blood lead concentrations greater than 10 µg/dl may ultimately lead to hematologic, neurologic, and renal disease. Elevated lead concentrations require prompt removal of the patient from the source of exposure and may require chelation therapy to acutely reduce body lead burden.



References

1. U.S. Environmental Protection Agency (29 January 1996). "EPA Takes Final Step in Phaseout of Leaded Gasoline". <http://www.epa.gov/history/topics/lead/02.htm>
2. U.S. Consumer Product Safety Commission (September 2, 1977) Release # 77-096. <http://www.cpsc.gov/cpscpub/prerel/prhtml/77/77096.html>.
3. Needleman H. Lead Poisoning. *Ann Rev Med* 2004. 55:209-22.
4. Centers for Disease Control and Prevention. Childhood Lead Poisoning Data, Statistics, and Surveillance. National Surveillance Data (1997-2007). <http://www.cdc.gov/nceh/lead/data/national.htm>.
5. Centers for Disease Control and Prevention. Lead: topic home. <http://www.cdc.gov/lead/>.
6. Wright, RO, Tsaih, SW, Schwartz, J, et al. Association between iron deficiency and blood lead level in a longitudinal analysis of children followed in an urban primary care clinic. *J Pediatr* 2003;142:9.
7. Agency for Toxic Substances and Disease Registry. Case Studies in Environmental Medicine (CSEM): Lead Toxicity Cover Page. http://www.atsdr.cdc.gov/csem/lead/bcover_page2.html.
8. Patrick L. Lead toxicity part I: Exposure, evaluation, and treatment. *Altern Med Rev*. 2006;11:2-22.
9. Alexander, FW, Clayton, BE, Delves, HT. Mineral and trace-metal balances in children receiving normal and synthetic diets. *Q J Med* 1974;43:89.
10. Ziegler, EE, Edwards, BB, Jensen, RL, et al. Absorption and retention of lead by infants. *Pediatr Res* 1978;12:29.
11. James, HM, Hilburn, ME, Blair, JA. Effects of meals and meal times on uptake of lead from the gastrointestinal tract in humans. *Hum Toxicol* 1985;4:401.
12. Mahaffey, KR. Environmental lead toxicity: nutrition as a component of intervention. *Environ Health Perspect* 1990;89:75.
13. Rabinowitz MB, Wetherill GW, Kopple JD. Kinetic analysis of lead metabolism in healthy humans. *J Clin Invest* 1976;58:260-270.
14. Silbergeld EK. Lead in bone: implications for toxicology during pregnancy and lactation. *Environ Health Perspect* 1991;91:63-70.
15. Silbergeld EK, Schwartz J, Mahaffey K. Lead and osteoporosis: mobilization of lead from bone in postmenopausal women. *Environ Res* 1988;47:79-94.
16. Klein M, Barbe R, Pascal V, et al. Lead poisoning secondary to hyperthyroidism: report of two cases. *Eur J Endocrinol* 1998;138:185-188.
17. Beaney RP, Buxton EJ, El-Sharkawi AM, et al. Cisplatin invoked lead mobilisation studies. *Br J Cancer* 1990;61:169-170.
18. Smith CM, Wang X, Hu H, Kelsey KT. A polymorphism in the delta-aminolevulinic acid dehydratase gene may modify the pharmacokinetics and toxicity of lead. *Environ Health Perspect* 1995;103:248-253.
19. McIntire MS, Angle CR. Air lead: relation to lead in blood of black school children deficient in glucose-6-phosphate dehydrogenase. *Science* 1972;177:520-522.
20. Bressler JP, Goldstein GW. Mechanisms of lead neurotoxicity. *Biochem Pharmacol* 1991;41:479-484.
21. Staessen JA, Lauwerys RR, Buchet JP, et al. Impairment of renal function with increasing blood lead concentrations in the general population. The Cadmibel Study Group. *N Engl J Med* 1992;327:151-156.
22. Payton M, Hu H, Sparrow D, Weiss ST. Low-level lead exposure and renal function in the Normative Aging Study. *Am J Epidemiol* 1994;140:821-829.
23. Kim R, Rotnitsky A, Sparrow D, et al. A longitudinal study of low-level lead exposure and impairment of renal function. The Normative Aging Study. *JAMA* 1996;275:1177-1181.
24. Muntner P, He J, Vupputuri S, et al. Blood lead and chronic kidney disease in the general United States population: results from NHANES III. *Kidney Int* 2003;63:1044-1050.
25. Kaplan LA, Pesce AJ. Clinical Chemistry: Theory, Analysis, Correlation. Methods of Analysis: Lead. Mosby; 5th edition. 2009.

Lead concentrations > 10 µg/dL require prompt removal of the patient from the source of exposure and may require chelation therapy to acutely reduce body lead burden.





Interview with an organic acid: Succinylacetone

Tijen Tanyalcin MD, PhD
Tanyalcin Medical Laboratory
Izmir Turkey

tyrosine and significant urinary excretion of other tyrosine metabolites such as *p*-hydroxyphenylpyruvate and *p*-hydroxyphenyllactate that can be detected by urine organic acid testing. Additionally, my structure closely resembles that of delta aminolevulinic acid (δ -ALA). I can competitively inhibit δ -ALA dehydratase, an enzyme involved in heme biosynthesis, and cause accumulation of δ -ALA which is neurotoxic. SAA and I can cause an inhibition of methionine-S-adenosyl transferase activity resulting with possible increase in methionine levels in tyrosinemia patients.

The M : Is it important to find you?

OA: Yes, it is. I am very special. I am being introduced as a biomarker for tyrosinemia type I in newborn screening laboratories. Infants with tyrosinemia usually have normal or only modestly elevated or blood concentrations of tyrosine. I can fix this problem.

The M: If someone wanted to find you, how would they accomplish this?

OA: You can find me both in blood and in urine. I can be extracted from blood spots and analyzed by tandem mass spectrometry. My inclusion into analysis of acylcarnitines and amino acids allows for rapid and cost effective detection of tyrosinemia. If you want to see me in urine, you have to extract me first and turn me into a volatile TMS derivative and shoot me through this really hot thing called a GC/MS used for other organic acids. I am not alone in urine, you will also see two of my cohorts, *p*-hydroxyphenyllactate and *p*-hydroxyphenylpyruvate. Together we sing the tyrosyluria song. It was a big hit here in Turkey.

The M: Well, Mr SUAC, it is a great pleasure to talk with you.

OA: Feelings are mutual, I am off to meet my friends because we are going to be practicing for the concert which is going to be held next week. I hope you can join us.

Today we interview succinylacetone (4,6-dioxoheptanoic acid). This molecule is an indicator of hereditary tyrosinemia type I, a severe autosomal recessive metabolic disorder associated with mutation in fumarylacetoacetate hydrolase (FAAH, EC 3.7.1.2.).

The M: Would you describe yourself for us?

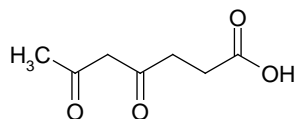
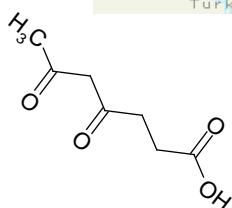
OA: I am a substance that nobody likes to see in the blood or urine of babies. Just call me SUAC for short. Indeed when you see me, I am not alone, I am always together with my partner in crime succinyl acetoacetate (SAA, 3,5-dioxooctanedioic acid). I am his decarboxylated cousin. We both stem from maleylacetoacetate or fumarylacetoacetate.

The M: What causes you to accumulate?

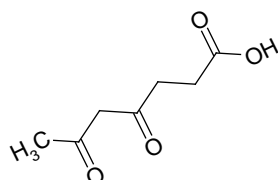
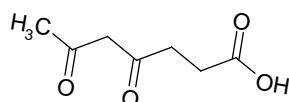
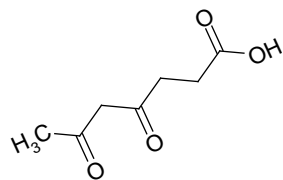
OA: There are some reasons that cause me to accumulate. When FAAH is not working, it cannot break down tyrosine. Tyrosine and other substances then build up in blood and in urine. I am found only in patients with tyrosinemia type I, no other disorders.

The M: Are you harmful ?

OA: Of course I am. I am notorious for destruction. I cause impairment of renal tubular absorption and liver damage. That is why the disease tyrosinemia type I is often referred to as hepatorenal tyrosinemia. I interfere with para-hydroxyphenylpyruvic acid dioxygenase (*p*-HPPD), resulting in a mild to moderate accumulation of plasma



Succinylacetone facts	
Molecular Formula	C ₇ H ₁₀ O ₄
Molecular Weight	158.15 g/mol
Density:	1.189 g/mL
Melting Point:	66-67°C
Boiling Point:	318.7 °C@1atm



Excerpts from the Literature

(Compiled by the editorial board)

1. Improved treatment options for Phenylketonuria. *Mol Gen Metab* 2010;99:S90-S95 (UCG)

Phenylketonuria (PKU) was the first inherited metabolic disorder for which dietary treatment was found to prevent major clinical features. Despite the establishment of dietary management almost 60 years ago, challenges in the treatment and outcome still remain. Although, dietary treatment still remains the mainstream therapy, in the last few years several promising treatment options have emerged. At the dietary level, new foods are being developed which are more palatable and practical to use. A protein called glycomacropeptide, derived from goat milk, is almost free of phenylalanine and has a better taste than current protein substitutes. However, this is not a full protein substitute as it lacks other amino acids such as tyrosine and tryptophan. At the gut level, the enzyme phenylalanine-ammonia lyase (PAL) which metabolizes phenylalanine is available to metabolize phenylalanine before absorption. In

addition, an injectable form of PAL modified with polyethylene glycol for stability has the potential to decrease both vascular and tissue phenylalanine. This enzyme is undergoing clinical trials with promising results. Large neutral amino acid intake (e.g., tryptophan, threonine, isoleucine, leucine, valine, methionine, and histidine) has also been found to decrease brain phenylalanine levels by inhibiting the transport of phenylalanine from gut to blood and blood to brain. Studies have shown that many patients with PKU, particularly mild forms, respond to tetrahydrobiopterin (BH₄) therapy. 6R-BH₄ (sapropterin, Kuvan®) has been approved by the FDA and EMEA (European Agency for Evaluation of Medicinal Products). In addition to these new promising therapies, preclinical work in the fields of gene and cellular therapy continues. Overall, the future of new therapies for the treatment of PKU appears bright.

Large neutral amino acid intake has also been found to decrease brain phenylalanine levels by inhibiting transport of phenylalanine from gut to blood and blood to brain.

2. Yield of lumbar puncture in complex febrile seizure. *Pediatrics* 2010;126:62-69 (DJD)

The objective of this study by Kimia et al was to assess the rate of bacterial meningitis in children who present with their first complex febrile seizure. Febrile seizures are the most common type of childhood seizure, affecting between 2-5% of all children. Complex seizures typically last longer and are more likely to recur compared to simple seizures. There is persistent concern that febrile seizures are the sole presenting manifestation of acute bacterial meningitis.

This study retrospectively enrolled 526 patients (44% female) between 6 and 60 months of age who presented with their first complex febrile seizure. Of these, 340 had a lumbar puncture. Fourteen patients had pleocytosis (>7 WBC/mm³). Only three of these patients had bacterial meningitis. Two had positive CSF culture for *S. pneumoniae* while a third had presumptive bacterial meningitis based on a positive blood culture for the same organism but negative CSF.

This study included patients both before and after the widespread practice of pneumococcal vaccination and

immunization for *Haemophilus influenzae* (Hib). Of the three patients identified with bacterial meningitis, two presented prior to introduction of vaccination. The overall rate of acute meningitis in children presenting with complex febrile seizure in the study is, therefore, <1% and only about <0.3% when considering only those patients who had been previously immunized.

Strengths of the study include the large cohort of ED visits that were available for examination (~650,000) and a setting that included children both before and after widespread immunization for common causes of bacterial meningitis. Weaknesses included the fact that CSF data were not available on a larger subset of patients and the fact that many patients may have been followed elsewhere after initial presentation. The study suggests that children whose only presenting feature is a complex febrile seizure, with no other signs and symptoms, are at extremely low risk for bacterial meningitis. Blanket recommendations for lumbar puncture in such patients are not well-founded.

The study suggests that children whose only presenting feature is a complex febrile seizure, with no other signs and symptoms, are at extremely low risk for bacterial meningitis.

3. Aminoglycosides and deafness during treatment of acute leukemia. Arch Dis Child 2010;95:153 (MPM)

The m.1555A>G mutation is associated with sensitivity to ototoxic medications such as aminoglycosides. This study suggests that it is perhaps time to look for such mitochondrial mutations in children diagnosed with a variety of disorders which dictate aminoglycoside therapy.

Therapeutic drug monitoring of aminoglycosides is a common practice in both pediatric and adult hospitals. It is often difficult and less than satisfying. Challenges arise in determining dose and timing of drug administration. Most aminoglycoside assays have analytical ranges that are inadequate for peak levels achieved with once daily dosing regimens and provide less than ideal performance for accuracy and precision. The demands of the pharmacy further complicate matters. Children commonly receive multiple courses of aminoglycosides. The poor correlation between drug levels and the risk of nephrotoxicity and ototoxicity adds to the overall challenge of TDM for aminoglycosides.

This is the report of three cases of deafness acquired after aminoglycoside treatment. The children were diagnosed with Acute Lymphocytic Leukemia and treated with a standard UK ALL protocol. As is common, they all received multiple courses of aminoglycosides (gentamicin, kanamycin, and other non-specified aminoglycosides) with levels in the therapeutic range. They developed profound deafness early in the course of their treatment. Interestingly, the children were of diverse ethnic origin, one was caucasian, one British-Caribbean, and one Asian. Each of these children carried the

m.1555A>G mutation in the mitochondria 12S rRNA gene.

Mitochondrial DNA mutations are recognized to be a common genetic cause of sensorineural hearing loss. A useful review of mitochondrial mutations and hearing loss is published in the International Journal of Audiology 2008;47:702 by Bindu et al. The m.1555A>G mutation has been reported to be an important cause of nonsyndromic hereditary hearing loss as well as exquisite sensitivity to ototoxic medications (particularly aminoglycosides). A single dose of aminoglycosides are reputed to be able to cause deafness in some carriers. Vandebona et al reported in NEJM 2009;360:642 their conclusion that the m.1555A>G mutation affects about 1 in 500 subjects in their population-based cohort.

The m.1555A>G mutation is associated with sensitivity to ototoxic medication (particularly aminoglycosides). The authors suggest that this mutation should be sought in at risk populations. Perhaps it is time to look for mitochondrial point mutations in children diagnosed with disorders whose treatment commonly involves multiple courses of aminoglycosides, including leukemia, cystic fibrosis & prematurity.

4. Monitoring children with Duarte Galactosemia: Clin Chem 2010;56:1177-82 + editorial:1045-1046 (SFL)

Ficicioglu et al. demonstrate that less commonly measured biomarkers, RBC galactitol and RBC galactonate are increased despite normal levels of RBC galactose 1-phosphate. This suggests that these new markers may be more sensitive metabolites for assessing galactose burden in DG patients.

An evaluation of biomarkers for Duarte galactosemic (DG) patients (ages 1-6 years) was undertaken by Ficicioglu and colleagues. The Duarte allele encodes for a ASP residue rather than the wild type ASN residue at peptide position 314. Patients who carry one Duarte mutation and one classical galactosemia mutation in the galactose-1-phosphate uridylyltransferase (GALT) gene have roughly 25% of normal GALT activity. The galactose burden placed upon DG children is monitored by measuring RBC galactose 1-phosphate. For children on a regular diet, this study shows that less commonly measured biomarkers such as RBC galactitol and RBC galactonate are increased despite normal levels of RBC galactose 1-phosphate. This suggests that these new markers may be more sensitive metabolites for

assessing galactose burden in DG patients. For the patients with increased RBC galactitol and galactonate, no physical or developmental complications were identified, however this does not rule out the development of secondary complications later in life.

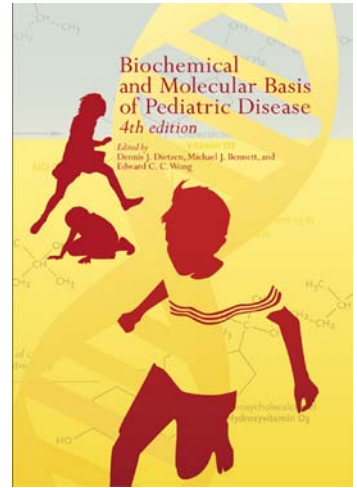
The clinical controversy (see editorial) is whether DG newborns require any dietary restriction of lactose and galactose intake. The time and expense to counsel and treat DG patients is considerable. A mistaken diagnosis of galactosemia may lead to many years of unnecessary dietary restriction and biochemical testing. Can these new biomarkers be useful in determining the correct treatment regimen for newborn DG patients? No one knows. The study to answer the question remains to be conducted.

IN OTHER NEWS.....

Book Alert: The Biochemical and Molecular Basis of Pediatric Disease. Fourth Edition.

This continuation of the series originally edited by Drs. Jocelyn Hicks, Steve Soldin, and Nader Rifai has a new slate of editors and a new slate of authors and topics. Much has transpired in pediatric laboratory medicine since the last edition in 1998. Dr. Ed Wong (Children's National Medical Center), Dr. Mike Bennett (Children's Hospital of Philadelphia), and your faithful newsletter editor (St. Louis Children's Hospital) have spanned the globe for experts in a wide array of subjects. All chapters have been updated with the

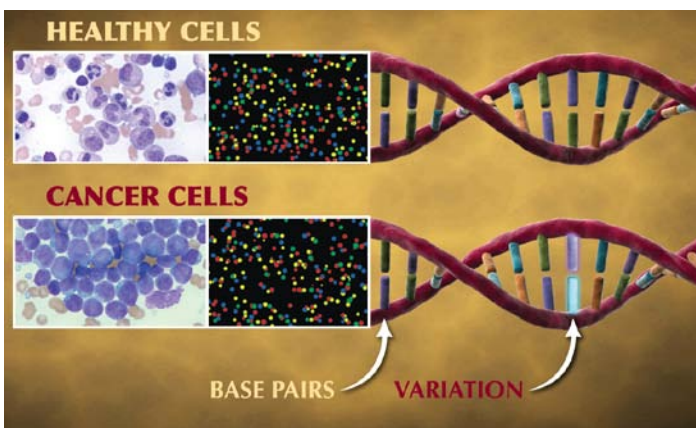
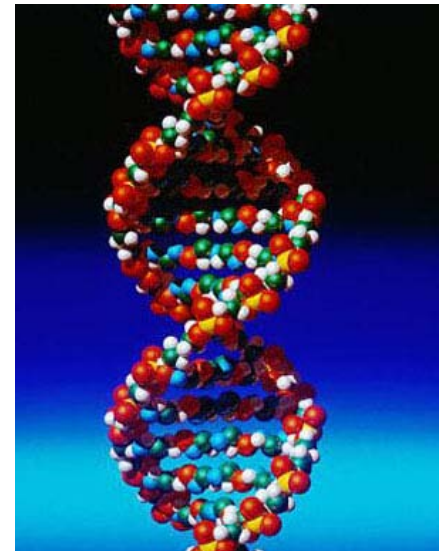
latest information in the field and many new chapters have been included including ones on iron metabolism, cystic fibrosis, endocrine emergencies, allergy, and coagulopathies. As much as possible, clinical and laboratory experts have been paired to provide the most comprehensive and up to date information about disorders that span the neonatal period through adolescence. The book will be available this summer. Look for it at the AACC bookstore in Anaheim. In my totally objective opinion, this book should be on all your bookshelves.



St. Jude's Children's Research Hospital and the Washington University Genome Center join forces to sequence pediatric cancer genomes.

In the last two years, scientists at the Washington University Genome Center have begun to sequence entire cancer genomes and compare them to those from normal tissue in the affected individual. The first sequence was obtained from a middle-aged female with AML (*Nature* 2008;456:66-72). In this manner new changes in cancer genomes that may improve detection and therapy are likely to be uncovered. Washington University and St. Jude's have now joined forces to sequence the genomes of childhood leukemia, brain tumors, and sarcomas. In this \$65

million dollar project, scientists will take advantage of the high-speed sequencing capabilities at Washington University and a repository of over 50,000 biological samples from children with cancer which have accumulated since the 1970's. The technological demands for the project are substantial as the groups plan to sequence 600 cases, 2 genomes per case, with 30X coverage to ensure accuracy. Future projects will pursue non-coding alterations to the cancer genome including those involving RNA and epigenetic changes that may alter gene expression.



GUESS WHAT?
IT'S ALMOST
HERE!



The 2010 Annual meeting promises to be among the biggest and baddest meetings in recent memory. Following are some of the scientific and social highlights for those of us in the Pediatric Division. As always, the Annual meeting will be once again.....an excuse to behave like children.

Highlights

Consult your program for session types, speakers, times, and locations.

Sunday, July 25

Opening Plenary: Wallace H. Coulter Lecture by John Trojanowski
"The Impact of Biomarkers on the Diagnosis of Alzheimer's Disease"

Monday, July 26

Plenary: Gail Wilensky, "The Changing and Challenging Healthcare Landscape."

- + hCG: Beyond a Test for Pregnancy
- + Glucose Meters and Tight Glycemic Control
- + The Practice and Promise of Metabolomics
- + Neonatal Bilirubin: Clinical Guidelines and Different Measurement Technologies
- + Pitfalls and Errors associated with Common POC Tests and Devices.



Tuesday, July 27

Plenary: James Thompson, "Human Induced Pluripotent Stem Cells Derived from Episomal Vectors"

- + Immunosuppression in Solid Organ Transplantation
- + Testosterone Measurements in Disorders of Androgen Excess and Deficiency
- + Drugs of Abuse Testing in Alternative Specimens
- + Sepsis as a Critical Illness: New Perspectives in Pathogenesis and Diagnosis
- + Vitamin D Revisited
- + Reference Intervals in Neonatal to Geriatric Populations

- ++ Update on Pediatric Reference Interval Initiative**
- ++ PMF, Molecular Pathology, and Industry Division Joint Mixer**

Wednesday, July 28

Plenary: Paul Ridker, "Inflammation, hsCRP, and Cardiovascular Prevention: A Paradigm Shift"

- + Estimated GFR: New Equations, New Markers, and New Applications
- + Infectious Disease Testing: Molecular Approaches
- + LC/MS/MS: Various Applications in the Clinical Laboratory
- + How to Avoid Errors in Laboratory Medicine
- + Personalized Medicine in Pediatric Neurology





Thursday, July 29

Plenary: Leroy Hood, “Systems Medicine, Transforming Technologies and the Emergence of P4 Medicine: Perspective, Personalized, Preventative, and Participatory”

- + Lipids in Nutrition: Role in Health and Cardiovascular Disease
- + Definitions in Endocrinology
- + Measuring Proteins in Clinical Samples Using LC/MS/MS

Thursday, July 29: Pediatric/Fetal Chemistry Poster Session, 9:00-12:00

- E-125: Can we use the glucose challenge test more efficiently?
- E-127: Prevalence of anemia and iron deficiency in children from Brasilia
- E-128: Compound heterozygous mutations in two classic CAH female patients
- E-129: 7 α -hydroxylase gene polymorphisms and lipid levels in Chinese children
- E-130: Ethnic differences in thyroid tests during the first trimester of pregnancy
- E-131: Value of detection of the fetal SRY gene during pregnancy by RT-qPCR
- E-132: Serologic screening of Korean pregnant women for toxoplasmosis
- E-133: Antibodies to diabetes antigens in children diagnosed with Type I diabetes
- E-134: Evaluation of thyroid function tests in the first and second trimester
- E-135: Plasma BNP and NT-proBNP levels in children with heart failure
- E-136: Cord blood and maternal serum neopterin levels in pre-eclampsia
- E-137: Assessment of status in pregnant women and newborns in South Spain
- E-138: Measurement of asymmetric dimethylarginine in pregnancy
- E-139: Multiplex assay for newborn screening of galactosemia using UPLC/MS
- E-140: Prevalence of risk factors and insulin resistance in overweight children
- E-141: Evaluation of measurement of nRBCs in neonatal blood
- E-142: Simultaneous detection of trisomy 21, 18, and 13 using PCR
- E-143: Time-resolved immunofluorometric assay for TSH using Eu(III) chelate
- E-144: Pediatric hematological reference values for central Ghana
- E-145: Pediatric reference intervals for 25 analytes on the Vitros 5600
- E-146: Evaluation of an in-line blood gas monitor in the NICU
- E-147: Validation of lamellar body counts using three hematology analyzers
- E-148: Utility of the “shake test”, L/S ratio, and PG to predict fetal lung maturity
- E-149: Validation of an assay standardized to GC/MS for unconjugated estriol
- E-150: Gestational diabetic patients have increased circulating leptin
- E-151: A panel of cervicovaginal biomarkers predicts gestational age at delivery
- E-152: Rubella seroepidemiology and immunization in Taiwan
- E-153: Prevalence and antibiotic sensitivity of bacteria in rural Africa





Award Winners

Pediatric and Maternal-Fetal



Outstanding Contributions to Pediatric and Maternal-Fetal Clinical Chemistry

Edward Ashwood, MD. ARUP Laboratories, Salt Lake City, Utah.

Dr. Ashwood is President and CEO of ARUP Laboratories and a Professor of Pathology at the University of Utah School of Medicine. He is best known for his role as co-editor of the Tietz Textbook of Clinical Chemistry and Molecular Diagnostics but has made many contributions in the clinical chemistry of pregnancy. He has maintained a longstanding interest in biomarkers of fetal lung maturity in amniotic fluid and has published multiple studies characterizing the accuracy of serum bilirubin measurements in his capacity on the Chemistry Resource Committee of the CAP. Dr. Ashwood will be presented with the Award at the PMF mixer on Tuesday, July 27, from 6-8 in the Carmel Room of the Hilton Anaheim Hotel.

Best Poster Award

J. He, University of British Columbia, Vancouver, BC, Canada

B-124: Genetic variants of transcription factor NF-Kappa B and atopic diseases.

Best Student and Young Faculty Award

Christina Lockwood, Washington University School of Medicine, St. Louis, MO.

E-147: Validation of lamellar body counts using three hematology analyzers

Finally, the Changing of the PMF Newsletter Guard.....



This edition of *The Monitor* will be my last as editor. I have enjoyed carrying you through the ABCs from F through L. I thought I would bow out well before we got to Q. I have had fun creating interviews with organic acids and am heartened to see this feature gaining an international following. My successor is Dr. Angela Ferguson (pictured at right) whom I know well because she was a fellow at Washington University and now directs the laboratories at Children's Mercy Hospital in Kansas City with Dr. Uttam Garg. She will have at her disposal the wise counsel of my stellar editorial board. I am sure she would appreciate newsworthy tidbits starting today. I wish her well. See you in Anaheim.



Dennis