

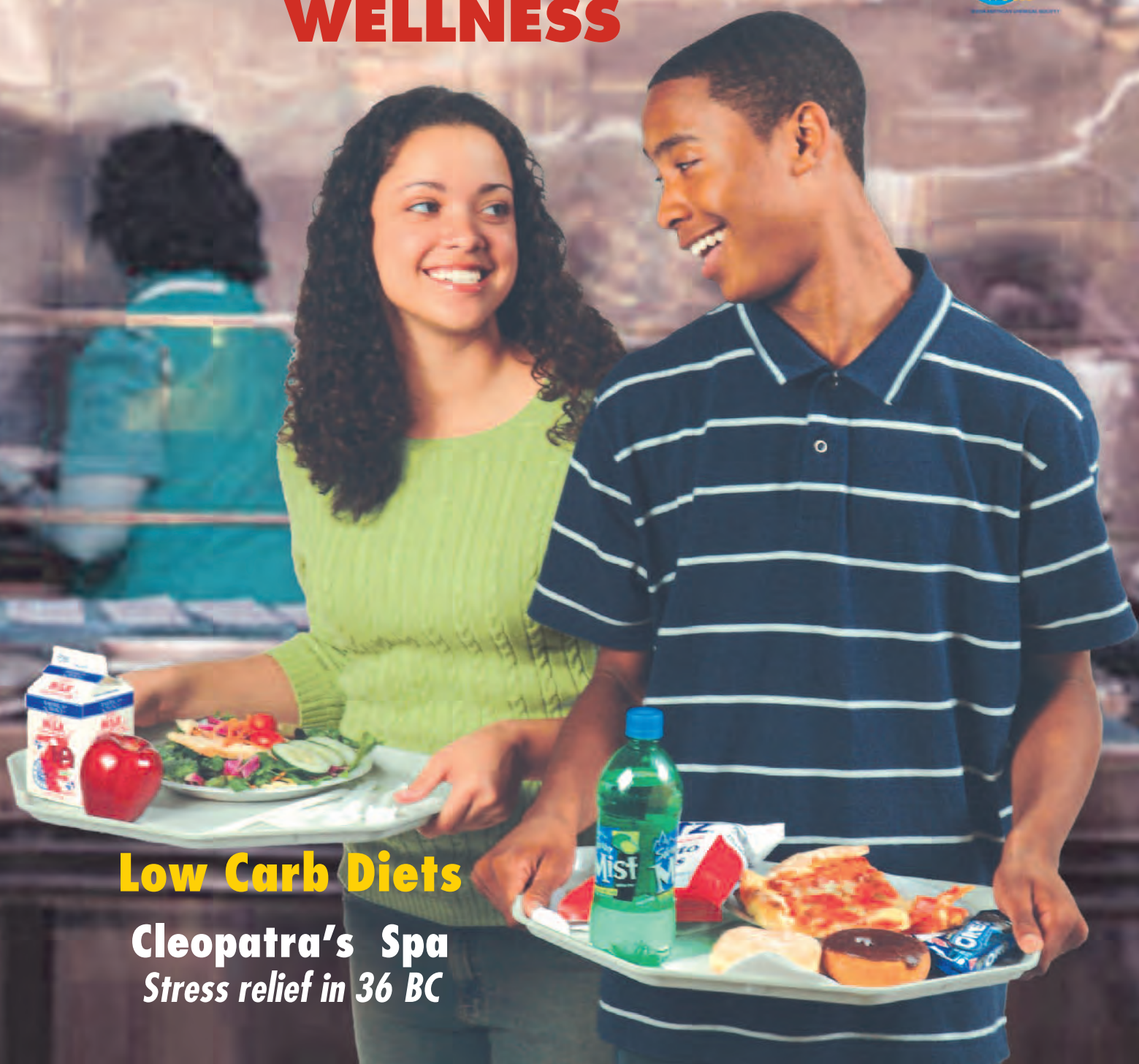
CHEM ¹MATTERS[®]

DEMYSTIFYING
EVERYDAY CHEMISTRY

OCTOBER 2004



HEALTH AND WELLNESS



Low Carb Diets

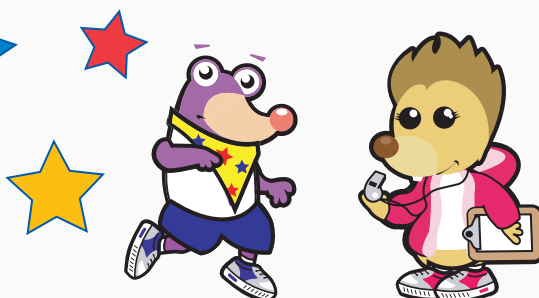
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EVERYDAY CHEMISTRY

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CHEM MATTERS[®]

Vol. 22, No. 3

OCTOBER 2004



MIKE CIESIELSKI

Question From the Classroom 4

Should soda vending machines in school be banned? You can test the sugar content in your favorite beverage with an activity that relates density to sugar content.

Carb Crazy 6

Low-carb diets and products are everywhere. What are carbs and why are some people cutting down on them?

Lab on a Stick 9

Meet the tiny paper strip with two enzymes and 16 reagents that can perform 10 urinalysis tests in under two minutes and help diagnose a host of medical conditions.

ChemHistory 13

Cleopatra's Perfume Factory and Day Spa

The famous young queen owned a perfume factory and recorded recipes for early cosmetics—many of which used early chemical techniques.

MysteryMatters 16

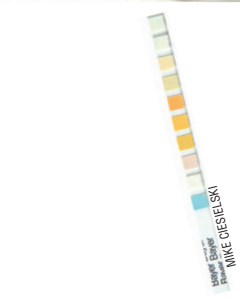
When Good Science Goes Bad!

Did a mother really poison her baby with ethylene glycol?

ChemShorts 19

In this edition: New high-tech bandages made from shrimp shells and a tiny sensor that gives blood glucose levels.

Chem.matters.links 20



MIKE CIESIELSKI



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STAFF

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Question From the Classroom

By Bob Becker

The Great Soda Sellout

Q. Why are some schools considering a ban on soda machines? They are in stores and offices all over town. Why should the school be any different? Besides, what's wrong with soda anyway?

A. This is certainly a hot topic right now in many state legislatures and at many school district board meetings. On the one hand, soda vending machines are bringing in much-needed money for schools. And in recent years, beverage companies have started offering even more lucrative contracts for schools that allow only their product to be sold. These added bonuses may have helped pay for the new landscaping at your school, or perhaps the new scoreboard for the football field.

Schools have grown increasingly dependent on these funds and most administrators consider these vending machines essential sources of income for the school's budget.

At the forefront of the controversy is a molecule composed of 6 carbon atoms, 12 hydrogen atoms, and 6 oxygen atoms: $C_6H_{12}O_6$ —also known as fructose, the sugar most commonly used in carbonated beverages. There are 40 grams of sugar in a typical 12 oz soda can, 67 grams in a 20 oz bottle.

All this added sugar in a student's diet translates into extra Calories. Combine this increase in Calorie intake with a general decline in physical activity, and the outcome is scary: Thirty years ago, the average American consumed 85 L of soda per year. One teenager in 17 was overweight. Today, the average American consumes over 220 L of soda per year, and one teenager in seven is overweight. Furthermore, soda offers virtually nothing in the way of nutritional value: no vitamins or minerals. When your parents were in high school, the average teenager drank twice as much milk as soda. Today that statistic has completely flipped around, with twice as much soda consumed as milk. This has prompted some health experts to nickname soda "liquid candy," and

has prompted some states to consider banning sales of soda in public schools, especially at the elementary level.

Soda proponents have argued that although soda consumption may have increased over the past few decades and obesity may have gone up as well, that does not mean that the extra soda was responsible. Certainly, many other factors have contributed to the rise in obesity, from a decline in physical activity to an increased preference for super-sized burgers and fries.

But what if you are concerned about sugar intake? How could you determine the sugar contents of some of your favorite beverages? The "total carbohydrates" percentage listed on the label is a good place to start, but is there a simple way to verify these values, so you don't have to take the beverage companies' word for it? When chemists want to measure a quantity or value such as sugar content, they often



What 67 and 40 grams of sugar look like!

do so by indirect means—measuring instead a related property and then comparing that to the values for prepared standardized samples. For sugar content, the most easily correlated property is that of density.

Density, as you may have already learned in class, is the ratio of a substance's mass to its volume: ($D = m/V$). In other words, density is the concentration of matter: how much material is compacted into how much space. Sugar-water solutions tend to have a slightly higher density than pure water, and the more sugar, the higher the density. This is true of most substances dissolved in water, but since sugar is by far the most abundant solute in most beverages, sugar content and density should show the highest correlation.

do so by indirect means—measuring instead a related property and then comparing that to the values for prepared standardized samples. For sugar content, the most easily correlated property is that of density.



Wow—density in action! Regular Coke sinks, but the new C2 (half-the-sugar cola) floats because its overall density is less than water ($< 1.0 \text{ g/cm}^3$).



What's going on here? Now the C2 cola is floating in the middle of the vase. What happened? It's the same can. What's changed? The answer is on our back page.



PHOTOS BY MIKE CHESLESKI

TRY THIS!

So, how well do you know the sugar content of the beverages you drink?

Rank the following four beverages from lowest sugar content to highest: Coke, Welch's 100% grape juice, Powerade, and orange soda.

Along with adding Calories to a beverage, dissolved sugar also increases the density of the solution. In this activity, you will first determine the density of five known (standardized) sugar solutions: 0% (which is just plain water), 5%, 10%, 15%, and 20%. You will then plot these densities on a density vs. sugar content graph. Finally, you will determine the densities of the four beverages, and then use the graph to approximate their sugar contents.

Safety note: Never drink a beverage that has been opened or used in the laboratory (or has been put in laboratory glassware). Discard all your solutions down the sink.

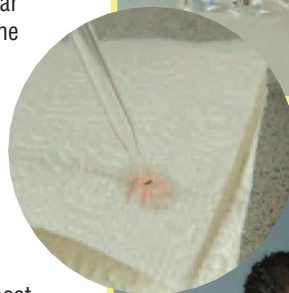
You will need:

- A cola, grape juice, sports beverage, and orange soda. **Note:** Carbonated beverages should be decarbonated (made flat) by pouring them back and forth several times between two cups and setting them out overnight.
- Standardized sugar solutions: 0%, 5%, 10%, 15%, and 20%. For example, to make up 5% sugar solution, dissolve 5 g of table sugar in 95 g of water. Note: The sugar found in soda is usually fructose; however, table sugar is sucrose. Since sucrose and fructose solutions have very similar densities, this substitution will have very little impact on your results.
- 10-mL volumetric pipet and bulb.
- 100-mL beaker.
- Electronic balance, paper towels, and graph paper.

What to do:

Your teacher will demonstrate the proper technique for using a volumetric pipet.

1. Place the beaker on the balance and hit the "tare" (rezero) button. The scale should read "0.00 g." Draw up a precisely measured 10.00 mL of 0% sugar solution into the pipet. Then empty it into the beaker, touching the tip of the pipet to the inside wall of the beaker to help get out most of the liquid in the tip. Do not try to shake out any liquid that remains there. The pipets are designed "TD" ("to deliver") 10.00 mL and that remaining drop should not be squeezed out. Since the beaker has already been zeroed out, the mass is that of the liquid alone. Record this mass reading, then push the "tare" button to rezero the scale for the next reading.
2. Touch the pipet to a paper towel to get rid of the excess liquid in the tip. Repeat step 1 with each of the remaining sugar solutions, and then with each of the four beverages. Do not put any of the solutions back into the cups from which they came, just leave them in the beaker. When the beaker gets full, simply empty it into the sink, set it back on the scale, and push the "tare" button.
3. Calculate the density of each solution. Because the volume is always 10.00 mL, this should be easy.



PHOTOS BY MIKE CIESIELSKI

4. Carefully plot your calculated densities versus sugar content (%) for each of the five sugar solutions. Then use a ruler to draw a best-fit straight line through the point (DO NOT PLAY CONNECT-THE-DOTS!!!). Then use the densities of the four beverages to approximate their sugar contents. To do this, start on the y axis at a density of one of the beverages; then follow the line over to where it hits the best fit line you drew; then go straight down to the x axis to determine the corresponding sugar content value.

The results might surprise you!



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**No-Carb?
Low-Carb?
High-Carb?**

Never before has the role of carbohydrates in our diet been so thoroughly discussed. We'll try to cut through the hype to give you the lowdown on...



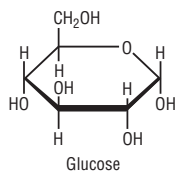
**CARBOHYDRATES
AND
LOW-CARB DIETS.**

By Brian Rohrig

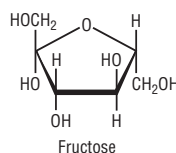
What are carbs?

The word carbohydrate reveals that all carbs are composed of three elements: carbon, hydrogen, and oxygen. There are three main categories of carbohydrates—monosaccharides, disaccharides, and polysaccharides.

Monosaccharides are composed of one sugar unit and are referred to as simple sugars. Their empirical formula is generally CH_2O . Some common monosaccharides are glucose and fructose. Although both have a formula of $\text{C}_6\text{H}_{12}\text{O}_6$, they have a different arrangement of atoms. Such compounds are called isomers. Glucose is produced by plants during photosynthesis. Glucose can be found



Glucose



Fructose

in sports drinks, providing quick energy when you need it. Glucose is the body's primary fuel source. It is broken down into energy through the process of cellular respiration. Carbon dioxide is released as a waste product. If glucose is not converted into energy, it is converted into glycogen to be stored.

Disaccharides, also known as double sugars, are composed of two simple sugar molecules. The most common disaccharide is **sucrose** or table sugar. In order to be used by our body as energy, sucrose must first be broken down into **glucose and fructose**. Food

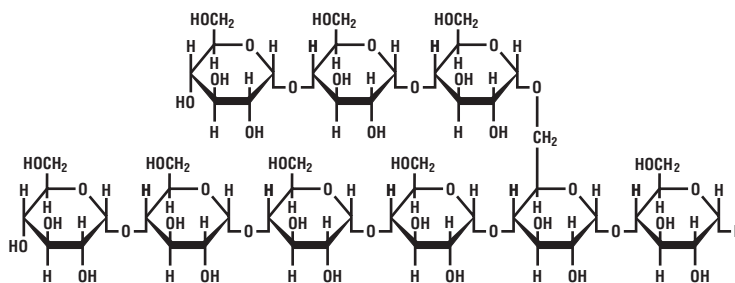
manufacturers are more often replacing sucrose in products with fructose, because it is cheaper to produce, and because it is sweeter, less needs to be used. Other common disaccharides are maltose and lactose (milk sugar).

Polysaccharides are complex carbohydrates. They are polymers composed of long chains of sugar units. A common polysaccharide is starch, which is composed of long chains of glucose molecules. Starch is used by plants as a way to store energy, and it can be found in foods such as potatoes, rice, corn, and wheat. Our body must break down starch into glucose, which it then uses for energy.

Other types of complex carbohydrates are not digestible by our body. Chief among these are cellulose, which forms the cell walls of plant cells, giving them structure and support. Its glucose molecules are linked together in such a way that our body lacks the necessary enzymes to break them down. These indigestible polysaccharides are known as fiber, and they contribute no calories because our bodies cannot convert them into energy. High-fiber foods—such as oats, bran, and whole grains—are an essential part of our diet, aiding in digestion.

Carbohydrates and blood sugar

To understand the effect of carbohydrates on our body, it is important to understand their role on blood sugar. Just like a car requires fuel, so does our body. The body's major fuel source is glucose, or blood sugar. However, this blood sugar is not automatically released into the cells. If a car's engine receives too much fuel, the engine becomes flooded, and it will not start. In the same way, the body must regulate how much blood sugar enters our cells. This occurs through the production of the hormone insulin, which is manufactured by the pancreas. Think of insulin as the gatekeeper to the cells—it opens the gates and allows glucose to leave the bloodstream and enter our cells.



The polysaccharide glycogen is a stored form of glucose.

Once sugar enters our cells, it can do one of three things. It can be converted into energy through cellular respiration. Or it can be converted into glycogen in the liver and muscles, for use as an emergency fuel. **Glycogen** is similar to starch but is more extensively branched. Finally, it can be converted into fat if there is more sugar available than is needed.

Consider what happens when you wash down a glazed doughnut with a sugary soda. As your bloodstream is inundated with sugar, a temporary spike in blood sugar will occur. Your pancreas responds by producing a surge of insulin to quickly rid the bloodstream of this excess sugar. This quick release of insulin will cause your blood sugar to then drop suddenly. The sudden drop in blood sugar can cause weakness, fatigue, and intense hunger—often leading to a craving for more delicious glazed donuts. This can create a vicious cycle, where our blood sugar constantly rises and falls, leading to overeating and eventual weight gain. A well-balanced diet can help to reduce these sudden peaks and falls in blood sugar.

Over the long term, constant spikes in blood sugar and insulin are not a good thing. When so much insulin is produced for so long, your body may become immune to the effects of it, creating a condition known as insulin resistance—often a precursor to type 2 diabetes. For reasons not yet fully understood, the cells can become desensitized to the effects of insulin, with the result that glucose is not effectively taken into the cells and converted into energy. The liver then takes over, taking this excess blood sugar and converting it into fat—leading to obesity. And the overworked pancreas may get worn out from producing so much insulin.



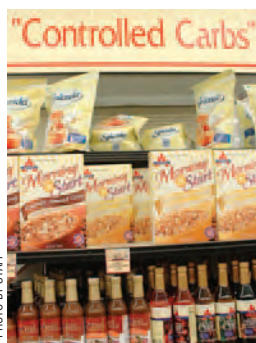
**In the United States
one teen in seven is
overweight.**

SOURCE: SURGEON GENERAL

DAN SHERRO

Why not radically cut carbohydrates from the diet?

Eat as much bacon and eggs as you want as long as you forget about the toast and orange juice?! Such a suggestion is at the very least culinary, if not dietary, heresy. But as a result of two decades of rising obesity rates and the sheer popularity of low-carb diets, researchers, doctors, and dietitians began re-examining the theories of Dr. Robert Atkins, who published Dr. Atkins' Diet Revolution in 1972. He advocated eating all of the fats and proteins you wanted, and said that if you only cut out all those carbohydrates, you would lose weight.



Numerous modifications of the Atkins' diet, such as the South Beach Diet and the Zone, have since appeared. Many of these modified diets have a more balanced approach, stressing the importance of avoiding unhealthy saturated fats found in red meat and dairy products, and emphasizing healthier unsaturated fats found in nuts, fish, and vegetable oils.

Millions have tried the diet with success and testimonials abound. By now, you've probably heard a friend or family member give the low-carb dieter litany: "I failed on other diets, but this diet worked for me because I wasn't hungry all the time." "I lost weight faster than on other diets, and I wasn't hungry all the time." Or even, "I failed on this diet, but at least I wasn't hungry all the time!"

How do low-carb diets work?

All diets work according to one basic principle: You must burn more calories than you consume. How many calories you burn depends on both metabolism and exercise. You cannot lose weight if you consume more calories than your body uses. By cutting out carbs, you are cutting back on a lot of calories. Cutting out just sugared sodas, potato chips, and candy bars can reduce your overall calorie consumption dramatically.

Another reason low-carb diets are successful is that fats and protein have better "staying power". Because carbohydrates are rapidly converted into glucose and then utilized by your body as energy, they do not keep us satiated for long. Fat and protein are absorbed more slowly by the body, so they stay with us longer. Even though low-carb diets claim you can eat all the fat and protein you want, in truth these foods fill us quicker, causing us to ultimately eat less.

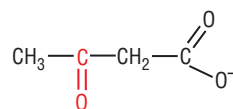
Ketosis: An inefficient way to burn fat

Although some claim that a reduced-carbohydrate diet is nothing more than a reduced-calorie diet in disguise, low-carb diets actually do one thing that is radically different than any other diet. The majority eliminate most, if not all, carbohydrates in the initial induction phase of the diet. When the body becomes carbohydrate-starved, it must find an alternate energy source.

The first source it taps is the glycogen found in the liver and muscle. But the body's glycogen stores can only last about two days. After this, our body turns to fat for energy. However, the breakdown of fat in this case does not produce glucose. Instead, the fat is broken down into ketones, through an unusual process known as ketosis. Normally, people burn fats without making ketones. Ketosis only occurs when people are carbohydrate-starved.

During ketosis, fat is not completely broken down and you don't receive the "normal" caloric value from burning it. The excess ketones produced during ketosis are secreted in our urine and breath. Sometimes this gives your breath a fruity odor, since acetone—a ketone—

may be released by the breath during ketosis. Because the kidneys flush out these ketones from the body, it is important to drink a lot of water on a low-carb diet. Dieters often confirm their body is in ketosis by checking their urine with ketone test strips (see "Lab on a Stick" in this issue).



Acetoacetate
(a ketone body)

Considerable debate has arisen among medical experts as to whether or not ketosis is dangerous. Some confuse ketosis with a more serious condition

known as ketoacidosis, which is an extreme form of ketosis sometimes suffered by type 1 diabetics. During ketoacidosis, the pH of the blood falls to dangerous levels, because of excessive buildup of acidic ketones. This can lead to coma and death if left untreated.

Some argue that the body functions even better when using ketones as fuel. Others claim that the brain cannot function as well on ketones, and the body will turn to muscle and organ tissue to try to scavenge glucose for fuel. However, most medical authorities today are

leaning toward the view that ketosis is a safe bodily process as long as ketones are not produced faster than the body can get rid of them.

Are low-carb diets good for you?

The low-carb diet phenomenon is still too new to judge its long-term effects. However, two studies recently published in the *New England Journal of Medicine* offered some promising news for adult low-carb dieters. These studies found that participants who lost weight on low-carb diets had higher levels of HDL (good) cholesterol and lower levels of triglycerides, or fats, than those who lost weight on different diets.

There's no specific research on teenagers, but you should be wary of going on the diet, especially if you are still growing. Many participants sacrifice the health benefits of the nutrients found in milk, fruit, and whole grains. Osteoporosis can result from a long-term deficiency of calcium in the diet, which could result if milk was totally eliminated from the diet. Pregnant women should definitely not be on the diet, because the developing fetus can be harmed by the lack of nutrients. Athletes should also avoid low-carb diets, since peak athletic performance is dependant on

the quick availability of glucose for energy, as well as relying on glycogen reserves.

Any diet is only as successful as the ability to remain on it for life. And make sure you incorporate plenty of exercise in any weight-loss plan you choose. ▲



MIKE GIEBELSKI

All diets work according to one basic principle: You must burn more calories than you consume.



PHOTO BY STAFF

Brian Rohrig is a chemistry teacher at Jonathan Alder High School in Plain City, OH. His last article for *ChemMatters*, "Lightning: Nature's Deadly Fireworks" appeared in the April 2004 issue.



MIKE GIESIELSKI

LAB ON A STICK

Within two minutes, one tiny strip, with two enzymes and 16 reagents, can perform 10 tests that help diagnose a host of medical conditions.

By Christen Brownlee

Imagine that you have just swallowed a rich and delicious chunk of sweet chocolate cake. Minutes later, carbohydrates from that cake will become glucose, the sugar your cells use for energy. If you're healthy, a hormone called insulin, produced by islet cells in your pancreas, will snatch glucose from your bloodstream and squirrel it away inside your liver and muscle cells in long chains for future use. But if you have diabetes, your body either doesn't make insulin (type I), or is no longer sensitive to its effects (type II), allowing glucose to build up in the bloodstream.

High blood sugar levels can be toxic to many types of cells, leading to poor circulation, kidney failure, blindness, or worse. Normally, the body naturally regulates the amount of insulin and a counteracting hormone called glucagon to keep blood sugar in check. Diabetics can keep their blood sugar under control by taking insulin or regulating their diets. But

there's only one catch—to know how much insulin to take or how to modify what they eat, people with diabetes need a way to keep track of exactly how high their blood sugar levels are.

Researchers struggled for decades to develop a test for glucose in urine that was easy enough for anyone to use. But it wasn't until the 1950s that former American Chemical Society president Helen Free and her husband, Al, developed the dip-and-read test strips called Clinistix. The tests were such an advance that researchers have since combined 10 urine tests—to check for ailments like liver failure, urinary tract infections, and others—onto one plastic stick. It's like having a team of chemists instantaneously at your disposal. For such a simple idea, urine dipsticks have revolutionized diabetes care and modern urinalysis. But just how did the Frees develop this "Lab on a Stick"?

Most people with type 1 diabetes are first diagnosed during their teen years. In the United States, more than 400,000 new cases of diabetes are reported every year.



COURTESY OF HELEN FREE

Helen and Al Free share a moment with a lab rat at the Miles-Ames Research Laboratory in 1948.

A rainbow of tests

Researchers have known for thousands of years that diabetics excrete sugar into their urine—a side effect of overwhelming the kidneys with too much blood glucose. So, in one of the first tests for diabetes, doctors poured urine on the ground to see whether it attracted insects. If insects crowded around the puddle, it meant they were attracted to sugar, a dead giveaway for diabetes.

Although this test was helpful for determining whether a patient had diabetes, it wasn't sensitive enough to detect how much sugar was present in the urine, an indicator of diabetes severity. So, in the early 1900s, researchers developed a method to estimate the level of glucose in urine. Doctors mixed a blue solution of cupric sulfate (CuSO_4) into a urine sample, then put in some alkali (strong base) and a complexing agent such as tartrate or ammonia to prevent precipitation of copper(II) hydroxide. Heating the mixture over a Bunsen burner or in a water bath caused any glucose, a strong reducing (electron donating) substance, to react with the blue cupric ions, changing them to copper(I), which precipitates as the orange-brown copper(I) oxide. The extent of the mixture's color change—from blue to green, brown, and red—gave doctors a rough

estimate of how much glucose was in a patient's blood. The test was "colorimetric"—it relied on a visible color change to track the presence of a chemical.

This analysis was better than pouring urine on the ground, but not great. It still required special equipment, harsh chemicals, and plenty of know-how to judge the results. In the 1930s, Walter Compton, the doctor whose family helped found Miles Laboratories, developed an improved version of the same test, with a lot of less mess and effort. He made a tablet with cupric sulfate, sodium hydroxide (the strong base), and citric acid, which he dubbed Clinitest. After putting the tablet in a test tube and adding several drops of water, it fizzed like Alka Seltzer. Heat from the reaction allowed any glucose present to reduce the cupric ions, and doctors compared the remaining mixture's color to a chart to determine the urine's glucose level.

Clinitest was easy enough for some diabetics to use outside the doctor's office, but it still wasn't perfect. Scientists knew that many chemicals, including some drugs, act as reducing substance in urine. So, patients with normal blood glucose levels frequently ended up with false positive results for diabetes. To weed out these bogus results, Helen and Al Free, along with other chemists at Miles Laboratories, developed a tablet test for ketone bodies, a byproduct in diabetics' urine caused by metabolizing fat instead of glucose. The white tablet contained alkali and nitroprusside, $[\text{Fe}(\text{CN})_5(\text{NO})]^{2-}$. If a drop of urine turned the tablet purple, the patient had diabetes.

Put it on paper

For years, doctors had to perform both tests and a blood test to get an accurate reading of a patient's blood sugar. But in 1953, diabetes diagnostics took a giant leap ahead. A factory owned by Miles Laboratories developed an enzyme called glucose oxidase, which reacted only with glucose. Al Free immediately noticed the potential for

a brand new type of glucose test. When glucose oxidase reacts with glucose, it forms two products, gluconic acid and hydrogen peroxide. Testing for gluconic acid proved too tricky for easy analysis, so the Miles chemists focused on a reaction to show the presence of hydrogen peroxide instead. The researchers added peroxidase to react with hydrogen peroxide, as well as a benzidine, a type of chromogen, or chemical that changes color when it becomes oxidized.

The reaction worked like a charm, turning shades of blue with different glucose levels. But the test was still too complicated

for most diabetics to use at home. After doing thousands of tests on spot plates and in test tubes, Al had an idea—if the same reagents were on a piece of paper, could you dip it into a urine sample and get the same results? After many more tests, the researchers found that the answer was yes.



COURTESY OF HELEN FREE

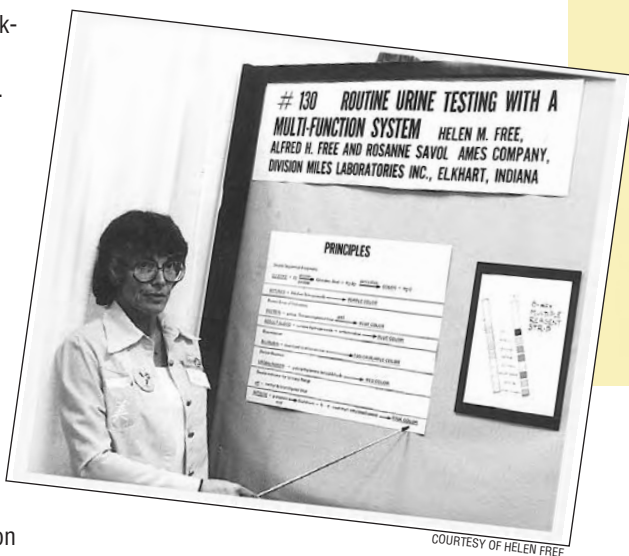


Colorimetric Test. Just like the universal pH paper you might have used in lab, readings for the Multistix-10SG are made by comparing the strip to a color chart on the container.

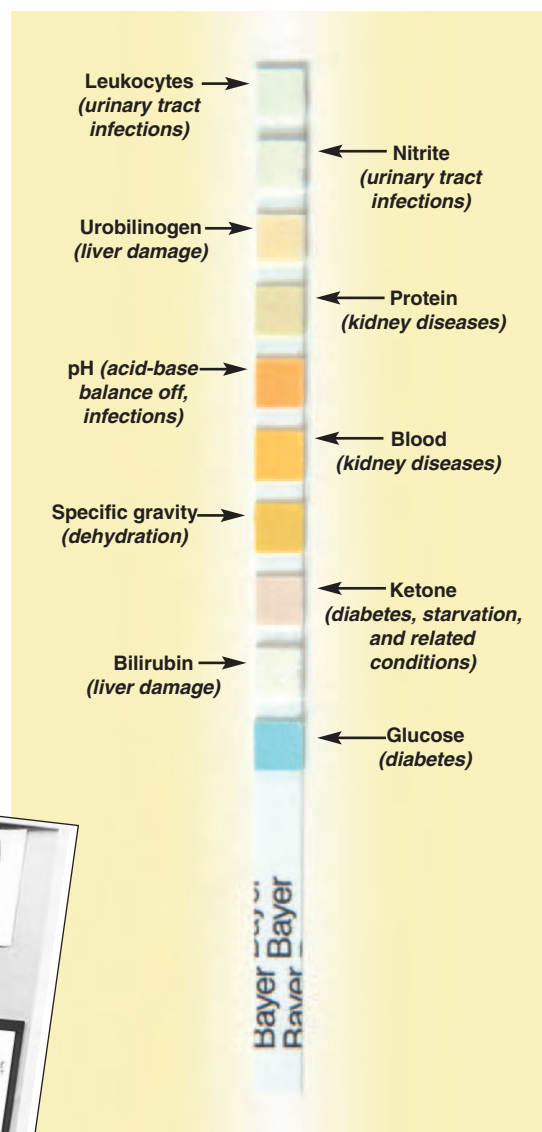
Ten tests in one

But the Frees and a hundred other researchers at the Miles Ames Research Laboratory couldn't stop quite yet. They developed a colorimetric paper test for albumin, a plasma protein that leaks into diabetics' urine when their kidneys fail. Since doctors frequently test for glucose and albumin at the same time, they decided to put the two tests on the same paper strip. They later incorporated the ketone test and added colorimetric analyses for bilirubin and urobilinogen, byproducts formed by the breakdown of red blood cells and good indicators for liver failure. Later came tests on the same strip for occult (hidden) blood and protein—two signs of kidney damage—as well as leukocytes and nitrite, signs of a urinary tract infection. The researchers rounded off the strips with reagents for pH and specific gravity, a measure of concentration.

The test strips were so easy to use that they became an instant hit and a big seller for the Ames division of Miles Laboratory (later to become Bayer). Clinical lab personnel simply dipped a strip into urine samples for a practically instant urinalysis, a window into diabetic, liver, or urinary tract health. Today, Bayer sells several varieties of urine dip-and-read tests, including special diabetic test strips, with just glucose and ketone tests, as well as strips called Multistix-10SG, with all 10 tests. Helen Free, still a consultant for Bayer, says that she frequently meets people her work has helped. "They'll say, 'My mother used those strips,' or 'My grandfather did,'" she said. "It's a wonderful feeling, knowing that your work changed people's lives—it gives me shivers when I think about it." ▲



COURTESY OF HELEN FREE



MIKE CIESIELSKI

Teachers!
Remember the free
Teacher's Guide
(chemistry.org/education/chemmatters.html) for
"Lab on a Stick" student
questions, additional
information, and activities!

Special interview with Helen Free on the next page!

Q&A With Helen Free

How did you decide to become a chemist?

My English teacher in high school, Miss Johnson, was my role model, and so I was going to be a Latin or English teacher. Then in December, the bombing of Pearl Harbor happened, setting off World War II. In order to avoid being drafted into the army, all the guys went out and joined up for the navy or the air force or wherever, and left college. We had house mothers in our college dorms back in those days, and our house mother's name was Harriet Kline. At dinner one night, she said, "You know, girls, you're going to have to take some science, because we don't know how long this war will last or when the guys will come back." She turned to me and said, "You're taking chemistry, aren't you, Helen? Do you like it? Are you getting good grades?" I answered yes to all of her questions. Then she asked, "Why don't you switch majors?" And I said okay. Boom, it happened just like that. It was a turning point, and such a lucky one that it happened.



COURTESY OF HELEN FREE

How did you meet your husband, Al Free?

I was hired in the control lab of Miles Laboratories, which is now Bayer, in 1944. At the time, we were devising methods to determine the amount of each vitamin in a multivitamin tablet, so each tablet would have the same amount. After we established the method, we were just analyzing vitamins day after day, and it got to be too routine. I kept bugging them to let me go into the research lab, because I thought research sounded like a glamorous thing to do. I was not offered the job in the research lab until 1946, after Miles had expanded, so they had a biochemistry lab. Al Free came from Cleveland to run the new lab. He was a professor of biochemistry at Western Reserve Medical School. My co-workers encouraged me to go interview with Al Free, so that maybe he would hire

me. And he did. Two years later, I married the boss—it was one of the smartest things I ever did. We made a team; we were married 53 years, and we just worked together and had a good time the whole time.

Did you get special treatment because you were married to the boss?

No, in fact, Al bent over backwards not to give me the plush experiments. He gave me experiments working with blood and tears and stools and all that kind of stuff.



Helen Free demonstrates the use of Hemastix and Combistix at a 1940's AMA meeting.

COURTESY OF HELEN FREE

How many patents have you held for your research? Did you earn extra money at Miles for each patent?

I've held just seven patents, and there were a whole bunch of people who contributed to each one—it wasn't just Al and me. I made a dollar for each

patent. People often will tell me, "Oh, that's terrible!" But I say, well, that's what Miles hired us to do. If you work in a research lab, you're supposed to invent things. That's your job. They pay you a salary, and they pay your salary even if you don't invent anything. So, why give you more if one of the things happens to earn a patent?

What advice would you give someone who is thinking of pursuing chemistry?

You can do a whole lot of other things besides working in a lab if you have a science degree—it's going to help you in no matter what kind of career you choose. The scientific approach to solving problems is helpful in almost any area of your life. If you do choose to do straight chemistry, they need chemists not only in the research lab and in the control laboratory, but they need chemists in manufacturing and chemists in the legal departments. People don't understand how important the field is. If you get an opportunity like I did to jump into a field you like, grab hold of it and take it. And if you work in a lab and decide that you don't like it, get out and do something else. The world is your oyster nowadays—there are so many kinds of jobs that you can do. I don't see how kids ever make up their minds. ▲

Christen Brownlee is a freelance science writer living in Washington, DC. Her last article, "Four Cool Chemistry Jobs", appeared in the December 2003 issue of *ChemMatters*.



CLEOPATRA'S PERFUME FACTORY AND DAY SPA

Nestled at the south end of the Dead Sea, Cleopatra's Day Spa is more than just a relaxing destination. It is an intoxicating experience designed to provide an escape from the pressures of everyday life. We hold an unmatched reputation and an elite clientele—Mark Antony marched his army to the Dead Sea to secure the spa for



beautiful Queen Cleopatra. If our services work for a Queen and Roman Consul, just imagine what they'll do for your health and wellness. Specially trained therapists assess your current level of fitness and discuss your goals. Our most popular packages:

Package 1: "The Cleopatra"

45 silver denarii

*Package includes full use of the spa's facilities and your choice of three of the following:

DEAD SEA MUD BODY POLISH deep cleans, purifies, and restores your skin, leaving you feeling smooth and radiant all over.

CROC EXCRETA FACIAL tightens the pores and stimulates circulation so your skin is left with a smooth, healthy glow.

DEAD SEA SALT SCRUB exfoliates and revitalizes the skin by drawing out toxins.

MORINGA MANICURE leaves your hands feeling fresh and revitalized, not greasy.

ASS-MILK BATH nourishes the skin and leaves it feeling soft and lithe.

OUR SIGNATURE MAKEOVER includes expertly applied green malachite and black galena eyeliner, red ocher and henna body paint, finishing with our exclusive crushed beetle-shell glitter for that Cleopatra look your own Mark Antony will love.

Package 2: "The Mark Antony"

30 silver denarii

*Package includes full use of the Cleopatra Day Spa facilities and your choice of two of the following:

DEAD SEA MUD BATH relieves aches and pains, reduces stiffness and muscle tension after exercise, and revitalizes the skin. Enriched with Dead Sea salts, our mud has a young, masculine fragrance.

ASPHALITE HAIR PACK alleviates dandruff and normalizes oily hair. Makes hair feel revitalized and moisturized. It will not overdry the scalp.

DEAD SEA SALT FOOT SCRUB is just the thing after marching with your legions.

HORSERADISH BODY POLISH revitalizes your skin without that smelly, greasy afterfeel.

BLACK-TAR MASK draws out toxins to clear up unsightly facial blemishes, purifies, and revitalizes the complexion.

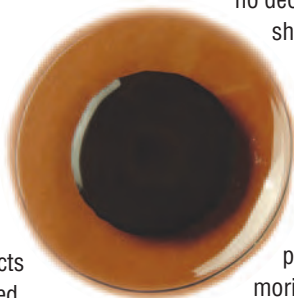
Cleopatra, famous Queen of Egypt, ascended to the throne at the age of 17. She later owned a perfume factory and recorded recipes for early cosmetics—many used early chemical techniques.

By Lois Fruen

These real treatments from our fictional ancient spa brochure, (on the previous page), may not seem appealing, but they were sought after in ancient times, and many are still available today, albeit with different names. It was Cleopatra, the famous queen of Egypt, who popularized skin care treatments in her book titled *Cleopatra Gynaeciarum Libri*. There, she recorded recipes for making cosmetics and perfumed ointments. She was so interested in spa treatments and perfumes that her lover, Mark Antony, gave her the gift of a spa and perfume factory that had been built by Herod the Great at the south end of the Dead Sea.

Asphalt skin care

Archaeologists unearthed jars at Cleopatra's spa that contained residues of ancient skincare products that Cleopatra likely used. When chemists analyzed the residue from one jar of Dead Sea mud treatment, they were surprised to find it had a similar chemical composition to asphalt, better known as tar or pitch, a complex mixture of high molecular weight hydrocarbons, with partially oxidized and sulfur-containing compounds mixed in for good measure. Their analysis was confirmed by Pliny the Elder, a first-century AD historian, whose histories tell us that ancient spa treatments started with an application of asphaltite mud followed by a treatment of Dead Sea salt.



Before you sneer at the spa treatment using asphaltite, Dead Sea mud skin care products and bath salts are still marketed today. In fact, some of the finest spas advertise wraps and facial masks made from mud and Dead Sea salt.

Imagine Cleopatra submitting to being smeared in muddy tar and then having the tar rubbed off her skin with bath salts. Pliny also tells us that perfume was used to cover the smells of the pitch and salts. Boy, would Cleopatra have needed perfume after a spa treatment like that!

Perfumes

For Cleopatra, perfumes were important not just for masking the smells of skin treatments but to cover offensive body odors. Cleopatra would have carried small containers of her perfumed ointments and powdered perfumes that she would have reapplied several times a day to keep her complexion looking fresh and her skin sweet smelling. Remember that there were no deodorants available in her time, and she lived in a hot climate.

Chemists have reconstructed a number of ancient perfumes using Cleopatra's own recipes and analysis of perfume residues found in jars from Cleopatra's spa. They discovered that Cleopatra favored perfumed ointments made from moringa oil or horseradish oil (*Moringa pterygosperma* or *M. aptera*). Those ointments would have disappeared into her



Two Minneapolis students from the Breck School, Stacy White and Caroline Kaylor, made a project of trying to recreate ancient perfumes. They used natural materials such as lard (as a nonpolar solvent) to extract fragrant organic molecules from rosemary and borneal heather.

skin quickly and left no greasy feeling behind. Moringa oil is still used in Persian perfumes today, and chemists at L'Oreal have recreated ancient Egyptian perfumes using moringa oil.

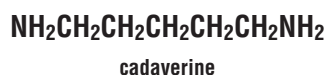
Other chemists have followed Cleopatra's ancient recipes that call for mixing herbs, flower petals, leaves, or seeds with hot vegetable oil made from pressed olives. They let the mixture soak for a week at 30–40°C. Then, they pressed the mixture through a cloth bag to extract the perfumed oil from the pressed olive mixture. Besides using perfumes made with olive oil to anoint herself, Cleopatra may also have added perfumed oils to her wine to give it a more pleasant smell since those made with olive or vegetable oils were edible.

You may have heard the saying, "Flies in the ointment." It comes from a very real problem in Cleopatra's spa. Flies were ever-present in Egypt and the near east, and they were attracted to the fats and oils used to make perfumes. The flies would get trapped in the perfumed oint-



PHOTODISC

ment and die. The ointment putrefied as the flies decomposed, giving off a foul odor that spoiled the batch. Even the Bible makes reference to this in Ecclesiastes 10:1. "Dead flies make the perfumer's ointment give off an evil odor ...". The odor is the result of the chemical breakdown of the proteins that produce diamines called **putrescine** and **cadaverine**. The names of these two amines are appropriate—they smell like rotting bodies. They can also contribute to bad breath and the less-than-desirable body odors that made the use of perfumes so necessary in ancient times.



When chemists used crystallographic and other chemical tests to analyze the residues of ancient eye makeup, they found that the green eye makeup contained malachite, which is hydrated copper(II) carbonate ($\text{CuCO}_3 \cdot 5\text{H}_2\text{O}$). They discovered that the black eye paint, called kohl, contained galena, a gray-lead ore of lead(II) sulfide (PbS), and cerussite, which is lead(II) carbonate (PbCO_3). The kohl also contained laurionite (PbOHCl) and phosgenite ($\text{Pb}_2\text{Cl}_2\text{CO}_3$). These last two chemicals were unexpected, because they do not occur naturally; ancient Egyptians had to synthesize them. Following recipes reported by Pliny,



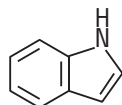
chemists duplicated ancient methods for making kohl by crushing PbO with natron (Na_2CO_3) or rock salt (NaCl) and then filtering the mixture and repeating the process over the course of several weeks. The rock salt produced PbOHCl , while the carbonate resulted in $\text{Pb}_2\text{Cl}_2\text{CO}_3$. Modern-day chemists say these syntheses developed by ancient Egyptians were the first "wet-bench" chemistry ever done.

Cleopatra would have dyed her nails, hands, and feet and perhaps her hair with henna from a shrub called Egyptian privet (*Lawsonia alba*). Henna is a reddish-brown organic dye that was used in Turkey as early as 7000 B.C. In Cleopatra's time, henna could have been applied as a paste or by a more complex formulation using oil, sugar, and citric acid. Henna is still used today for temporary tattoos and by a variety of cultures to signify a woman's fertility.



The results of their project? Good ... if you like fragrant lard! Stacy and Caroline suggest that further work exploring the effects of temperature, light, and other solvents such as olive oil is necessary.

Besides perfumed essential oils, Cleopatra used powdered excrement from Egyptian crocodiles to clean and embellish her complexion. This is not as odd as it sounds.



indole

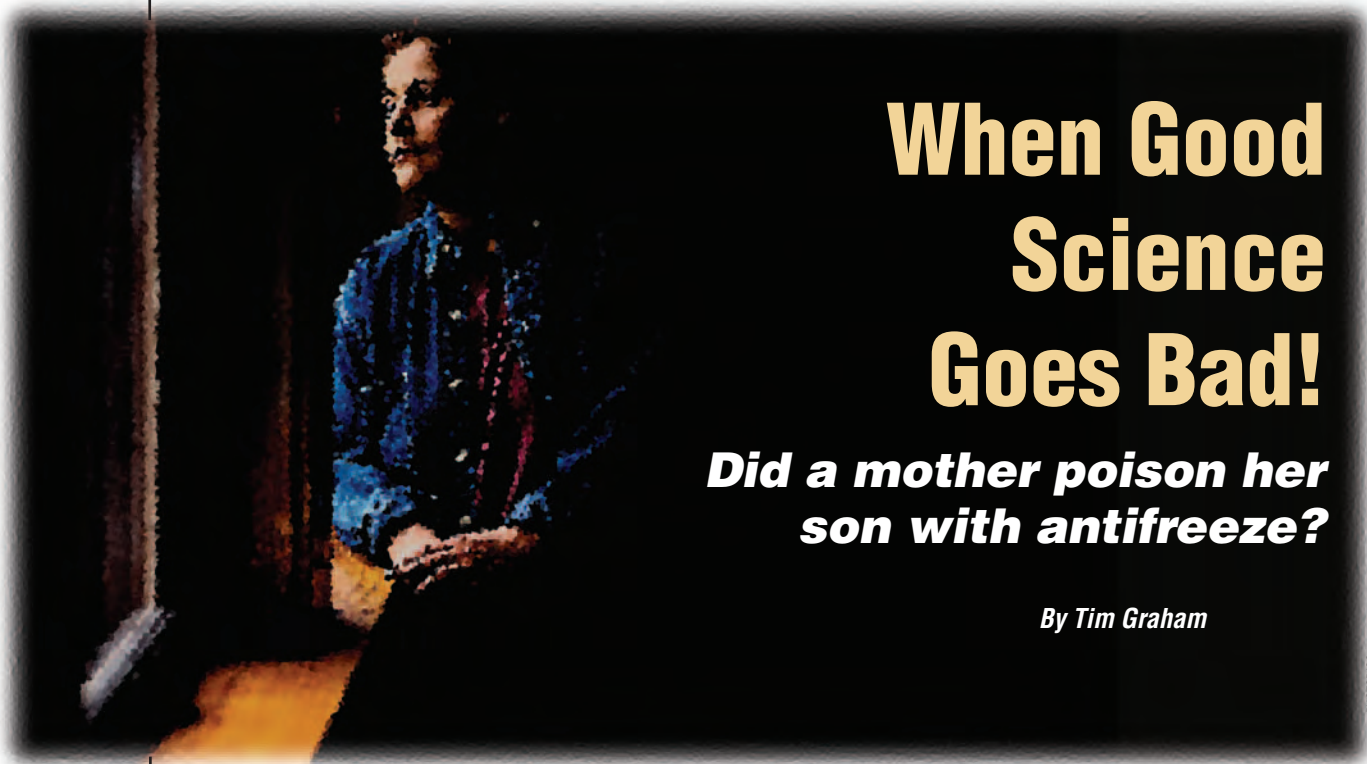
Indole, used to moderate modern-day perfumes, is derived from feces, and urea, a component of urine, is used in modern skin-care products.

To keep her skin soft and supple, Cleopatra bathed in milk of asses, having discovered an important property of protein in a fatty emulsion—it contains lactic acid that is an alpha-hydroxy acid which breaks down dead skin cells. Today, you can buy foaming milk baths,



Finally, Cleopatra would have stored her perfumed oils and cosmetics in attractive jars that were designed to hold skin care products and pigments. Archaeologists have discovered hieroglyphs on similar jars that advertise the benefits of using the product. Some things just don't change! ▲

Lois Fruen teaches chemistry at the Breck School in Minneapolis, MN. Her article "Copper Verdigris: A Women's Art" appeared in the February 2003 issue of *ChemMatters*.



When Good Science Goes Bad!

Did a mother poison her son with antifreeze?

By Tim Graham

PHOTO: ISC

Every mother's worst nightmare began for Patricia Stallings on Friday, July 7, 1989. Her 4-month-old son, Ryan, took his bottle just before bedtime but soon vomited. This set in motion a series of events that would forever change the lives of the Stallings family.

By Sunday morning, it was obvious that Ryan was quite sick. His breathing was labored and he couldn't keep any food down. Patricia loaded Ryan into the car and rushed to a local St. Louis hospital. After three days of testing, doctors concluded that Ryan's illness was due to high levels of ethylene glycol found in his blood. Ethylene glycol is a primary component of automobile antifreeze/coolant (See *ChemMatters*, October 1996). Ryan was too young to have drunk the toxic liquid on his own ... someone must have poisoned him! So, on July 17, 1989, the Missouri Division of Family Services took custody of Ryan. It wasn't long before Patricia became

the primary suspect in the poisoning of her son.

Early in September, Patricia was granted supervised visits during which she was allowed to feed Ryan a bottle. Three days after one of these visits, Ryan was again hospitalized. This time his condition was much worse and doctors were unable to save him. Ryan Stallings died on September 7, 1989. Patricia found out about Ryan's death the next day when she was arrested and charged with murder. Patricia

spent the next seven months in jail while prosecutors built their case against her.



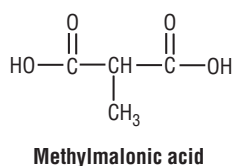
The mounting evidence was damning: a gallon of antifreeze was found in the basement of the Stallings's home, two independent labs confirmed the presence of ethylene glycol in Ryan's blood serum, and postmortem analysis of Ryan's brain tissue confirmed the presence of calcium oxalate crystals, all consistent with ethylene glycol poisoning. Patricia Stallings was convicted of 1st-degree murder in the death of her son and sentenced to life in prison.

But here's where the story takes an interesting twist. Three months after Ryan's death, Patricia Stallings learned she was pregnant! Her second son, David Jr., born five months after Ryan's death, was taken by Family Services and placed into foster care. A few weeks later, David Jr. experienced seizures, vomiting, and lethargy ... the very same symptoms that his brother Ryan had suffered previous to his death. But experts at a different hospital diagnosed David Jr. with rare metabolic disease known as methylmalonic acidemia, or MMA, and were able to treat him. It would be this diagnosis that would ultimately unravel the conviction against Patricia Stallings.

What is MMA? Methylmalonic acidemia is an inherited illness that inhibits the body's ability to metabolize protein correctly. Most babies are diagnosed with the disease only after they get sick. MMA results from a deficiency of

enzymes responsible for the metabolism of **methylmalonic acid**. When a sufferer of

methylmalonic acidemia eats too much protein (mother's milk and baby formula are high in protein), the capacity to digest and use the protein overloads the deficient enzyme system. Too little dietary protein triggers the body to break down its own protein, which can also overwhelm the enzyme. The disorder is potentially life threatening, but once the condition has been diagnosed, careful monitoring of diet along with a prescribed medical regimen can minimize the symptoms.



A new trial

Six months after her conviction, Patricia was granted a new trial by the state of Missouri. The toxicology lab at St. Louis University provided samples of Ryan's blood serum and it was determined that Ryan had also suffered from MMA. But would this new information be enough to exonerate Patricia Stallings? The original laboratory work had found ethylene glycol present in Ryan's blood serum. Since experts concluded that there could be no mistaking any of the metabolic products of MMA with ethylene glycol, there was not sufficient evidence to reverse the decision.



Patricia's case soon gained national exposure when it aired on an episode of the NBC television series, "Unsolved Mysteries." One of the viewers of this episode was Dr. William S. Sly, a professor of Biochemistry/Molecular Biology at St. Louis University. Both Sly and a colleague, Dr. James Shoemaker, had been following the case in the news media. They were convinced that a compound called propionic acid was misidentified as ethylene glycol.

Was the blood test correct?

The laboratories that did the original analytical work relied on a technique known as GC-MS. GC-MS is actually a combination of two technologies: the gas chromatograph and the mass spectrometer. The gas chromatograph is a device for separating a mixture of compounds according to their relative attractions to a material called an adsorbent. In

chromatography, the adsorbent is known as the stationary phase. Typically, the adsorbent is silica or alumina gel lining the column inside the machine. The sample is injected into a stream of carrier gas (known as the mobile phase) inside the machine where it goes through the column that is heated by an oven. The separations occur on the column. The separated compounds in the mixture pass by a detector, which causes a signal to be emitted.

This signal is sent to a recorder, which prints a chromatogram, which records the relative retention times and amounts of the compounds present in the mixture. After separation on the column in the GC, the materials

then pass through to the mass spectrometer. In this machine the materials are fragmented in the presence of a strong magnetic field. Fragments of the materials are then analyzed according to their molecular weight. The results can be compared against a database library of known compounds and their fragmentation patterns.

The combination of information about the physical properties of the compounds (as measured by its retention time) and its chemical composition (as measured by its mass spectrum) is extremely powerful, almost like measuring a fingerprint. GC-MS is practically a "gold standard" for the identification of compounds; it is extremely rare that two different compounds will have both the same retention time and the same mass spectrum. For example, not only do propionic acid and ethylene glycol differ in their retention time by 33 seconds on a standard column, but their mass spectra look completely different. You would think, as the experts testified in the second trial, that there would be no possibility of confusing them!

But Sly and Shoemaker were not so sure. After all, the accuracy of the results depends on the technicians' skill in interpreting the data. It is easy to deceive oneself about the identity of a compound present in a

complex mixture such as blood, particularly if one expects to find ethylene glycol. So Sly and Shoemaker applied a *qualitative* test that would give a yes-or-no answer less subject to interpretation. One such test uses a simple colorimetric method. Periodic acid (H_5IO_6) oxidizes ethylene glycol to formaldehyde, which then is reacted with a chemical called Purpald (4-amino-3-hydrazino-5-mercapto-1,2,4-triazole). A purple-colored complex forms if ethylene glycol is present. The pair of scientists was able to secure a sample of Ryan's blood and their additional tests confirmed their suspicions. Propionic acid, expected in the blood serum of a person suffering with MMA, was indeed misidentified as ethylene glycol by both laboratories involved in the original testing. Sly and Shoemaker were so certain of their conclusions that they sent samples doped with propionic acid to seven independent commercial laboratories for analysis. In fact, three of the seven labs misidentified the propionic acid as ethylene glycol. It would seem that few analytical labs, employing only GC-MS techniques, are capable of making a reliable determination when it comes to ethylene glycol!

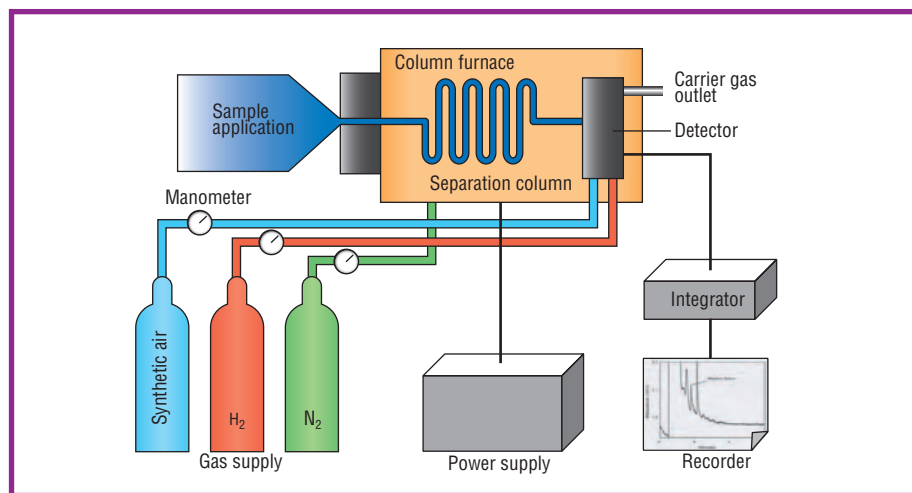


Diagram of a gas chromatograph. Mixtures of compounds injected into the GC are separated by their relative attraction to a stationary material in the separation column. The separated (or partially separated) compounds then pass through a detector, and their signal is recorded.

Prosecutors, now a bit more uncertain about Patricia's guilt, had to take a new look at the mounting evidence that suggested that Stallings might be innocent. They decided to consult with a world-renowned genetics expert from Yale University named Dr. Piero

Rinaldo. Rinaldo agreed with Sly and Shoemaker's findings and concluded that Ryan's symptoms were due to MMA and not ethylene glycol poisoning. The original tests run on Ryan's blood were also scrutinized and found to be insufficient to condemn a person to life in prison.

What about the calcium oxalate crystals?

Rinaldo also concluded that Ryan's death might have been the result of his treatment for ethylene glycol poisoning. The treatment, an ethyl alcohol drip, while appropriate for most patients suffering from ethylene glycol poisoning, was totally inappropriate for a patient with MMA.

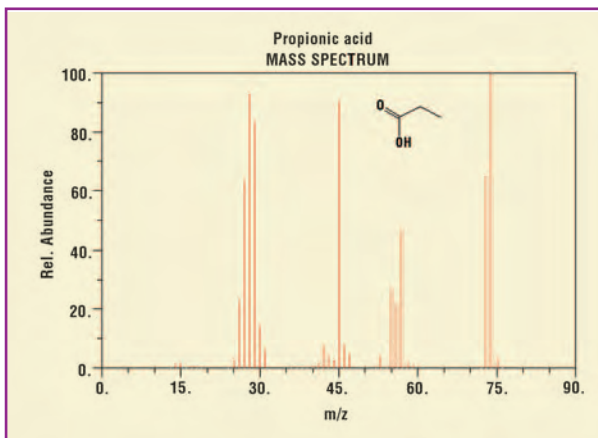
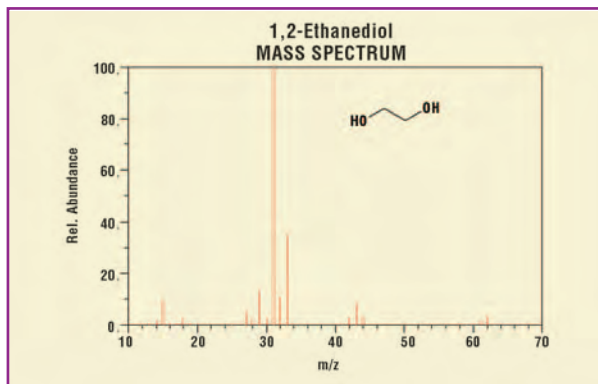
Once ethylene glycol has entered the bloodstream, an enzyme called alcohol dehy-

drogenase (ADH) breaks it down into several organic acids, one of which is oxalic acid. In an attempt to rid the body of a potentially harmful chemical, the enzyme makes the situation worse. Oxalic acid reacts with calcium ions in the blood to produce the insoluble salt, calcium oxalate. It is this substance that does much of the damage to organs like the brain and kidneys.

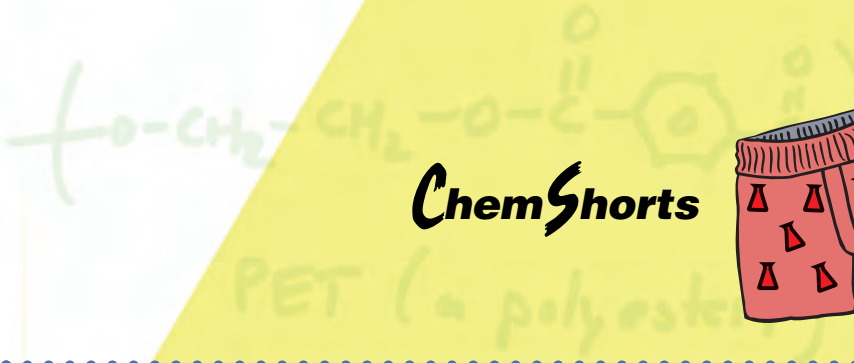
But ethanol treatment for ethylene glycol poisoning must be carefully monitored because of the unpredictable behavior of ethanol when subjected to the human body. In small children, especially ones vulnerable because of poor health, ethanol can prove to be just as harmful as it is helpful. Excess ethanol exposure is known to increase the precipitation of calcium oxalate resulting in a calcium deficiency in the blood (hypocalcemia). This may well explain the crystals found in Ryan's brain tissue post-mortem.

Faced with the new evidence collected by the two very determined chemists, prosecutors for the state of Missouri dropped all charges against Patricia Stallings. Patricia's conviction was overturned, and she was released from prison 14 months after her nightmare began. ▲

Tim Graham teaches chemistry at Roosevelt High School in Wyandotte, MI. His most recent article, "Scanning Electron Microscopy Solves a Mystery!", appeared in the December 2003 issue of *ChemMatters*.



The mass spectra of ethylene glycol and propionic acid look completely different. You would think, as the experts testified in the second trial, that there would be no possibility of confusing them.



Shrimp bandages



In Iraq, Army medics are carrying a new bandage that can stop hemorrhaging better than conventional gauze dressings. And it's made from a familiar menu item!

The need for a new bandage is pressing; approximately 90% of combat deaths occur before a patient gets to a hospital. Not surprisingly, bleeding is the single most frequent cause of death. But given the high tech nature of the modern battlefield, what is surprising is that field medics have basically the same tools—namely, gauze dressings—to



CREDIT: HEMCON, INC

Don't call this new 4" × 4" bandage a shrimp. It stops bleeding, seals wounds, and kills germs to boot!

stop bleeding that they had 100 years ago.

That was until a group of researchers discovered a property of *chitosan*—a polymer derived from shrimp shells. When chitosan comes in contact with blood, it induces clotting. It also has antimicrobial proper-

ties—something desirable under dirty conditions.

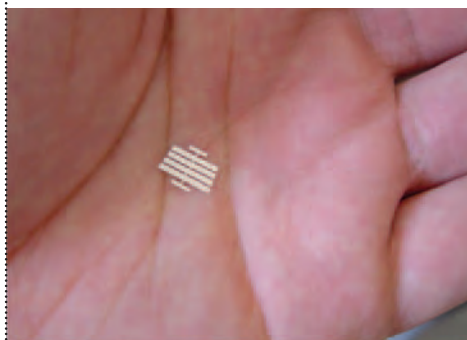
How do you go from shrimp shell to bandage? The raw material is *chitin*, a biodegradable polysaccharide, similar to cellulose, found in shrimp and crab shells. Chitosan is made from chitin by removing acetyl groups (CH_3-CO) from the chitin polymer chain with dilute acid. Like cellulose, chitin and chitosan are fibrous materials. By controlling the number of acetyl groups removed along the chain or by adding new groups, chemists can tune the properties of chitosan to make it more versatile. Using a modified form of chitosan, the researchers developed a durable and flexible field dressing that sticks to and seals wounds.

Could shrimp bandages be coming to an ambulance near you? It's possible. Trauma caused by a car accident can be similar to what's seen on the battlefield. There's no official word on its performance, but the Army has ordered over 50,000 of the 4" × 4" bandages so far and awarded the manufacturer \$6 million to expand production. If the bandages save lives, there's no doubt that paramedics will want to start carrying them. For now, only the military can use the bandages and only on extremities, but the manufacturer of the new dressing, Hem-Con, Inc, is confident that it can

win FDA approval for a multitude of uses.

Measuring blood sugar with a wave of the arm

People with diabetes could soon be waving goodbye to the pain and hassle of needles,



KEFENG ZENG

Implanted in a hand or arm, this sensor could soon make monitoring blood sugar levels as easy as waving your arm.

thanks to a new under-skin sensor that monitors blood sugar levels with a simple wave of the arm.

The research was recently described in a paper in the ACS journal of *Analytical Chemistry*.

Designed as an inexpensive device to continually monitor the blood glucose levels of people with diabetes, the passive sensor requires no internal power supply and no connections outside the body. "Whenever a reading is needed, a person can wave his or her hand or arm in front of a

reader that will automatically detect the sensor," says Craig Grimes, lead author of the paper.

The sensor is based on "magnetoelastic" technology, the kind of technology used in plastic security tags on store merchandise. These tags are sensed wirelessly if someone passes a tagged item through an exit.

Magnetoelastic sensors are the magnetic equivalent of an

acoustic bell. Grimes explains, "If you hit a bell with a hammer, the bell rings at a characteristic frequency. If you coat the bell with a layer of paint, the frequency changes." Likewise, the molecules in a magnetoelastic sensor vibrate in the presence of a magnetic field, and the frequency varies with

different chemical coatings.

Grimes's glucose sensor is first coated with a polymer that responds to changes in acidity, and then it is coated with the chemical glucose oxidase. The glucose oxidase reacts with blood glucose to produce an acid, which causes the polymer to swell, thereby changing the frequency of the sensor. The reader then interprets these changes as blood glucose levels.

Grimes hasn't found a developer to commercialize the sensor. ▲

From a news release by Jason Gorss from the ACS Office of Communications.



Solve a mystery!

Here's how you can put all that chemistry you've been learning to use—solve a murder mystery! Download a video game that puts you in this scene: A student dies under mysterious circumstances at a university reception and you are brought in to solve the crime. Using interactive menus, you interview suspects, search for clues, and analyze evidence. Along the way, you'll use your knowledge of formula weights, stoichiometry, and the scientific method to solve the crime.

The Chemistry Collective at Carnegie Mellon University developed the new activity with a grant from the National Science Foundation. The software is free. To download "Mixed Reception" or to get more information, visit <http://iry.chem.cmu.edu/mr/>.

Bacteria battery

Phil Palko (teacher) and Nikki McDonald (student) from Indiana Senior High School in Indiana, PA, submitted this working battery in response to our challenge in "Bacteria Power" (April 2004). You can still take on the challenge and earn your teacher a free copy of our 20-year CD. We have four more CDs to give away.

The battery in our article used anaerobic soil. Since then another microbial fuel cell was reported that's been popularly



PHIL PALKO

referred to as using "poop power". Read about it at <http://www.newscientist.com/news/news.jsp?id=ns99994761>.

Slide rules do rule!



JOHN BRUGMAN

John Brugman's class at Toulte Lake High School submitted these homemade slide rules from the activity we included in "Slide Rules Rule" (April 2004). Not easily seen is the giant slide rule underneath the pink slide rules.

Why was the can floating in the center of the vase on page 5?



The answer lies with the small bottle to the left of the tank. Just like diet sodas, cans of the new low-sugar sodas (like C2) float in water. That's because the effective density of the can is less than the density of water. Here, we've added just enough isopropyl alcohol (density = 0.79 g/cm^3) to the surrounding water to lower the density of the surrounding solution. We've created a situation known as *neutral buoyancy*, where the density of the can and the solution are the same. It's the same condition that allows a submarine to stay at the same depth. ▲

MIKE CIESIELSKI

CHEM MATTERS

1155 Sixteenth Street, NW
Washington, DC 20036-4800

Reach Us on the Web at
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