Sniffing Out Cancer

By Justin Warner

The dog—a friendly Doberman Pincher-Border Collie mix—had always been well-behaved. So why wouldn't she stop sniffing that mole on her owner's leg? No matter how many times her master shooed her away, the dog would not leave the mole alone. The annoying habit turned alarming one day while the dog's owner worked in the garden. She wore shorts, and the mole on her leg was fully exposed. The dog wandered out, pounced on her leg, and tried to bite off the mole.

That was enough to make the woman start thinking about the mole, rather than the dog's behavior. Her primary care doctor told her the mole was harmless. But after the gardening incident, she went to a dermatologist, who diagnosed **malignant melanoma**. Had the tumor gone undetected longer, it could have spread and killed her. But thanks to the dog's nose, it was caught early and removed.

Stories such as this one—which was reported in a 1989 issue of the British medical journal *The Lancet*—eventually led to a widespread effort to turn tumor sniffing into a life-saving diagnostic tool. Some researchers train dogs, while others design machines to do the smelling. In the past two decades, both lines of research have come a long way toward reliably catching the scent of cancer.

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Chemical cancer clues

Cancer's odor comes from a complex combination of volatile organic compounds (VOCs), molecules that are released by tumors into the breath, urine, stool, and other body products. VOCs evaporate or sublimate from a liquid or solid phase of the same substance. This happens constantly in virtually any substance, but at room temperature, volatile compounds spontaneously evaporate more readily than they condense back into the solid or liquid phase.

The volatility of an organic compound depends on its vapor pressure at room temperature. Vapor pressure is the pressure at which vaporized molecules reach equilibrium with the solid or liquid phase of the same substance in a closed system (Fig. 1).

The weaker the intermolecular forces between the molecules of a compound, the higher the vapor pressure. In other words, the more strongly the molecules are held to one another in the liquid or solid phases, the more difficult it is for these molecules to escape and enter the vapor phase.

Cancerous cells release distinctive mixes of VOCs in different concentrations than VOCs released from healthy cells. The process starts when cells produce excessive reactive oxygen species. These molecules, such as superoxide (O_2^-) and hydroxyl radical ($\cdot OH$), have unpaired valence electrons that make them highly reactive with surrounding biological materials.

Healthy cells constantly produce reactive oxygen species as a byproduct of oxygen metabolism. In limited quantities, they perform positive functions, such as triggering the death of unhealthy or damaged cells. Also, antioxidant molecules, such as vitamins C and E, keep the amount of reactive oxygen species in check. But when that balance

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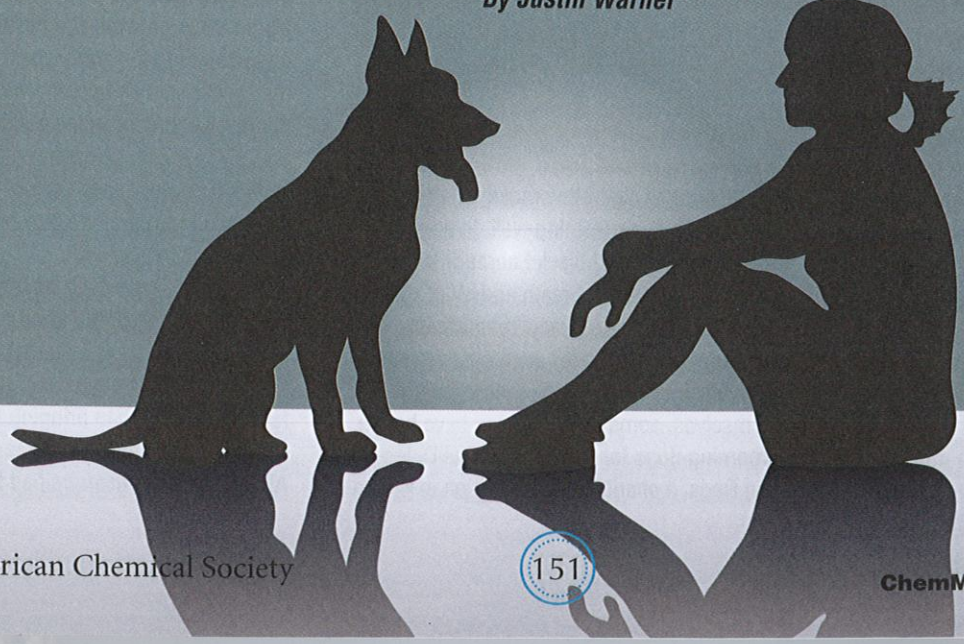
Figure 1. Vapor pressure is a measure of the force exerted by a gas above a liquid in a sealed container.



becomes disturbed—for example, by low levels of antioxidants—reactive oxygen species can damage DNA and healthy cell tissue. This damaging process is known as oxidative stress, and it is a known precursor of cancer and other illnesses.

Under oxidative stress, reactive oxygen species oxidize fats in cell membranes, resulting in increased emissions of VOCs, such as aldehydes—organic compounds containing a formyl group (R–C(=O)–H)—and ketones (R–C(=O)–R'). In basic research studies, dozens of specific VOCs have been linked to various types of cancer, including cancers of the lung, skin, prostate, colon, breast, and ovaries. However, tumors produce changes in not just one type of VOC, but many—possibly dozens. What's more, many cancer-linked VOCs are probably produced by the body under normal conditions, but in different quantities.

But how do you detect VOCs? A complex mixture of volatile chemicals is actually what makes up an odor, and nature has created noses to detect them. But noses, and the brain areas that process smell, vary from species to species, and from individual to individual. While some doctors, nurses, and patients have informally described cancer-related smells, dogs or scientific instruments can detect VOCs more reliably, in much lower concentrations.

A doctor's best friend?

Dogs have two key advantages over machines. First, their noses are highly efficient chemical sensors built by nature and evolution—no assembly required. Second, dogs can be taught to distinguish between different odors even if the researchers have no clue what volatile chemicals the odors actually contain. If a dog can learn to smell it, then something's there.

So far, dogs have been trained to sniff out cancer of the skin, breast, lung, prostate, colon, bladder, and ovaries. **In a recent study at Schillerhoehe Hospital in Gerlingen, Germany, dogs learned to distinguish breath samples of lung cancer patients from those of healthy volunteers and those of patients with a noncancerous lung disease.** Even the strong odor of cigarette smoke didn't prevent the dogs from performing well. In another study, an 8-year-old black Labrador learned to spot colon cancer in breath and stool samples with 91 and 97% accuracy, respectively.

Although the dogs for such experiments are typically trained by the researchers themselves, some organizations have begun grooming dogs for the job. Medical Detection Dogs, a charitable organization in Milton Keynes, England, trains cancer-sniffing dogs. According to a 2011 study, these dogs correctly picked out urine samples from bladder cancer patients 64% of the time, on average, when random guessing would have resulted in 14% accuracy.

Psychologist Claire Guest, chief executive officer of Medical Detection Dogs, became interested in the topic long ago, when a young colleague's dog discovered a cancerous mole on her leg, much like the dog mentioned earlier. Years later, she teamed up with British surgeon John Church, and they began training dogs to detect bladder cancer from urine. "We needed to give the dogs something to sniff that had had contact with the cancer tumor but wasn't part of the tumor," Guest said. Once tumors separate from the body, they die and begin releasing different odors. Urine seemed like a good starting point—something that dogs are used to smelling.

Teaching a dog to sniff out cancer is not very different from teaching them to sniff residues from explosives or drugs. **The key difference, as Guest explains, is that trainers of bomb- or drug-sniffing dogs know which chemicals they are training their dogs to smell, but trainers of cancer-sniffing dogs just know that one sample came from a cancer patient and the other didn't.**

The trick is to try to eliminate any other means that the dog might have to distinguish the samples, apart from the actual cancer-related volatiles. "For example, if I am presenting the dogs with a cancer sample that has blood in it, I have to make sure that when I am teaching dogs, I then put blood in the controls, as well," says Guest.

The training procedure varies from one experiment to another, but in a nutshell, it involves presenting dogs with known samples from cancer patients and healthy volunteers, and rewarding the dogs when they indicate the cancer sample in some way. For instance, the black Labrador in the colon cancer study was given breath samples from patients, collected in covered paper cups. When the dog sat down in front of the correct cup, she was given a ball to play with.

Of course, even the best-trained canine does not exactly fit the mold of a sterile medical facility. Guest expects that in the short term, samples could be sent to dog-sniffing facilities, rather than bringing the dog to the doctor's office. And she notes that in South Africa, rats have been trained to sniff out

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A dog's sense of smell can be up to 100,000 times better than ours. This amazing ability allows (a) hunting dogs to smell game at a distance, (b) drug-sniffing dogs to find small amounts of illegal drugs mixed in a powder, and (c) cancer-sniffing dogs to detect the presence of chemicals released by people who have cancer.

tuberculosis. Because rats are cheaper to raise and house than dogs, they could provide a low-cost screening solution in developing countries. But eventually, most researchers expect that machines will ultimately take over the work.

Artificial noses

One of the most advanced artificial noses is being developed by Metabolomx, a small company in Mountain View, Calif. It consists of a horizontal breathing tube, connected to a Thermos-like canister, which, in turn, connects to a machine the size of a desktop computer. A patient simply breathes in and out into the tube for about four minutes, while the machine analyzes the VOCs present in the breath.

The VOCs can be detected in a person's breath because VOCs present in a tumor first pass into the bloodstream and then into the lungs, which act as a bridge between the bloodstream and airways. So the breath exhaled by a patient carry the VOCs expected from a tumor, if one is present.

Volatile Organic Compounds Are All Around Us

Many types of volatile organic compounds exist in our environment. Some, such as isoprene (CH_2=C(CH_3)CH=CH_2), a component of human breath and plant emissions, are common, natural, and generally harmless. Others come mainly from man-made sources and may cause health risks to people and animals, as well as environmental damage. **Among the most common and toxic of these is benzene (C_6H_6), a known carcinogen. People can be exposed to benzene through chemical and manufacturing processes, tobacco smoke, and fumes from some consumer products, such as paint, furniture polish, and gasoline.**

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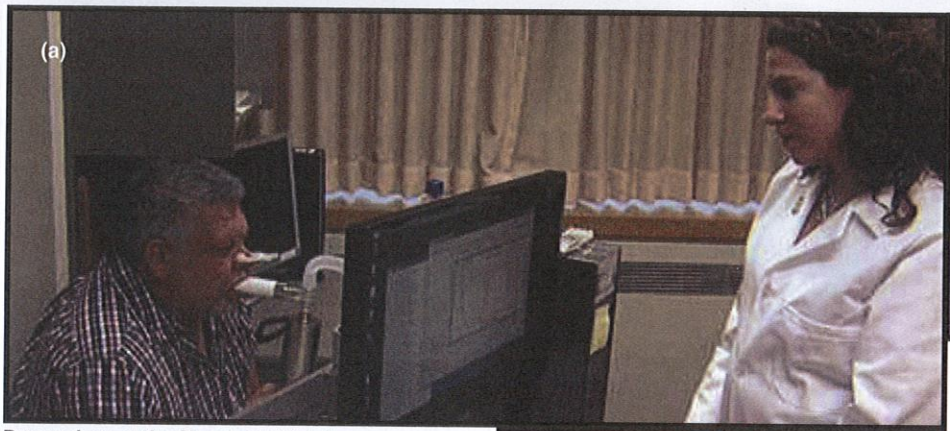
The sensor provides information on about 130 different VOCs, chosen because they, or compounds like them, had been linked to cancer in previous studies. "In time, we may find out that only 20 are necessary to get the information we need," Mazzone explains, but for now, they are casting as wide a net as possible.

In his last round of clinical trials, an artificial nose correctly identified breath samples from lung cancer patients 80% of the time, a performance comparable to a CT scan. Mazzone hopes to beat those numbers significantly with the new Metabolomx machine, in trials at the Cleveland Clinic and at the National Jewish Health hospital in Denver, Colo. Mazzone says the device is at least a couple of years from being ready for widespread use. Whether in the form of a machine or an eager canine, sniff tests hold the promise of an accurate, noninvasive screening test that could possibly help in the early detection of cancer. *CM*



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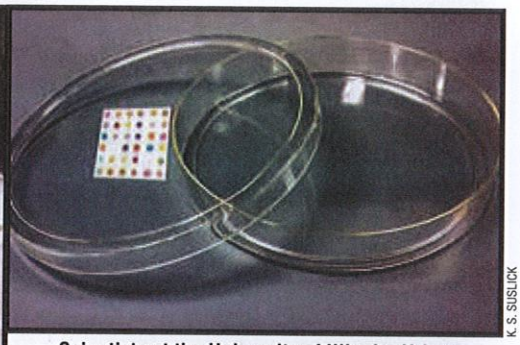
Researchers at the Cleveland Clinic have developed a device (a) that can detect distinctive chemicals in the smell of people who have cancer; (b) when such chemicals are detected, they cause pigments present in test samples to change color.



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Scientists at the University of Illinois, Urbana-Champaign, have created a sensor array (multicolored white device) that can detect telltale gases emitted by bacteria (present in the petri dish). A variation of this device is used to detect telltale chemicals in the breath of people who have cancer.

The technology within the machine stems from the work of Ken Suslick, a chemistry professor at the University of Illinois, Urbana-Champaign. Suslick and his colleagues printed tiny drops of dye on a glass, paper, or plastic film. Each drop of dye was engineered to change color in response to a particular volatilized chemical. By putting dozens of these dyes in an array, the researchers could distinguish complex gas mixtures by the multicolored dot patterns they created—a pattern sometimes called a "smellprint." Suslick used early versions of artificial nose machines to identify toxic gases and signs of bacterial infections and even to distinguish between different brands of cola, beer, and coffee.

According to Peter Mazzone, an oncologist at the Cleveland Clinic in Cleveland, Ohio, the new Metabolomx machine is much more sensitive than previous versions, rivaling a dog's sense of smell. "We get about 910 data elements out of each picture from the sensor, and the dog's olfactory system has around 900 or so olfactory neurons," he explains.

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