

BEWARE THE MUTANT VEGGIE DOG

Mutagenicity and Plant Proteins

It all started with charcoal. Ryan Hayes, associate professor of chemistry, worked briefly with local company NeoBiotech on charcoal patches in 2011 and started asking questions like, “Why are some burned things good, like charcoal, but other burned things, like grilled meat, cause cancer?” Hayes posed the question to his General Chemistry class, piquing the interest of student Tyler Pender. The two discussed possible theories and a research project was born.

“Charcoal is made from carbonaceous material, usually coconut husk, that is burned at very high temperatures. Carbon is all that is left. Charcoal is non-polar and passes through your system. It’s harmless,” explains Hayes.

Charred food is another matter. “Food is not burned at a high enough temperature for it to be reduced to purely carbon atoms.” In burned meat, molecules called heterocyclic amines (HCAs) are formed when creatine reacts with various amino acids. HCAs are carcinogenic, meaning that they cause cancer.

HCAs are so named because they contain other elements besides carbon (hetero), have a ring-like structure (cyclic), and contain nitrogen (amine). Researchers have isolated about 25 different compounds, which have been shown to cause cancer in animals.

According to the National Cancer Institute, meat cooked at temperatures above 300°F or cooked for long periods of time “tend to form more HCAs.” Epidemiologic studies have confirmed that, “high consumption of well-done, fried, or barbecued meats is associated with increased risks of colorectal, pancreatic, and prostate cancer.”¹

RIGHT: Ryan Hayes, associate professor of chemistry, supervises Irene Hwang, senior biochemistry/pre-dentistry major, as she sets up an experiment to burn amino acids from plants (soy protein powder), that will be analyzed for possible mutagenic molecules after the burning process.

Hayes and Pender wondered what would happen if non-meat protein was burned. “All the research identified that creatine, which comes from the muscle tissue of animals, had to be present in order to form these molecules. Plants don’t have creatine, but they do have other amino acids, so we asked, is it possible to get carcinogens from burnt plant protein?”

They began reading relevant literature and were able to find previous research that suggested arginine, a plant amino acid, might react similarly to creatine. This is of particular interest to vegetarians since arginine, along with all the other major amino acids, is found in soy protein.

As they narrowed down their literature search to arginine-based studies, Hayes and Pender came across the 1994 research papers

of James Felton and his research group. “The researchers found that mutagenicity can occur when you burn arginine with other amino acids,” says Hayes. Mutagenic molecules alter DNA, which means they may be carcinogenic.

Felton and his colleagues discovered that some burned grains developed mutagens, “but they did not continue the research to isolate any of the molecules or identify chemical structures.” It was a research project waiting to be continued. “No one was looking at the chemical structures of these potentially mutagenic and potentially carcinogenic molecules from burnt plant proteins,” says Hayes.

“Tyler and I started looking at the methods used in the creatine-based research for burning the amino acids, separating the molecules, and honing in on heterocyclic amines,” he



says. As they learned the various methods and developed them for their specific project, they came across a surprising discovery.

Initially, they thought that arginine might create the same compounds as creatine when burned, since they share a similar structure. However, “what Tyler and subsequent students have shown is that we are making something different,” says Hayes. He refers to these new compounds as arginine-based heterocyclic amines.

As the research developed, Hayes incorporated more students, funded by Undergraduate Research Scholar awards, into various aspects of the project and received internal Faculty Research Grant funding. The research team, known as the HCA (heterocyclic amines) Group, has spent the last several years acquiring the necessary equipment and refining their



methods of burning, extracting and isolating various arginine-based heterocyclic amines. “We knew what the problem was,” says Hayes, “but getting the methods in place and bringing in new equipment was important.”

This last school year, the HCA group began their experiments by combining arginine with the amino acid phenylalanine. The process begins by burning the mixture at a high temperature. Once the mixture is burned, they extract the desired material using a number of different solvents, and then separate the molecules using a newly acquired preparative scale High Pressure Liquid Chromatography (HPLC) machine. Finally, they analyze the



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molecular structure using nuclear magnetic resonance and mass spectroscopy. Each different molecule has to be isolated and analyzed separately.

Hayes is anxious to get structural

information on all the various molecules. David Alonso, a former Andrews University chemistry professor who now works at LECO in St. Joseph, Michigan, has offered to help the HCA group with the structural analysis aspect of the research. LECO is a manufacturer of chemical analysis equipment, “and they have some very specialized equipment that could help us figure out the structure,” says Hayes. Once the structural data has been analyzed and they can demonstrate that the molecules they are finding are a new class of heterocyclic amines, the team can publish their material.

Besides analyzing the structure, one of the most important aspects of the research is the Ames test, a mutagenicity test used by previous researchers that is conducted at the same stage as the spectroscopic analysis. Robert Zdor, professor of biology, has been working with the HCA Group to refine the test.

Eventually, tests will need to be run on the molecules to determine if those compounds found to be mutagenic are also carcinogenic. However, that would require animal testing, the approval of the Institutional Animal Care and Use Committee and additional funding. For now, the project

is limited to studying mutagenicity.

“We’ve had one compound go through the Ames test and show that it was as mutagenic as the molecules created by burned creatine,” says Hayes. While the researchers are excited, they are also a little concerned. Soy protein isolates, which are used in vegetarian soy products, contain arginine. Soy is “high in arginine and in all the amino acids,” Hayes explains. “It’s the complete protein source.”

Most scientists see meat as a larger problem, but vegetarians may find that burning a veggie hot dog is no safer than burning one made of meat. “We may be exposed to something toxic that we don’t know about,” says Hayes. “I think one of the lessons coming out of this is that you have to be careful. You can’t just do whatever you want to plant based food.”

“The other thing that we will probably find out is that when you isolate these amino acids away from the plant product, they are more susceptible to becoming mutagenic and carcinogenic under heating conditions than the whole plant.” The one bright spot in the

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meat studies was the finding that herbs and seasonings on the meat reduced carcinogens. This implies that the whole plant, due to its natural antioxidants, has built-in barriers to becoming carcinogenic.

“I think we may find that if we burn a whole soybean, it may not produce these heterocyclic amines.” Burning a veggie hot dog that has been processed and contains protein taken away from the structure of the plant, and its carcinogen-blocking antioxidants, may be another matter. Hayes hopes to involve the Department of Public Health, Nutrition & Wellness in investigating the cooking conditions of soy-based foods and the possible effects of frying and grilling.

So far, the team has only analyzed the compounds resulting from mixing arginine and phenylalanine. All the other amino acids remain to be tested with arginine, but Hayes is optimistic about long road ahead. “It’s a lot of work to run these tests,” he admits, but he has formed a team of capable students who understand the literature and methods and are invested in the project. “It’s so rewarding to see the students take ownership of the project,” he says. “You have a real partnership with the student at that point.”

Members of the Hayes group, like Tyler Pender, J.C. Lynch, Zach Reichert, Michael Plantak and Andrew Stewart, have developed the research into Honors theses and independent research, presenting at the Honors Scholars & Undergraduate Researchers Poster Symposium and the Michigan Academy of Science, Arts & Letters.

Although most of these students have now graduated, they have trained successors to continue the work. For Hayes and his students, this research project “shows what Andrews University can do. We can address and inform health-related issues.”

¹ <http://www.cancer.gov/about-cancer/causes-prevention/risk/diet/cooked-meats-fact-sheet>. Accessed July 15, 2016