GETTING RISK RIGHT

Understanding the Science of Elusive Health Risks

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Deadly Remedy

A Mysterious Disease, a Medicinal Herb, and the Recognition of a Worldwide Public Health Threat

> Just because something is natural it does not mean that it is good, and just because something is unnatural it does not mean that it is bad. Arsenic, cobra poison, nuclear radiation, earthquakes, and the Ebola virus can all be found in nature, whereas vaccines, spectacles, and artificial hips are all man-made.
>
> —SIMON SINGH AND EDZARD ERNST

As head of the nephrology service at the Erasmus Hospital of the Free University of Brussels, Dr. Jean-Louis Vanherweghem had seen many cases of chronic kidney disease. Usually this condition occurs in older people and is most commonly associated with diabetes and hypertension. But he and his colleagues were at loss when, in the early 1990s, relatively young women started showing up at hospitals in the city with unexplained and rapidly progressing kidney disease. Tests showed anemia and elevated creatinine levels, indicating that the kidneys were not doing their job of filtering toxins and waste products from the blood. In a matter of months, many of the women went on to develop life-threatening end-stage renal disease and had to go on dialysis or have a kidney transplant. Most were in their forties, and none had a history of medical conditions that would have put them at increased risk.

One day, as Dr. Vanherweghem came out of his office into the waiting room, he noticed that several of the women were chatting. Asking how they

were acquainted, he learned that they had all attended the same weight-loss clinic. Over the following months and years, the number of young women with kidney damage coming in to his and other clinics in the city continued to grow.

To pinpoint the cause of this epidemic of kidney failure, Vanherweghem and colleagues contacted nephrology centers throughout Brussels to identify all cases of renal failure occurring in women under fifty. In their initial report published in the *Lancet* in 1993, they described the results of nine cases of nephropathy in young women and examined details of the weight-loss regimen and the various medications the women were taking.[1] They learned that the weight-loss clinic had been in operation for fifteen years—from 1975 until May 1990—with no apparent ill effects. During that period the slimming regimen had consisted of a mixture of thirteen compounds given in capsule form or by injection. In May 1990, however, the regime had been modified, with the addition of two Chinese herbs believed to be *Stephania tetendra* and *Magnolia officinalis*. This regimen was in place for the next two years. While the authors were appropriately circumspect regarding the specific ingredient responsible for the kidney damage, they emphasized the striking connection between the unusual pathology and a slimming treatment involving Chinese herbs.

As time went on, more women from the weight-loss clinic sought medical attention for renal disease, and the syndrome was given the name "Chinese herbs nephropathy."[2] Early descriptions of the renal pathology had been based on biopsies, which provided only very small amounts of tissue. But in 1994 Jean-Pierre Cosyns, a pathologist at the hospital of the Catholic University of Louvain, across town from the Erasmus Hospital, used three whole kidneys from patients with Chinese herbs nephropathy to give the first detailed description of what the pathology looked like.[3] He described a distinctive fibrosis, or scarring, of the renal tubules, the structures that are responsible for reabsorbing electrolytes and excreting wastes. The fibrosis is most prominent in the outer layers of the kidney (the cortex) and works its way inward. Something similar is seen only with cadmium poisoning. Cosyns pointed out that, on "morphological and clinical grounds," the lesions seen in the Belgian women were "very similar to those described in Balkan endemic nephropathy," and he and his coauthors suggested that a "common agent" might be involved in both diseases.[4] In addition, both Cosyns and the nephrologists at Erasmus Hospital noted changes in the cells of the renal pelvis (the funnel-shaped part of the kidney where urine collects after filtration from the blood) and the ureters signifying the early stages of

cancer. In a separate paper in 1994, he reported the first case of urothelial malignancy among women with Chinese herbs nephropathy.[5] "Urothelial" refers to the distinctive type of cells lining the urinary tract, including the renal pelvis, ureters, and bladder. This cell type is distinct from the type of cells in the renal cortex, in which 90 percent of *kidney cancer* arises.

By 1995 eighty cases of Chinese herbs nephropathy in Brussels had come to light. Because exposed women appeared to be at high risk of developing urothelial cancer as well as kidney failure, Vanherweghem recommended regular cystoscopic examinations and the prophylactic removal of the kidneys and ureters in all his patients with end-stage Chinese herbs nephropathy. By the time he and his colleagues published their findings regarding cancer in the *New England Journal of Medicine* in 2000, thirty-nine patients had agreed to undergo prophylactic surgery.[6] Microscopic examination of the upper urothelial tissues from eighteen of the patients revealed cancer, and those of another nineteen patients showed mild to moderate urothelial dysplasia, a precursor to cancer. Thus the clinicians' aggressive response proved to be well-founded.

Further investigation revealed the exact nature of the change in the mixture of powdered herbs used at the weight-loss clinic that had occurred in May 1990. Instead of *Stefania tetendra* and *Magnolia*, the company that supplied the Chinese herbs had substituted *Aristolochia*. The tragic mix-up was facilitated by the similarity of the names for the two herbs in Chinese.[7] *Aristolochia* is *fangchi*, whereas *Stephania* is *fangji*. In contrast to the benign *Stephania*, *Aristolochia fangchi* contains aristolochic acid—a powerful nephrotoxin and carcinogen—which belongs to the class of chemicals called nitrophenanthrenes. (Diesel fuel contains nitrophenantrenes.)

Aristolochiaceae are a family of flowering plants with over five hundred species, which are found in diverse climates worldwide. The European birthwort (*Aristolochia clematitis*) is so named because its flower resembles a birth canal. *Aristolochiaceae* have been used in different cultures in the ancient Mediterranean world, in Europe, South America, India, and China, and in other countries in East Asia going back at least two thousand years.[8] *Aristolochia clematitis* was highly valued as a medicinal plant in ancient Greece and Rome and on into the early modern era. Owing to its resemblance to the uterus, birthwort was believed to be useful in childbirth. Many *Aristolochia* species are widely used in Chinese traditional medicine, including *Aristolochia manshuriensis*, which, as Guanmutong, was widely used for the treatment of urinary tract and cardiovascular diseases. Other preparations including *Aristolochia* herbs are used in

traditional Chinese medicine to alleviate gastrointestinal symptoms and as antirheumatics, diuretics, and liver tonics.

As is often the case in the history of medicine, it turned out that aristolochic acid had been a topic of considerable interest decades earlier, in a very different context. From the 1950s to the 1970s the National Cancer Institute had conducted a major program to screen plant compounds for antitumor activity. Virtually all the chemotherapeutic agents in use today are the result of that program. In the late 1960s Morris Kupchan, the head of the program, had declared that aristolochic acid was "the most potent antitumor agent" of all the compounds screened. In the late 1970s a German pharmaceutical entrepreneur named Rolf Madaus synthesized the compound in the laboratory and tested it in volunteers in order to study its anti-infection properties, with a view to developing it as a drug.[9] It was, indeed, effective, but then in the early 1980s a German toxicologist showed definitively that aristolochic acid was a carcinogen in rats.[10] At that point Madaus stopped drug development, but his company was able to provide pure aristolochic acid to other researchers.

Prompted by the evidence of carcinogenicity, in the mid-1980s Heinz Schmeiser, a biochemist at the German Cancer Research Center in Heidelberg, demonstrated that aristolochic acid was mutagenic. By 1990 he and his colleagues had published results showing that it could bind to DNA, forming *adducts*, which, if they persist, could lead to the development of cancer.[11] Thus, before the first report about Chinese herbs nephropathy in Belgian women appeared in the *Lancet* in 1993, all the analytic methods for detecting aristolochic acid–DNA adducts had been worked out in Heidelberg.

The two groups of nephrologists—at the Protestant Erasmus Hospital and at the Catholic University of Louvain Hospital—were aware of each other's findings as well as the work of Schmeiser in Heidelberg. Cosyns at Louvain Hospital was first to initiate a collaboration with Schmeiser. The resulting chemical analyses, published in 1996, showed that all renal tissue samples from the Belgian women contained aristolochic acid–DNA adducts and that the cumulative dose of *Aristolochia* was a significant risk factor for kidney disease and urothelial cancer.[12] This provided confirmatory evidence for the substitution of the nephrotoxic and carcinogenic *Aristolochia fangchi* for the benign *Stephania tetranda* and documented exposure in the actual tissues. Vanherweghem and his group also collaborated with Schmeiser, and the landmark *New England Journal of Medicine* paper in 2000 by Nortier and Vanherweghem listed Schmeiser and his colleague as coauthors.[13]

In 2001 the U.S. Food and Drug Administration issued an advisory alerting consumers to immediately discontinue the use of products containing aristolochic acid. Other countries took similar actions. And in 2002 the International Agency for Research on Cancer classified aristolochic acid as "probably carcinogenic to humans."[14]

* * *

It was not until early 2002 that Arthur Grollman, a molecular pharmacologist and head of the Department of Pharmacological Sciences at the School of Medicine at Stony Brook University, came across the *New England Journal of Medicine* article describing the similarity between Chinese herbs nephropathy in the Belgian women and Balkan endemic nephropathy. That linkage immediately "piqued his interest," as he told me when I interviewed him in his office ten years later, and set him on a course of research combining epidemiologic investigations with powerful molecular and genomic techniques. His research would take him to the Balkans and Taiwan and would contribute new insights to our understanding of the mechanisms underlying the development of urothelial cancer and cancer in general. No less important, it would draw attention to a worldwide public health problem.

I knew Arthur from the 1990s when I was in the Department of Preventive Medicine at Stony Brook. As a molecular biologist interested in chemical carcinogenesis, he was always alert to opportunities to study the effects of environmental and occupational exposures on the development of cancer—such as the extensive exposure of workers and residents in the Techa River area in the former Soviet Union to high levels of radiation from a nuclear plant disaster that occurred in the 1950s. For this reason, he has always been eager to collaborate with epidemiologists. On a number of occasions, we had met to discuss possible projects in his office, which showcased striking photographs of his trekking expeditions in the Himalayas.

Today, in his early eighties, Grollman is trim and energetic and totally immersed in his research program. When explaining the intricacies of his work, his manner is low-key and unhurried, and one detects in his speech a trace of his childhood growing up in Texas as the son of an eminent pharmacologist. He smiles benignly as he highlights the twists and turns in the research, the false paths, and the competing claims of different groups. In an age of extreme specialization, he is willing to immerse himself in unfamiliar disciplines and cultures and to learn new technologies in order to pursue a problem that interests him. He has numerous collaborations both

within his own institution and with clinicians and scientists in Europe and Asia, and he travels widely to attend meetings and give lectures on his work. His work on the molecular toxicology of aristolochic acid and cancer has become a poster child at the National Institute of Environmental Health Sciences for *translational research*, a term that refers to basic research that can be utilized to develop new treatments.

Grollman has devoted much of his career to studying how specific molecules damage DNA, and the consequences of such damage. Humans—and indeed all animals—have an exquisite system for repairing damage to DNA, and most such damage is repaired. However, when the damage affects key segments of our genetic material—such as tumor suppressor genes or oncogenes—and when the resulting lesions elude repair, this can lead to a mutation that gets perpetuated and eventually develops into a cancer. Tackling the mystery of Balkan nephropathy using the most advanced techniques in molecular genomics would turn out to be the culmination of a career studded with accomplishments. However, Grollman would probably never have gotten involved with this obscure disease if it hadn't been for his interest in an issue that had attracted his attention closer to home.

By the early 2000s Grollman had become aware of the huge and largely unrecognized problem created by the widespread availability of herbal supplements, which had come into vogue in the 1960s and had continued to grow since then. The popularity of these products was reflected in the sales of *Prevention* magazine and the spread of megacompanies like GNC. With the rise of the Internet, their availability and popularity continued to expand. In 2001, $17.8 billion was spent in the United States on dietary supplements, $4.2 billion of it for herbs and other botanicals. Many consumers tend to assume that, because these products are "natural" and are advertised and marketed legally, they must be safe, and that the claims of beneficial effects must have some basis. The reality is quite different. In fact, owing to the growing clout of the dietary and herbal supplements industry, in 1994 Congress had passed the Dietary Supplement Health and Education Act (DSHEA). By defining herbal supplements and botanicals as "dietary supplements," DSHEA exempted them from the more rigorous standards used by the FDA in regulating prescription and over-the-counter drugs and medical devices, essentially leaving it up to the industry to regulate itself.

Soon after DSHEA opened the floodgates for herbal supplements, Grollman and his Baylor College of Medicine colleague Donald Marcus started drawing attention to this alarming state of affairs. Their first effort was to organize a symposium at the national meeting of the Association of

American Medical Colleges. The following year, in 2002, they published an article in the *New England Journal of Medicine* drawing attention to the fact that "natural" is not necessarily safe.[15] Their message was clear and unambiguous: since botanicals are "complex mixtures of chemicals," some of which are potentially toxic, in order to protect the public, these products should be subject to the same rigorous regulation as applies to food and drugs. In 2003, as a case in point, they documented the toxicity of the popular botanical Ma Huang, better known as ephedra, in the journal *Science*.[16] As a result of their writings, Grollman was asked to testify before Congress and the White House Commission on Alternative and Complementary Medicine on the topic of herbal supplements.

As academics, Grollman and Marcus thought that their arguments would carry weight with their colleagues, particularly if they focused on botanicals, which they predicted would have toxicities and little or no reliable evidence of therapeutic value. However, they soon became aware that Complementary and Alternative Medicine, or CAM, and an uncritical attitude toward the use of botanicals were making inroads within the academic community itself. They were taken aback that the deans at prestigious medical schools, including Johns Hopkins, Columbia, Duke, and Harvard, had been persuaded of the value of establishing programs in CAM at their institutions. At Stony Brook in 1997, the dean of the School of Medicine and the director of the University Hospital decided to set up a center for CAM as a way of bringing in funds from this emerging, if academically dubious, discipline. Grollman and several other department heads voiced their opposition to the university's engaging in this area. In spite of their objections, however, the dean hired a pediatrician named Sam Benjamin to head up the new program. When Benjamin gave a lecture, Grollman would attend and ask probing questions, and it got to the point where Benjamin would appear to shrink when he saw Grollman enter the room. By this time, Grollman's annual lecture on pharmacology for the medical students and residents was devoted to a critical examination of the toxicity of botanicals and herbal supplements.[17]

The Stony Brook CAM Center proved to be short-lived and closed down after three years. But because of it, Grollman had become even more aware of what he now saw as a national problem, and he continued to be on the lookout for new material for his lecture and for "ammunition" to expose the facile and dangerous misrepresentations of the purveyors of CAM. It was in this heightened state of alert that in 2002 he came across the *New England Journal of Medicine* article from 2000 by the group from

the Erasmus Hospital in Brussels. What piqued Grollman's interest in the article describing Chinese herbs nephropathy in the Belgian women was the likening of the Belgian syndrome to Balkan nephropathy. He had heard about Balkan endemic nephropathy as a medical student at Johns Hopkins, and now he became aware of the long-standing failure to make progress in identifying its cause since it was first recognized forty years earlier.

* * *

In the late 1950s throughout the Balkans (in Bosnia, Serbia, and Croatia, which were then part of the former Yugoslavia, as well as in Romania and Bulgaria), physicians had noticed a mysterious renal disease in certain rural farming villages located along tributaries of the Danube River, the Sava, the Drava, the Morava, and the Kolubara[18] (fig. 6.1). It was documented independently in the different countries, but its features were the same everywhere. The disease was characterized by a unique type of renal

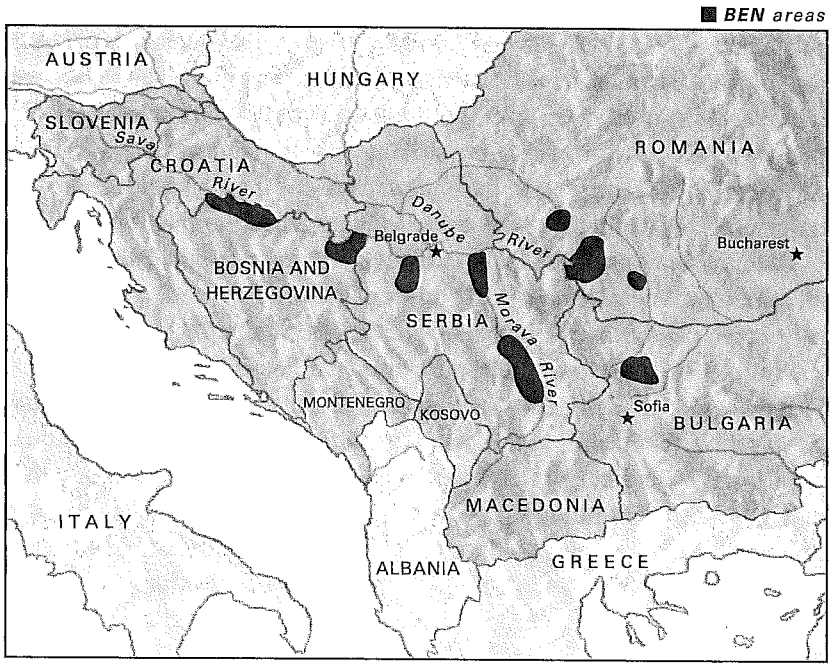

Figure 6.1
Map showing distribution of Balkan endemic nephropathy regions.
Source: Maharaj et al. 2014. By permission Springer Publishing Company.

pathology—fibrosis, or scarring, of the renal tubules, progressing invariably to end-stage renal failure. The glomeruli—the capillaries that perform the first step in filtering the blood—remained untouched until the kidney was shrunken with fibrosis, so patients didn't show symptoms until the very late stages of the disease. The geographic distribution of the disease was also striking. It was limited to rural areas and to families engaged in farming, and it presented a "mosaic pattern"—one village would have it, while another nearby village did not. Endemic villages were almost always ones whose fields were located in the floodplain of a river. The disease affected adults, often in the same household, but never occurred in those less than 18 years of age. The female-to-male ratio was 1.0 or somewhat higher.[19]

Over the next thirty or forty years, clinicians and public health scientists in these countries carried out solid epidemiologic studies with minimal financial support. By conducting surveys of villages—including those with and without the disease—in defined endemic areas over a number of decades, researchers were able to describe the distinctive features of the condition. Because the disease tended to cluster in families, it was logical to surmise that the condition was hereditary. However, these early epidemiologic investigations pointed strongly to an environmental cause. This was suggested by the fact that when women in an endemic village who were not affected moved to an unaffected village, after about fifteen years they developed the disease. Even more striking was the experience of Ukrainian migrants who had moved to the endemic area of Croatia at the end of the nineteenth and beginning of the twentieth centuries. They had been farmers in Ukraine, and they made up nearly half of the farming population in some villages in the endemic region. Their way of life was very similar in almost all respects to that of their Croatian neighbors, except that each group went to their own churches. Endemic nephropathy did not exist in Ukraine; by the 1950s, however, the migrants had levels of the disease comparable to their Croatian neighbors. This amounted to what epidemiologists call a "natural experiment." The fact that two different populations—the indigenous Croats and the Ukrainian immigrants—both had comparable rates of nephropathy in the affected areas suggested strongly that the disease was not hereditary and that some common environmental exposure was involved.[20]

The mysterious condition, which had never appeared anywhere else in this particular form except in these five countries, was given the generic appellation of "Balkan endemic nephropathy," or simply "endemic nephropathy." (Grollman remarked that no people wish to have a disease associated with their name, and people in the Balkans were delighted

when he and his colleagues eventually showed that this condition was not limited to the Balkans.)

By the 1960s it had also been noticed, first in Bulgaria, that in addition to the occurrence of nephropathy, these same endemic villages had a high incidence of cancer of the upper urinary tract (renal pelvis and ureter), and the cancers frequently occurred in the same patients who had the nephropathy.[21] The vast majority of urothelial cancers worldwide occur in the bladder, with less than 5 percent of tumors involving the upper urinary tract. So it became clear that the unusual form of kidney damage and the rare urothelial cancer of the upper urinary tract associated with it were two features of the same syndrome.

(It is important to clarify that damage to the tubules of the kidney is unrelated to the development of the cancer, which occurs in a different tissue—that which lines the upper urinary tract, which runs from the renal pelvis to the bladder. However, the aristolochic acid–DNA adducts are best measured in the cortex of the kidney because they are concentrated there twentyfold.)

Then in 1964 the World Health Organization organized an international symposium in Dubrovnik at which the different aspects of Balkan nephropathy—clinical, pathological, and epidemiologic—were comprehensively reviewed by scientists from the region and from many other countries as well.[22] Participants considered all the possible explanations, including genetics, viruses, bacteria, immunologic disorders, heavy metals such as lead and cadmium, lignites from coal, and ochratoxin—a fungal toxin. And they could pretty well rule out most of them over the next few years. But the one they eventually focused on was ochratoxin, in part because there were high levels in the blood of the farmers in the villages. In retrospect, it is easy to see that ochratoxin didn't really make sense. The fact is that high levels of the toxin are found in farmers in certain parts of Europe and elsewhere throughout the world, where the incidence of nephropathy is unremarkable. But they chose to overlook that inconvenient fact. Scientists focusing primarily on endemic nephropathy wanted very much to believe that ochratoxin must be involved, and for the next thirty years that was the only theory that got attention.[23]

Actually, an astute Serbian microbiologist named Milenko Ivić had published a paper in 1969 proposing that aristolochic acid toxicity might be responsible for Balkan nephropathy and its associated cancer.[24] Based on his own observations of farming life and unpublished work he had done on aristolochic acid toxicity as a graduate student, Ivić made a compelling argument that all the available facts pointed to contamination of wheat with the seeds

of the weed *Aristolochia clematitis*. These facts included the distinctive distribution of the disease (occurring only in villages, never in cities, and often among multiple members of the same household); the plant's renal toxicity, which had also been demonstrated in horses accidentally and experimentally poisoned with *Aristolochia*; and the carcinogenic properties of aristolochic acid, which Ivić himself first demonstrated in rabbits. Other scientists working in the field sometimes cited his paper, but it was always referred to as just another hypothesis. Not one attempt was made to test it, and Professor Ivić died long before Grollman was to prove his hypothesis to be correct.

* * *

Forty years after the initial recognition of Balkan nephropathy, the incidence of the disease had hardly changed. And despite the publication of hundreds of scientific papers and the holding of numerous symposiums, there was no clue as to its cause. This is where things stood in 2002, when Grollman, after reading the article drawing attention to the similarity of Chinese herbs nephropathy to Balkan nephropathy, was seized by the urge to delve into this long-standing unresolved conundrum. The usual way a scientist would go about exploring a new idea would be to apply for a grant from the National Institutes of Health. But this would mean that, even in the best possible case, a year or more would elapse before he could obtain funding, and then, realistically, it would take another two or more years to carry out the study.

Instead, Grollman walked over to his computer and pulled up Google Earth and studied the pattern of the fields and farming villages laid out on either side of the Sava River, several hours east of Zagreb. He had no idea how to go about conducting an epidemiologic study in a remote and unfamiliar region, but it so happened that he and his wife were close friends with a couple from Croatia, the Prelećs. Krsto Preleć was a physicist at Brookhaven National Laboratory, and his wife was a librarian at Stony Brook. They put him in touch with Bojan Jelaković, head of nephrology at the Zagreb University School of Medicine. Thanks to this connection, Grollman was able to arrange a quick visit to Zagreb in the spring of 2002, and Dr. Jelaković, who was to become his main collaborator, drove him to the endemic area. When they got to the village of Kaniža in the focal area of Brodska Posavina, Jelaković said, "Let me show you the black houses." This was the local term for houses that had been abandoned when their owners died of endemic nephropathy. They walked through the village, which had fifty or sixty homes. Several of them were completely run-down, with gaping doors and windows and at least one with the branches of a tree growing

through the roof. And Jelaković remarked only half-jokingly that one could determine the local prevalence of nephropathy by counting the number of black houses. Six black houses, 10 percent of the village.

From the endemic village, Jelaković took Grollman to the dialysis clinic at the hospital in Slavonski Brod, the main city in the county. Since the Belgian women had been taking herbs, and since this was a rural area and Grollman knew from his reading that *Aristolochia* had been used as an herb known as "birthwort" for thousands of years in Europe, he had the idea that the patients must have been taking it. With Jelalović acting as translator, he interviewed a number of patients who were tethered to the dialysis machines for up to six hours. The patients were highly cooperative. They and their families were living with the scourge of this inexplicable disease, and they were eager to help anyone who took an interest in it and were comfortable answering probing questions about their way of life. But after interviewing a number of patients, Grollman was convinced that none of them had used any form of herbal medicines. "It was a good idea," he remarked, but it simply didn't fit their medical history.

Grollman's wife had come along on the trip, and they had planned to visit Dubrovnik on the Dalmatian coast. But before they left, he went back to the medical school library in Zagreb, where he happened to know the librarian, who had spent time at Stony Brook as a visiting scholar. She showed him a section of the library that had dust-covered books relating to kidney disease, among which he found a 1956 article from the veterinary school at the university. "Now you would never pick this article up on PubMed, I can tell you," Grollman said. The article, which was in German, described kidney disease in horses, which the authors had linked to their ingesting *Aristolochia clematitis*, a weed that grows in many cultivated fields. Grollman remarked that "the vets knew their subject," having recognized in 1920s that *Aristolochia* plants were toxic for horses. They had published several papers on this topic and had even fed horses *Aristolochia* and studied its toxic effects. "But since these reports were from the veterinary school, their colleagues in the nearby school of medicine didn't pay attention." But what caught Grollman's eye was that the article displayed pictures of fields with *Aristolochia clematitis* growing abundantly. And when he looked at the histopathology of the *Aristolochia*-induced kidney disease in horses, it resembled what was seen in humans. At this point Grollman realized that Balkan nephropathy might have something to do with the wheat used in making bread, which, he had learned on his visit to the endemic region, makes up about 50 or 60 percent of the diet in these poor, rural areas.

Grollman reasoned that the *Aristolochia* had to comingle with the wheat and that it had to be pretty strong to withstand the baking process. He knew that you could pick up evidence of exposure and genetic damage by measuring adducts to aristolochic acid in kidney tissues, as Schmeiser had done in the Belgian women.

To test his hypothesis that aristolochic acid was actually the cause of endemic nephropathy, he asked Jelaković for tissue samples to take back with him. The pathology department in Slavonski Brod had samples stored in formalin, going back forty years, and Jelaković obtained a couple of paraffin-embedded blocks from endemic nephropathy patients, which, after visiting Dubrovnik, Grollman took back to New York. In addition to the age of the samples, they had been whittled down by other investigators who had dug out much of the tissue. In spite of the poor condition of the specimens, Grollman's long-term colleague, Shinya Shibutani, was nevertheless able to tentatively identify aristolactam-DNA adducts using a method he had developed called P32-postlabelling, a type of radioisotope analysis. (This method was a modification of that used to detect aristolochic acid–DNA adducts in tissues from the Belgian women.) But, owing to the treatment of the samples with formaldehyde, the image was blurry, and they could not be 100 percent confident of their conclusion.

Grollman and his collaborators were eager to obtain fresh frozen kidney tissue from patients with endemic nephropathy to confirm their finding, and they got very excited on hearing that two such kidneys were stored frozen in Bulgaria. After lengthy correspondence to get cooperation from the Bulgarian clinicians, they were about to send a collaborator from Croatia to pick up the samples when they learned that the freezer storing the tissues had failed, and the specimens were irrevocably damaged.

Just when he thought all was lost, Grollman was contacted by a malpractice attorney in Providence, Rhode Island. Somehow the attorney had heard about Grollman's work on aristolochic acid, and he told him about a woman who had been given herbs by a local practitioner of alternative medicine. An astute pathologist at the University Hospital in Providence had made the tentative diagnosis of aristolochic acid nephropathy on the woman's biopsy, and he wanted Grollman to confirm it.

Grollman innocently asked whether any of the patient's renal tissue had been saved in pathology and learned that it was standard practice to leave the damaged kidney in place when performing a renal transplant. This particular operation had been performed by a prominent transplant surgeon in Providence. Grollman informed the attorney, and later the transplant

surgeon, that it was highly advisable that they operate again to remove the damaged kidneys, since they were very likely to develop urothelial cancer over time. The transplant surgeon agreed, and Grollman was asked about his consultation fee. He replied "none," provided that he would be given access to the fresh frozen tissue when the kidney was received. To make sure he got it, Grollman asked to come to the operating room at the time of the repeat surgery. So on a snowy night in 2003, a few days before Christmas, Grollman flew up to Providence and picked up the kidney personally. (As if to further heighten the drama surrounding this serendipitous acquisition of the crucial tissues, while waiting for his return flight, there was a bomb scare at the airport and the terminal was evacuated. Grollman was worried that airport security would not allow him through with his samples marked "Biohazard—Biological Materials." However, his explanation was accepted and he caught his flight.) It was this fresh kidney tissue that he used to definitively identify aristolactam-DNA adducts, as described in a landmark paper in the *Proceedings of the National Academy of Sciences* (2007).[25]

While Grollman and his colleagues were refining their methods for detecting aristolochic acid-adducts, by coincidence Tjaša Hranjec, a Stony Brook medical student who was fluent in Serbo-Croatian, came to him looking for a summer research project. Under his supervision (through frequent telephone calls and e-mails) and with assistance from Dr. Jelaković, Tjaša provided the "boots on the ground" necessary to carry out an initial case-control study of Balkan endemic nephropathy in the endemic region of Croatia. She conducted interviews with patients and controls, obtained all the needed specimens, and helped solve the logistical problems that arose. She met with the patients Grollman had interviewed in the dialysis clinic, including a farmer who took her out to his fields. It was after the harvest, and she saw *Aristolochia clematitis* growing scattered throughout the wheat fields, just the way the horse paper had described fifty years earlier. The seeds come to fruition at the height of the summer, and the farmers use very primitive methods—little beyond the scythe—to harvest the wheat. She asked the farmer why he didn't get rid of this weed. And he said, "Doc, it's very hot out here, and it's not gonna do you any harm. Look at all the weeds."[26]

While in the endemic area, Tjaša had visited a retired miller and his old-fashioned mill that local farmers had used for generations. After harvesting the wheat each year, farmers would take it back to their homes and store it in the attic. Every two weeks they would take grain to the miller, have it ground, give the miller 10 percent in payment, and bring the flour home.

The women would then bake five-pound loaves of bread. The first week the bread would be fresh, but by the second week, it would be stale and they would feed it to the animals. But bread constituted 50 percent of the farm diet. And the aristolochic acid in the seeds is very stable and therefore survives the temperature of the baking oven.

The initial study included twenty-eight cases who met the criteria for endemic nephropathy, thirty individuals with other forms of renal disease, and thirty healthy controls. Using a detailed questionnaire, the researchers collected information on demographics, exposure to potentially toxic substances, diet, agricultural practices, and other factors that might contribute to endemic nephropathy. In addition, seeds of *Aristolochia clematitis*, obtained from plants growing in the endemic region, were analyzed for their aristolochic acid content.

The results of this initial epidemiologic study showed that twenty to thirty years earlier, patients with endemic nephropathy had encountered *Aristolochia clematitis* in the fields much more frequently than controls did. All groups reported that since that time there had been a significant increase in the use of herbicides, leading to a reduction in the prevalence of the weed in recent years. Chemical analysis established that the seeds of *A. clematitis* contained 0.65 percent aristolochic acid and that it was likely that the seeds had mingled with the wheat grain during harvesting. The results were published in the *Croatian Medical Journal* in order to get the word out quickly to clinicians in Balkan countries who were taking care of endemic nephropathy patients.[27]

Now Grollman had a hypothesis. Ingestion of aristolochic acid combined with individual susceptibility accounts for all the epidemiologic and clinical features of endemic nephropathy. And the hypothesis had a corollary: Balkan endemic nephropathy, Chinese herbs nephropathy, and aristolochic acid nephropathy were one and the same disease. Or, in Grollman's notation: BEN = CHN = AAN.

* * *

Grollman was undertaking his study in Croatia just after the end of the wars among Balkan countries with their widespread atrocities, and he remarked on the geopolitics he and Dr. Jelaković had to contend with. "The five physician groups who had studied this rarely talked to each other, even when three of them were part of one country—Yugoslavia—much less collaborate on medical research." He knew that—in a perverse reflection of nationalism—they

would all say that "their" disease was different. He realized that, if they were going to solve the riddle of Balkan endemic nephropathy, they were going to have to work together. It took a good deal of informal diplomacy to succeed in getting tissue samples from Serbia, Bosnia, and Croatia. In addition to the political animosities, Grollman and his collaborators had to overcome distrust on the part of some clinicians and deal with very different medical practices. For example, he needed tissue specimens from the cancer patients to analyze for DNA adducts and mutations. But when he asked for biopsy specimens, he learned that the nephrologists did not perform biopsies on patients with suspected endemic nephropathy. Nor did they any longer perform autopsies on patients dying of the disease. It looked like he was never going to get the kidney samples he needed for a systematic analysis of adducts in human kidneys. But then he realized that the urothelium extends into the kidney pelvis and it's a curable cancer—the surgeon removes the affected kidney, so he could get both tissues into the bargain. The urologists were doing two or three operations per month. So he asked, "What happens to the kidney?" "Oh, we throw that away." He told them, "Please don't throw it away anymore." Since the cancer was so common in this area, all one had to do was get the cooperation of the urologists who did the surgery in Slavonski Brod and the pathologist, who were pleased to provide the samples. So he got both the kidneys and the urothelium at the same time.

Encouraged by these developments, Grollman and colleagues went on to conduct molecular studies of upper urothelial cancer including cases from endemic areas in Bosnia and Serbia, as well as Croatia, and using patients with upper urinary tract cancers from nonendemic areas as controls. They detected adducts to aristolochic acid in 85 percent of nonsmoking patients with nephropathy and upper urothelial cancer living in endemic regions. These adducts persist in the renal cortex for decades, making it likely that people with the exposure would eventually develop cancer. Significantly, adducts were not detected in patients with upper urinary tract cancer living in Zagreb or Belgrade. The investigators concluded that aristolochic acid–DNA adducts provide a robust "biomarker" of exposure to aristolochic acid.[28]

The comparison between the effects of exposure to aristolochic acid in the Brussels weight-loss spa and those of long-term dietary exposure in the Balkans was instructive. On average the women in Brussels were exposed to their regimen for twenty months, and progression to end-stage renal disease also occurred within months. In the Balkans, where both men and women were affected, lower-dose exposure to the aristolochic acid–contaminated bread had occurred over decades, and the average age of onset

of nephropathy occurred in the fourth or fifth decade of life. The Belgian women developed upper urothelial cancer within two to six years following the end of their exposure, in contrast to a much longer interval in the Balkans, ranging from twenty to thirty years and roughly ten years after the onset of nephropathy. To a large extent, these differences were likely due to the fact that the Belgian women ingested a much higher dose of aristolochic acid over a short period of time, whereas in the Balkans the typical dose of aristolochic acid was about one-tenth that of the Belgian women, and typically exposure extended from childhood over the better part of a lifetime.[29] Regarding the potency of *Aristolochia*, Grollman commented that ten seeds of *Aristolochia* scattered among perhaps ten thousand seeds of wheat in a loaf of homemade bread was enough to cause disease. The fact that aristolochic acid is both a kidney toxin and a carcinogen, together with the persistence of the damage over a lifetime, make it stand out among environmental mutagens.

The much shorter "induction period" for nephropathy among the Belgian women and the fact that they had all attended the same clinic allowed alert clinicians to quickly pinpoint Chinese herbs as the probable cause, whereas in the Balkans, owing to the chronic exposure to a lower dose, the disease developed slowly and insidiously, and it took forty years to identify the causative exposure.

* * *

The presence of DNA adducts to aristolochic acid in tissues from patients with upper urothelial cancers who were long-term residents of endemic areas suggested that aristolochic acid–induced mutations might play a role in causing the cancer. However, the nature of the damage and how it led to the development of cancer were unclear. Grollman and colleagues proceeded to make a novel contribution to understanding the mechanism by which aristolochic acid induces upper urothelial cancer.

Over the past thirty years discoveries in molecular biology have transformed our understanding of how cancer develops. This new understanding can be stated simply: cancer is a genetic disease. Every individual has a unique genetic identity inscribed in the DNA in every cell in his or her body. Within the DNA, segments of four nucleic acids, or "bases," specify every protein that is made and every physiologic process. The four bases are adenine, guanine, thymine, and cytosine (A, G, T, C). Errors in DNA occur routinely, but most are corrected thanks to our exquisite "copyediting" machinery. If, however, a

change in single nucleic acid base in a key gene eludes repair, this can lead to the development of cancer. It is now believed that a handful of mutations to key genes drive the complex, multifactorial, multistep carcinogenic process. Among the most important events are the inactivation of tumor suppressor genes and the activation of oncogenes. Mutations in these genes may be caused by physical agents (e.g., ultraviolet radiation, X-rays), chemical agents (such as benzene, arsenic, benzo(a)pyrene, and vinyl chloride), viruses, and bacteria, or may be inherited. p53 is a major tumor suppressor gene, which is often referred to as part of the "braking system" that protects against cancer. Mutations of the p53 gene are present in roughly 50 percent of all human cancers and occur in different locations along the gene.

Roughly twenty years ago, the discovery of so-called signature or fingerprint mutations had caused great excitement among cancer researchers. This referred to alterations in the sequence of nucleic acid bases in the p53 gene that could serve as a marker of exposure to a specific agent, which plays a role in the induction of a specific type of cancer.[30] Bert Vogelstein, a leading figure in the field of carcinogenesis, at Johns Hopkins, was coauthor on the first paper that made a strong case for a signature mutation in the p53 gene specifically associated with exposure to aflatoxin, a chemical produced by a fungus that grows on peanuts and corn in southern Africa and China, and that plays a role in primary cancer of the liver in those regions. This work generated enormous enthusiasm for the identification of other signature mutations associated with other carcinogenic exposures. However, few comparable fingerprint mutations have been identified in the past twenty years.

The fact that mutations in p53 are also present in about 50 percent of upper urothelial cancers led Grollman and colleagues to examine specific mutations in tissues from patients with endemic nephropathy who had developed upper urothelial cancer. Performing genomic analysis, they identified a unique signature mutation in p53, involving the substitution of thymine for adenine, referred to as an "A T transversion."[31] (Changes of this type have particularly drastic effects because they involve a dramatic change in the chemical structure of DNA.) They also showed that, owing to its location on the nontranscribed stand of the p53 gene, this change eluded repair. This clarified at the molecular level why these adducts persist for decades and eventually lead to cancer. More recent work has shown that the overall mutation rate in aristolochic acid–associated cancers is several times higher than that caused by other carcinogens, such as tobacco and ultraviolet light.

By 2007 the International Agency for Research on Cancer in Lyon had compiled a worldwide databank of genetic sequences of different cancers

that researchers can consult.[32] When Grollman compared the p53 mutation pattern in patients with upper urothelial cancer from endemic areas with that of all urothelial cancer cases worldwide in the IARC databank, 78 percent of the former had the A → T transversion compared to only 5 percent of the twenty-five thousand urothelial cancers in the databank. If one limits the comparison to upper urothelial cancers in the IARC database, less than 1 percent have the A → T transversion. As Grollman put it, "So, the game's over right there. This mutation clearly is dominant and a signature of aristolochic acid–associated upper urothelial cancer." What this means is that the way in which aristolochic acid induces cancer is distinct from the way in which other agents such as tobacco, aflatoxin, or X-rays induce cancer. According to Grollman, after aflatoxin, this is the first truly distinctive signature associated with a major chemical exposure to be identified in many years.

With this work that was published in the *Proceedings of the National Academy of Sciences USA* in 2007, Grollman and colleagues had confirmed their predictions. Seeds of *A. clematitis* comingle with the wheat grain used to prepare home-baked bread. Aristolochic acid–DNA adducts are present in the renal cortex and in urothelial tumor tissue of patients with Balkan endemic nephropathy. And finally, a single, specific signature mutation is the most common p53 mutation in upper urothelial cancer associated with endemic nephropathy. They had demonstrated that BEN = CHN = AAN.[33]

When the results of the study in the Balkans were complete, each national group of collaborators had to organize a separate symposium—one in Zagreb, one in Belgrade, and one in Sarajevo—for Grollman to present the work before physicians and researchers from each Balkan country were willing to accept that "we had proved that something other than ochratoxin was responsible for Balkan endemic nephropathy."

* * *

After working out the mechanism by which aristolochic acid modifies DNA and identifying the unique mutational signature in the p53 gene present in the majority of aristolochic acid–associated upper urothelial cancers, Grollman saw that another critical question needed to be answered. In spite of the strong link between ingestion of aristolochic acid, whether in powdered Chinese herbs or in bread contaminated with seeds from *Aristolochia clematitis*, not everyone who was exposed became ill. In Belgium, 105 women developed nephropathy out of 1,800 who were exposed, or about

5 percent. And in endemic villages in Croatia, 5–10 percent of residents of these villages develop endemic nephropathy. This suggested that genetic susceptibility, or resistance, to the effects of aristolochic acid influenced one's risk of developing the disease. In laboratory experiments with mice, Rosenquist and Grollman had confirmed the existence of genes governing susceptibility or resistance to aristolochic acid–induced nephropathy. Thus an important question that remains to be answered is, what is the genetic basis for human sensitivity to aristolochic acid?

Over the past three decades a major thrust of biomedical research has been to identify the genes and genetic variants that make someone either susceptible or resistant to chronic disease, including cancer. During this period, at an ever-increasing rate, scientists had been examining "candidate genes" suspected of playing a role in susceptibility to specific diseases. When the rough version of the human genome was announced in 2000 and featured on the covers of *Nature* and *Science*, this search for candidate genes only intensified. Grollman pointed out to me that "if you look back, during the years before 2007, hundreds of papers were published in leading journals saying, 'We found this gene that contributes to susceptibility.' But if you ask, 'Which studies were replicated?' the answer is: very few—perhaps only one or two each year! Everyone had their favorite gene, but no one did the statistics to remind themselves that there are 23,000 genes, so you are going to get a lot of false positives."

In 2007 the field moved away from the approach of looking for hypothesis-driven candidate genes and embraced "genome-wide association studies" in which whole genome sequences are compared between those with a disease and those without. Large sample sizes are required for these studies—thousands or tens of thousands of patients—and the requirement for replication of results is built into the new approach. As Grollman put it, "genome-wide, non-hypothesis testing trumps candidate genes." By the time Grollman turned to the question of susceptibility in 2009, the methods for sequencing whole genomes and identifying all potentially relevant genes had been fundamentally transformed, and so-called "next-generation sequencing" had become possible.

Grollman is collaborating with Bert Vogelstein and Ken Kinzler at Johns Hopkins, using advanced DNA sequencing techniques to identify genes that influence a person's risk of developing upper urothelial cancer, given exposure to aristolochic acid. This new work has revealed that exposure to aristolochic acid is associated with a number of somatic mutations throughout the genome, in addition to the ones in TP53. Nearly

three-quarters of these mutations exhibit the distinctive signature A → T transversions. The pattern of mutations in aristolochic acid–associated upper urothelial cancer contrasts starkly with that seen in smoking-associated upper urothelial cancer cases.[34]

* * *

The outbreak of Chinese herbs nephropathy in Brussels resulted from the unfortunate substitution of one Chinese herb for another. And Balkan endemic nephropathy proved to be a long-standing environmental disease due to the unrecognized presence of the toxic weed *Aristolochia clematitis* growing in the local wheat fields, which led to contamination of the grain used in preparing homemade bread. But it now became clear to Grollman that the potential impact of the toxic and carcinogenic effects of *Aristolochia* was likely to be much greater than suggested by these two localized episodes, since in various forms this herb has been used on virtually every continent going back thousands of years. It now occurred to him that *Aristolochia*-caused nephropathy and cancer might be global diseases.

When he looked for reported cases of *Aristolochia*-associated upper urothelial cancer, however, there were no systematically recorded statistics. All he found were small numbers based on recent case reports: 4 in both the United Kingdom and France, 1 in both Spain and Germany, 128 in Belgium, 1 in South Korea, 6 in Japan, 33 in Taiwan, and 116 in China. These were individual cases where there was some indication that the person had used Chinese herbs, but there was no objective evidence of exposure, such as aristolochic acid–DNA adducts. Grollman realized that his two biomarkers—for aristolochic acid–DNA adducts and for the signature mutation—provided a robust means of determining the prevalence of aristolochic acid–induced urothelial cancer in different populations with a high degree of accuracy.

Aware that Taiwan had the highest incidence of upper urothelial cancer, as well as one of the highest rates of kidney disease in the world, Grollman contacted urologists at the National University Hospital in Taipei and suggested that *Aristolochia* might be a contributing factor. The urologists were skeptical, but they agreed to collaborate. In 2010 a group of Taiwanese researchers had published the results of a countrywide case-control study of Chinese herbal products containing aristolochic acid and risk of urinary tract cancer.[35] Owing to the existence of a national health insurance system that covers 96 percent of the Taiwanese population, they were able to access all prescriptions for Chinese herbs filled between January 1, 1997,

and December 31, 2002. Comparing the prescription histories of nearly 4,600 urinary tract cancer cases enrolled during a one-year period to those of 174,701 controls, the authors showed that the risk of urinary tract cancer increased in a dose-dependent manner with increasing intake of Chinese herbs containing aristolochic acid. The scale of use of herbal supplements—and their potential impact on kidney disease and urinary tract cancer—was driven home by a systematic analysis of prescriptions filled by a 200,000-person random sample of the entire insured population of Taiwan between 1997 and 2003. Approximately one-third of the sample consumed herbs containing, or likely to contain, aristolochic acid. Approximately 140,000 pounds of one of these herbs, *Aristolochia debilis* (Quing-Muxiang), are imported annually into Taiwan.

Grollman proceeded to carry out a molecular epidemiologic study to learn whether exposure to aristolochic acid, found in all *Aristolochia* herbal remedies, contributed to the high incidence of upper urothelial cancer in Taiwan. The study design was similar to that used in the Balkans. The study included 151 patients with upper urothelial cancer and 25 patients with renal cell cancer (the most common type of kidney cancer) serving as a control group. Both groups were equally exposed to the toxin, based on the presence of aristolochic acid–DNA adducts. However, similar to the results in the Balkans, the pattern of p53 mutational spectra in Taiwanese patients with upper urothelial cancer showed a predominance of the rare A → T transversions, whereas this mutation was absent in the controls. Furthermore, the combination of aristolochic acid–DNA adducts and presence of the signature mutation underscored the close association between exposure to aristolochic acid and its carcinogenic effect. These results were published in *Proceedings of the National Academy of Sciences* in April 2012, shortly before I visited Grollman, and they had generated interest in the scientific community as well as millions of hits on the Internet, particularly in Asia.[36]

As Grollman put it, describing the p53 mutation results from the Balkans and those from Taiwan, "when you put them side-by-side, they're almost mirror images. The important thing is to compare the two. When you look at those mutations in a single base pair in DNA—in the Balkans and Taiwan—they go absolutely on top of each other. It's not even one base off. You have different ethnic groups, different environments, and different routes of exposure. To have that degree of specificity—that is solid evidence for the global nature of this disease."

In addition, the researchers noted that the prevalence of adducts and of the signature mutation was slightly higher in female compared to male

cases, and women in Taiwan are more likely than men to obtain prescriptions for herbal supplements. Thus the higher incidence of upper urothelial cancer among Taiwanese women may reflect, in part, their more extensive exposure to *Aristolochia*-containing herbal remedies.

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Referring to the hundred-plus case reports of *Aristolochia*-induced upper urothelial cancer in all of China, Grollman said, "A hundred cases! Either the Han Chinese in China have different genes—which seems very unlikely—or they are not recognizing or reporting it." *Aristolochia* has been used as an herbal remedy in China since at least the Han dynasty, two thousand years ago. In the 1500s the Chinese herbalist Li Shizen assembled all previous *materia medica* from China, which included various herbs in the *Aristolochia* family. This was around the time of Paracelsus in Europe, who, by the way, also used *Aristolochia*, where it was known as "birthwort." But whereas Western medicine has advanced dramatically since Paracelsus, discarding his remedies, Li Shizen's herbal compendium was still being used until recently as a primary reference by schools of Chinese Traditional Medicine. Grollman remarked, "It's important in terms of the Chinese cultural traditions to realize that everything that needed to be known about Chinese traditional medicine practiced today was known hundreds of years ago." If you use herbal medicine in China, Li Shizen is still a preeminent authority to consult, just as we would go to Goodman and Gilman's indispensable *The Pharmacological Basis of Therapeutics*, now in its twelfth edition.

In presenting data from Taiwan, Grollman and his Taiwanese collaborators reported that the incidence of upper urothelial cancer in Taiwan had increased about fourfold from 1983 to 2007, whereas its incidence in other countries had remained at the same level over this time period. How was one to explain the sharp increase in the incidence of the cancer in Taiwan, if, in fact, use of *Aristolochia*-containing Chinese herbs had been an important factor all along? By examining the production and use of *Aristolochia* herbs in China, particularly since the 1930s, the authors were able to correlate the progressive increase in upper urothelial cancer with the systematic replacement of traditionally used Mutong herbs with *Aristolochia manchuriensis*. In mainland China, this practice appears to have begun in the 1930s, when, owing to the Japanese occupation, the usual sources of Mutong in southern provinces were cut off. The practice had become widespread by

the 1950s and continued until 2003, when these substitutions were outlawed by the Chinese government. The presence of aristolochic acid in *A. manchuriensis* exported from China to Taiwan between 1995 and 2003, as well as to other Asian countries, Great Britain, and the Netherlands, has been documented by chemical analyses. Thus, assuming a latency period of thirty years, the carcinogenic effects of aristolochic acid would be expected to have become increasingly manifest in Taiwan starting in the mid-1980s, as in fact they are.

The concluding sentence in the *PNAS* paper in 2012 delivered a sobering message regarding the implications for the future. Given the "the lifelong persistence" of the aristolochic acid–DNA adducts in target tissues and the "irreversible damage to the proximal . . . renal tubules caused by aristolochic acid, persons treated with *Aristolochia* herbal preparations at any time in their life are at significant risk of developing upper urothelial carcinoma and/or chronic renal disease, thereby creating an international public health problem of considerable magnitude."[37]

Since the traditional practice of Chinese herbal medicine in Taiwan mirrors that in China and other Asian countries, Grollman surmised that upper urothelial cancer and its attendant aristolochic acid nephropathy must also be prevalent in these countries where *Aristolochia* herbs have long been widely used for the treatment and prevention of disease. But when he contacted clinicians in China, he quickly became aware of the psychology and culture surrounding the use of traditional Chinese herbs. Many clinicians were reluctant to discuss the issue. In China, the government controls the distribution of traditional herbs, and people don't want to be seen criticizing the government—or traditional Chinese medicine. At a nephrology meeting, he encountered the head of nephrology at a major hospital that treats patients with *Aristolochia* poisoning. This individual confirmed that several *Aristolochia* herbs were still listed in the Chinese pharmacopeia—twelve years after the first report about the women in Belgium and two years after the Chinese government had outlawed the use of most *Aristolochia* herbs. Grollman asked what was being done in the way of public health measures to prevent the now well-documented consequences of exposure to the herbs: "You know it, I know it, the world knows it." The nephrologist replied, "All I can do is take care of my patients."

A number of Chinese nationals with kidney disease deduced that they had been poisoned by *Aristolochia* plants, and they reasoned that, if the government controls industry, the government should be responsible for their adverse effects. In fact, a class-action suit—apparently the first in

Chinese history—was brought against the government in 2004.[38] However, other than a single article in the *China Times* nothing more has been heard about the case.

The large herb company based in Hong Kong, Tong Ren Tang, sells herbs not only in China but elsewhere in Asia and throughout the world. In 2003 the Chinese government banned the use of *Aristolochia* herbs in the popular product Longdan Xiegan Wan, although it continued to be marketed under the same name. Grollman analyzed samples of Longdan Xiegan Wan before and after the ban, and he could see the aristolochic acid content of this product had disappeared. Since one manufacturer dominated the market for industrially produced herbals, the government was able to stop exposure to aristolochic acid in the form of Longdan Xiegan Wan. As he noted, however, several other forms of *Aristolochia*, including the toxic and carcinogenic varieties, are still listed in the official pharmacopeia, and throughout China it is relatively easy to obtain them. Furthermore, as of 2003, more than one hundred *Aristolochia* products were still available on the Internet.[39]

Data on production of *Aristolochia* species in China are available, and in one report, the amount produced was enough to cause toxic effects in one hundred million people. As Grollman commented, "There is nothing else you use medicinal herbs for, so, unless they discarded it, which seems very unlikely—by a conservative estimate, approximately one hundred million people in China and elsewhere have been exposed to the toxin, and those that are susceptible are at risk of developing aristolochic acid–induced upper urothelial carcinoma and chronic kidney disease."

After encountering bureaucratic resistance, Grollman finally succeeded in initiating a collaborative study of upper urothelial tract cancer at the Shanghai Cancer Hospital. As expected, the great majority of patients with upper urothelial cancer (over 85 percent) showed evidence of exposure to aristolochic acid in the form of adducts and the signature mutation.[40]

The latest development in the unfolding story of aristolochic acid–associated cancer entails new work from Europe and Asia suggesting that aristolochic acid–induced carcinogenesis may not be limited to upper urothelial cancer but may play a role in some liver cancers and renal cell cancer (the most common type of kidney cancer).[41]

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It was only due to the fortuitous presentation of multiple women from the same weight loss spa at clinics in Brussels that the harmful effects of

Aristolochia came to light. The discovery of a cluster of young women with kidney fibrosis set in motion a twenty-year research effort that has shed new light on Balkan nephropathy, the mechanisms of cancer causation, and a serious international public health problem. Had it not been for the concentration of exposed women with similar pathology in a single city, the effects of *Aristolochia* might well have gone unnoticed. What does this mean for people in the United States? This takes us back to the short-lived program at Stony Brook devoted to Complementary and Alternative Medicine.

Today Americans spend more than $32 billion a year on different combinations of vitamins, minerals, botanicals, probiotics, amino acids, and other supplement ingredients, and more than half of American adults use these products.[42] Herbal supplements account for roughly one-fifth of the total. A majority of consumers believe, wrongly, that the government requires manufacturers to report all adverse effects and that the FDA must approve supplements before they are sold.[43] Few consumers of supplements are aware of the implications of the Dietary Supplements and Health Education Act, which was passed by Congress in 1994 with strong support from the supplements industry and its political allies. By defining herbal supplements and botanicals as "dietary supplements," DSHEA excluded them from the more rigorous standards used in regulating prescription, and even over-the-counter, drugs. Unlike prescription drugs, supplements do not have to undergo premarket testing before they can be sold to consumers. Rather, they are assumed to be safe based until proven otherwise. The FDA has the unrealistic charge of identifying and recalling dangerous supplements only after they have caused harm.[44]

Since DSHEA was enacted, the number of dietary supplements on the market has surged from roughly four thousand to more than fifty-five thousand.[45] However, of the fifty-one thousand products introduced since 1994, only 170 (0.3 percent) have any documentation of their safety.[46] Major deficiencies in the oversight of dietary supplements include the lack of standardization to guard against adulteration and to ensure a consistent level of the active ingredients;[47] adverse interactions between herbal supplements and prescribed drugs, including chemotherapy; the absence of premarketing testing for safety, as is required for prescription and over-the-counter drugs; deceptive marketing by producers of dietary supplements and lack of adequate labeling to inform consumers about the nature and regulation of these products; and the failure to require reporting of all adverse effects promptly to the FDA.[48]

Owing to the lack of a proper surveillance system for reporting adverse events promptly and directly to the FDA, harm from supplements is seriously underreported, and in a number of cases the FDA has been woefully slow to act. According to Marcus and Grollman, "It took the agency more than ten years to remove from the market ephedra-containing herbal weight-loss products that had caused hundreds of deaths and thousands of adverse events."[49] More recently, in 2011, the Department of Defense banned supplements containing the stimulant DMAA from military bases because of safety concerns, but it took the FDA an additional sixteen months to alert consumers about DMAA's risks, and despite the agency's efforts the stimulant is still present in dozens of supplements.[50]

In the most recent manifestation of the dangers of inadequate oversight of dietary supplements, as of March 2014, the Centers for Disease Control and Prevention have documented an outbreak of hepatitis involving ninety-seven cases and one death in sixteen states linked to the "fat-burning" sports supplement OxyElitePro.[51] Most of the cases were adolescents, and roughly half occurred in Hawaii, where, in 2015, local officials reported one death and two liver transplants. The effects of OxyElitePro were picked up only because of an alert transplant surgeon in Hawaii.[52]

An example of a more systematic effort to gauge the extent of adverse events linked to use of supplements is the Drug-Induced Liver Injury Network, which includes eight U.S. referral centers.[53] Between 2004 and 2013 patients presenting with liver damage at these centers were evaluated for use of medications and herbal and dietary supplements and were followed to ascertain outcomes, including deaths and transplantations. Sixteen percent of all cases of liver damage were attributed to supplements. The most commonly used products implicated were bodybuilding supplements. During the ten-year period the frequency of liver injury caused by supplements increased from 7 percent to 20 percent. This one effort—focused on only one of many types of harm—represents only a first step in documenting the effects of supplements.

A recent study used nationally representative surveillance data from sixty-three emergency departments from 2004 through 2013 to estimate the number of visits because of adverse events related to dietary supplements.[54] The authors estimated that 23,000 emergency department visits in the United States every year were attributable to adverse events involving dietary supplements. The most common problems were cardiac symptoms from weight-loss or energy products among young adults and swallowing problems, often associated with micronutrients, among older adults.

Manufacturers of dietary supplements, their trade associations, and their political supporters in Congress claim that the industry is being unfairly branded owing to the misconduct of a small number of supplement producers. However, this position reflects either cynicism motivated by self-interest—the supplements industry is hugely profitable—or an ideological opposition to tighter regulation, or both. Opponents of tighter oversight of supplements rely on the fundamental confusions and misunderstandings that are widespread regarding these products. First, documented harm and the potential for harm from supplements need to be balanced against the benefits conferred by these products. In spite of claims that are made for a wide range of beneficial effects, in the majority of cases in which popular supplements have been evaluated in clinical trials, no evidence of a benefit was found.[55] Second, while many supplements may indeed be harmless, even if a small percentage of the fifty-five thousand products on the market pose a risk of serious harm, this could affect thousands of consumers.[56]

It should be clear from the record that the problem goes much deeper than the malfeasance of a few rogue supplement manufacturers and that the stakes are not trivial. Those who argue that the current system is adequate to protect consumers should remember that people failed to recognize the nephrotoxic effects of *Aristolochia* in spite of its use in many cultures worldwide over thousands of years. In my interview with him, Grollman explained why: "The reason, of course, is quite simple. It's painless, and the damage happens much later, so you don't put together the fact that you took this medicine and ten years later, you have kidney failure. It's been part of Ayurvedic, European, Chinese, and South American medicine for centuries. All of the great civilizations have used it. And not one reported its toxicity until the Belgians did twenty years ago. There are certain things that tradition can't tell you."

Commenting on the disturbing lack of oversight and regulation of these poorly studied herbal products, many of which have known toxicities, Grollman referred to the thalidomide episode in the 1950s in Europe and to the Belgian women: "The next time we may not be lucky enough to have observations on women from a Brussels spa to alert us to a danger." His take-home message: "DSHEA needs to be amended, and it needs to be amended fast."

6. DEADLY REMEDY: A MYSTERIOUS DISEASE, A MEDICINAL HERB, AND THE RECOGNITION OF A WORLDWIDE PUBLIC HEALTH THREAT

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42. Cohen PA. Hazards of hindsight—monitoring the safety of nutritional supplements. *New England Journal of Medicine* 2014;370:1277–80.

43. Offit PA. *Do You Believe in Magic? The Sense and Nonsense of Alternative Medicine*. New York: HarperCollins, 2013.

44. Cohen. Hazards of hindsight.

45. Ibid.

46. Ibid.; Offit. *Do You Believe in Magic?*, 91.

47. Adulteration of supplements with unapproved, banned, or untested drugs is a major problem, documented in more than five hundred instances. The contaminants include stimulants, anabolic steroids, antidepressants, weight-loss medications, and Viagra analogues (Cohen. Hazards of hindsight). Both adulterants and legal ingredients present in supplements have been linked to a wide range of potential adverse reactions, from arrhythmias, cancer, and liver damage to heart attacks and strokes (ibid.).

48. Marcus DM, Grollman AP. The consequences of ineffective regulation of dietary supplements. *Archives of Internal Medicine* 2012;172:1035–36.

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50. Cohen. Hazards of hindsight.

51. Acute hepatitis and liver failure following the use of a dietary supplement intended for weight loss or muscle building—May–October, 2013. *MMWR Morbidity and Mortality Weekly* 2013;62:817–19.

52. Cohen. Hazards of hindsight. Grollman explained something that was not mentioned in the news reports, or even in medical articles, discussing the OxyElitePro incident. For OxyElitePro to qualify as an herbal supplement, the manufacturer USPLabs added a natural ingredient, aegeline, derived from the bael fruit, which is popular in China and India. However, the concentration used in OxyElitePro was many times higher than in the bael fruit. In effect, aegeline was being used as a drug. Unbelievably, an earlier formulation of OxyElitePro and another product, Jack3d, that contained the stimulant DMAA were linked to one hundred cases of illness and six deaths. After destroying the stocks of these products, USPLabs reformulated OxyElitePro and rereleased it. A month later the first reports of liver toxicity turned up in Hawaii.

53. Navarro VJ et al. Liver injury from herbals and dietary supplements in the U.S. drug-induced liver injury network. *Hepatology* 2014;60:1399–1408.

54. Geller AI et al. Emergency department visits for adverse events related to dietary supplements. *New England Journal of Medicine* 2015;373:1531–40.

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56. Another argument used by supporters of dietary supplements is that there are well-documented cases in which medications approved by the FDA have been shown to cause harm. But this argument doesn't stand up since, first, in order to be approved, medications have to have some evidence of effectiveness, and, second, due to regulation, drugs that show adverse effects are removed from the shelves. In fact, this argument favors tighter oversight of supplements.