ALL IN GOOD TIME

MAURA C. FLANNERY, DEPARTMENT EDITOR

Science is usually seen as a progressive enterprise, with one discovery leading on to the next and then the next. However, this progress isn't always as smooth as it's sometimes assumed to be. Take for example the case of thalidomide, that infamous drug from the 1950s. For those not old enough to remember the story, thalidomide was marketed as a sedative and treatment for morning sickness. While it was never approved for sale in the United States, it was considered so safe in Germany that it was sold as an over-the-counter medication. Then obstetricians began to see an increase in babies born with phocomelia or "seal limb," where the long bones of one or both pairs of limbs fail to develop correctly. These children had limbs that looked like seal flippers: hands or feet attached to the trunk.

Phocomelia can be caused by a genetic abnormality, but it is rare. If an obstetrician saw a patient with this defect, it was the type of unusual event to share with a colleague, and the coincidence of one or more colleagues also recently seeing such children led to an investigation into why the abnormality was suddenly becoming more common. It turned out that almost every woman who gave birth to a baby with phocomelia had taken thalidomide early in pregnancy. In all, about 10,000 children, mostly in Europe, were born with the defect before the drug was taken off the market. In the United States, a physician at the Food and Drug Administration had questioned the drug's safety on the grounds of its nervous-system side effects during trials. This slowed its approval long enough that its other flaws were discovered before it could be distributed.

The lingering effects of thalidomide are still seen in the now older adults who have lived their lives with its consequences. In many cases, they learned to compensate well, but are now suffering from chronic pain due to years of overuse of their functional muscles and limbs. In the meantime, thalidomide has been found to be an excellent treatment for leprosy, reducing inflammation, and for the cancer of the bone marrow called multiple myeloma. It's also being tested for use against HIV and Crohn's disease. When given to women of child-bearing age, the use of contraceptives is required in the United States, but in other countries administration of the drug has led to the birth of more children with phocomelia. It's because of the continuing use of therapeutic thalidomide that research also continues into why it causes limb malformations. Although this research has been going on intermittently for over 50 years, it is only now bearing useful results (Zimmer, 2010).

The approach that finally paid off was a very methodical one, based on the fact that when thalidomide is taken, it's quickly broken down into 18 metabolites. Researchers at the National Cancer Institute purified each of them and tested them individually on chicken embryos. Only one, called CPS49, caused a defect in wing development. But how? Observation of the developing embryo revealed that within minutes of exposure, CPS49 began to destroy developing blood vessels. Without a blood supply, the wing elements couldn't grow properly. Further research identified the protein involved as cereblon, one whose function hadn't yet been discovered and whose role in limb development hadn't been suspected. When zebrafish were used as a model animal, embryos that were prevented from making cereblon didn't develop fins as happens when these fish are exposed to thalidomide.

This finding is significant for several reasons. First, it identifies a key protein in limb development that hadn't been previously known. In addition, it provides a focus for work on attempting to create a situation where thalidomide can be effective therapeutically and yet not cause developmental abnormalities. Researchers have already found that if they alter cereblon at two sites, the resulting protein does not bind thalidomide and yet still allows for relatively normal limb development in chick embryos. Obviously a great deal more work is needed to find a therapeutic solution, and how rapidly this will occur is anyone's guess. Things are likely to proceed relatively quickly since there is such medical interest in the problem, but the roadblocks ahead are unknown. That's the way of science; yes it's predictable in that there will be progress, but unpredictable in terms of when. In this case, part of the reason for the long delay was that research methods had to catch up with the problem. Protein sequencing was only beginning in the 1950s, and the idea of making pinpoint changes in protein structure was hardly imagined. Yes, chick embryos had been studied for decades, but zebrafish as model organisms were still in the future. The thalidomide story is a beautiful example of the interplay of tools and problems, along with a medical impetus to move a research question forward.

The Great Apes

The story of time and thalidomide is one of starts and stops along a 50-year road. Another tale of time and research is more in line with the traditional view of gradual progress made on a scientific question. This involves the work of Jane Goodall and those who followed her into the study of the great apes. Jon Cohen (2010b) begins his article on her legacy with a vignette, not about Goodall, but about an African behavioral ecologist named Emily Otali who earned a master's degree from Makerere University in Kampala, Uganda, in 1999. She had studied mongoose behavior and wanted to continue this work while pursuing a doctorate, but the only fellowship she could find involved work with blue monkeys. She balked and did everything she could to get back to her mongooses. A researcher Otali knew invited her to spend a day with him studying chimps to see if she would find them more agreeable, but she wasn't too interested. Hadn't everything about chimps already been studied? After all, Jane Goodall and her associates had been at it since 1960. He assured Otali that this was hardly the case, pointing out that

The American Biology Teacher, Vol. 73, No. 7, pages 426–429. ISSN 0002-7685, electronic ISSN 1938–4211.
DOI: 10.1525/abt.2011.73.7.10

like any good research field, this one expanded with exploration – questions answered just led to ever more questions being asked.

On the day of her visit, Otali found chimp observation rather boring since she only spotted one mother and baby out of a band of 50. However, she did something she wasn't supposed to do: make eye contact with the apes. When she did, it changed everything for her. She suddenly wanted to know as much as possible about these creatures. Otali went on to earn her doctorate doing research on chimpanzees and to become field manager of the Chimpanzee Project at Kibale National Park in Uganda, the place she visited that fateful day. She is representative of the new breed of African researchers trained in Africa and continuing the work Goodall began. Otali's story is told in the first of several articles Cohen wrote for a special Focus section of *Science* on "Chimpanzee Research Today." Obviously, Goodall and her research are at the center of this tale, but there are many strands to it. It is a wonderful story that could only take place over a significant period of time. It's a great example of how a field can develop and mature and be built into a major scientific enterprise.

As Goodall soon discovered, just beginning the work took both time and patience. Since chimps are essentially tree dwellers, they are difficult to observe. Yet strategies used to make them easier to see, like leaving food in a clearing, quickly led to unwanted behavioral changes – chimps are smart enough to opt for an easy meal over hours of foraging. So by trial and error Goodall forged a form of observation that attempted to study behavior without altering it. Soon others were drawn to follow her – to Gombe, where she continued her research on chimps, and to other areas to study other apes using techniques similar to hers.

The long-term nature of this research is suggested by Tim Clutton-Brock and Ben Sheldon (2010: p. 1207):

> [T]o understand the network of social relationships between individuals, you need to know their ages, kin relations, and relative dominance rank. That will take at least one decade, or more likely, two. However, other important questions will take three or four decades of systematic data collection: how and why groups increase or decline in size; how genetic differences interact with environmental factors to affect breeding success and survival; how population density is regulated.

Obviously, the benefits in terms of fascinating findings are huge. However, carrying out such a research program requires investigators committed to long-term field work and needs long-term funding as well. Much of this work has been funded by universities, but now field stations are turning more to conservation groups for support. The latter see this research as part of their larger mission of saving environments where primate species, most of whom are endangered, can continue to live in the wild.

One factor working against long-term research seems to be the human attention span. Just as with dress and automobile design, research areas go in and out of fashion. In the 1950s and 1960s, studies of ape language-acquisition were the rage, but they are now considered passé, and there is only one center, the Great Ape Trust, continuing this work in the United States (Cohen, 2010a). At the same time, the increasing ease with which scans of brain activity and structure can be done means that ape brains, like those of humans, can be probed and their similarities and differences explored. MRI has been used, but at the Yerkes National Primate Research Center at Emory University scientists are employing an even more advanced technique called diffusion tensor imaging (DTI). It detects the direction in which water molecules move, and this aids in locating fiber tracts in the white matter and helps researchers figure out how the various parts of the brain are connected to each other in chimps compared with humans (Cohen, 2010c). With more time, and more new techniques, who knows where this research will lead.

Mushroom Clouds

In a sense, this column is a walk down memory lane, as I recall learning about Jane Goodall's work in the 1960s when I was also hearing about thalidomide's awful effects. This was also the time of the cold war and atmospheric testing of atomic bombs. It is difficult to accept now that the extent of fallout from atmospheric testing took several years to be fully appreciated and assessed. Only in the mid-1950s, after testing had been going on for about 10 years, was it confirmed that fallout was much higher in some areas than in others and that weather patterns played a significant role in the distribution. Revelations like this aroused enough indignation and political pressure to lead to the signing of the Limited Test Ban Treaty in 1963. Even that didn't end the practice, because some countries, including China and France, continued to detonate bombs in the atmosphere, while the United States and the Soviet Union took their testing underground.

Among the elements in the fallout was carbon-14 (^{14}C), which is often used to date bodies found at archaeological sites. Ordinarily, the technique is only accurate to within a few hundred years. However, the post–World War II "bomb pulse" of ^{14}C allowed researchers to date much more accurately in part because there are good records of how much ^{14}C was in the atmosphere since the 1950s. Measurement of ^{14}C is being used to tackle a number of problems, including one that has arisen over the past 20 years or so: how to determine whether or not humans generate new brain cells as adults. It was assumed for a long time that all our brain cells grew early in life, and after that, they just made new connections; those that died weren't replaced. However, research on bird song revealed that canaries made new brain cells as adults. This finding spurred the search for similar phenomena in other species, including humans, in which the results have been equivocal (Grimm, 2008).

To explore this problem, Kristy Spalding, a doctoral student, checked the amount of ^{14}C in tissues from both the human neocortex and occipital cortex. She found about the same ^{14}C levels in all the cells, indicating that they were all generated at about the same time, that none were younger than the others. In another study, on fat cells, which are also supposed to remain for life, she found that the ^{14}C levels varied, and she estimated that the fat cells turn over about once every 8 years. In a very different but equally clever use of the technique, Australian winemakers employed it to verify the age of their vintages in the face of threats from European regulators who have questioned the age of wines as a way of slowing down Australian imports into Europe. The tests indicate that the wines are as old as they have been claimed to be, and although the technique is too costly to be used routinely, it has quieted the Europeans.

A Long Time Coming

It's not uncommon to find science articles beginning with a statement that the discovery under review settles a "long-standing question," because there are a lot of such questions in science. There are only so many problems that can be investigated; resources are limited, so some questions just have to wait in line. Sometimes it's a matter of the question being in a field in which there is little interest, but in others,

it's a case of there just not being enough information or the right tools to tackle it. For example, there is the issue of what makes a particular honeybee (*Apis mellifera*) become a queen? Well, a simple but imprecise answer is that royal jelly does the job, because this is fed to the larvae from which the queen arises. But the jelly is a complex, water-based blend of lipids, sugars, minerals, and proteins. As Gene Robinson (2011: p. 454) notes: "Despite intense scientific and economic interest, the specific substances in royal jelly that cause this remarkable transformation had, up to now, escaped detection."

One clue that helped solve the problem was the discovery that there were temperature-related differences in the jelly's potency, with gradual loss of effect if it's stored at 40°C until all activity is lost after 30 days. When the various constituents were separated from each other and stored under these conditions, one protein deteriorated in just the way the jelly's potency did. The protein is called royalactin, and when it's fed to larvae it causes classic queenly effects: shortened development time, increased mass, and perhaps most importantly, larger ovaries. The odd thing is that when *Drosophila* larvae are fed royalactin, they too develop into queens, even though there are no queens in this species. But what possessed researchers to even give the protein to flies? So much work has been done on *Drosophila* genetics and development that the researchers thought this system might give them some clues as to how royalactin produced its effects, and they were right. Using a combination of RNA interference of gene expression and other genetic control factors, they found that royalactin stimulates signaling through the epidermal growth factor receptor (EGFR) in fat bodies. Then EGFR activates enzymes that control cell size and life span. Finally, they used RNA interference of the EGFR gene in bees to show that royalactin works through EGFR in this species as well.

In his review of this research, Robinson describes the twists and turns that studies on queen honeybee development have taken over the years, including a 1948 study using *Drosophila*. There were several hints that royal jelly was involved, but they never led to definitive results on caste determination. Then the research focus moved to juvenile hormone, and even to insulin-related signaling pathways. This is a great example of the twists and turns investigations can take, inching forward but still not getting to the heart of the matter. Research really does mean groping in the dark and can sometimes lead away from the target as well as toward it, making it difficult to predict where the breakthrough will come. As with the work on thalidomide using chickens and zebrafish, the importance of model organisms such as *Drosophila* is again highlighted. There is a reason why researchers keep focusing on the same creatures: knowledge really can beget more knowledge.

No Substitute for Time

In the case of the honeybees, it took time to finally hit upon the correct answer; in other situations it takes time to accumulate data. That is, time can be a major factor in the data. An example is phenology, the study of periodic events in nature, such as when the first robin appears in the spring or when a particular tree species flowers. Keeping records on such events year after year builds up a valuable store of information, but there is no way it can be rushed; there is no substitute for the passage of time in this type of work. In one sense, phenology seems terribly old-fashioned, and not even very scientific. This is something farmers and gardeners have been doing for centuries, if not millennia. Still, it is a valuable activity, and one that's coming to be appreciated anew in this age of climate change. One example of its usefulness is a recent article on shifts in the flowering times of plants in the northern Great Plains. From 2007 to 2010, Kelsey Dunnell and Steven Travers (2011) collected a data set on first flowering dates (FFDs) of native prairie plants and then compared it with historical data that O. A. Stevens, a botany professor at North Dakota State University, had amassed between 1910 and 1961 – an impressive record in itself.

Dunnell and Travers also looked at weather records for the same periods; these indicated, not surprisingly, that annual average temperatures had increased as had the length of the growing season. For 2010, they found that ~41% of the species studied shifted their flowering times, with spring flowers blooming earlier and fall flowers blooming later. This high percentage came in the year with the warmest average temperature. In the cooler years, only about a quarter of the species had shifted flowering times. It is significant that over the stretch of ~50 years between the last of the older data and the new data, FFDs had changed so much for so many species.

There are several other things to note here. First, the number of species that Stevens observed was much greater – 178 – than the 93 Dunnell and Travers studied. This is partially due to deterioration of the prairie flora, but also to the fact that the latter collected for only 4 years as opposed to 50. This brings up another point: isn't this study really comparing apples and oranges, a rich data set with a much poorer one? The latter is based not only on fewer species, which may just be a result of environmental change and unavoidable, but also on a much shorter time span. One of my favorite songs from the 1960s is "You Can't Hurry Love," sung by the Supremes. Well, flowering is another thing you can't hurry. You have to wait for the next growing season to see when those blooms will first appear. No amount of technology is going to give you 50 years of phenological data without waiting 50 years for it. That's what makes records, such as those of Stevens, so valuable.

Citizen Science

If Durrell and Travers want to make a contribution comparable to Stevens's, they will just have to keep at their observations, or perhaps interest others in continuing this work. Last year, there was a short article in *BioScience* on phenology and citizen science by Amy Mayer (2010). She describes the work of volunteers who record when plants in their area bloom, or what bird species show up at their feeders, or when they spot the first of a bird species arriving for its breeding season. These data may not seem very "scientific," but if enough such data are amassed, they can provide powerful information about environmental change. There is strength in numbers. The Audubon Society has proved this with its continuing Christmas Bird Count, which has become more organized and with greater participation – meaning more data – as the years go on.

This spring, I assigned Mayer's article to my students. Phenology was an unfamiliar term to them, and when they saw it in the title, they were a little worried: if they couldn't even figure out what the title meant, then this article was going to be a tough one. But then they discovered that the concept of phenology is almost ridiculously simple. With even a little observation, they could figure out when the first tulip bloomed on campus. This made them feel a little more "scientific." This is an "ology" they can handle, and for the rest of the semester, the word "phenology" popped up from time to time in their essays. I cannot claim to have made any of them into citizen scientists, but at least I introduced them to the fact that such people exist, and they might want to join these ranks in the future.

The future is the time frame we can't do anything about. Who knows when an HIV vaccine will be available, as one example of something that has been a long time coming (Altman, 2011). To say nothing of definitively answering the question of whether or not there is life

on Mars (Kerr, 2010) – that debate was going on long before HIV was even discovered. Patience isn't a very popular virtue today. "ASAP" is one of our favorite battle cries. But "you can't hurry biology" or, really, most of science. Yes, you can focus on a particular problem and thus make it more likely that it will crack open, but as examples like that of royal jelly illustrate, even that doesn't always work. Sometimes, it just takes time, and maybe this is not a bad thing, because along the way biologists often make other interesting discoveries.

References

Altman, L. (2011). 30 years in, we are still learning from AIDS. *New York Times*, 31 May, D1, D6.

Clutton-Brock, T. & Sheldon, B. (2010). The seven ages of *Pan*. *Science*, *327*, 1207–1208.

Cohen, J. (2010a). Boxed about the ears, ape language research field is still standing. *Science*, *328*, 38–39.

Cohen, J. (2010b). In the shadow of Jane Goodall. *Science*, *328*, 30–35.

Cohen, J. (2010c). The inner workings of the chimpanzee brain. *Science*, *328*, 40–41.

Dunnell, K. & Travers, S. (2011). Shifts in the flowering phenology of the northern Great Plains: patterns over 100 years. *American Journal of Botany*, *98*, 945–955.

Grimm, D. (2008). The mushroom cloud's silver lining. *Science*, *321*, 1434–1437.

Kerr, R. (2010). Growing prospects for life on Mars divide astrobiologists. *Science*, *330*, 26–27.

Mayer, A. (2010). Phenology and citizen science. *BioScience*, *60*, 172–175.

Robinson, G. (2011). Royal aspirations. *Nature*, *473*, 454–455.

Zimmer, C. (2010). Answers begin to emerge on how thalidomide caused defects. *New York Times*, 16 March, D3.

MAURA C. FLANNERY is Professor of Biology and Director of the Center for Teaching and Learning at St. John's University, Jamaica, NY 11439; e-mail: flannerm@stjohns.edu. She earned a B.S. in biology from Marymount Manhattan College; an M.S., also in biology, from Boston College; and a Ph.D. in science education from New York University. Her major interests are in communicating science to the nonscientist and in the relationship between biology and art.