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Archaeological coprolite science: The legacy of Eric O. Callen (1912–1970)

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Abstract

The detailed analysis of human coprolites as a recognized field of archaeological science is barely 40 years old. Dr. Eric O. Callen, the founder and developer of the discipline, has been dead for more than 30 years, yet the ideas he developed and techniques he perfected continue to guide the discipline today as it widens analysis into more areas than he ever dreamed possible. Callen would be gratified to learn that others have extended his initial research efforts to include the routine analysis of plant macrofossils, pollen concentration values, fauna and insects, phytoliths, and more recently, immunological proteins, trace elements, gas chromatography, and the extraction and identification of DNA from prehistoric human feces.

Keywords: Coprolites; Palynology; Pollen analysis; Pollen statistics; Archaeology; Archaeobotany

1. Introduction

During my undergraduate studies in the early 1960s, I (Bryant) visited my first archaeological site: a dusty rockshelter perched in the side of a canyon wall in west Texas near the Rio Grande. I noticed that each morning during the screening process the workers found dozens of flat, cow patty-shaped human coprolites (dried human feces), which they would carefully remove and pile up at the foot of the screens. These were considered worthless junk and a nuisance because the smaller pieces clogged the screens and delayed the process of looking for what they considered to be far more important artifacts. Later, we were treated to after-lunch entertainment when the screeners gathered at the edge of the shelter for their daily game: "Frisbee throwing." As each coprolite sailed out over the canyon the crowd would cheer or laugh, depending on how far the thermal updrafts carried each coprolite. It was great sport and I even tried my luck at throwing along with the rest. I did not know it then, but we were discarding some of the most valuable data being excavated from that site.

It was the early 1960s and few people had ever heard of human coprolites and few archaeologists realized the importance of saving them for analysis. James H. Word, an avocational archaeologist who conducted excavations at Baker Cave intermittently from 1962 to 1965, was a notable exception (Word and Douglas, 1970). In the early 1970s, I (Dean) enrolled as a graduate student at Texas A&M University where I hoped to study

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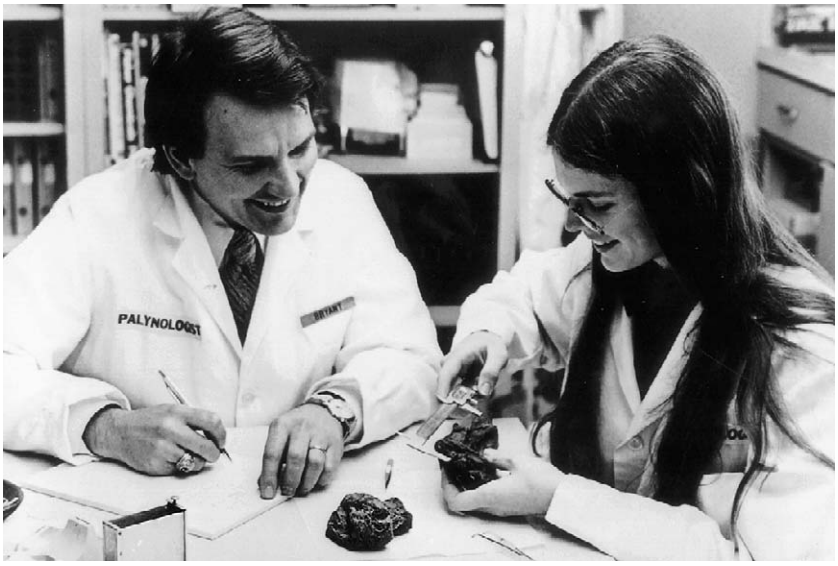

Fig. 1. Vaughn M. Bryant and Glenna W. Dean working on coprolites in June of 1976, at Texas A&M University.

prehistoric diets with Professor Vaughn Bryant (Fig. 1). Not long after beginning my doctoral program I (Dean) asked to study the few coprolites that had been entered into curation from the Baker Cave excavations located near Del Rio, Texas. I learned that the specimens had unaccountably disappeared, their value finally recognized by archaeologists but unrecognized by others. A few years later Bryant and I would both return to another dry rockshelter in that same region of west Texas to excavate and collect ancient human coprolites that would become part of my doctoral dissertation research.

Few people were conducting research on coprolites during the mid-1960s because, as a research interest, it was fairly new and not well known. Aside from a few short articles, the first major publications that brought attention to coprolite research were in 1967. First, was the article written by Eric O. Callen in *American Antiquity* (Callen, 1967a), then his chapter (Callen, 1967b) on the coprolite evidence of early Mexican diets that was published in *The Prehistory of the Tehuacan Valley: Environment, and Subsistence*.

During 1967 and 1968, I (Bryant) corresponded with Callen and spoke with him by telephone on many occasions while I was conducting my first attempt to process and analyze more than 40 Late Archaic age coprolites from Conejo Shelter, located near the Pecos River a few miles west of Comstock, Texas (Bryant, 1974a). In April of 1970, I first met Dr. Callen at the Society for American Archaeology meeting in Mexico City (Fig. 2). Two months later, I drove to Canada to visit Dr. Callen and to work with him at his lab in MacDonald College of McGill University in Montreal. During my short visit, Callen was flattered that someone interested in coprolites had traveled all the way to Canada to see him, and he was eager to share his techniques and ideas about the potentials of coprolite research. He was also excited about his forthcoming trip that summer to begin work on the coprolites Dr. Richard S. MacNeish was finding at the Andean site of Pikimachay near Ayacucho, Peru. Both Callen and MacNeish believed that the data from those coprolites would contain the earliest records of plant cultigens in South America.

During one of our talks in his small lab, which was no larger than the size of one of today's average-sized bathrooms, he expressed his pessimistic views about the acceptance of coprolite studies and about the minor impact coprolite studies seemed to be having on other fields of science such as botany and archaeology (Callen, 1970). He lamented that his colleagues at McGill University considered his work a "waste of time," and that in the decade since he and Cameron first published a short paper on the subject (Callen and Cameron, 1960), less than a dozen other researchers had collected or tried to examine human coprolites. The reality that very few of those individuals continued to show any interest in pursuing other coprolite research also saddened Callen.

1.1. Callen's legacy

Less than 2 months after my visit with Callen, he died of a heart attack in Ayacucho, Peru while working on the

Fig. 2. Vaughn M. Bryant (left) and Eric O. Callen (right) at the Society for American Archaeology Meeting in Mexico City, April, 1970.

coprolites found at the site of Pikimachay. Although he had ushered in the new age of coprolite analysis, Callen's death went unnoticed in the world of archaeology and he was quietly buried in Ayacucho, Peru. Because the city of Ayacucho is located high in the Andes and has limited access by road or air transportation, returning his body to Canada was impossible, even though that was his wife's request (Fig. 3). A few months later, in December 1970, Richard MacNeish asked Dr. Bryant to continue Dr. Callen's work in Peru the following summer.

Callen had converted his bedroom in Ayacucho into a coprolite laboratory. Because the reconstitution of coprolites generally produces strong odors, Callen had the only bedroom on the third floor of the house that served as the archaeological headquarters for the project. After he died, his bedroom was locked and was not opened again for nearly a year.

Reaching Ayacucho the next summer was an experience, especially flying over the Andes in a non-pressurized propeller-driven airplane with motors that were leaking oil so badly that droplets smeared the

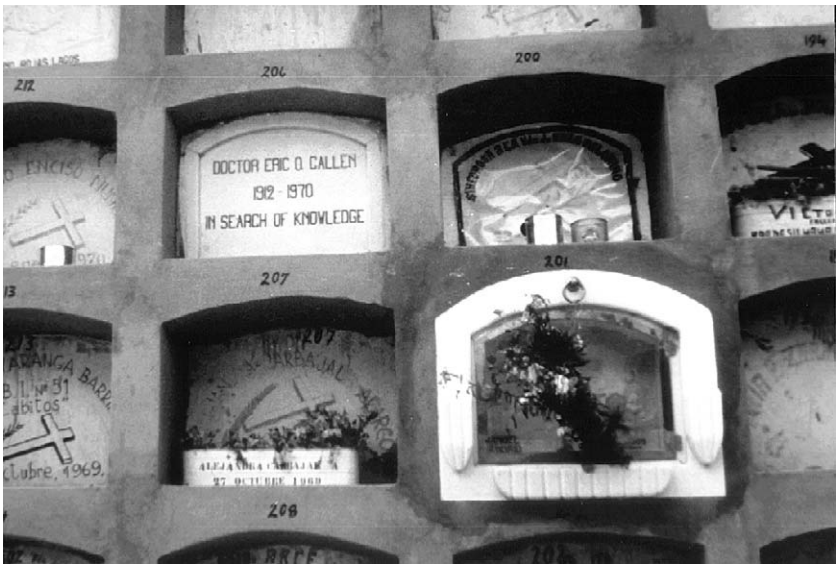

Fig. 3. The grave of Dr. Eric O. Callen located in Ayacucho, Peru. Photo taken in July of 1971.

window and the view of the terrain below. After I arrived, I became the first person to reenter Callen's bedroom and laboratory since his death nearly a year before (Fig. 4).

There were dusty cardboard boxes on the floor containing paper bags with faded labels written in Spanish. Each contained a coprolite or a fragment of a suspected coprolite. Scattered around on wooden tables was an array of items including dozens of wide-mouth jam jars with their contents long since dried. Each jar had several thin, brown bands around the inside walls and in the bottom of each was a mat of hardened, varnish-like material. Index cards, pencils, microscope slides, plastic bags containing dried plant materials, and small white envelopes lay on every surface. Forceps and probes were scattered like pick-up-sticks near a microscope that was covered with a dusty plastic bag. Two notebooks and a Spanish/English dictionary lay open on a chair next to the bed. One open notebook had a pencil sketch of a chili-pepper seed (*Capsicum*) that was only half finished, as if Callen had put it aside to complete it the next day.

I sat on what was once Callen's chair, blew the dust off one of his notebooks, and began reading pages filled with cryptic notes about the many coprolite samples he had begun to analyze. At first, many of Callen's notes and the citations made little sense to me. But then, who leaves complete notes of a day's work thinking that someone else might have to complete an experiment only just begun and left unfinished? In the margin of various pages were tragic reminders to himself of how many nitroglycerin tablets he had taken, when he had chest pains, and even one, short, underlined message dated a week before he died saying, "I must leave soon."

I closed the notebook, looked at the cluttered bedroom, and wondered why Callen had refused to leave his fieldwork even when a fatal heart attack seemed imminent. I glanced at the table covered with half-processed specimens and wondered, "Are these really worth a man's life?"

For the rest of that first day I continued searching for answers. I sifted through Callen's notes, poked at trays and beakers containing macrobotanical remains, and looked through a box of microscope slides he had already prepared from some specimens. Each day for the rest of my stay in Ayacucho, I lived in awe of Callen's work and wondered what legacy he had left for us who wanted to follow in his footsteps.

Eric O. Callen ushered in the modern age of human coprolite research during the late 1950s and early 1960s (Callen and Cameron, 1955, 1960). During the next decade, until his untimely death in 1970, Dr. Callen worked with missionary zeal to convince archaeologists, botanists, zooarchaeologists, and anyone else who would listen of the importance of archaeological fecal research. Although his initial research successes were limited, he would feel vindicated today by the acceptance and growing importance of coprolite studies.

He was an unlikely person to become the "father" of coprolite analysis. After receiving a doctorate in botany from the University of Edinburgh in Scotland, he spent his professional career as a professor of plant pathology

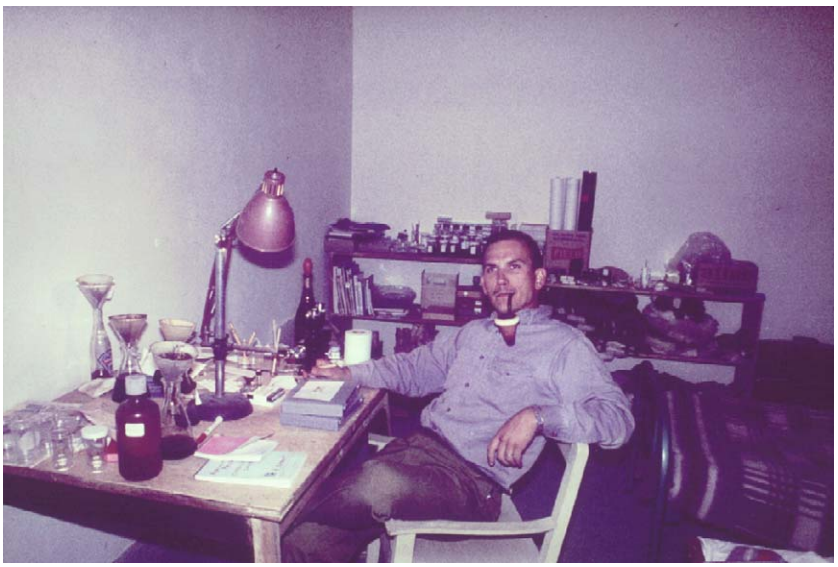

Fig. 4. Vaughn M. Bryant sitting in Dr. Callen's bedroom and laboratory. Photo taken on the first day after Dr. Bryant arrived in Ayacucho, Peru, in July of 1971.

at McGill University in Canada where he researched cereal pathogens. Callen's first exposure to coprolites occurred during the early 1950s after a conversation with Dr. T. Cameron, a member of the McGill University parasitology faculty.

The story begins with archaeologist Junius Bird. In the late 1940s, Bird excavated the site of Huaca Prieta de Chicama in the coastal region of Peru (Bird, 1948). During his excavation, he collected what he believed were dried specimens of ancient human feces. He also collected a few fecal samples from the preserved intestines of a human mummy that had been buried at the site. During a visit to McGill University in 1951, Bird gave the Peruvian fecal samples to Dr. Cameron and asked if he would be willing to examine them and search for evidence of ancient human parasites. Dr. Callen, who was searching for early records of fungal pathogens that infect cereals, such as maize, learned of the Peruvian coprolite specimens and asked Dr. Cameron for a few samples to study.

It is ironic that the first detailed study of ancient human coprolites was begun by two scientists looking for human parasites on the one hand and for traces of fungi that infect and destroy maize on the other. Neither of them had any formal training as anthropologists.

When Callen and Cameron began their study, the greatest problem they faced was finding a way to hydrate the dried coprolites without injuring the delicate parasite remains that they might contain. Previously, techniques used in coprolite analysis included using pliers or rubber hammers to break open dried coprolites, grinding dried coprolite specimens through coarse screens, or, when possible, pulling samples apart by hand. Callen's first contribution was solving the hydration problem (Callen and Cameron, 1955). Callen discovered that by soaking dried coprolites in a weak solution (0.5%) of trisodium phosphate, a technique he learned from the research of several zoologists and a botanist (Bennington, 1947; van Cleave and Ross, 1947), dried fecal material would hydrate and in the process would not harm even the most delicate plant or animal tissues. He also discovered that a side effect of the hydration procedure was the release of the original foul odors associated with fresh fecal remains (including methane, indole, skatole, methylmercaptan, and hydrogen sulfide).

Junius Bird's Peruvian coprolites contained no useful information about human parasites or evidence of early maize pathogens, but the specimens did contain a wide variety of macrofossils that reflected ancient dietary preferences. This discovery convinced both scientists to alter their research goals and instead report on the dietary contents of those coprolite samples. They also realized that coprolites, more than any other type of prehistoric material, could provide unique data keys to understanding ancient human diet and nutrition (Callen and Cameron, 1955, 1960).

After his initial Peruvian study, Callen devoted the next decade to examining human coprolites from other important sites. By the mid-1960s, Callen developed what he believed was an ideal technique for concentrating insect remains in coprolites by using benzene (Callen, 1965). It was effective, but his technique is no longer used today because benzene is a carcinogen. He studied coprolite specimens from Dr. Richard S. MacNeish's work at the Ocampo caves in Mexico (Callen, 1968). After that, Dr. MacNeish invited Callen to work with coprolites from sites in the Tehuacan Valley of Mexico (Callen, 1967b). Next came a study of six, 90,000-year-old coprolites that had been found at the Neanderthal site of Lazaret in France (Callen, 1969). His last completed coprolite study was an examination of 10 specimens from a site in the Glen Canyon region of the American Southwest (Callen and Martin, 1969).

During the decade that Callen worked on coprolites, he never once taught a course on coprolite analysis, he never mentored a graduate student working on a study of human coprolites, and he endured the frequent ridicule of colleagues in botany and archaeology. Other faculty members at McGill University chastised him for doing coprolite research, which they believed was of little scientific benefit. Although never openly bitter about his situation, Callen often confided that he wished someone in the academic world would just once publicly acknowledge his work, or at least give public recognition to the importance of human coprolite studies.

2. Callen's impact on coprolite studies

In 1968, Häntzschel et al. published an annotated bibliography of coprolite studies that focused on fossilized geological specimens and referenced little work with desiccated human specimens other than Callen's. Just a few years later, Wilke and Hall (1975) published an annotated bibliography of research conducted specifically with desiccated human feces that referenced nearly 150 studies.

It has been 36 years since Callen died and he would be pleased to discover how far the field of coprolite analysis has advanced since Wilke and Hall's (1975) compilation (e.g., Bryant, 1974b; Reinhard and Bryant, 1992; Sobolik, 2000). The number of individual studies has increased exponentially around the world, and the

quality of the science has evolved. In many cases, phrasing questions about previous assumptions has led to new perspectives, while in other cases conducting simple experiments has facilitated new understandings.

3. Aspects of Callen's coprolite research

3.1. Determining human origin

One of the questions that confronted Callen, and the other early analysts of his day, was how to be certain a coprolite was of human origin (Callen, 1968). Some would say that this problem is still with us today. Callen was convinced that the smell of a coprolite in a trisodium-phosphate solution was one proof of its origin and could reveal certain items that were eaten (Callen, 1963). For example, he believed he could tell whether or not the individuals living in Mexico more than 2000 years ago had been drinking maguey beer just from the odor of their hydrated coprolites. My own observations (Williams-Dean, 1978, pp. 91–94), and those of others including Janet Stock (1983, pp. 59–61) and Kristin Sobolik (1988a, p. 34), who have examined human coprolites ranging in age from about 1100 to 8300 years, suggest that odor is variable and therefore a poorly understood indicator of either the human origin of coprolites or of their food contents.

As a second test, Callen believed that if the solution during the hydration process turned black, the coprolite was of human origin (Callen, 1968). In the late 1960s, Gary Fry conducted an experiment to determine if this conclusion was correct. Fry examined the feces produced by a number of animals housed at the Salt Lake City zoo. His purpose was to see which animals, if any, might produce feces that mimic the color reported by Callen for human coprolites when placed in dilute trisodium phosphate. Fry found that the feces of most animals did not produce the color reported by Callen for human coprolites. However, one animal, the coatimundi (*Nasua nasua*), a tropical New World mammal, produced feces that mimicked the color produced by human coprolites when placed in trisodium phosphate. Fry concluded that the color of the feces determined the color of the trisodium-phosphate fluid (Fry, 1970a,b, p. 18), implying that light-colored feces would produce a light-colored hydration fluid and that dark-colored feces would produce a dark-colored fluid. As discussed by Lemberg and Legge (1949), however, the color of human feces results from the oxidation of urobilinogen (a product of the breakdown of red blood cells) to urobilin. In an experiment designed to test Fry's specific conclusion, I (Williams-Dean, 1978, pp. 88–91) demonstrated that even pale human feces contain enough urobilinogen to subsequently color the hydration fluid a characteristic "human" reddish–brownish black if the feces are at least partially air-dried, oxidizing the urobilinogen to urobilin. Thus, it seems that neither the color of dried feces (light or dark) nor the origin of its constituents (meat or vegetable) determines the color of the trisodium phosphate fluid. Rather, the oxidation of metabolic products (urobilinogen and urobilin) formed by the breakdown of red blood cells is the determining factor, with metabolic pathways apparently differing among humans, coatimundi, and other animals. Importantly, the feces must be dried for the maximum color development in trisodium phosphate. Fortunately for studies of North American archaeological coprolites, at least, there seems to be little potential for confusing the color produced by human coprolites in the trisodium phosphate fluid with that produced by non-human coprolites.

Other attempts to identify the certainty of human origin of coprolites include experiments during the early 1980s by John Jones with immunological techniques. He used the double agar diffusion test, to see if the results could be used to verify the origin of coprolites as human (Jones, 1985). He found the results were inconclusive and abandoned his attempts without reporting them. He also stated that the technique would have been too time consuming and too expensive for routine use, even if it had worked. In another study, Robert Yohe et al. (1991) used immunological techniques such as the crossover electrophoresis test to search for protein traces that could identify the genus of the animal producing a coprolite. During their study, they also discovered that coprolites contain traces of other proteins that they believed could be linked to specific animals that were eaten as part of the diet.

3.2. "Invisible" dietary items

Callen also searched for a reliable way to determine when a coprolite contained traces of meat protein. He noted that when meat protein is eaten it often leaves no visible physical evidence (Callen, 1967a). He felt that even bone and hair remains in coprolites only "implied" meat eating but was not certain proof, although few coprolite analysts working today would agree with this last point.

When hydrating coprolite samples from early Mexican sites, Callen noted that some samples produced a thin crust, or "chemical skin" as he called it, on the surface of the trisodium-phosphate solution. That, he believed, signaled the presence of consumed meat

protein (Callen, 1967b). After his death, I (Bryant, 1974a) experimented with his technique, but I was never convinced that it was an accurate or reliable indicator of meat consumption).

Today, Callen's original link between a chemical skin and meat protein has been discarded. Subsequent observations by Williams-Dean (1978, pp. 94–96), Stock (1983, pp. 61–62), and Sobolik (1988a, p. 34) reveal that the film consists of phytoliths, fungal material, probable bacteria, and other material likely unrelated to the ingestion of meat. Further, I (Dean) demonstrated that the abundance, color, and character of the film changed dramatically after the coprolite was completely hydrated, then stirred and allowed to settle again (Williams-Dean, 1978, pp. 94–95).

3.3. Microscopy

To Callen, determining what was in a coprolite seemed like a fairly simple task. He relied on the visual signs he could see with the naked eye and through a dissecting microscope. Callen believed that the way seeds were broken could offer clues about how they were milled, ground, or chewed and could even suggest how the seeds were prepared as food (Callen, 1967a). Occasionally, he would examine some materials under the higher magnification levels provided by compound light microscopes.

By the early 1970s, we began using the increased resolution of scanning electron microscopes (SEM) in coprolite analyses (Bryant and Williams-Dean, 1975). Whereas resolution levels in a light microscope are limited by the wavelength of light to about 0.4 μm, scanning electron microscopes can bend light sources and thus increase the resolution limits down to about 0.0025 μm.

The importance of increased resolution provided by SEM helped Kate Rylander (1994) in her study of tiny chewed and ground fragments of maize kernels recovered in Basketmaker-period prehistoric coprolites from the American Southwest. The SEM resolution was so good that Rylander was able to identify different types of cooking, chewing, and grinding techniques in various coprolites. Using those data, Rylander was able to reconstruct probable food preparation techniques and the potential nutritional value of the specific maize types that were consumed.

3.4. Size of study sample

From the beginning of coprolite research, specialists have debated the amount of material that one should examine from a single coprolite. The initial coprolite samples that Junius Bird presented to Cameron and Callen were small fragments of coprolites with none of them weighing more than a few grams. For that first study, and in all of his later studies, Callen believed that one should examine the contents of an entire coprolite. He believed that technique, combined with reporting the ubiquity of coprolite contents, was the ideal method for understanding ancient dietary preferences as they are recorded in coprolites (Callen, 1967a).

Analysts still do not agree on how much material to examine from a given coprolite. Some researchers believe that it is sufficient to extract a small sub-sample, such as 1 cm^3 or a few grams (Reinhard, 1988; Edwards, 1990; Reinhard et al., 1991; Sutton and Reinhard, 1995; Reinhard et al., 2002). Others have pointed out that one should cut each coprolite in half along the longest axis and examine one half and save the other portion for future studies (Bryant, 1969; Williams-Dean and Bryant, 1975; Kelso, 1976; Williams-Dean, 1978; Stock, 1983; Sobolik, 1988a; Gremillion and Sobolik, 1996).

Reasons given by those on either side of this debate usually cite the fact that a different pollen spectrum is obtained from a coprolite depending on how much and where it is sampled. Martin and Sharrock (1964) first demonstrated this point. Gerald Kelso (1976) carefully documented the phenomenon by deliberately sub-sampling modern coprolites at intervals along their length. We have every reason to suspect that the same is true for macrofossil evidence as well. Certainly, a more complete understanding of the contents of a coprolite is gained when at least half of the specimen is analyzed as a unit. Because of this, some researchers, such as Kate Aasen (1984) in her studies of coprolites from Turkey Pen Ruin, believed it is essential to examine the contents of entire coprolites. Reinhard and Bryant (1992) agree that destructive analysis of a complete specimen might be appropriate for small or poorly preserved coprolites (e.g., Clary, 1975, 1984).

Callen and many subsequent coprolite analysts believed that realistic dietary reconstructions and adequate insights into food habits could not be thoroughly understood unless a large number of coprolite specimens from a single site or from a single time period were examined. This, they argue, is the only way to develop a reliable reconstruction of dietary and nutritional data from the coprolite record of a given culture because individuals do not eat the same things every day. In an effort to disprove this proposition, Reinhard (1988) examined a number of Anasazi-age coprolites from the American Southwest and concluded that only 18 to 20 coprolites need to be examined from a

single time period at a given site to yield 80% to 90% of the potential taxa present. He argued that studies of additional specimens are costly and time-consuming yet will add little additional knowledge to the coprolite information already yielded.

On the other hand, Jones (1988) mentions that examining as few as 18 to 20 coprolites per time period would not have provided an adequate record of ancient Peruvian diets. I had shown earlier (Williams-Dean, 1978) that 86% of identified food components (25 out of 29 taxa) appear in 50% or fewer of 100 Hinds Cave coprolites from a single latrine deposit. These same data reveal that a given pollen taxon does not regularly co-occur with a given macrofossil taxon, demonstrating that both data sets require complete analysis for the "major taxa" to appear. Analysts probably should not be surprised at the different data yielded by archaeological coprolites resulting from different subsistence practices developed over time in different ecological settings. Hunter–gatherers, of necessity, are likely to have a more varied dietary intake than do settled farmers. The ability to "predict" uniformity of diet in the past, and hence the need to examine only a given percentage of a coprolite collection, might be a worthy goal for a variety of reasons, but it is likely unrealistic to expect that goal to be achievable a priori. It is probably wise to analyze some portion of each coprolite in a collection if only to account for individual dietary idiosyncrasies.

3.5. Chemical and biological approaches

Chemical analysis of coprolites is an analytical technique that developed after the death of Callen. A number of different techniques using chemical applications have been tried on coprolite materials. However, the value and reliability of some of these chemical techniques have yet to be confirmed. For example, during the mid 1970s, John Moore et al. (1984) reported that, through the use of gas chromatography, one could successfully identify plants that had been eaten even though no visible trace of the plant's remains could be found in the coprolites during microscopic analyses. To our knowledge, this claim has never been confirmed by other studies.

Immunological protein studies have also been applied to human coprolites. During the early 1990s, Newman et al. (1993) examined protein residues they recovered from human coprolites in Lovelock Cave, Nevada. They were able to isolate human protein residues in six of the seven samples they examined. In addition, they found other types of animal protein in some of the samples, such as the protein from pronghorn sheep in four of the seven samples.

Early studies by Lin et al. (1978) were among the first to focus on a search for steroids in human coprolites. In their initial study, they proved that meaningful data could be derived from residual steroid traces. More recently, others have used steroids found in human coprolites to identify the sex of the individual who produced the feces (Sobolik et al., 1996). Sobolik and her colleagues examined 12 human coprolites ranging in age from 2700 to 2300 years old that were recovered from Mammoth and Salts Caves in Kentucky. Based on levels of testosterone in the coprolites, they concluded that males produced all 12.

Poinar et al. (2001) successfully extracted DNA using PCR amplification techniques from five human coprolites recovered from Hinds Cave in Southwest Texas. The macrofossil and pollen contents of the coprolites, radiocarbon dated to approximately 2500 years ago, were originally identified by one of the authors (Sobolik). Her list of the contents was then compared with the DNA evidence. Only the DNA evidence revealed some of the items eaten. Other items were identified by the macrofossil or microfossil remains, but were not noted by the DNA studies. Based on the cloned DNA sequences they identified that the coprolites contained traces of plants including cacti (*Opuntia*), sunflowers (*Helianthus*), ocotillo (*Foquieria*), various legumes (*Acacia*, *Prosopis*, *Sophora*), acorns (*Quercus*), hackberries (*Celtis*), members of the nightshade family (*Datura* or *Nicotiana*), and members of the lily family (*Allium*, *Dasylirion*, *Nolina*, or *Yucca*). Some of the animals represented by DNA in the coprolites included pronghorn antelope (*Antilocapra*) and cottontail rabbits (*Sylvilagus*). Other animal remains, including packrats (*Neotoma*), cotton rats (*Sigmodon*) and squirrels (*Citellus*) were identified only from the macrofossil remains in the coprolites. In addition to the plant and animal DNA, the researchers also amplified traces of mtDNA fragments from the coprolites. Those amplifications revealed 9-bp direct repeats of variable lengths, which define haplogroups A–D. As a group, the A–D haplogroups account for 95–100% of contemporary Native Americans (Poinar et al., 2001). Three of these four different haplogroups are represented by the coprolites they studied.

In a new study currently in progress, Poinar (personal communication, 2005) and his research group are examining 12 additional coprolites from Hinds Cave dating to a much earlier period around 6000 years ago. As with the first study, Sobolik, Working with Bryant have conducted a preliminary pollen and macrofossil

analysis so that those results can be compared with the ongoing DNA studies of the same specimens. Thus far, the PCR amplification technique has revealed DNA from many of the same plants and animals identified in the earlier study of coprolites dating to about 2500 years ago (Poinar et al., 2001). However, their new study is adding to that list of economically important plants. Preliminary results already reveal that the 12 coprolites from the earlier group that occupied Hinds Cave contain PCR clones of DNA indicating they ate some species of mustard (Brassicaceae) greens or seeds, amaranths (Amaranthaceae), pinyon nuts (*Pinus* sp.), walnuts (*Juglans* sp.), grass seeds such as millets (Poaceae), and dewberries (*Rubus* sp.), confirming in many cases the earlier observations of coprolite pollen and macroremains made by Williams-Dean (1978) and others. As Poiner's group continues their studies, they hope to add more names to the list of plants and animals eaten by this earlier cultural group that lived in Southwest Texas.

3.6. Phytoliths

Callen was the first to recognize the importance and potential value of identifying phytoliths in coprolite samples. He believed that phytoliths could identify plant foods and used the presence of specific phytolith crystals in Tehuacan coprolites to suggest that the ancient inhabitants of that region had eaten two different types of cacti (*Opuntia* and *Lemairocereus*). Callen also experimented with cooking maguey (*Agave*) leaves and found that roasting caused their raphide crystals to shatter in a unique pattern. During his coprolite analysis he found shattered raphide phytoliths, which he reported as proof that the Indians had roasted and then eaten maguey leaves (Callen, 1967b).

I (Bryant, 1969) followed Callen's example mentioned in his Tehuacan study (Callen, 1967a) and identified agave and prickly pear phytoliths in Late Archaic coprolites from Conejo Shelter, Texas). I (Williams-Dean, 1978, p. 73) also identified prickly pear phytoliths in 6000-year-old coprolites from Hinds Cave, Texas, although the phytoliths did not receive a separate discussion. Stock (1983) and Sobolik (1988a,b, 1991) also discussed phytoliths recovered from Hinds Cave and Bakers Cave coprolites. More than a decade later Cummings (1990) conducted a comprehensive study of phytoliths recovered from 49 Medieval-age coprolites recovered from the island of Kulubnarti in Nubia. Cummings and Puseman (submitted for publication) conducted a similar type of analysis when they examined coprolites from Mesa Verde and used both pollen and phytoliths to identify the diets of Indians that had lived in that region of Colorado. Horrocks et al. (2003) analyzed pollen, phytoliths, and diatoms from prehistoric dog coprolites to extrapolate Maori diet in New Zealand.

3.7. Pollen

Callen made no attempt to recover pollen from the coprolites he examined, although he occasionally saw pollen grains during his analyses and recognized their potential importance. After learning of Callen's earlier studies, it was palynologist Paul S. Martin who was the first to search for pollen in human coprolites. During the mid 1960s, Martin and Floyd Sharrock (1964) published their pollen study of samples from human coprolites recovered in a site in the Glen Canyon region of Arizona. Many others followed Martin and Sharrock's example and have used pollen found in coprolites to suggest certain dietary preferences, changes in dietary patterns, and at times, the season of the year in which some coprolites were deposited.

The need to understand the movements of pollen grains through the human digestive tract was addressed in the late 1970s when Gerald Kelso (1976) and I (Williams-Dean, 1978) conducted experimental studies with volunteers. In separate studies, Kelso and I documented the travel of pollen grains through the digestive tract. Because my experiment lasted months longer than Kelso's, the data suggest that size and shape of pollen grains appear to be essential features that result in their leaving the human system at different rates. I found that some large pollen grains remained in the digestive tract for only a few days while other smaller types, such as mustard pollen (*Brassica*) became trapped in intestinal folds and continued to be emitted in feces for nearly a month after ingestion of the food that contained the pollen type. This mirrors the findings of early workers who tracked the time required for a known number of ingested glass or gold beads to be recovered from human volunteers (Alvarez and Freedlander, 1924; Alvarez, 1940).

Routine calculation of pollen concentration values for the analysis of archaeological specimens will make such experimental pollen data immensely useful in the future. Coprolite pollen analysts have gradually adopted the pollen concentration method of analyzing pollen spectra, following the lead of Kelso (1976). But pollen concentration data have been used in isolation from experimental data. For example, Sobolik (1988b, 2000) and Reinhard et al. (1991, p. 123) propose that coprolite pollen concentrations in excess of 100,000 pollen grains per gram signal intentional consumption. This is an

arbitrary number based on the extremely large pollen concentrations calculated for specimens in those studies and is not based on any experimental data.

Recently I (Dean, 2002) added *Lycopodium* marker grains to the original pollen residues from that initial, lengthy modern coprolite experiment and then derived pollen concentration data that were previously unavailable. Some of the results are discussed at length in Dean (2002). Important for the present discussion is the fact that known foods, such as grapes and strawberries, do carry small amounts of insect-borne pollen, meaning that any appearance of their pollen grains in a coprolite should be interpreted as intentional ingestion regardless of the pollen concentration. This supports the statement of Sobolik and Gerick (1992, p. 207) that the amount of pollen produced by a plant as well as pollen dispersal methods should be considered in assessing the significance of recovered pollen in coprolites. We do not believe that there is any way to predict a "threshold" below which intentional ingestion is unlikely, nor should there be any such expectation. Close corollaries are that there is no way to say with certainty that the total contents of a single coprolite were ingested during one meal or even one day, nor that all items consumed at the same time will appear in the same coprolite. Reinhard (1993) apparently continues to hold an opposing view.

3.8. Parasites

Callen and Cameron (1955, 1960) were the first to search for endoparasites in human coprolites. Their pioneering efforts were followed by Henry Hall (e.g., 1969, 1972, 1977), Gary Fry and Hall (e.g., 1969, 1975, submitted for publication), Fry and John Moore (1969), Moore et al. (1969, 1974), and Fry (1970a,b). I (Dean) initiated the first search for human parasites in archaeological coprolites recovered in Texas (Williams-Dean, 1975, 1978, pp. 75–77, 222–223, 259–260). Stock (1983) and Sobolik (1988a,b) continued that effort with additional coprolites from Hinds Cave and Baker Cave. Karl Reinhard (e.g., 1985, 1988, 2006), Peter Warnock and Reinhard (1992), Reinhard and Warnock (1996), and others (e.g., Bouchet et al. 2003) have since widened the search for endoparasites in human coprolites throughout the world.

3.9. Algae

I (Williams-Dean 1978, pp. 217–222) recovered the remains of algae in coprolites I examined from Hinds Cave, Texas. Specifically, I found the remains of several 32-celled *Pediastrum* and also a diatom. To my knowledge, this is the first report of algae recovered from ancient coprolites. Since then this seems to be an aspect that is rarely searched for in coprolite studies. However, recently a seacrh for diatoms was mentioned as part of a study of ancient coprolites from New Zealand (Horrocks et al., 2003).

3.10. Viruses

Inquiries by Dr. D. O. Cliver of the Food Research Institute, Madison, Wisconsin, in 1976 led me (Dean) to send him 13 coprolites from various Hinds Cave deposits dating from about 1800 to 3700 years old. A specimen from an undated deposit sandwiched between deposits dated at 2300 and 3700 years yielded a biologically active, yet difficult to culture, taxonomically unknown virus (Kostenbader, 1975–1977). The Institute never published this discovery and additional details are apparently unavailable. However, the recovery of viable viruses from ancient coprolites is an endeavor that will benefit from additional study.

4. Callen's data presentation

4.1. Quantification of macrofossils

Callen believed that the reporting of coprolite contents in terms of ubiquity was sufficient to characterize dietary components (Callen, 1965) and he did not explore other techniques that made use of weight, volume, or counting. When I (Callen, personal communication to Bryant, 1970) discussed this technique with him, he remarked that he believed the contents of any specimen would be thoroughly revealed after examining between 50 and 100 microscope slides made from the residue of that coprolite. His usual technique was to hydrate the entire coprolite and then use forceps to pick up small pieces of plant tissue, feathers, hairs, tiny bones, seeds, or insect parts. Each small item was placed on a separate microscope slide and then gently teased apart while examining it under a dissecting microscope. Next, each item was covered with hot glycerin jelly and a cover slip before being allowed to cool and harden. From each coprolite, Callen generally made between 50 and 100 separate slides, each containing some tiny fragment of information. Others before and after Callen have used different methods of removing individual specimens from coprolites, yet all are reported in terms of ubiquity (Smith and Jones, 1910; Young, 1910; Ruffer, 1921; Laudermilk and Munz, 1938; Heizer and Napton, 1969; Riskind, 1970; Stiger 1977; Fry and Hall, submitted for publication).

Is there a better way to quantify the contents of coprolites? Others began using techniques that they

believed would more nearly reflect the true importance of each dietary food. Richard Yarnell (1969), for example, introduced the percentage-estimate method, in which he assigned one of five broad percentage categories to each item he recovered in the coprolites he examined from Salts Cave in Kentucky. Yarnell later modified this technique somewhat in two subsequent studies of coprolites from Salts Cave (Yarnell, 1974a,b) and I (Bryant, 1974a) also began using a variation of this latter technique.

Sutton (1998) took the percentage-estimate method to new lengths in his examination of data from 137 coprolites recovered from five sites located in the northern part of the Coachella Valley of California. Sutton assigned an abundance rating from 0 (absent) to 4 (abundant) for each component in each coprolite sample, and then added the values for each resource to arrive at a total for each site. Those totals were then used to assign a percentage to each resource at each site. When the coprolite data from the five sites were examined using cluster analysis, Sutton identified 12 different clusters, each of which suggested the utilization of different foods or combination of foods at different seasons. His cluster analyses suggest the region was very lightly occupied during the winter months and heavily occupied during the spring and summer seasons, refuting Wilke's (1978) original model that suggested most of the sites represented villages with permanent populations.

Other researchers, including Fry, Lewis Napton, and Robert Stewart, expressed coprolite contents in their studies in terms of weight (Fry, 1968, 1969; Heizer, 1969; Napton, 1969; Fry, 1970a,b; Hall, 1972; Stewart, 1974; Fry and Hall, 1975). Many of these studies and others (Cummings, 1994) summarized the results in terms of quantity as well as ubiquity. William Marquardt (1974), using correlation coefficients and factor analysis, attempted to reconcile the differences in data presentation between Yarnell's percentage-estimates and Stewart's weights for Mammoth Cave coprolites, but was required to reduce the data to ubiquity to discern meaningful patterns of dietary intake.

Napton (1970) used a combination of visual-estimation of percentage-volume, volume, and weight, but recommended visual-estimation of percentage-volume as a standard method. This requires all the macrofossils to be identified and grouped in a large flat container; the proportion of 100% that each group represents is then visually estimated. I (Williams-Dean, 1978, pp. 98–112) used this visual-estimation of percentage-volume in my study of 100 early- to middle-archaic coprolites from southwest Texas. Jones (1988) used a slightly different method to quantify coprolite contents in his studies. After separation of each group of macrofossils into components, all were placed in the petri dish with a grid pattern in the bottom. The total number of grid squares each component covered was used to estimate that component's percentage of the total sample.

Regardless of the method, the aim of quantification is to portray the relative importance of each item in the *diet*. Quantification by weight favors heavier items. Quantification by volume favors bulky items. In truth, the question of quantification begs an obvious fact: coprolites contain the remains of what was *indigestible*, not everything that was *eaten* (Korschgen, 1971). As Holden (1994) wonders, how can one translate the *amount* of a food item eaten based only on the undigested portion of that same food product? An interesting study by Little and Little (1997) proposes the use of bone collagen isotope values and linear equations to derive possible proportions of various foods that could have been part of the diet. To our knowledge, no study has made use of this approach. Meanwhile, literal quantification and comparison of coprolite contents as "dietary items" overemphasizes the contribution of seeds, plant epidermis, fiber, bones, feathers, shell, and other indigestible items to the diet at the expense of completely digested dietary items such as seedless plant tissue, boneless animal tissue, soups, teas, and stews. It is possible that trying to accurately measure a coprolite's contents, other than by ubiquity, is an unproductive concern with precision. Precise measurements will be accurate and will be reproducible by others, but because of differential digestion and the vagaries of the digestive process, those precise quantities probably have little meaning in terms of specific dietary practices.

4.2. Quantification of pollen

Martin and Sharrock (1964) reported the first analysis of pollen grains from human coprolites and used relative percentages as their mode of data presentation. Relative percentages (relative frequencies), along with a saw-tooth diagram or a histogram, are used by environmental palynologists to present pollen data from soil samples taken to explore evidence of past vegetation and, by inference, paleoclimate. Except for Kelso (1976), and more recently Dean (1993, 2002), all subsequent coprolite pollen analyses have faithfully followed Martin and Sharrock's lead and have used relative percentages of taxa, an environmental technique, to analyze coprolites, quintessential products of human behavior.

The underlying flaw in all of these pollen analyses is the fact that, because the pollen percentages must sum to 100, all pollen taxa in a sample must increase or decrease in response to a decrease or increase in any other taxon in the same sample (Birks and Gordon, 1985, p. 11). In other words, relative percentages smooth the data, offer large-scale views of environmental reconstructions, and treat slight fluctuations in the total number of pollen grains from each pollen type as not being too important from sample to sample.

Kelso (1976, pp. 33–35) was the first to propose using *Eucalyptus* and *Lycopodium* marker grains and the calculation of pollen concentrations to analyze the pollen fraction of coprolites by taxon. He deliberately contrasted the results of his archaeological and experimental analyses by expressing them as relative percentages and as pollen concentrations. He concluded that the use of pollen concentration removed the statistical constraint imposed by relative percentages and allowed actual increases and decreases in abundance of individual pollen taxa to be seen (Kelso, 1976, p. 23). But the method languished because it was new and poorly understood by others.

Later coprolite analysts, including Aasen (1984), Reinhard (1993), Reinhard et al. (1991), Sobolik (1988b), and Sobolik and Gerick (1992), used *Lycopodium* marker grains to allow pollen concentration values to be calculated for coprolite samples. All presented total pollen concentrations for coprolite specimens but, unlike Kelso, conducted their analyses of individual taxa using only traditional pollen percentages. I (Dean, 1993) presented a reanalysis, using pollen concentrations, of the data provided by Reinhard et al. (1991) and arrived at conclusions recognized by Reinhard as importantly different (Reinhard, 1993). My recent work (Dean, 2002) transforms relative percentage coprolite pollen data gathered and presented in Williams-Dean (1978), drawing on more than a decade of using pollen concentrations to analyze archaeological soil samples. As seconded by Louis Maher (personal communication to Dean, 2002), using pollen concentrations in coprolite analysis to see the actual number of pollen grains that were ingested at the taxon level affords an opportunity for fine-grained analysis that is lost when the data are expressed as relative percentages.

5. The future of human coprolite study

Are coprolite studies becoming an accepted discipline? What can be done to encourage new students and other researchers to pursue studies in coprolite analysis? Where will the future generation of coprolite analysts be trained? What problems still remain to be solved? Does the field of coprolite studies have a future? These questions are just some of the concerns facing the discipline today.

5.1. Challenges in contemporary coprolite analysis

As a discipline, coprolite studies are somewhat more accepted today than they were several decades ago. Nevertheless, a problem still facing many coprolite analysts is "discipline identity." When Callen began his studies during the 1960s, his colleagues never appreciated his pioneering research efforts in fecal analysis and often criticized him for "wasting valuable time" examining coprolites. Even most other scientists outside his discipline of plant pathology considered his work a passing curiosity, and few thought it had much of a future in either botany or archaeology. However, this failure to recognize the value of disciplines that bridge several fields is not limited to those who work with coprolites. For several decades between 1940 and 1960, another scientist, Volney Jones, pioneered the discipline of paleoethnobotany, and, like Callen, his research efforts often went unrewarded and misunderstood. In a speech presented in 1957, Jones offered the following observation about his career that bridged the fields of botany and archaeology. He said, "Being something of an anthropologist and something of a botanist, one is looked upon as not quite either. One goes through life feeling miscellaneous" (Pearsall, 1989).

The view of coprolite analysts as "jacks of all trades and masters of none" is one reason why most researchers who complete their first coprolite study then move on to other more traditional research topics and never return to conduct a second study. Another reason that often deters researchers from pursuing coprolite studies is time and training. Coprolite analysts need to be broadly trained. Because of the diverse diets consumed by humans and because eating has important cultural significance, analyzing coprolites requires an expertise in many fields such as archaeology, anthropology, botany, zoology, palynology, entomology, parasitology, genetics, chemistry, ichthyology, ornithology, microscopy, internal medicine, pharmacology, and a myriad of other new and emerging specialties. Many graduate students are either not interested in learning all the skills needed to conduct thorough coprolite studies, or they do not feel they can afford the academic and research time that is required. Nevertheless, a few students still choose to pursue coprolite studies although the opportunities for training and employment remain limited. Until coprolite specialists are accepted for being

more than "miscellaneous," we doubt there will be a surge in either new students or new academic facilities where coprolite specialists can be trained. As the scientists cited in this paper illustrate, the programs in the United States where coprolite analysis can be learned are limited. Occasionally, a graduate student at some university will focus his or her thesis work on the study of coprolites. Nevertheless, as of this writing there are only four universities that have established major doctoral programs with an emphasis on training students in the study of human coprolites: Texas A&M University, The University of Nebraska–Lincoln, Washington State University, and The University of Maine-Orono.

In the past, a serious problem for coprolite specialists has been finding an academic or research position. We do not know of a single coprolite specialist who was ever hired primarily for his or her expertise in fecal studies. Instead, those who are employed have been hired for one or more of their other professional skills. Today, most placement ads want "traditionally trained" researchers and teachers with traditional doctoral degrees in the discipline. Because of this trend, there is an unwritten, but well-understood rule that graduate students quickly learn to follow: "learn and pursue traditional areas of research." There is often a high risk for those who stray too far into "strange areas of research," such as coprolite studies.

Our own careers are examples of the problems that coprolite specialists can face. I (Bryant) entered a graduate program in anthropology after a bachelor's degree in geography, and was tolerated by my fellow graduate students and faculty because I knew a skill they considered important: map making. As some in my anthropology department suspected, I soon pursued a non-traditional course of study: combining and using geographical and botanical data in archaeology. Fellow graduate students and some of the faculty told me I did not belong in anthropology because my research in geography and botany detracted from my becoming a "good archaeologist." Later, as a doctoral student in botany, I was told that my archaeological interests, and especially my coprolite studies, prevented me from becoming a "good botanist." After graduation, I was hired as an anthropologist at a time when there was a severe shortage of new teachers; but I was not hired for my skills in coprolite analysis!

I (Dean) had a similar experience. After two degrees in anthropology/archaeology, I changed universities to continue my studies in coprolite pollen analysis, but no doctoral degree in anthropology was yet available. Choosing the next logical major for pollen studies, I found myself tackling the entire field of botany at an advanced graduate level. Fellow students and faculty alike told me I was "too anthropological to be a botanist." Most could not understand how my botanical dissertation research could focus on a study of human coprolites that I had excavated from an archaeological site. Now, even as a Registered Professional Archaeologist employed as an archaeologist, I still must justify to others how I can be a "real archaeologist" when I earned a doctorate in botany some 30 years ago.

5.2. Opportunities in contemporary coprolite analysis

Even for those practitioners who proudly identify themselves as "coprolite analysts," there are problems and fundamental disagreements that need resolution (Sutton, 1994). For example coprolite analysts have summarized their data in many different ways during the past 40 years, including ubiquity, volume, weight, numerical totals, percentage, percentage ranges, and visual-estimates of percentage-volume. Anyone trying to synthesize coprolite data from diverse studies knows firsthand how difficult the task becomes because of the lack of agreement on how data should be presented. Unfortunately, the very nature of coprolite macrofossils, those small solid remains of different volumes and weights, resists the easy creation of a meaningful reporting format. Even more important is the difficulty of equating pieces of macrofossil evidence with the quantity of an item eaten.

For example, when one finds the physical remains of pollen, macrofossils, DNA, other types of chemical traces, or even dirt in a human coprolite, what do those data tell us about what was eaten and when, why, and how much was eaten, and whether any of the items were eaten together? What does one seed or a hundred seeds mean in terms of the amount of an item consumed? What does a single animal hair, a few fish or reptile scales, or even a large number of tiny fish or mammal bones reveal about how much meat was actually eaten? How reliable are the traces of DNA in coprolites? What do the identification of steroids, or protein residues recovered in coprolites really mean? Answers to these, and likely other, issues lie in the biological aspects of coprolite creation and the logic of coprolite analysis.

Using coprolite data from a site to derive a potential nutritional intake and caloric balance is an area where we believe advances can and should be possible. By recognizing that coprolites contain the remains of *undigested* items, it should be possible to determine the nutritional aspects and caloric values of

parent items that were originally eaten. If macrofossil data can be combined with evidence of *digested* items, as revealed from DNA, steroids, and protein residues, then deriving a richer picture of ancient nutrition, health, diet, and cultural ecology should be possible. Even determining the sex of a coprolite's donor through the study of fecal hormones is becoming routine, although finding a quick and inexpensive method for these tests is needed. If we could point to a pair of techniques that are likely to spark renewed interest in the discipline and encourage a new crop of students to become specialists, it would be the recent advances in chemical and genetic analyses. These new advances, when combined with traditional studies of coprolite macrofossil and microfossil analyses, will yield new avenues of understanding, and may even help us trace the origins and migration patterns of the earliest Americans through their coprolite DNA.

6. Conclusions

The desire of the public to know and to touch their ancestors, anybody's ancestors, whether dead for one century or for millennia, is almost insatiable. Recreating the very being of individual men and women from DNA and hormonal evidence left in coprolites, those most personal of artifacts, resonates with the public in a way that even facial reconstruction of skulls cannot. For non-specialists, coprolite data are fascinating. It is reassuring to learn that people have been challenged to find ways to feed themselves, as we are today, and that people have always found effective ways to meet that need.

If Callen were alive today he would be astonished and gratified to know that the lonely studies he began at McGill University during the mid-1950s, and that ended abruptly in his Ayacucho, Peru bedroom fifteen years later, have grown to include many branches of science. We remain optimistic for the future of coprolite studies. With new discoveries on the horizon in areas of chemical and genetic testing, and with a growing public fascination about who they are and how their ancestors might have lived, we believe that coprolite analysts will eventually graduate from being the butt of humor to recognition as scientists in the spotlight on center stage.

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