### Acid Dissolution of Fossil Dinosaur Eggs [1]

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Acid dissolution is a technique of removing a fossil from the surrounding rock matrix in which it is encased by dissolving that matrix with acid. Fossilized bone, though strong enough to be preserved for thousands or millions of years, is often more delicate than rock. Once a fossil is discovered, scientists must remove the fossil from its surroundings without damaging the fossil itself. Scientists have used chemicals to expose vertebrate fossils since the 1930s, and in the late 1990s Terry Manning, an amateur scientist and technician working in England, adapted the technology to dinosaur eggs. Manning used acid dissolution on dinosaur eggs to expose the embryos beneath the rock and fossil shell. Manning's acid dissolution enabled scientists to better study the remains of dinosaur embryos otherwise hidden beneath layers of eggshell and rock, revealing previously unrecorded aspects of dinosaur growth and development.

Acid dissolution is a technique that scientists have used since the mid-nineteenth century, first used to expose invertebrate fossils. In the 1930s, scientists Harry Ashley Toombs and Errol Ivor White of the British Museum of Natural History [3] in London, England, began to experiment with acid dissolution to prepare the remains of vertebrate fossils for viewing. White and Toombs experimented with different levels of acid to remove the red sandstone matrix from around the ancient armored jawless fish [4] (ostracoderms [5]) that they were studying. They discovered that acetic acid, when diluted in water, was the most successful in dissolving the matrix of calcium carbonate without compromising the fossilized bone, which was composed of calcium phosphate. Toombs published the methods of acid dissolution for vertebrate fossils in 1948, and afterwards many scientists used similar methods to prepare fossils.

Since the 1930s, paleontologists experimented with a variety of acid dissolution techniques to remove fossils from various kinds of stone matrices, including fossilized eggs containing potential embryos. Scientists estimated that fewer than one in 500 eggs contain the remains of an embryo. Bones of dinosaur embryos are not well developed (ossified), and therefore are more fragile and more likely to decay over millions of years than a fossil of an adult dinosaur with a fully developed skeleton. Most dinosaur eggs are crushed and eroded due to scavenging by predators or as a result of having hatched.

Prior to the use of acid dissolution, scientists attempted a number of unsuccessful methods to view embryos within eggs, including the use of x-ray [6] and CT scan technology to attempt to peer through the bone matrix and eggshell. However, those technologies could detect only differences in density within the egg [7], but not the structures within the egg [7]. Scientists hypothesized that those differences indicated embryonic bones contrasted against other parts of the fossilized egg [7]. Even when later methods revealed the presence of embryonic remains, the density differences picked up by CT scans and x-rays were often either pieces of eggshell inside the egg [7] or differential mineralization of the matrix within the egg [7].

In the 1990s, Manning turned to acid dissolution as an alternative method to reveal fossilized dinosaur embryos. During that decade, thousands of dinosaur eggs were exported and sold from China, and Manning purchased eggs that were the least crushed and most likely to contain embryonic remains. Manning aimed to dissolve both the eggshell and the stone matrix without damaging the delicate embryonic bones within the egg [7]. In his 1995 co-authored publication *The Dinosaur Egg and Embryo Project: Exhibition Guide*, Manning described his technique for acid dissolution using dilute acetic acid to dissolve the matrix composed of calcite and silt without dissolving the fossilized bones.

To conduct acid dissolution, Manning first partially submerged a whole dinosaur egg [7] in a solution of 5 percent acetic acid for several weeks. During that time, he removed the egg [7] every few days and soaked it in distilled water to remove any acid that seeped deep into the egg [7]. The egg [7] was then air dried for several days and examined under a microscope [8]. If any bone was present, Manning cleaned it using a small paint brush or a needle. He then coated the bone with a clear preservative, such as acryloid b67, to ensure that the bone didn't decay when he returned the eggs to the acid bath and the process was repeated. Those steps continued for several months, until the entire embryo was visible. Fewer than twenty percent of the eggs that went through Manning's acid dissolution process revealed an embryo.

In 1997 Manning described his acid dissolution technique in "Observations of Microstructures within Dinosaur Eggs from Henan Province, Peoples' Republic of China," which examined an egg [7] previously assigned to the family *Dendroolithidae* [9]. Through acid dissolution, Manning discovered that the egg [7] was from Late Cretaceous period, 94 to 66 million years ago. It belonged to a long-necked Therizinosaurid dinosaur. The egg [7] had been excavated from the Nanyang Valley of the Henan Province, China, along with thousands of other dinosaur eggs during the late twentieth century. Upon receiving the egg [7], Manning first attempted to x-ray [9] and CAT-scan the egg [7], but after the images proved inconclusive, he turned to acid dissolution to reveal the embryo within.

In the paper Manning described how he began with the standard technique of acid dissolution described in a widely used
once bone was exposed, Manning and his team covered it with a dilute solution of less than 1 percent of Paraloid B72 in acetone. That procedure sealed part of the embryo so that when the egg was re-immersed in the acid, the fossilized bone did not disintegrate. Additionally, the team photographed the embryos during each stage of the acid dissolution process. The 1997 paper notes that in addition to uncovering embryonic bones, that process can uncover fossilized soft tissue like muscle and cartilage, as well as parts of the egg like the surrounding yolk.

The embryos Manning uncovered using acid dissolution enabled scientists to explain much about dinosaur evolution and development. In 2008, a team of scientists led by biologist Martin Kundrát at McGill University in Montreal, Canada, published a paper analyzing the embryos he had processed. The team describes the features of the two Theropod embryos, including the toothless beak and massive face, larger than in any other described dinosaur egg, indicating that embryos of that species spent a longer time incubating than other kinds of dinosaurs. Kundrát also argued that the Theropod embryos appeared to have reached a more advanced stage of ossification than other embryos previously found, with the dinosaurs born relatively mature and able to move around on their own immediately upon hatching.

In the early decades of the twentieth century, Manning was the primary technician using acid dissolution on dinosaur eggs to reveal potential embryos, with the majority of other technicians preparing embryos by manually removing stone matrices, under microscopes, one grain at a time.

Sources


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