Blood clots in bones of dinosaurs and Permian tetrapods—evidence of asphyxiation?

Mark Armitage

Blood clots occluding microvascular bone canals have been reported in Cretaceous dinosaur remains, including those of separate individuals of *Triceratops* and *Nanotyrannus*,[1–3] and in *Dimetrodon*, an early Permian synapsid.[4] We report here newly discovered blood clots in *Edmontosaurus* (a Cretaceous hadrosaur), *Camarasaurus* (a Jurassic sauropod), and four Permian early tetrapod individuals, *Cacops, Eryops, Varanops,* and *Captorhinus*.

Materials and methods

We collected *Edmontosaurus* post-cranial elements of rib, vertebrae, and scapula at the Hell Creek Formation in Glendive, MT, and limb bone elements from *Camarasaurus* at the Morrison Formation in CO. We subjected them to fixation in formalin at the site for transport to the lab. We also secured museum limb specimens of *Cacops, Eryops, Varanops*, and *Captorhinus* from the Oklahoma Sam Nobel Museum of Natural History. These were fixed in formalin upon arrival. Bones were rinsed in pure water, then air-dried, and ground thin sections were made to 40 and 80-micron thickness on glass slides. Sections were examined without coverslip under reflected light UV fluorescence microscopy[2] for the presence of auto-fluorescing clots within microvascular bone canals.

Most clots presented as uniformly dark masses under brightfield microscopy, often filling the canal lumen, except for Permian specimens, which appeared diffuse in comparison. Crystalline shaped structures were evident in all clots, however (figures 1a, 2a, 3a, 4a,11a).

All clots autofluoresced brightly under UV illumination (275–290 nm), indicating massive presence of iron—probably from remains of heme in blood from the once-present vasculature (figures 1b, 2b, 3b, 4b, 5b, 6b, 7b, 8b, 9b, 10b, 11b, 12b). Permian specimens were less reactive in UV fluorescence, probably because they were fixed and dehydrated long after removal from deposits, but also because they exhibit thinner compact bone. We fix our specimens at the deposits to retard any potential degradation post-removal. Despite environmental factors (erosion, water infiltration, annual freeze-thaw cycle) and predation by bacteria, fungi, microbes, worms, and rodents over a substantial period of time, clots adhered tenaciously to bone matrix walls and often completely occluded blood canals the entire depth of our sections (both thick and thin). We were shocked that intense mechanical vibration during grinding and polishing for thin sectioning did not dislodge clots from canals.

We also cut rectangular planks of Permian bone and partially decalcified them in EDTA, which exposed solid tubes of clots extending several millimetres from the remaining undecalcified bone (unpublished results).

Clots adhered unwaveringly to microvascular bone canals and were characterized by embedded, dark and non-fluorescing crystallized objects surrounded by the brightly fluorescing iron signal (figures 1b, 2b, 3b, 4b, 5b, 6b, 7b, 8b, 10b, 11b, 12b, 13b). Clots did not infiltrate into bone (figures 1b, 2b, 3b, 6b, 8b, 10b, 11b, 12b, 13b, 14b, 15), thus any tissues buried deeply in bone matrix (osteocytes, long canalicular filipodia, collagen) were unperturbed by iron.

We examined clots on a Hitachi SU3900 SEM in BSE mode and performed elemental analysis. Clots returned a very bright backscattered electron signal, strikingly like the autofluorescence images in UVFL (figure 15). The BSE image confirms that clots did not infiltrate the bone, except for minimal intrusions into very small cracks. EDS confirmed that clots were high in calcium, iron, and oxygen, and were phosphorus rich (figure 15) and lacked significant presence of aluminum, carbon, chlorine, magnesium, manganese potassium, sodium, silica and sulfur (unpublished results).

Discussion and conclusions

We reason that the astonishingly persistent clotted microvascular bone canals observed in these specimens are characteristic of disseminated intravascular coagulation (DIC) or hypercoagulopathy, a process that results in clotting of blood throughout the vascular system as a result of severe trauma.[2,5–8] Systemic clotting leads to obstruction of blood vessels, organ failure, and death.

All thin sections are 40 microns thick, and magnification is 250X.
UVFL = ultraviolet fluorescence; HC = Hell Creek formation, MT; MO = Morrison formation; CO, LP = Lower Permian; OK; SN = Sam Nobel Museum, OK.

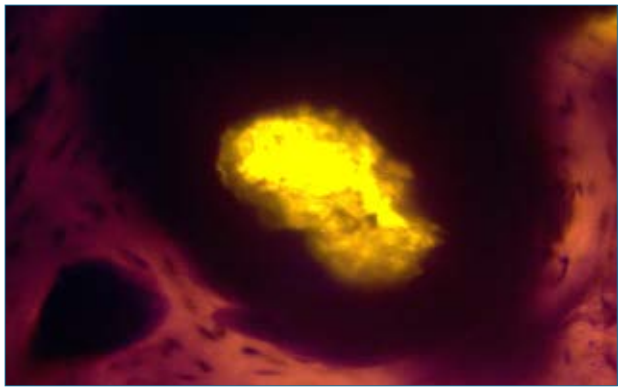

Figure 1a. *Edmontosaurus* rib, #DSTRI-5622B. Brightfield image, collected HC deposit, MT

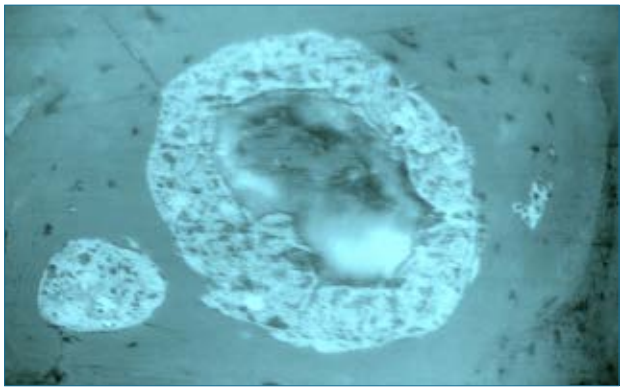

Figure 1b. *Edmontosaurus* rib, #DSTRI-5622B. UVFL image

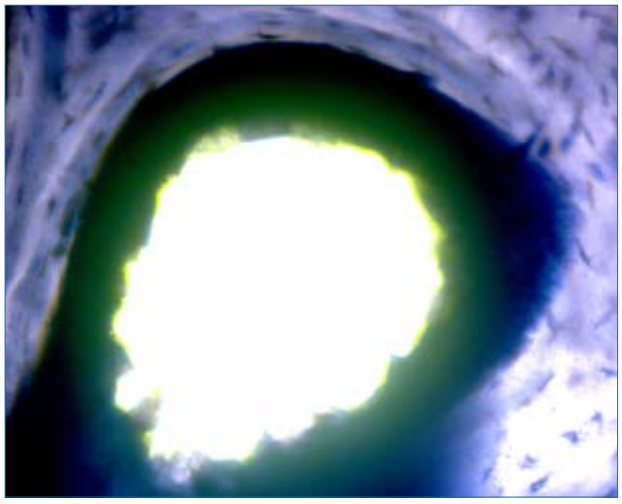

Figure 2a. *Edmontosaurus* scapula, #DSTRI-5622E, Brightfield, collected HC deposit, MT

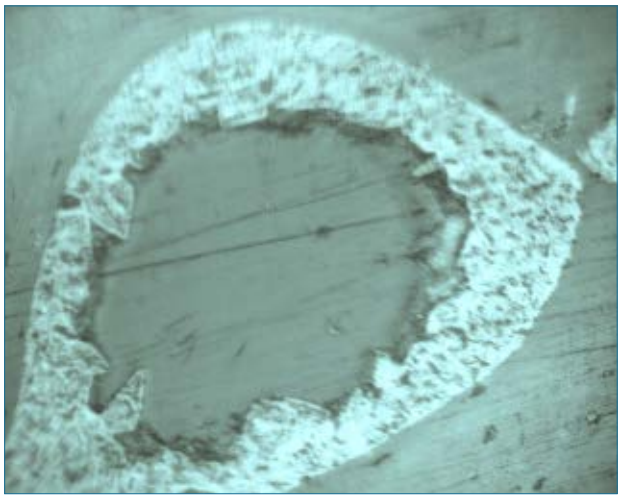

Figure 2b. *Edmontosaurus* scapula, #DSTRI-5622E UVFL image

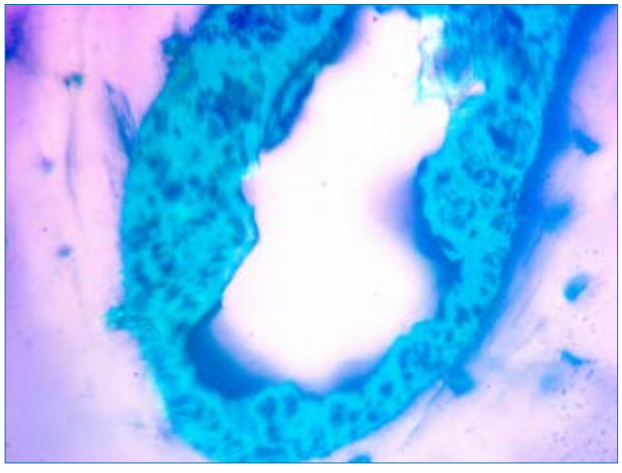

Figure 3a. *Nanotyrannus* vertebra, #DSTRI-91C, Brightfield image, collected HC deposit, MT

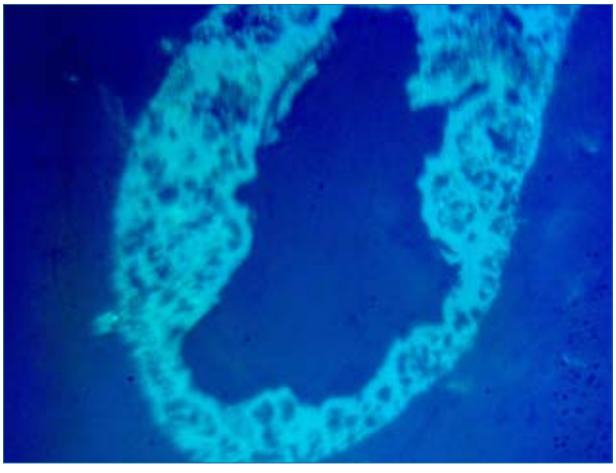

Figure 3b. *Nanotyrannus* vertebra, #DSTRI-91C, UVFL image

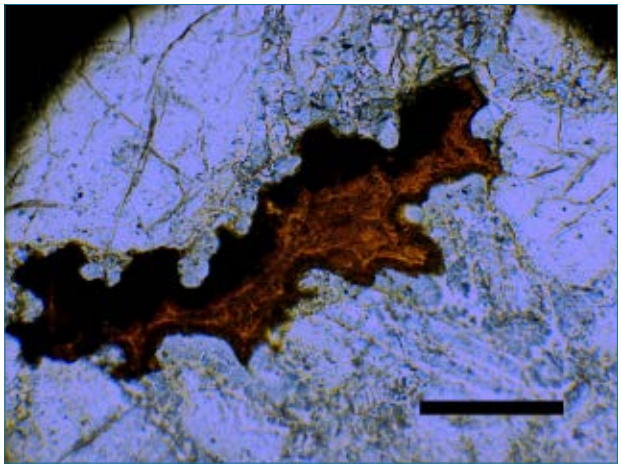

Figure 4a. *Camarasaurus* limb, #DSTRI-9420, Brightfield/FL image, MO, OK

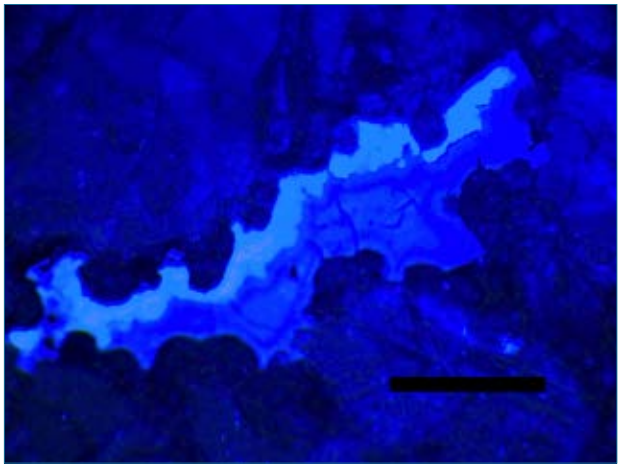

Figure 4b. *Camarasaurus* limb, #DSTRI-9420, UVFL image collected

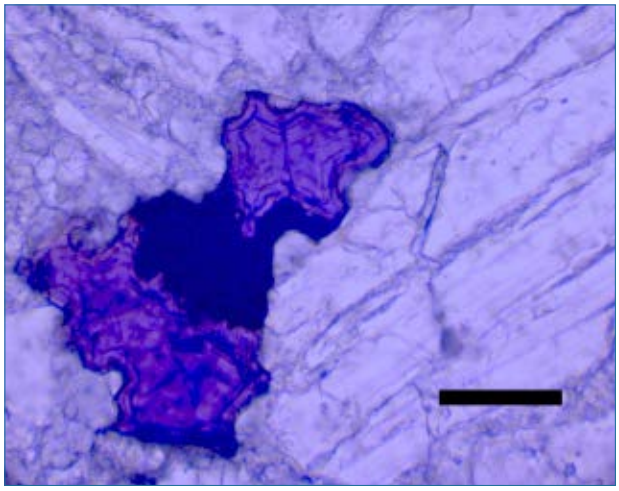

Figure 5a. *Camarasaurus* limb, #DSTRI-9421, Brightfield image, collected HC deposit, MT

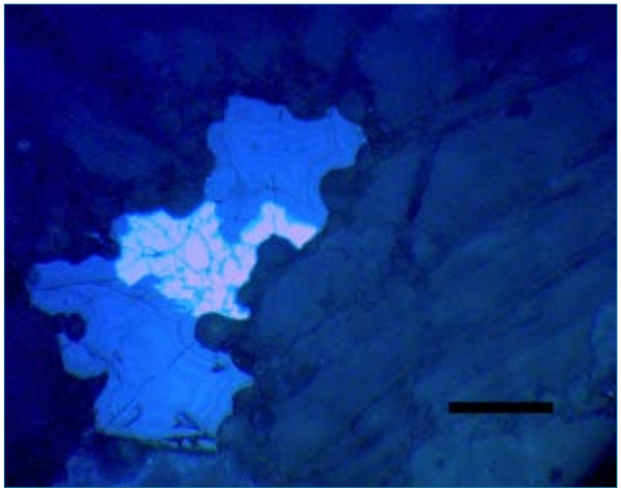

Figure 5b. *Camarasaurus* limb, #DSTRI-9421, UVFL image

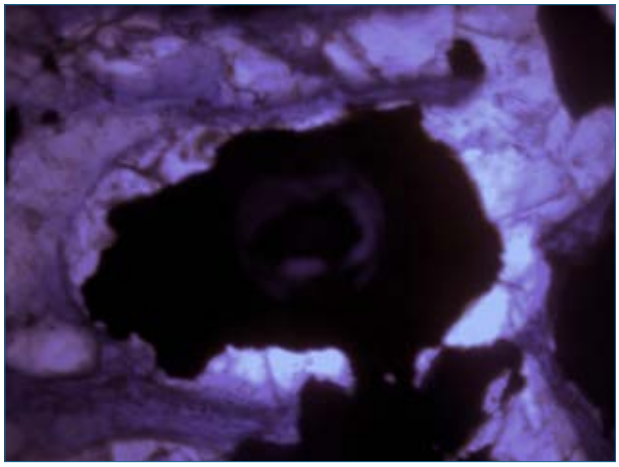

Figure 6a. *Cacops* humerus, #DSTRI-6923, Brightfield image, collected SN Museum, OK

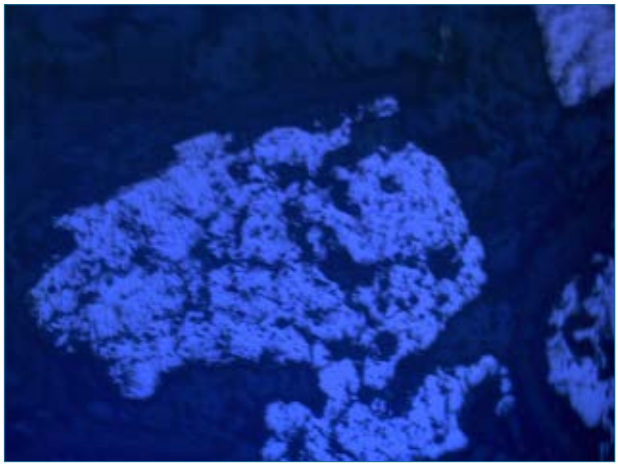

Figure 6b. *Cacops* humerus, #DSTRI-6923, UVFL image

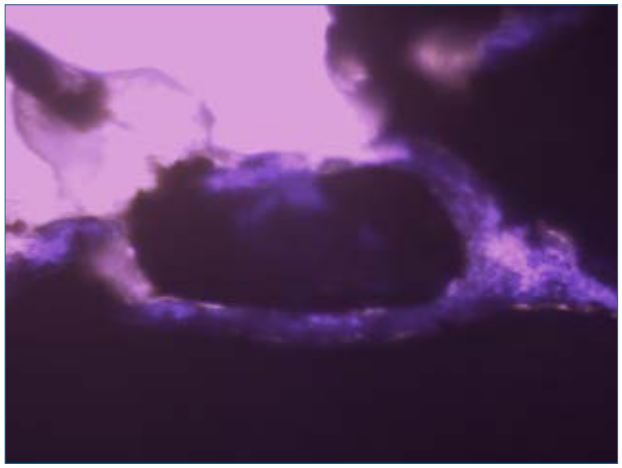

Figure 7a. *Cacops* humerus, #DSTRI-6923, Brightfield image, collected SN Museum, OK

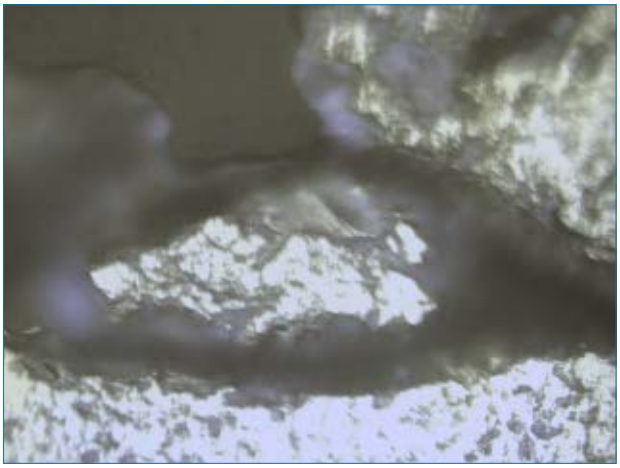

Figure 7b. *Cacops* humerus, #DSTRI-6923, UVFL image

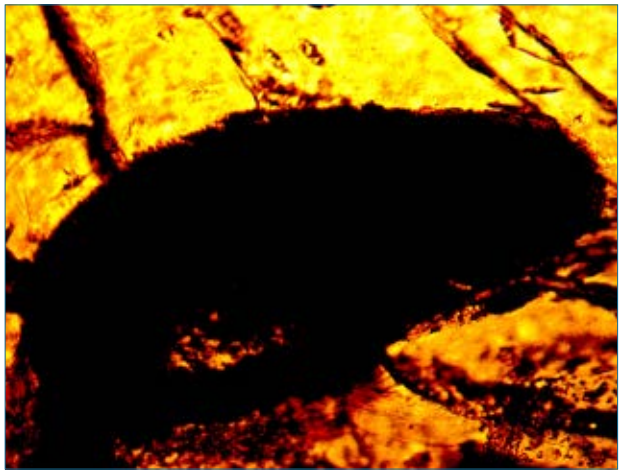

Figure 8a. *Eryops* humerus, #DSTRI-11223, Brightfield image, collected SN Museum, OK

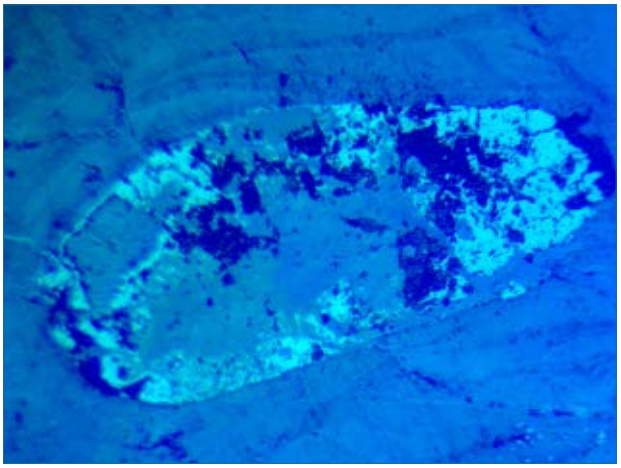

Figure 8b. *Eryops* humerus, #DSTRI-11223, UVFL image

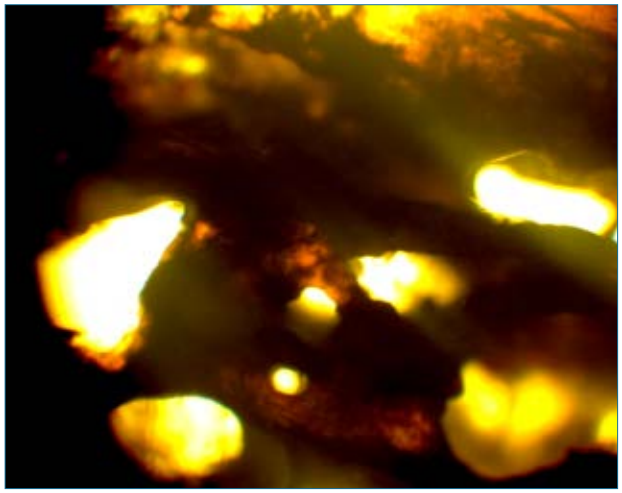

Figure 9a. *Varanops* femur, #DSTRI-11323, Brightfield image, collected SN, OK

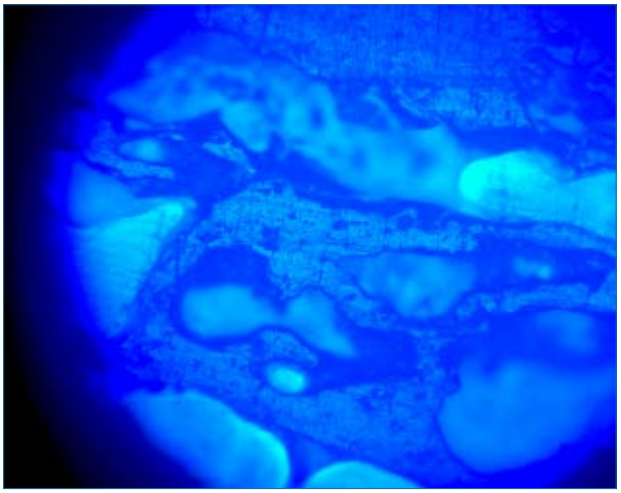

Figure 9b. *Varanops* femur, #DSTRI-11323, UVFL image

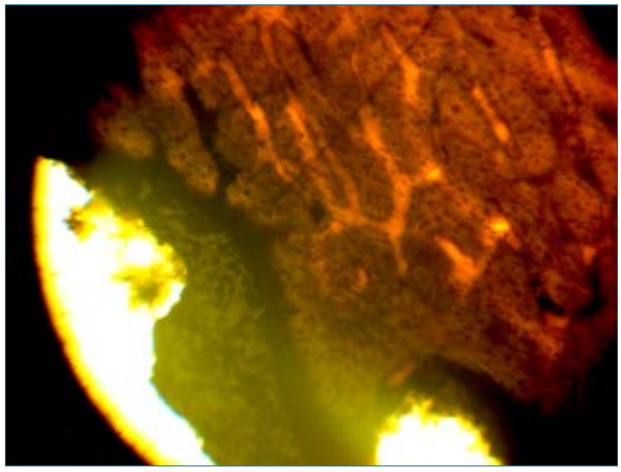

Figure 10a. *Captorhinus* humerus, #DSTRI-11423, Brightfield image, collected SN, OK

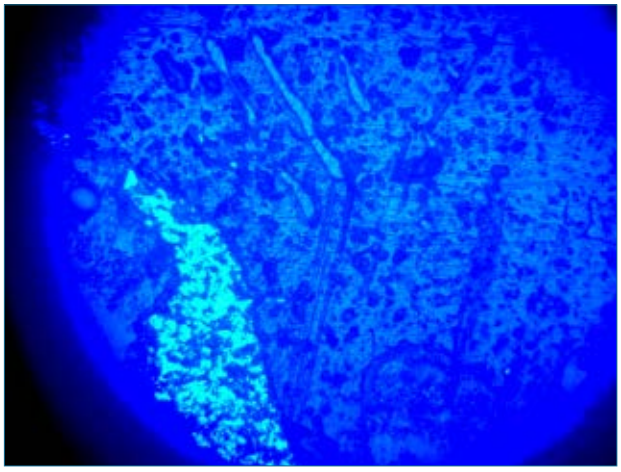

Figure 10b. *Captorhinus* humerus, #DSTRI-11423, UVFL image

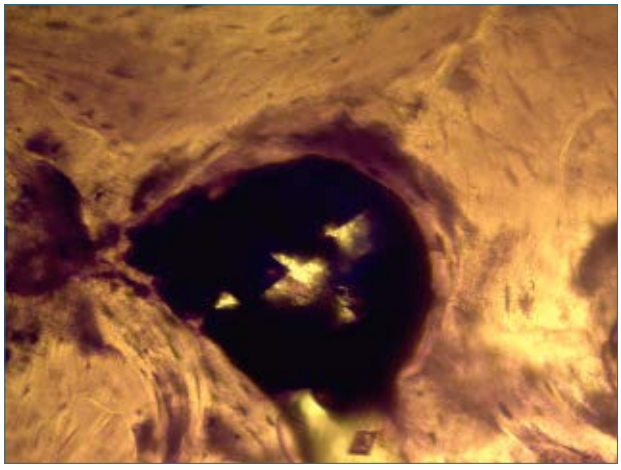

Figure 11a. *Dimetrodon* femur, #DSTRI-54G, Brightfield image, collected LP, OK

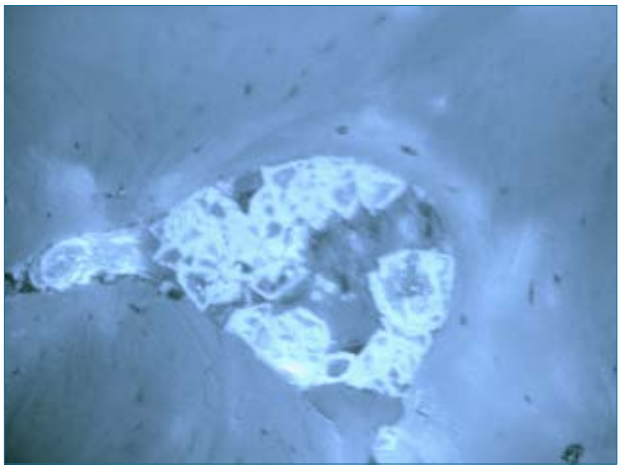

Figure 11b. *Dimetrodon* femur, #DSTRI-54G, UVFL image

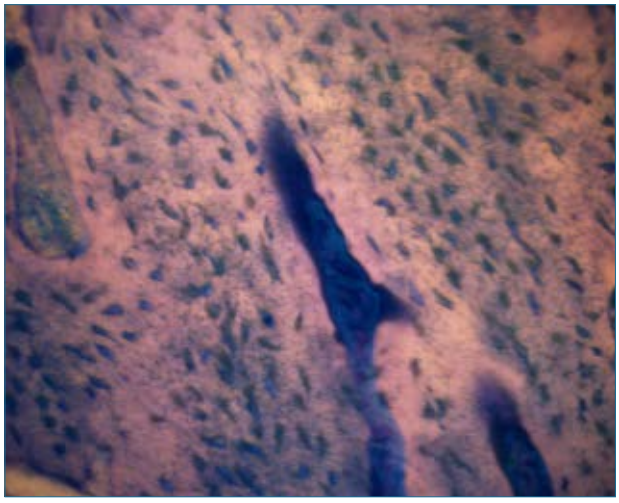

Figure 12a. *Dimetrodon* jaw, #DSTRI-54J, Brightfield image, collected LP, OK

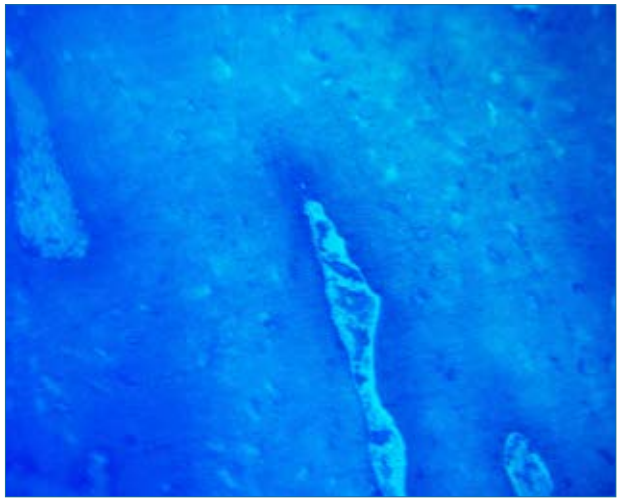

Figure 12b. *Dimetrodon* jaw, #DSTRI-54J, UVFL image

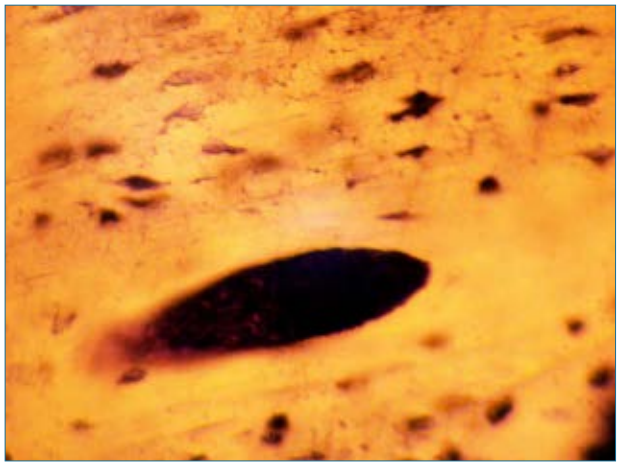

Figure 13a. *Dimetrodon* rib, #DSTRI-54K, Brightfield image, collected LP, OK

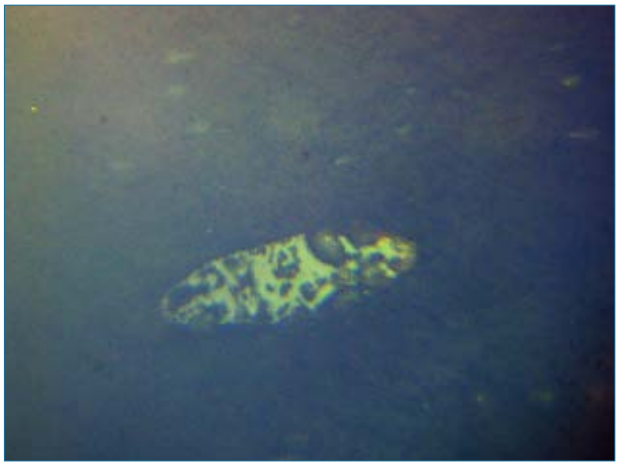

Figure 13b. *Dimetrodon* rib, #DSTRI054K, UVFL image

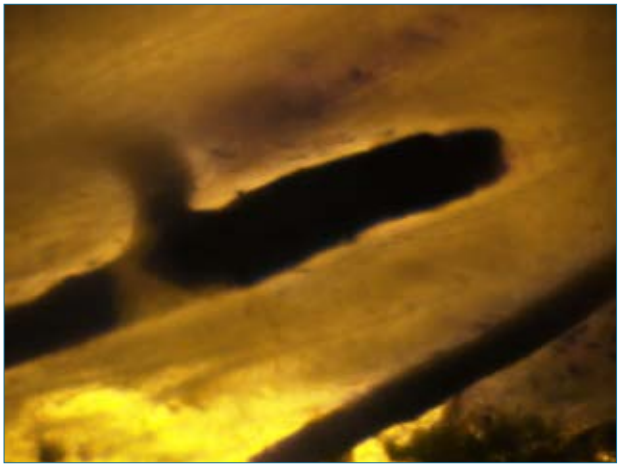

Figure 14a. *Dimetrodon* rib, #DSTRI-54K, Brightfield image, collected LP, OK

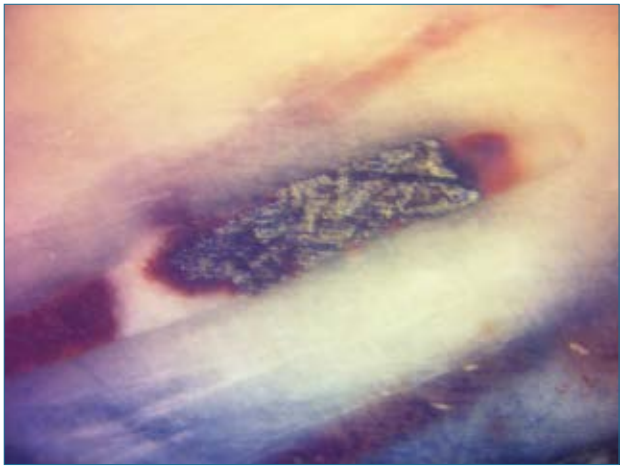

Figure 14b. *Dimetrodon* rib, #DSTRI054K, UVFL image

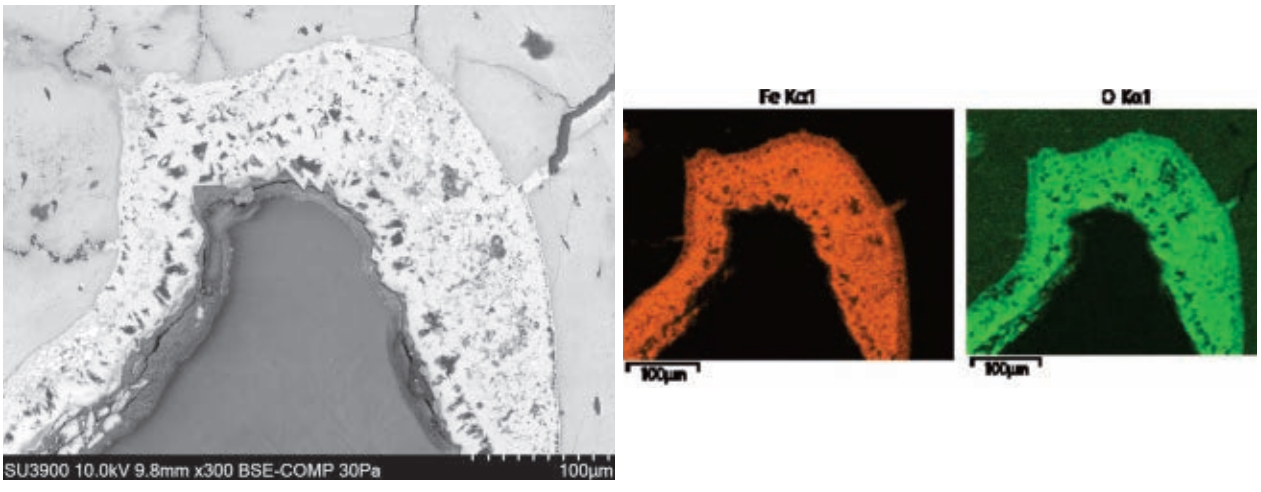

Figure 15. *Triceratops* vertebra #DSTRIHCTV22, SEM, BSE image, collected HC MT

Reports generally indicate that DIC develops due to severe trauma, acute injury, sepsis, cancer, leukemia, aneurysms, infections, and complications with obstetrics and other conditions, even Covid-19.[5–8] Our conclusion is that death by drowning is indicated by these microvascular clots.

A direct challenge to our conclusion is noted.[9] That author failed to note that our work in 2013 presented the first ever SEM micrograph of a clearly crystallized blood clot in a dinosaur bone blood vessel (*Triceratops* horn).[10]

Additionally, we published a higher resolution SEM micrograph of a *Triceratops* clot within a vessel in 2015 showing fine detail of the clotted blood products.[11] These crystalline clots show no evidence of infill from the surrounding soil matrix. One could argue that they are simply crystallized calcite, however the elemental map of a *Triceratops* vascular crystalline clot shows otherwise (figure 15).

Also, we have observed clots in histological reports of Permian amphibian bone thin sections and have commented accordingly.[4] Furthermore, we also recognize clots in dozens of reports of dinosaur bone histology.[12–23]

Senter,[9] provides a list of causative factors for DIC similar to those listed above, and includes others like cardiovascular disease, anemia, and heat stroke. He concludes that this "means that DIC is not diagnostic of drowning." However, the link between DIC and asphyxia/drowning is certainly supported. One recent study lamented "no study has investigated coagulopathy following drowning", and found that 80% of patients with drowning-induced asphyxia developed overt DIC within one day after hospitalization. They concluded, "Overt DIC occurs in the vast majority of drowning patients."[6]

It is also well established that humans resuscitated in the E.R. after a traumatic drowning event experience profuse bleeding, since clotting factors are exhausted during the trauma event.[5,6] Senter makes note of this profuse hemorrhaging presented by drowning victims and states, "the kind of DIC that drowning causes ... [means that] clots that begin to form are immediately destroyed so that a patient's blood cannot clot."[9]

This betrays the fact that most DIC reports are in the *medical* literature, specifically related to *human patients* who have been *rescued* from a drowning event and are *being treated* in the emergency room when they present with hemorrhaging. It is likely that treatment began the very moment they were rescued. Humans don't drown in the E.R., and no dinosaur was rescued or ever received transfusions, heparin, or CPR.

The medical literature on this subject cannot adequately explain the conditions which produced the abundant clots we observe in vertebrates buried at Hell Creek, especially with the obvious benefits of medical therapies employed to save humans from near-death drowning events. Additionally, the literature is unclear if resuscitation of human drowning victims impacts the onset of profuse bleeding, or if hemorrhaging is a natural consequence after acute traumatic systemic thrombosis, as Senter surmises.[9]

In the case of the bones studied here, no profuse bleeding seems to have occurred post-mortem because most of the microvascular canals are still fully obstructed by clots that did not dissolve. Furthermore, the clots remain unperturbed to this day and seem impervious to the mechanical vibrations of sectioning and polishing.

The typical death scenario for dinosaurs presented in the paleontology literature relies on natural causes of death prior to burial in sediments.[24] This scenario even anticipates mineralization of bone during the burial process.[24] Most of the dinosaur bones we have removed from deposits are still bone (thus are non-mineralized). They behave as modern bone during standard decalcification, practiced routinely in hospital histology labs. Moreover, the tissues we recover after decalcification take up standard tissue and nucleic acid stains, and are surprisingly pliable.

If buried dinosaurs died by natural means, blood would have pooled to the decumbent part of the body (*livor mortis*) thus clots or pooled blood should be *absent* in skeletal elements closer to the surface than other (deeper) bones in the same buried animal. The presence of systemic clots throughout these skeletal remains points strongly to a traumatic event leading to clotting of blood within microvascular bone canals during a drowning event, and not a death characterized by postmortem blood pooling.[2]

The fact that systemic bone clots are found in specimens that are considered to be in deposits ranging in conventional ages of 65 million to 290 million years suggests that this was one mass burial event.

As mentioned, histological studies of dinosaur bone feature thin sections to illustrate bone physiology and growth. Paleontology workers must undertake an examination of those specimens using UV autofluorescence microscopy to identify the presence of thrombosis in dinosaur microvascular canals, which we recognize as clots. This could extend the range and prevalence of clots across taxa and deposits globally. It might also confirm that *livor mortis* was unlikely in death due to asphyxiation and disseminated intravascular coagulation.

References

1. Armitage, M.H. and Anderson, K.L., Soft sheets of fibrillar bone from a fossil of the supraorbital horn of the dinosaur *Triceratops horridus*, *Acta Histochemica* **115**(6):603–608, 2013 | doi:10.1016/j.acthis.2013.01.001.
2. Armitage, M.H. and Solliday, J., UV autofluorescence microscopy of dinosaur bone reveals encapsulation of blood clots within vessel canals, *Microscopy Today* **28**(5):30–38, 2020 | doi:10.1017/S1551929520001340.
3. Armitage, M.H., Ultraviolet autofluorescence microscopy of *Nanotyrannus lancensis* sections reveals blood clots in vessel canals, *Microscopy Today* **30**(6):34–39, 2022 | doi:10.1017/S1551929522001262.
4. Armitage, M.H., UV autofluorescence microscopy of oklahoma permian synapsid femur (*Dimetrodon* cope, 1878) reveals blood clots in vascular canals, *Microscopy Today* **30** (1):18–23, 2022 | doi:10.1017/S1551929521001565.
5. Wada, H., Matsumoto, T., and Yamashita, Y., Diagnosis and treatment of disseminated intravascular coagulation (DIC) according to four DIC guidelines, *J. Intensive Care* **2**(15):1–8, 2014 | doi:10.1186/2052-0492-2-15.
6. Schwameis M., Schober, A., Schörgenhofer, C. *et al.*, Asphyxia by drowning induces massive bleeding due to hyperfibrinolytic disseminated intravascular coagulation, *Critical Care Medicine* **43**(11):2394–2402, 2015 | doi:10.1097/CCM.0000000000001273.
7. Iba, T., Levy, J.H., Raj, A., and Warkentin, T.E., Advance in the management of sepsis-induced coagulopathy and disseminated intravascular coagulation, *J. Clinical Medicine* **8**(728):1–16, 2019 | doi:10.3390/jcm8050728.
8. Ceriz, T., Lagarteira, J., Alves, S.R., Carrascal, A., and Terras Alexandre, R., Disseminated intravascular coagulation in Covid-19 setting: a clinical case description, *Cureus* **15**(6):e39941, 2023 | doi:10.7759/cureus.39941.
9. Senter, P.J., Soft tissues in fossil bone, *Palaeontogia Electronica*, 25.3.a34, Misconception 8, 2022 | doi:10.26879/1248.
10. Armitage and Anderson, ref. 1, p. 607, figure 16.
11. Armitage, M.H., Soft bone material from a brow horn of a *Triceratops horridus* from Hell Creek Formation, Montana, CRSQ **51**(4):248–258, 2015.
12. Ericksen, G.M. and Tumanova, T.A., Growth curve of *Psittacosaurus mongoliensis Osborn* (Ceratopsia: Psittacosauridae) inferred from long bone histology, *Zoological J. Linnean Society* **130**:551–566, 2008 | doi:10.1006/zjls.2000.0243.
13. Horner, J.R., Padian, K., and de Ricqlès, A., Comparative osteohistology of some embryonic and perinatal archosaurs: developmental and behavioral implications for dinosaurs. *Paleobiology* **27**(1):39–58, 2016 | doi:10.1666/0094-8373(2001)027<0039:COOSEA>2.0.CO;2.
14. Horner, J.R. and Padian, K., Age and growth dynamics of *Tyrannosaurus rex*, *Proceedings of the Royal Society of London* **271**:1875–1880, 2004 | doi:10.1098/rspb.2004.2829.
15. Padian, K., Horner, J.R., and de Ricqlès, A., Growth in small dinosaurs and pterosaurs: the evolution of archosaurian growth strategies, *J. Vertebrate Paleontology* **24**(3):555–571, 2004 | doi:10.1671/0272-4634(2004)024[0555:GISDAP]2.0.CO;2.
16. Klein, N. and Sander, M., Ontogenetic stages in the long bone histology of sauropod dinosaurs, *Paleobiology* **34**(2):247–263, 2008 | doi:10.1038/nature04633.
17. Zhao, Q., Benton, J.M., Sullivan, C., Sander, P.M., and Xu, X., Histology and postural change during the growth of the ceratopsian dinosaur *Psittacosaurus lujiatunensis*, *Nature Communications* **4**:2079, 2013 | doi:10.1038ncomms3079.
18. Lee, Y.C., Chiang, C.C., Huang, P.-Y. *et al.*, Evidence of preserved collagen in an early Jurassic sauropodomorph dinosaur revealed by synchrotron FTIR microspectroscopy, *Nature Communications* **8**, 14220, 2017 | doi:10.1038/ncomms14220.
19. Qin, Z., Clark, J., Choiniere, J., and Xu, X., A new alvarezsaurian theropod from the Upper Jurassic Shishugou Formation of western China, *Scientific Reports* **9**, 11727, 2019 | doi:10.1038/s41598-019-48148-7.
20. Woodward, H.N., Tremaine, K., Williams, S.A. *et al.*, Growing up *Tyrannosaurus rex*: osteohistology refutes the pygmy '*Nanotyrannus*' and supports ontogenetic niche partitioning in juvenile *Tyrannosaurus*, *Science Advances* **6**(1), 2020 | doi:10.1126/sciadv.aax6250.
21. Skutschas, P.P., Morozov, S.S., Averianov A.O. *et al.*, Femoral histology and growth patterns of the ceratopsian dinosaur *Psittacosaurus sibiricus* from the Early Cretaceous of Western Siberia, *Acta Palaeontologica Polonica* **66**(2):437–447, 2021 | doi:10.4202/app.00819.2020.
22. Aureliano, T. Nascimento, C.S.I., Fernandes, M.A., Ricardi-Branco, F., and Ghilardi, A.M., Blood parasites and acute osteomyelitis in a non-avian dinosaur (Sauropoda, Titanosauria) from the Upper Cretaceous Adamantina Formation, Bauru Basin, Southeast Brazil, *Cretaceous Research* **118**, 104672, 2021 | doi:10.1016/j.cretres.2020.104672.
23. Cullen, T.M., Canale, J.I., Apesteguía, S. *et al.*, Osteohistological analyses reveal diverse strategies of theropod dinosaur body-size evolution, *Proceedings of the Royal Society B* **287**, 20202258, 2020 | doi:10.1098/rspb.2020.2258.
24. Schweitzer, M.H., Blood from stone, *Scientific American* **303**(6):62–69, 2010.

***Mark Armitage**, Ed.D., is senior microscopist at the Dinosaur Soft Tissue Research Institute, a 501c3 organization headquartered in Mansfield, TX.*