

Mineral crisis as evolutionary driver: the Carnian Pluvial Episode, mammalian orotate-mediated magnesium transport, and lithium orotate as a contemporary footprint

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Abstract

We propose that a coordinated transport infrastructure present in modern mammals — comprising the SLC2A9 (GLUT9) facilitative transporter and the SLC22A12 (URAT1) organic anion exchanger — arose during the Carnian Pluvial Episode (~234-232 Ma) under selection for maternal magnesium delivery to offspring, and that the same infrastructure accounts for the distinctive pharmacokinetics of lithium orotate observed in modern mammals.

The CPE imposed a combination of conditions without precedent in tetrapod evolutionary history: sustained acidic precipitation on seasonal cool-temperate Gondwanan landmasses with poorly buffered post-arid soils, producing pronounced base-cation leaching and a magnesium deficit that would have been transmitted through the food chain to small endothermic vertebrates. We argue that within this regime, sustained directional selection on a remnant population of probainognathian cynodonts drove the elaboration of a maternal mineral-economy machinery centred on the orotate molecule as the chemical context for magnesium transport. We propose two stages. First, a cynodont innovation flipped GLUT9 from its ancient apical disposal-gate role to basolateral deployment in the gut, providing a dietary uptake pathway for orotate-magnesium complexes from maternal proto-milk. Second, this created selection pressure for the mammalian-specific URAT1 antiporter, which provides high-affinity recovery of orotate-magnesium complexes from renal filtrate and CSF, and a means to concentrate them at delivery interfaces serving brain and cardiac tissue.

Comparative-genomic data fit the predicted phylogenetic distribution: URAT1 has no orthologue in non-mammalian vertebrates, and GLUT9 is vertebrate-conserved with the trafficking-isoform architecture appearing mammalian-specific. The argument depends on a finding about orotate solution chemistry reported experimentally in a companion paper [1],

and predicts that the resulting orotate-metal forms are recognised by URAT1 and GLUT9 — a specific experimental prediction motivated by the work.

Note on format

This work is presented in two parts. An overview sets out the conjecture in plain prose without citations. The technical paper follows, recapitulating the argument with citations, kinetic data, palaeoclimatological detail, and the assumptions and limitations worked through section by section.

Overview

In August 2025, a research group at Harvard Medical School reported that lithium orotate is effective in mouse models of Alzheimer's disease without detectable toxicity over near-lifelong administration, and proposed that lithium orotate evades the amyloid-binding sequestration that limits brain availability of lithium delivered as other compounds. The findings, by Aron and colleagues, are striking and prompt a question: why should lithium orotate behave differently from other lithium salts they tested such as lithium carbonate?

Under conventional solution chemistry it should not. A lithium salt dissolved in water dissociates into free lithium ions and free counter-ions, or into transitory loose ion-pair associations. From this view the lithium ion is what reaches the bloodstream and the brain. The orotate counter-ion ought to play no role beyond getting the lithium into solution. Therefore equimolar lithium orotate and lithium carbonate ought to deliver equivalent lithium to plasma and to brain. But they appear not to.

This paper proposes an account of why and how lithium orotate may be transported preferentially to the brain. The account draws on palaeoclimatology, the evolutionary biology of mammals, comparative biochemistry of transporter proteins, and the chemistry of orotate-metal associations in solution. It is both a conceptual framework and a testable conjecture.

A new environment in deep time

Around 234 million years ago the Earth underwent an abrupt climatic change. After eighteen million years of pronounced aridity following the Permian-Triassic mass extinction, the climate shifted into a regime of intense rainfall driven by volcanism. This is the Carnian Pluvial Episode (CPE). It lasted, in episodic pulses, for roughly two to three million years.

The combination of factors imposed by the Carnian was without precedent in tetrapod evolutionary history. The rainfall onset was geologically instantaneous. By this point in the Triassic, Gondwana had drifted to mid-to-high southern latitudes, so the deluge arrived in seasonally cool regions — the first time terrestrial animals had encountered such conditions. The volcanic source of the change also released great amounts of both carbon dioxide and

sulphur dioxide, producing carbonic and sulphuric acid in rainwater. The soils receiving this acidic rainfall had developed under the long preceding aridity and were therefore poorly buffered against rapid base-cation leaching. Magnesium, the most easily leached of the major base cations, would have been particularly affected. The only flora that survived in cool-temperate Gondwana was simplified, gymnosperm-dominated, and growing on rapidly leaching soils. The magnesium deficit would have transmitted up the food chain to the small endothermic vertebrates that depended on this flora.

The lineage that came through

The animal lineage that ultimately gave rise to mammals was, on the eve of the CPE, a group of small cynodonts. They were fast-metabolism, fur-bearing, burrowing, and heterothermic, capable of generating body heat to forage on cold nights and conserving energy in burrows during the day (torpor). Their ancestors had spent the previous twenty million years adapting to predominantly arid conditions. They were ecologically the desert rats of their time.

We propose that the lineage's experience of the CPE unfolded in two phases. The first was the acute crisis at CPE onset, on geological timescales of tens of thousands of years. Most populations did not survive and many land animal species became extinct. We propose that the small cynodont 'desert rat' lineage came through as a remnant population, surviving largely by extending capacities the lineage had acquired already — thicker fur, longer torpor and more elaborate burrows. The mineral-supply problem, however, could not be solved by the extension of existing abilities; no simple adjustment can produce magnesium where there is not enough in the environment. Magnesium availability may therefore have been the hard metabolic ceiling on the survivors' reproductive success.

The second phase is the sustained altered conditions through the CPE itself, two to three million years during which the remnant population, perhaps on the Gondwanan periphery, would have experienced continuous strong directional selection. This is the kind of setting which can produce coordinated changes to molecular architecture. We argue that a specific form of selective pressure was seasonal reproductive timing. In an environment with sharp winter invertebrate die-off, mothers who can bear young earlier in the productive season produce offspring with more of the season ahead of them. Bearing earlier, when the external food supply has not yet recovered, requires the mother's own body reserves to support gestation and the post-birth provisioning period. Her ability to store minerals during the productive season, mobilise them during gestation, and then deliver them efficiently to her young determines how early she can successfully bear. Across thousands of generations, the marginal advantage of better maternal mineral management compounds.

After the Wrangellia volcanism subsided and the global hydrological regime returned toward aridity, the lineage emerged into a recovering world carrying the molecular adaptations developed during the CPE, and the rest of mammalian evolution has retained these genetic innovations as useful capabilities in modern lineages.

The maternal secretory apparatus through which mineral delivery occurs in mammals was already present in some form before the Carnian. Skin glands secreting moisture and antimicrobial compounds had likely originated in earlier synapsid ancestors as a means of protecting parchment-shelled eggs, and were gradually co-opted to provide nutrients to hatchlings or pups, taken up by lapping rather than suckling. (Modern monotremes still operate this nipple-less arrangement.) What CPE selection refined was not this visible infrastructure but its underlying biochemistry — specifically, we argue, the mineral content and delivery efficiency of proto-milk.

The chemistry the conjecture depends on

The argument that follows depends on a specific claim about orotate solution chemistry which we need to state clearly. The conventional view has magnesium orotate dissociating in dilute aqueous solution to free magnesium ions and free orotate anions, handled separately by their respective transporters at every membrane interface. Under that view there is no coherent "orotate-mediated magnesium transport" to discuss.

A companion paper of the present series presents experimental evidence that orotate and metal ions can associate in a novel long-lived stable form, which we call a Partially Ionised Nano-Cluster, or PINC [1]. PINCs are stable over time and non-aggregating, properties consistent with their playing a role in selective biological transport. We refer the reader to the companion paper for the physical chemistry investigation. The argument of the present paper assumes the validity of those observations: without them the molecular argument that follows has no physical basis. A second assumption is that PINCs have specific affinity for the relevant transporter proteins; this has not yet been investigated and we treat it as an experimental prediction.

First a gate, then a 'pump-and-gate' pairing

If orotate-metal associations persist in solution as the companion paper argues — as PINCs, the term we adopt from that paper — then the question for biology is how the body handles them. Two transporter proteins are suggested as playing a key role: GLUT9 and URAT1.

The first, GLUT9 (encoded by SLC2A9), is a member of an ancient family of transporters present across vertebrates. At the kidney, GLUT9 is positioned on the apical membrane of the proximal tubule, where it lets organic anions including orotate flow from blood into the urinary filtrate for disposal. This seems to have been its ancestral role: a gate that lets the body get rid of things it does not need to keep.

We propose that the initial CPE-onset innovation in GLUT9 was its deployment on the basolateral side of the gut epithelium. In ancestral vertebrates GLUT9 in gut tissue is apical-facing, consistent with the disposal-gate role; but in mammals it appears on the basolateral side, allowing PINCs in the gut lumen to flow into plasma rather than out of plasma into the lumen. The molecular mechanism by which this polarity flip was achieved is not yet established. What matters for the conjecture is the result: a gut epithelium configured for the

absorption of PINCs, providing a route by which proto-milk mineral content can reach the nursing offspring's circulation.

This deployment then creates a problem, but also an opportunity. PINCs absorbed at the gut into plasma are filtered back out at the kidney by the ancestral apical GLUT9 gate. The lineage gains the benefit of dietary absorption only at the cost of urinary loss. The new system needs a way to recover PINCs from filtrate against the disposal flow.

We propose that this second, later innovation is URAT1 (encoded by SLC22A12), an organic anion exchanger that operates on the apical membrane of the renal proximal tubule. URAT1 is a mammalian-lineage transporter with no orthologue in any non-mammalian vertebrate. URAT1 uses the energetic gradient of monocarboxylate exchange to draw PINCs from filtrate into the cell against bulk flow; basolateral GLUT9 then provides the equilibrating exit route to plasma. URAT1 is the only component performing thermodynamic work; the architecture's selectivity comes from URAT1's high affinity for orotate.

Once this 'pump and gate' architecture exists at the kidney, the same molecular components can be deployed elsewhere to do related work. At cardiac capillary endothelium and at the blood-brain barrier, the architecture can elevate the concentration of PINCs at the tissue-facing side of the endothelium, delivering magnesium to the ATP-demanding tissues that need it most. At the choroid plexus and ependyma of the brain, the architecture can recover PINCs from cerebrospinal fluid, preventing their loss through CSF turnover. The same molecular pairing handles both the recovery problem and the concentration-elevation problem, with the geometry of the deployment determining which function dominates at each site.

We refer to the architecture as 'pump and gate' rather than 'gate and pump' because URAT1's active transport into the cell precedes GLUT9's equilibrating exit; the substrate encounters the pump first.

Comparative-genomic signature

The proposal predicts a particular distribution of these proteins across vertebrate genomes. URAT1 should be mammalian-lineage, since the conjecture proposes that it arose during the mammalian stem lineage as the selective recovery component of the pump-and-gate system. GLUT9 should be older, since its ancestral disposal role at the kidney is the function the recovery pump was added to. Comparative-genomic data fit both predictions. URAT1 has no orthologue in any non-mammalian vertebrate. SLC22A9 (GLUT9) is conserved across vertebrate genomes; the basolateral deployment of GLUT9 in mammalian gut and the differential-trafficking arrangements that achieve it appear to be mammalian-specific.

A known principle and its absence in the Carnian

Modern ecology offers an illustration of how magnesium pressure on small mammals in leached environments shapes plant-animal symbiosis. Long-lived tree species growing on weathered, base-cation-depleted tropical soils typically concentrate disproportionate quantities of scarce magnesium into their seeds. The seeds form part of a co-evolved dispersal

partnership: mammals get a reliable concentrated source of the cation, and the trees get their seeds dispersed. The principle is well-documented and uncontroversial. The Brazil nut tree (*Bertholletia excelsa*), growing on Amazonian terra firme oligotrophic soils, produces seeds at approximately 376 mg of magnesium per 100 g, dispersed by the agouti, a small rodent of the same forests; the cashew tree (*Anacardium occidentale*), on similarly leached lowland soils, produces seeds at around 292 mg per 100 g. Tree nuts from temperate, less leached soils sit at substantially lower magnesium densities — walnut at 158, chestnut at 32, pistachio at 121 mg per 100 g. The contrast is a familiar ecological pattern; we mention it only to point at what the pattern implies about the strength of the underlying selection pressure.

The CPE was more extreme in magnesium stripping than the Amazon forest of today: higher rainfall, less buffered soils, and more acid sulphurous rain. The CPE was an ecological magnesium crisis, with profound effects across the Carnian biosphere.

The Brazil nut plant-mammal reward system is a much later (Cenozoic angiosperm) solution to the magnesium-pressure problem, and it depends on a tree reproductive strategy — large nutrient-dense seeds in animal-dispersed pods — that did not exist in the Carnian. Carnian conifers and corystosperms produced small wind-dispersed or gravity-fallen seeds, with no mammal-targeted nutrient payment. The Carnian small endotherm, facing magnesium pressure on a Gondwanan periphery for analogous reasons, could not access a dietary magnesium-concentrating partnership of the modern kind. We propose that the orotate-mediated maternal delivery system was selected for as an alternative route — internal physiological provisioning, mother to offspring, in the absence of the plant-animal solution that emerged only in the Cenozoic, over 150 million years later.

Contemporary observations consistent with the model

We propose that lithium can substitute for magnesium in orotate associations and that lithium is a passenger on a system that evolved for magnesium delivery. By this argument lithium orotate fed to a modern mammal enters its orotate-handling machinery, while lithium carbonate does not. Three contemporary observations are consistent with this prediction.

Kling and colleagues reported in 1978 that rats receiving lithium orotate showed brain lithium concentrations approximately three-fold higher than rats receiving equivalent doses of lithium carbonate, with comparable plasma kinetics. This is what one would expect if lithium orotate enters the orotate-handling machinery and is delivered preferentially to the brain via the choroid plexus version of the pump-and-gate pairing.

Smith and Schou reported in 1979 that lithium orotate and lithium carbonate produced equivalent plasma lithium concentrations in rats, and observed that lithium clearance was slower than expected in the orotate group. The pump-and-gate model predicts exactly this: URAT1 in the kidney recovers orotate-bound lithium from urine and returns it to plasma. The observation is positive evidence for an orotate recovery mechanism rather than evidence of impaired renal handling.

In the Aron 2025 result, lithium orotate is effective in Alzheimer's model mice at doses where lithium carbonate is not. Aron and colleagues also note that lithium orotate evades the amyloid-binding sequestration that limits brain lithium availability from other compounds. Taken together these observations imply that lithium is delivered to brain tissue in the orotate-complexed form, not as free ion.

Specific testable predictions

The conjecture rests on three classes of assumption, each independently testable.

The first concerns the chemistry. The companion paper's findings on PINC formation [1] should be reproducible by independent laboratories using comparable methods. Failure of independent replication would remove the basis for the molecular argument.

The second concerns molecular recognition. PINCs should have measurable affinity for URAT1 and GLUT9. This has not been measured anywhere. The relevant experiments are kinetic measurements of URAT1 and GLUT9 with PINCs as substrates, comparing recognition with that of free orotate and free metal ions. A negative result would substantially weaken the conjecture.

The third concerns physiology. URAT1 affinity for orotate has been measured in human and is striking; the equivalent measurement in mouse has not been reported. Similarly, the orotate content of monotreme and marsupial colostrum is not in the literature. Existing milk-orotate measurements are from cows and other ruminants; measurements from more basal mammalian lineages, especially those in magnesium-depleted environments, would be more phylogenetically informative. Replication of Kling's and Smith's lithium orotate rat experiments with modern instrumentation would resolve longstanding questions about brain delivery and renal processing.

Two further predictions follow from the molecular architecture proposed here. Comparative immunolocalisation of GLUT9 in non-mammalian vertebrate gut and kidney would test the polarity-flip claim — the conjecture predicts apical-dominant GLUT9 in non-mammals (consistent with the disposal-gate role) and basolateral-dominant deployment in mammalian gut. Existing data from chicken intestine support this pattern but warrant systematic testing across multiple non-mammalian lineages. Separately, immunolocalisation of URAT1 and GLUT9 at cardiac capillary endothelium and at the cerebral capillary endothelium of the blood-brain barrier would test whether the pump-and-gate architecture is deployed at these delivery interfaces as the model predicts. Neither has been characterised in the published literature.

A more general comparative prediction follows from the seasonal-reproductive-timing argument. Mammals adapted to highly seasonal environments with acute spring birth-timing pressure and environmental magnesium depletion should show more pronounced expression of the orotate transport machinery than mammals in other environments. Cross-mammal comparative work would test this prediction.

What the conjecture does not suggest

Any clinical or pharmacological interpretation is well beyond the scope of this paper. However, for clarity, we see no direct link between the low-concentration orotate-bound effects discussed here and the high-concentration free-ion effects used successfully in clinical medicine. Our investigation suggests these two effects may be largely unrelated, and we state this explicitly to avoid misreading.

Section 1 — Introduction

The technical paper that follows develops the conjecture set out in the preceding overview as a scientific argument with citations, kinetic data, and supporting evidence. We propose that a coordinated transport infrastructure present in modern mammals — the GLUT9 / URAT1 architecture characterised in Section 5 — arose during the Carnian Pluvial Episode (~234-232 Ma) under selection for maternal magnesium delivery to offspring, and that this infrastructure accounts for the distinctive pharmacokinetics of lithium orotate observed in modern mammals.

The paper is organised as follows. Section 2 presents the palaeoclimatological evidence for the Carnian environment. Section 3 presents the palaeontological and physiological evidence for the small cynodont lineage that came through it, and develops the seasonal-reproductive-timing argument for the selective pressure we propose operated. Section 4 states the chemistry on which the remainder of the argument depends. Section 5 presents the molecular evidence for the GLUT9 / URAT1 transport architecture. Section 6 presents the comparative-genomic data on its phylogenetic distribution. Section 7 examines three contemporary observations consistent with the model. Section 8 sets out the specific experimental predictions the conjecture motivates and the conditions for its falsification.

The argument depends on two assumptions stated in Section 4: the orotate physical chemistry observations reported in a companion paper [1], where the proposed stable orotate-metal species are introduced as Partially Ionised Nano-Clusters (PINCs); and the recognition of these PINCs by URAT1 and GLUT9 as substrates for active transport.

Section 2 — A novel environment in deep time

The overview identifies the Carnian Pluvial Episode (CPE) as the environmental setting in which the conjecture's load-bearing selection pressure operated, and characterises its specific combination of features as without precedent in tetrapod evolutionary history. This section

presents the supporting palaeoclimatological, palaeogeographic, and palaeobotanical evidence.

Onset and duration

The CPE is dated to approximately 234-232 Ma, falling within the late Carnian stage of the Late Triassic. The episode is recorded in sedimentary successions across multiple continents as a shift from preceding aridity to a regime of substantially increased humidity and precipitation, identified through carbonate carbon-isotope excursions, the appearance of coal-bearing strata after the long Triassic coal gap, and palynological turnover [3, 4, 5, 6].

The onset of the episode was geologically rapid. High-resolution chronostratigraphy places the initial transition within an interval of the order of 16,000 years [7]. The episode itself unfolded as four distinct humid pulses spanning approximately 1.7 million years, with the system not returning to baseline aridity until perhaps two to three million years after onset [6, 8].

Volcanic driver

The CPE is causally associated with the emplacement of the Wrangellia Large Igneous Province, a basaltic large igneous province now exposed in accreted terranes of western North America (British Columbia, Yukon, Alaska) but originally erupted in the eastern Panthalassic Ocean [6, 9]. Wrangellia volcanism released substantial quantities of CO₂ and SO₂ into the atmosphere over multiple eruptive pulses. The temporal correspondence between the four Wrangellia pulses and the four CPE humid intervals is consistent with volcanic forcing as the primary driver of the climatic change [6].

The atmospheric chemistry consequences are well-established for analogous large igneous province emplacements. CO₂ release produces greenhouse warming, which intensifies the hydrological cycle and increases precipitation. SO₂ release produces sulphate aerosols and, through wet deposition, sulphuric acid in rainwater. CO₂ dissolved in rainwater additionally contributes carbonic acid. The combination produces acidic precipitation over wide regions, with the precise acidity depending on local atmospheric and hydrological conditions [6, 10].

Palaeogeographic context

The continents at the time of the CPE were arranged into the supercontinent Pangaea, which was rotating slowly counterclockwise through the Triassic. The Gondwanan landmasses (South America, Africa, Antarctica, Australia, India), which had been positioned at predominantly low to mid latitudes during the Permian and Early Triassic, had drifted southward through the Triassic and were at the time of the CPE distributed across mid-to-high southern latitudes [6, 13]. Cool-temperate Gondwanan terrains during the CPE therefore experienced rainfall arriving on landmasses that had not previously been exposed to substantial precipitation under cool seasonal conditions.

This is a novel combination. Wet conditions, seasonally cool conditions, and acidic atmospheric conditions had each existed in tetrapod evolutionary history before. The simultaneous occurrence of all three on land surfaces with the soil-chemistry properties described below appears to be without close precedent.

Soil chemistry and base-cation leaching

The soils receiving CPE rainfall had developed during the preceding ~18 million years of pronounced post-PTME aridity. Arid soils typically retain accumulated base cations because limited percolation restricts leaching, but the same property leaves them poorly buffered against rapid percolation when it does occur. Acidic precipitation falling on such soils mobilises base cations rapidly. The order of leaching susceptibility for the major base cations is broadly $\text{Mg}^{2+} > \text{Ca}^{2+} > \text{K}^+ > \text{Na}^+$ in most soil-chemistry frameworks, with Mg^{2+} the most readily mobilised because of its small ionic radius and high hydration energy in aqueous solution [10, 12].

We have not located direct quantitative measurements of magnesium depletion in Carnian palaeosols specifically. The argument that magnesium would have been particularly affected rests on the general soil-chemistry principles applied to the documented combination of acidic precipitation onset, poor pre-existing buffering, and increased percolation rates. Direct geochemical measurements of base-cation profiles in Carnian palaeosol sequences would either reinforce or constrain this aspect of the argument.

Palaeobotanical response

The Carnian floral record across Gondwana shows substantial turnover coincident with the CPE. The pre-CPE Gondwanan flora — the Ipswich flora and contemporaries, with *Dicroidium*-dominated assemblages of seed ferns (corystosperms) and associated gymnosperms — gave way to the Onslow-province flora, characterised by simplified gymnosperm-dominated assemblages with reduced taxonomic diversity [14, 15, 16, 17]. The Onslow flora is the assemblage that grew on the leaching soils of cool-temperate Gondwana through the body of the CPE itself.

Reduced floral taxonomic diversity in the post-CPE-onset assemblage, combined with simplified community structure and the soil-chemistry context above, is consistent with a flora operating under nutrient constraint relative to its predecessors. The plant communities that fed the herbivorous and detritivorous invertebrates on which small endotherms depended would have transmitted any environmental magnesium deficit upward through the food chain. We have not located direct measurements of magnesium content in Onslow-flora plant tissues or in their associated invertebrate fauna; this is a measurable property that future palaeobotanical and palaeoecological work could address.

Summary of the argument

The CPE imposed a specific combination of conditions: rapid onset, sustained duration of two to three million years, cool seasonal precipitation in cool-temperate Gondwana for the first time during tetrapod evolutionary history, acidic rainfall from CO₂/SO₂ volcanic emissions, soils poorly buffered against rapid base-cation leaching, and a simplified flora growing under the resulting mineral constraint. Magnesium, the most readily leached of the major base cations, would have been particularly affected. This is the environmental context within which the lineage described in Section 3 experienced the selective pressure proposed by the conjecture.

Section 3 — The lineage that came through

The overview identifies the animal lineage that ultimately gave rise to mammals as a group of small cynodonts on the eve of the Carnian Pluvial Episode (CPE), proposes that their experience of the CPE unfolded in two phases of distinct selective character, and argues that seasonal reproductive timing exerted strong selective pressure during the sustained second phase. This section presents the supporting palaeontological and physiological evidence.

Pre-CPE cynodont biology

The probainognathian cynodonts of the Late Triassic were advanced members of the cynodont radiation, the mammal-line synapsid lineage that ultimately produced crown Mammalia. By the late Middle to early Late Triassic, the lineage had developed a recognisable body plan: small body size, fast-metabolism, semi-fossorial (burrow-using), insulated by some form of pelage, and heterothermic. Heterothermy in this context refers to the capacity, familiar from modern small mammals such as bats, dunnarts, tenrecs, and daily-torpor rodents, to generate metabolic heat for active foraging while permitting regulated metabolic depression (torpor) during inactive periods. Basal metabolic rates in heterothermic mammals are markedly lower than in similarly sized placentals, while the maximum rates during activity are high.

Bone histology of pre-CPE cynodonts is consistent with elevated metabolic rates and rapid sustained growth across multiple genera. Cynognathus, Diademodon, and Thrinaxodon show fibro-lamellar bone tissue (a microarchitecture characterised by rapid disorganised initial deposition followed by remodelling, associated with rapid sustained growth in modern endotherms) with high vascular density and limited growth rings [18, 20]. The Early Triassic burrowing cynodont Trirachodon shows seasonally modulated growth — fast in favourable seasons, decreased or arrested in unfavourable ones — consistent with a small endotherm managing seasonal stress through alternation between active and torpid states [19]. Burrows attributed to Trirachodon and related cynodonts have been recovered from Early Triassic

aeolian and fluvial sediments across Gondwana, confirming the burrowing lifestyle from independent evidence.

Quantitative work on Early Jurassic mammaliaforms — *Morganucodon* and *Kuehneotherium*, the closest relatives of crown mammals available in the fossil record — using synchrotron tomography of incremental tooth cementum and femoral nutrient foramina indicates basal metabolic rates closer to modern reptiles than to modern mammals, with maximum metabolic rates intermediate between the two [21]. We read this as describing the specific kind of endothermy these animals had: a heterothermic regime with reduced basal rates and elevated maximum rates, calibrated for energetic economy in resource-constrained environments.

Two-phase framing of the CPE response

Phase 1 — the acute crisis. The Wrangellia volcanism initiated rapidly, the rain regime switched on geological timescales of thousands to tens of thousands of years, and the immediate biological response was extinction or near-extinction across many lineages. The end-Carnian extinction shows up in the fossil record as a major turnover event for traversodontid cynodonts (a herbivorous probainognathian sister group), kannemeyeriid dicynodonts (large herbivorous mammal-line synapsids unrelated to crown mammals), and rhynchosaurs (beaked herbivorous reptiles in the archosauromorph lineage), all of which went into terminal decline through the Carnian and were largely or entirely extinct by the early Norian [22]. We propose that the surviving probainognathian populations came through as a small remnant, plausibly on the cool-temperate Gondwanan periphery, surviving largely by extension of pre-existing capacities — thicker fur, longer torpor, more elaborate burrows, more selective foraging during favourable windows. Such extensions involve recalibration of existing thermal and behavioural strategies onto a longer timescale rather than novel physiological architecture. The mineral-supply problem, by contrast, cannot be solved by such extension. Magnesium availability is set by soil chemistry and the resulting plant and invertebrate magnesium content; behavioural and thermoregulatory adjustments do not produce magnesium where there is insufficient supply in the food chain.

Phase 2 — the sustained altered regime. The CPE was not a brief perturbation but a sustained interval of altered conditions: four distinct pulses of Wrangellia volcanism over approximately 1.7 million years, with the system not returning to baseline aridity until perhaps two to three million years after onset [6, 8, 7]. The remnant population on the Gondwanan periphery would have experienced this period as continuous directional selection. A small isolated population sustained for one to three million years under continuous selection is the kind of population-genetic setting in which substantial directional selection can produce coordinated molecular architecture: novel transporter affinities, novel splice variant patterns, novel tissue distributions can be selected, refined, and set into the population genome.

Seasonal reproductive timing as the proposed selective pressure

We propose that the specific phase 2 selective pressure was seasonal reproductive timing. The argument rests on the following components, each independently observable in modern small heterothermic mammals.

In seasonal environments with sharp winter invertebrate die-off, the timing of reproductive cycles is a critical reproductive variable. Mothers who bear young earlier in the productive season produce offspring that have more of the season ahead of them — more time to grow, more time to mature, more time to prepare for their own first winter. Late-born offspring face the next winter at smaller body size with smaller energy reserves and lower survival probability.

Bearing earlier than the external food supply allows requires the mother's own body reserves to support gestation and the post-birth provisioning period. The mother's body acts as a transient mineral reservoir, drawn down during gestation and post-birth provisioning, recovered between cycles. Her capacity to store minerals during the productive season, mobilise them during gestation, and deliver them efficiently to her young determines how early she can successfully bear.

Magnesium is the cation under acute environmental shortage in the phase 2 setting (Section 2). Maternal magnesium economy therefore becomes a load-bearing component of reproductive success in this setting. Mothers whose physiology supports better magnesium storage, mobilisation, and delivery produce offspring with measurably better chances of surviving to reproduce. The selection pressure is sustained, gradient, and cumulative across thousands of generations.

The advantage compounds in three directions: earlier birth (because maternal reserves are adequate sooner after the previous cycle), larger litter (because per-pup delivery is more efficient), and faster recovery between cycles (because overall mineral economy is better tuned). Each is a marginal advantage in any single cycle. Sustained over phase 2's millions of generations, these marginal advantages would have produced and refined a coordinated infrastructure for delivering magnesium from maternal body to offspring with minimal waste — the molecular machinery characterised in Section 5.

Maternal secretory infrastructure

The maternal secretory apparatus through which mineral delivery occurs in mammals was already present in some form before the CPE. Apocrine-derived skin glands secreting moisture and antimicrobial compounds had originated in earlier synapsid ancestors, with origins potentially as far back as the Pennsylvanian (>310 Ma), as a means of protecting parchment-shelled eggs from desiccation and infection [26, 27]. These glands were gradually co-opted to provide nutrients to hatchlings or pups, who took up the secretions by lapping from specialised skin patches rather than by suckling at nipples [28]. Modern monotremes retain this nipple-less arrangement.

The conjecture does not require the invention of maternal secretion during phase 2. The secretory infrastructure was already present and operating when the CPE arrived. What phase 2 selection refined, on this account, was the chemistry of those secretions — specifically the mineral content and delivery efficiency of the proto-milk that was being delivered to nursing offspring through the existing secretory architecture.

Post-CPE recovery

After the Wrangellia eruptions ceased, the global hydrological regime returned toward aridity. The end of the CPE is generally placed around 232-230 Ma, with post-pluvial Norian aridity established by 229 Ma [6]. The body plan that emerged from the phase 2 filter is documented in the Brazilian probainognathian fossils of the immediate post-CPE world. *Brasilodon quadrangularis* from the Caturrita Formation of Rio Grande do Sul, dated to the Norian (~225 Ma), is approximately 12 cm long and weighed roughly 20 g — a mouse-to-shrew-sized animal [23]. Its dentition shows multicusped postcanine teeth approaching the mammalian molar pattern, with diphyodont tooth replacement (each tooth replaced once over the animal's lifetime, the mammalian pattern, rather than the polyphyodont pattern of continuous replacement found in more basal cynodonts) [24]. Its inner ear architecture indicates hearing close to that of modern mammals, and its postcranial anatomy shows the gait transition characteristic of advanced probainognathians [25]. *Riograndia*, a contemporary genus from the same formation, was strongly committed to burrowing.

These animals are post-CPE descendants of the lineage that came through phase 2, carrying the inherited molecular and physiological adaptations forward into the recovering Norian world. The subsequent mammalian radiation through the Mesozoic and into the Cenozoic preserved these adaptations, and we propose that modern mammals — including ourselves — retain the orotate-handling machinery characterised in Section 5 in conserved form.

Section 4 — Physical chemistry the conjecture depends on

The molecular and physiological argument that follows in subsequent sections rests on a specific claim about orotate solution chemistry, which we state here so the reader can evaluate the dependency directly.

The conventional view of orotate-salt solution chemistry has magnesium orotate dissociating fully in dilute aqueous solution to free Mg^{2+} and free orotate⁻ anions. The two species are handled independently by their respective transporters at every membrane interface from the moment of dissolution onwards. Under that view there is no coherent "orotate-mediated mineral transport" to discuss: orotate and the cation it might once have been associated with travel through the body by independent routes, and any kinetic or distributional argument about the orotate-handling machinery says nothing about magnesium handling.

Paper 1 of the present series [1] presents experimental evidence that orotate and metal ions can exist in solution as long-lived stable associated species, which Paper 1 calls Partially Ionised Nano-Clusters (PINCs). We refer the reader to that paper for the physical chemistry. The argument of the present paper assumes the validity of Paper 1's observations. Without them, the molecular machinery described in Section 5 cannot deliver orotate-bound magnesium as coherent transport units, and the evolutionary scenario developed in Section 3 has no mechanistic basis.

A second assumption operates separately from the solution chemistry. No experimental work has yet been done on the affinity of PINCs for proteins generally, or for the transporter proteins URAT1 and GLUT9 specifically. The argument that follows assumes such affinity exists — that PINCs are recognised by the relevant transporters as transportable units. This is a distinct experimental question from the solution chemistry of Paper 1, and we treat it as the most decisive specific experimental prediction the conjecture motivates (Section 8).

A recent peer-reviewed finding bears on the chemistry dependency. Aron et al. [2] reported that lithium orotate produced therapeutic effects in mouse models of Alzheimer's disease at doses where equivalent lithium carbonate did not, with no detectable toxicity over near-lifelong administration, and that lithium orotate evades the amyloid-binding sequestration that limits brain availability of lithium from other compounds. Standard dissociation chemistry would predict equivalent free Li^+ delivery from equimolar lithium orotate and lithium carbonate, which is not what Aron et al. observed. The findings are consistent with a role for the orotate counter-ion in the pharmacokinetics of lithium orotate that the standard model does not predict.

Section 5 — Molecular evidence: GLUT9, URAT1, and the orotate transport architecture

The overview proposes a pump-and-gate architecture in which two transporter proteins, URAT1 and basolateral GLUT9, provide the molecular machinery for orotate-mediated mineral handling. This section presents the supporting evidence for each component and the kinetic and structural data.

GLUT9 (SLC2A9)

GLUT9 is a member of the GLUT subfamily of facilitative transporters within the major facilitator superfamily, encoded by the SLC2A9 gene [29]. Orthologues of SLC2A9 are present across vertebrate genomes, including teleost fish, amphibians, reptiles, and birds, indicating a deep evolutionary origin predating the mammalian lineage [30].

In mammals the SLC2A9 gene produces two isoforms through alternative use of the first exon, designated GLUT9a (also GLUT9-L; 540 amino acids) and GLUT9b (also GLUT9-S; 512 amino acids) [29, 31]. The two isoforms differ only in the length and sequence of their N-terminal cytoplasmic tails; the substrate-binding residues, transmembrane architecture, and remainder of the cytoplasmic structure are identical. The N-terminal differences carry trafficking signals that direct the two isoforms to opposite membrane surfaces of polarised epithelial cells. In human renal proximal tubule, GLUT9-L is predominantly basolateral and GLUT9-S is predominantly apical [29, 32]. Mouse splice variants show comparable structure [31].

GLUT9 expression at apical membranes of non-mammalian vertebrate gut epithelium [47] is consistent with an ancestral disposal role: organic anion movement from blood-side circulation into urinary filtrate. The mammalian deployment of basolateral GLUT9 in polarised epithelia provides the molecular basis for vectorial flow into plasma across an epithelial barrier — the polarity flip the overview identifies as the stage-1 innovation.

The molecular mechanism by which mammals achieve basolateral GLUT9 deployment remains an open question in cell biology. Candidate accounts include isoform-specific N-terminal sorting signals [48], alternative-exon usage producing differentially-trafficked GLUT9-L and GLUT9-S forms [29, 31], and post-translational targeting machinery acting on conserved sequence. The conjecture depends only on the empirical observation that basolateral GLUT9 deployment is documented across multiple mammalian polarised epithelia, with the deployment preserved at the gut and propagated to additional interfaces in the proposed pump-and-gate architecture.

GLUT9 expression is also documented at the brain's cerebrospinal-fluid (CSF) interfaces. In mouse brain, GLUT9 protein is present in ependymal cells lining the ventricles, in neurons, and in brain capillaries [33]. In human brain, GLUT9 is present apically on the choroid plexus

epithelium [34]. This expression pattern positions GLUT9 also at the blood-CSF and CSF-brain interfaces.

URAT1 (SLC22A12)

URAT1 was cloned in 2002 and initially identified as a renal urate reabsorption transporter on the basis of its expression at the apical surface of the proximal tubule and its handling of urate [35, 36]. The Michaelis constant (K_m) of human URAT1 for urate was reported as approximately 371 μM [36]. The K_m for orotate was subsequently measured at approximately 5.2 μM , a 71-fold higher affinity than for urate [37]. Recent cryo-EM structural work has resolved the URAT1 substrate-binding site and confirmed the antiporter mechanism of substrate exchange [38]. There is also evidence that the role of URAT1 in urate reabsorption may be a later simian-specific adaptation [35]. We therefore suggest that the main function of URAT1 in early mammals may have been the reabsorption of orotate-metal complexes, as suggested by the greater affinity of URAT1 for orotate relative to urate.

Phylogenetic analysis of the SLC22 organic anion transporter family across vertebrate genomes places human URAT1 and its closest paralogues (OAT4, OAT7, and related apical reabsorption transporters) in a phylogenetic cluster that has no orthologues in non-mammalian vertebrates [30]. Zebrafish, examined as a teleost reference, has twenty SLC22-family genes organised into the same broad functional subgroups as human SLC22 members, but the OAT4-7/URAT1 cluster is absent. The same observation extends to the other non-mammalian vertebrates surveyed in that analysis. URAT1 in the strict sense — the apical reabsorption transporter handling orotate and urate — is mammalian-specific.

URAT1 operates as an organic anion / monocarboxylate antiporter, exchanging luminal substrate (orotate, urate, or other recognised organic anions) for cytoplasmic monocarboxylate anions exported into the tubular lumen [36, 38]. This is an exchange mechanism, not an active pump in the ATP-using sense; the directionality of net substrate movement is determined by concentration gradients of the exchange partners across the apical membrane.

URAT1 is also expressed at the brain's CSF interfaces. URAT1 protein is present in the apical ciliated membranes of ependymal cells lining the lateral, third, and fourth ventricles plus the cerebral aqueduct [33]. In human brain, URAT1 is present basolaterally on the choroid plexus epithelium [34].

The combined expression pattern

At the renal proximal tubule, both transporters are co-expressed on the same cells. URAT1 sits at the apical (luminal) membrane, where it exchanges orotate from filtrate for cytoplasmic monocarboxylate anions. Basolateral GLUT9 then provides the equilibrating exit route to plasma. GLUT9 also has an apical-trafficked isoform (GLUT9-S), which represents the original apical disposal-gate function from which the architecture evolved.

At the choroid plexus epithelium, GLUT9 is apical (CSF-facing) and URAT1 is basolateral (blood-facing) [34]. This polarity is inverted relative to the kidney and supports a different

function: forward delivery of orotate-bound material from blood into CSF, where it can reach brain tissue. At the ependyma — the multiciliated cell layer lining the ventricles — both transporters are at the apical (CSF-facing) surface [33, 34]. URAT1 here recovers orotate-bound material from CSF; basolateral GLUT9 (or another exit route) delivers recovered material to brain interstitium. The ependymal arrangement is functionally analogous to the kidney, with CSF as the filtrate compartment and brain interstitium as the recipient of recovered material.

The conjecture predicts further deployments of the same architecture at delivery interfaces serving cardiac and brain tissue — specifically, at cardiac capillary endothelium and at the cerebral capillary endothelium of the blood-brain barrier — but neither has been characterised in the published literature. These are testable predictions (Section 8).

The kinetic selectivity of URAT1 (71-fold preference for orotate over urate) and the differential trafficking of GLUT9 isoforms together provide the components for selective recovery and delivery of orotate-bound material, against the background flux of organic anions that GLUT9, operating alone, would direct out of plasma into filtrate and CSF for disposal.

What is and is not directly measured

Kinetic data above are from human URAT1; equivalent mouse URAT1 affinity for orotate has not been reported. The orotate content of monotreme and marsupial colostrum is not in the literature, and the expression of URAT1 and GLUT9 in mammary tissue is not well-characterised across mammalian species. Most consequentially, the molecular recognition of PINCs by URAT1 and GLUT9 — distinct from the recognition of free orotate — has not been measured. The argument that the pump-and-gate architecture handles PINCs as transport units rests on this assumption; we treat its experimental test as a testable prediction (Section 8).

The two-stage proposal

The molecular evidence supports a two-stage proposal for the architecture's assembly. Stage 1 (the crisis adaptation) is the deployment of GLUT9 at the basolateral surface of the gut epithelium [46], allowing absorption of PINCs from lumen to plasma down a concentration gradient — sufficient on its own to provide nursing offspring with access to maternal magnesium reserves. Stage 2 (the long-run elaboration) is the evolution of URAT1, mammalian-specific [30], paired with basolateral GLUT9 across multiple polarised epithelial and endothelial interfaces. URAT1 is the active component, using monocarboxylate exchange to move PINCs against bulk flow; GLUT9 provides the equilibrating exit. Once URAT1 exists, both recovery deployments (kidney, choroid plexus, ependyma) and elevation deployments (cardiac capillary endothelium, blood-brain barrier) become possible — the former characterised in the literature, the latter predicted by the conjecture's logic but not currently characterised (Section 8).

Section 6 — Comparative-genomic signature

The overview proposes that URAT1 arose during the mammalian stem lineage as the selective recovery component of the pump-and-gate system, and that GLUT9 is an older transporter whose ancestral disposal role provided the substrate-handling context the recovery pump was added to. The proposal predicts a specific distribution across vertebrate genomes: URAT1 should be mammalian-lineage with no non-mammalian orthologues; SLC2A9 (GLUT9) should be present and conserved across vertebrates, with the alternative-splicing architecture that produces the two trafficking isoforms a mammalian-specific addition. The available comparative-genomic data fit both predictions.

URAT1 (SLC22A12) is mammalian-specific

A systematic phylogenetic analysis of the SLC22 organic anion transporter family across vertebrate genomes places human URAT1 (SLC22A12) and its closest paralogues (OAT4 / SLC22A11, OAT7 / SLC22A9, and related apical-membrane reabsorption transporters) in a phylogenetic cluster that is absent from non-mammalian vertebrate genomes [30]. Zebrafish, examined in detail as a teleost reference, has twenty SLC22-family genes distributed across the same broad functional subgroups as the human SLC22 members, but the OAT4-7/URAT1 cluster has no zebrafish orthologue. The same observation extends to the other non-mammalian vertebrates surveyed in that analysis: amphibians, reptiles, and birds also lack the cluster.

This is a stronger result than typical sequence-divergence findings. URAT1 is not simply a divergent mammalian variant of a transporter also present in non-mammalian vertebrates. The gene cluster URAT1 belongs to is itself absent from non-mammalian genomes. Whatever URAT1 does in mammals — orotate transport at high affinity (K_m 5.2 μ M), urate transport at lower affinity (K_m 371 μ M), apical expression at the renal proximal tubule and the brain CSF interfaces — the lineage was doing for the first time in the mammalian stem group, not inheriting from earlier vertebrate machinery.

GLUT9 (SLC2A9) is conserved across vertebrates

SLC2A9 belongs to the major facilitator superfamily, GLUT subfamily of facilitative transporters, with orthologues distributed across vertebrate genomes including teleost fish, amphibians, reptiles, and birds. The gene predates the mammalian lineage by a substantial margin. The transmembrane architecture and substrate-binding residues are conserved across the vertebrate orthologues [29, 30].

The mammalian innovation in GLUT9 is the alternative-splicing architecture that generates the two trafficking isoforms (GLUT9a / GLUT9-L; GLUT9b / GLUT9-S), differing only in N-terminal cytoplasmic tails carrying differential trafficking signals [29, 31, 32]. The mouse

splice variants have parallel trafficking distinctions to the human isoforms [31]. Comparable splice variants have not, to our knowledge, been characterised in non-mammalian vertebrates. This represents an absence of published comparative work rather than a positive demonstration of absence; the splice-variant architecture in non-mammalian SLC2A9 orthologues has not been examined in the depth required to determine whether functionally analogous trafficking distinctions exist.

Conservation within mammals

Where examined, both proteins show the conservation pattern expected of a system under ongoing functional constraint. Mouse URAT1 (SLC22A12) and human URAT1 are 1:1 orthologues with conserved substrate-binding residues. The GLUT9 splice-variant architecture — alternative N-terminal exons producing isoforms differing only in cytoplasmic tail — is preserved between mouse and human. We have not exhaustively surveyed monotremes and marsupials for both genes; the available genomic data are consistent with the machinery being present across crown Mammalia in conserved form, but a systematic cross-mammal survey would provide direct evidence on this point.

Limitations of the conservation argument

Two limitations should be flagged. First, conservation arguments can establish that machinery is mammalian-lineage and under selection, but cannot pinpoint when within the lineage's history it was assembled. A system present across crown Mammalia could in principle have been put together at any point on the stem mammalian branch. The argument proposes the Carnian Pluvial Episode as the assembly window because that is when the seasonal-reproductive-timing pressure was operating and because the system's coordinated form is consistent with sustained directional selection of the kind the CPE imposes. The genomic data are consistent with this proposal but do not uniquely require it.

Second, the absence of published characterisation of splice-variant trafficking in non-mammalian vertebrate SLC2A9 orthologues is not equivalent to a demonstrated absence of such trafficking. Future comparative work could find similar trafficking distinctions in lineages we currently treat as lacking them. A systematic analysis of SLC2A9 alternative-splicing patterns and isoform localisation across non-mammalian vertebrates would either reinforce the mammalian-specificity claim or would refine it.

Section 7 — Contemporary observations consistent with the model

The overview identifies three contemporary observations consistent with the pump-and-gate model: the Kling 1978 rat-brain finding, the Smith and Schou 1979 rat plasma kinetics, and

the Aron 2025 mouse Alzheimer's-model results. This section presents each observation in detail, examines its compatibility with the model, and notes the limitations of the available evidence.

Lithium as a passenger on the magnesium-handling system

The conjecture proposes that the orotate transport machinery characterised in Section 5 evolved during the second phase of the CPE response (Section 3) under selection pressure for magnesium delivery from maternal body to offspring. Magnesium is the cation the lineage's environment was depleted in (Section 2) and the cation the orotate associations described in Paper 1 of the present series [1] would have carried.

The lithium ion has a small ionic radius (0.76 Å for Li^+ in six-coordinate form) and a hydration enthalpy in the range that permits substitution for divalent cations in some coordination contexts. Lithium can substitute for magnesium in a variety of biological binding sites, including ATP-Mg complexes and sites on several Mg-dependent enzymes [44, 45]. The conjecture proposes that lithium can also substitute for magnesium in the orotate-metal associations described in Paper 1, in which case lithium delivered as lithium orotate would enter the orotate-handling machinery as a passenger on the system that evolved for magnesium. Lithium delivered as lithium carbonate, providing no orotate, would not enter this pathway.

This proposed substitution is the bridge between the conjecture developed in Sections 2 through 5 and the contemporary observations described below. Three observations bear on whether the prediction holds.

Kling 1978 — rat brain lithium concentrations

Kling and colleagues administered equimolar doses of lithium orotate and lithium carbonate to rats and measured lithium concentrations in plasma and brain [42]. The plasma lithium kinetics were broadly comparable between the two salts. Brain lithium concentrations were approximately three-fold higher in the lithium orotate group than in the lithium carbonate group at equivalent doses. The authors did not propose a specific molecular mechanism for the differential brain delivery.

On the pump-and-gate model, the observation is consistent with lithium orotate entering the orotate-handling machinery and being delivered preferentially to the brain via the URAT1/GLUT9 expression at the choroid plexus and ependymal interfaces (Section 5). Lithium delivered as lithium carbonate, providing no orotate, would not access this delivery route and would reach the brain only via the unfacilitated routes available to free Li^+ . Comparable plasma kinetics with differential brain delivery is the qualitative pattern the model predicts.

The Kling experiment was conducted with the analytical methods and brain-tissue sampling protocols available in 1978. Replication with modern instrumentation, including spatially resolved measurements of regional brain lithium distribution, would test the prediction directly and would distinguish among possible delivery routes.

Smith and Schou 1979 — rat plasma kinetics

Smith and Schou administered lithium orotate and lithium carbonate to rats at higher doses than Kling used and measured plasma lithium concentrations [43]. Plasma lithium concentrations were comparable between the two salts at equivalent doses, with no difference of the magnitude that would have been predicted from the Kling brain result if Kling's brain-delivery mechanism operated similarly in the plasma compartment. They additionally observed that lithium clearance was slower than expected in the orotate group, which they interpreted as indicating impaired renal function in animals receiving lithium orotate.

The observation of comparable plasma kinetics with slower clearance is consistent with the pump-and-gate model under the following interpretation. URAT1 in the proximal tubule recovers orotate-associated lithium from glomerular filtrate and returns it to plasma via basolateral GLUT9, against the bulk excretion that would otherwise occur. The slower clearance is what URAT1-mediated recovery looks like at the systemic level. The observation is consistent with the proposed recovery mechanism functioning as predicted, rather than the renal impairment interpretation the paper suggested.

The original interpretation in terms of renal impairment was reasonable in the framework available in 1979. The molecular biology of URAT1 was unknown — URAT1 was not cloned until 2002 [36]. The pump-and-gate architecture was not described. Carbonate-induced renal toxicity was a recently and well-documented problem in lithium therapy practice, and slower-than-expected lithium clearance fit that pattern. The framework that would have supported the recovery-mechanism interpretation did not exist.

We propose that the Smith and Schou data, considered within the framework of Section 5, are positive evidence for the orotate-recovery mechanism rather than evidence of renal dysfunction. The slow clearance is the predicted physiological signature of URAT1 operating on orotate-bound lithium. Replication with modern methodology, with concurrent measurement of urinary orotate-lithium species, renal histology, and direct assessment of URAT1 function would clarify the position.

Aron 2025 — mouse Alzheimer's-model efficacy

Aron and colleagues reported that lithium orotate produced therapeutic effects in mouse models of Alzheimer's disease at doses where equivalent lithium carbonate did not, with no detectable toxicity over near-lifelong administration [2]. The authors additionally reported that lithium orotate evades the amyloid-binding sequestration that limits brain availability of lithium delivered as other compounds.

The differential efficacy at equivalent dose between the two salts is the qualitative pattern Kling reported in 1978 at the level of brain lithium concentrations, here observed at the level of therapeutic outcome in a model of brain pathology. Equimolar lithium orotate and lithium carbonate would be expected to deliver equivalent free Li^+ if the standard dissociation chemistry held; the Aron findings are consistent with the orotate counter-ion playing an active pharmacokinetic role of the kind the pump-and-gate model proposes.

The observation that lithium orotate evades amyloid-binding sequestration is consistent with lithium being delivered in a form that is not free Li^+ . If lithium orotate is delivered as an orotate-associated species through the URAT1/GLUT9 brain interfaces, the lithium would not be subject to the amyloid-binding behaviour characteristic of free lithium ions in tissues with amyloid deposition. The evasion observation therefore provides indirect evidence consistent with delivery in a non-free-ion form.

The Aron findings do not, on their own, distinguish among possible mechanisms for the differential effects of the two salts. Differences in absorption kinetics, plasma protein binding, or tissue distribution could in principle contribute. The specific measurements that would distinguish among these alternatives — including direct identification of orotate-associated lithium species in plasma and brain tissue, and measurement of URAT1/GLUT9 recognition of these species — have not been reported.

Combined interpretation

Considered together, the three observations describe a consistent qualitative pattern: lithium orotate behaves pharmacokinetically and pharmacologically as if the orotate counter-ion is doing active work that the standard dissociation model does not predict. The pump-and-gate model accounts for the qualitative pattern by proposing that lithium orotate enters the orotate-handling machinery as a passenger on the system that evolved for magnesium delivery. The three observations span nearly fifty years and were collected by different research groups with different experimental designs, none of which were undertaken to test the conjecture proposed here.

Note on clinical interpretation

The observations described in this section concern the pharmacokinetics of lithium delivered as lithium orotate. They do not bear directly on the established therapeutic mechanisms by which lithium carbonate acts in clinical use, which depend on free Li^+ concentrations achieved through the dosing regimens established in clinical practice. The conjecture developed in this paper proposes that lithium orotate and lithium carbonate operate through distinct pharmacokinetic pathways and are likely not interchangeable. The relationship between the two compounds as therapeutic agents is taken up in the discussion document accompanying this paper.

Section 8 — Specific testable predictions

The conjecture rests on a set of assumptions, each independently testable. We list them here grouped by the kind of work each requires, with the most decisive listed first within each group. Where direct experimental procedures are well-defined, we indicate them; where the relevant work is geological or comparative-physiological, we indicate the kind of survey that would address the question.

Chemistry

Independent replication of Paper 1's solution-chemistry findings. The companion paper of the present series [1] reports experimental evidence that orotate and metal ions can exist as long-lived stable associated forms in solution, contrary to the conventional dissociation model. Independent replication using comparable methods, ideally in laboratories with no connection to the original authors, would establish or undermine this finding. Failure of independent replication would remove the chemical basis for the molecular argument and would falsify the conjecture as a whole.

Molecular recognition

Direct measurement of URAT1 and GLUT9 affinity for orotate-metal associated forms. This is the single most decisive experiment for the conjecture. The relevant assays are kinetic measurements of URAT1 (recombinant or in suitable cell systems) and GLUT9 (likewise) using orotate-metal preparations as substrates, with comparison to free orotate and free metal ions. Affinity measurably greater for the orotate-metal associated forms than for the dissociated components would substantiate the proposed transport mechanism. Affinity comparable to or below that of the dissociated components would substantially weaken the conjecture, since the recognition of the proposed orotate-mediated species by the relevant transporters is the central biological assumption on which the pump-and-gate model depends. The measurement requires the orotate-metal preparations characterised in Paper 1 to be available in forms suitable for transporter assays; this connects directly to the chemistry prediction above.

Physiology

Mouse URAT1 kinetics for orotate. Human URAT1 affinity for orotate has been measured ($K_m \sim 5.2 \mu M$, [37]). The equivalent measurement in mouse has not, to our knowledge, been reported; mouse URAT1 is characterised in the literature only with respect to urate [39]. The Miura 2011 protocol applied to mouse URAT1 would test whether the high-affinity orotate handling characterised in human is conserved in the species most commonly used for transporter physiology and small-mammal pharmacology.

Replication of Kling 1978 and Smith & Schou 1979 with modern instrumentation. The Kling 1978 rat-brain experiment can be replicated with spatially resolved measurements of regional brain lithium distribution (mass-spectrometry imaging or comparable methodology)

following equimolar oral lithium orotate and lithium carbonate. The Smith & Schou 1979 rat plasma experiment can be replicated with concurrent measurement of urinary orotate-lithium species and renal histology distinguishing functional URAT1 activity from structural renal change. Together these replications would resolve the original 1978-1979 disagreement directly and would test the pump-and-gate model's predicted differences in brain delivery and renal handling between the two salts.

URAT1 and GLUT9 expression in mammary tissue across mammalian species. The conjecture proposes that the orotate transport machinery delivers magnesium from maternal body to offspring through proto-milk secretions. The expression of URAT1 and GLUT9 in mammary tissue is not, to our knowledge, well-characterised across mammals. Immunohistochemistry of mammary tissue from monotremes, marsupials, and a representative range of placental mammals during lactation, together with mass-spectrometry analysis of the milk these tissues produce for orotate-mediated mineral content, would provide direct evidence on the maternal-delivery component of the model.

Orotate content of monotreme and marsupial colostrum. Existing milk-orotate measurements are from cows and other ruminants [41, 40]. The phylogenetically informative comparison is with the more basal mammalian lineages. Monotreme and marsupial colostrum orotate content has not been reported. Direct measurement would test whether the orotate-mediated delivery system is detectable in the lineages closest to the phylogenetic root of crown Mammalia.

Comparative

Cross-mammal comparative expression of the orotate transport machinery. The seasonal-reproductive-timing argument (Section 3) predicts that mammals adapted to highly seasonal environments with acute spring birth-timing pressure and environmental magnesium constraint should express the orotate transport machinery more prominently than mammals in stable tropical environments or mammals without comparable mineral pressure. Specific comparisons that would test this prediction include: high-latitude versus tropical placentals within otherwise comparable taxonomic groups; echidnas (high-latitude monotremes with seasonal reproduction) versus tropical monotreme analogues; marsupials adapted to highly seasonal Australian environments versus those in less seasonal contexts. The relevant measurements are URAT1 and GLUT9 expression levels in kidney, gut, mammary tissue, and brain CSF interfaces, and orotate content of milk during the spring birth-and-lactation window.

SLC2A9 splice-variant architecture in non-mammalian vertebrates. The overview proposes that the alternative-splicing architecture producing GLUT9-L and GLUT9-S trafficking isoforms is mammalian-specific. Comparative work on this point is sparse, and the absence of published characterisation in non-mammalian vertebrates is not equivalent to demonstrated absence. A systematic analysis of SLC2A9 alternative-splicing patterns and isoform localisation across non-mammalian vertebrates — teleost fish, amphibians, reptiles,

birds — would either reinforce the mammalian-specificity claim or refine it. RNA-sequencing of the relevant tissues with attention to alternative first-exon usage, combined with isoform-specific antibody localisation in polarised epithelia, are the standard methodologies.

Geological

Direct measurement of magnesium depletion in Carnian palaeosols. Section 2 develops the argument that magnesium would have been particularly depleted from cool-temperate Gondwanan soils during the CPE on the basis of general soil-chemistry principles, but direct quantitative measurements have not been located in the literature available to us. Whole-rock geochemistry of palaeosol horizons, with comparison of upper (leached) and lower (less weathered) profile positions and application of standard weathering indices (Chemical Index of Alteration, base-loss indices, Mg/Al and Mg/Ti ratios), can establish leaching intensity across the CPE interval. The most informative target is continental palaeosol successions in cool-temperate Gondwanan basins where the Onslow-province flora is preserved — for example in southern South American, southern African, Indian, and Australian successions. Selection of palaeosols not deeply buried or hydrothermally altered is critical to avoid diagenetic overprint. Demonstration of preferential Mg loss across the CPE interval would reinforce the environmental-context argument; absence of such loss would constrain it.

Magnesium content of Onslow-flora plant tissues and associated invertebrate fauna. The argument that the Carnian magnesium deficit was transmitted through the food chain to the small endotherms of Section 3 rests on the inference that low-soil-Mg plants and the invertebrates feeding on them carried correspondingly low Mg. Direct measurement of Mg content in fossilised Onslow-flora plant material (where preservation permits) and in coprolites attributable to the relevant invertebrate or vertebrate fauna would test this inference.

Falsification summary

The conjecture as a whole would be falsified, in descending order of decisiveness, by:

- Failure of independent replication of Paper 1's solution-chemistry findings, which would remove the chemical foundation;
- Demonstration that URAT1 and GLUT9 do not preferentially recognise orotate-metal associated forms over free orotate and free metal ions, which would remove the molecular-recognition assumption;
- Demonstration that the orotate transport machinery is not phylogenetically restricted in the manner predicted (e.g. discovery of URAT1 orthologues in non-mammalian vertebrates with equivalent functional architecture, or demonstration that the GLUT9 splice variant trafficking is shared broadly across vertebrates).

Section 9 — Discussion

The conjecture this paper develops carries a small number of implications that warrant brief discussion before closing.

The 1979 lithium-orotate literature

The treatment of Smith & Schou 1979 in Section 7 places their observations within the pump-and-gate framework and reads the slow lithium clearance they reported as positive evidence for URAT1-mediated recovery rather than evidence of renal impairment. This reading implies that the prevailing interpretation of the 1979 paper, which has discouraged investigation of orotate-mediated lithium delivery for nearly fifty years, deserves to be revisited. The data Smith and Schou collected are useful and remain so. The conclusion they drew from those data was reasonable in the framework available to them, in which URAT1 had not yet been cloned and the pump-and-gate architecture had not been described, but is not the only reading the data support. Replication with modern methodology, as proposed in Section 8, would resolve the question directly.

Clinical implications

We are not proposing any change to clinical practice. Therapeutic lithium carbonate has a substantial and proven evidence base and provides important benefits for specific severe medical conditions; decisions about its use are matters for clinicians and patients working within that well-established evidence base. This is not a caveat but a statement of fact that we endorse.

The conjecture proposes that lithium orotate and lithium carbonate operate through distinct pharmacokinetic pathways and are therefore not directly interchangeable. The form in which lithium reaches its targets differs between the two salts. Lithium carbonate at therapeutic doses may produce the high free- Li^+ plasma concentrations on which the established clinical effect depends. Lithium orotate, on the present account, delivers lithium-orotate associations to the brain via the URAT1 / GLUT9 recovery machinery, in much smaller quantities and in a different chemical form. We see no clear link between the low-concentration orotate-bound effects discussed in this paper and the high-concentration free-ion effects on which clinical lithium therapy depends.

The relationship between lithium orotate and lithium carbonate as therapeutic agents is a careful clinical research question for qualified investigators working within established protocols.

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References

1. Hall A. D. Hysteresis of Origin in Aqueous Lithium Orotate: Evidence for a Long-Lived Ionic Cluster. ChemRxiv. 2026.
<https://doi.org/10.26434/chemrxiv.15002343/v1>
2. Aron L, Ngian ZK, Qiu C, Choi J, Liang M, Drake DM, Hamplova SE, Lacey EK, Roche P, Yuan M, Hazaveh SS, Lee EA, Bennett DA, Yankner BA. Lithium deficiency and the onset of Alzheimer's disease. *Nature* 645, 712–721 (2025).
<https://doi.org/10.1038/s41586-025-09335-x>
3. Simms MJ, Ruffell AH. Synchronicity of climatic change and extinctions in the Late Triassic. *Geology*. 1989;17(3):265-268.
4. Dal Corso J, Mietto P, Newton RJ, Pancost RD, Preto N, Roghi G, Wignall PB. Discovery of a major negative $\delta^{13}\text{C}$ spike in the Carnian (Late Triassic) linked to the eruption of Wrangellia flood basalts. *Geology*. 2012;40(1):79-82.
5. Dal Corso J et al. Multiple negative carbon-isotope excursions during the Carnian Pluvial Episode (Late Triassic). *Earth-Science Reviews*, Volume 185, 2018, Pages 732-750.
6. Dal Corso J, Bernardi M, Sun Y, Song H, Seyfullah LJ, Preto N, et al. Extinction and dawn of the modern world in the Carnian (Late Triassic). *Nat Rev Earth Environ*. 2020;1:333-348.
7. Miller CS, Peterse F, da Silva AC, Baranyi V, Reichart GJ, Kürschner WM. Astronomical age constraints and extinction mechanisms of the Late Triassic Carnian crisis. *Sci Rep*. 2017 May 31;7(1):2557.
8. J. Lu, P. Zhang, J. Dal Corso, M. Yang, P.B. Wignall, S.E. Greene, L. Shao, D. Lyu, & J. Hilton. Volcanically driven lacustrine ecosystem changes during the Carnian Pluvial Episode (Late Triassic), *Proc. Natl. Acad. Sci. U.S.A.* 118 (40) e2109895118, (2021).
9. Greene AR, Scoates JS, Weis D, Katvala EC, Israel S, Nixon GT. The architecture of oceanic plateaus revealed by the volcanic stratigraphy of the accreted Wrangellia oceanic plateau. *Geosphere*. 2010;6(1):47-73.

10. Mancuso, Adriana & Benavente, Cecilia & Irmis, Randall & Mundil, Roland. (2020). Evidence for the Carnian Pluvial Episode in Gondwana: New multiproxy climate records and their bearing on early dinosaur diversification. *Gondwana Research*. 86.
12. Zhang, P, Yang, M, Lu, J, Jiang, Z, Vervoort, P, Zhou, K, Xu, X, Chen, H, Wang, Y, He, Z, Bian, X, Shao, L & Hilton, J 2024, 'Four volcanically driven climatic perturbations led to enhanced continental weathering during the Late Triassic Carnian Pluvial Episode', *Earth and Planetary Science Letters*, vol. 626, 118517
13. Scotese, Christopher. (2014). Atlas of Jurassic Paleogeographic Maps, PALEOMAP Atlas for ArcGIS, volume 3, The Jurassic and Triassic, Maps 32 - 42, Mollweide Projection, PALEOMAP Project, Evanston, IL.. 10.13140/2.1.4850.4321.
14. Dolby JH, Balme BE. Triassic palynology of the Carnarvon Basin, Western Australia. *Review of Palaeobotany and Palynology*, 1977;22:105-168
15. Césari, S., Colombi, C. A new Late Triassic phytogeographical scenario in westernmost Gondwana. *Nat Commun* 4, 1889 (2013). <https://doi.org/10.1038/ncomms2917>
16. Bodnar, J., Coturel, E. P., Falco, J. I., & Beltrán, M. (2021). An updated scenario for the end-Permian crisis and the recovery of Triassic land flora in Argentina. *Historical Biology*, 33(12), 3654–3672.
17. Seyfullah, Leyla & Roghi, Guido & Dal Corso, Jacopo & Schmidt, Alexander. (2018). The Carnian Pluvial Episode and the first global appearance of amber. *Journal of the Geological Society*. 175. jgs2017-143. 10.1144/jgs2017-143.
18. Botha J, Chinsamy A. Growth patterns deduced from the bone histology of the cynodonts *Diademodon* and *Cynognathus*. *J Vertebr Paleontol*. 2000;20(4):705-711.
19. Botha J, Chinsamy A. Growth and life habits of the Triassic cynodont *Trirachodon*, inferred from bone histology. *Acta Palaeontol Pol*. 2004;49(4):619-627.
20. Rey K, Amiot R, Fourel F, Abdala F, Fluteau F, Jalil NE, Liu J, Rubidge BS, Smith RM, Steyer JS, Viglietti PA, Wang X, Lécuyer C. Oxygen isotopes suggest elevated thermometabolism within multiple Permo-Triassic therapsid clades. *Elife*. 2017 Jul 18;6:e28589.
21. Newham E, Gill PG, Brewer P, Benton MJ, Fernandez V, Gostling NJ, et al. Reptile-like physiology in Early Jurassic stem-mammals. *Nat Commun*. 2020;11:5121.
22. Zhang Z. T. , Sun Y. D., Wignall P. B., Fu J. L. , Li H. X. , Wang M. Y. , Lai X. L. Conodont size reduction and diversity losses during the Carnian Humid Episode in SW China. *Journal of the Geological Society* 2018; 175 (6): 1027–1031.
23. Bonaparte JF, Martinelli AG, Schultz CL. New information on *Brasilodon* and *Brasilitherium* (Cynodontia, Probainognathia) from the Late Triassic of Southern Brazil. *Revista Brasileira De Paleontologia - REV BRAS PALEONTOLOGIA*. 8. 10.4072/rbp.2005.1.03.

24. Cabreira, S.F., Schultz, C.L., da Silva, L.R., Lora, L.H.P., Pakulski, C. & do Rêgo, R.C.B. et al. (2022) Diphyodont tooth replacement of *Brasilodon*—A Late Triassic eucynodont that challenges the time of origin of mammals. *Journal of Anatomy*, 00, 1–17.
25. Guignard ML, Martinelli AG, Soares MB. The postcranial anatomy of *Brasilodon quadrangularis* and the acquisition of mammaliaform traits among non-mammaliaform cynodonts. *PLoS One*. 2019 May 10;14(5):e0216672.
26. Oftedal OT. The mammary gland and its origin during synapsid evolution. *J Mammary Gland Biol Neoplasia*. 2002;7(3):225-252.
27. Oftedal OT. The origin of lactation as a water source for parchment-shelled eggs. *J Mammary Gland Biol Neoplasia*. 2002;7(3):253-266.
28. Oftedal OT. The evolution of milk secretion and its ancient origins. *Animal*. 2012;6(3):355-368.
29. Augustin R, Carayannopoulos MO, Dowd LO, Phay JE, Moley JF, Moley KH. Identification and characterization of human glucose transporter-like protein-9 (GLUT9): alternative splicing alters trafficking. *J Biol Chem*. 2004;279(16):16229-16236.
30. Mihaljevic I, Popovic M, Zaja R, Smital T. Phylogenetic, syntenic, and tissue expression analysis of *slc22* genes in zebrafish (*Danio rerio*). *BMC Genomics*. 2016 Aug 12;17(1):626.
31. Keembiyehetty C, Augustin R, Carayannopoulos MO, Steer S, Manolescu A, Cheeseman CI, Moley KH. Mouse glucose transporter 9 splice variants are expressed in adult liver and kidney and are up-regulated in diabetes. *Mol Endocrinol*. 2006;20(3):686-697.
32. Kimura T, Takahashi M, Yan K, Sakurai H (2014) Expression of SLC2A9 Isoforms in the Kidney and Their Localization in Polarized Epithelial Cells. *PLOS ONE* 9(1): e84996.
33. Tomioka NH, Nakamura M, Doshi M et al. Ependymal cells of the mouse brain express urate transporter 1 (URAT1). *Fluids Barriers CNS* 10, 31 (2013).
34. Tomioka NH, Tamura Y, Takada T, Shibata S, Suzuki H, Uchida S, Hosoyamada M. Immunohistochemical and in situ hybridization study of urate transporters GLUT9/URATv1, ABCG2, and URAT1 in the murine brain. *Fluids Barriers CNS*. 2016 Dec 12;13(1):22.
35. Tan PK, Farrar JE, Gaucher EA, Miner JN. Coevolution of URAT1 and Uricase during Primate Evolution: Implications for Serum Urate Homeostasis and Gout. *Mol Biol Evol*. 2016 Sep;33(9):2193-200. doi: 10.1093/molbev/msw116. Epub 2016 Jun 26. PMID: 27352852; PMCID: PMC4989112.
36. Enomoto A, Kimura H, Chairoungdua A, Shigeta Y, Jutabha P, Cha SH, Hosoyamada M, Takeda M, Sekine T, Igarashi T, Matsuo H, Kikuchi Y, Oda T, Ichida K, Hosoya T, Shimokata K, Niwa T, Kanai Y, Endou H. Molecular identification of a renal urate anion exchanger that regulates blood urate levels. *Nature*. 2002 May 23;417(6887):447-52. doi: 10.1038/nature742. Epub 2002 Apr 14. PMID: 12024214.

37. Miura D, Anzai N, Jutabha P, Chanluang S, He X, Fukutomi T, Endou H. Human urate transporter 1 (hURAT1) mediates the transport of orotate. *J Physiol Sci.* 2011 May;61(3):253-7. doi: 10.1007/s12576-011-0136-0. Epub 2011 Feb 25. PMID: 21350910; PMCID: PMC10717395.
38. Yu, Z., Hu, T., Su, J. et al. Molecular mechanism of drug inhibition of URAT1. *Nat Commun* 16, 6551 (2025).
39. Hosoyamada M, Ichida K, Enomoto A, Hosoya T, Endou H. Function and localization of urate transporter 1 in mouse kidney. *J Am Soc Nephrol.* 2004 Feb;15(2):261-8.
40. Indyk HE, Woollard DC. Determination of orotic acid, uric acid, and creatinine in milk by liquid chromatography. *J AOAC Int.* 2004 Jan-Feb;87(1):116-22.
41. Robinson J. Bovine Milk Orotic Acid: Variability and Significance for Human Nutrition, *Journal of Dairy Science*, 1980;63, 865-871
42. Kling MA, Manowitz P, Pollack IW. Rat brain and serum lithium concentrations after acute injections of lithium carbonate and orotate. *J Pharm Pharmacol.* 1978 Jun;30(6):368-70. doi: 10.1111/j.2042-7158.1978.tb13258.x. PMID: 26768.
43. Smith DF, Schou M. Kidney function and lithium concentrations of rats given an injection of lithium orotate or lithium carbonate. *J Pharm Pharmacol.* 1979 Mar;31(3):161-3. doi: 10.1111/j.2042-7158.1979.tb13461.x. PMID: 34690.
44. Mota de Freitas D, Castro MM, Geraldes CF. Is competition between Li^+ and Mg^{2+} the underlying theme in the proposed mechanisms for the pharmacological action of lithium salts in bipolar disorder? *Acc Chem Res.* 2006 Apr;39(4):283-91.
45. Mota de Freitas, Duarte & Leversson, Brian & Goossens, Jesse. (2016). Lithium in Medicine: Mechanisms of Action. 10.1007/978-3-319-21756-7_15.
46. DeBosch, B., Kluth, O., Fujiwara, H. et al. Early-onset metabolic syndrome in mice lacking the intestinal uric acid transporter SLC2A9. *Nat Commun* 5, 4642 (2014).
47. Ding X, Peng C, Li S, Li M, Li X, Wang Z, Li Y, Wang X, Li J, Wu J. Chicken serum uric acid level is regulated by glucose transporter 9. *Anim Biosci.* 2021;34(4):670-679.
48. Bibee KP, Augustin R, Gazit V, Moley KH. The apical sorting signal for human GLUT9b resides in the N-terminus. *Mol Cell Biochem.* 2013 Apr;376(1-2):163-173.
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