

Fig. 2 Biological assay for determining trialkyl lead concentrations in evaporated rainwater samples. Pork brain tubulin, prepared as in ref. 2 but not separated from microtubule-associated proteins (1-ml samples, 15 μM , in a quartz cuvette, in buffer containing 0.1 M PIPES, 1 mM MgCl_2 , 0.1 mM EDTA and 1 mM EGTA, pH 6.9), were polymerized by adding GTP to a final concentration of 1 mM and warming the samples from 0 to 37 °C. Polymerization was monitored by absorbance at 350 nm on an Aminco DW 2 spectrophotometer. Aliquots of evaporated rainwater samples (10–50 μl) were added to the tubulin immediately before polymerization began. For calibration, Et_3PbCl (Ventron, Karlsruhe; purified by column chromatography on silica gel with benzene containing 5% acetic acid) was used. During evaporation of the rainwater samples (~1:200), total lead content decreased due to the adsorption of R_3PbX to the glass surface, probably as reaction (1a) formed in anhydrous conditions. Repeated washings of the flasks recovered trialkyl lead with 90% yield. The evaporated samples were assayed in at least two dilutions corresponding to concentrations between 2.5 and 7.5 μM Et_3PbCl . Each of the solid bars in Fig. 1 represents a value interpolated from at least two of the inhibition curves shown here.

degradation of R_3PbX to PbX_2 certainly occurs, but must be slower than generation of R_3PbX from R_4Pb .

Most of the Feldberg samples containing R_3PbX in concentrations $\geq 1 \times 10^{-7}$ M were collected in typical weather conditions, that is, with wind from the south-west. However, the number of samples was limited, and wind from the south-west prevails on most days (70%) with precipitation. Therefore, the answer to the question of whether wind from this direction is concomitant with high organolead pollution in rain, will require a longer period of observation.

The analytical procedure recently described¹³ does not differentiate between the various possible species of R_3PbX such as Et_3PbX , Et_2MePbX , EtMe_2PbX and Me_3PbX . These compounds, however, differ in their cytotoxic activity, as can be shown in experiments with algae¹⁴, in which Et_3PbX is considerably more toxic than Me_3PbX . Accordingly, the measurement of organolead concentration will provide reliable information on cytotoxicity only in cases where the composition of the mixture is known. On the other hand, considerable evidence has accumulated suggesting that antimicrotubular activity is closely related, or even identical, to cytotoxicity. Such a correlation has been shown for mammalian cells^{2,4} as well as for plant cells such as algae¹⁴. Similarly, the decrease of the mitotic index, as observed in the tissue of onion^{15,16} (*Allium cepa*), is most easily explained by assuming a disturbance of the microtubular system. Therefore, measuring the antimicrotubular activity is thought to give the most reliable information on the ecological menace residing in a lead-polluted rain sample.

The highest concentrations of R_3PbX determined in rainwater were only three times lower than the concentration of Et_3PbCl that is toxic for neuroblastoma or soybean cells. In this context, it is interesting that needles of coniferous trees, such as fir and spruce, accumulate Et_3PbCl several-fold from dilute aqueous solutions¹. For example, 1 g of fresh plant tissue incorporated 1.6 μg of Et_3PbCl from a 0.3 μM solution within minutes. Provided the toxin is freely distributed between the extracellular and intracellular space, the concentration of Et_3PbCl in the

aqueous compartments of the plant tissue may rise to $>1 \mu\text{M}$, that is, to potentially phytotoxic concentrations.

We thank the staff of the Meteorological Station on top of the Feldberg and the community of Loffenau for their collaboration, and Dr K. H. Doenges for the gift of tubulin.

Received 17 May; accepted 12 August 1985.

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Archosaur ankles and the relationships of the thecodontian and dinosaurian reptiles

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Several attempts have been made to outline the early evolution and radiation of the archosaurs through a study of the morphology and function of their ankle joints^{1–6} (Figs 1a, 2). Here we present new interpretations of the evidence which show that several aspects of archosaur ankle anatomy have been misunderstood, and we re-state the case for the 'advanced mesotarsal' (AM) ankle of the dinosaurs being derived from an intermediate stage and not directly from the 'primitive mesotarsal' ankle of a proterosuchid thecodontian (Fig. 1b). Two modifications to the original scheme² are required. First, that *Euparkeria*-like ankles are basically 'proterosuchian', digitigrade and unlikely to be ancestral to any other form; the term 'modified primitive mesotarsal' is proposed to describe this pattern of ankle. The second modification is to recognize that no 'advanced mesotarsal (reversed)' ankle² has been described to date, and that previous allocations of forms to this type have been in error. Therefore, all forms with the AM ankle (Fig. 2) probably share a common ancestry. The 'advanced mesotarsal (normal)' ankle seems to be present in all known dinosaurs. The 'crocodile reversed' ankle is at present known only in the Ornithosuchidae². This supports earlier suggestions that the dinosaurs are a monophyletic group^{7–9}, a view not generally accepted². The character state used here to define the dinosaurs is acquisition of the 'fully improved' gait, exemplified by the AM ankle¹⁰.

At the earliest level of organization in the archosaurs, in the thecodontian Proterosuchidae, the astragalus and calcaneum met in two pairs of articulation facets separated by a perforating foramen. The ankle hinge was mesotarsal, and the term primitive mesotarsal was used to describe this condition (Fig. 2, PM). Cruickshank² proposed that one or other of the complementary facets had later been elaborated into distinct but analogous rotating joints, and used the terms crocodile normal (CN) and crocodile reversed (CR) as suggested by Chatterjee¹¹ to describe these two conditions. In the CN state, a process on the astragalus

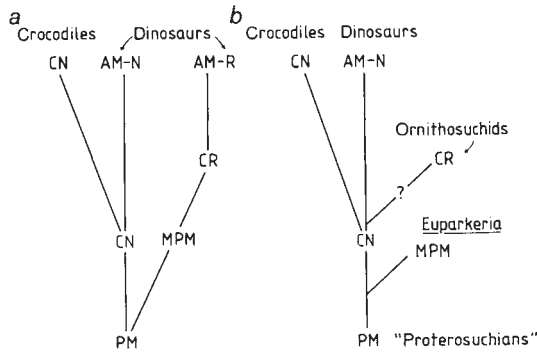


Fig. 1 *a*, Relationship of archosaurs by ankle pattern as suggested by Cruickshank². *b*, Revised relationships of archosaurs by ankle pattern as suggested in the present paper. PM, primitive mesotarsal; CN, crocodile normal; AM, advanced mesotarsal; AM-N, advanced mesotarsal (normal); AM-R, advanced mesotarsal (reversed); MPM, modified primitive mesotarsal; CR, crocodile reversed.

fits into a recess formed on the calcaneum, in front of and lateral to a 'tongue' which helps to stabilize the rotating joint (Fig. 2*a*, CN). The calcaneum grows a tuber calcis which points to the rear of the foot, a structure not seen in primitive archosaurs. This joint corresponds to the distal pair of facets joining astragalus and calcaneum in the earliest archosaurs (Fig. 2, CN). In the CR condition, the calcaneum develops a medial process which comes to lie in a hemicylindrical recess on the distal face of the astragalus. This joint corresponds to the proximal articulation facets of the primitive form. Once again a tuber calcis is formed (Fig. 2, CR). In both cases the other pairs of facets fail to make contact with each other and therefore the foramen is not enclosed; in each case the joint is intra-tarsal (cruro-tarsal).

There was a radiation of thecodontian lineages in the early to middle Triassic, the ankles of which were based on the proterosuchid PM ankle joint and which we call here modified primitive mesotarsal. This type of ankle is seen in the families Euparkeriidae, Erythrosuchidae and Proterochampsidae, none

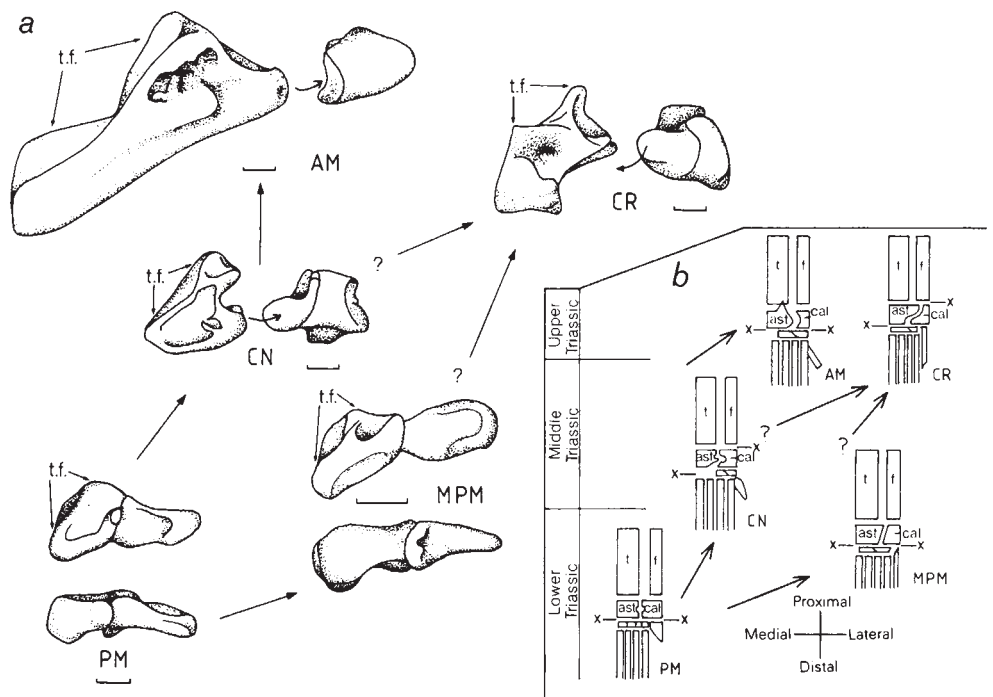
of which we believe had any descendants (Fig. 2, MPM). On the other hand, the major radiation that descended from the Proterosuchidae includes the crocodiles and all the dinosaurs, all of which possess a CN pattern of ankle (Fig. 2, CN). An analogous CR ankle is seen only in the Ornithosuchidae of the Upper Triassic (Fig. 2). Elaborating on the descriptions of these crocoid ankles, Cruickshank² suggested that several of the dinosaur lineages had CN ankles and postulated that others had CR ankles.

Three locomotory stages of the archosaurs⁶⁻¹² could be linked to the three morphological stages of ankle and foot. The earliest, present in the Proterosuchidae, was sprawling and PM in pattern. The intermediate condition was seen in the semi-erect CN and CR types, and the ultimate, fully erect (AM) type was seen only in the dinosaurs. In the original scheme² there were two AM ankles, AM-N (for CN-derived types) and AM-R (for the complementary forms)^{2,5}. The MPM type was believed then to fit in evolutionary terms between PM and CR ankles² (Fig. 1*a*).

The evolution of the AM-N ankle joint has been described in detail elsewhere^{2,13,14} and accepted by Chatterjee in a recent review⁵, thus confirming the apparent natural progression of ankle types from PM through CN to AM-N (Figs 1*b*, 2, AM). It is the status of the analogous AM-R ankle which is of importance here, as the resolution of this pattern of ankle joint has important implications for archosaur evolution in general and the dinosaurs in particular. If, as has been suggested^{2,5}, there are two patterns of AM archosaur ankle, the dinosaurs cannot be a monophyletic group, because the two patterns of ankle are not capable of changing, the one into the other, except in very special conditions^{2,4,5}.

Two taxa are particularly important in this study, *Euparkeria*^{2,15} and *Riojasuchus*^{2,16}. The anatomy of the ankle of *Euparkeria* is superficially what might be expected in an early example of a CR form. The calcaneum fits into a recess on the distal surface of the astragalus, and the perforating foramen is eliminated. However, the calcaneum retains the medial, posterior pyramid and the lateral tuber of the same pattern as in *Proterosuchus* and earlier forms². In this respect the illustration in Brinkman⁴ is at fault, for he gives the impression of a 'backwardly' pointing tuber calcis, whereas it should be laterally directed (ref. 2, Figs 6-8; ref. 15, Fig. 13). In *Euparkeria*, with

Fig. 2 *a*, Astragalus and calcaneum from left ankles of: *Proterosuchus* (PM), anterior (upper) and distal (lower) views; *Neoaeosauroides* (CN), astragalus in anterior view, calcaneum rotated to show recess (arrowed) for reception of astragalar 'peg' (the medially pointing, spoon-shaped protuberance is the 'tongue', which stabilizes the calcaneum when rotating on the astragalus; see text); prosauropod gen. et sp. indet. (AM), detail as for CN; *Chanaresuchus* (MPM), detail as for PM; *Riojasuchus* (CR), calcaneum rotated to show 'peg'. *b*, Diagrammatic representations of the left limbs of the above taxa, as lettered, with timescale. All illustrations in *a* are redrawn from originals used in ref. 2 and have the tibial facets on the astragalus orientated in the same plane. Scale bar represents 1 cm in all cases. *Chanaresuchus* ankle (MPM) reproduced here for closer comparison with the others, as it is nearer their size than that of *Euparkeria*. ast, astragalus; cal, calcaneum; f, fibula; t, tibia; t.f., tibial facet; x-x, axes of ankle flexure; heavy arrows indicate lines of possible relationships.



a laterally directed tuber, any movement between astragalus and calcaneum would involve the calcaneum rotating on the axis of the posterior pyramid, in a proximo-distal direction; such movement would, however, be only minimal because of the stabilizing effects of the fibula proximally, and of the distal tarsals. Moreover, movement here is functionally unnecessary if it is assumed that *Euparkeria* was facultatively digitigrade¹⁵. Thus, *Euparkeria* has an ankle only slightly modified from the PM condition. The other forms with MPM ankles are *Erythrosuchus*, *Chanaresuchus* and their relatives¹⁷⁻¹⁹ which, along with *Euparkeria*, may represent an entirely separate radiation of early archosaurs (Figs 1b, MPM).

The CR ankle of *Riojasuchus* (and by implication, of the Ornithosuchidae in general) is structurally and functionally superficially similar to the CN type. However, the calcaneum is placed in a horizontal, hemicylindrical recess on the distal face of the astragalus. There is a strong possibility that mechanical advantage on the tuber calcis could only be achieved with the foot in a digitigrade pose, as is indicated in the figure reconstruction of Bonaparte¹⁶, and unlike the modern crocodile. We believe it is unlikely that the antero-posterior-deepened calcaneum-astragalus joint in the MPM ankle could be elaborated into the CR condition, although Brinkman⁴ has described an evolutionary pathway which can derive the CR ankle from a CN ancestor. We disagree with this solution, as it is difficult to match homologies of the detailed structures in both types. The origin of the CR ankle must remain obscure for the present, nor do we believe that any convincing AM-R ankle has been described to date. The difficulty in this respect is that many of the AM ankles described in the literature^{2-5,13,14,20} have interlocking processes arising from both calcaneum and astragalus. Therefore, if a process arising from the calcaneum seems larger than any arising from the astragalus, the temptation would be to regard it as representing the CR condition. This arbitrary technique does not identify true homologies and can be refuted in the descriptions of the ornithischian and coelurosaurian ankles given by Cruickshank, who notes² that in the former "The astragalar-calcaneal joint is a series of interlocking processes...". In the coelurosaur ankle a less obvious but similar situation was also illustrated (ref. 2, Fig. 18). These two ankles are of AM-N type, and it thus follows that any ankle of AM morphology which has the calcaneum fitting up against the astragalus laterally must be of CN derivation. Therefore, the assignment of *Vulcanodon*, and by association the sauropods, to the CR condition is wrong^{13,14} and, whatever the relationship of the Ornithosuchidae to other groups, the Carnosauria are probably not of AM-R type either². The ankle of *Allosaurus*²⁰ is essentially similar to that of *Syntarsus*². Although the astragali and calcanea of dinosaurs show a variable pattern of pegs and sockets, these do not function in any way like those of CN and CR ankles. The shared similarities of all AM ankles are so overwhelming that we must assume that this pattern arose once only. The dinosaurs that have been described as having AM-R ankles are really of CN-derived type. If the AM-R ankle existed, it should have the calcaneum-astragalus relationship illustrated by Brinkman (ref. 4, Fig. 8D), with the calcaneum fitting into a notch or recess on the distal face of the astragalus, and not fitting into a recess on the lateral side of the astragalus as is implied elsewhere^{2,5} (Fig. 2, CR). In the CR ankle the calcaneal articulation is distal, as in *Riojasuchus*^{2,16}, and not lateral. Any AM-R ankle must also have the same relationship of astragalus and calcaneum.

We thank several colleagues for reviewing the draft manuscript, particularly Drs Alec Panchen and Alan Charig, and Elizabeth Lawson for drawing the figures.

Received 13 June; accepted 6 September 1985.

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Glutamate stimulates inositol phosphate formation in striatal neurones

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The major excitatory amino acids, glutamate (Glu) and aspartate (Asp), are thought to act at three receptor subtypes¹ in the mammalian central nervous system (CNS). These are termed quisqualate (QA), *N*-methyl-D-aspartate (NMDA) and kainate (KA) receptors according to the specific agonist properties of these compounds revealed by electrophysiological studies^{1,2}. Although Glu has been shown to stimulate cyclic GMP formation in brain slices³, direct regulation of second messenger systems (cyclic AMP, Ca²⁺ or inositol phosphates) subsequent to activation of excitatory amino-acid receptors, has not been extensively studied. Here we demonstrate that in striatal neurones, excitatory amino acids, but not inhibitory or non-neuroactive amino acids, induce a three- to fourfold increase in inositol mono-, di- and triphosphate (IP₁, IP₂, IP₃) formation with the relative potency QA > Glu > NMDA, KA. The Glu-evoked formation of inositol phosphates appears to result principally from actions at QA as well as NMDA receptors on striatal neurones. Our results suggest that excitatory amino acids stimulate inositol phosphate formation directly, rather than indirectly by the evoked release and subsequent actions of adenosine⁴ or acetylcholine⁵.

All experiments were performed on striatal neurones in primary culture, devoid of non-neuronal cell types. The cells were generated from fetal mouse brain and these neuronal cultures provide a good model for the examination of neurotransmitter actions on striatal signal transduction mechanisms⁶. Neurones were raised in the presence of 5 µCi ml⁻¹ ³H-inositol. After 11-14 days *in vitro*, cells were washed, exposed to 10 mM Li⁺ for 10 min to block IP degradation⁷, and putative amino acid neurotransmitters were added for 30 min. The formation of inositol phosphates was followed by sequential chromatography on Dowex-formate columns^{8,18} and liquid scintillation spectrometry.

All putative excitatory amino acids tested evoked increases in inositol phosphate formation (Fig. 1), whereas inhibitory amino-acid transmitter candidates, such as γ-aminobutyric acid (GABA), taurine and hypotaurine, were without effect. Other amino acids with little or no neuroactivity (valine, isoleucine, serine, threonine, methionine, arginine, histidine, tyrosine, tryptophan and proline) did not stimulate inositol phosphate formation. The exposure of striatal neurones to various excitatory amino acids resulted in a dose-dependent and saturable increase in inositol phosphate accumulation (Fig. 2); Glu stimulated inositol phosphate production three- to fourfold; half-maximal

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