

A Molecular Model for the Evolution of Endothermy in the Theropod-Bird Lineage

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ABSTRACT Ectothermy is a primitive state; therefore, a shared common ancestor of crocodiles, dinosaurs, and birds was at some point ectothermic. Birds, the extant descendants of the dinosaurs, are endothermic. Neither the metabolic transition within this lineage nor the place the dinosaurs held along the ectothermic-endothermic continuum is defined. This paper presents a conceptual model for the evolution of endothermy in the theropod-bird lineage. It is recognized that other animals (some fish, insects, etc.) are functionally endothermic. However, endothermy in other clades is beyond the scope of this paper, and we address the onset of endothermy in only the theropod/bird clade. The model begins with simple changes in a single gene of a common ancestor, and it includes a series of concomitant physiological and morphological changes, beginning perhaps as early as the first archosaurian common ancestor of dinosaurs and crocodiles. These changes continued to accumulate within the theropod-avian lineage, were maintained and refined through selective forces, and culminated in extant birds. Metabolic convergence or homoplasy is evident in the inherent differences between the endothermy of mammals and the endothermy of extant birds. The strength and usefulness of this model lie in the phylogenetic, genetic, evolutionary, and adaptive plausibility of each of the suggested developmental steps toward endothermy. The model, although conceptual in nature, relies on an extensive knowledge base developed by numerous workers in each of these areas. In addition, the model integrates known genetic, metabolic, and developmental aspects of extant taxa that phylogenetically bracket theropod dinosaurs for comparison with information derived from the fossil record of related extinct taxa. *J. Exp. Zool. (Mol. Dev. Evol.)* 291:317-338, 2001. © 2001 Wiley-Liss, Inc.

Metabolic strategies employed by dinosaurs have been the subject of much speculation. Implicit in Richard Owen's classification of dinosaurs as reptiles was an assumption of ectothermic lifestyles for these animals (Desmond, '79). Hence, they were portrayed in art and science as cold-blooded and slow moving, despite Owen's identification of characteristics suggestive of a more active lifestyle (Desmond, '79). In the 1970s, the pendulum swung the other way, with some researchers attributing activity patterns, growth rates, and consequently metabolic rates to dinosaurs equal to or even greater than those of extant endotherms, the mammals and birds (e.g., Bakker and Galton, '74). Since patterns of bone deposition may be an indicator of metabolic strategies, histological data were used to suggest that dinosaurs employed a spectrum of growth strategies (Ricqlès, '74, '80, '92; Chinsamy, '90, '93; Chinsamy et al., '95; Reid, '81, '84a, '84b, '87, '90, '97c). With increasing evidence that the most likely ancestor of birds was a theropod dinosaur,

the question of dinosaur metabolism has become the focus of much debate and intense research. However, hypotheses regarding acquisition of a physiological trait such as endothermy are limited by interpretations of the fossilized remains of extinct taxa.

Cladistic analysis identifies over 120 shared derived characters supporting the ancestry of birds within theropod dinosaurs (Gauthier, '86; Chiappe, '95; Holtz, '96; Currie, '97; Sereno, '97, '99; Forster et al., '98), and crocodylians are the closest living sister taxon to both (Fig. 1). Because the interrelationships of the Archosauria are well supported, phylogenetic bracketing, or the use of characters possessed by the two extant sister taxa of non-avian dinosaurs (crocodiles and birds), may be

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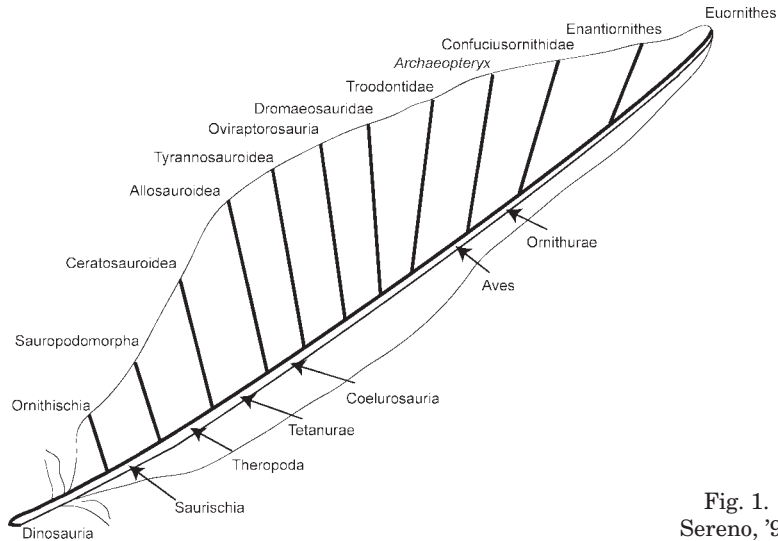


Fig. 1. Cladogram of theropod-bird lineage. Modified from Sereno, '99.

used to infer or compare physiological characteristics of extinct theropods (Bryant and Russell, '92; Witmer, '95).

Conversely, the 225 million years of independent evolution between crocodiles and birds since their divergence in the Triassic have allowed extensive modifications in each clade. Therefore, it cannot be assumed that either extant crocodiles or birds retain the physiological attributes of their most recent common ancestor (Gans, '78; Pough, '80).

Evolutionarily significant novelties initially arise by alterations in the genome and are maintained by selection acting upon the resulting phenotypic changes. Selection can act only when genetic mutations result in phenotypic expressions. However, there are no specific genes for endothermy in the same sense as having genes for eye color. Rather, endothermy appears to be an "emergent" trait, deriving from higher-level organismal integration. Nonetheless, certain genetically determined cellular and molecular traits are tightly linked to higher metabolic regimes. For example, all living endotherms, regardless of phylogenetic affinity, have a ten-fold increase in mitochondrial membrane surface area within tissues over similarly sized extant ectotherms (Bennett, '72; Ricqlès, '75; Ruben et al., '95). Cellular metabolic reactions are regulated by enzymes located on these membrane surfaces, and greater membrane surface area correlates directly with higher levels of energy released per unit body mass (Else and Hulbert, '81). Consequently, endotherms require a ten-fold or higher increase per unit body mass in food intake and oxygen consumption over ectotherms (Pough, '79). Genetic mechanisms are involved in these endothermy-correlated traits be-

cause they are inherited, and evolutionary changes within them are both incremental and integrated.

For example, enzymes that regulate cellular processes, movements across cellular membranes, or even rates of muscle contractions in ectotherms are adapted to work across a wide range of temperatures. However, they do not function with the same intensity or efficiency as those found in endotherms, whose enzymes are adapted to work optimally within narrow temperature windows (Hohtola and Visser, '98). Because organismal systems are integrated, significant change must occur in incremental stages because too many systems would be affected deleteriously by great alterations.

Organisms fit for one environment can become fit for another if they possess certain traits that provide phenotypic flexibility (i.e., "preadaption," *sensu* Lee and Doughty, '97). The same must be true of metabolic strategies. Increased metabolic rates would allow access to new environments over time, both geographic and temporal, with consequent exposure to new selective forces. With physiological changes, one could predict that certain recognizable morphological changes would follow. At least some of the physiological and morphological changes that began the trend toward endothermy may have arisen first in the archosaurian common ancestor of dinosaurs and crocodiles (Ricqlès, '78). Further unique advances may then have accumulated within the theropod and/or avian lineages.

This paper puts forth a working model that begins with a relatively minor (four-base) change in a single gene and that consists of sequential and interrelated stages that may account for charac-

teristics of dinosaurs, birds, and their ancestors and can be observed both in the fossil record and in living descendants.

Many terms have been used, often in different ways, to describe metabolic regulation. To avoid confusion, terms used in this paper will follow definitions in Ricqlès ('78) and Reid ('97b).

Endothermy is the use of metabolically generated energy to maintain body temperature within a narrow range despite wide fluctuations in ambient environmental temperatures. Endothermic metabolism affords organisms an active lifestyle, even at low ambient temperatures, particularly when coupled with behavioral responses to environmental changes. The alternate metabolic strategy is *ectothermy*, in which energy for physiological processes depends largely upon ambient sources.

Homeothermy refers to an organism's ability to maintain body temperatures within a very narrow range, regardless of variation in external environments or activity levels. Body core temperatures in animals that exhibit homeothermic metabolism generally do not vary more than a degree or two. This allows temperature-dependent physiological processes to occur at stable rates across a wide range of ambient temperatures. *Endothermy* and *homeothermy* are often used interchangeably, but they are separate and distinct, and if an ectotherm is large, it can attain a state of homeothermy (Seebacher et al., '99). Almost all mammals are homeothermic endotherms; however, at least one species of mammal, the "naked mole rat" (*Heterocephalus glaber*), is functionally ectothermic, with its body temperatures varying in accordance with ambient temperatures (Jarvis, '78).

Poikilothermy or *heterothermy*, on the other hand, denotes wide variation in body temperatures, usually corresponding to ambient fluctuations. Poikilothermy is most often associated with amphibians and reptiles; however, this strategy is also employed by some endotherms, such as animals that lower body temperatures and metabolic rates during hibernation or torpor.

Although these terms seem to imply distinct and separate states, metabolic strategies of organisms actually occupy a continuum. For example, ectothermic organisms regulate their body temperatures almost as precisely as endotherms, primarily behaviorally rather than metabolically. Even though all living reptiles have overall low-energy lifestyles, they are capable of efficient bursts of activity. To ward off infections, some lizards can raise their body temperature significantly, and some snakes generate heat for limited "brooding"

through isotonic contractions of skeletal muscles (Benedict, '32; van Mierop and Barnard, '78). However, these animals rely upon anaerobic metabolism to sustain these short-term high activity levels (Pough, '80), rather than on the aerobic strategies employed by endotherms. Except for rare exceptions, metabolic processes and rates in ectotherms are otherwise affected by changes in ambient temperatures.

Birds, on the other hand, can be described as heterothermic endotherms, in that they are capable of significant variations in overall body temperatures. Some birds, such as the Poor-will (*Phalaenoptilus nuttallii*), may vary body temperatures by as much as 30°C or more over the course of hours (Bartholomew et al., '57).

Endothermic animals can function consistently in a wide range of environments and can also function earlier in the morning, later at night, or be totally nocturnal, thus opening niches unavailable to ectothermic animals. On the other hand, because ectotherms are more restricted to diurnal cycles and equable habitats, they have a ten-fold or greater decrease in food and oxygen requirements than endotherms. Therefore, ectotherms are able to occupy habitats where food resources and/or oxygen may be limited. The heterothermic endothermy exhibited by birds may similarly have advantages in terms of energy conservation. Allowing body temperatures to drop in unfavorable ambient conditions reduces temperature gradients and lessens heat loss to the environment, resulting in considerable energy savings (Wilson and Gremillet, '96; Hohtola and Visser, '98). It is generally assumed that the primitive (plesiomorphic) state for vertebrates is poikilothermic ectothermy and that any increase or modification of metabolic rate over this is by definition derived (Ricqlès, '92).

THE EVOLUTION OF ENDOTHERMY

A change in metabolic strategy within a lineage must have been accomplished over time and must incorporate many discrete steps, some of which may be semi-autonomous, but which are causally connected and depend, in an evolutionary sense, on the other steps. The steps enumerated below represent modular units that are not necessarily sequential. Not all steps are genetic in origin but may be epigenetic or a selectively favored consequence of preceding steps. Additionally, because lineages diverge, all traits may not appear in all descendant taxa within a clade. These steps incorporate molecular and morphological information obtained from the study of extant and extinct

organisms that share common ancestry with birds (Fig. 2).

1. *Mutations in the gene coding for the beta (β) chains of the hemoglobin protein in the Archosaurian common ancestor involved the substitution of positively charged amino acids for neutral ones in the region of the protein that includes the organophosphate binding pocket. These mutations would favor the binding of more negatively charged organophosphates, changing the affinity of the hemoglobin molecule for oxygen and improving the efficiency of oxygen delivery to the tissues.*

Hemoglobin, a multichain globular protein complexed to four iron-containing heme prosthetic groups, is the major component of red blood cells and carries oxygen in the circulatory system of all vertebrates because it has a high affinity for molecular oxygen. The affinity of hemoglobin for oxygen is so great that each molecule will be fully saturated with oxygen at less than ambient pressures and will not release oxygen at even the relatively low pressures existing in oxygen-poor tissues. At a distance from the binding site of oxygen in the three-dimensional hemoglobin molecule is a second site, which binds an allosteric effector, or "helper molecule." When the hemoglobin is deoxygenated, the effector stabilizes this configuration and shifts the equilibrium between the oxygenated and deoxygenated states. This, in turn, lowers hemoglobin oxygen affinity and allows the release of oxygen at higher PO_2 than would occur without the presence of the effector (Isaacks and Harkness, '80; Weber, '92). The effector is both

phylogenetically determined and physiologically significant. The ancestral effector molecule is generally accepted to be ATP because it is so widely distributed among vertebrate taxa in ontogeny and phylogeny (Perutz, '83; Kemp, '88). This organophosphate, familiar for its role as the currency of cellular energy exchange, also alters hemoglobin affinity for oxygen (Fig. 3) and is the primary effector of embryonic forms of many tetrapods; it is also the effector molecule for most fish hemoglobin (Bauer and Jelkmann, '77).

Bis-phosphoglycerate (BPG) is the primary effector found in mammals (Bartlett, '76, '80). BPG is a more efficient regulator of oxygen downloading than ATP because it imparts greater stability to the deoxygenated hemoglobin, thus lowering oxygen affinity to a greater degree. It is transitionally present in embryonic birds but is not present in their sister taxon, the crocodiles (Bartlett, '76, '80). Although embryonic crocodiles use ATP as an effector, adult crocodiles are unique among vertebrates in that their hemoglobins have lost sensitivity to any organophosphate as a regulator of oxygen delivery. Instead, they use bicarbonate ions (Bauer and Jelkmann, '77; Perutz et al., '81), formed as a result of CO_2 metabolism, to modulate hemoglobin function. Oxygen delivery modulation by bicarbonate is a derived trait restricted among extant animals to crocodylians. It is the result of the replacement of two charged amino acids with neutral ones, thus leaving no region to which charged organophosphates may bind (Perutz et al., '81). Significantly, when bicar-

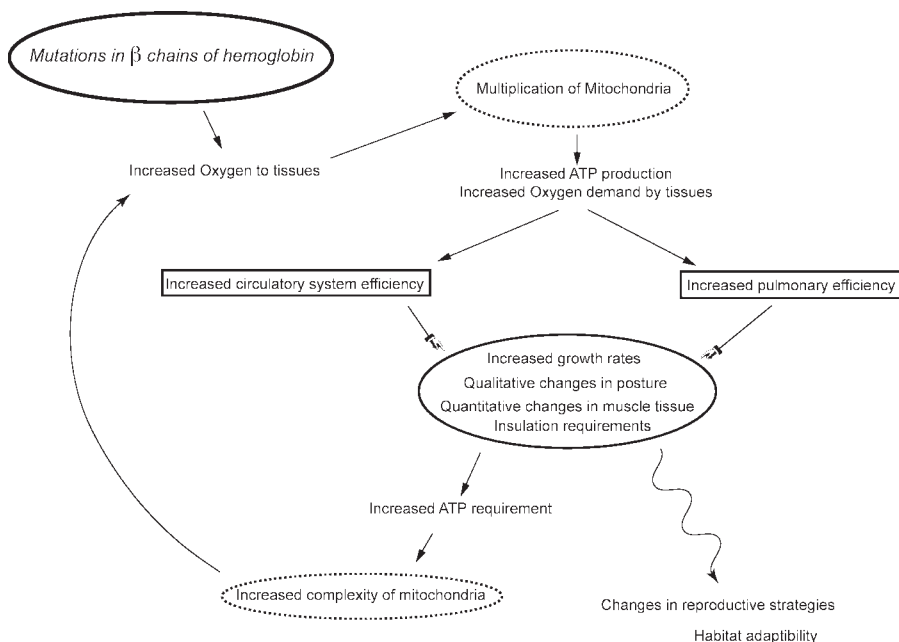


Fig. 2. Schematic diagram illustrating genetic and epigenetic changes and the possible consequences of these changes as presented in the text.

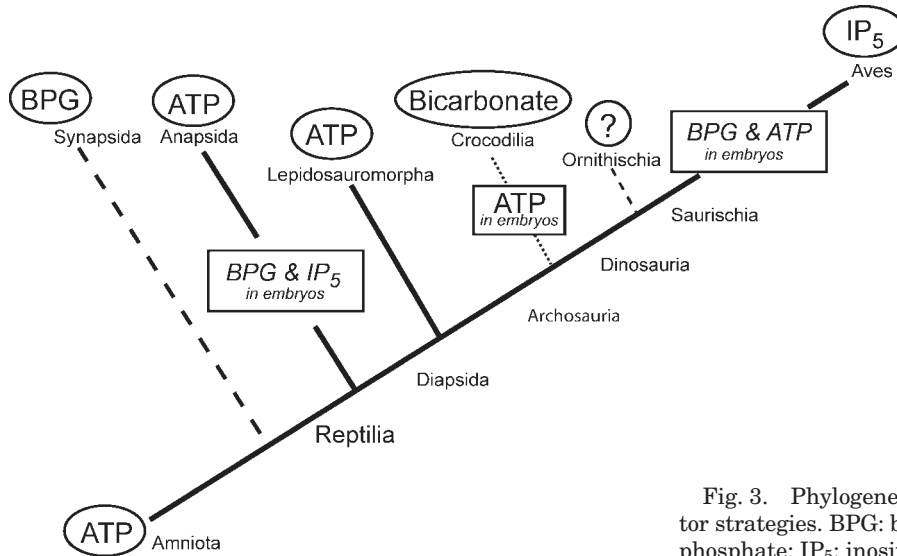


Fig. 3. Phylogenetic relationships of oxygen-binding effector strategies. BPG: bis-phosphoglycerate; ATP: adenosine triphosphate; IP₅: inositol pentaphosphate.

bonate binds to crocodile hemoglobin, it does so in a different region of the molecule than where the organophosphates bind, and both could, in theory, bind at the same time. This provides a means for the gradual replacement of one effector by another in a lineage, without deleterious results (Komiyama et al., '95).

The genetic changes that brought about the bicarbonate binding character state in crocodiles most likely occurred after their divergence from the bird-dinosaur lineage, for it is not found either in other reptile taxa or in birds. Alternatively, it could have been acquired by the common ancestor of birds and crocodiles, and thus would be possessed by the ancestor of theropods as well. This scenario, however, would require a reversal in the bird lineage. Because the blood of all other vertebrates is sensitive to organophosphate effectors, it is more reasonable to infer that this is the primitive state and that bicarbonate binding in crocodylians is apomorphic for this clade. Sequence data do not shed light upon when such regional mutations may have occurred in these taxa (Perutz et al., '81).

In contrast to crocodylians, all living birds use one of the inositol phosphates—inositol tetraphosphate (IP₄) in ratites, inositol pentaphosphate (IP₅) in all other birds—as an effector molecule post hatching (Isaacks and Harkness, '80; Weber, '92). In the presence of CO₂, IPs have a greater effect on hemoglobin oxygen affinity than any other effector (Isaacks and Harkness, '80; Bauer et al., '81) because of their greater overall negative charge. The efficiency of bird blood as an oxygen delivery system is greater than that of any

other extant taxon (Zaidi and Sultana, '89) and explains, in part, the ability of birds to maintain a highly efficient aerobic metabolism.

The difference in the effector binding pockets (regions in the hemoglobin molecule where effectors bind) between bird and crocodile hemoglobins is only four amino acids (Perutz, '83; Schnek et al., '85), requiring mutations in four bases of the gene. Two of the charged residues in this region of hemoglobin have been replaced in crocodiles with neutral residues, thus changing the overall charge of the binding pocket and leaving no residues to which charged organophosphates may bind (Perutz et al., '81). Another change disrupts hydrogen bonding necessary to stabilize this region of the molecule. In birds, amino acids that are neutral in other taxa have been replaced with positively charged ones at two sites, increasing the overall charge of this region and favoring the binding of the more negatively charged IP₅ over DPG (Perutz, '83). These relatively minor genetic changes result in a major functional effect (Perutz et al., '81). Thus, a change of only a few amino acids is sufficient to alter the sensitivity of the hemoglobin molecules to effectors. These changes seem to be a result of Darwinian selection acting to maintain mutations in the molecule, resulting in increased oxygen delivery (Bauer et al., '78).

Mammalian blood is also highly sensitive to IP₅, and when in the presence of this organophosphate, oxygen downloading is comparably increased in this taxon as well. However, the precursor molecule of IP₅, phytic acid, is a metabolic byproduct of birds and is not present in mammals in sufficient quantities to be co-opted as the primary al-

losteric effector (Isaacks et al., '76, '82; Roncales et al., '82).

2. *Increased oxygen delivery to the tissues favors the multiplication of mitochondria within the cells of these tissues, primarily because excess oxygen results in the formation of free radicals that are toxic to cells and tissues. Mitochondrial enzymes, necessary to reduce oxygen to water, prevent the formation of free radicals and decrease oxygen toxicity.*

Although metabolic rates in extant animals are, to a large degree, governed by mitochondrial capacity, the question remains, what drove the multiplication of mitochondria in the "protoendotherms" of antiquity? The metabolic breakdown of oxygen results in the production of free radicals, which are deleterious to many cellular processes. Increased oxygen delivery to the tissues would require a compensatory cellular mechanism to reduce the toxic effects of oxygen. Therefore, the cell has developed a way to reduce oxygen to water through chemical reactions that occur on the inner membranes of the mitochondria. Even though the mitochondrial and cellular genomes are functionally integrated, mitochondria retain their own DNA and can replicate independently from the cells in which they reside. Like all cellular processes, replication of mitochondria is energetically expensive and is favored by selective factors such as the necessity of toxic free radical reduction.

The more mitochondria, the more aerobic chemical reactions occur, and they occur more rapidly. However, ATP production by mitochondria is inherently inefficient, and the result of this biochemical inefficiency is metabolically generated heat and a consequent increase in organ-specific metabolic rates (Hulbert and Else, '89; Ruben, '95). Independent measurements on living tissues show that when more mitochondria are contained within these tissues, the greater is the oxygen consumption and the higher the basal metabolic rate (resting, or inactive rate, BMR) of those tissues (Else and Hulbert, '85; Hulbert and Else, '89). Studies have shown that there is basically no difference in the metabolic capacities or overall enzyme functions of mitochondria in the viscera of ectotherms versus endotherms. The metabolic differences of these tissues can be attributed directly to the existence of more mitochondrial enzymes per unit tissue in the former (Else and Hulbert, '85).

Multiplication of mitochondria and the subsequent increase in ATP production would allow the tissues to increase in mass and would therefore

increase both the function and capacity of these tissues. The viscera of endothermic organisms are more massive per unit body weight than are those of ectotherms (Ruben, '95). In addition, they contain more and larger mitochondria. The metabolism of visceral tissues is primarily responsible for an animal's BMR (as opposed to active or peak rates).

Although increased BMR is not equivalent to endothermy, it could have given these animals the ability to use metabolic energy to achieve and sustain elevated core temperatures (Ricqlès, '74, '92), allowing increased activity levels. Greater activity levels would require the production of more energy, thus setting up a positive feedback loop (Fig. 2).

3. *Increased numbers of mitochondria per unit tissue (step 2) results in increased ATP production and the accompanying production of waste heat. As numbers of mitochondria increase, the physiological consequence is an increased demand for oxygen. To meet this demand, more efficient circulatory systems evolved within archosaurs.*

A four-chambered heart (e.g., Seymour, '76; Reid, '97b) allows full separation of oxygenated and deoxygenated blood. This separation is essential to maintain high metabolic levels. Additionally, only a four-chambered heart can establish pressure differentials sufficient to supply blood to distal organs when an animal holds itself more erect, as did the dinosaurs and some basal archosaurs. Both components—separation of oxygenated and deoxygenated blood, and strong pressure differentials—would be necessary to supply oxygen at levels required for aerobic metabolism. Both birds and crocodiles possess a fully separated four-chambered heart, an adaptation that occurred in the common ancestor of crocodiles and birds (Grigg, '92) and that may support the hypothesis that elevated metabolic rates may be primitive for archosaurs (Nicholas Fraser, personal communication; Ricqlès, '78).

In crocodilians an aortic shunt allows limited mixing of oxygenated and deoxygenated blood. This adaptation favors prolonged apnea because it allows for total depletion of hemoglobin-borne oxygen; thus, it is advantageous to animals with low respiratory frequency or as an adaptation to diving. Aortic shunting is most likely a derived feature in crocodilians, increasing their fitness for an amphibious habitat (White, '78; Grigg, '92).

The circulatory system of early theropods, and perhaps their ornithomimid ancestors, was almost certainly more efficient than that of extant croco-

diles. The demands of both large size and erect, vertical body posture necessitate a more powerful and efficient circulation to supply the brain (Seymour, '76; Bakker, '87; Ostrom, '97; Reid, '97b). A circulatory system capable of supplying oxygen and metabolites to tissues efficiently would not only allow for the advent of bipedality; it would also allow an organism to grow larger and more rapidly, since maximum growth rate is constrained by the ability of the vascular system to supply metabolic substances to the growing tissues (Ricqlès, '75, '78; Reid, '97b).

4. *Selective forces set in motion by increased oxygen demand result in a more efficient pulmonary system. This would include both increased complexity (greater number of divisions for expansion of surface area of exchange) and increased vascularization of lung tissues to facilitate hemoglobin saturation.*

Extant reptiles use several ventilation mechanisms and lung types, and they have responded to diverse environmental demands in a variety of ways. It cannot be assumed that pulmonary structures and functions in any living reptile represent ancestral conditions. Indeed, because their low-energy lifestyles demand less oxygen, reptiles may have more plasticity in their respiratory systems than do more active animals. The phylogenetic trend among reptiles may have been toward the reduction of the absolute cost of respiration, rather than toward maximal efficiency (Gans, '78).

Some extant lepidosaurs possess the simplest tetrapod lung, a single-chambered organ firmly attached to the body wall and partitioned into thin-walled faveoli that contain a network of capillaries. Since this lung type is similar to that seen in most adult amphibians, it may approximate in structure those found in early reptiles (Duncker, '78). In larger animals, lungs are septated to increase surface area for gas exchange, and in some cases, they may also possess larger chambers that do not function in respiration. Similar structures may have been the precursors for avian caudal air sacs. In extant crocodiles, the surface across which gas exchange occurs is more densely partitioned and inspiratory movements are coupled to abdominal musculature (Duncker, '78).

As in their reptilian ancestors, avian lungs are fixed to the body wall. However, this fixation is more complete than in other reptiles, resulting in lungs that are "pre-expanded" and that maintain a constant volume (Duncker, '78). In addition, the avian respiratory system incorporates a unique system where air capillaries surround the dense

blood capillaries in the lungs, allowing more direct exchange of gases over a greatly increased surface area and approaching a ten-fold increase over the mammalian lung (Perry, '92). These adaptations allow birds to meet the greater oxygen needs their higher metabolism demands (Perry, '92).

The avian respiratory system is also unique in its unidirectional airflow and complex system of air sacs, some of which extend into the pneumatic spaces of skeletal elements. Pneumatic spaces preserved in the skeletal elements of most theropods and some of their archosaurian precursors support the hypothesis that these animals possessed some characteristics of this complex respiratory system. A plausible mechanism for the evolution of this lung, including various intermediate states, is included in a discussion by Perry ('92, 2001).

5. *A physiological consequence of increased metabolic rates may be rapid growth.*

Patterns of bone deposition, along with ontogenetic modifications, are faithfully recorded in fossilized skeletal elements. These growth strategies may be correlated to some degree to metabolic regimes (Ricqlès, '74, '78; Ricqlès et al., 2000). Much of the histological evidence available for theropods is interpreted by some to suggest a growth strategy that incorporates characteristics found in both ectotherms and endotherms (Reid, '84a, '87, '90, '97b; Chinsamy et al., '94, '95), although this interpretation is contested (Ricqlès et al., 2000; Padian et al., 2001). Extant ectotherms most often demonstrate a lamellar-zonal (*sensu* Ricqlès, '74) histological pattern, in which poorly vascularized zones of lamellar bone are punctuated by annuli (thin strata of avascular bone), or lines of arrested growth (LAGs) representing periods where bone deposition slowed or ceased (Fig. 4a). This pattern is generally correlated to slow rates of bone deposition and thus has been linked to a lower organismal metabolic state. As the animals approach adult size, these cycles are spaced more closely together and the intervening lamellar layers become narrower, with decreasing vascularization. Ectotherms usually grow throughout their lifespans, and this "indeterminate" growth pattern extends to the superficial layers of the cortical bone regions. Because this type of bone deposition is widely distributed among vertebrates, lamellar-zonal deposition interrupted by LAGs reflects a primitive (pleisiomorphic) state (Ricqlès, '92; Horner et al., '99).

Extant endotherms, on the other hand, most often demonstrate a bone microstructural pattern that is fibrolamellar, a pattern closely correlated

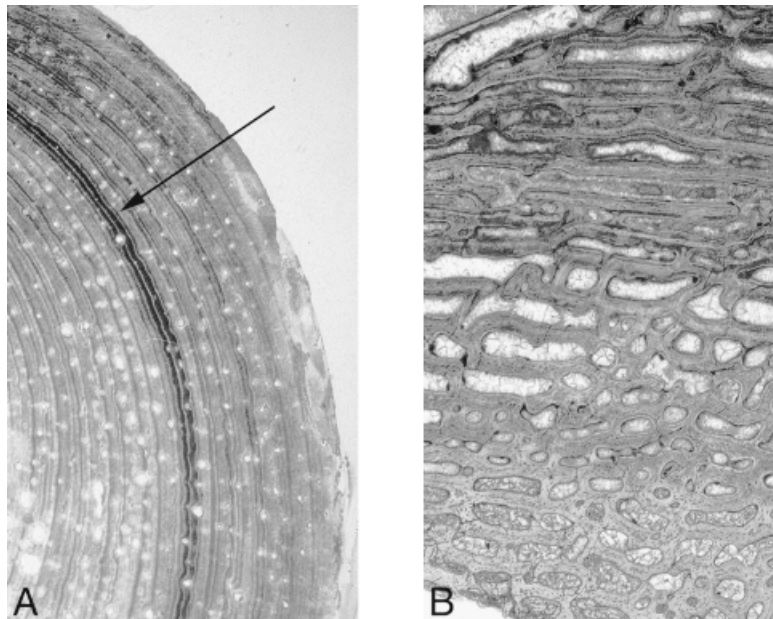


Fig. 4. **a.** Photomicrograph of cross-section of crocodilian long-bone, showing characteristic lamellar-zonal pattern of bone deposition. Arrow indicates a LAG. **b.** Cross-section of bone from the lambeosaurine dinosaur *Hypacrosaurus* demonstrating a fibrolamellar microstructural pattern.

with rapid rates of bone deposition (Fig. 4b). However, these various bone patterns are observed in animals of either metabolic regime under certain circumstances, and therefore cannot be directly correlated with any particular physiological pattern (Horner et al., '99). Nevertheless, a strong contrast exists between endo- and ectotherm bone microstructure, even among extant tetrapods. For instance, fibrolamellar bone is found to some degree in crocodiles raised under ideal conditions of uniform temperature and ample availability of food resources, indicating that these animals are capable of sustained and rapid, but still cyclical, growth, even though they are ectothermic (Horner et al., '99). Conversely, LAGs have been observed in histological sections of endothermic mammals (De Buffrenil, '82; Chinsamy et al., '98; Horner et al., '99) and birds (Van Soest and Utrecht, '71; Koubek and Hrabe, '84). This clearly indicates that LAGs can form in response to a variety of factors, including phylogenetic legacy, seasonal habitat, hibernation, or extreme environmental conditions (Reid, '97a; Horner et al., '99). Because the ability to form LAGs is a primitive rather than a derived characteristic, their retention in histology is not useful for either phylogenetic or physiological hypotheses, but may best be used to interpret growth characteristics of individual elements (Horner et al., '99; Ricqlès et al., 2000).

Histological studies support the hypothesis that sauropods, ornithopods, and theropods grew rapidly (Ricqlès, '80; Reid, '84a, '84b, '87, '97b; Chinsamy, '90, '93; Varricchio, '93; Erickson et al.,

2001; Padian et al., 2001). These dinosaurs exhibit a fibrolamellar histological pattern, indicating bone deposition at rates not seen in living ectotherms except temporarily under artificial conditions (Ricqlès et al., 2000). Most dinosaur bone also displays deposition of LAGs. One interpretation of these histological data is that upregulated metabolism might have made it possible for dinosaurs to grow rapidly and continuously at rates approaching extant endotherms; however, when seasonal variation or other stressors produced less equitable conditions, their metabolic threshold may have been too low to offset heat loss to the environment (e.g., Ricqlès, '83, '92). This would have required that metabolic energy that had been used for bone deposition (growth) would have to be shunted to heat production or metabolic maintenance, resulting in a slowing or cessation of growth until favorable conditions resumed. Additionally, the onset of flight in precocial birds could demand a diversion of energy that was previously devoted to growth, causing a decrease in growth rates (Chinsamy and Elzanowski, 2001).

Such a growth slowdown has been observed to some degree in bird hatchlings. When metabolic energy must be expended by the chicks to maintain body temperatures in the face of reduced ambient temperatures, growth either ceases or slows remarkably (Starck, '93; Eppley, '96; Pearson, '98; Yahav et al., '98). Growth rates are inversely correlated to the onset of endothermic homeothermy in extant birds (see later). Altricial birds, in whom the energy to maintain body temperatures is sup-

plied by brooding parents rather than their own metabolism (Dunnington and Siegel, '84; Starck, '93; Pearson, '98), may grow up to four times faster than precocial birds, which must use metabolic energy to maintain body temperatures (Starck, '93). Finally, in genetic variants of chickens that do not develop sufficient feathery coverings to provide insulation for offsetting heat loss to the environment, exposure to cold results in some quantifiable shifts in physiological responses, including growth rates (Yahav et al., '98).

Despite the many lines of evidence supporting the hypothesis that metabolic rates significantly above ectothermy had been attained by the theropod ancestors of birds, one intriguing example from the fossil record has been interpreted to suggest the possibility of a metabolic reversal in one group of birds. Although *Archaeopteryx* is recognized as the first true bird by most scientists (Chiappe, '95; Padian and Chiappe, '98; Sereno, '99), partly because of its possession of flight feathers and well-developed wings, the origin of birds must predate the appearance of this fossil. Avian lineages arising after *Archaeopteryx* include the enantiornithines, which became extinct at the end of the Cretaceous, and ornithurines, which include all living birds (Fig. 1).

Histological studies of the bones of enantiornithine birds show a pattern not seen in either theropods or extant birds. LAGs are present throughout the cortex of the limb bones, indicating periods of growth cessation. These LAGs interrupt dense regions of avascular bone (Chinsamy et al., '94, '95; Chinsamy and Elzanowski, 2001), which are correlated with very slow growth in extant taxa. One interpretation of these histological data is that this lineage of birds did not possess a metabolism that allowed them to grow as rapidly as modern birds (Chinsamy et al., '94, '95), a hypothesis that is supported by the preservation in the fossil record of skeletal elements representing various growth stages. If members of this taxon grew as rapidly as modern birds, one would expect very few examples of anything other than either hatchlings or adult forms (Chiappe, pers. com. '98).

This pattern of avascularity is not seen in modern birds, although LAGs are known to occur (Van Soest and Utrecht, '71; Koubek and Hrabe, '84). An alternative explanation exists for this apparently contradictory suite of characteristics in enantiornithines. Some taxa of extant birds have secondarily selected slow growth rates (Starck, '93). This strategy is seen to be adaptive in seabirds,

for example, where parental foraging times are necessarily longer than in other taxa, resulting in longer periods during which energy resources are unavailable to the young. Therefore, slower growth of offspring is selectively advantageous because it reduces energy demand (Starck, '93). Secondarily slowed growth strategies are also evident in birds living at extremely high altitudes (Monge and Leon-Velarde, '91). Additionally, the earlier the onset of endothermic homeothermy in avian ontogeny, the slower the growth rate, because when more energy is required for temperature maintenance, less is available for growth (Starck, '93). Finally, decreases in body mass in organisms with increased metabolic capacity are correlated with increased energy demands (Hulbert and Else, '89), partly because of heat loss due to changes in surface area/volume ratios, and may have played a role in driving the selection to the smaller size that is seen in some lineages of theropods, undoubtedly including the theropod clade that gave rise to birds (Sereno, '97). All of these factors (decrease in mass from theropod ancestors, early onset of endothermy, high altitude adaptations in some taxa, and sporadic access to energy resources and/or brooding provided by parents) may have been important factors in secondary selection for slower growth in enantiornithines than is seen in their theropod ancestors. A secondary reduction in the growth rate in these birds is not accompanied by decreases in overall metabolic rate (McNab, '78; Ricqlès 2000; Ricqlès et al., 2001) and would not compromise muscle activity required for flight and other high-energy activities in adult birds. Enantiornithines, according to fossil evidence, were more widespread and diverse than the coexistent ornithurines, the ancestors of extant birds (Chiappe, pers. com., '99) during the Cretaceous. Secondarily slow growth rates are more consistent with the widely acknowledged flight capabilities of enantiornithines, which could not be supported by an ectothermic metabolism as exemplified by extant taxa (Ruben et al., '97).

6. *Changes in ATP production, circulation, and pulmonary exchange (steps 2, 3, and 4) would allow increased periods of sustained activity. An increase in sustained, metabolically demanding activities would make any morphological changes that increased efficiency of movement advantageous, and thus likely to be maintained, including qualitative changes in posture and gait and quantitative changes in the size and numbers of muscle fibers that participate in and maintain sustained exercise.*

Since at least the Permian, the archosauroid

morph ancestors of crocodiles and dinosaurs exhibited an increasing trend toward more gracile forms and limb movements in a more fore-and-aft pattern. These features imply that members of these taxa were active and occupied terrestrial habits (Carroll, '88). Similarly, gracile skeletal morphologies and, in some taxa, facultative bipedality suggest that like the earliest dinosaurs, some Triassic crocodiles were capable of much higher activity levels than extant members of this group (Ricqlès, '78; Carroll, '88; Sereno, '91). Indeed, the basal members of the clade were most probably active, terrestrially adapted organisms rather than amphibious in habit as are all living crocodiles (Carroll, '88).

Most basal eusuchians retained long limbs, suggesting terrestriality, but others were amphibious or aquatic. Outgroup analysis shows a phyletic trend toward more amphibious regime, and perhaps a lower-energy lifestyle. It is assumed that modern crocodiles have acquired specialized features that adapt them to low-energy lifestyles, thus secondarily reversing some of the metabolic advances of their ancestors while retaining others, such as the fully divided heart acquired by the common ancestor of crocodiles, dinosaurs, and birds (Ricqlès, '78; Reid, '97b).

Fully erect posture (legs held directly under the body, rather than splayed outward as in lizards and crocodiles), parasagittal gait, and the inability to sprawl were characteristic of all ornithodiran taxa, including pterosaurs and lagosuchids (Gauthier, '86; Holtz, '96). In addition to allowing concurrent breathing and locomotion for increased stamina (Carrier, '87, '98), it has been shown that the muscles responsible for postural maintenance are capable of producing metabolic heat through shivering (Heath, '68; Ricqlès, '78; Hohtola, '81; Ostrom, '97; Hohtola and Visser, '98), even before locomotor abilities of these muscle groups develop ontogenetically (Eppley, '87). Both of these adaptations would drive a further increase in metabolic rates. Although some mammals may employ a secondarily derived sprawling posture, the primitive state for mammals is an erect posture with a parasagittal gait.

In contrast, all theropods, including birds, are obligate bipeds (with the possible exception of *Segnosaurus*), a condition that is probably primitive for ornithodira (Gauthier, '86; Sereno, '91). This condition is limited in extant taxa to endotherms, and it is not found in any ectotherm (Ricqlès, '74; Ostrom, '80). These traits are strongly correlated with endothermy and support

the hypothesis that in some primitive archosauromorphs, and almost certainly in theropods, metabolic rates were elevated sufficiently to support and sustain a more active lifestyle.

7. *Changes in both muscle content and posture require increased production of ATP. Because muscle cells are tightly packed with actin, myosin, and other proteins, the absolute number of mitochondria that can be added to these cells is constrained. In extant endotherms, this is reflected by the fact that the mitochondria present in skeletal muscle tissues have an increased complexity of form over those of ectotherms.*

While the metabolic rate of the visceral organs is primarily responsible for increases in BMR, it is the metabolism of the skeletal muscles, including the postural muscles, which generates the active metabolic rates (Ruben, '95; Duchamp and Barre, '98). To maintain an elevated body temperature (the byproduct of increased rates of cellular metabolism), an organism must compensate for heat loss to the environment. This is accomplished efficiently in living endotherms by, among other things, accessory thermogenesis: the use of muscle contractions (shivering) to elevate metabolic heat generation over basal or resting rates.

The ontogenetic study of neonate and hatchling birds may approximate the phylogenetic acquisition of temperature regulation in this taxon. Birds are unable to maintain a stable body temperature in the face of changing ambient temperatures (poikilothermic) at hatch (Grav et al., '88; Eppley and Russell, '95; Hohtola and Visser, '98; Pearson, '98; Visser, '98). Additionally, some birds are also essentially ectothermic at hatch, incapable of generating sufficient metabolic heat to keep body temperatures above ambient. As birds mature and gain more control over body temperatures, skeletal muscle tissues are the primary sites of such thermogenesis (Eppley, '96; Duchamp and Barre, '98). Muscle involvement in heat generation is supported by the observation that skeletal muscle mass in endotherms is proportionately over twice as great as that seen in similarly sized ectotherms (Else and Hulbert, '81). Muscle mass increases not by cellular division, as in other tissues, but by expansion in diameter of the individual muscle fibers or by increasing fiber length through the addition of individual muscle units called sarcomeres (Hohtola and Visser, '98). The two muscle groups primarily responsible for thermogenesis in neonate and hatchling birds are the flight and the leg muscles (Visser, '98). Precocial forms have relatively more massive leg muscles, because they run

before flight is attained. For these birds, metabolic heat production via shivering occurs in muscles of the legs. However, in other groups, the pectoral muscles are proportionately more massive and play the primary role in shivering thermogenesis, even though the onset of flight does not occur until much later (Visser, '98).

The increase in muscle mass in endotherms is accompanied by a proportional increase in mitochondria within these tissues and concomitant increase in ATP production. However, it has been noted that mitochondria within endothermic skeletal muscle are also qualitatively different from those seen in ectotherms, with greater membrane infolding and much more complex overall structure (Akhmerov, '86; Grav et al., '88; Eppley and Russell, '95).

An increase in mitochondrial membrane surface area is directly related to oxidative capacity in these skeletal muscles, allowing the generation of more ATP per mitochondrion than is seen in visceral mitochondria (Grav et al., '88; Hulbert and Else, '89; Eppley and Russell, '95). Increased production of ATP increases the work capacity of these muscles, allowing them to contract more efficiently over longer periods before exhaustion, thus creating a positive feedback loop (Schuh, '51). Accessory thermogenesis to support a mildly elevated body temperature would require increased ATP production. This in turn would supply energy to the skeletal muscles, allowing an increase in work efficiency and favoring greater efficiency of posture. With these adaptations, additional refinement of the lungs and heart would be required, as oxidative metabolism and longer time spent in sustained activities increases the demand for oxygen delivery (Bennett and Ruben, '79; Bennett, '91; Ruben et al., '97). The correlation between the onset of endothermy and qualitative changes in mitochondrial characters within skeletal muscle tissues of birds is demonstrated in electron microscopy studies of newly hatched bird before and after the onset of endothermy (Eppley and Russell, '95).

Evidence for increased mitochondrial number and/or complexity early in the theropod lineage may also be inferred from the seemingly characteristic death pose of these animals. Like birds, theropod fossils are found with the dorsal neck muscles contracted, drawing the head and neck caudally downwards over the back of the animal (Fig. 5). This apparently occurs when all muscles contract at death and the stronger weight-bearing muscles of the neck "win out" over the weaker, ventrally contracting ones. Although this posture

is present in mammals, it is rarely, if ever, seen in ectotherm carcasses. It may be possible that the "dead bird" posture is due to the increased capacity of the muscles of endotherms to contract and maintain contractions under the anaerobic conditions that prevail in the tissues after death.

8. *The changes outlined in the preceding steps would allow an expansion of activity levels, resulting in the ability to occupy new territories where competition would be decreased. Cooler territories, or those where hours of darkness increasingly predominated, would require cellular compensation to drive increased energy and/or heat production.*

Animals that are capable of sustained activity over longer periods can range over a wider variety of temporal and geographic environments. If such expanded distributions are linked to metabolic changes possessed by some taxa and not others, they may be favored by a decreased competition. More active animals would be free to move into environments that would be considered "marginal" for ectotherms, whose activity levels are tied to ambient temperatures.

Movement into less than equable environments would require an increased tolerance to cooler temperatures and longer time spent in darkness, with subsequent increased energy demands. Simple changes in quantity and quality of mitochondria are insufficient to meet these needs. One molecular means by which such an increase in overall metabolic rates may have occurred is through changes in cellular membranes. Movement of essential ions out of endothermic cells is seen at levels not observed in ectotherms. Cell membranes of endotherms are significantly more permeable to sodium and potassium ions than those of ectotherms because of increased desaturation of membrane phospholipids (Hulbert and Else, '89, '90; Ruben, '95). This requires the expenditure of increased metabolic energy (and consequent increase in heat production) to return these needed ions to the cell's interior against a concentration gradient. Animals adapted to cold environments produce greater amounts of enzymes needed for phospholipid desaturation, and these "leaky membranes" have been proposed as a cellular adaptation to decreased temperatures (Gracey et al., '96). Increased permeability of membranes to essential ions as a cellular adaptation to cold would contribute to nonshivering thermogenesis as a result rather than a causal agent of incipient or emerging endothermy. In other words, as dinosaurs established a metabolic rate sufficient for migration, invasions of harsher



Fig. 5. *Albertosaurus* displaying characteristic “death pose.” This pose is frequently exhibited by animals purported to have higher metabolic strategies, such as mammals,

theropods, pterosaurs, and birds. This pose is not reported in ectothermic organisms. (Photo by D. Tanke, RTMP.)

territories may have provided selective pressures for increased output of metabolic heat and membrane “leakiness,” with consequent generation of metabolic heat through cellular pumps as an adaptive mechanism.

In contrast to crocodiles, champsosaurs, and other obviously ectothermic Mesozoic vertebrates, dinosaur remains have been found on all continents, including those that would have been north of the Cretaceous Arctic Circle (Clemens and Nelms, '93; Rich et al., '97; Chinsamy et al., '98), giving them a wider latitudinal range and distribution than any extant or coexistent ectotherm (Molnar and Wiffen, '94; Rich, '96; Wiffen, '96; Molnar, '97). This supports the hypothesis that dinosaur metabolism allowed for the invasion of territories that were not favorable to vertebrate ectotherms (Ostrom, '69), and it has led to the

proposal that some dinosaurs may have undertaken seasonal migrations that may indeed have exposed them to cooler temperatures (Currie, '89; Paul, '97; Rich et al., '97).

Additionally, some dinosaurs may have lived in large herds (Currie, '81; Currie and Dodson, '84; Varricchio and Horner, '93). The evidence for migratory and herding behaviors comes in the form of trackways (Farlow and Chapman, '97; Lockley, '97) and mass death sites (Currie and Dodson, '84; Varricchio and Horner, '93) and suggests that some dinosaurs both lived and moved in groups (although this evidence is subject to other interpretations because of the likelihood of time-averaging). Among extant, strictly terrestrial vertebrates, only the endothermic birds and mammals contain taxa that exhibit either herding or migration, and although ectotherms such as some lizards may live in small

groups (temporarily, during mating season), the metabolic and circulatory requirements of long-distance migration are not supported by ectothermic metabolic rates, and it has not yet been demonstrated that these behaviors existed in either Permian or Triassic crocodylomorphs or their ancestors.

9. *Even a relatively small increase in BMR and its concurrent heat production is too metabolically expensive to be maintained in small animals and/or neonates because of unfavorable surface area/volume ratios. External insulation would provide a selective advantage in ontogenetically and/or phylogenetically small animals by decreasing heat loss and reducing energy requirements.*

Integumentary structures that function as insulators are not known for any ectothermic vertebrate group and are restricted among extant taxa to animals that generate heat through aerobic metabolic reactions (birds and mammals). Whereas in mammals this insulatory requirement is satisfied by hair, feathers serve this role in living birds.

Some have argued that feathers, the most complex integumentary structure known, evolved for flight (Feduccia and Martin, '96). However, flight has evolved independently in three vertebrate lineages (birds, pterosaurs, and mammals) and several insect taxa, but among these flying lineages, feathers are only present in birds, and therefore are not absolutely necessary for flight (Cena et al., '86). An alternative and more plausible explanation for the origin of feathers is that they arose for insulating purposes (Schuh, '51).

Insulating structures derived from the integument need not be complex to be effective. One of the best forms of insulation in mammals is the hair of the polar bear, which can be described as a tube of keratin proteins, hollow at the core to trap air (Misky, '88). Hair is phylogenetically constrained to mammals, but it is not inconceivable that the first feathers were simple, hollow cylinders that functioned in a manner similar to polar bear hair (Prum, '99; Brush, 2001).

If such hollow "protofeathers" functioned first for insulation, it is possible that they were primitively present only in hatchlings. The need for insulation would lessen as the animal became larger (increased heat retention due to more favorable surface area/volume ratios) and as adult capacities of thermoregulation were attained. Evidence for external insulation has so far only been identified in some theropods (Chen et al., '98; Ji et al., '98, 2001), not in all dinosaur skin. Addition-

ally, although no evidence of feathers has been seen in examination of embryonic sauropod skin (Chiappe et al., '98), there are no examples of embryonic skin preserved in the dinosaurs most closely related to birds, the theropods. Two possibilities are suggested by these data if feathers were indeed lacking in these embryos, rather than lost due to diagenesis. Insulatory coverings may have evolved after the divergence of sauropods and other saurischians from theropods, and absence of feathers in sauropod embryonic skin may imply that the threshold of metabolic output requiring compensation for heat loss was not reached in the dinosaurs until after the divergence of sauropods. An alternate possibility is that feathers are a primitive trait for dinosaurs but were secondarily lost in some lineages. An increase in mass is accompanied by a decrease in mass-specific metabolic rates (Hulbert and Else, '89) as well as favorable surface area/volume ratios, and it may have lessened the need for insulation in sauropods. However, the opposite is also true, that a phylogenetic trend toward decreasing size was accompanied by a proportionate increase in mass-specific metabolic rates (Hulbert and Else, '89). Therefore, feathers, which may have originated to compensate for heat loss in early ontogeny, may have been a paedomorphic trait retained in some lineages that demonstrated a trend toward clade-specific reduction in size.

10. *Increased basal metabolic rates would necessitate changes in reproductive strategies from those exhibited by extant ectotherms, favoring those more independent of ambient temperatures (Farmer, '98).*

Crocodiles, along with most reptiles, exhibit temperature-dependent sex determination (TSD) (Bull, '83). Females are produced at both temperature extremes, whereas males are produced at intermediate temperatures (Woodward and Murray, '93). A variation in this pattern exists in alligators, where cooler temperatures produce females and warmer temperatures produce males (Bull, '83). All extant birds, on the other hand, have genetically determined sex differentiation (GSD) (Harry et al., '93). Avian eggs are incubated at a relatively higher and consistently more stable temperature than reptile eggs. Either one sex or the other would be greatly favored if birds exhibited TSD.

It has been proposed that the temperature-dependent pattern of sexual differentiation exhibited by crocodylians may be primitive for reptiles (Webb and Smith, '84; Deeming and Ferguson, '88;

Lang and Andrews, '94). However, the acquisition of endothermy may have necessitated fundamental changes in the determination of gender in progeny. That organisms with TSD can share recent common ancestry with those exhibiting GSD is demonstrated by the existence of both mechanisms in squamate taxa related at the family level or lower (Viets et al., '94), and increasing evidence points to genetic influences in sex determination in reptiles, including crocodiles, even though specific genes for sex determination have not been identified. For example, incubation temperature has been shown to activate the synthesis of aromatase, the enzyme responsible for the generation of estrogen from lipid-based cholesterol precursors in the yolk (Pieau et al., '99, 2001). These precursors are metabolized differently at different temperatures by the embryo (Lance, '97), providing a means for regulating estrogen concentrations and thereby imposing environmental controls on gender determination. Interestingly, even though birds exhibit genetic sex determination, estrogen synthesis plays a crucial role in sex determination, and excess estrogen can even negate the effects of GSD (Lance, '97; Smith et al., '97; Burke and Henry, '99).

One consequence of imposing increased metabolic rates on the reptilian pattern of reproduction may have been to prevent the evolution of viviparity in the theropod/bird lineage (Dunbrack and Ramsay, '89). The avian clade is unique in that no taxon gives birth to live young. Viviparity is thought to have arisen in mammals by increasingly longer periods of egg retention (Dunbrack and Ramsay, '89), but increased retention of eggs is accompanied by a need to overcome oxygen supply constraints to the developing embryo. Dunbrack and Ramsay ('89) argue that the reduction of egg size necessary to overcome oxygen-supply constraints during prolonged egg retention resulted in extremely small, altricial young in primitive mammals. Care of these very immature and helpless young was made possible by the development of lactation in the mammalian lineage, whereas no analogous method evolved in reptiles. Another factor in the lack of viviparity in birds may be the result of decreasing fecundity as maternal mortality increases and paternal involvement decreases when egg retention is prolonged (Blackburn and Evans, '86). Finally, the development and subsequent demands of flight may have made viviparity disadvantageous from a payload standpoint.

The reptilian mode of reproduction incorporates

sperm storage as a means of increasing reproduction rates. Long breeding cycles, scarcity of mates, and fluctuations in resource availability may make the disassociation of mating from fertilization adaptively advantageous (Birkhead and Moller, '93). Sperm storage is a common phenomenon in reptiles, where sperm stored in the female reproductive tract can be viable for up to 7 years (Birkhead and Moller, '93).

In most species of mammals, on the other hand, sperm viability in the female reproductive tract is very short, less than 24 hours (Birkhead and Moller, '93), since sperm viability is compromised with prolonged storage at the higher body temperatures of endotherms, especially beyond 100 days. However, viviparity permits regulation of other mechanisms that can accomplish the same purpose, such as delayed implantation or delayed development of the embryo. Avian reproductive strategies reflect the superimposition of endothermy on a reptilian reproductive system. Length of sperm storage in birds seems to be intermediate between the reptile and mammal patterns (maximum ~ 100 days) and accommodates the sequential fertilization and egg-laying pattern of birds (Birkhead and Moller, '93). Sperm from one mating event can survive long enough to fertilize even a large, serially produced, clutch of eggs.

DISCUSSION

Evolutionary processes result in a branching pattern between ancestral taxa and descendant lineages. The acquisition of an evolutionary novelty in a lineage, therefore, would likely be followed by subsequent divergences. Consequently, the morphological or physiological adaptations outlined herein may not be equally distributed among all descendant taxa. Not all of the adaptations we discuss necessarily apply to all of the archosaurian taxa, or, for that matter, to all of the dinosaurian and/or bird lineages that may have arisen from them. A major shift in something so fundamental as the metabolic rates of organisms demands that it occur incrementally, because all body systems must accommodate such a shift (Fig. 2). The incorporation of the characteristics outlined here allows for the incremental onset of endothermic metabolic strategies from the ectothermic state in three fundamental ways.

First, as argued by Ruben ('95), the accumulation of greater numbers and greater complexity of mitochondria per unit tissue, made possible by increased access to oxygen, could have been accomplished over a relatively long period of time,

and not necessarily in all body tissues concurrently. Elevations in metabolic energy have been shown to be the summation of incremental increases in the mitochondrial membrane surface available for energy-producing reactions, rather than quantum increases (Else and Hulbert, '81).

Second, if two or more functional hemoglobin types were present in the common ancestor, as is the case in some extant reptiles and birds (birds adapted to high altitudes may express up to four functional hemoglobin types), the amino acid replacements facilitating the switch to inositol pentaphosphate allosteric binding could have occurred in the minor component, while the major component continued to function as before (Lutz, '80; Zaidi and Sultana, '89; Monge and Leon-Velarde, '91). As the increased accessibility of oxygen provided greater metabolic capacity, the expression of this mutated hemoglobin may have become favored until it exceeded that of the hemoglobin with higher affinity. Once in place, expression of the mutated and more efficient hemoglobin would be favored and would gradually become dominant in circulating blood.

Finally, birds can regulate the concentration of inositol pentaphosphate within the cell to some degree in response to environmental changes (Isaacks et al., '83). In living animals, organophosphate regulation of oxygen affinity is seen with acclimation to high altitudes (Monge and Leon-Velarde, '91), but phylogenetically this may also have been a means of incremental increases in the efficiency of oxygen delivery once the mutation that allowed the binding of this effector was in place.

Because birds and mammals are the only extant endotherms, they are often compared physiologically. However, endothermy (more accurately, heterothermy) as expressed in modern birds is qualitatively and quantitatively different than the endothermy of mammals in the following ways.

Birds have been noted to increase body core temperatures during periods of activity, such as flight, over resting metabolism by as much as 4–5°C (Lutz, '80; Wilson and Gremillet, '96), while newly hatched avian neonates are capable of remaining active with much lower body and/or environmental temperatures (Hohtola and Visser, '98; Visser, '98).

Some birds are more tolerant of both higher and lower ambient temperatures than correspondingly sized mammals (Grav et al., '88; Giardina et al., '90; Wilson and Gremillet, '96), and they exhibit a far wider range of body temperature fluctuations than do mammals (Dumonteil et al., '94).

Birds are also capable of lowering their metabolic rates significantly in response to prolonged exposure to extreme cold in order to reduce thermal gradients, thereby lessening heat loss to the environment (Dumonteil et al., '94; Wilson and Gremillet, '96). Alternatively, they are capable of raising BMR by a factor of four or higher to produce metabolic heat sufficient to offset loss (Grav et al., '88). Likewise, birds are capable of storing metabolically generated heat, raising body core temperatures to levels beyond what would be lethal in mammals (Brackenbury, '84). Given the phylogenetic distance between these two clades, it is unlikely that birds and mammals followed the same steps in attaining advanced metabolic strategies. As such, the study of ontogenetic acquisition of temperature regulation may hold insights into the phylogenetic acquisition of these abilities.

In birds, endothermic homeothermy is established between 24 hr and 12–13 days post hatch, sooner in precocial than in altricial birds (Dunnington and Siegel, '84; Starck, '93; Eppley and Russell, '95; Pearson, '98). The onset of homeothermy is generally paralleled by a rapid rise in IP_5 concentration, from relatively low at hatching to approximately 75% that found in the adult over a 5-day period (Isaacks and Harkness, '80), when it is the primary factor in determining oxygen affinity and therefore oxygen delivery to the tissues. Thus, biochemical evidence supports the role of hemoglobin in the acquisition of endothermic homeothermy in developing birds.

It is certain that the initial biochemical and physiological changes leading to an increased metabolic rate, whatever they were, preceded the subsequent anatomical and/or biological consequences (Ricqlès, '75). In fact, animals can respond to excess oxygen either by decreasing respiratory rate or increasing heat output. It is likely that, at first, the genetic changes making excess oxygen available to the tissues were accompanied only by decreased respiratory rates and no other changes. However, these changes would have allowed increased consumption, and therefore increased energy output, upon demand, as in "fight or flight" situations, giving these animals an evolutionary advantage. Thus, it may be that some of these changes may have already been in place in some of the earliest ancestors of this clade. However, even if this is so, some fundamental questions remain with respect to how the acquisition of this advanced physiological state might be recognized in the fossil record.

When in the archosaur/avian lineage can we be confident that endothermy was attained? It is not likely that endothermic capacity would be attained more than once in a lineage, although it may have been lost multiple times.

Was the onset of endothermy marked by postural changes, including a parasagittal gait? All dinosaurs, as well as some early crocodylomorphs, possess upright posture. Therefore, it is possible that a metabolic strategy approximating endothermy may have been primitive for archosaurians and may have been lost in the lineage leading to modern crocodiles (Ricqlès, '78; Carrier, '87, '98). Indeed, based upon some studies (Olson, '59; Ricqlès, pers. com., '99), one could argue that a "trend" toward endothermy may have both preceded and favored the acquisition of upright posture. Alternatively, it may be that although upright posture is strongly correlated with an increased metabolic rate, it may not necessarily be linked to full endothermic capacity.

Can the attainment of endothermy be marked by the onset of fibrolamellar bone characteristics? Although this bone type is found only in rapidly growing animals, it can and does occur in animals that are not functional endotherms (e.g., crocodiles raised under ideal conditions, Horner et al., '99). It is also found in sauropods (Curry, '99), a taxon for which continued metabolic generation of heat (as is demonstrated by birds) is unlikely. Therefore, although this bone type may be correlated with a metabolism sufficiently advanced to support rapid, sustained growth, its presence alone may not be conclusive evidence for full endothermy.

Is evidence for full endothermy found in the onset of herding behavior or long-distance migration? There have been many arguments that endothermy may have been maladaptive for very large animals because surface area/volume ratios would not allow for the efficient dissipation of metabolically generated heat in these large animals, and body temperatures would soon reach deleterious levels if they possessed the metabolic levels exhibited by extant endotherms (Spotila, '80). Fossil evidence supports the hypothesis that sauropods may have lived or moved in groups and probably migrated over distances not achieved in any extant ectothermy. Migration and herding may have been possible and even likely with metabolic strategies consistent with mass homeothermy (Rimblot-Baly et al., '95). Mass homeothermy is the ability of animals with ectothermic metabolism to be functionally endo- and/or homeothermic simply be-

cause of high mass (Weaver, '83). Arguments for mass homeothermy may also be made for the smaller but still massive hadrosaurs and ceratopsians that are thought to have existed in very large herds (Ostrom, '72; Lockley and Pittman, '89; Brett-Surman, '97).

The possession of integumentary insulation is a stronger argument for the attainment of endothermy. It has been demonstrated ontogenetically that, in some birds, metabolic rates increase before the development of any insulation ontogenetically, suggesting the possibility that, phylogenetically, increased resting metabolic rates were attained before the development of integumentary insulation (Pearson, '98; Visser, '98). However, if insulation were first employed only in the very young of rapidly growing animals, its retention may be a paedomorphic trait. Selection for smaller adult body size (Sereno, '97) would necessitate the retention of insulation longer in small animals with elevated metabolism to offset environmental heat loss. Integumentary insulation seems to be more tightly linked to elevated metabolic levels than some other characteristics mentioned herein. New fossil finds from China strongly indicate that some sort of integument-derived filaments were found in adult forms of some small theropods. Members of at least four different dinosaur taxa have been identified to possess these structures (Chen et al., '98; Ji et al., '98, 2001). Among extant vertebrate taxa, no ectotherm possesses any form of integumentary insulation, at any point in ontogeny (Schuh, '51).

Brooding and parenting behaviors similar to those observed in extant birds may also indicate that some metabolic threshold was breached in at least some lineages of dinosaurs. However, not all modern birds incubate their young through bodily contact (Clark, '60); therefore, this behavior may have evolved first for some reason other than to supply the eggs with consistent heat and later co-opted for metabolic reasons. Based upon fossil evidence, brooding has been established for several theropod dinosaur groups (Norell et al., '95; Dong and Currie, '96; Varricchio et al., '97, '99).

Finally, because ectothermic anaerobic metabolism is not sufficient to sustain flight, the onset of powered flight must have been accompanied by metabolic levels approaching true endothermy (Ruben, '95; Ruben et al., '97; Hohtola and Visser, '98). The respiratory systems of adult birds that are active fliers must be uniquely adapted to meet the high metabolic demands and conditions of hypoxia characteristic of this lifestyle. Adaptations

that are evidenced in modern birds include a complex system of air sacs protruding from the lungs into the abdominal cavity, which are ventilated by movements of expanded sternal plates during inhalation. In these birds, the abdomen plays an active role in ventilation during flight. Additionally, all birds, both active fliers and those secondarily flightless, exhibit pneumatic bones filled with air sacs which assist the respiratory system in ventilation. Pneumatic bones are present in theropods (Gauthier, '86), supporting the hypothesis that some elements of this complex respiratory system existed in this clade, possibly because of the need for increased efficiency of oxygen intake to meet increased energy requirements.

The most direct test of the hypothesis we propose, that a mutation in the hemoglobin genes that facilitated more efficient oxygen delivery led to physiological changes that eventually culminated in endothermic capacity, would be the ability to recover molecular fragments from fossil specimens. Detection of inositol pentaphosphate from dinosaur and early bird fossils, but not in coexistent, obviously ectothermic animals would provide the most direct support for the key innovation proposed. However, this molecule may be too labile for preservation over these time periods, and such recovery may not ever be possible, since, in addition to molecular lability, recovery would be highly dependent on diagenetic factors and the chemistry of the depositional environment. Similarly, recovery of hemoglobin fragments sufficient for mass spectrometric sequencing may reveal the amino acid replacements in the region corresponding to the effector-binding pocket. However, even though there is some evidence to suggest that degradative products of hemoglobin may persist across geological time (Schweitzer et al., '97), protein sequencing has not been attempted on fossil specimens of this antiquity, and the hypothesis of molecular preservation is still very controversial.

However, other aspects of this proposal may be directly or indirectly tested. Exposure of muscle tissue to excess oxygen has not resulted in measurable increases in metabolic rates in lab tests (Grassi et al., '98). However, these are of extremely short duration, and metabolic responses of isolated muscle cells may not correspond with those of an integrated organism. Since it has already been demonstrated that numbers and complexity of mitochondria change as metabolic rates increase, both phylogenetically and ontogenetically, *in vitro* monitoring of ectothermic muscle tissues supplied with excess oxygen may show increases in mito-

chondria at the microscopic level, if such infusions were carried out over long periods. However, evolutionary changes are, by definition, slow accumulations; no changes may be observed under these conditions in tissue-specific metabolic rates.

The role of insulation in endothermy has been tested, both by using birds with genetic mutations that make them unable to produce feathers (Yahav et al., '98), and by supplying reptiles with insulation-providing coats (Cowles, '58). In the first case, metabolic rates and temperatures were indeed affected by the lack of feathers for insulation. In the second, no effect was seen on the metabolic rates of lizards supplied with little fur jackets.

A much more comprehensive histological study of fossil organisms would provide information on the growth strategies of multiple taxa and would allow some inferences to be made regarding the possible link between these growth strategies and metabolic rates. It has been shown that growth strategies as reflected by histological characters recorded in bone tissues vary within a single organism, both ontogenetically and between various bones of a single organism (Horner et al., '99). Not only is there a paucity of histological data for extinct birds, but the data are also lacking in comprehensiveness with respect to basal nondinosaurian taxa. For example, histological studies of the gracile sphenosuchians and other members of ancestral archosaurs and ornithomirans have rarely been carried out. Exceptions are histological studies by de Ricqlès, who examined the bones of early archosaurs (Ricqlès, '74, '75, '78). If these specimens show the pattern of fibrolamellar bone interrupted by LAGs, it may provide evidence that elevated metabolic rates capable of sustained rapid growth existed in these taxa, and may support the hypothesis that these elevated rates may be pleisiomorphic for archosaurs, secondarily lowered in one of the two extant groups of archosaurs, the crocodiles. The history of bone histology from earliest synapsids (Upper Carboniferous) to extant mammals is extremely suggestive of similar developments and concurs with anatomical and other evidence (Ricqlès, pers. com., '99).

Finally, molecular techniques may provide a most promising avenue for testing aspects of this model. In 1995, it was demonstrated that changes in amino acid residues at particular regions of the human hemoglobin molecule resulted in a strong bicarbonate effect, similar to what is seen in crocodiles (Komiya et al., '95). This could be taken further with gene knockout technologies that would allow the replacement of one hemoglobin

type for another. Inserting human or avian hemoglobin genes into cells from ectotherms and then supplying these cells with the appropriate organophosphate effectors may verify the role of these hemoglobins in the elevation of metabolic rates. A similar but much more technically challenging experiment would be to replace reptilian hemoglobin genes with avian genes in the embryos of crocodiles or other reptiles, and then measure metabolic rates between these organisms and controls from the same clutch. In vivo substitutions of hemoglobin components would be far more accurate and reflective of evolutionary processes than perfusion experiments on isolated muscle cells or external applications of organophosphates in measuring tissue metabolic shifts, since the whole organism must adapt to increased oxygen supply, not just isolated cells or tissues.

It is recognized that many factors affect thermoregulation in living taxa and would almost certainly have done so in dinosaurs and their ancestors as well. For example, the pineal gland (George, '82) and the hypothalamus have profound effects on metabolism and temperature regulation. Additionally, levels of various hormones, including adrenaline and nor-adrenaline, thyroid, and parathyroid hormones, have a significant impact on temperature regulation. However, although these factors do indeed affect thermoregulation and homeostasis, they seem to be more important in regulation of existing strategies rather than in acquisition of novel ones, and they are beyond the scope of this paper.

Likewise, metabolic strategies employed by living taxa, either bird or mammal, vary greatly and are affected by body size, body temperature, climate, and altitude, to name only a few. Once endothermy was acquired in a lineage, it was no doubt modified as acclimation to new conditions required. For example, metabolic energy may have been used in sauropods to achieve tremendous size, and once the size necessary for mass homeothermy was attained, the generated energy could be devoted to further growth and maintenance of body tissues rather than toward maintaining body temperatures. Theropods may have used metabolically generated energy to maintain higher activity levels and, as selection favored a trend toward small sizes in some taxa, to maintain body temperatures and offset heat loss to the environment.

SUMMARY

This paper describes a ten-point model for the evolutionary onset of endothermy in the bird-

theropod lineage. The model uses a genetic change as the starting point, resulting in an enhanced oxygen delivery system. This genetic change could have occurred without any immediate morphological or physiological changes, because animals can compensate for increased exposure to oxygen by decreasing breathing rates. However, possessing a more efficient oxygen delivery system would allow the animal to have a more ready supply of aerobic energy in "fight or flight" situations than animals without this trait, thus contributing to survivability. As increased activity levels became adopted in the lineage, more rapid breathing would expose tissues to greater oxygen levels and the concurrent toxic effects of oxygen free radicals, causing the compensatory multiplication of mitochondria in these tissues. A side product of the reduction of free radicals to harmless water is the generation of ATP and waste heat, which would be utilized by the organism to increase basal metabolic rates. Epigenetic and/or morphological changes would follow as a natural consequence of these physiological changes. The changes outlined herein would not work in isolation, and changes in addition to the ones proposed would no doubt follow. The model provides a mechanism for the gradual onset of metabolic change in a lineage, a necessary factor when discussing such an encompassing transition. Thermoregulation and thermal strategies in living taxa are complex and are affected by many factors. The acquisition of endothermy from an ectothermic state was no doubt equally complex.

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