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# Selective factors in the origin of the mammalian diaphragm

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*Abstract.*—The origin of endothermic homeothermy and of high metabolic rate in mammals is currently believed to be the result of early (Mesozoic) selection in advanced cynodont therapsids and/or early mammals for either (1) enhanced thermoregulatory capacity or (2) increased powers of endurance and stamina. Selective factors underlying the origin of specialized respiration/ventilation-support systems in mammals are possible indices of the validity of these two hypotheses. One such support structure is the diaphragm, a specialized muscle that facilitates lung ventilation. We tested capacity for maintenance of resting metabolic rate, thermoregulation, and for extended, intense exercise in laboratory rats (*Rattus rattus*) in which diaphragm function had been completely ablated. The results were virtual elimination of aeroboic scope (active metabolic rate − resting metabolic rate) but resting metabolic rate was unaffected. Thermoregulatory capacity was unimpaired to at least 8° below lower critical temperature. These and other data suggest that the origin of the mammalian diaphragm, as well as mammalian metabolic rates, may have been related to selection for greater levels of sustainable activity rather than for functions associated with thermoregulation.

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## Introduction

The persistent maintenance of high and stable body temperature in the presence of often greatly fluctuating ambient temperature is one of the most distinctive and unique features of mammals and birds. Such "warm-bloodedness," or, more correctly, endothermic homeothermy, generally results from a combination of high resting, aerobically supported heat production rates (aerobic metabolism) in virtually all soft tissues, sensitive internal "thermostats," and insulation sufficient to retard excessive heat loss.

High avian and mammalian resting aerobic metabolic rates appear linked to greatly enhanced powers of aerobic metabolism during periods of physical activity. Virtually all vertebrates, ectotherms and endotherms alike, exhibit fairly constant ratios between resting and maximally active rates of oxygen consumption. Consequently, in addition to elevated resting oxygen consumption rates, birds and mammals have markedly expanded powers of aerobically supported endurance and stamina as well as higher levels of "routine" activity (Bennett and Ruben 1979; Gordon et al. 1982).

Mammalian and avian endothermic homeothermy is, however, energetically expensive: laboratory measurements indicate resting birds and mammals expend about 5–10× the calories metabolized by resting ectotherms at similar body temperatures (Gordon et al. 1982). Energetic requirements for birds and mammals in the wild may be as high as 30× that of equivalent-sized amphibians and reptiles (Nagy 1982).

A few genera of pythons (e.g., *Python*) and some large-size fish (including some tunas and sharks) are able to maintain somewhat greater than ambient deep body temperatures. This is achieved in brooding female pythons by enhanced heat production via powerful, spasmodic axial muscle contractions. Fish combine high activity levels with well-developed vascular countercurrent heat exchange ("rete-mirable") systems.

However, "endothermy" in those taxa is not truly comparable to that of birds and mammals: in the absence of constant heat-generating axial muscle contractions in the fish and pythons, it is unlikely that sufficient heat is generated to maintain elevated body temperatures. In any case, body temperature in these taxa is not known to exceed ambient temperature by more than about 10° (Eckert and Randall 1983; Harlow and Grigg 1984). Thus, the source and magnitude



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of caloric expenditure as well as the stability and marked elevation of body temperature associated with avian and mammalian endothermy is truly unique among the Metazoa.

It is hardly surprising, therefore, that factors responsible for the origin of avian and, especially, mammalian endothermy have historically been the subject of considerable speculation and debate. Traditionally, the evolution of high resting metabolic rate in mammals has been attributed to selection for endothermically based thermoregulation in early (Mesozoic) mammals and their immediate ancestors, the cynodont therapsid reptiles (Hopson 1973; Crompton et al. 1978; McNab 1978). Similarly, a number of specialized ventilation/respiration-support structures in mammals (four-chambered heart, secondary palate, etc.) seem to have evolved contemporaneously with endothermy and are thought to have originated, at least in part, in association with selection for increased thermoregulatory capacity in those groups (e.g., Brink 1956; Gunderson 1976; Stahl 1974). For a discussion of thermoregulatory and metabolic capacities of therapsids, see Bennett and Ruben (1986).

However, on the basis of a relatively constant 10-fold ratio of maximal/minimal oxygen consumption rates in a variety of ecto- and endothermic vertebrates, Bennett and Ruben (1979) suggest the initial factor responsible for the evolution of high mammalian resting metabolic rate was, instead, selection for increased powers of sustainable, aerobically supported physical activity (see also Taigen 1983). According to this hypothesis (the "aerobic capacity" model), elevated therapsid or mammalian metabolic rates were initially advantageous for generation of higher levels of sustainable activity. Therefore, original selective advantages of elevated aerobic metabolic rates had little or nothing to do with selection for increased resting metabolic rates or thermoregulatory powers. However, chronic selection for expansion of aerobically supported activity levels, combined with the described linkage of resting and active aerobic metabolic rates, led simultaneously to elevated resting rates of oxygen consumption. Eventually resting oxygen consumption rates were sufficiently increased to maintain endothermic homeothermy.

Clarification of selective factors in the origin of ventilation/respiration-support structures would seem particularly instructive in evaluating the validity of these two hypotheses. Accordingly, we describe here a series of experiments, the results of which seem to illuminate elements responsible for the origin and evolution of the mammalian diaphragm—a structure whose origin is in fact, frequently assumed by other authors to have been related to selection for increased thermoregulatory powers (Brink 1956; Stahl 1974; Gunderson 1976). Our experiments involved assessment of the relative importance of the diaphragm in helping to maintain endothermy and/or expanded capacity for aerobiosis in extant mammals.

The diaphragm is a transversely situated muscle that forms the posterior border of the thorax. It functions, along with the intercostal muscles, to help ventilate the lungs during both rest and exercise. Significantly, diaphragm ontogeny, morphology, and function are identical in both prototherian and therian mammals—lineages believed to have diverged at the very outset of mammalian evolution, about 185 ma B.P. (Lillegraven et al. 1979). Thus, the diaphragm may well have been completely developed in the very earliest mammals, the late Triassic Morganucodontidae, and seems to have been evolving or fully developed in the late cynodont therapsids (Brink 1956).

The mammalian diaphragm is innervated solely by the phrenic nerves (which arise from cervical vertebrae #4–6), and interruption of those nerves results in complete paralysis of the diaphragm (Sant'Ambrogio et al. 1963). Our experiments involved ablation of the phrenic nerves (phrenicotomy), which allowed us to measure resting and active metabolic rates in unrestricted laboratory rats with paralyzed diaphragms, as well as in unrestricted intact individuals.

## Materials and Methods

Twelve *Rattus rattus* (mean mass = 431 g) which had been fasted for 24 h previous to experimentation were used to determine resting and active oxygen consumption rates. Such measurements constitute reliable indirect indices of aerobic metabolic rates. Determinations were conducted on individual animals placed in an airtight, transparent lucite chamber measuring 52 × 30 × 15 cm that had been covered by black

cloth. Individuals were placed in the container at 1200 PDT. Air was metered through the chamber at the rate of 1 liter/min. Measurement of resting metabolic rate, which was recorded at 2200 PDT, involved stopping the air flow through the chamber for 5 min. Immediately following this period a peristaltic pump mounted at the excurrent air port of the chamber was utilized to pump air from the chamber into an evacuated balloon. Approximately 500 cc of air from the chamber were collected in about 10 s; air flow through the chamber was then restored immediately. The air sample from the balloon was passed through columns of Drierite (anhydrous $CaSO_4$) and Ascarite (Na-hydrate asbestos) and then metered through a Beckman paramagnetic oxygen analyzer (Model E-2; range = 20–21% $O_2$). Readings from obviously active animals were discarded, hence these values approximate true resting metabolic rates.

Oxygen consumption during exercise (active metabolic rate) was determined after sufficient time had lapsed to allow the oxygen concentration in the chamber to return to ambient room levels. Air flow through the chamber was then stopped for a period of 5–7 min, during which the animal was induced to activity by administration of a series of 2–8-v electrical shocks via gold-plated safety-pin electrodes which had been implanted into the hind limbs before resting metabolic rates had been measured. The transparency of the chamber allowed observation of the animal's behavior so that shocks were administered just frequently enough to stimulate the animal to movement at all times during the activity period. The animal's response to stimulation was intense and was assumed to represent near-maximal activity levels. Immediately following the activity period, a sample of air was removed from the chamber as described above, and analyzed for oxygen content.

Oxygen content in the chamber never fell below 20.5% during either resting or active oxygen consumption determinations. Measurement of resting and active oxygen consumption rates were determined from the percent decrement in oxygen content of the chamber given the initial and final fractional concentration of oxygen in the chamber, the animal's volume, pressure, and humidity (with a Hygrodynamics humidity sensor). All reported values are corrected for STP and include compensation for $CO_2$ removal by the method of Hill (1972). All values for resting metabolic rates represent the minimum of three such daily determinations for each animal. Active oxygen consumption rates represent maximal values obtained during at least two such determinations for each individual.

Following determinations of resting and active metabolic rates in intact individuals, the paired phrenic nerves in eight animals were completely severed. Phrenicotomy was accomplished by etherizing experimental subjects and exposing the ventral aspect of the cervico-thoracic region. A 2 mm section of both the left and right phrenic nerves was then removed at approximately the level of the seventh cervical vertebra. Identical surgical procedures were conducted on four "sham" operated subjects, except that the phrenic nerves were exposed but not severed. Immediately following phrenicotomy, the wound was sterilized and closed with "butterfly" bandages. Animals regained consciousness about 10–15 min after completion of surgical procedures. All experimental subjects recovered fully within 24–28 h after surgery and appeared to behave normally except that phrenicotomized animals exhibited an exaggerated expansion and contraction of the rib cage during each breathing cycle.

Approximately 7 days following surgery, resting and active measurements were again recorded for both phrenicotomized and sham-operated experimental subjects. Following this set of measurements, all rats were humanely killed by etherization. Autopsies were then conducted on surgically altered individuals to verify complete and continued disruption of the phrenic nerves in these subjects.

Resting and active measurements were taken at $T_a$ = 30°C. An additional set of experiments was conducted to determine the effect of phrenicotomy on thermoregulatory capacity. Anally inserted (to 1 cm) copper-constantin thermocouples, connected to a Honeywell Class 15 recorder, were used to monitor $T_b$ at $T_a$ = 20°, 25°, and 30°C. Readings were taken from intact ($N$ = 6) and phrenicotomized ($N$ = 5) animals exposed to ambient temperatures for 4 h.

## Results

During stimulation, intact individuals underwent an initial period of vigorous activity lasting

about 1.5–2.5 min. This activity consisted of attempts to escape by running or jumping. Following this initial period of intense exercise, all individuals exhibited reduced activity levels. Escape attempts became more sluggish and less coordinated. By the end of the exercise period, animals were close to total exhaustion. Escape attempts continued, but these consisted of slow, almost spasmodic movements.

Resting and active oxygen consumption measurements for intact individuals at $T_a = 30°$ are reported in Fig. 1. Mean aerobic scope (active metabolic rate − resting metabolic rate) for these subjects was 1.66 cc $O_2/g \times h$.

Activity patterns exhibited by phrenicotomized animals were essentially similar to those previously described. However, phrenicotomized animals appeared to tire markedly faster than intact individuals following the initial 1.5–2.5 min of exercise.

Resting and active oxygen consumption measurements at $T_a = 30°$ for phrenicotomized animals are reported in Fig. 1. Basal rates were virtually identical to those of intact animals ($P > .5$, $t$-test). However active rates of oxygen consumption in phrenicotomized animals were significantly lower than those of intact animals ($P < .05$, $t$-test) and, in comparison with intact animals, aerobic scope was reduced by about 80%.

Both resting and active oxygen consumption rates of sham-operated individuals were indistinguishable from those of intact animals ($P > .5$, $t$-test).

All experimental individuals retained normal thermoregulatory capacity over the range of ambient temperatures investigated. $T_b$ remained at 37.7° (±.3°C) from $T_a$ = 20–30°C ($P < .01$, $t$-test).

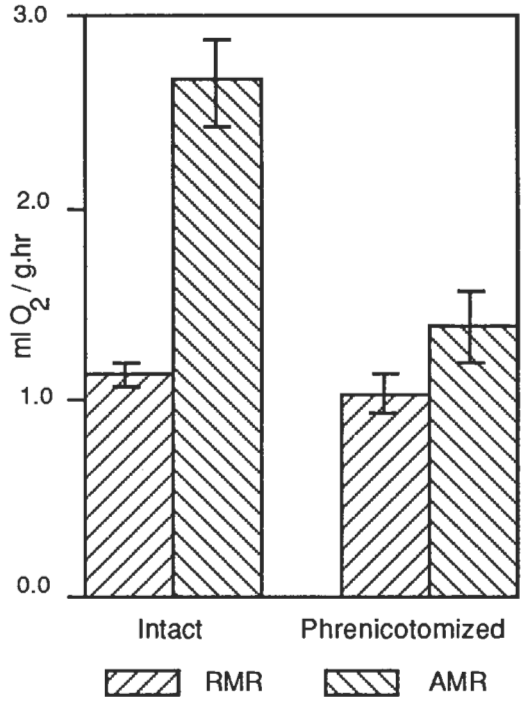


FIGURE 1. Resting (RMR) and active (AMR) oxygen consumption rates of intact and phrenicotomized laboratory rats. Mean values are represented; vertical lines represent standard errors. $N = 8$ for all categories.

## Discussion

According to the "aerobic capacity" model, mammalian metabolic rate is merely the final outcome of selection for expanded maximal rates of aerobiosis to facilitate increased stamina and overall activity levels in the therapsid-mammal lineage. Increments in endurance and routine activity levels are assumed to have been advantageous to late therapsids and early mammals, animals that appear to have been relatively active predators and foragers (Kemp 1982). The linkage of resting and active rates of aerobic metabolism would, presumably, have resulted in simultaneously expanded resting metabolic rates. Thus, selection for increasingly active life-style in mammals and their immediate ancestors was fortuitously responsible for elevated rates of resting metabolism until the latter had reached sufficient levels to support high and constant body temperature.

Data reported here lend support to such a scenario. The diaphragm, one of those respiration-ventilation support structures that probably appeared about the time of origin of endothermy, seems essential to facilitate rates of aerobiosis required during periods of intense exercise. It is, however, superfluous for maintenance of endothermic homeothermy, at least at ambient temperatures of 20°–30°C. Such a range of temperatures is similar to that hypothesized for worldwide terrestrial environments of the Late Triassic-Early Jurassic, the time of origin of the mammals (A. J. Boucot, pers. comm.).

Thus, resting metabolic rate and thermoreg-

ulatory competency in phrenicotomized individuals was unaffected, but the capacity for increased aerobic metabolism during activity was severely restricted (Fig. 1). Significantly, humans who have suffered accidental paralysis of the diaphragm apparently remain capable of maintaining normal resting metabolic rate (Strauss 1933), as do phrenicotomized dogs (Schlaepfer 1926) and goats (Jansen 1931).

Other, independent observations suggest the diaphragm would not have been necessary for the establishment of endothermic homeothermy in mammalian ancestors. Some saurians (e.g., varanid lizards) attain maximal rates of oxygen consumption equal to, or greater than, resting metabolic rate of mammals (Bennett 1972). Thus even the reptilian ventilatory apparatus appears sufficient to support mammalian resting oxygen consumption rates. It seems quite reasonable, therefore, to assume that even without a diaphragm, therapsids or early mammals would have been quite capable of achieving lung ventilation sufficient to support mammalian resting metabolic rates. These observations tend to confirm our observations regarding the role and origin of the mammalian diaphragm. They also raise serious doubts about long-held assumptions that mammalian specializations such as the four-chambered heart, secondary palate, or diaphragm evolved as a result of selection for higher resting metabolic rate and endothermic thermoregulation.

The preceding implies that the reptilian ancestors of mammals, with or without a diaphragm, would probably have possessed ventilatory capacity sufficient to maintain resting metabolic rate consistent with endothermic homeothermy. However, the elimination of locomotory-related aerobic scope in phrenicotomized animals may indicate the diaphragm was and is essential to facilitate elevated rates of oxygen consumption during physical activity in mammals. Similarly, Alexander (1975) has previously suggested that the origin of the mammalian diaphragm may have been associated with locomotor-related mechanical constraints on rib-cage function. Such constraints may have arisen from the development of a distinct lumbar region and the use of sustained asymmetric gaits in early mammals or their immediate ancestors. Moreover, recent studies have indicated an obligatory integration of respiratory and locomotor mechanics in a variety of mammals (Bramble and Carrier 1983).

Bennett and Ruben (1979) have previously suggested that unrelated data from comparative physiology and paleoclimatology indicate that mammalian resting and active metabolic rates evolved in response to selection for increased stamina and elevated rates of sustainable activity, not for increased capacity for thermoregulation. It is not surprising that the diaphragm might have evolved for similar reasons.

Finally, in light of reduced locomotory-related aerobic scope in phrenicotomized animals, it seems paradoxical that these individuals should be able to thermoregulate normally down to $T_a$ = 20°. Maintenance of $T_b$ below $T_a$ = 28°, the lower critical temperature for *Rattus* (Hart 1971), implies oxygen consumption rates greater than resting metabolic rate. Perhaps intercostal musculature is capable of supporting greater rates of oxygen consumption during nonlocomotory periods in these animals. Indeed, this does seem to be the case in at least some lizards (Bramble, pers. comm.).

Further investigations into factors involved with the origin of such structures as the mammalian secondary palate and four-chambered heart seem warranted. Data presented here seem to raise reasonable doubt that these structures must necessarily have evolved for thermoregulatory purposes.

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