ANAEROBIC METABOLISM IN A LIZARD (ANOLIS BONAIRENSIS) UNDER NATURAL CONDITIONS[1]

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Lactate contents of *Anolis bonairensis* (Sauria: Iguanidae) were measured to determine the extent of anaerobic metabolism under a variety of laboratory and field conditions. Groups of lizards in the laboratory which were resting quietly or active for 3 min contained an average of 0.55 and 1.45 mg lactate/g body mass, respectively. Anoles sampled in the field at four different times of day had a mean lactate content of 0.75 mg lactate/g, a value significantly greater than that of resting animals in the laboratory. Individual lizards in each field group exceeded lactate contents of 1.0 mg/g at all times of the day. Lactate accumulation was proportional to intensity of territorial defense in a separate series of territorial intrusion experiments. This anole commonly undertakes bouts of anaerobic metabolism under natural field conditions, and its behavioral repertoire is considerably extended by this anaerobic ability.

INTRODUCTION

Reptiles, along with many other vertebrate ectotherms, have a relatively low capacity for sustained activity. Walking or running speeds which can be maintained by lizards are generally less than 1.0 km/h (Moberly 1968; Bakker 1972; Bennett and Gleeson 1979; Gleeson 1979; Gleeson, Mitchell, and Bennett 1980). These limitations are the result of a limited ability to support high levels of aerobic metabolism. Additional behavioral capacity may be provided by anaerobic metabolism, principally the production of lactic acid in the locomotory muscles. While this supplementary mechanism of energy production permits higher levels of performance, it is also accompanied by rapid fatigue and loss of activity. Thus the utilization of anaerobic metabolism presents these organisms with a dilemma: the opportunities of intense and rapid activity versus the debilitating effects of exhaustion.

The extent of the utilization of anaerobic metabolism by terrestrial lizards freely active in the field has not been investigated. Numerous laboratory studies (Bennett and Dawson 1972; Bennett and Licht 1972; Bennett and Gleeson 1976) have indicated that anaerobic scope and capacity are very high in these animals. Great amounts of lactic acid can be produced over short-term intervals. The relevance of these experimental laboratory observations to the behavior of animals active under natural circumstances is not known. Are these anaerobic abilities used under field conditions, or are the disruptive effects on functional capacities and behavior so great that anaerobic metabolism is normally avoided?

The subject of this study is the iguanid lizard *Anolis bonairensis*, an endemic species on the arid Caribbean island of Bonaire in the Netherlands Antilles. Aspects of the natural history, energetics, thermoregulation, and water relations of this lizard have been reported elsewhere (Hillman and Gorman 1977; Bennett and Gorman 1979).

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It is a small (2–10 g) shrub and tree-dwelling anole which is very abundant, territorial, and site specific. It is strictly insectivorous, eating primarily ants which forage on and around the trees. It is not a highly active animal, remains immobile for more than half of its diurnal period, and moves an average of only 0.4 m/min when active (Bennett and Gorman 1979). This sit-and-wait type of foraging behavior is typical of many lizards.

We have measured lactic acid formation by *A. bonairensis* in its natural environment. Animals were quick-frozen directly upon capture in the field, and their lactate contents were compared to those of resting controls. Lactic acid contents of field-caught animals which were greater than these resting levels were assumed to indicate recent, anaerobically supported activity in these animals since lactic acid in lizards persists in the body for periods of several minutes to hours after its formation (see Bennett 1978). We also examined the effect of territorial defense on lactic acid accumulation in adult male anoles.

MATERIAL AND METHODS

Our measurements were made during early June 1976 at Fontien, Bonaire, a natural spring area with many large trees and shrubs and abundant anoles. A total of 60 specimens of *Anolis bonairensis* were used, including 23 adult males (4.4–11.8 g), 32 adult females and young males (2.0–4.2 g), and five juveniles (1.3–1.9 g). All animals were captured by hand.

Lizards were quick-frozen under a variety of natural and laboratory conditions, and the carcasses were later analyzed for lactate content. Animals were frozen by immersion into a 1-liter Thermos flask containing 95% ethanol and dry ice, a mixture with a temperature of −55 to −60 C. The carcasses froze in approximately 1–2 s, and no muscle movements were observed. They were kept frozen and submerged in the liquid until returned to the laboratory. Each was then removed with long forceps, weighed rapidly on a pan balance, and homogenized immediately in 30 ml of 0.6 N perchloric acid in a Sorvall Omnimixer. Care must be taken that the carcasses do not thaw during this procedure prior to homogenization since large quantities of lactic acid are produced after freeze-thaw activation of the glycolytic enzymes. The supernatant liquid was stored and transported to the United States for analysis of lactate content according to the spectrophotometric method of Bennett and Licht (1972). Prior validation studies on resting lizards indicated that resting animals which were quick-frozen by this method had lactate contents equivalent to those of another group which were decapitated and homogenized immediately without freezing. The homogenization of animals of different sizes in a constant volume of perchloric acid did not result in any apparent size-dependent artifacts: lactate concentrations in field animals were independent of body size ($r = .08$, 37 df).

Our study consisted of three parts: (1) lactate concentrations of animals resting and exercised under laboratory conditions; (2) field lactate contents of undisturbed animals; and (3) lactate content and territorial defense. Sixteen lizards were collected in the field in the afternoon and transported to the laboratory for experimentation later in the evening. They were divided into two groups for determination of lactate concentrations in resting and maximally active animals. Each lizard was placed individually in a darkened container in the laboratory. After 7 h (1500–2200 local time) at 28 C, the normal nocturnal body temperature of these lizards, eight animals were removed individually and were frozen immediately and eight were stimulated to intense activity for 3 min before freezing. The latter lizards were placed in a small box, and they ran and jumped until exhausted, attempting to escape from the experimenter. Lizards of both groups were then homogenized immediately.

Lactate concentrations were determined in animals taken directly in their natural environments. At four different times during the day (0620–0730, 1200–1300, 1700–1840, 2330–0015 local time), nine to 10 animals were captured individually by hand and frozen immediately. Lizards were sighted from a distance and kept in view until seizure. Any animal that moved was not captured; any animal that struggled after capture was released. Previous behavior was not monitored. The frozen carcasses were transported to the laboratory and homogenized about 1 h after capture.

A short series of experiments was undertaken on lactate accumulation as a result of territorial defense by five adult male anoles. An adult male *Anolis* was tethered about the pelvic region with nylon monofilament line attached to a fishing pole, and the tethered male was introduced into the territory of a resident male. This technique of territorial intrusion has been utilized previously to elicit territorial and aggressive displays in *Anolis* (Gorman 1968). A similar range of responses, varying in magnitude from uninterest to prolonged aggressive interactions by the resident male, have been observed during natural encounters by these lizards in the field (Gorman 1968). In our experiments, we recorded the behavior of the resident male for several minutes. Behavior prior to introduction of the tethered male was not observed. The resident was then captured and frozen immediately. The carcasses were transported to the laboratory and homogenized.

RESULTS

The anoles resting in the laboratory had a mean lactate concentration of 0.55 mg lactate/g mass (±0.053 SE, no. = 8, range = 0.31–0.77). Lizards stimulated to maximal activity underwent very active evasive behavior for only about 1 min ($\bar{x}$ = 65 s, range = 40–105 s). The righting response was lost after 2 min, and the lizards appeared thoroughly exhausted and unresponsive after 3 min. Mean lactate concentration of these lizards after 3 min of exhaustive activity was 1.45 mg/g (±0.050 SE, no. = 8, range = 1.29–1.75).

Lactate contents of anoles captured in the field are reported in figure 1. Mean values of each time group range between 0.69 and 0.81 mg/g. None of these groups is significantly different from the others (ANOVA, P = .77). Consequently, they are combined to yield a mean lactate concentration of 0.75 mg/g (±0.040 SE, no. = 39, range = 0.40–1.60). The lactate content of this aggregate sample of field-caught animals is significantly greater than that of the anoles resting in the laboratory (Student's one-tailed t-test, P = .020). In each of the field-sampled groups, three or more animals had lactate concentrations exceeding the maximal observed for laboratory resting animals. In each of the field groups, at least one animal had a concentration in excess of 1.0 mg lactate/g.

Lactate concentrations of resident animals in the territorial intrusion experiments are reported in table 1. Lactate content is generally correlated with intensity of defensive response. Lizards which exhibited little movement or display behavior had lactate contents which were characteristic of those sampled randomly in the former field observations. Anoles with long and vigorous displays, involving much movement and many attacks on the intruder, had lactate concentrations of 1.06 and 1.12 mg lactate/g.

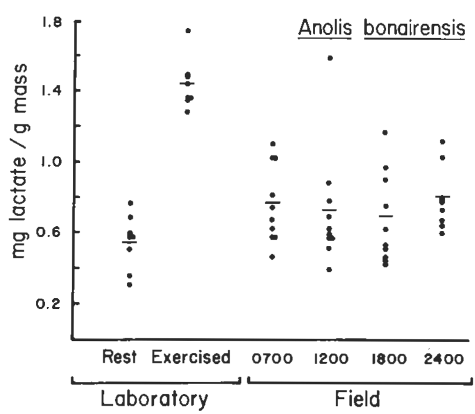

FIG. 1.—Lactate contents of *Anolis bonairensis* under laboratory and field conditions. Horizontal bars indicate mean values for each group. Experimental protocol is described in text.

TABLE 1

LACTATE CONCENTRATION AND TERRITORIAL DEFENSE IN ADULT MALE "ANOLIS BONAIRENSIS"

Animal	Mass (g)	[Lactate] (mg/g)	Behavioral Response (min Observed)
A.........	10.5	.78	Little display, little movement. 2 throat fan displays, 2 brief attacks (6 min)
B.........	8.3	.87	No display. Moved 0.3 m (2 min)
C.........	9.3	1.06	No display. Moved 2 m (2 min)
D........	11.8	1.06	Long vigorous display. Ran and jumped 3.5 m, 6 throat fan displays, 3 attacks (2.4 min)
E.........	9.7	1.12	Long vigorous display. Ran and jumped 2.7 m, 7 throat fan displays, 8 attacks, 1 fall from tree (2.5 min)

DISCUSSION

These observations indicate that natural activity in the field by this small anole may involve a degree of anaerobic metabolism and result in the accumulation of lactic acid. The difference in lactate content between resting and field-active animals suggests that anaerobic metabolism makes a significant contribution to daily activity in this lizard. Each of the field groups contained animals with lactate contents similar to those of lizards resting quietly in the laboratory. However, each of the field groups also contained animals with lactate contents well above those of resting animals, some exceeding 1.0 mg lactate/g mass. Similarly high levels of blood lactic acid are generally associated with prolonged and vigorous activity by reptiles in the laboratory (Bennett and Dawson 1976). The presence at all times of the day of individuals with high lactate contents indicates either that anaerobically supported activity is undertaken throughout the day, even at night, or that lactate can persist for long periods after its formation.

The territorial intrusion experiments support the association of lactic acid formation with vigorous activity under field conditions. The highest lactate levels were achieved in the most active territorial defenders. Some individuals in each of the field groups (fig. 1) had lactate contents which were equivalent to the territorial lizards which were observed to undergo vigorous activity. Consequently, similarly intense exertion, supported anaerobically, may have occurred in these animals prior to sighting and capture.

Although these anoles may undergo significant bouts of anaerobically supported activity, it is not possible on the basis of these observations to quantify their frequency or energetic contribution. The rate of lactic acid turnover and elimination under these circumstances is not known. The lactic acid may have been formed in only a single burst of activity or may be the result of low-level continuous production. Anaerobic metabolism certainly accounts for only a minor proportion of the total daily energy utilization of these lizards. Anaerobic energy utilization, however, permits a behavioral capacity beyond that provided by aerobiosis. Consequently, its importance cannot truly be measured in caloric or temporal terms alone. The capacity for anaerobic activity metabolism will be strongly selected even if it is used only a single time in the life of the animal, if its use permits survival and later successful reproduction.

Few comparable data on levels of field anaerobic metabolism are available for reptiles or for any animals. Female green sea turtles have been reported to accumulate moderate lactic acid concentrations (45 mg/ml blood) during nest-building and egg-laying activities (Jackson and Prange 1979). This anaerobic capacity permits additional energy utilization and activity at a crucial period of reproduction for this turtle. Marine iguanas do not generally utilize supplementary anaerobic metabolism during the low-level, intermittent activity which occurs during basking (Gleeson 1980). Some observations are available on the utilization of anaerobiosis during diving in reptiles. It is somewhat ironic that the original associations of lactic acid production were made with diving metabolism. Although reptiles exhibit extensive lactic acid formation during forced dives (Andersen 1961; Murdaugh and Jackson 1962; Robin et al. 1964; Berkson 1966; Bartholomew, Bennett, and Dawson 1976), unrestrained free dives undertaken voluntarily generally do not involve lactate accumulation and are presumably supported aerobically (Wood and Johansen 1974; Seymour and Webster 1975; Seymour 1979; Gleeson 1980). Anaerobic metabolism appears to be reserved in free-diving animals for situations where additional escape or pursuit capacity is required.

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