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HEAT PRODUCTION BY THE EASTERN BOX TURTLE (*TERRAPENE CAROLINA CAROLINA*) AS MEASURED BY DIRECT CALORIMETRY.[1]

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ABSTRACT. - Heat production by eastern box turtles (*Terrapene carolina carolina*) has been measured directly. The diet was a mixture of animal and plant foods with vitamins and bone meal. The calorimeter was a four liter Dewar flask kept in an insulated box at 24° ± 1°C. After a fast of three days each of ten animals was studied by hourly thermometer readings for a twelve hour period. The mean heat production was steady (linear) at 0.12 cal $h^{-1}g^{-1}$ for fasting animals resting in the dark at 24°C ± 1°C.

The purpose of this study has been twofold. First, the production of heat by the eastern box turtle has been observed under controlled conditions to test the reliability of the direct calorimetric methods. Second, inasmuch as this method appears to have been little used, if at all, then a comparison between the records obtained from the direct method with those reported by others who have made use of indirect calorimetry is an appropriate goal. Animals whose oxygen consumption or carbon dioxide production have been used to calculate heat production include: lizards (Martin, 1903); frogs (Krogh, 1914); alligators, large snakes, rattlesnakes, land tortoises (Benedict, 1932); painted turtles (Baldwin, 1926); red-eared turtles (Hutton *et al*, 1960); and Galapagos turtles (Dawes and Mestyan, 1963). Kaplan (1974) has summarized this literature. In other words, if one assumes that O_2 and CO_2 measurements can be used as indices of heat production in laboratory animals, and if these results agree with data directly obtained by the use of thermometers, then a simple method for direct calorimetry has utility.

MATERIALS AND METHODS

Animals. Ten eastern box turtles (*Terrapene carolina carolina*) were collected from their natural woodland habitats in eastern and southern Illinois. The species and sex were ascertained by the key given in Smith (1961). Their mean weight was 437 g (range 245 to 565 g) and the mean

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carapace length was 126 mm (range 111 to 138 mm). (See Rothschild, 1915).

The animals were housed in environmentally controlled rooms for four months before this study was started. Throughout the investigation lights, coming from 3-40 watt GE cool white fluorescent bulbs, were on from 6 AM to 6 PM local time. The temperatures were 27°C by day and 22°C by night. Dr. J. Wallach, Brookfield Zoo, has reported 27°C as being within a suitable range for laboratory adaptation by turtles. Sturbaum and Riedesel (1974) agree. The turtles in the present study have lived for eighteen months in our laboratory without a single death; each has gained weight. Health was maintained by the use of uncooked base diet consisting of two parts freshly ground beef, one part ground beef liver, and one part beef bone meal, beef fat, and beef bone marrow. This was supplemented with cod liver oil and a pediatric vitamin formula. The turtles were kept in pairs matched by size. The vitamin weekly supplement for each pair of turtles provided: iron 10 mg, A 0.45 mg, D_2 10 mcg, C 30 mg, B_1 0.4 mg, B_2 0.6 mg, B_6 0.4 mg, and niacinamide 6 mg. Fresh water was available at all times. In addition, sun lamp treatment, exercise routines and cage -animal cleanliness were maintained each week. Each pair was offered 50 g of meat diet each Monday and green vegetables with fruit each Thursday. The animals were housed in polycarbonate tubs with medium page bark (Far West Forests, Sacramento, California) as a substrate. Each animal had no food for three days before the initiation of this study.

Materials. The calorimeter consisted of a four liter Pope Dewar flask with an insulated top. A wet bulb thermometer, accurate to one-tenth degree C, was inserted through the calorimeter top. To allow for pressure equalization within the calorimeter, two catheters were also inserted through the insulated top and placed down inside the Dewar flask. Two additional thermometers were used to measure the ambient temperature and were positioned next to and at equal distances from the calorimeter. The calorimeter was placed inside an insulated box; the whole system (calorimeter, insulated box, and thermometers), was housed in another environmentally controlled room kept at 24°C $\pm$ 1. Prior to use, all thermometers were standardized with reference to one selected thermometer, namely the one inserted through the calorimeter top. The whole system was further insulated from air currents by placement under a hood consisting of polystyrene panels covered with insulating blankets.

Methods. Calibration of the calorimeter was done by using both hot (28°C) and cold (17°C) water. An amount of water equal to the weight of a corresponding turtle was placed inside the calorimeter. Temperature readings were taken every hour for twelve hours from all three thermometers. In this manner the thermal properties and heat flow into or out of the calorimeter were determined, permitting the calculation of a temperature gradient defined as follows: (calorimeter temperature - mean ambient temperature). Prior to actual experimentation all of the materials were allowed to equilibrate in the environmentally controlled room for a twelve hour period. During the experimental phase a turtle was placed inside the calorimeter along with twenty milliliters of deionized water. The heat production of the turtle was measured directly by readings taken from the one reference thermometer. Temperature readings were made every hour for a twelve hour period. The first two hours of experimentation were discarded in order to allow the turtles to adjust to the calorimeter.

Statistical Considerations. The generalized equation for heat balance, as stated in 1945 by Brody, has been used:

(Heat Stored) = (Metabolic Heat) - (Evaporative Heat) ± (Conduction) ± (Radiation) ± (Convection) ± (Heat Equivalent of Work) Eq. 1

By experimental design the last five variables in Eq. 1 become zero. The heat equation therefore became:

(Heat Stored) = (Metabolic Heat) Eq. 2

RESULTS

The mean heat production of these turtles (*Terrapene carolina carolina*) fasting, at rest, and in the dark at 24°C, was 0.12 cal $h^{-1}g^{-1}$, with a standard deviation of ± 0.05 cal $h^{-1}g^{-1}$ (Table 1). In international SI units these numbers are 0.52 J $h^{-1}g^{-1}$ ± 0.20 J $h^{-1}g^{-1}$, or 0.14mWg^{-1} ± 0.06 mWg^{-1}.)

TABLE 1. Heat Production of the Eastern Box Turtle (*Terrapene carolina carolina*) at 24°C Measured by Direct Calorimetry.

Sex	Body Weight grams	Heat Production cal $h^{-1}g^{-1}$	Heat Production J $h^{-1}g^{-1}$
F	245	0.11	0.46
F	401	0.14	0.59
M	356	0.12	0.50
F	503	0.16	0.67
F	459	0.07	0.29
M	421	0.25	1.05
F	453	0.06	0.25
F	488	0.10	0.42
F	476	0.08	0.33
F	565	0.15	0.63
Mean	437	0.12	0.52
Standard Deviation	84	0.05	0.22

One important feature of the heat production was the consistency and regularity of the curves during the last 10 hours of each experiment (Figure 1).

The larger animals should produce heat faster than the smaller.

An x-y plot of slope m against body weight (Figure 2) showed a correlation (r = -0.27) between weight and slope. This justifies expressing heat production on a gram basis. There was no sex-related difference.

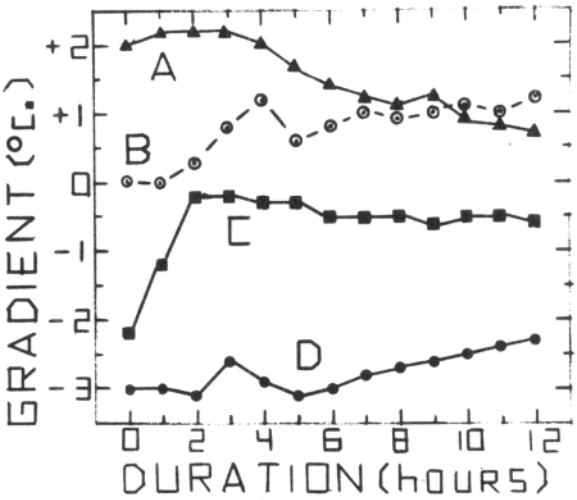

FIGURE 1. Changes in temperature gradients (inside to outside the Dewar flask) over 12 hours, with warm water at 28°C (A), water at room temperature 23°C (C), cool water at 17°C (D), and a female eastern box turtle (Terrapene carolina carolina) weighing 245 g, Code 1 (B).

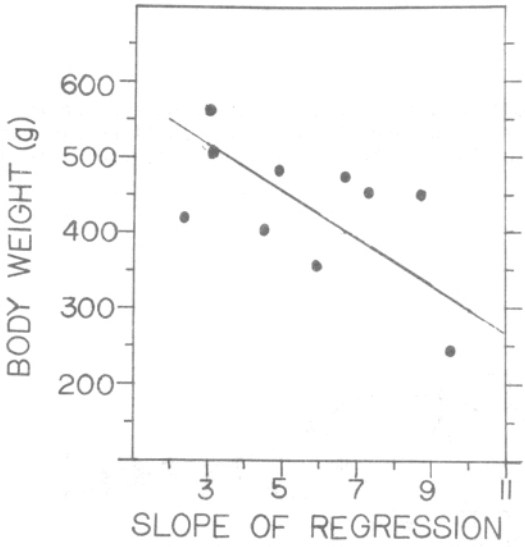

FIGURE 2. Body weight and slope of the regression equation for rise in temperature due to metabolic heat in eastern box turtles (Terrapene carolina carolina). The regression equation Y = -20X + 549 was fitted by the method of least squares (Moroney, 1965). The correlation coefficient was -0.27.

DISCUSSION

We have found no reports of direct measurement of the energy metabolism of the eastern box turtle (Terrapene carolina carolina) or for that matter any other chelonian. Workers (even Rubner, 1924) say "heat production" when all they have measured is oxygen consumption, carbon dioxide production, total weight loss, or some combination of these. This could be an important error if cold blooded animals produced a significant proportion of their heat through anaerobic pathways, and not exclusively aerobic. Our data do not bear on this point, and some authors who have studied heat regulation in turtles have not mentioned this possibility (Templeton, 1960; Sturbaum and Riedesel, 1974; Morgareidge and Hammel, 1975).

Among the data for chelonians, Benedict (1932) reported the results of indirect calorimetry on two species of large turtles, the giant Galapagos tortoise (Testudo vicina) and the South American gopher tortoise (Testudo denticulata). For three of the former he gave values ranging from about 1.2 to 4.2 kcal per kilogram total weight for 24 hours, and for five of the latter, values ranging from 1.3 to 9.7. Expressed in terms of gram calories per gram and hour, these become 0.05 to 0.18 for the former, and 0.05 to 0.11 for the latter. Our total range was 0.06

to 0.25 for 10 eastern box turtles (Terrapene carolina carolina). Benedict (1932) stated that he had tried, but failed, to measure heat production directly, and therefore used indirect calorimetry, calculating from the CO_2 production.

We believe that this is the first report of the use of direct calorimetry in chelonians. Our values are of the same order of magnitude as those reported by other workers using indirect calorimetry.

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