used to investigate the response to ionizing radiation (*7*) and ultraviolet light (*15*) of cells irradiated in various parts of the cell cycle and to determine the capacity of cells to repair sublethal radiation damage as a function of their age in the cell cycle (*7*).

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25 October 1965

Thermoregulation in a Brooding Female Indian Python, Python molurus bivittatus

Abstract. *At varying environmental temperatures, measurements of body temperatures and gas exchange of a female Indian python* (Python molurus bivittatus) *show that during the brooding period this animal can regulate its body temperature by physiological means analogous to those in endotherms. Ambient temperatures below 33°C result in spasmodic contractions of the body musculature with a consequent increase in metabolism and body temperature.*

Internal heat production and temperature regulation in the Indian python during incubation have been suspected since 1832 when Lamarre-Picquot read a communication before the French Academy in which he stated that the python, after laying eggs, coils about them and produces sensible heat as an aid to incubation (*1*). A committee of the French Academy rejected Lamarre-Picquot's statements as being "hazardous and questionable." Later observations in zoological gardens appeared to support the original observations (*2, 3*), but the lack of adequate thermometers and the failure to consider all external sources of heat cast doubt on these earlier studies. Temperature measurements by one of us (H.G.D.) on brooding pythons in the New York Zoological Park in 1960 and 1961 showed conclusively that these snakes were able to maintain, for extended periods, body temperatures up to 7.3°C higher than the substrate or ambient air temperatures and that the maintenance of this temperature differential was correlated with the rate of spasmodic contractions of the body musculature (*4*).

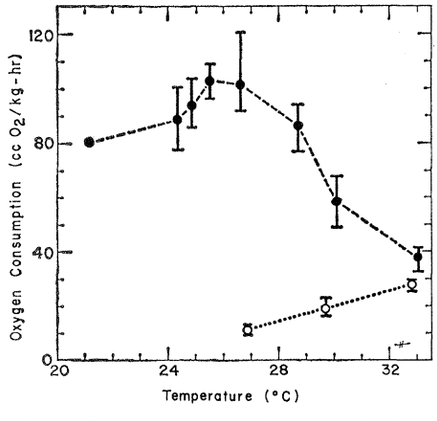

Fig. 1. Oxygen consumption of a *Python molurus* at different ambient temperatures. Upper curve: animal during brooding. Lower curve: the same animal during nonbrooding periods. Vertical lines, range; circles, means.

About 15 February 1965 a 14.25-kg, 2.7-m Indian python (NYZP specimen No. 630514) laid 23 eggs, all of which later proved to be infertile. The eggs with the snake coiled about them were transferred to a respiration chamber located in a temperature-controlled (± 1°C) room on 18 February. Oxygen consumption and carbon dioxide production were recorded constantly in an open circuit system by a Beckman Model F3A3 Paramagnetic Oxygen Analyzer and a Model 15A Infrared Carbon Dioxide Analyzer (*5*). Temperatures were recorded from copper-constantan thermocouples taped to the skin at several points so that some of the thermocouples would lie between tightly appressed coils of the snake. Spasmodic contractions of the body musculature were counted visually. The animal was allowed to acclimate (as evidenced by a new steady rate of gas exchange and new level of body temperature) for at least 48 hours after each change in the temperature of the room before data were taken.

The female remained coiled around the eggs for a period of approximately 30 days. We placed the same individual in the apparatus 40 days after the completion of the brooding period; data taken during this period were used for "nonbrooding" values. After oviposition and the incubation period the animal weighed 10.34 kg, a decrease in weight of 27.4 percent. Calculations of oxygen consumption were based on the weight of the snake at 14.25 kg during brooding; during nonbrooding, 12.37 kg.

The oxygen consumption of the nonbrooding python was characteristic of an ectothermic animal, decreasing with decreasing temperature (Fig. 1, lower curve); but the oxygen consumption of

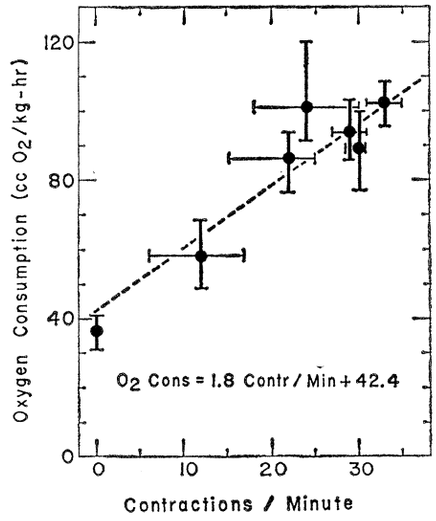

Fig. 2. Correlation of the rate of spasmodic body contractions with the rate of oxygen consumption in a brooding Indian python. Dashed line and regression equation calculated by method of least squares. Vertical lines, range of oxygen consumption; horizontal lines, range of contraction rate; circles, means.

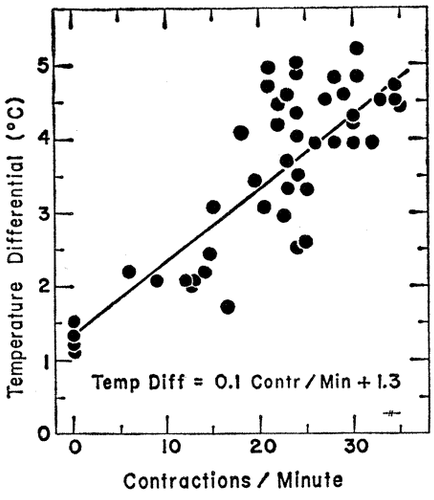

Fig. 3. Correlation of contraction rate with temperature differential in a brooding Indian python.

the same animal during brooding was similar to that of endothermic animals (Fig. 1, upper curve). The metabolic rates of the animal during and after brooding were essentially identical at 33°C. However, as the ambient temperature was lowered from 33° to 25.5°C the brooding animal increased its rate of oxygen consumption. Thus, 33°C appears to be analogous to the "lower critical temperature" of birds and mammals.

The temperature coefficient, Q_{10}, for brooding metabolism between 25.5° and 33°C was 0.26. This represents a decrease in oxygen consumption of 8.5 percent per degree increase in temperature. Benedict (*2*) found a Q_{10} of 2.5 between 26° and 36°C for the change in metabolism in nonbrooding pythons. We obtained a Q_{10} of 4.7 in this Indian python at temperatures of 26.8° to 32.8°C when it was not brooding. However, these values represent increasing metabolism with increasing ambient temperature, while our value for the brooding animal represents decreasing metabolism with increasing temperature. This may be termed "facultative endothermy" because it occurs only during brooding.

At 25.5°C the oxygen consumption during brooding was 9.3 times higher than during the nonbrooding period. From 25.5° to 21.2°C the rate of oxygen consumption declined from a mean of 102.56 to 80.67 cm^3 of oxygen per kilogram-hour. This animal apparently was unable to increase its metabolic rate further at temperatures below 25.5°C, or even to maintain the level obtained at that temperature.

Carbon dioxide production closely followed oxygen consumption at all temperatures and resulted in mean respiratory quotients during brooding of: 21.2°, 0.92; 24.3°, 0.91; 24.8°, 0.90; 25.5°, 0.81; 26.6°, 0.81; 28.7°, 0.92; 30.1°, 0.91; 33.0°C, 0.98. The corresponding respiratory quotients obtained from the nonbrooding animal fell within the same general range: 26.8°, 0.23; 29.7°, 0.94; 32.8°C, 0.90.

The increased rate of oxygen consumption at lower environmental temperatures is correlated with an increase in the rate of contractions of the body musculature (Fig. 2). Similarly, the contraction rate is correlated with the temperature differential between the animal and the surrounding air (Fig. 3). We suggest that these contractions are probably analogous in function to the shivering of birds and mammals at temperatures below the lower critical temperature. The linear relationship between an increasing contraction rate and oxygen consumption suggests that thermogenesis in the absence of shivering probably does not occur in the Indian python. If thermogenesis without shivering were present, the relation between contraction rate and metabolism (Fig. 2) would not be linear, but curvilinear (*6*). Thermogenesis without shivering has been described in mammals (*7*) but is apparently lacking in birds (*6*, *8*).

The increased contractions and oxygen consumption at lower ambient temperatures resulted in a temperature differential of up to 4.7°C at an ambient temperature of 24.8°C (Fig. 4). The decreasing temperature of the animal at the lower ambient temperatures indicates that at these levels the heat produced by the animal is not sufficient to offset that lost to the environment. The maximum temperature differential of 4.7°C found here is less than the 7.3°C previously recorded for brooding pythons by Dowling (*4*); this is probably a result of the difference in size of the animals used. The data in this report are taken from a 14.25-kg snake in her 2nd year of breeding, whereas Dowling recorded data from 43.41- and 53.07-kg females. These differences in temperature differentials may be simply a result of the large differences in mass and surface areas of the animals.

Thermoregulation by endogenous heat production and by changes in heat transport by the circulatory system has been described in lizards (*9*). The endogenous heat production in lizards, however, is appreciably less than in brooding Indian pythons. Varanid lizards can elevate body temperature as much as 2°C above ambient in a temperature-controlled chamber, compared to a 7.3°C elevation possible in a brooding python; also, lizards appear not to increase endogenous heat production as ambient temperature is lowered. Thus, the physiological temperature regulation of the brooding Indian python approaches that of mammals more closely than it does that of varanid lizards. The gap in metabolic rate between reptiles and mammals that previously had been assumed to exist now is bridged by at least one species of snake as well as by certain lizards.

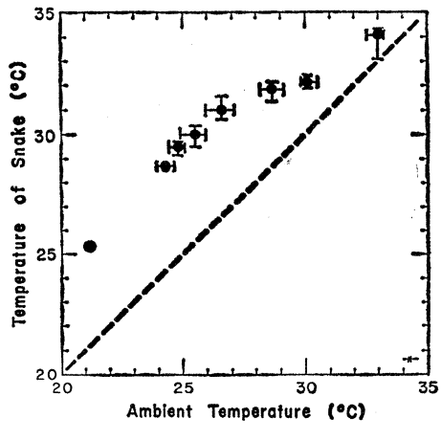

Fig. 4. Relation of the body temperature of a brooding python to ambient temperature. Dashed line indicates equal ambient and animal temperatures. Symbols as in Fig. 2.

The report (*10*) of spasmodic body contractions in a brooding green python, *Chondropython viridis*, and our observations on other species indicate that thermoregulation of the type reported here may occur in still other pythons. Pythons belong to the Boidae, generally considered to be one of the most primitive families of snakes. The demonstration of physiological temperature regulation in varanid lizards and of both physiological temperature regulation and thermogenesis in pythons suggest that mechanisms of physiological thermoregulation occurred in some of the large primitive reptiles and did not originate *de novo* in mammals and birds.

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Morphogenesis in Trichoderma: Suppression of Photoinduction by 5-Fluorouracil

Abstract. *Sporulation in the fungus* Trichoderma viride *is inducible with a short light pulse. 5-Fluorouracil applied prior to photoinduction and removed thereafter suppressed sporulation without greatly affecting growth. This compound also halved the rate of incorporation of uracil-C^{14} into RNA but did not change the ratio of uridylic to cytidylic acid. The effect of 5-fluorouracil was counteracted by uracil but not by thymidine. This supports the hypothesis that 5-fluorouracil affects RNA rather than DNA.*

The biochemical control of differentiation can be studied best in systems in which differentiation can be artificially induced. In these systems, the changes caused by external stimuli can be followed and causal relationships between induction, biochemical change, and differentiation can be established. Although the correlation between induced enzyme synthesis and new specific messenger ribonucleic acid (mRNA) synthesis was made a few years ago (*1*), it was only recently found that, in *Bacillus* species, there exists a transcription of new and different mRNA's upon morphogenetic change (sporulation and germination) (*2*).

Most isolates of the fungus *Trichoderma viride* Pers. ex Fries produce flat, septate, hyaline mycelia when cultured in the dark. They sporulate when illuminated for more than 30 seconds, but only the narrow region of the mycelium produced just prior to illumination sporulates. The requirement of light for morphogenesis is continuous, as a short illumination given to a round colony of *T. viride* induces only a ring of mycelia bearing dark green spores (*3*). We therefore chose this organism to study the role of RNA in induced morphogenesis.

5-Fluorouracil (FU) has been shown to prevent induced enzyme synthesis without decreasing the synthesis of constitutive enzymes in *Escherichia coli* (*4*). Champe and Benzer (*5*) concluded that "FU acts mainly by incorporation into mRNA in place of uracil, there acting partially like cytosine." As this compound has such a pronounced effect on induced enzyme synthesis, we tested its effect on induced morphogenesis.

The culture conditions were as follows. Sterilized liquid potato-dextrose medium (2.7 ml) was soaked into a 7-cm sheet of sterilized Whatman No. 1 filter paper in each 10-cm petri dish, and a sheet of sterilized hardened filter paper (Whatman No. 50) was placed on the lower sheet. A small agar block was removed from an actively growing region of a culture of *T. viride*, isolate No. M 2042 (*6*), and put in the center of the hardened filter paper. Cultures were kept in the dark at 24°C. A 3-minute induction (about 5500 lu/m^2 of fluorescent lighting) was given after 26 to 36 hours of growth (culture diameters, 30 to 50 mm). Under these conditions a ring of conidiophores with dark green conidia (spores) was visible the following day; growth and sporulation were very uniform. Four cultures were used for each treatment. Since the mycelia adhered firmly to the hardened filter paper, rinsing the cultures and transferring them from medium to medium was facilitated.

We found that when cultures were transferred to FU 30 minutes before induction and left in it for 1 day, photoinduced sporulation was prevented. Sporulation was completely prevented but growth was only slightly affected by $7 \times 10^{-6}M$ FU. Even $7 \times 10^{-3}M$ FU did not stop growth completely.

Table 1. Uracil-2-C^{14} incorporation in *Trichoderma viride* RNA. Mycelia were grown to a diameter of about 5 cm in basal medium, then transferred to a dish with 2.5 μc uracil-2-C^{14} ($5 \times 10^{-5}M$), with or without $10^{-4}M$ FU, and kept there for either 1 or 6 hours. The mycelia, attached to the hardened filter paper, were then fixed in cold ethanol–acetic acid (3:1), rinsed with 70 percent ethanol, and air dried. Four half sheets per treatment were cut up in pieces (1 cm^2) and incubated 16 hours at 37°C in 6 ml of 0.3*N* KOH to which 1.5 mg of yeast RNA was added as carrier. After hydrolysis the KOH was neutralized and the product was precipitated with cold $HClO_4$. A portion was removed for total RNA determination and uridylic and cytidylic acids were separated on Dowex 50W × 4 according to the method of Katz and Comb (*14*). The $KClO_4$ precipitate (which included the DNA) was dissolved and found to contain less than 3 percent of the counts. Incubation of the mycelia on the filter paper with deoxyribonuclease did not reduce radioactivity, whereas incubation with ribonuclease removed over 95 percent of it. Results in the table are expressed in counts per minute per dish. FU, 5-fluorouracil; UMP, uridylic acid; CMP, cytidylic acid.

Incubation (hr)	Incorporation in: RNA (count/min)	UMP (%)	CMP (%)
	Control mycelia		
1	23,930	78	22
	Mycelia plus FU		
1	11,365	79	21
	Control mycelia		
6	143,550	56	44
	Mycelia plus FU		
6	68,140	63	37

Other inhibitors of nucleic acid, protein, and steroid synthesis were tested on this system. These included actinomycin D, azaguanine, azauridine, thiouracil, puromycin, and tris-(2-diethylaminoethyl)-phosphate trihydrochloride. Of these, only azaguanine differentially inhibits sporulation, with little effect on growth, in a manner similar to FU.

In later experiments, colonies were subjected to 5-hour treatment with FU beginning 30 minutes before induction. They were then rinsed, transferred to new dishes with medium, and incubated for a day. The results (Fig. 1*A*) indicated that even a temporary exposure of the culture to $10^{-4}M$ FU prevented photoinduced sporulation, although growth was only slightly affected.

If the photoinduced sporulation is not mediated through *de novo* synthesis of RNA, there should be little difference in the effect of FU when it is applied before, during, or after photoinduction. If FU retarded sporulation only when applied during induction,