

OBSERVATIONS ON RESPIRATORY METABOLISM  
IN THE RED-SPOTTED GARTER SNAKE  
THAMNOPHIS SIRTALIS CONCINNUS HALLOWELL

by

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INTRODUCTION

When we observe a particular animal in its "ecological niche," we frequently speculate as to why it has taken up its peculiar way of life, what limitations are imposed upon it, how well it adjusts to new situations, and how it compares in these respects to related forms. Many of the questions raised here may be partially elucidated by exhaustive field studies, but until the inner workings of the animal itself--its physiological mechanisms--are investigated, some of the questions will go unanswered. It is essential, therefore, that intensive field studies be interpreted in the light of physiological investigations if we are to gain some understanding of an animal's relationship to its environment. However, investigations of specific physiological mechanisms and metabolic pathways are not particularly instructive, when ecological interpretations are sought, until they can be related to the general physiological state of the animal. Under a given set of conditions, a composite indicator of the latter will be the rate at which metabolism is proceeding, for metabolism is conceived as being the sum total of all physiochemical reactions in the body.

It is well known that the metabolic rate of animals is greatly influenced by a number of factors. Those which seem to be the most important, from an ecological point of view, are temperature and size. Homiothermic or "warm-blooded" animals typically have a relatively high rate of metabolism which maintains their body temperatures at rather constant levels. Their activities are generally regarded as being nearly independent of the environmental temperature, although there are many exceptions. The studies of Scholander et al. (41), Hart (24) (25), and Hart and Heroux (26) have shown that they may exhibit definite adaptive responses in metabolic rate, insulation and behavior.

Poikilothermic or "cold-blooded" animals, on the other hand, are decidedly less independent of environmental temperature, their metabolism not being geared to consistently high heat production. Since their activities are considerably restricted by the temperature factor, which is easily measured and controlled under laboratory conditions, it is not surprising that they have been studied much more extensively. Most of the voluminous literature on temperature-induced physiological responses in poikilotherms has been thoroughly reviewed by Bullock (6) and Prosser (32). More recent literature includes the papers by Vance (44), Lowe and Vance (29), Tashian (42),

Dawson and Bartholomew (16) (17), Tashian and Ray (43) and Roberts (35) (36).

One of the physiological responses frequently observed in thermal studies with poikilotherms is acclimation.<sup>1</sup> It may take the form of temperature hardiness (tolerance, resistance) and/or metabolic compensation. For example, cold-acclimated animals may have increased tolerance for cold and increased sensitivity to heat, or vice versa. Furthermore, cold-acclimated animals usually exhibit a higher metabolic rate than warm-acclimated ones when the two are compared at some temperature intermediate to the acclimation temperatures. The survival value of such responses is obvious. No doubt they are related, for it is easy to see how a rise in the resting metabolism of muscle could increase cold resistance due to greater heat production. Fisher (18, p. 3-49), however, stresses the point that acclimations sometimes do not have obvious survival value, and Precht (33, p. 50-78) discusses cases where resistance acclimation may not be a "reasonable" adaptation, i.e., resistance is acquired in both directions with

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<sup>1</sup> This term is sometimes given special meaning, referring only to phenotypic adaptive alterations produced in the laboratory. The term acclimatization is then used to distinguish genotypic alterations induced under natural conditions. In this paper, the terms are considered synonymous, and the former will be used for consistency.

acclimation to either cold or heat. If the animal were acclimated to cold, for instance, only increased cold tolerance would be "reasonable," but the animal might also have greater heat tolerance.

Another response of poikilotherms that has aroused considerable interest is the acceleration of metabolism with rising temperature in the biological range. A convenient and frequently used expression of the magnitude of the temperature effect is  $Q_{10}$ . This is the factor by which the velocity of a chemical reaction is increased for a temperature rise of 10° C. According to the Van't Hoff rule, the  $Q_{10}$  will be 2 or 3 for inorganic reactions (5, p. 267). In biological systems, similar values might be expected, but often they are higher and vary depending upon the temperature range studied (31, p. 240). Also, since reaction velocities in enzyme systems are not a linear function of temperature,  $Q_{10}$  diminishes at higher temperatures (31, p. 343). The  $Q_{10}$  concept is still useful, nevertheless, and some attention has been given to the effect of acclimation and size on  $Q_{10}$ . Rao and Bullock (34) have reviewed much of the pertinent literature. The general tendency seems to be an increase in  $Q_{10}$ , both with acclimation temperature and size. However, neither relation appears to be necessarily adaptive, and exceptions have been pointed out.

For many years, the influence of size on metabolic rate has been the object of extensive investigation. Very early it was recognized that small homoiotherms had higher rates of metabolism than large ones, and the relationship appeared to be proportional to the external body surface. Originally, the contention of several early workers (5, p. 354-357) was that, while heat production is dependent on body mass, heat loss is governed by the radiating surface. Then, since surface area is proportional to  $\sqrt[3]{W^2}$  or  $W^{2/3}$  (where W is weight), it naturally follows that metabolic rate is also proportional to  $W^{2/3}$ . This is the "surface law." With the accumulation of more and more data, its propriety has been questioned. In the first place, as discussed by Brody (5, p. 352-403), the surface law does not apply to both classes of homoiotherms. The metabolic rate of mammals is more nearly proportional to  $W^{.73}$ , though  $W^{.67}$  is applicable to birds. Secondly, the surface law is no less applicable to poikilotherms, for which, of course, heat regulation considerations cannot be used to explain the phenomenon. The classic paper of Zeuthen (49, p. 17-161) presents an excellent coverage of the literature, together with original work. Among the more recent papers having a bearing on the subject are those of Davison (15) and Roberts (35) (36).

Ecologically, the surface law is not without significance. Bergman's rule states that homoiotherms tend to be larger in cold climates than their relatives in warm climates (1, p. 119-120), the interpretation being in terms of heat conservation and radiation. When terrestrial poikilotherms are considered, the situation is reversed, with the largest species and individuals tending to occur in warm climates. Here, the surface-mass relationship enables small forms to utilize more efficiently lesser amounts of heat and radiant energy to raise their temperatures to the physiological optimum.

In spite of the general abundance of information on physiological phenomena related to temperature and size in poikilotherms, it becomes evident from a brief survey of the literature that very little work has been done on reptiles. Fish and amphibians have received considerable attention, but except for the classic work of Benedict (3) on large reptiles and a few special studies (39) (37), reptiles seem to have been neglected by physiologists.

Most investigations on reptiles have been concerned with determining body temperatures and tolerance levels and describing behavioral responses.<sup>2</sup> Such studies have,

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<sup>2</sup> Particularly interesting in this respect are the papers of Cowles (12), Cowles and Bogert (13), Bogert (4), and Saint-Girons and Saint-Girons (38). Additional

indeed, made valuable contributions to our knowledge of reptilian ecology. Nevertheless, the question is still raised as to whether reptiles also make metabolic compensations and are subject to the surface law. In his 1955 review, Bullock (6) could only "feel confident in predicting that reptiles will also yield examples of physiological adaptation," and he noted that work just appearing, (29) (16)<sup>3</sup>, was in accord with his prediction. These papers, together with a later paper by Dawson and Bartholomew (17) and the earlier papers of Cook (11) and Vance (44), seem to account for most of the studies conducted with small reptiles in this country.<sup>4</sup> All of these studies have dealt with lizards. While there is no reason to suspect that small snakes may have any basically different physiological characteristics, the present author considers that information on them is desirable, if for no other reason than that they constitute a blank spot in the record.

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information will be found in the papers of Cole (10), Bailey (2), Carpenter (7) (8) (9), Fitch (19), Wilhoft (46), Inger (27), and Wilhoft and Anderson (48).

<sup>3</sup> Bullock cites another paper by Lowe which did not appear in the literature.

<sup>4</sup> The French workers, Gelineo and Gelineo (22) (23), have been cited by Dawson and Bartholomew (16).

Garter snakes of several species have been shown to be extremely cold hardy (2) (8) (30) (14). Often they may emerge to sun themselves during mild spells in the winter. In Kansas, Fitch (19) notes that the common garter snake, Thamnophis sirtalis, may be the latest to enter hibernation sites and the earliest to emerge. Our local form, the red-spotted garter snake (Thamnophis sirtalis concinnus), behaves in much the same way. This subspecies occurs abundantly in Oregon's Willamette Valley, ranging westward from the Cascades to the coast, and it is not unusual to see them abroad on mild winter days. Moreover, they definitely seem to be active for a greater part of the season than either sympatric species, the northwestern garter snake (Thamnophis ordinoides) or the western garter snake (Thamnophis elegans).

These observations indicate that T. s. concinnus is more cold hardy than associated species, and the speculation arises that one factor contributing to its cold hardiness might be a higher metabolic rate. It was hoped that this hypothesis could be tested by a comparative study of respiratory metabolism in the three species, particular attention being given to acclimation responses and different size categories. Unfortunately, circumstances prevented the comparative aspect from being carried out. Thus the investigation was limited to T. s. concinnus.

#### METHODS AND MATERIALS

The snakes used in this study were collected at a marsh six miles east of Corvallis owned by Mr. Orville O. Doerfler. The marsh covers some 90 acres of land and harbors a heavy population of red-spotted garter snakes. They are most abundant along the southeast edge of the marsh where clumps of wild rose (Figure 1) provide shelter to which they immediately retire when disturbed. Most of the snakes seem to hibernate and spend the nights in holes under the roots of the rose bushes, and in the numerous burrows of meadow mice (Microtus).

On collecting trips, a few observations were made regarding the activities and body temperatures of snakes. Cloacal and air temperatures were recorded with a fast-reading Schultheis thermometer graduated in 0.2° C. intervals. The snakes were grasped by the tail away from the cloacal region, and the reading was taken within 15 to 20 seconds. Air temperatures were recorded in the observer's shadow about four feet above the ground.

Experimental work was conducted during the months of February and March, 1960. Twelve male snakes<sup>5</sup> were collected as follows: 30 January, six; 7 February, three; and

<sup>5</sup> Female snakes were not used because experiments with them indicated that their physiological condition was different than that of males.



Figure 1. Garter snake habitat along southeast edge of marsh. Note clumps of rose bushes in foreground.

7 March, three. When brought into the laboratory, they were weighed and measured, and arranged in three groups approximately equal in total weight and variation of individual weights. Initially, the weights ranged from 32 to 72 grams. The snakes were kept in fish bowls (diameter 9 inches), one or two in each bowl. Paper towels covered the bottoms of the bowls and facilitated cleaning. All snakes were kept at room temperature ( $18^{\circ}$ - $24^{\circ}$  C.) except during acclimation periods.

Experiments were started with the nine snakes collected in January and February prior to the collection of the last three in March. One of the latter was added to each group, making a total of four per group. The animals in one group were acclimated at  $4^{\circ}$ - $8^{\circ}$  C. for three to ten days before their respiratory metabolism was measured. Those in another group were acclimated for three to seven days at  $26^{\circ}$ - $30^{\circ}$  C. The third group was kept at room temperature throughout the study.

Snakes in the hot and control (room temperature) groups were fed weekly on tree frogs (Hyla regilla) and long-toed salamanders (Ambystoma macrodactylum). One of each was given to each snake, the size of the individuals being roughly proportional to that of the snake. The respiratory metabolism of these snakes was not measured

for three days or more after feeding, during which time they were being acclimated. Snakes acclimated to cold were not fed so frequently as the others, and were allowed to digest their food for at least three days at 25°- 27° C. before starting acclimation. A finger bowl of water was provided for the snakes acclimating at 26°- 30° C. At feeding time, all snakes were left overnight in bowls containing about two inches of water and an exposed rock surface on which they could rest.

Following acclimation to their respective temperatures, the respiratory metabolism of all snakes was measured at three different temperatures: 18.5° C., 24.5° C., and 34.5° C. These temperatures were maintained by submerging the respirometer in a constant-temperature water bath. After a snake had been run through an experiment at one temperature, it was either run at a new temperature or returned to the animal room to await reacclimation for a later experiment. If another experiment was begun immediately at a new temperature, the respirometer was not removed from the water bath, and the snake was simply given time to adjust to the new temperature. A snake was never run in more than two consecutive experiments, and the second experimental temperature was always higher than the first.

Various types of respirometers have been devised to measure gaseous metabolism, a number of them being similar in principle to the direct reading volumetric microrespirometers of Scholander (40). The modification described by Flemister and Flemister (20) was adapted for use in the present investigation. The apparatus is shown in Figure 2.

The animal chamber (1)<sup>6</sup> was constructed from a small bowl,  $5\frac{1}{2}$  inches in diameter and  $3\frac{1}{4}$  inches deep (volume approximately 1000 cc.). The lid of the animal chamber (2) was made from a .312 inch thick sheet of "plexiglass" plastic. Three holes were drilled in it and fitted with rubber stoppers and glass tubing. A tube from one stopper opened the chamber directly to the outside. Two millimeter bore capillary tubing from a second stopper linked the animal chamber to a compensating jar (3) via a "U" tube (4) of the same bore containing Brodie's manometer fluid. The compensating jar was  $3\frac{1}{4}$  inches in diameter and 6 inches deep (volume approximately 880 cc.). It was closed by a large rubber stopper through which a glass tube opened to the outside.

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<sup>6</sup> The original design of the apparatus included a large bowl, 10 inches in diameter and  $4\frac{1}{2}$  inches deep, as the animal chamber. In trial runs, its volume immediately proved to be too great for accurate measurements, and it was subsequently replaced by a smaller bowl.

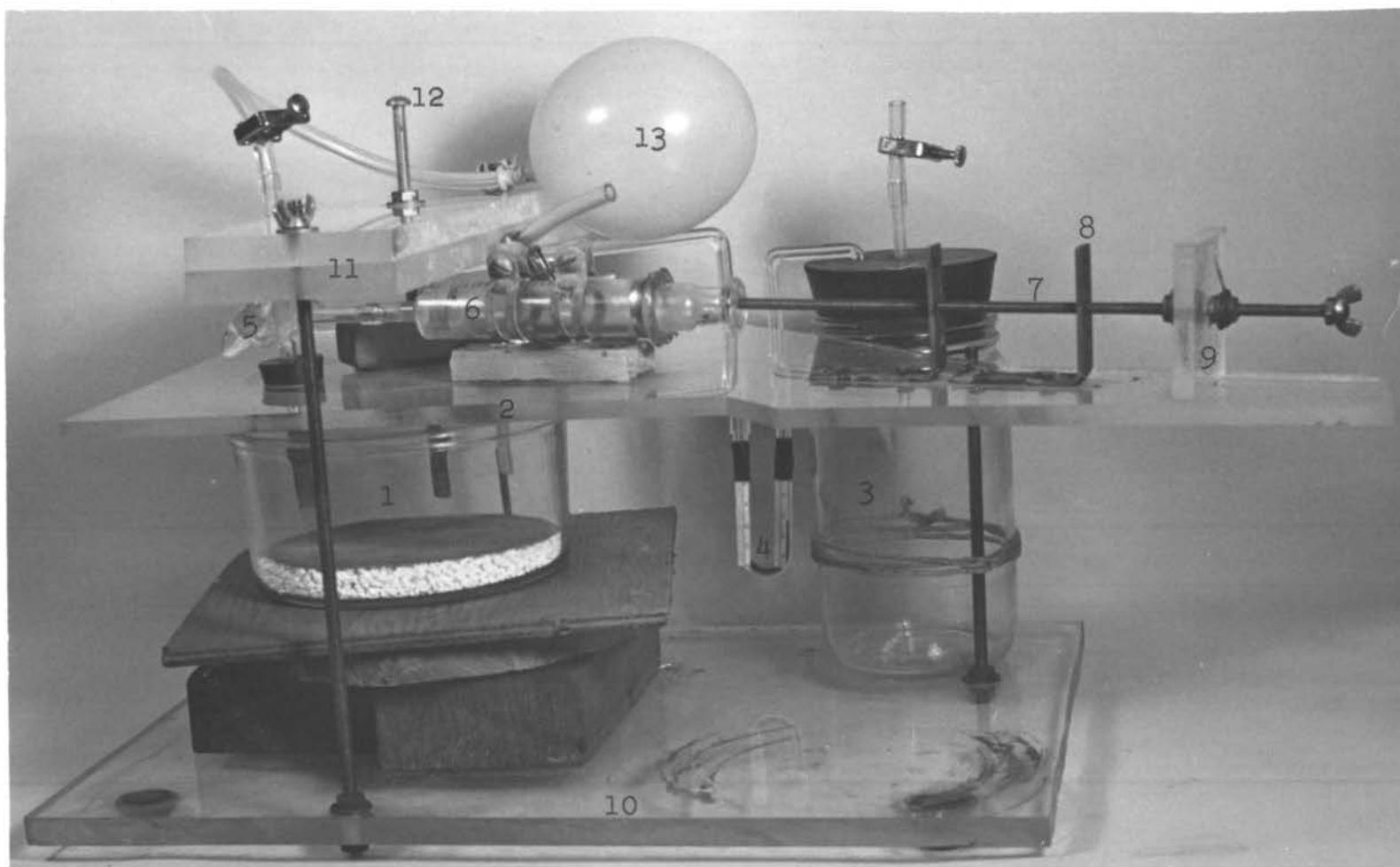


Figure 2. A volumetric respirometer.

A "T" tube with a three-way stopcock (5) was thrust into a third stopper in the lid of the animal chamber and connected to a 20 cc. syringe (6). The syringe was clamped to a wooden block which was cemented to the lid. The plunger of the syringe could be adjusted inward by a 3/16-inch screw (7) running through two steel brackets (8) bolted to an extension of the lid. The screw made contact with the center of the plunger head. Behind the brackets, the screw ran through the center of a sliding plastic block (9) on which a dial was engraved. By watching a pointer attached to the screw, the number of revolutions could be counted. Exactly three revolutions of the screw advanced the plunger one cubic centimeter mark on the syringe.

The lid of the animal chamber was closed onto the bowl by a stirrup clamp. Both animal chamber and compensating jar rested on a 12-inch by 17-inch base of  $\frac{1}{2}$ -inch "plexiglas" (10). The base was drilled and tapped to take two threaded brass rods, one on each side of the animal chamber. A crossmember (11) consisting of two strips of  $\frac{1}{2}$ -inch "plexiglas" was drilled to pass the threads of the rods at each end. Also, it was drilled and tapped in the center to take the threads of a  $\frac{1}{4}$ -inch wall screw (12). The latter was bolted securely to the crossmember. With crossmember in position, pressure was applied by tightening

wing nuts on the rods. The center screw bore directly on a block of wood which distributed the pressure over the lid. A gasket of neoprene rubber between the lid and rim of the bowl made an air-tight seal. The compensating jar was held in place by tying it to a third brass rod set in the base.

Oxygen was stored in a balloon (13) attached to the top end of the "T" tube by a length of  $\frac{1}{4}$ -inch polyethylene tubing. A piece of rubber tubing on the inside end of the "T" tube facilitated inflation of the balloon from an oxygen tank. Polyethylene tubing was used for all joint connections and outside extensions, the latter being closed when necessary by light weight hose clamps. A  $\frac{1}{2}$ -inch layer of #4-8 mesh soda lime covered the bottom of the animal chamber, serving as a carbon dioxide absorbent and dessicant. Two discs of plastic screen were laid over the soda lime to prevent the animal from coming in contact with it. During an experiment, air inside the chamber was assumed to be dry.

The procedure in running an experiment was as follows: a snake was taken directly from its temperature-acclimation room, weighed, and placed in the animal chamber. The lid with freshly inflated oxygen balloon was tightened in position, and the stopper with capillary tubing linked to the

manometer and compensating jar was set in its hole. With both outside extensions open, the apparatus was submerged in the water bath. The animal was given  $2\frac{1}{2}$  to  $3\frac{1}{2}$  hours to adjust to the conditions of the experiment before oxygen consumption was measured. This seemed to be sufficient time for the animal to reach a constant temperature and end its exploratory activity.

At the end of the adjustment period, 10 cc. of oxygen were drawn from the balloon into the syringe, the outside extensions were closed, and the run was begun. As oxygen was consumed, it was replaced by oxygen from the syringe, the plunger being advanced just enough to maintain the menisci of the manometer at a constant level. The stopcock closed the passage from syringe to animal chamber except when oxygen was added. Two strips cut from a white plastic ruler and taped to the arms of the "U" tube were useful in reading the manometer. The smallest change in pressure that could be read on the manometer was equivalent to a volume decrease of  $1/6$  cc. or an advancement of the plunger by one half revolution of the screw.

Oxygen consumption was recorded at the end of every 30 minutes for  $1\frac{1}{2}$  to 3 hours. If necessary, the syringe could be refilled at the end of a measurement period without stopping the experiment. When the snake was quiet,

and oxygen consumption did not vary more than 1/3 cc. in the first three periods,<sup>7</sup> the experiment was terminated and an average of the three values was determined and used in calculating the oxygen consumption per hour. In some experiments, the snakes were not perfectly quiet. Then, periods in which the animals were active were not counted, and averages for such experiments were computed from the three or four most similar values of non-active periods. Some snakes were rerun as a check. In these cases, the average oxygen consumption for the two experiments was computed and used in subsequent calculations. Since a blank run showed no appreciable pressure change over a period of eight hours, it was considered that no error could be attributed to oxygen consumption by the respirometer.

Metabolic rate was expressed in terms of the cubic centimeters of oxygen consumed per kilogram of body weight

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<sup>7</sup> The smaller volume and cylindrical shape of the compensating jar caused it to be more greatly affected than the animal chamber by any change in temperature of the water bath, i.e., in terms of expansion and contraction of the contained air. The temperature of the water bath fluctuated about 1° C., accounting for some variation in oxygen consumption from one period to the next. A compensating chamber is desirable because it makes the respirometer much less sensitive to changes in barometric pressure, humidity and temperature than uncompensated systems (46). But to be most effective in the latter respect, it must be the same size and shape as the animal chamber.

per hour (cc./kg./hr.). It was calculated from the equation

$$\text{Rate } O_2 \text{ consumption} = C/W \times T$$

where C is the total amount of oxygen consumed, W is the weight of the snake at the start of the experiment, and T is the time factor. Using the average of the metabolic rates calculated for the four snakes in each acclimation group,  $Q_{10}$  values for each group were calculated for the temperature ranges  $18.5^{\circ}$ -  $24.5^{\circ}$  C.,  $24.5^{\circ}$ - $34.5^{\circ}$  C., and  $18.5^{\circ}$ -  $34.5^{\circ}$  C. The equation is

$$Q_{10} = (k_1/k_2)^{10/t_1-t_2}$$

where  $k_1$  and  $k_2$  are the averages of the metabolic rates of four snakes at the respective temperatures  $t_1$  and  $t_2$ . The logarithmic equation becomes

$$\log Q_{10} = 10/t_1-t_2 \times \log k_1 - \log k_2.$$

Other calculations and statistical methods will be discussed with the presentation of the data.

## RESULTS

The body temperature data for snakes in the field is summarized in Table I. Although the weather information is incomplete, it will be noted that snakes checked late in the morning or in the afternoon had body temperatures consistently averaging above 21° C. Seven snakes checked on afternoon trips had body temperatures above 26° C., the highest being 30.2° C. Only the two animals checked earlier on an overcast morning had body temperatures below 21° C. In all cases, the body temperatures were higher than the air temperatures four feet above the ground at the point of capture. For 64 snakes checked on seven trips to the field between 9 October, 1959, and 7 February, 1960, the mean body temperature was 22.0° C., and the mean difference between the mean body temperatures and mean air temperatures was 5.7° C.

Data on the relation of oxygen consumption and metabolic rate to body weight, temperature acclimation, and temperature are summarized in Tables II, III, IV, V and VI, and presented graphically in Figures 3, 4, 5 and 6. With increasing body weight, oxygen consumption increases also, but the rate of increase seems to be affected by acclimation and apparently does not necessarily conform to the surface law. Regression coefficients expressing the slopes

Table I

## Body Temperatures of Snakes in the Field

Date and Time	Weather and Average Air Temp. - °C.	No. Snakes Checked	Ave. Body Temp. °C.	Difference Snakes-Air
10/9/59 10-11 a.m.	Overcast 14.0	2	16.8	2.8
10/13/59 2-3 p.m.	Hazy sun 17.0	7	21.1	4.1
10/17/59 11 a.m. - 12:30 p.m.	Clear 19.4	7	23.2	3.8
10/25/59 12 noon - 4 p.m.	- - - - - 16.2	6	23.4	7.2
10/26/59 1-3 p.m.	- - - - - 18.4	5	23.9	5.5
1/31/60 12 noon - 4 p.m.	- - - - - 14.5	23	23.9	9.4
2/7/60 12 noon - 4 p.m.	- - - - - 14.5	14	21.6	7.1
	Ave. 16.3	T. 64	Ave. 22.0	Ave. 5.7

of the lines of double logarithmic curves (Figures 3, 4, 5) for the three acclimation groups at each experimental temperature were calculated according to the method described by Li (28, p. 244-268). Because the March animals were physiologically different than those collected previously, regression coefficients were calculated both with and without them. The values are given in Table II.

Table II

Coefficients for the regression of oxygen consumption on body weight in snakes acclimated to three different temperatures and run at 18.5° C., 24.5° C., and 34.5° C. Values in parentheses are for three snakes not including the animals collected in March

Experimental Temperature °C.	Acclimation Group		
	4°- 8° C.	18°- 24° C.	26°- 30° C.
18.5	0.94 (0.71)	0.89 (0.80)	0.45 (0.45)
24.5	1.10 (1.07)	0.90 (0.74)	0.40 (0.38)
34.5	0.95 (0.80)	0.67 (0.75)	0.54 (0.56)

Each of the animals collected in March had a higher rate of oxygen consumption and metabolism at 18.5° C. and 24.5° C. than the others in its group, i.e., in proportion to its weight (Figures 3, 4; Tables III, IV). At 34.5° C., however, these rate expressions for the March animals fall below the levels for the others except in the 4°- 8° C. acclimation group (Figure 5, Table V). If the March

animals are ignored, it will be noted that metabolic rates decrease with increasing size, except in the 4°- 8° C. group where one animal had a higher rate of metabolism than expected at 24.5° C. and 34.5° C. (Figures 4, 5; Tables IV, V).

From an examination of Table II and Figures 3, 4 and 5, it may be seen that the regression coefficients for the 18°- 24° C. and 26°- 30° C. acclimation groups are rather similar within the respective groups at the three experimental temperatures, regardless as to whether the values are calculated on the basis of three or four snakes. Furthermore, the difference between the coefficients for these two groups is quite noticeable. In comparing the 18°- 24° C. group with the 4°-8° C. group, however, only a slight difference may be observed when all four animals are compared, and no difference is evident when three animals are compared, except for the still exceptionally high coefficient calculated at 24.5° C.

An analysis of covariance (28, p. 344-349) revealed that the regression coefficients of the three acclimation groups, calculated on the basis of four animals, are not significantly different at the 5 per cent significance level ( $F = 5.14$  with 2 and 6 degrees of freedom) at any of the three experimental temperatures (18.5° C.,  $F = 1.23$ ;

Table III

Weight, oxygen consumption, and metabolic rate of snakes acclimated at three different temperatures. Experiments run at 18.5° C.

	Animal Number	Weight kg.	O <sub>2</sub> Consumption cc./hr.	Metabolic Rate cc./kg./hr.
Acclimated at 26° - 30° C.	1H	0.0369*	1.42*	38.6*
	3H	0.0531*	1.67*	31.5*
	6H <sup>X</sup>	0.0543	2.22	40.9
	4H	0.0796	2.00	25.1
Average		0.0560	1.83	34.0
Acclimated at 18° - 24° C.	2C	0.0375	1.61	42.9
	3C	0.0587*	2.33*	39.7*
	5C <sup>X</sup>	0.0627	2.83	45.1
	4C	0.0710	2.67	37.6
Average		0.0557	2.36	41.3
Acclimated at 4° - 8° C.	1CD	0.0396	2.00	50.5
	2CD	0.0482	2.34	48.6
	3CD	0.0594	2.67	45.0
	4CD <sup>X</sup>	0.0651	3.34	51.3
Average		0.0531	2.59	48.9

\* Values represent average of two experiments.

<sup>X</sup> Collected March 7, 1960.

Table IV

Weight, Oxygen Consumption, and Metabolic Rate  
of Snakes Acclimated at Three Different  
Temperatures. Experiments run at 24.5° C.

	Animal Number	Weight kg.	O <sub>2</sub> Consumption cc./hr.	Metabolic Rate cc./kg./hr.
Acclimated at 26°- 30° C.	LH	0.0367	2.67	72.8
	3H	0.0530	3.00	56.6
	6H <sup>X</sup>	0.0545*	3.55*	65.1*
	4H	<u>0.0744*</u>	<u>3.50*</u>	<u>47.0*</u>
Average		0.0547	3.18	60.4
Acclimated at 18°- 24° C.	2C	0.0338*	2.53*	74.9*
	3C	0.0465	3.00	64.5
	5C <sup>X</sup>	0.0614	4.66	75.9
	4C	<u>0.0629</u>	<u>4.00</u>	<u>63.6</u>
Average		0.0512	3.55	69.7
Acclimated at 4°- 8° C.	1CD	0.0400	3.22	80.5
	2CD	0.0477	3.66	76.7
	3CD	0.0591	4.88	82.6
	4CD <sup>X</sup>	<u>0.0644</u>	<u>5.34</u>	<u>82.9</u>
Average		0.0644	4.28	80.7

\* Values represent average of two experiments.

<sup>X</sup> Collected March 7, 1960.

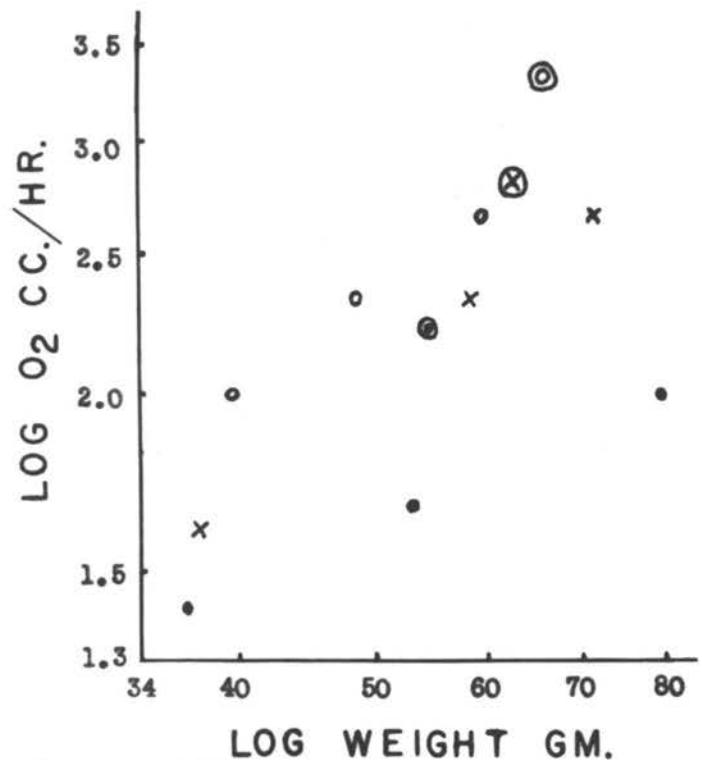


Figure 3. Increasing oxygen consumption with weight. Snakes run at 18.5° C.  
• acclimated 26°- 30° C.; x acclimated 18°- 24° C.; o acclimated 4°- 8° C. Each symbol represents one animal. Circled symbols are March animals.

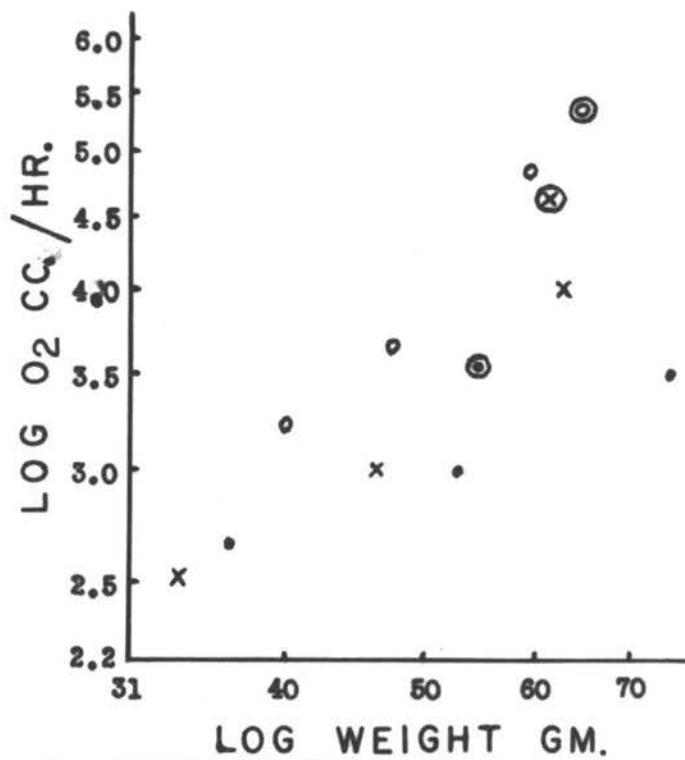


Figure 4. Increasing oxygen consumption with weight. Snakes run at 24.5° C. Symbols and individuals same as in Figure 3.

Table V

Weight, Oxygen Consumption, and Metabolic Rate  
of Snakes Acclimated at Three Different  
Temperatures. Experiments run at 34.5° C.

	Animal Number	Weight kg.	O <sub>2</sub> Consumption cc./hr.	Metabolic Rate cc./kg./hr.
Acclimated at 26°- 30° C.	1H	0.0363	6.42	176.9
	3H	0.0536*	7.45*	139.0*
	6H <sup>X</sup>	0.0555	7.00	126.1
	4H	<u>0.0743</u>	<u>9.50</u>	<u>127.9</u>
Average		0.0549	7.59	142.5
Acclimated at 18°- 24° C.	2C	0.0380	7.14	187.9
	3C	0.0572	10.00	174.8
	5C <sup>X</sup>	0.0627	9.16	146.1
	4C	<u>0.0684</u>	<u>11.00</u>	<u>160.8</u>
Average		0.0566	9.33	167.4
Acclimated at 4°- 8° C.	1CD	0.0397	8.00	201.5
	2CD	0.0482	8.44	175.1
	3CD	0.0594	11.00	185.2
	4CD <sup>X</sup>	<u>0.0651</u>	<u>12.66</u>	<u>194.5</u>
Average		0.0531	10.03	189.1

\* Values represent average of two experiments.

<sup>X</sup> Collected March 7, 1960.

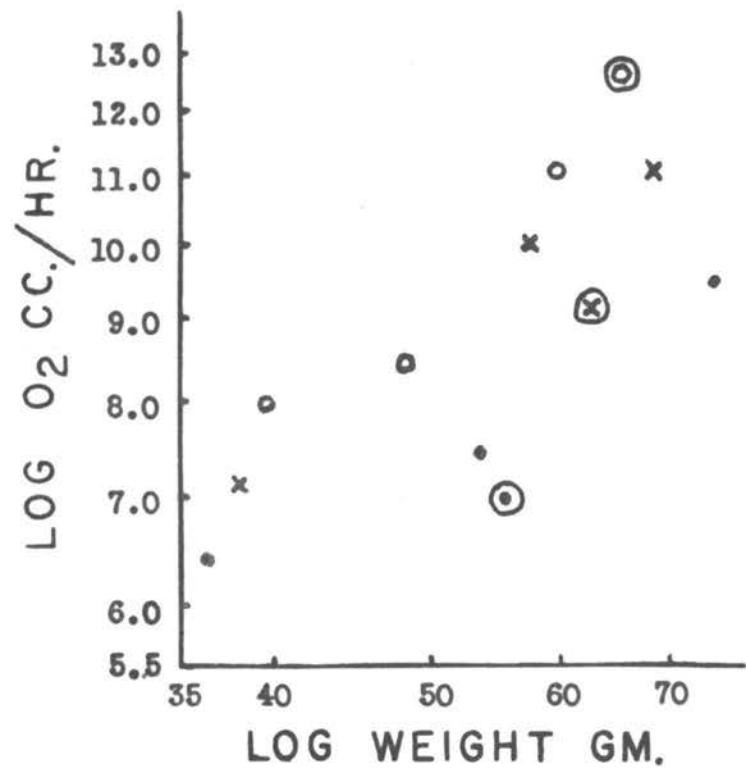


Figure 5. Increasing oxygen consumption with weight. Snakes run at 34.5° C. Symbols and individuals same as in Figures 3 and 4.

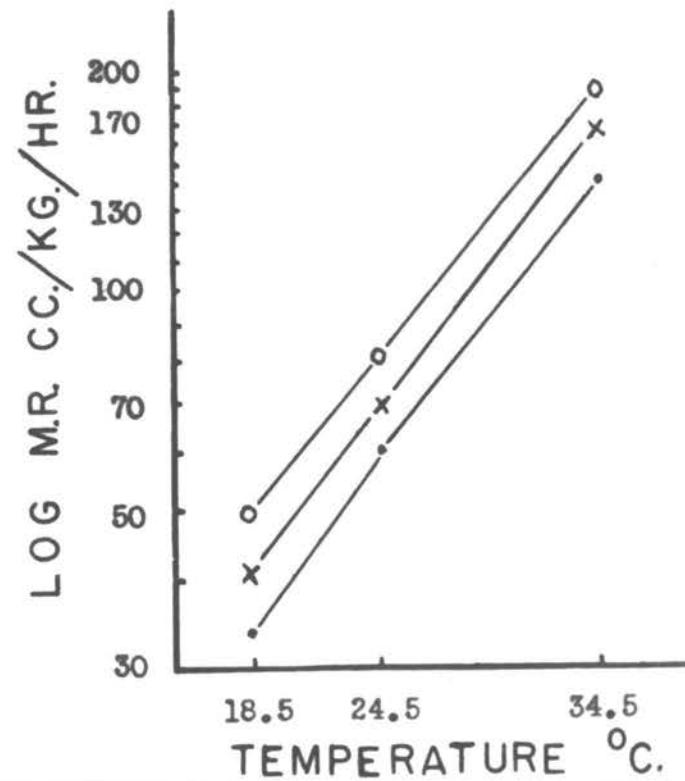


Figure 6. Increasing metabolic rate with temperature. Symbols same as in foregoing figures. Each symbol represents the average M.R. for the four snakes in each acclimation group.

24.5° C.,  $F = 3.81$ ; 34.5° C.,  $F = 1.24$ ). No statistical comparison was made for regression coefficients based on data for three snakes, but it is doubtful that they would be significantly different. While not statistically significant, the results indicate that there may be a tendency for animals acclimated at temperatures in the upper part of the tolerable range to have lower regression coefficients for oxygen consumption on body weight. There is no apparent trend in change of regression coefficients with experimental temperatures.

When the averages of the metabolic rates for the four snakes in each acclimation group (Tables III, IV, V) are plotted semilogarithmically (Figure 6), curves are obtained which are nearly straight and parallel. The  $Q_{10}$  values for these curves are presented in Table VI. For the temperature ranges 18.5°- 24.5° C. and 18.5°- 34.5° C.,  $Q_{10}$  values become somewhat greater as the acclimation temperature increases.  $Q_{10}$  values are not diminished in the higher 24.5°-34.5° C. range except in the 26°- 30° C. acclimation group.

Definite compensatory changes in metabolic rate were attained in three or four days of acclimation, and at all experimental temperatures the effect of acclimation is quite evident (Figure 6). Animals acclimated at 26°- 30° C.

Table VI

$Q_{10}$  Values for Metabolic Rates of Snakes Acclimated to Three Different Temperatures and Run at 18.5° C., 24.5° C., and 34.5° C.

Temperature Range - °C.	Acclimation Group		
	4°- 8° C.	18°- 24° C.	26°- 30° C.
18.5-24.5	2.31	2.39	2.61
24.5-34.5	2.34	2.40	2.36
18.5-34.5	2.33	2.40	2.45

have the lowest metabolic rates; those acclimated at 4°- 8° C. have the highest metabolic rates; and the animals acclimated at 18°- 24° C. have metabolic rates more or less intermediate to the others. Student's t-test (28, p. 119-135) was used in an analysis of the differences between the means. The differences between the means of the 4°- 8° C. and 26°- 30° C. groups were significant at the 5 per cent significance level ( $t < -2.45$  and  $t > 2.45$  with 6 degrees of freedom) at all three experimental temperatures (18.5° C.,  $t = -4.17$ ; 24.5° C.,  $t = -3.54$ ; 34.5° C.,  $t = -3.55$ ). The differences between the 18°- 24° C. and 4°- 8° C. groups were significant at the 5 per cent level at 18.5° C. ( $t = -3.49$ ) and 24.5° C. ( $t = -3.06$ ), but not at 34.5° C. ( $t = -2.03$ ). The differences between the 18°- 24° C. and 4°- 8° C. groups were not significant at any

experimental temperature ( $18.5^{\circ}$  C.,  $t = -1.85$ ;  $24.5^{\circ}$  C.,  
 $t = -1.44$ ;  $34.5^{\circ}$  C.,  $t = -1.68$ ).

## DISCUSSION

The tendency for related groups of reptiles to maintain more or less characteristic body temperatures irrespective of geographical distribution and environmental conditions has attracted considerable attention in recent years.<sup>8</sup> It is interesting, therefore, to compare briefly the information obtained in this study of Thamnophis sirtalis concinnus with that recorded for T. s. sirtalis by Carpenter (9) in Michigan. The average body temperature recorded for T. s. concinnus in the present study (22.0° C.) is somewhat lower than that recorded for T. s. sirtalis (25.6° C.). Also, the maximum body temperature recorded here (30.2° C.) is less than Carpenter's maximum record (35.0° C.). These differences do not necessarily indicate a lower preferred body temperature for T. s. concinnus, but more likely reflect the season during which the records were obtained. Carpenter's records were taken from April to September, during which time the mean air temperature was 21.6° C., and he notes that from April to June the body temperatures averaged lower than from July

<sup>8</sup> The papers of Bogert (4), Cowles (12), Cowles and Bogert (13), and Saint-Girons and Saint-Girons (38) present much of the current information on this subject.

to September. The records of the present study were taken from October to February when the mean air temperature was  $16.3^{\circ}$  C. From Carpenter's data it may be calculated that the mean snake-air temperature difference was approximately  $4.0^{\circ}$  C., which compares favorably with the mean  $5.7^{\circ}$  C. difference observed in this study.

It must be realized that air temperature is not being implicated as the factor determining body temperature of the snakes. Although Lueth (30) considers it to be an important factor for snakes, together with radiation, the works of Cole (10), Carpenter (9), Bogert (4), Cowles (12) and Cowles and Bogert (13) indicate that, under natural conditions, direct absorption of radiant energy and conduction from the substratum are the most important means by which snakes and lizards warm their bodies. Furthermore, behavioral patterns enable these animals to utilize most efficiently these sources of heat. Reference to air temperature in this paper is made because more detailed observations were not recorded. The fact that body temperatures were higher than surrounding air temperatures is in accord with information presented in the literature.

Several authors have studied the regression curves for oxygen consumption on weight in amphibians and reptiles. Cook (11) studied the lizards Xantusia vigilis,

Uma notata, Dipsosaurus dorsalis, and Cnemidophorus tessellatus at 20° C. and obtained rather high regression coefficients ranging from 0.892 to 0.984. For the salamander Aneides lugubris he gives a coefficient of 0.725. The significance of Cook's data is difficult to ascertain since he gives no acclimation history or other information for the animals. Vance (44) studied acclimation and weight regression in the lizard Urosaurus ornatus and found that animals acclimated at 8° C. had a higher regression coefficient (0.775) than those acclimated at 35° C. (0.683) when compared at 15° C. This difference was highly significant. Tashian and Ray (43) have also found the weight regression curves for certain anurans to be affected by acclimation. But temperate and boreal forms ("cold acclimated") had lower regression coefficients (approx. 0.70) than tropical ("warm acclimated") forms (approx. 0.84). Different experimental temperatures did not greatly affect the coefficients. The lizards Uta stansburiana and Sceloporus occidentalis have been studied by Dawson and Bartholomew (16). They obtained regression coefficients ranging from 0.47 to 0.68 for animals kept at 20°- 24° C. and run at 20° C., 30° C., 35° C., and 40° C. The differences were not statistically significant, and no trend with temperature was apparent.

Regarding the effect of acclimation on the weight regression curves of Thamnophis sirtalis concinnus, the trend seems to be similar to that described for Urosaurus ornatus (44), although the difference in regression coefficients is more striking between the 26°- 30° C. and 18°- 24° C. acclimation groups than between the 18°- 24° C. and 4°- 8° C. groups. That the differences are not statistically significant is undoubtedly due to the small sample size and the variation introduced by the animals collected in March. It is possible that the generally higher metabolic rates of the latter may be due to endocrine changes associated with the beginning of reproductive activity. But the reason for the drop in metabolic rates observed at 34.5° C. for March animals acclimated at 18°- 24° C. and 26°-30° C. can not be determined. Female snakes were not abroad during January when the first males were collected. The first females were collected late in February, and they had metabolic rates higher than January males. Investigations on frogs (21) and salamanders (45) have shown pronounced increases in metabolism associated with the reproductive season. A thorough investigation on these garter snakes might reveal similar seasonal changes.

Two sources of variation which might have affected the metabolic rates of the snakes have been pretty well ruled out after a careful examination of the feeding and acclimation history of each snake in each experiment. No correlation could be discerned as to individuals having higher rates of metabolism when run only three days after feeding. Furthermore, while Vance (44) indicates that the degree of acclimation to cold may continue to increase over a longer period than acclimation to heat, this interpretation could account for but little of the variation observed in the present data. Thus, inherent variation among the individuals or other undetermined variables must account for much of it.

The coefficients greater than one obtained at 24.5° C. are unusual and probably not normal, though they are not much higher than some of the values obtained by Cook (11). Such high coefficients indicate that oxygen consumption is increasing directly with body weight, i.e., a 100 per cent increase in weight brings a 100 per cent increase in oxygen consumption, rather than a 67 per cent increase as would be the case if the surface law were followed. In seeking to explain these unusual coefficients, as well as the failure of metabolic rate to decrease consistently with increasing body weight (Tables IV, V), the

disadvantages of a small sample size become plainly evident, and any conclusions must be deferred until more experiments are run.

It would be unwise, indeed, to attempt any broad interpretations on the basis of the small amount of uncertain data obtained in this study regarding the relation of weight regression to temperature acclimation. However, it does appear that larger snakes may be more greatly affected by acclimation than smaller ones; i.e., there is a greater spread between larger individuals in the different acclimation groups than between smaller individuals (Figures 3, 4, 5). This may be why the regression coefficients are different for the various acclimation groups. For poikilotherms in general, there has been a great deal of variation observed in the regression coefficients for oxygen consumption on body weight (or for other rate functions). Some of the calculated values come very close to agreeing with the surface law, but others do not. It is beyond the scope of this paper to enter into a discussion of the voluminous literature on the subject. However, it is suggested that some of this variation may be due to differences in acclimation history of the animals as well as the experimental temperatures at which they were studied.

The direct effect of temperature on metabolism in poikilotherms has also been extensively investigated. Again, it is not the purpose of the present paper to review the existing literature. For this the reader is referred to the papers of Rao and Bullock (34), Bullock (6), Roberts (36), and Prosser (31, p. 341-374).  $Q_{10}$  values calculated for the snakes for the temperature ranges  $18.5^{\circ}$ -  $24.5^{\circ}$  C. and  $18.5^{\circ}$ -  $34.5^{\circ}$  C. increase with increasing acclimation temperature (Table VI), following the trend which Rao and Bullock (34) consider to be usual in most poikilothermic species. These authors point out, nevertheless, that there are exceptions to this rule, and the more recent papers by Dawson and Bartholomew (16), Roberts (36) and Tashian and Ray (43) describe additional exceptions. Also, they emphasize the importance of acclimation history in seeking an interpretation of rate-temperature curves. The significance of the  $Q_{10}$  values obtained in this investigation was not tested statistically and no conclusive statements can be made regarding the results. It is interesting to note that the values fall within the range predicted by the Van't Hoff rule, and that they are not necessarily diminished toward the upper limits of the tolerable range.

As far as acclimation itself is concerned, the results of this study (Tables III, IV, V; Figure 6) clearly demonstrate that Thamnophis sirtalis concinnus is able to make compensatory changes in metabolism when maintained at different temperatures even for relatively short periods of time. No doubt the differences between the means of the 18°- 24° C. acclimation group when compared with those of the other two groups are not statistically significant in all cases because of the small sample size. However, the significant differences demonstrated at all three experimental temperatures when the 4°- 8° C. and 26°- 30° C. groups are compared, in spite of the small sample size, are quite convincing. Moreover, the absolute weight-specific rates of oxygen consumption of the snakes agree quite well with those presented by Cook (11) and Dawson and Bartholomew (17) for lizards of comparable size. Thus, another example of metabolic compensation for temperature in reptiles can be added to those described by Vance (44) and Dawson and Bartholomew (16).

## SUMMARY

1. Metabolic compensation for temperature has been demonstrated in a great variety of poikilothermic animals, but papers describing acclimation responses in reptiles have only recently appeared in the literature. None of these papers has dealt with snakes. In order that some information on them might be obtained, the present study was conducted using a species abundant in the Willamette Valley, the red-spotted garter snake (Thamnophis sirtalis concinnus). Since there has been considerable interest regarding the effect of size on the metabolic rate of poikilotherms, experiments were designed to provide information on this subject also. In addition, a limited amount of data was recorded for body temperatures of snakes in the field.

2. Twelve snakes were collected in February and March, 1960. They were arranged in three groups approximately equal in total weight and variation of individual weights. One group was acclimated to warm temperature (26°- 30° C.), another to room temperature (18°- 24° C.), and the third to cold temperature (4°- 8° C.). After acclimation for three to ten days, the oxygen consumption of all snakes was measured in a direct-reading volumetric

respirometer. Measurements were made at 18.5° C., 24.5° C., and 34.5° C.

3. Typical acclimation responses were observed. Snakes acclimated at 4°- 8° C. had significantly higher metabolic rates at all experimental temperatures than those acclimated at 26°- 30° C. The differences between the metabolic rates of snakes acclimated at 18°- 24° C. and those acclimated at the other temperatures were not statistically significant in all cases, probably due to the small sample size.

4.  $Q_{10}$  values for the temperature ranges 18.5°- 24.5° C., 24.5°- 34.5° C., and 18.5°- 34.5° C. fell between 2.31 and 2.61. Cold acclimated animals had lower  $Q_{10}$ 's than those acclimated to heat.  $Q_{10}$  was not diminished in the higher temperature range (24.5°- 34.5° C.).

5. Small animals generally had higher metabolic rates (cc./kg./hr.) than large ones. However, acclimation at the different temperatures seems to have affected the rate at which total oxygen consumption increased with increasing body weight. Animals acclimated at 26°- 30° C. had coefficients for the regression of oxygen consumption on body weight ranging from 0.40 to 0.56; those acclimated at 18°- 24° C. and 4°- 8° C. had coefficients ranging from 0.67 to 1.10. The differences are not statistically significant

because of the small sample size and the variation introduced by animals collected in March.

6. For 64 snakes checked on seven trips to the field between 9 October, 1959, and 7 February, 1960, the mean body temperature was  $22.0^{\circ}$  C. The mean difference between the mean body temperatures and mean air temperatures recorded on the field trips was  $5.7^{\circ}$  C.

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