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The Development of Two Apparatuses and Techniques for the Measurement of the Basil Respiration Rate of a Bull Snake at Various Temperatures

Rowand R. Chaffee

Chaffee — Development of Two Apparatuses

THE DEVELOPMENT OF TWO APPARATUSES AND TECHNIQUES FOR THE MEASUREMENT OF THE BASAL RESPIRATION RATE OF A BULL SNAKE AT VARIOUS TEMPERATURES

by

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MASTER OF SCIENCE

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The literature is extensive with respect to mammalian physiology, but extremely sparse concerning reptilian physiology. According to Cook (1949) very little comprehensive work concerning the fundamental aspects of reptilian physiology has been done since Benedict's work in 1932.

Originally the author of this thesis intended to investigate the effect at various temperatures of certain hormones on the metabolic activity of the reptiles. Because metabolic activity is indicated by pulmonary gaseous exchanges it was believed by this author that the basal respiration rates could be employed to great advantage as an indicator of the effect of hormones on the metabolism of reptiles at various temperatures. A survey of the literature revealed that no inexpensive, wholly satisfactory apparatus has been designed for the measurement of the respiration rate in the reptiles. Also, no complete description of the method to be employed in determining these respiration rates, was described. Therefore, this investigation was made for the purpose of establishing a simple, practical, and inexpensive method of obtaining the respiration rate of a representative reptile.

Two bull snakes (*Pituophis sayi*) were used as representative reptiles. This bull snake is a desirable experimental animal from many standpoints. It is an animal representative of the reptiles prevalent in the southwestern part of the United States, it is easy to work with and care for in the laboratory and can be contained in a relatively small respiration chamber so that its movements are restricted, thus making metabolic conditions more nearly basal.

The data obtained using these two snakes would indicate the consistency, and, to a certain extent, the accuracy of the method. Of course, the data in itself would not be of significance for the reason that not enough animals would have been used. However, it is the belief of this author that if reproducible results could be gotten using only two bull snakes, the method could legitimately be used in future investigations.

According to Vernon (1894), Moeshott reported that the metabolic rate in some reptiles was lower between 10°C. and 15°C. than it was at 5°C. These findings were not in keeping with a later report by Vernon (1894) which indicated the metabolic rate of poikilothermic animals is a function of the temperature. Subsequent workers have since confirmed this relationship.

Baldwin (1928) wrote a paper on the oxygen consumption of certain snakes, including the bull snake, (Pituophis sayi) at 20° C. His apparatus was not internally compensated for temperature variation or barometric changes during the experiment.

Benedict (1929) did some work concerning the effects of handling on the metabolic rate of certain snakes, not including the bull snake, at room temperature. He reported that the metabolic rate of these snakes was temporarily increased by handling.

Dill (1929) worked on the respiration and metabolism of the crocodile and reported anoxia, rather than carbon dioxide accumulation, is the stimulus for pulmonary respiration in the crocodile. There is a possibility that this may also be the case in the snakes, but there is nothing in the literature to confirm this.

Benedict (1932) wrote a very comprehensive book concerning his experiments on the physiology of the large reptiles. He reported the relationship of the metabolic rate to temperature for snakes including some snakes of the genus Python, some snakes of the genus Boa, some rattle snakes of the genus Lachesis, and some gopher snakes of the genus Drymarchon.

He did not, however, use any of the snakes of the genus Pituophis in his experiment. He states that most of his data concerning these metabolic rates were calculated from direct volumetric measurement of the oxygen consumed. His apparatus was a modification of the original Haldane (1892), but instead of adding dehydrated, carbon dioxide-free air by means of an absorption inlet chain, he added oxygen directly by means of a spirometer. These data indicated that the metabolic rate increased, in general, with the temperature, but that there was not a linear relationship between the two.

The effect of aggregation on the consumption of oxygen by the brown snake, (Storeria dekayi) was reported by Clausen (1936). His technique and apparatus were similar to those of Benedict (1932). He introduced a constant temperature burette, but his gas analysis was carried out on only 10 cc. samples of gas, which assumed complete homogeneity of the gas mixture.

Roth (1942) established the respiratory quotient of the bull snake (Pituophis sayi) and others at room temperature. The apparatus that he used was a modification of that used by Benedict (1932) equipped with a revolving drum to record the movements of a dual-piston spirometer with respect to time. However, he unfortunately did not publish the actual oxygen con-

So [illegible], however, [illegible] of the [illegible]

Pimephales [illegible]

data concerning the [illegible]

from [illegible]

[illegible]

(1962), [illegible]

[illegible]

[illegible]

indicated that [illegible]

with the [illegible]

[illegible]

The effect of [illegible]

[illegible]

by Gibson (1953) [illegible]

similar to those of [illegible]

constant temperature [illegible]

carried out on only [illegible]

complete independently of [illegible] temperatures.

Heath (1963) [illegible]

of the [illegible]

Compensation [illegible]

[illegible]

[illegible]

[illegible]

[illegible]

sumption nor the carbon dioxide and water exhaled.

Cook, 1949 published a paper on the relationship of the oxygen consumption of certain lizards to various temperatures. His apparatus was similar to that of Baldwin (1928) but he had a constant temperature regulating system for the respiration chamber.

In this investigation two apparatuses have been designed to analyze the respiratory gases and determine the oxygen consumption respectively in the bull snake (Pituophis sayi) at various temperatures. Each of these apparatuses has features which have not been applied in similar apparatuses, but are modified forms of respirometers employed by former workers. One of these is a modification of the original Haldane (1892), wherein the production of respiratory gas was analyzed. However, instead of using a gravimetric determination of the carbon dioxide produced, a titration method was used for greater accuracy in determining small amounts of gas. The other apparatus is a modification of the Sholander (1942) micro-respirometer, wherein the respiration chamber has been increased in size sufficiently to contain the bull snake.

A different apparatus was used for each set of data, i.e., respiratory gas analysis and the oxygen consumption, respectively, because of the simplicity

of operations and the introduction of a minimum possibility for error. Either one of these sets of data could be used as an indication of the respiration rate in a given temperature range for comparative purposes in future investigations. The necessity for using the two different apparatuses lies in the fact that one was used as a check on the other and vice versa. The two procedures were very different and it was believed that if the results correlated it would indicate that no gross source of error in either procedure was likely. The snakes were subjected only to temperature variation well within their normal temperature limits. Therefore their physical condition due to use in experiments was not subject to change.

MATERIALS AND METHODS

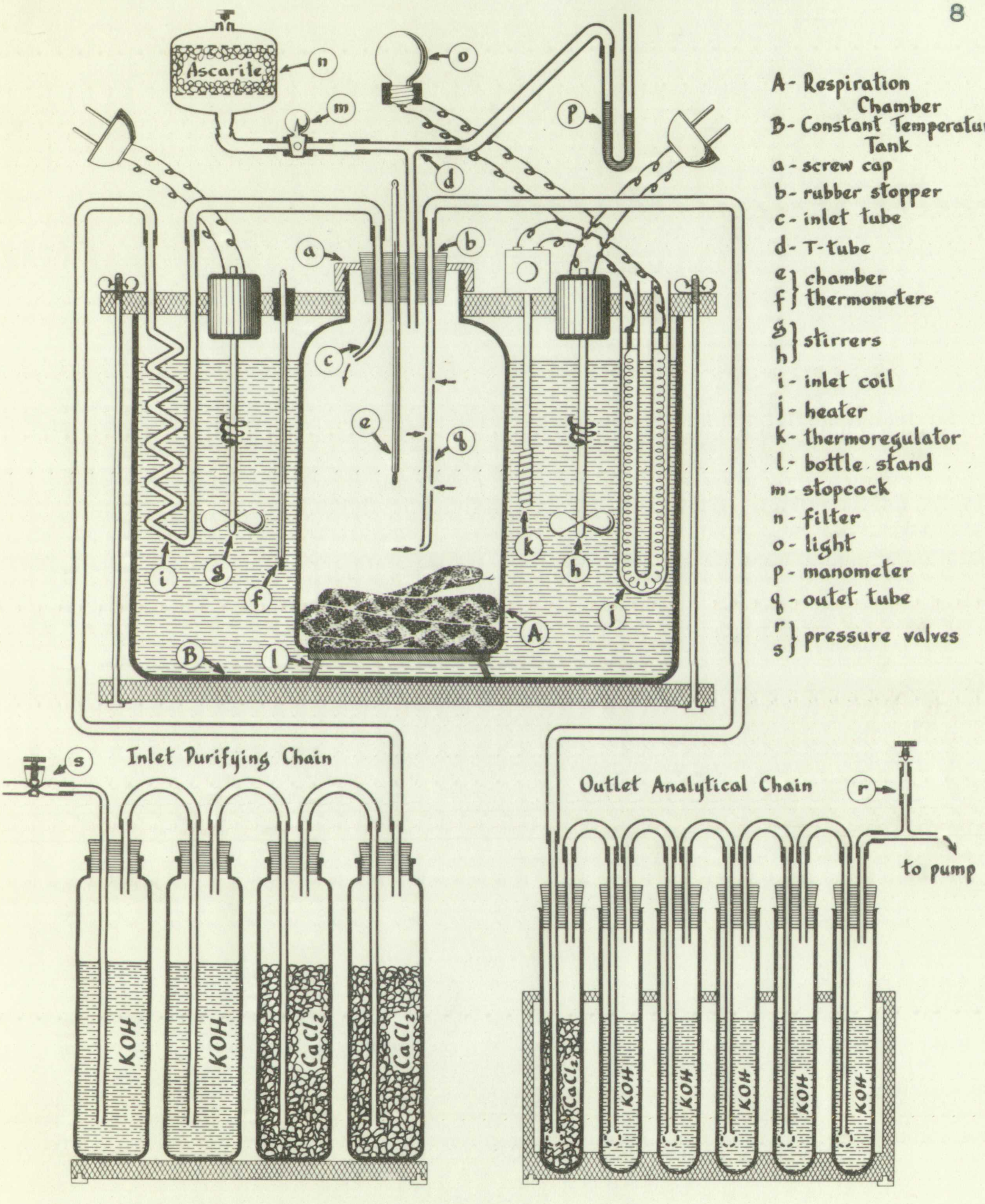
Description of the Apparatus. Two apparatuses, I and II, used in studying the respiratory rate of the bull snake, at various temperatures, are shown on the following pages.

Apparatus I has a purifying inlet chain (see Fig. 1) which consists of a series of 450 cc. bottles, some of which are filled with concentrated potassium hydroxide solution which takes out all of the carbon dioxide in the room-air as it enters the air-tight respiration chamber, D. Other bottles in this inlet chain contain sufficient amounts of calcium chloride to remove all of the moisture from the incoming air. As this air passes through the coiled copper tubing, 1, which is twelve feet long, its temperature is brought to that of the water bath before it enters the respiration chamber. As the air in the respiration chamber, sucked through by the means of an air-suction pump, passes out, it goes through the outlet analytical chain (see Fig. 1). This outlet chain consists of a weighed, calcium chloride-filled test tube and a series of 100 cc. test tubes containing a known volume and concentration of potassium hydroxide. This concentration of potassium hydroxide is more than sufficient to remove

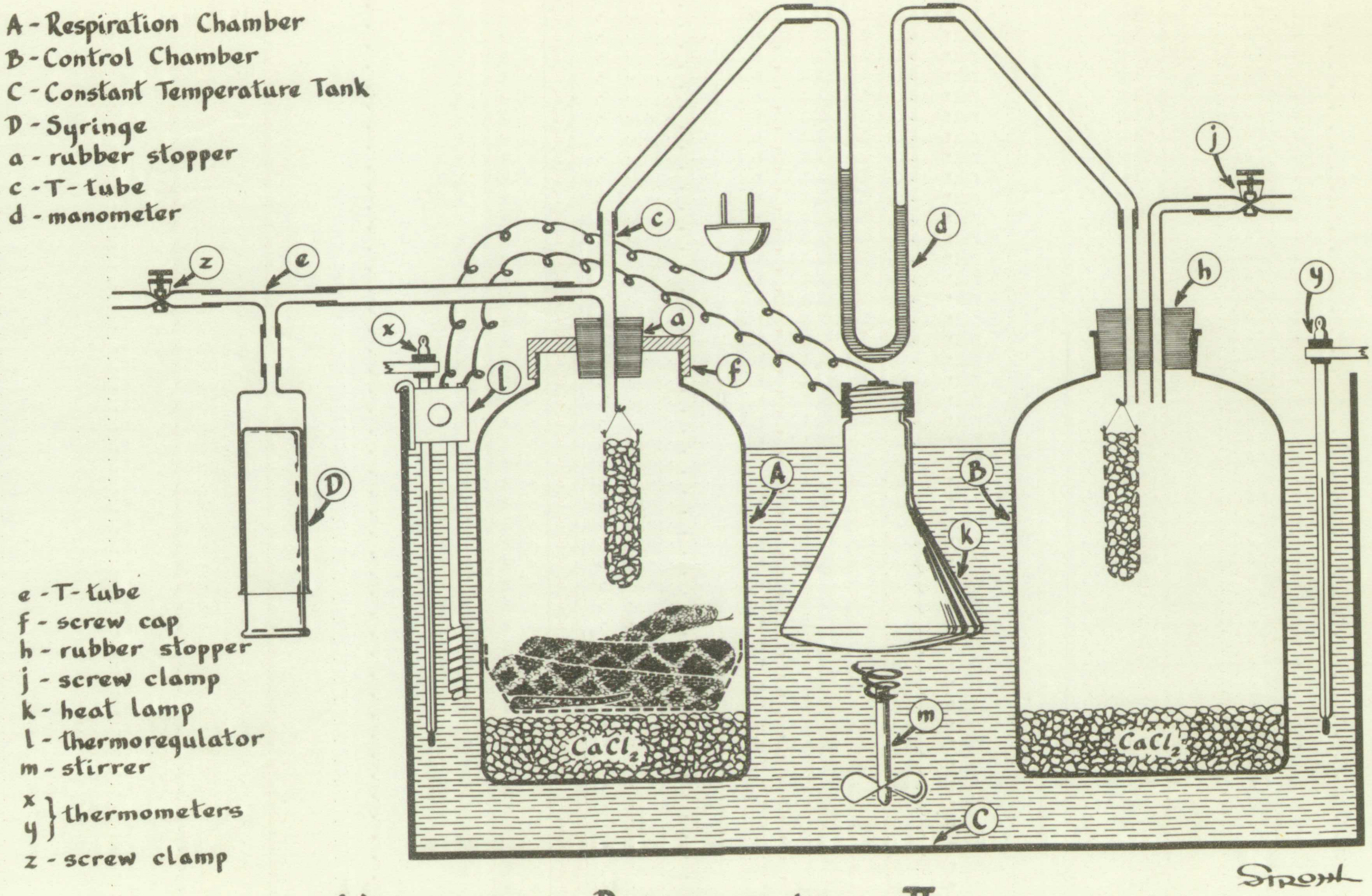
MATERIALS AND METHODS

Description of the Apparatus. [illegible]
I and II, used in studying [illegible] of the
bull [illegible], [illegible] are shown on the
following pages.

Apparatus I [illegible]
Fig. 1) which consists of [illegible] cm. [illegible]
some of [illegible] and [illegible]
hydroxide solution [illegible] the carbon
dioxide [illegible]
respiration [illegible]
chain [illegible] to
remove all of the [illegible] from the [illegible]
this air passes through the [illegible]
which is [illegible] to [illegible]
to that of the [illegible] the respira-
tion chamber. [illegible]
[illegible]
passes [illegible] through the [illegible]
(see Fig. 1). [illegible]
calcium chloride [illegible]
test [illegible] solution
[illegible] potassium hydroxide [illegible]
potassium hydroxide [illegible]



Titrometric Respirometer I
for CO_2 and H_2O Analysis
fig 1



Volumetric Respirometer II

for measurement of Oxygen Consumption

Fig. 2

all of the carbon dioxide in the exhaled air.

To insure that during the evacuation, and concomitant refilling of the respiratory chamber, the snake is not subjected to a vacuum of more than one and one half inches of mercury below atmospheric pressure, at this altitude, a manometer, p, was used to record the magnitube of the vacuum. If the vacuum became too great, a pressure-equalizing valve, m, was immediately opened. The room-air rushing through this valve passed through an ascarite filter, n, and all of the carbon dioxide in this incoming air was removed.

The glass inlet tube, c, was tapered and bent at an angle to increase the washing power of the incoming jet of air. The outlet tube, q, had four small holes at different levels to extract the air in the respiration chamber at these four levels and thus reduce the possibility of having significant amounts of carbon dioxide left in the respiration chamber.

The temperature of the water in the constant temperature bath, B, was controlled by the thermo-regulator, k, which was connected in series with the heater, j. Low temperatures were obtained by the addition of varying amounts of ice to the water bath. Connected and in parallel with the heater was a light, o, which turned on

all of the carbon dioxide in the entering air.

To insure that during the evaporation, and [illegible] ant refilling of the [illegible] [illegible], the [illegible] not subjected to [illegible] of more than [illegible] inches of mercury [illegible] [illegible] altitude, a [illegible] [illegible] tube of the vacuum. If the vacuum [illegible] pressure-equalizing [illegible] [illegible] the room-air rushing through this [illegible] an asbarite filter, [illegible] all of the carbon dioxide in this incoming air was removed.

The glass inlet tube, [illegible] an angle to increase the [illegible] jet of air. The outlet tube, [illegible] at different levels [illegible] chamber, at [illegible] possibility of [illegible] dioxide left in the [illegible]

The temperature of the [illegible] ture bath, [illegible] controlled by [illegible] which was connected in [illegible] temperatures [illegible] amounts of ice to the water [illegible] parallel with the [illegible]

automatically when the heater was in operation. The light functioned as a safety signal to warn the operator if the thermo-regulator should be operating with too much margin. Two thermometers, e, and f, were used to record the temperatures of the air in the respiration chamber and the water in the water bath, respectively. Two one-fiftieth horse-power, series wound, electric stirrers, g, and h, circulated the water in the constant temperature bath.

The speed of the air flow and, to a certain extent, the air pressure in the system were controlled by the inlet and outlet valves, r and s, respectively.

The respiration chamber, A, consisted of a large glass jar with a screw cap, a, which allowed the passage of the snake. This screw-cap had a large central hole which was filled by the rubber stopper, b, through which the inlet tube, the outlet tube, and a control tube, d, passed.

<u>Description of the use of Apparatus I</u>. The snake was put into the respiration chamber and the screw-cap was screwed into place using stopcock grease to insure an air-tight seal. The stopper, containing the inlet, outlet, and control tube, was then placed into the screw-cap hole tightly. A rubber tube which shunted the outlet chain was then connected directly with the air suction

automatically when the [illegible]

light [illegible] if [illegible]

if the [illegible] should [illegible]

such margin. [illegible] to

record the temperatures of [illegible]

chamber and the water in the [illegible]

Two one-fiftieth horse-power [illegible]

stirrers, [illegible] and [illegible]

temperature [illegible].

The speed of the [illegible]

the air pressure [illegible]

inlet and outlet [illegible]

The [illegible]

glass jar with [illegible]

of the snake. [illegible]

which was [illegible]

the inlet tube [illegible]

passed.

Description of the [illegible] was put into the [illegible]

was screwed into place [illegible]

an air-tight seal. [illegible]

outlet, and [illegible]

cap hole tightly [illegible]

chain was then screwed [illegible]

pump. The heater and stirrers were then turned on and the respiration chamber and snake were brought to the desired temperature. The air in the respiration chamber was then removed directly by means of a shunting rubber tube thru the air-suction pump, as fresh, carbon dioxide-free, water-bath-temperature-air entered thru the coil, i. Since there was sufficient air in the respiration chamber to supply oxygen to the snake for about half an hour or more without unduly decreasing the oxygen percentage in the respiration chamber, the air was removed therefrom at given time intervals, thereafter thru the analytical outlet chain. The total time that the snake could stay in the respiration chamber in any given experiment was unlimited, except for possible cramping of the snake over a long period of time, because the outlet chain was in duplicate. The refilling of an outlet chain was only a matter of minutes once the titration of the potassium hydroxide for the day had been done.

Method of gas analysis in Apparatus I. As the air from the respiration chamber was drawn thru the outlet chain the carbon dioxide reacted with the potassium hydroxide to form potassium bicarbonate, water, and potassium carbonate, as shown by the following formula:

pump. The [illegible] and [illegible] [illegible]

the respiration [illegible] [illegible] [illegible]

desired temperature. [illegible]

was then removed [illegible]

tube [illegible] the [illegible]

free, water-[illegible]

Since there was [illegible]

to supply [illegible]

more without [illegible]

the respiration [illegible]

at given time intervals [illegible]

outlet chain. The [illegible]

in the respiration [illegible]

unlimited, except [illegible]

over a long period of time, [illegible]

was in duplicate. The [illegible]

only a matter of minutes [illegible]

potassium hydroxide [illegible]

Method of [illegible] analysis [illegible]

From the respiration [illegible]

drain the [illegible]

hydroxide to form [illegible]

potassium carbonate [illegible]

$$CO_2 + 2KOH \rightarrow K_2CO_3 \underset{}{\overset{HOH}{\rightleftarrows}} KHCO_3 + KOH$$

Since there was an excess of potassium hydroxide (amount established by correlating the works of others on snakes at twenty degrees, and by trial and error) and the air was drawn through the outlet chain at a fairly slow rate, there was very little chance for carbon dioxide to escape. The last tube of the outlet chain was checked periodically and each time it was found devoid of any appreciable amount of carbonates.

After each experiment the tubes in the outlet chain were emptied quickly, so that atmospheric carbon dioxide would not react with potassium hydroxide, and washed out with distilled water into a 1000cc. Volumetric flask containing an excess of barium chloride. The level of the solution was brought to the 1000 cc. mark by adding carbon dioxide-free distilled water. The flask was then plugged with an air-tight ground glass, greased stopper. The chemical reactions involved are:

$$\begin{array}{ccccccc}
H_2O & + & K_2CO_3 & + BaCl_2 \longrightarrow & BaCO_3 & + 2KCl + HOH & \\
\uparrow & & \upharpoonleft\downharpoonright & & \downarrow & & \\
 & & & & ppt. & & \\
CO_2 + 2KOH & & KHCO_3 + KOH & & & & \text{Neutral}
\end{array}$$

The barium chloride reacted with the carbonate and bicarbonate of potassium to form the insoluble barium carbonate. After the precipitate settled out, two aliquots of fifty cc. each of the supernatant solution of potassium hydroxide were analyzed for the remaining hydroxyl concentration. In the formation of a carbonate ion two hydroxyl ions are removed from the original potassium hydroxide solution.

$$CO_2 + 2KOH \longrightarrow K_2CO_3 + H_2O$$

Before each experiment the potassium hydroxide was titrated against sulphuric acid of a known normality. The same acid was used to titrate the above mentioned aliquots after the experiment. Phenolphthalein was used as the indicator.

Methods used in the calibration of Apparatus I. A given amount of carbon dioxide gas was introduced into the inlet tube, by means of a gas burette, and allowed to remain in the respiration chamber for a period of one hour. At the end of this period the gas was removed through the analytical outlet chain and each tube was individually analyzed. The last tube in the chain always proved devoid of carbonates. The respiration chamber was flushed with carbon dioxide-free-air for six minute intervals. The first six minute interval resulted in the removal of eighty-nine per cent of the injected carbon dioxide. Repeating the check and doubling the interval to twelve minutes removed ninety-six per cent of the injected carbon dioxide. Running the check for twenty-four minutes did not show any appreciable increase in the percentage of recovery. Thus it is assumed that about four per cent of 200 cc., or 8cc., of carbon dioxide remained in the respiration chamber. During an experimental run, since the system is flushed about ten times, this amount is relatively insignificant as compared to the amount of carbon dioxide produced by the snake during any experiment. The total time involved in an experimental run at lower temperatures was purposely increased in an attempt to minimize this source of error.

The purifying effect of the inlet chain, and a good indication that the apparatus was not leaking, was evidenced in the lack of change in the hydroxyl ion concentration in the outlet chain when the apparatus was run for a period of two hours, at a slow rate, without the snake being in the chamber. Another check which showed that the system was not leaking was made by sucking a vacuum of six inches of mercury on the system and clamping off the inlet and outlet valve. The manometer showed no change in level although the apparatus was left frequently for a period of more than an hour.

The efficiency of the water vapor removing system of the inlet chain was checked by weighing the tube of calcium chloride in the outlet chain before and after allowing the system to run for an hour or more. At a normal rate the calcium chloride tube in the outlet chain did not take on any appreciable weight. However, if the air was drawn through at a very fast rate the moisture was not properly removed by the inlet dehydrating bottles of calcium chloride. Therefore, during the experiments the rate was kept fairly slow in order that all the water be removed in the inlet chain, and also because faster rates caused a greater vacuum in the respiration chamber. The checking of the efficiency of complete moisture

The purifying effect of the inlet [illegible], an [illegible] indication that the [illegible] evidenced in the [illegible] of [illegible] in the [illegible] concentration in the outlet [illegible] when the [illegible] for a period of the [illegible] snake being in the [illegible]. Another [illegible] that the system was not [illegible] vacuum of six liters [illegible] [illegible] no change in [illegible] [illegible] for a period of [illegible].

The efficiency of [illegible] of the inlet [illegible] by [illegible] the [illegible] of calcium chloride in the [illegible] allowing the system to run [illegible]. [illegible] normal rate the [illegible] did not [illegible] any [illegible] air was drawn through it. [illegible] was not properly removed by the inlet [illegible] of calcium chloride. [illegible] the rate was [illegible] be removed in the inlet [illegible] rates [illegible]. The meaning of [illegible].

absorption by the outlet tube of calcium chloride was done by adding an additional drying tube in the line of air flow, beyond the original drying tube. There was no increase in weight in the second tube whatsoever even at a very fast flow. During this last checking procedure the room air was passed directly into the respiration chamber without the use of the inlet chain.

Description of Apparatus II. Apparatus II was a greatly enlarged modification of the Sholander micro-respirometer. A large glass jar, A, was the respiration chamber. One leg of t-tube, c, was connected by means of rubber pressure tubing with the manometer, d, which was filled with "Tween 80", which has a low specific gravity and contains a wetting agent to insure sensitivity to pressure changes. The other leg of the t-tube, c, was connected to one leg of another t-tube, e. The stem of t-tube, e, was connected to a 30 cc. syringe, D. The other leg of t-tube, e, was connected to a spirometer, which was filled with oxygen via screw clamp, z. The manometer was also connected to a pressure control chamber, B, which was a large glass bottle (larger than jar, A). This bottle had a rubber stopper, h, which allowed for the air-tight passage of two glass tubes. One of these was attached to the manometer, as stated above, and the other connected, via screw clamp, j, with the room-atmosphere.

absorption by the [illegible] of [illegible] [illegible] [illegible] [illegible] by adding an [illegible] [illegible] [illegible] [illegible] beyond the [illegible] [illegible] [illegible] [illegible] in weight in the [illegible] [illegible] [illegible] [illegible] foot flow. During this [illegible] [illegible] [illegible] air was passed directly into the [illegible] [illegible] without the use of the [illegible].

Description of [illegible] [illegible] [illegible] [illegible] greatly enlarged [illegible] of [illegible] respirometer. A [illegible] [illegible] [illegible] chamber. One leg of [illegible] [illegible] [illegible] of rubber pressure tubing with [illegible] was filled with [illegible] [illegible] [illegible] gravity and contains a [illegible] [illegible] [illegible] to pressure changes. The other leg of [illegible] [illegible] connected to one leg of [illegible] [illegible] [illegible] t-tube, t, was connected to a [illegible] [illegible] [illegible] other leg of t-tube, [illegible] connected [illegible] which was filled [illegible] [illegible] [illegible] manometer was [illegible] [illegible] [illegible] c, which was a large glass bottle [illegible] [illegible] This bottle has a [illegible] [illegible] [illegible] the air-tight [illegible] of the [illegible] [illegible] was [illegible] by [illegible] [illegible] other connected, via [illegible] [illegible] [illegible]

The constant temperature bath consisted of an eight gallon aluminum tank, D. The heating system for the bath was a 200 watt silver painted heat lamp, k, connected in series with a thermoregulator, l, which was sensitive to plus or minus five-tenths of a degree. Low temperatures were obtained by adding varying amounts of ice to the water bath and draining the excess water. The water was kept in constant circulation by a stirrer, m, which was centrally located in the bath. Two thermometers, x and y, were kept in the water bath at opposite ends of the tank and uniform temperature was observed throughout the water bath.

In respiration chamber, a, was a wire gauze tube filled with ascarite to absorb the carbon dioxide exhaled by the snake. To absorb water vapor and thus do away with any vapor pressure build-up, calcium chloride was placed in the bottom of the respiration chamber. The snake was kept from coming in contact with the calcium chloride by means of two layers of heavy wire gauze on which the snake remained during the experiments. The pressure chamber, b, also contained sufficient ascarite to remove any carbon dioxide originally present, and calcium chloride to remove any water that might be present in the air.

The constant temperature [illegible] of a [illegible] gallon aluminum [illegible]. The [illegible] was a 200 watt [illegible] in series with a [illegible] pile or [illegible] by [illegible] were obtained by [illegible] water bath and [illegible] kept in constant [illegible] centrally located in the [illegible] F. were kept in the water [illegible] tank and uniform temperature [illegible] water bath.

In respiration [illegible] filled with [illegible] to absorb the [illegible] by the snake. [illegible] with any vapor [illegible] placed in the [illegible] snake was [illegible] chloride by means of [illegible] which the snake [illegible] pressure [illegible] to remove [illegible] calcium chloride [illegible] sent in the [illegible]

A description of the use of Apparatus II. The snake was put into the respiration chamber and the total system was allowed to come to the desired temperature. The syringe was then filled with oxygen from the spirometer and the screw clamps were then closed. The exact time, barometric pressure, and room temperature were recorded. At given time intervals the manometer indicated a decrease in pressure in chamber, A. Oxygen was added by means of the syringe until the manometer returned to the level position. The volume of the oxygen added was recorded and converted to standard conditions.

Checking Apparatus II. Apparatus II was checked by adding a given amount of carbon dioxide gas by means of a gas burette and observing its absorption rate by the ascarite. Observations were also made to determine whether or not the manometer returned to its level position. The carbon dioxide was removed in seven minutes after its introduction into the respiration chamber, However, the manometer never completely returned (in a three hour period) to the level position indicating that a slight amount of carbon dioxide was not absorbed. The total pressure difference in chamber, A, and chamber, B, at the end of seven minutes was about three-sixteenths of an inch of "Tween 80". When large amounts of carbon

dioxide were added the time of absorption did not increase above seven minutes nor did the final level difference vary. In order to be sure that there were no leaks in the system two hundred cc. of oxygen were added to the respiration chamber and the column difference in the manometer marked. After a period of ten minutes, to insure complete absorption of any carbon dioxide contained in the chamber, the levels of the manometer fluid were again marked and the apparatus was allowed to stand for one hour. The total difference between the two fluid levels in the manometer, originally about eight inches of fluid, remained the same.

Laboratory Care of the Bull Snakes. The bull snakes were kept in cages two and one half feet long, two and one half feet wide, and one foot high. The temperature of the cages was about 22° Centigrade and did not fluctuate more than 3° Centigrade up or down. Fresh water was available to the snakes at all times. The snakes were fed, or offered, mice once every three weeks. However, even though the mice were left in the cages with the snakes, the snakes did not eat at any regular intervals. Frequent handling of the snakes at times other than the actual time of the experiments tended to minimize excitement during manipulation previous to actual experimentation, Benedict (1929).

dioxide were above the time of [illegible]

above seven minutes [illegible]

vary. In order to insure that there was no leak in

the system two hundred cc. of oxygen were [illegible]

respiration chamber and the system, [illegible]

manometer [illegible]. After a period of [illegible] minutes, to

insure complete absorption of [illegible] carbon dioxide [illegible]

in the chamber, the levels of the manometer fluid were

again marked and the [illegible]

one hour. The total difference between the

levels in the manometer, [illegible]

of fluid, remained the same.

Laboratory Care of the [illegible] Snakes. The [illegible]

were kept in cages one and one half feet long, [illegible]

one half feet wide, [illegible] feet high. [illegible]

of the cages was about 2[illegible] [illegible]

more than 30 [illegible]

available to the snakes at all times. [illegible]

fed, or offered, [illegible]

even though the [illegible]

snakes, the snakes [illegible]

[illegible]

actual time of [illegible]

excitement during [illegible]

mentation, [illegible]

EXPERIMENTAL RESULTS

The results obtained using these two different apparatuses on the two bull snakes are examples of the results which can be obtained using these apparatuses.

Tables I and II on the following pages each consist of the carbon dioxide production and calculated oxygen consumption of two bull snakes, Bull Snakes 1 and 2, respectively, at various temperatures in Apparatus I. Those experiments made while snakes were shedding were marked with asterisks. The period of shedding was considered to be that period commencing with the first physical signs of the early stages of shedding, and ending with the loss of the outer skin, Benedict (1932). The bull snake does not eat during the shedding period, King (1941).

The values shown in the tables for the respiratory gases, expressed in cubic centimeters at standard conditions per gram of bull snake per hour, were obtained from bull snakes under basal conditions during periods of fasting. The values for the carbon dioxide (see Tables I and II) were calculated from each of the titrations made during an experiment. During the course of the experiment the potassium hydroxide in the outlet chain was replaced one or more times. The solution which had absorbed the carbon dioxide in the air from the respiration chamber for a time indicated by "Hours between Titrations" (see Tables I and II)

TABLE I

OXYGEN CONSUMPTION IN CUBIC CENTIMETERS AT STANDARD CONDITIONS PER GRAM OF BULL SNAKE 1 PER HOUR USING APPARATUS I

20°C

	Hrs. between Titrations	CO_2	O_2	R.Q.
	2	.138		
	3	.135		
	6	.137		
Total Hrs. for Expr. 1 ---	11			
Average Values for CO_2, O_2, and R.Q. for Expr. 1 ---		.137	.193	.710
	2	.139		
	3	.141		
	4	.141		
Total Hrs. for Expr. 2 ---	9			
Average Values for CO_2, O_2, and R.Q. for Expr. 2 ---		.141	.200	.705
Average Values for CO_2, O_2, and R.Q. for Exprs. 1 and 2 -----------		.139	.196	.708
Total Hrs. for Expr. 3 ---	*8	.161		
Average Values for CO_2, O_2, and R.Q. for Expr. 3 ---		.161	.224	.717

25°C

	Hrs. between Titrations	CO_2	O_2	R.Q.
	3	.160		
	4	.161		
Total Hrs. for Expr. 1 --	7			
Average Values for CO_2, O_2, and R.Q. for Expr. 1 --		.161	.226	.712
	2	.164		
	2	.163		
	4	.166		
Total Hrs. for Expr. 2 --	8			
Average Values for CO_2, O_2, and R.Q. for Expr. 2 --		.165	.233	.712
Average Values for CO_2, O_2, and R.Q. for Exprs. 1 and 2 ---------		.164	.229	.712
Total Hrs. for Expr. 3 --	*6	.165		
Average Values for CO_2, O_2, and R.Q. for Expr. 3 --		.165	.282	.720

30°C

	Hrs. between Titrations	CO_2	O_2	R.Q.
	4	.204		
	4	.202		
Total Hrs. for Expr. 1 ---	8			
Average Values for CO_2, O_2, and R.Q. for Expr. 1 ---		.203	.285	.713
	4	.204		
	4	.199		
Total Hrs. for Expr. 2 ---	8			
Average Values for CO_2, O_2, and R.Q. for Expr. 2 ---		.202	.281	.719
	4	.204		
	5	.200		
Total Hrs. for Expr. 3------	9			
Average Values for CO_2, O_2, and R.Q. for Expr. 3 ---		.202	.281	.719
Average Values for CO_2, O_2, and R.Q. for Exprs. 1, 2, and 3 ---------		.202	.282	.717
	*6	.251		
	*1½	.269		
Total Hrs. for Expr. 4 ----	*7½			
Average Values for CO_2, O_2, and R.Q. for Expr. 4 --		.255	.350	.728

35°C

	Hrs. between Titrations	CO_2	O_2	R.Q.
	2	.249		
	2	.256		
	2	.257		
	2	.255		
Total Hrs. for Expr. 1 --	8			
Average Values for CO_2, O_2, and R.Q. for Expr. 1 --		.243	.354	.718
	3	.259		
	4	.254		
	6	.256		
Total Hrs. for Expr. 2 --	13			
Average Values for CO_2, O_2, and R.Q. for Expr. 2 --		.256	.356	.720
Average Values for CO_2, O_2, and R.Q. for Exprs. 1 and 2 ---------		.255	.355	.719
	*2	.309		
	*3	.319		
	*4	.327		
Total Hrs. for Expr. 3---*	9			
Average Values for CO_2, O_2, and R.Q. for Expr. 3 --		.320	.433	.739

—— Points plotted on graphs (normal) *Shedding
—— Points plotted on graphs (shedding)

TABLE II

OXYGEN CONSUMPTION IN CUBIC CENTIMETERS AT STANDARD CONDITIONS
PER GRAM OF BULL SNAKE 2 PER HOUR USING APPARATUS I

5°C

	Hrs. between Titrations	CO_2	O_2	R.Q.
	3	.084		
	4	.079		
	4	.078		
	6	.082		
Total Hrs. For Expr. 1---	17			
Average Values for CO_2, O_2, and R.Q. for Expr. 1--		.081	.116	.698
	2	.083		
	2	.079		
	4	.078		
	8	.080		
Total Hrs. for Expr. 2---	16			
Average Values for CO_2, O_2, and R.Q. for Expr. 2--		.080	.114	.702
Average Values for CO_2, O_2, and R.Q. for Exprs.1 and 2		.080	.115	.700
	*4	.073		
	*8	.074		
Total Hrs. for Expr. 3---	*12			
Average Values for CO_2, O_2, and R.Q. for Expr. 3 --		.074	.110	.671

10°C

	Hrs. between Titrations	CO_2	O_2	R.Q.
	4	.103		
	4	.098		
	6	.115		
Total Hrs. For Expr. 1-	14			
Average Values for CO_2, O_2, and R.Q. for Expr. 1-		.107	.152	.704
	3	.108		
	4	.104		
	6	.102		
Total Hrs. For Expr. 2-	13			
Average Values for CO_2, O_2, and R.Q. for Expr. 2-		.104	.148	.703
Average Values for CO_2, O_2, and R.Q. for Exprs. 1 and 2		.106	.150	.704
	*6	.103		
	*6	.107		
Total Hrs. for Expr. 3-	*12			
Average Values for CO_2, O_2, and R.Q. for Expr. 3-		.105	.150	.700

15°C

	Hrs. between Titrations	CO_2	O_2	R.Q.
	3	.121		
	4	.119		
	6	.120		
Total Hrs. for Expr. 1---	13			
Average Values for CO_2, O_2, and R.Q. for Expr. 1---		.120	.170	.706
	4	.125		
	8	.120		
Total Hrs. for Expr. 2---	12			
Average Values for CO_2, O_2, and R.Q. for Expr. 2 ---		.122	.172	.709
Average Values for CO_2, O_2, and R.Q. for Exprs. 1 and 2 ------------		.121	.171	.706
	*3	.122		
	*8	.125		
Total Hrs. for Expr. 3---	*11			
Average Values for CO_2, O_2, and R.Q. for Expr. 3---		.124	.175	.709

20°C

	Hrs. between Titrations	CO_2	O_2	R.Q.
	4	.137		
	4	.138		
	4	.132		
Total Hrs. for Expr.1--	12			
Average Values for CO_2, O_2, and R.Q. for Expr. 1----		.136	.191	.712
	4	.139		
	6	.136		
Total Hrs. for Expr. 2-	10			
Average Values for CO_2, O_2, and R.Q. for Expr.2		.137	.194	.707
Average Values for CO_2, O_2, and R.Q. for Exprs. 1 and 2 --- ------		.137	.192	.710
	*4	.155		
	*6	.163		
Total Hrs. for Expr. 3-	*10			
Average Values for CO_2, O_2, and R.Q. for Expr. 3--		.160	.223	.717

25°C

	Hrs. between Titrations	CO_2	O_2	R.Q.
	4	.153		
	6	.155		
Total Hrs. for Expr. 1 ---	10			
Average Values for CO_2, O_2, and R.Q. for Expr. 1 ---		.154	.216	.713
	6	.158		
	6	.156		
Total Hrs. for Expr. 2 --	12			
Average Values for CO_2, O_2, and R.Q. for Expr. 2 ---		.157	.220	.714
	4	.157		
	4	.159		
Total Hrs. for Expr. 3 --	8			
Average Values for CO_2, O_2, and R.Q. for Expr. 3 ---		.158	.222	.712
Average Values for CO_2, O_2, and R.Q. for Exprs. 1, 2, and 3 -----		.156	.219	.713
	*4	.179		
	*6	.181		
Total Hrs. for Expr. 4 --	*10			
Average Values for CO_2, O_2, and R.Q. for Expr. 4 ----		.179	.249	.723

30°C

	Hrs. between Titrations	CO_2	O_2	R.Q.
	4	.184		
	6	.176		
Total Hrs. for Expr. 1	10			
Average Values for CO_2, O_2, and R.Q. for Expr. 1 ---		.180	.252	.714
	3	.181		
	8	.178		
Total Hrs. for Expr. 2 --	11			
Average Values for CO_2, O_2, and R.Q. for Expr. 2 ---		.179	.251	.713
	4	.182		
	4	.184		
Total Hrs. for Expr. 3 ---	8			
Average Values for CO_2, O_2, and R.Q. for Expr. 3 ---		.183	.255	.718
Average Values for CO_2, O_2, and R.Q. for Exprs. 1, 2, and 3 -----		.181	.253	.715
	*10	.200		
	* 4	.205		
Total Hrs. for Expr. 4 --	*14			
Average Values for CO_2, O_2, and R.Q. for Expr. 4 ---		.202	.279	.724

35°C

	Hrs. between Titrations	CO_2	O_2	R.Q.
	3	.245		
	5	.241		
Total Hrs. for Expr. 1 --	8			
Average Values for CO_2, O_2, and R.Q. for Expr. 1 --		.242	.338	.716
	4	.242		
	4	.246		
Total Hrs. for Expr. 2 ---	8			
Average Values for CO_2, O_2, and R.Q. for Expr. 2 --		.244	.340	.718
Average Values for CO_2, O_2, and R.Q. for Exprs. 1 and 2 ----------		.243	.339	.717
	*7	.287		
	*6	.303		
Total Hrs. for Expr. 3 --	*13			
Average Values for CO_2, O_2, and R.Q. for Expr. 3--		.294	.404	.731

*Shedding

Points plotted on graphs (normal)

Points plotted on graphs (shedding)

was titrated. Then the carbon dioxide production for that time interval was calculated and converted to cubic centimeters at standard conditions per gram of bull snake per hour. In order to obtain the average carbon dioxide production value for an experiment the individual values obtained for each titration, were multiplied by the "Hours between Titrations"; then the products were added together and divided by the total hours involved in the experiment. The average value of the oxygen consumption for an experiment was obtained by calculation (see Formula (1), page 13).

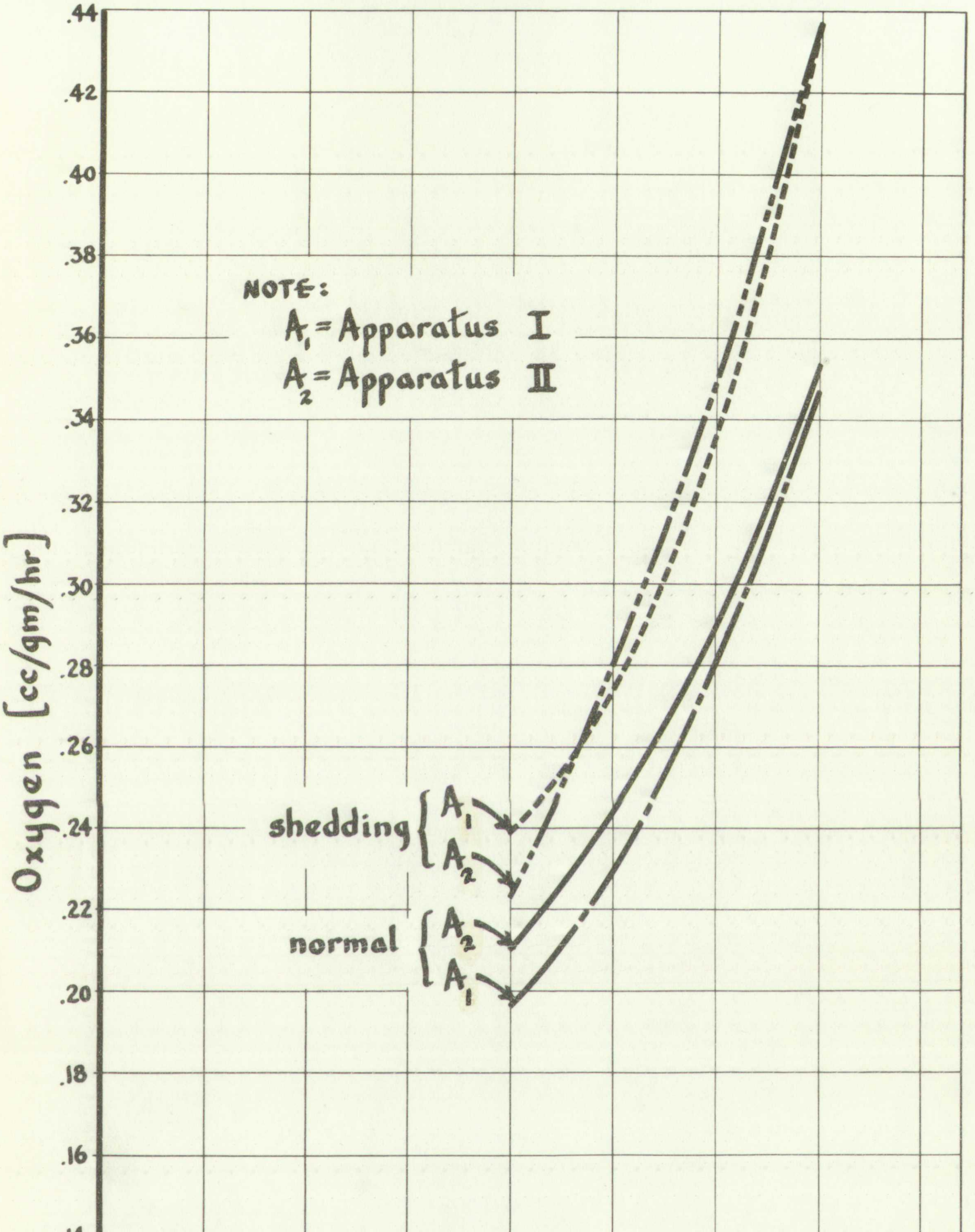
The respiratory quotient, or R.Q., for each experiment was obtained by dividing the average carbon dioxide value for an experiment by the calculated average oxygen consumption value for that experiment.

The values plotted on the graphs, Figures 3 and 4, for Apparatus I, on the following pages, were the average of the calculated oxygen consumption values for all the experiments carried on at a given temperature for each snake. These were calculated by totalling the average oxygen consumption values of each experiment and dividing by the number of experiments for that snake at that temperature using Apparatus I. This was justi-

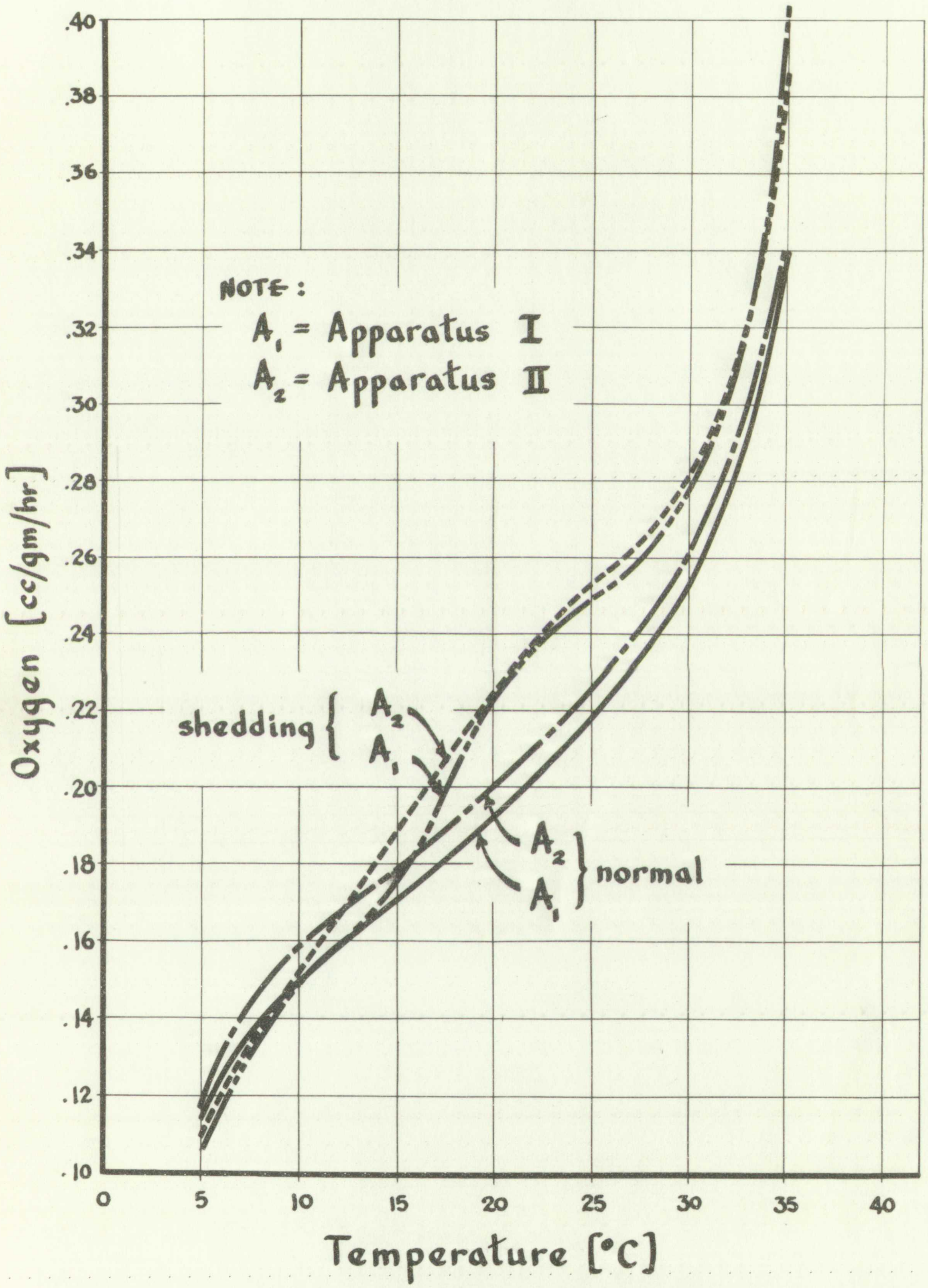
was titrated. Also the [illegible] of [illegible] [illegible] [illegible] that time interval was calculated [illegible] [illegible] cubic centimeters of [illegible] [illegible] [illegible] per bull snake per hour. In order to obtain the average carbon dioxide production value for an experiment the individual values obtained for each titration were multiplied by the hours between titrations, then the products were added together and divided by the total hours of the experiment. The average value of [illegible] for each experiment was obtained by [illegible] [illegible] formula (2), page 13.

The respiratory quotient, or R.Q., for each experiment was obtained by dividing the average carbon dioxide value for an experiment by the calculated average oxygen consumption value for that experiment.

The values plotted on the graphs, Figures [illegible] and [illegible] for Apparatus I, on the following pages, were the average of the calculated oxygen consumption values for all the experiments carried out at a given temperature for each snake. These were calculated by totaling the average oxygen consumption values of each experiment and dividing by the number of experiments [illegible] at that temperature [illegible] [illegible] [illegible] [illegible]



Oxygen Consumption of Bull Snake 1
at Various Temperatures
Fig. 3



Oxygen Consumption of Bull Snake 2
at Various Temperatures
Fig. 4

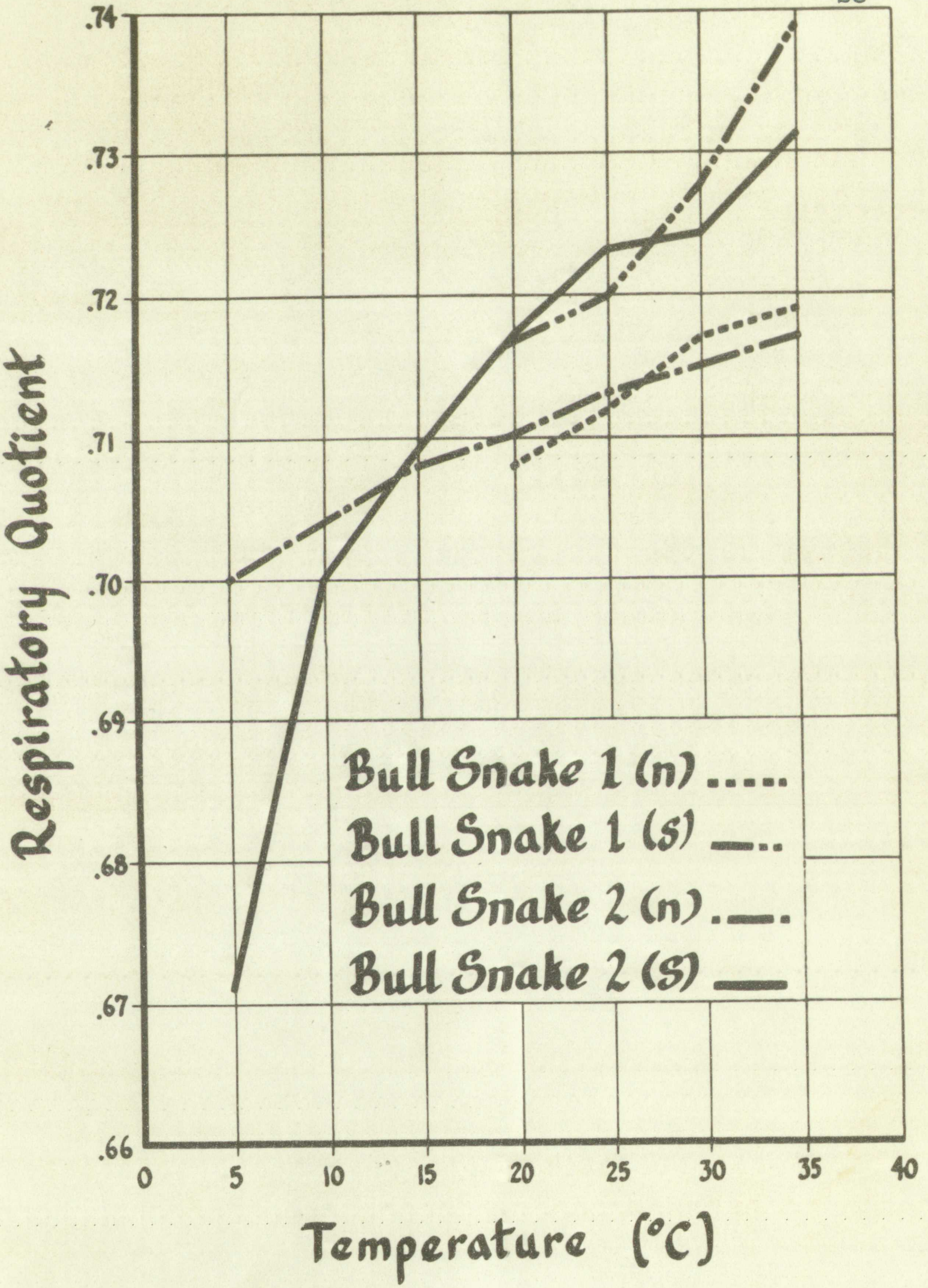
fied because the results of each experiment represented the actual oxygen consumption of the snake at the time of each experiment.

In all cases the experiments run during the shedding period were averaged separately and appear on different curves of the same graph.

The average R.Q.s shown on the graph, Figure 5, on the following page, were obtained by adding the R.Q. of each experiment and dividing by the total number of experiments for that temperature. The R. Q.s obtained during periods of shedding were averaged separately and appear on different curves of the same graph.

Tables III and IV shown on the following pages consist of the oxygen consumption in cubic centimeters at standard conditions per hour per gram of Bull Snakes 1 and 2, respectively, at various temperatures, obtained using Apparatus II. The averages at each temperature were obtained by adding the oxygen consumption values obtained during each separate experiment and dividing the total by the number of experiments. In these tables as in Tables I and II the numerical values obtained during the shedding period were averaged separately.

Shown in Figure 3 are four curves. Each curve re-



Respiratory Quotients of Bull Snakes 1 & 2 at Various Temperatures Using Apparatus I

TABLE III

OXYGEN CONSUMPTION IN CUBIC CENTIMETERS AT STANDARD CONDITIONS
PER GRAM OF BULL SNAKE 1 PER HOUR USING APPARATUS II

	20°C		25°C		30°C		35°C	
	Total Hrs. of Experiment	O_2	Total Hrs. of Experiment	O_2	Total Hrs. of Experiment	O_2	Total Hrs. of Experiment	O_2
	-	.---	-	.---	-	.---	1½	.381
	2	.226	-	.---	2	.301	-	.---
	2	.229	2	.247	2	.294	-	.---
	-	.---	2	.253	2	.298	2	.373
	-	.---	3	.247	3	.281	3	.361
	3	.218	3	.249	3	.309	3	.358
	3	.217	3	.248	-	.---	3	.342
	4	.209	4	.239	4	.288	-	.---
	-	.---	6	.222	-	.---	1	.---
	6	.203	6	.251	6	.277	6	.361
	6	.194	6	.239	6	.274	6	.338
	7	.201	-	.---	-	.---	-	.---
	8	.177	-	.---	8	.273	8	.341
Total		1.874		2.195		2.595		2.855
Average		.208		.244		.288		.356
	#3	.239	-	.---	#3	.351	-	.---
	-	.---	#4	.274	#4	.321	#4	.378
	-	.---	-	.---	-	.---	#6	.496
#Average		.239		.274		.336		.437

——— Plotted on graph (normal)
——— Plotted on graph (shedding)

#Shedding

TABLE IV

OXYGEN CONSUMPTION IN CUBIC CENTIMETERS AT STANDARD CONDITIONS PER GRAM OF BULL SNAKE 2 PER HOUR USING APPARATUS II

5°C		10°C		15°C		20°C	
Total Hrs. of Experiment	O_2	Total Hrs. of Experiment	O_2	Total Hrs. of Experiment	O_2	Total Hrs. of Experiment	O_2
4	.121	4	.163	4	.181	4	.199
4	.124	4	.161	-	.---	-	.---
4	.117	-	.---	-	.---	-	.---
6	.107	6	.161	6	.179	6	.206
-	.---	6	.158	6	.176	6	.196
-	.---	6	.152	6	.177	-	.---
Total	.469		.795		.713		.601
Average	.117		.159		.178		.200
*4	.109	*4	.151	-	.---	*4	.227
-	.---	-	.---	*6	.189	*6	.221
*Average	.109		.151		.189		.224

25°C		30°C		35°C	
Total Hrs. of Experiment	O_2	Total Hrs. of Experiment	O_2	Total Hrs. of Experiment	O_2
4	.229	-	.---	4	.352
6	.221	6	.269	6	.323
6	.226	6	.251	6	.339
-	.---	8	.261	-	.---
Total	.676		.781		1.014
Average	.225		.260		.338
-	.---	*4	.281	*4	.401
-	.---	*4	.283	-	.---
*6	.253	-	.---	*6	.371
*Average	.253		.282		.386

Plotted on graph (normal) *Shedding
Plotted on graph (shedding)

presents the average oxygen consumption rate of Bull Snake I plotted against temperature in degrees Centigrade. Thus the curve marked "normal A_1" (see Figure 3) was drawn through the points resulting from plotting the average oxygen consumption rate, at each temperature, of Bull Snake 1 against temperature, during a period of normal fasting, using Apparatus I. The curve marked "normal A_2" (see Figure 3) was produced in the same manner as the former, except that the points were obtained by the use of Apparatus II during a period of normal fasting. The curves marked "shedding A_1 and A_2" were constructed in a manner similar to the normal fasting curves but the values plotted were obtained during a shedding period of the same bull snake (Bull Snake 1) in the same two apparatuses. The temperature range used for Bull Snake 1 was from 20°-35° Centigrade. Data on the metabolic rate of Bull Snake 1 at other temperatures were not obtained because the snake was accidently killed.

In Figure 4 the curves were constructed in a manner similar to those in Figure 3 except that the values plotted were those obtained by using Bull Snake 2, instead of Bull Snake 1, in the two apparatuses (see Figure 4). Both the normal and shedding curves were shown for each apparatus. Rates were obtained from 5° - 35° Centigrade

presents the average oxygen consumption rate of [illegible] these 4 plotted against temperature [illegible] from the curve derived [illegible] drawn through the points [illegible] average oxygen consumption rate of [illegible] Bell-Shape 4 against temperature, [illegible] normal feeding [illegible] "normal A_5" (see Figure [illegible]) [illegible] manner as the [illegible] tained by the [illegible] normal feeding. The [illegible] A_5" were constructed [illegible] feeding curves [illegible] during a shedding period of the [illegible] (Bell Shape 1) [illegible] temperature [illegible] Centigrade. [illegible] at other temperatures [illegible] snake was accidentally killed.

In Figure [illegible] similar to those [illegible] were those obtained [illegible] Bell Shape [illegible] the normal and [illegible] apparatus, [illegible]

in both apparatuses, using Bull Snake 2.

The curves shown in Figure 5 were drawn through points produced by plotting the average respiratory quotients shown in Tables 1 and 2 at various temperatures, against temperatures in degrees Centigrade for Bull Snakes 1 and 2 using Apparatus I. Apparatus II was not adapted for obtaining the respiratory quotient.

In using Apparatus I the oxygen consumption was calculated by the method of Haldane (1892). This method is accepted as a valid procedure in determining the respiration rates of small animals, Fulton (1946). The method consists of collecting the carbon dioxide and water exhaled by the animal after the experiment, and subtracting from this sum the initial weight of the animal before the experiment in order to find the grams of oxygen consumed. Since renal and intestinal excretions occur not more than once in six weeks, if that frequently, during the fasting period, and it has been shown that no nitrogenous material is exhaled (Haldane (1892) this method was used to calculate the oxygen consumption values shown in Tables 1 and 2.

The following formula was used to determine the oxygen consumption rate from the carbon dioxide and water production which were obtained using Apparatus I, and the change of weight of the snake.

$$\text{gms. } O_2 = (S_{w_2} + \text{gm } CO_2 + \text{gms } H_2O) - S_{w_1}$$

In expressing O_2 in cc./gm./hr. the formula was used in the following form.

$$O_2 \text{ in cc(STP)/gm/hr.} = \frac{[(S_{w_2} - S_{w_1}) + \text{gms } CO_2 + \text{gms } H_2O] \times 22{,}400 \text{ cc}}{\text{No. Hrs. in Expr.} \times \text{gms Mole wt. of } O_2 \times \left(\frac{S_{w_1} + S_{w_2}}{2}\right)}$$

where S_{w_1} = initial weight of snake before the experiment in gms.

and S_{w_2} = finall weight of snake after the experiment in gms.

The carbon dioxide was converted from moles to grams in the titration procedure (see page 14). For the tables the moles were directly converted to cubic centimeters at standard conditions per gram per hour, as shown.

The snakes were weighed carefully in a glass jar with a lid. The tare weight of the jar was ascertained before each weighing. The respiration chambers of Apparatuses I and II were kept very clean and carefully observed before and after the snakes were put into them during experiments. In cases where excretion occurred or where there was loss of skin from the snakes, such as sometimes occurred in the last stages of shedding, the experiment was considered invalid.

In using Apparatus II the values of the oxygen consumption rates were converted directly from the volume of oxygen introduced at room temperature to standard conditions. A spring barometer calibrated with the barometer at the University of New Mexico Chemistry Department afforded accurate barometric readings. The oxygen was introduced at room temperature which was recorded each time oxygen was added to the system. The room temperature remained fairly constant. Probably an error of at least 5 or 6% was introduced in this operation because of the impossibility of making all the readings simultaneously and because of the great variation of gas volumes with small changes in temperature and pressure.

The curves shown in Figure 3 for the normal variation of Bull Snake 1 correlate with a maximum variation of about thirteen per cent. The average difference between the two curves is about six per cent. Considering the fact that experiments were made at different times, because the same snake was involved in each type of experiment, this difference in itself would not have indicated more than a normal experimental variation. However, the fact that the results obtained when using Apparatus II were consistently higher than those using Apparatus I (see Figures 2 and 4) seemed to indicate that one or more

In using Apparatus, [illegible] values of [illegible] oxygen consumption rates [illegible] [illegible], the volume of oxygen introduced at room [illegible] [illegible] conditions. A spring barometer [illegible] [illegible] barometer of the [illegible] [illegible] pertment [illegible] [illegible] oxygen was introduced [illegible] [illegible] corded each time oxygen was [illegible] to the system. The room temperature [illegible] [illegible] ency of at least [illegible] [illegible] because of the impossibility of [illegible] simultaneously and because of [illegible] of gas volumes with small changes in temperature and pressure.

The [illegible] [illegible] of bull [illegible] [illegible] about thirteen [illegible] the two curves is about [illegible] per cent. [illegible] fact that [illegible] cause the [illegible] lement, this difference [illegible] more than [illegible] fact that [illegible] were [illegible] (see Figure [illegible]

of the steps in the procedure involved in one or both techniques employed introduced a more or less constant error. Nevertheless, the total accuracy of either apparatus, which will be further discussed, is probably not more than ninety per cent so the correlation is within the limits of the accuracy of this experiment. Also the consistency of the individual curves during the normal fasting periods makes each useful.

The curve shown in Figure 3 for the shedding snake did not have a total variation as great as the curve for the normal snake, but the correlation between the curves was not consistent. This was probably due to the inconsistent respiration of the snake during the shedding period as has been reported by Benedict (1932) for other snakes.

In Figure 4 the correlation of the normal respiration curves for Bull Snake 2 from 20° - 35° Centigrade (see Figure 4) is better than the correlation of the two normal respiration curves of Bull Snake 1. The difference between the respiration curves of Bull Snake 2 is also consistent with the results obtained using Bull Snake 1 in that the values for the oxygen consumption using Apparatus II were again higher than those obtained using Apparatus I. From 5° - 20° Centigrade the variation was

of the stage in the procedure reached on the [illegible] technique employed [illegible] given. Nevertheless, the [illegible] which will be [illegible] ninety per cent, or [illegible] of the accuracy of [illegible] of the individual [illegible] makes each useful.

The curve shown in Figure [illegible] alcohol gave a total [illegible] the normal smoke, but the [illegible] was not consistent. This was probably due to the [illegible] consistent respiration of the [illegible] period as has been reported by [illegible] smoked.

In Figure 4 the [illegible] curves for [illegible] (Figure 4) is [illegible] normal respiration [illegible] between the respiration curves of [illegible] consistent with the [illegible] in that the values for the [illegible] Apparatus [illegible] were again [illegible] Apparatus [illegible]

slightly greater than from 20° - 35° Centigrade, but still consistent, with Apparatus II showing the greater oxygen production. The respiration rate during the shedding period of Bull Snake 2, produced two rather inconsistent curves which at a temperature of about 13° Centigrade both went below the two normal respiration curves.

The reason for the greater oxygen consumption indicated by Apparatus II was not ascertained, but the results indicated a consistent inaccuracy, which will be discussed further, of one or more steps in obtaining the oxygen consumption by one or both procedures. This inaccuracy, however, as was mentioned before, was not as great as the probable inherent inaccuracies of either apparatus. It is felt that the same procedure and apparatus could be used in future investigations for obtaining good comparative results.

In Figure 5 the respiratory quotient curves were not very consistent, however, the general increase of the respiratory quotient as the temperature increased was consistent. The respiratory quotient of .671 in Table II at 5° Centigrade is below respiratory quotients of known fats. This low respiratory quotient, however, was considered valid in that Roth (1942) and Cook (1949) have frequently observed even lower respiratory quotients in other reptiles. The reason for these low respiratory quotients has not been established, Roth (1942).

Some of the more important sources of error considered to be most significant were as follows:

(1) The impossibility of obtaining absolute basal conditions at warmer temperatures (this was especially noticeable at 35° Centigrade, at which temperature the snake moved occassionally) ± ?

(2) The inaccuracy in measuring the exact oxygen input into the respiration chamber in Apparatus II ± 6%

(3) The variation in the time involved in each experiment which was necessitated because of the schedule of the worker ± ?

(4) Weighing the snake - ?

In considering the accuracies of the two apparatuses, it is impossible to ascertain the exact magnitude of the errors involved until more experimental animals are used. Since the inherent errors of the apparatuses and the technique in each step in each procedure must of necessity be considered together the magnitude of error involved for each separately would be purely conjecture. However, the use of artificially induced gases in the two apparatuses, explained in the Materials and Methods section of this thesis would indicate that a relatively high degree of accuracy could be obtained using both apparatuses, and

it is believed by the author that the total error involved in either technique is not more than ten per cent.

DISCUSSION

The purpose of this investigation was to establish a practical method and apparatus for determining the effects of temperature on the respiratory rate of a normal bull snake. The usefulness of knowing more about this relationship lies in the possibilities that present themselves once this relationship is better known.

Since the bull snake is a poikilotherm, and yet is in a class of animals relatively close to the mammals, it should in the future lend itself very well to the study of various mammalian hormones (some of which are readily available) at various temperatures using Apparatus I and / or II. The effects of some mammalian hormones in reptiles is similar to their effects in mammals, Shaeffer (1933). This has also been shown to be the case in some amphibians, Furchner (1951). The possibility of the varied activity of certain hormones with changes in temperature as being the cause of certain seasonal variations in some cold blooded animals might conceivably offer a fairly fertile field of investigation. However, investigation of this nature cannot be made on very many poikilotherms because as yet there is a great lack of information concerning the normal respiration rates at different temperatures. The problem of ascertaining the effects of these hormones

at different temperatures might also be investigated simultaneously from a histological standpoint.

According to Cowles (1944) many reptiles are able to regulate their body temperature, within limits, by selecting a position in or out of the soil where heat through direct conduction can be had, or by varying the exposure of their bodies by moving from sunny to shady spots and vice versa. He observed that during a sunny day the body temperature of a rattle snake did not vary more than 2° Centigrade. This mechanism might be instrumental in sustaining the activity of certain hormones which become inactive during long cold periods.

In Table II at 5° Centigrade is a respiratory quotient of .671. This respiratory quotient is below the respiratory quotient given during the metabolizing of normal animal fats which is between .70 and .71, Fulton (1946), and is considered by the author as being a correct finding because previous instances of even lower respiratory quotients have been reported in reptiles by Benedict (1932), Roth (1942), and Cook (1949). Roth (1942) states that these low respiratory quotients are actualities and because of their frequency of occurrence cannot be considered experimental errors. Cook (1949) reported that at a

at different temperatures might all be investigated simultaneously from a physiological standpoint.

According to Cowles (1962) many reptiles are able to regulate their body temperature, within limits, by selecting a position in or out of the sun, whose rays produce direct conduction can be made, and by varying the exposure of their bodies by moving from sunny to shady spots and vice versa. He observed that on a sunny day the body temperature of a turtle [illegible] more than 6° centigrade. This mechanism might be [illegible] keeping the activity of enzymes [illegible] which become inactive during [illegible] periods.

In Table 11 at 6° the [illegible] a respiratory quotient of .971. This respiratory quotient is below the respiratory quotient given during the metabolism of normal animal data which is between .70 and .71. Hutton (1960), and is considered by [illegible] being a somewhat [illegible] previous [illegible] of even lower respiratory quotients have been reported in reptiles by Benedict (1932), Hoch (1949), and Cook (1949). Hoch (1962) states that these low respiratory quotients are unreliable and because of the infrequency of occurrence cannot be considered experimental errors. Cook (1949) reported that [illegible]

low temperature certain lizards maintained a respiratory quotient of .56 for an extended period of time, and was able to show experimentally that this was not due to absorption of carbon dioxide by the body fluids at lower temperatures. This was shown by heating up the lizards after keeping them at a low temperature over a period of time during an experiment when their respective respiratory quotients were low, and finding no great increase in the carbon dioxide output. He did not attempt to explain the reason for these low respiratory quotients but stated that this phenomenon might indicate the presence in reptiles of a stored food substance different from any of the presently known metabolites.

The increase of the respiratory quotient in this investigation from .70 to .73 as the temperature increased might indicate a shift from the metabolism of fats to a metabolism of fats and relatively small amounts of proteins. However, more experiments on more experimental animals will be necessary in order to lend laboratory strength to this assumption. Since the respiratory quotient for proteins is about .8, and the highest recorded respiratory quotient reported in this investigation is only .74, the metabolism of fats as a primary source of energy in these bull snakes might be indicated. During

low temperature curtails rigidly [illegible]
quotient of .90 for an extended period of time, [illegible] was
able to show experimentally that this was due to the
absorption of carbon dioxide by the body fluids at
lower temperatures. This was shown by [illegible] the
lizards after keeping them at a low temperature for a
period of time during which [illegible] their respir-
respiratory quotients were low, [illegible]
crease in the [illegible] carbon dioxide [illegible]
to explain the reason for these low results [illegible]
but stated that this phenomenon [illegible]
ence in reptiles of a [illegible]
from any of the presently known [illegible]

The increase of the respiratory quotient in this
investigation from .70 to .80 [illegible]
creases might indicate a shift from [illegible]
fats to a [illegible] of fats and carbohydrates [illegible]
of proteins. However, more [illegible]
methods will be necessary in order to [illegible]
strength to this [illegible]
for proteins is about .80 and the [illegible]
respiratory quotient [illegible]
only .74, the [illegible]
energy in these [illegible]

periods of shedding the respiratory quotient was higher than it was during normal periods at temperatures of 17° Centigrade and above. This was reversed, however, at lower temperatures (see Figure 5) where the shedding respir atory quotient curve dropped below the normal respiratory quotient curve. The possibility of the snake metabolizing carbohydrates did not present itself because bull snakes are carnivrous animals and were fed only mice. The small amounts of carbohydrates contained in the mice according to Roth (1942) would probably be utilized in the snake within a matter of a few days. Therefore,no experiments were made for at least five days after the feeding of either of the bull snakes. Benedict (1932) measured the basal metabolism of a snake of the genus Python which had not eaten for two years and found it to be metabolizing fats. In this investigation the longest period during which either snake fasted was about two and a half months.

The oxygen production rates found in this investigation even though only two snakes were used correlate rather well with the work of Clausen (1936) who worked on the basal metabolism of the brown snake, *Storeria dekayi*, at temperatures of 10°, 20°, and 30° Centrigrade. His results were generally higher than those obtained for these two bull snakes. However, *Storeria dekayi* are

considerably smaller than Pituophis sayi; and according to Benedict (1932) larger snakes usually metabolize more slowly than smaller ones. Clausen (1936) did not publish any respiratory quotients in his report.

The respiratory quotients for Pituophis sayi at room temperature (20° Centigrade) reported by Roth (1942) were slightly lower than those found for the two snakes in this experiment at that temperature. Unfortunately, Roth (1942) did not measure the respiration rate at any other temperature.

The operation of Apparatus II was much more simple and direct than that of Apparatus I, and during the course of this investigation the deviation between the results of the two apparatuses was small. Since both the normal respiration curves obtained using Apparatus II correlated quite well with the normal respiration curves obtained using Apparatus I the use of Apparatus II with an occasional check using Apparatus I would afford a means of ascertaining the respiration rate of the bull snake in future experiments.

Because of the rather inconsistent result obtained during the periods of shedding it would seem advisable to experiment during normal fasting periods.

As has been shown by former workers on the metabolism

of cold blooded animals this investigation indicated that the respiration rate of the bull snake, _Pituophis sayi_, increases as the temperature increases. This relationship was not a straight line function, but varies with the range of temperature involved. The respiration rate for shedding snakes was generally higher than for snakes in a normal fasting period above 13° Centigrade; below this temperature the reverse was the case. It would appear as though the respiration rate of a shedding snake was more affected by temperature changes than the respiration rate of a normal fasting snake.

of cold arduous winter, while it was found (p. [illegible]) that the respiration rate of the [illegible] *[illegible] dayi*) increased as the temperature decreased. This relationship was not a straight line however, but varied with the range of temperature involved. The respiration rate for [illegible] was consistently higher than for animals in a normal state at [illegible] 10° centigrade, but at a temperature of [illegible] was the case. It would appear as though the respiration rate of [illegible] [illegible] [illegible]

[illegible]

SUMMARY

Two apparatuses have been designed and constructed for the measurement of the oxygen consumption rate of the bull snake, *Pituophis sayi*, at various temperatures. One of these apparatuses has been adapted to measure the carbon dioxide and water output of the snake. The results obtained using two apparatuses have been correlated. Experiments have been conducted to determine the effect of temperature on the respiration rate of two bull snakes. It was found that the respiration rate of these two snakes increases with temperature. This relationship was not a straight line function but varied throughout the temperature range approximating a straight line between 17° and 28° Centigrade.

The respiratory quotients at various temperatures have been ascertained and the findings indicate that there is an increase in the respiratory quotient with an increase in temperature.

Experiments conducted during the shedding period of these bull snakes indicated that above 13° Centigrade the respiration rate is higher than during normal periods, but below this temperature, is lower than the normal rate. A respiratory quotient of .671 was recorded. This is in keeping with previous reports by Benedict, Roth, and Cook.

SUMMARY

Two modifications [illegible] have been designed and [illegible] for the measurement of the oxygen consumption rate of the Bull snake, *Pituophis sayi*, at various temperatures. One of these apparatuses has been adapted to measure the carbon dioxide and water output of the snake. The results obtained using the two apparatuses have been reported.

Experiments have been conducted to determine the effect of temperature on the respiration rate of [illegible] snakes. It was found that the respiration rate of these two snakes increases with temperature. This [illegible] was not a straight line function but [illegible] the temperature range approximating [illegible] between 25° and 30° Centigrade.

The respiratory quotients at various temperatures have been calculated [illegible] that there is an increase in the respiratory quotient with an increase in temperature.

Experiments conducted during the shedding phase of these bull snakes indicated that [illegible] the respiration rate is higher than during normal periods but [illegible] the respiratory quotient [illegible]. This is in accordance with [illegible] Roth, and others.

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