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Title: AERIAL AND AQUATIC RESPIRATION IN THE NEOTENIC AND TRANSFORMED PACIFIC GIANT SALAMANDER, DICAMPTODON ENSATUS (ESCHSCHOLTZ)

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Aerial and aquatic respiration of transformed and neotenic D. ensatus were studied from September 1969 through July 1970. The relationship of body weight and metabolism, and the relative importance of the cutaneous, pulmonary, and branchial respiratory mechanisms were investigated. Moreover, an attempt was made to relate these findings to the ecology of the two life forms.

Pulmonary (lung and buccopharyngeal) and cutaneous gas exchange were measured separately and simultaneously in a closed-system respirometer using standard manometric techniques. Controls were also run to determine the effect of restraining the animals. The Micro-Winkler technique was used to test oxygen consumed by individuals held under water. In addition, gas exchange was measured while the animals were simultaneously respiring aerially and aquatically.

Acclimation and experimental temperatures were 5 ° C, 10 ° C, and 15 ° C. The photoperiod used was ten hours light and 14 hours dark with the light period centered at 1200 Pacific Standard Time. Experiments were conducted between 0700 and 1600 hours.

It was found that:

1. Total aerial oxygen uptake of the unrestrained neotene increased with temperature and exhibited a significant difference between 5 ° C and 15 ° C.
2. Total aerial oxygen uptake of the unrestrained transformed Dicamptodon showed no significant difference between 5 ° C and 10 ° C, but the metabolic rate increased appreciably at 15 ° C.
3. Restraining the animals put them under stress and increased their metabolic rate significantly. Stress seemed to affect the cutaneous system of the neotene and the pulmonary system of the metamorphosed Dicamptodon.
4. The neotene released an average of 70. 7 percent of the total carbon dioxide from the skin and an average of 62. 97 percent of the total oxygen was obtained through the skin.
5. The transformed adult released an average of 72. 40 percent of the carbon dioxide through the skin, and an average of 65. 90 percent of the total oxygen was obtained through the skin.
6. The neotene obtained significantly more oxygen from water

than air at 5 °C and 15 °C, but there was no significant difference in the amount of oxygen obtained from air and water by the transformed adult at the three experimental temperatures.

7. In both life forms, aquatic respiratory mechanisms became more important as the temperature decreased and aerial respiratory mechanisms became more important as the temperature increased.

8. The transformed adult has a more efficient pulmonary system at temperatures above 5 °C than that of the neotene. The transformed adult respired exclusively by aquatic means at 5 °C.

9. There was a correlation between oxygen consumption and weight at 15 °C. The exponential value of b in the equation $M = aW^b$ was 0.87 ($r=+0.8196$, $p < 0.01$) and 0.68 ($r=+0.9488$, $p < 0.01$) for neotenic and transformed Dicamptodon respectively, which was respiring aquatically. A b value of 0.39 ($r=+0.8694$, $p < 0.01$) was obtained for metamorphosed adults respiring aerially.

10. The findings of the study correlate well with the ecology of D. ensatus.

Aerial and Aquatic Respiration in the Neotenic and Transformed Pacific Giant Salamander, Dicamptodon ensatus (Eschscholtz)

by

Glen Warren Clothier

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TABLE OF CONTENTS

INTRODUCTION 1

MATERIALS AND METHODS 5

- Collecting and Acclimation 5
- Aerial Respiration 5
- Aquatic Respiration 8
- Simultaneous Measurement of Pulmonary and Aquatic Respiration 9
- Role of Gills in Respiration 10
- Data Computation 10

RESULTS 11

- Aerial Respiration 11
- Aquatic Respiration 14
- Comparison of Total Aerial and Aquatic Respiration in Neotenes and Total Aerial and Aquatic Respiration in Transformed Dicamptodon 15
- Simultaneous Measurement of Aerial and Aquatic Respiration 15
- Role of Gills in Respiration 17
- Metabolism and Body Weight 17
- Breathing Rates 18
  - Aerial Respiration 18
  - Aquatic Respiration 19

DISCUSSION AND CONCLUSIONS 21

- Aerial Respiration 21
- Aquatic Respiration 27
- Comparison of Total Aerial and Aquatic Respiration in Neotenes and Total Aerial and Aquatic Respiration in Transformed Dicamptodon 28
- Simultaneous Measurement of Aerial and Aquatic Respiration 29
- Branchial Respiration 32
- Metabolism and Body Weight 33
- Temperature Coefficients 37
- Respiratory Quoteints 38
- Breathing Rates 38

SUMMARY 41

Aerial Respiration 41
Aquatic Respiration 42
Comparison of Total Aerial and Aquatic Respiration in Neotenes and Total Aerial and Aquatic Respiration in Transformed Dicamptodon 42
Role of the Gills in Respiration 43
Simultaneous Measurement of Aerial and Aquatic Respiration 43
Body Size and Metabolic Rate 43
Respiratory Quotients 44
Temperature Coefficients 44
Ecological Significance 44

BIBLIOGRAPHY 64

APPENDICES 68

LIST OF FIGURES

Figure		Page
1.	Respirometer used to measure simultaneously cutaneous and pulmonary gas exchange in *D. ensatus*.	46
2.	Total aerial oxygen uptake in unrestrained neotenic *D. ensatus* at 5°C, 10°C, and 15°C.	47
3.	Total aerial gas exchange in unrestrained neotenic *D. ensatus* at 5°C, 10°C, and 15°C.	47
4.	Total aerial gas exchange in restrained transformed *D. ensatus* at 5°C, 10°C, and 15°C.	48
5.	Total aerial gas exchange in unrestrained transformed D. ensatus at 5°C, 10°C, and 15°C.	48
6.	Total aerial oxygen uptake in unrestrained transformed *D. ensatus* at 5°C, 10°C, and 15°C.	49
7.	Cutaneous gas exchange in restrained transformed *D. ensatus* at 5°C, 10°C, and 15°C.	50
8.	Total oxygen uptake in restrained neotenic *D. ensatus* at 5°C, 10°C, and 15°C.	51
9.	Total gas exchange in restrained neotenic *D. ensatus* at 5°C, 10°C, and 15°C.	51
10.	Total oxygen uptake of restrained transformed *D. ensatus* at 5°C, 10°C, and 15°C.	52
11.	Total aquatic oxygen uptake of neotenic *D. ensatus* at 5°C, 10°C, and 15°C.	52
12.	Total aquatic oxygen uptake in transformed *D. ensatus* at 5°C, 10°C, and 15°C.	53
13.	Pulmonary gas exchange in restrained transformed *D. ensatus* at 5°C, 10°C, and 15°C.	53
14.	Cutaneous oxygen uptake in restrained neotenic *D. ensatus* at 5°C, 10°C, and 15°C.	54

Figure		Page
15.	Cutaneous gas exchange in restrained neotenic *D. ensatus* at 5 °C, 10 °C, and 15 °C.	54
16.	Cutaneous oxygen uptake in restrained transformed *D. ensatus* at 5 °C, 10 °C, and 15 °C.	55
17.	Pulmonary oxygen uptake in restrained transformed *D. ensatus* at 5 °C, 10 °C, and 15 °C.	55
18.	Pulmonary oxygen uptake in restrained neotenic *D. ensatus* at 5 °C, 10 °C, and 15 °C.	56
19.	Pulmonary gas exchange in restrained neotenic *D. ensatus* at 5 °C, 10 °C, and 15 °C.	56
20.	Comparison of total oxygen uptake from air and water in transformed *D. ensatus* at 5 °C, 10 °C, and 15 °C.	57
21.	Comparison of total oxygen uptake from air and water in neotenic *D. ensatus* at 5 °C, 10 °C, and 15 °C.	57
22.	Oxygen uptake from air and water and total oxygen consumption in neotenic and transformed *D. ensatus* at 5 °C, 10 °C, and 15 °C.	58
23.	The relationship between body weight and oxygen uptake in transformed *D. ensatus* respiring aerially at 15 °C.	59
24.	Total aquatic oxygen uptake in neotenic *D. ensatus* with and without gills at 15 °C.	59
25.	The relationship between body weights and oxygen uptake in neotenic *D. ensatus* respiring aquatically at 15 °C.	60
26.	The relationship between body weight and oxygen uptake in transformed *D. ensatus* respiring aquatically at 15 °C.	60

LIST OF TABLES

Table		Page
1.	Value of b in the equation $M = aW^b$ for various vertebrate groups.	61
2.	Percent of total gas exchange through cutaneous and pulmonary system of neotenic and transformed Dicamptodon at 5 °C, 10 °C, and 15 °C.	62
3.	Percent of total oxygen obtained from water and air by neotenic and transformed Dicamptodon.	63

AERIAL AND AQUATIC RESPIRATION IN THE NEOTENIC AND TRANSFORMED PACIFIC GIANT SALAMANDER, DICAMPTODON ENSATUS (ESCHSCHOLTZ)

INTRODUCTION

Amphibians respire by several moist surfaces: lungs, skin, gills and buccopharyngeal mucosa. These are used in various combinations by different species. The first quantitative study of pulmonary and cutaneous respiration in amphibians was performed by Krogh (1904), who worked with the European frogs, Rana esculenta and R. temporaria. He separated pulmonary from cutaneous respiration by canulating the lungs, forcing air through them, and simultaneously analyzing this air and that surrounding the frog. Krogh discovered that oxygen was taken up chiefly by the lungs, while carbon dioxide was released predominantly through the integument. Dolk and Postma (1927), working with Rana temporaria, substantiated Krogh's work. Neither of the above workers acclimated their animals to constant temperature and photoperiod. These parameters are now known to influence amphibian respiration (Vinegar and Hutchison, 1965; Guimond and Hutchison, 1968). Moreover, another experimental error was introduced when they pumped air in and out of the lungs, preventing the frogs from carrying out their normal breathing movements (Scholten, 1942; Cherian, 1958).

Lapicque and Petetin (1910), working with the lungless

salamander, Euproctus montanus, found that cutaneous respiration may be more important than lung and/or buccopharyngeal respiration; when the salamander was submerged in vaseline with its head exposed to the air, it quickly died. It lived, however, when the buccopharyngeal oxygen uptake was eliminated by placing the head in vaseline. Whitford and Hutchison (1965), studying a number of lungless salamanders, Desmognathus quadramaculatus, D. monticola, Gyrinophilus porphyriticus, Pseudotriton ruber, and Plethodon glutinosus confirmed the above conclusion.

Information pertaining to the relative importance of the different respiratory mechanisms in amphibians has been based predominantly on the anatomical investigations of respiratory surfaces (Czopek, 1962; Czopek and Czopek, 1959; Bieniak and Watka, 1962). Czopek (1962) found that an adult Dicamptodon from Saratoga Springs, Santa Cruz county, California had the following distributions of respiratory capillaries: 54.04% in the skin, 42.49% in the lungs, and 3.47% in the buccal cavity.

As Foxon (1964) has noted in his review, one should be careful in interpretation of anatomical studies, for the breathing habits are especially important in the renewal of lung air on which gas exchange with the lung capillaries is dependent. Moreover, certain environmental parameters, particularly temperature and day length, affect the respiratory exchanges and relative function of the respiratory

membranes.

Not until recently were the relative roles of pulmonary and cutaneous respiration in urodeles investigated thoroughly. The first works, using improved methods, were those of Whitford and Hutchison (1963, 1965, 1966). Anuran respiration was also studied (Vinegar and Hutchison, 1965; Guimond and Hutchison, 1968; Hutchison, Whitford and Kohl, 1968). These studies were concerned with the analysis of gas exchange in an air environment. Whitford and Sherman (1968) investigated both aerial and aquatic respiration in neotenic and transformed Ambystoma tigrinum. They determined the total aquatic and pulmonary oxygen uptake but did not evaluate the relative importance of the respiratory surfaces. Guimond (1970), however, determined the relative role of the skin, lungs, and gills of Amphiuma means, Cryptobranchus alleganiensis, Necturus maculosus, and Siren lacertina. Furthermore, Smith (1967) evaluated the respiratory significance of the lungs, buccal mucosa, and skin of adult Taricha granulosa.

Body size in relation to metabolism has been studied in amphibians (Whitford and Hutchison, 1967; Smith, 1967; Hutchison, Whitford and Kohl, 1968; Whitford and Sherman, 1968). When large and small individuals are compared in relation to oxygen uptake, it is found that small organisms consume more oxygen per unit weight than large ones. The reader is referred to the reviews of Brody (1945),

Kleiber (1947), Zeuthen (1953), and Scholander et al. (1953) for a comprehensive treatment of the subject.

The objective of the present study, conducted from September 1969 through July 1970, was to compare the aerial and aquatic respiration of transformed and neotenic Dicamptodon ensatus. The relationship of animal weight and metabolism, and the relative importance of the cutaneous, pulmonary, and branchial respiratory mechanisms were investigated. Moreover, an attempt was made to relate these findings to the ecology of the two life forms.

MATERIALS AND METHODS

Collecting and Acclimation

Collection sites, weights, and volume displacements of the salamanders used in this study are listed in Appendix I. Some of the adults were transformed in the laboratory, but all had metamorphosed at least one year prior to experimentation. For collecting methods see Clothier, 1966.

Nineteen neotenes and 21 transformed Dicamptodon were maintained in constant temperature-photoperiod environmental chambers for at least two weeks prior to experimentation. A number of determinations were made on each animal. Acclimation and experimental temperatures were 5°, 10°, and 15° C. The photoperiod used was ten hours light and 14 hours dark (10L; 14D). The light period was centered at 1200 Pacific Standard Time, and experiments were conducted between 0700 and 1600 hours. The animals were not fed for two weeks before experimentation.

Aerial Respiration

Pulmonary (lung and buccopharyngeal) and cutaneous gas exchange were measured separately and simultaneously in a closed-system respirometer for three consecutive hours. The respirometer,

constructed of 0.25 in. acrylic plastic, consisted of four equal-volume chambers (Figure 1).

The animal, secured to a piece of styrofoam by rubber tubing, was placed in one of the front chambers with its head projecting through a rubber membrane stretched over a one and one-half inch plastic tube which was connected to the other front chamber. Ten ml. of sodium hydroxide (in a 30 ml. beaker) was placed in each chamber to serve as a carbon dioxide absorbent. The lid was then sealed on with Lubriseal. Each front chamber was connected through a manometer to a rear chamber which served as a thermobarometer, correcting for minor fluctuations in temperature and barometric pressure. The respirometer was then submerged in a constant temperature (± 0.1° C) water bath and allowed to stabilize for 20 minutes. A 10 cc. graduated glass syringe was filled with 100% oxygen and attached to each front chamber, stop-cocks closed, and the experiment begun. As the animal used oxygen, the manometer fluid became unbalanced. It was rebalanced by introducing oxygen from the syringe and the amount utilized over the three hour period read directly. At the end of the experiment, the sodium hydroxide was titrated with standardized 1 N hydrochloric acid to determine the amount of carbon dioxide released.

The method is described in more detail by Whitford and Hutchison (1963) and differs from their technique in two ways:

1) Sodium hydroxide was used as a carbon dioxide absorbent rather than barium hydroxide; and 2) a rubber membrane was used rather than a tygon tubing mask to separate pulmonary from cutaneous gas exchange. A soluble carbonate is formed with sodium hydroxide; this eliminates the necessity of breaking the insoluble film of barium carbonate formed when barium hydroxide is used. The rubber membrane was utilized because a tygon tubing mask would allow communication between the two front chambers via the gill slits of the neotene and prevent separation of pulmonary and cutaneous gas exchange. Moreover, since Dicamptodon is a large, strong animal, the sewing on of a mask would necessitate anesthetization and thus affect its respiration.

Before the partitioning experiment described above was conducted, total oxygen consumption in air was measured. The procedure was essentially the same except that the plastic tube connecting the two front chambers was plugged and an animal placed in each front chamber. Oxygen uptake by all the respiratory surfaces was determined while the animal was resting quietly. Moreover, the effect of restraining the animal and placing its head through a rubber membrane could be determined by comparing the data of these two methods.

Visual counts of buccal pumps and lung inspirations were made periodically.

Aquatic Respiration

The Micro-Winkler technique (see Appendix II), a modification of the Winkler method, was used to test oxygen consumed by Dicamptodon under water. The procedure is described in detail by Fox and Wingfield (1938). It is convenient in that it allows one to measure the sample of water and run the Winkler reaction in the same syringe. Moreover, small, manageable samples may be analyzed.

The large and small animals were placed in 1940 ml. and 1000 ml. Kerr jars respectively. The jars were then filled with dechlorinated water which had been stabilized overnight in the environmental chambers. Five animals were run at a time. Besides the five jars containing animals, an additional jar containing water only was maintained as a control. Just before an experiment, the dissolved oxygen in one jar was ascertained for later reference. Kerr canning lids were sealed on with Lubriseal and the jars submerged in a water bath. The rate of buccal pumping was periodically recorded.

At the end of one hour, the water in each jar was tested for residual dissolved oxygen. The difference between the initial and terminal dissolved oxygen in the control jar was taken as oxygen utilized by micro-organisms in the water. By using this correction and subtracting the terminal from the initial dissolved oxygen in each of the five jars, the amount of dissolved oxygen utilized by each of

the Dicamptodon could be ascertained.

Simultaneous Measurement of Pulmonary and Aquatic Respiration

The method used in this experiment is a modification of that employed by Whitford and Sherman (1968).

The four-chambered respirometer was prepared as described for total oxygen consumption in air, except that each chamber contained 1000 ml. of dechlorinated water and the air-water interface was covered with a layer of mineral oil 12 mm. thick (88 ml.). The mineral oil effectively prevented both the diffusion of gases into and out of the water. One animal was placed in each of the two front chambers. The salamanders would lie passively on the bottom of the chamber and respire aquatically but would periodically, with minimal effort (as the water was only 6 cm. deep), rise to the surface through the mineral oil layer and exhibit pulmonary respiration. The mineral oil did not adhere to the skin or gills.

Aquatic oxygen consumption was ascertained by the Micro-Winkler method by taking an initial and terminal dissolved oxygen on the water. Pulmonary oxygen consumption was determined by the standard manometric technique already described.

Role of Gills in Respiration

A series of neotenes was acclimated at 15° C and 100 determinations of two hours duration each were made to ascertain their oxygen consumption under water.

To make the gills more evident, the neotenes were placed in a pan of water. The gills were then excised with scissors; very little bleeding occurred. One hundred determinations were made subsequent to gill removal. The role of the gills in respiration could be deduced by comparing the two sets of data.

Data Computation

Calculations were made by the CDC 3300 computer located in the Oregon State University Computer Center. The data were entered on a patch teletype (#17). Three computer programs were utilized:

1. *SIMLIN--To fit lines to data in the weight metabolism study by the method of least squares.
2. *SDD--Gives sum of x, sample size, mean of x, standard deviation, standard error of mean, maximum value of x, minimum value of x, and range of x.
3. *Cloth--To compute raw data from the aquatic respiration experiments. *Cloth is not a canned program and was entered through the teletype. *Cloth is given in Appendix III.

RESULTS

Aerial Respiration

Data are first presented for the unrestrained salamanders. The animals were not held in place with the rubber tubing, nor were their heads placed through rubber membranes; they were merely put in the respirometer and the total atmospheric oxygen uptake measured.

Total aerial oxygen consumption of the neotene increased from 12.8 µl/gm/hr at 5 °C to 20.0 µl/gm/hr at 10 °C, and to 23.3 µl/gm/hr at 15 °C (Figure 3). There was a significant difference in total aerial oxygen uptake between 5 °C and 15 °C (Figure 2).

Total aerial carbon dioxide production of the neotene increased linearly from 7.4 µl/gm/hr at 5 °C to 16.3 µl/gm/hr at 10 °C, and to 17.9 µl/gm/hr at 15 °C (Figure 3).

Total aerial oxygen consumption in the transformed adult was 16.9 µl/gm/hr at 5 °C and 24.9 µl/gm/hr at 10 °C. It then increased to 45.8 µl/gm/hr at 15 °C (Figure 5). There was a significant difference in oxygen uptake between 5 °C and 15 °C but not between 5 °C and 10 °C and between 10 °C and 15 °C (Figure 6).

Total aerial carbon dioxide release of the transformed Dicamptodon increased from 9.8 µl/gm/hr at 5 °C to 16.1 µl/gm/hr at 10 °C, and then rose sharply to 27.8 µl/gm/hr at 15 °C (Figure 5).

The remaining data presented are for the restrained animals. The neotene showed an almost linear increase in total aerial oxygen

consumption with decreasing temperature (Figure 9). Oxygen uptake was 40.9 µl/gm/hr at 5° C, 35.8 µl/gm/hr at 10° C, and 27.5 µl/gm/hr at 15° C (Figure 9), being significantly different between 5° C and 15° C (Figure 8).

Total aerial carbon dioxide production in the restrained neotene increased from 16.2 µl/gm/hr at 5° C to 24.2 µl/gm/hr at 10° C, and then decreased slightly to 23.5 µl/gm/hr at 15° C (Figure 9).

Total aerial oxygen consumption of the transformed Dicamptodon was 51.5 µl/gm/hr at 5° C, 66.5 µl/gm/hr at 10° C and 66.9 µl/gm/hr at 15° C (Figure 4), showing no significant difference between 5° C, 10° C, or 15° C (Figure 10).

Total aerial carbon dioxide production of the metamorphosed salamanders increased from 39.5 µl/gm/hr at 5° C to 44.0 µl/gm/hr at 10° C, and then decreased slightly to 43.7 µl/gm/hr at 15° C (Figure 4).

Aerial oxygen uptake through the skin of the non-transformed Dicamptodon decreased from 31.8 µl/gm/hr at 5° C to 23.8 µl/gm/hr at 10° C, and to 12.3 µl/gm/hr at 15° C (Figure 15). There was a significant difference in the cutaneous respiration of the neotene between 5° C and 15° C (Figure 14).

Carbon dioxide released by the neotene's skin was 11.7 µl/gm/hr at 5° C, 15.1 µl/gm/hr at 10° C and 18.2 µl/gm/hr at 15° C (Figure 15).

Aerial cutaneous oxygen consumption of the transformed animals was 38.0 µl/gm/hr at 5° C, 40.0 µl/gm/hr at 10° C, and 40.1 µl/gm/hr at 15° C (Figure 7), exhibiting no significant difference between the three temperatures (Figure 16).

Carbon dioxide released through the skin of the metamorphosed salamanders decreased from 31.0 µl/gm/hr at 5° C to 28.7 µl/gm/hr at 10° C, and then increased to 32.3 µl/gm/hr at 15° C (Figure 7).

Pulmonary respiration in the neotenic Dicamptodon increased linearly with increasing temperature (Figure 19). Pulmonary oxygen consumption was 9.1 µl/gm/hr at 5° C, 11.9 µl/gm/hr at 10° C, 15.2 µl/gm/hr at 15° C, being significantly different between 5° C and 15° C (Figure 18).

Carbon dioxide released through the neotene pulmonary system was 4.5 µl/gm/hr at 5° C, 9.1 µl/gm/hr at 10° C, and 5.4 µl/gm/hr at 15° C (Figure 19).

Pulmonary oxygen uptake by the transformed animals was 11.8 µl/gm/hr at 5° C, 26.4 µl/gm/hr at 10° C, and 26.6 µl/gm/hr at 15° C (Figure 13), showing a significant difference between 5° C and 10° C, and between 5° C and 15° C (Figure 17).

The carbon dioxide given off by the lungs and buccopharyngeal mucosa increased from 8.5 µl/gm/hr at 5° C to 15.3 µl/gm/hr at 10° C, and then decreased to 11.5 µl/gm/hr at 15° C (Figure 13).

Table 2 summarizes the percent of oxygen taken up and carbon dioxide released through the cutaneous and pulmonary surfaces of the restrained neotenic and transformed Dicamptodon.

The temperature coefficients (Q_{10}) of the unrestrained neotenic and transformed animals were 1.82 and 2.71 respectively. Values of -1.49 and 1.30 were obtained for the restrained neotenic and metamorphosed Dicamptodon.

The average respiratory quotients for the unrestrained neotenes were 0.58 at 5° C, 0.82 at 10° C, and 0.77 at 15° C. The unrestrained transformed salamanders yielded values of 0.58 at 5° C, 0.65 at 10° C, and 0.61 at 15° C. Neotenes which were restrained gave values of 0.40 at 5° C, 0.68 at 10° C, and 0.85 at 15° C. Transformed adults which were restrained gave average respiratory quotients of 0.77 at 5° C, 0.66 at 10° C, and 0.65 at 15° C.

Aquatic Respiration

The data on total aquatic oxygen consumption in transformed and neotenic Dicamptodon are given in Figures 11, 12, 20, and 21. In the neotene the mean aquatic oxygen uptake increased from 20.5 µl/gm/hr at 5° C to 26.3 µl/gm/hr at 10° C, and to 30.9 µl/gm/hr at 15° C (Figure 21). There was a significant difference in aquatic oxygen consumption between 5° C and 10° C, but not between 10° C and 15° C (Figure 11).

In the transformed Dicamptodon the mean aquatic oxygen consumption increased from 15.4 µl/gm/hr at 5 °C to 21.6 µl/gm/hr at 10 °C, and to 32.4 µl/gm/hr at 15 °C (Figure 20). There was a significant difference in the aquatic oxygen uptake between 5 °C and 10 °C, but not between 10 °C and 15 °C (Figure 12).

The temperature coefficients (Q_{10}) of neotenic and metamorphosed Dicamptodon were 1.45 and 2.10 respectively.

Comparison of Total Aerial and Aquatic Respiration in Neotenes and Total Aerial and Aquatic Respiration in Transformed Dicamptodon

Figures 20 and 21 summarize the total oxygen consumption from air and water by neotenes and metamorphosed adults. The neotene obtained significantly more oxygen from water than from air at 5 °C and 15 °C. (Compare Figures 2 and 11.) There was no significant difference in the amount of oxygen obtained from air and water by the transformed Dicamptodon at 5 °C, 10 °C, and 15 °C. (Compare Figures 6 and 12.)

Simultaneous Measurement of Aerial and Aquatic Respiration

Neotenes and transformed salamanders were given the opportunity to respire aquatically and aerially, or by a combination of these methods. The data are summarized in Figure 22.

The neotene showed a significant difference in total oxygen consumption between 5 °C and 15 °C, but not between 5 °C and 10 °C nor between 10 °C and 15 °C. Aquatic oxygen uptake increased from 8.7 µl/gm/hr at 5 °C to 12.4 µl/gm/hr at 10 °C and 12.8 µl/gm/hr at 15 °C, exhibiting no significant difference between the three temperatures. Aerial oxygen consumption in the non-transformed animals increased from 3.9 µl/gm/hr at 5 °C to 7.8 µl/gm/hr at 10 °C, and to 17.8 µl/gm/hr at 15 °C. There was a significant difference in aerial oxygen uptake between 5 °C and 15 °C.

Total oxygen consumption in transformed Dicamptodon was significantly different between 5 °C, 10 °C, and 15 °C. Oxygen taken from the water decreased from 14.5 µl/gm/hr at 5 °C to 10.6 µl/gm/hr at 10 °C and then increased to 14.3 µl/gm/hr at 15 °C. There was no significant difference in aquatic respiration between the three temperatures. Aerial oxygen consumption was zero µl/gm/hr at 5 °C, 17.2 µl/gm/hr at 10 °C, and 34.0 µl/gm/hr at 15 °C, showing no significant difference between 10 °C and 15 °C.

The percent of oxygen obtained from air and water by the two life forms is presented in Table 3. As the temperature decreased, the amount of oxygen obtained from the water increased in the neotene and transformed Dicamptodon. Conversely, as the temperature increased, the role of pulmonary respiration became increasingly

important.

The temperature coefficients (Q_{10}) of neotenic and metamorphosed Dicamptodon were 2.33 and 3.33 respectively.

Role of Gills in Respiration

Neotenes with and without gills were tested at 15° C for total aquatic oxygen uptake. The gilled animals utilized an average of 21.6 µl/gm/hr, while those without gills consumed an average of 17.1 µl/gm/hr. As shown in Figure 24, there was a significant difference between aquatic oxygen uptake of the gilled and non-gilled neotenes. The gills accounted for 44 percent of the total aquatic oxygen consumption.

Metabolism and Body Weight

Metabolism varies to an exponential power of body weight, rather than increasing directly with weight. The following equation has been used to describe the relation between oxygen uptake and body weight in a wide variety of animals:

$$M = aW^b$$

M = oxygen consumption in µl/hr.

W = body weight in grams

b = exponential power of increase in oxygen uptake with weight.

a = constant at a given experimental temperature.

No correlation between metabolism and body weight was obtained for neotenic and transformed Dicamptodon at 5° C, 10° C, and 15° C; it is felt that this resulted because there were not enough weight differences in the animals held at a given temperature. In order to test this possibility, a series of salamanders was acclimated at 15° C and their weight metabolism relationship investigated. In these animals a highly significant correlation between metabolism and body weight was obtained. Neotenic and transformed Dicamptodon respiring aquatically gave a b value of 0.87 ($r = +0.8196$, $N = 16$, $P < 0.01$), and 0.68 ($r = +0.9488$, $N = 9$, $P < 0.01$) respectively. Transformed adults exhibiting aerial respiration gave a b value of 0.39 ($r = 0.8694$, $N = 11$, $P < 0.01$). Regression lines were fitted to the data by the method of least squares. The data were then plotted on log-log graph paper (Figures 23, 25, and 26).

Breathing Rates

Aerial Respiration

At 5° C the neotene exhibited 12.83 buccal pumps per minute. No lung inspirations were observed except when the animals were

active. At 10 ° C 23.70 buccal pumps and 1.16 lung inspirations occurred each minute, while at 15 ° C 26.27 buccal pumps and 1.15 lung inspirations were observed per minute. At 10 ° C only 50% of the animals utilized their lungs but at 15 ° C all salamanders showed lung inspirations.

The transformed salamanders showed 45.61 buccal pumps per minute at 5 ° C, but no lung inspirations were observed in quiescent Dicamptodon at this temperature. At 10 ° C 87.64 buccal pumps and .333 lung inspirations occurred per minute. At 15 ° C 103.09 and .981 buccal pumps and lung inspirations respectively were exhibited each minute. One half of the salamanders showed no lung inspirations at 10 ° C, but at 15 ° C all of them utilized their lungs.

Aquatic Respiration

The neotene exhibited 14.5 buccal pumps at 5 ° C, 19.9 buccal pumps at 10 ° C, and 22.4 buccal pumps at 15 ° C.

The transformed Dicamptodon pumped water in and out of their buccal cavities in two ways. The first method is termed "deep" buccal pumping for the gular membrane is raised and lowered to its full capacity. The second method is called "shallow" buccal pumping as only the anterior one-third of the gular membrane is utilized. Metamorphosed animals exhibited 8.18 shallow and 6.33 deep buccal pumps per minute at 5 ° C. At 10 ° C 6.75 shallow and deep buccal pumps

were observed per minute, but at 15° C 19.91 shallow and 11.37 deep buccal pumps were shown each minute. At 5° C about 75% of the animals showed shallow buccal pumping. One animal exhibited no buccal pumping and only one animal was observed to use both shallow and deep buccal pumps when a determination was being made. At 10° C approximately 50% of the salamanders exhibited shallow buccal pumping and one animal received all its oxygen through the skin. At 15° C about 66% of the transformed Dicamptodon showed shallow buccal pumping. No animals received oxygen exclusively through their skin.

DISCUSSION AND CONCLUSION

Aerial Respiration

The unrestrained neotenes increased in their respiratory rate with an increase in temperature, showing a significant difference between 5° C and 15° C. There was no difference in the oxygen uptake of the transformed Dicamptodon between 5° C and 10° C, but the metabolic rate increased appreciably at 15° C. This marked increase may be expected, for other workers have reported this phenomenon in many species of temperate zone amphibians (Whitford and Hutchison, 1963, 1965, and 1967; Whitford and Kohl, 1968; Whitford, 1968). There has, however, been no satisfactory explanation for this significant increase in metabolic rate at 15° C. Whitford and Sherman (1968) hypothesized at 15° C may represent the optimal temperature for activity in temperate zone amphibians and that an increase in oxygen consumption might result from greater activity of animals whose activity cannot be controlled during experimentation.

Aerial oxygen consumption in the restrained neotene showed a most unusual relationship with temperature. As the temperature decreased, the metabolic rate increased almost linearly. One would expect this in a homeotherm but not in a poikilotherm.

The metabolic rate of the restrained animals was significantly higher than that of the unrestrained salamanders. Thus, restraining the animals and placing their heads through the rubber membrane had a marked effect on their respiration. Whitford and Hutchison (1963) reported that oxygen consumption of restrained Ambystoma maculatum was only five percent higher than that of unrestrained A. maculatum. Guimond and Hutchison (1968), working with Rana pipiens, stated "masked animals showed a proportionately greater consumption than the unmasked controls." Most workers run no controls and evidently assume that restraining and masking the animals has no effect on their metabolism.

The increased metabolic rate at a given temperature and the inverse relationship between temperature and oxygen uptake in restrained neotenic Dicamptodon was probably due to stress. Neotenes are extremely excitable animals. They are capable of moving very swiftly and react in this manner when touched or handled. Once restrained, however, they did not struggle unduly against their bonds. Most were very quiescent while in the respirometer and the elevated metabolic rate could not be accounted for by an increase in activity. It is felt that merely restraining the animals put them under a great deal of stress. Hutchison (1963) demonstrated that a decrease in oxygen consumption occurred in A. maculatum because of temperature stress. He found that cutaneous oxygen consumption increased

linearly between 5 ° C and 15 ° C, and then dropped to lower values at 25 ° C and 30 ° C. There was an almost linear increase in pulmonary oxygen uptake. In neotenic Dicamptodon the cutaneous oxygen consumption decreased almost linearly with increasing temperature but the pulmonary oxygen uptake increased linearly with increasing temperature. Perhaps the cutaneous blood vessels were progressively shut down as the temperature increased. Neotenes do take short forays on land (see page 30) when the weather is cool and moist. If the animal were stressed and subjected to relatively high temperature, the lessening of blood flow through its dermal capillaries would help to prevent desiccation.

The restrained transformed adults also showed signs of stress. The total oxygen uptake increased between 5 ° C and 10 ° C but then leveled off between 10 ° C and 15 ° C. The increase in oxygen consumption between 5 ° C and 10 ° C was due primarily to the pulmonary system, as the cutaneous uptake remained relatively constant at all three temperatures. Whitford and Hutchison (1965) found that in A. tigrinum and T. granulosa pulmonary oxygen consumption increased linearly with temperature, while oxygen uptake by the skin remained almost constant. Smith (1967) confirmed the findings of Whitford and Hutchison in T. granulosa. That the stress in transformed Dicamptodon was not as great as that in the neotene is not surprising, for the metamorphosed form is much more docile and

amenable to handling. Mild stress is indicated, however, as the pulmonary respiration leveled off at 10° C and did not increase linearly with rising temperature. Moreover, the metabolic rate was significantly higher when the salamanders were restrained.

Even though the restrained Dicamptodon were under stress, when one considers the percent of carbon dioxide release and oxygen uptake through the cutaneous and pulmonary respiratory surfaces, the results are generally comparable to those found by other investigators.

The skin of amphibians is important in respiration, especially at low environmental temperatures. It generally plays a greater role in carbon dioxide release than in oxygen uptake (Hutchison, 1965; Guimond and Hutchison, 1968; Vinegar and Hutchison, 1965; Hutchison, Whitford and Kohl, 1968; Smith, 1967; Krogh, 1904; Dolk and Postma, 1927). Between 70 percent and 90 percent of the carbon dioxide is released through the skin in most species. Whitford and Hutchison (1965) found the following average values in urodeles at 15° C: Ambystoma maculatum, 78.6%; Taricha granulosa, 86.4%; Salamandra salamandra, 79.7%; Ambystoma tigrinum, 82.9%; Pseudotritin ruber, 85.6%; Gyrinophilus porphyriticus, 85.6%; Desmognathus quadramaculatus, 81.7%; Plethodon glutinosus, 88.7%; and Desmognathus monticola, 89.1%. The average value of carbon dioxide released through the skin of neotenic and transformed Dicamptodon at

15 ° C was 77.2% and 73.8% respectively. Although these values are lower than those reported by Whitford and Hutchison, they fall within the range reported for amphibians.

Oxygen uptake through the skin in lunged salamanders remains relatively constant with increasing temperature (Whitford and Hutchison, 1965). The neotenic Dicamptodon exhibited a significant difference in cutaneous oxygen uptake at 5 ° C and 15 ° C; oxygen consumption decreased with increasing temperature. Recall that the animals were highly stressed and probably shut down their cutaneous system to prevent desiccation. The transformed Dicamptodon seemed to show less stress and exhibited the expected relationship between temperature and oxygen uptake through the skin. That is, there was no significant difference in the cutaneous oxygen uptake at the three experimental temperatures.

Whitford and Hutchison (1965) reported that oxygen uptake through the pulmonary system increased at higher temperature. Smith (1967), working with T. granulosa, confirmed the findings of Whitford and Hutchison. Even though the neotenic Dicamptodon was highly stressed, the pulmonary oxygen uptake increased linearly between 5 ° C and 15 ° C. The pulmonary oxygen consumption of the transformed Dicamptodon increased between 5 ° C and 10 ° C and then leveled off between 10 ° C and 15 ° C. Stress seemed to affect the cutaneous system in the neotene and the pulmonary system in the

metamorphosed adult.

In view of the above comparison, one must conclude that the pattern of cutaneous and pulmonary respiration of Dicamptodon does not differ markedly from that of other lunged urodeles.

Czopek (1962) determined the average percent of respiratory capillaries in the skin (54.04%), lungs (42.49%), and buccal cavity (3.47%) of one transformed Dicamptodon. Adding the above values for the lungs and buccal cavity, one finds that 45.96 percent of the total respiratory capillaries are located in the pulmonary system. The present study determined that an average of 34.1 percent of the total oxygen was obtained through the lungs and buccopharyngeal mucosa of transformed Dicamptodon. This is a lower value than expected from Czopek's findings. Moreover, 65.90 percent of the total oxygen was obtained through the skin, a value higher than that expected by considering Czopek's findings. One must remember that Czopek studied a single individual and that other factors, such as temperature, heart rate, breathing rate, size, and the physiological state of an animal will all have an effect on its oxygen consumption. Thus, one cannot expect a perfect correlation between anatomical and physiological data.

The average percent of total oxygen obtained through the pulmonary (37.03%) and cutaneous (62.97%) respiratory surfaces of the neotene does not differ greatly from that of the metamorphosed Dicamptodon.

Aquatic Respiration

Workers report conflicting results concerning the comparison of the metabolic rate between larval and metamorphosed amphibians. Abelin and Scheinfinkel (in Helff, 1927) found that an artificially metamorphosed Tigrinum mexicanum [sic.] had a metabolic rate 50 to 70 percent lower than that of the normal larval rate. They also discovered that the oxygen consumption of Rana esculenta tadpoles decreased during metamorphosis. This same relationship was reported by Groebbels (in Helff, 1927) for Rana temporaria larvae. Conversely, Helff (1923) reported that Rana pipiens tadpoles increased their metabolic rate as much as 79 percent above the normal larval level as they approached the end of metamorphosis. Gahlenbeck and Bartels (1970) showed that the metabolic rate of transformed Ambystoma mexicanum increased four times that of the neotenous form. Furthermore, Helff (1927) found no significant difference in the total oxygen uptake of neotenic and larval Ambystoma tigrinum.

The significant increase in metabolic rate in Ambystoma mexicanum may result from weight loss during transformation. The body weight decreased from 44 to 26 gms. and the oxygen consumption increased from 22 to 96 µl/gm/hr. A smaller animal would be expected to use more oxygen per unit weight than a larger animal. In Dicamptodon a change in metabolic rate due to weight dependent

factors would not be expected, for they undergo no significant weight loss during transformation. The present study found no significant difference in aquatic oxygen uptake of neotenic and transformed Dicamptodon at 15° C.

Comparison of Total Aerial and Aquatic Respiration in Neotenes and Total Aerial and Aquatic Respiration in Transformed Dicamptodon

That the neotene was more efficient in obtaining oxygen from the water than from the air is not surprising, for they are primarily aquatic animals. The present study shows that the gills alone account for 44 percent of the total aquatic oxygen uptake at 15° C. While the animals were in the air, the gills adhered closely to the sides of the neck and probably did not contribute appreciably to the total aerial oxygen consumption. There are no data in the literature for comparison.

There was no significant difference in the amount of oxygen obtained from air and water by the transformed adult. Perhaps they are more aquatic than previously believed. It is now known that they spend considerable time in the water while attending the eggs (see page 29). Moreover, transformed individuals have been taken from under large rocks in streams during the winter months (Nussbaum, personal communication), and they have also been taken in streams during the dry summer months. Thus one should expect them to be quite flexible in their respiratory abilities.

Simultaneous Measurement of Aerial and Aquatic Respiration

The metamorphosed salamanders obtained no oxygen from the air at 5 ° C. That the transformed Dicamptodon respired solely by aquatic means at lower temperatures is not surprising if one considers its life history. Nussbaum (1969) reported that the incubation period for Dicamptodon is 275 days. Furthermore, all evidence suggests that there is maternal care of developing embryos in this species. Since the nest sites were discovered in underground waterways, the attending female would be under water for extensive periods of time; males were also found near the nest sites. A low metabolic rate would also be advantageous under these circumstances, for the female would probably have a limited food supply while attending the eggs.

The neotene obtained more oxygen from water than air at 5 ° C and 10 ° C. At 15 ° C more oxygen was extracted from air than water. Since the neotenic form is principally aquatic and cold adapted, one would expect it to be more efficient in obtaining oxygen from the water than from the air at low temperatures.

Table 3 summarizes the percent of total oxygen obtained from water and air by the two life forms. There is an inverse relationship

between temperature and percent of total oxygen uptake from the water, while there is a direct relationship between temperature and percent of oxygen consumption from the air. Thus, aquatic respiratory mechanisms become increasingly important as the temperature decreases and aerial respiratory mechanisms become increasingly important as the temperature rises. Table 3 shows that the transformed Dicamptodon has a more efficient pulmonary system at temperatures above 5° C than that of the neotene. Recall that the metamorphosed adult may remain as long as nine months underwater while attending the eggs. There is no evidence, however, that breeding occurs every year, for large nonbreeding animals have been taken at all times of the year. It is presumed, therefore, that transformed Dicamptodon are primarily terrestrial but capable of spending extended periods of time underwater, and would benefit by possessing a highly efficient pulmonary system.

Table 3 shows that neotenes also are capable of extracting a considerable amount of oxygen from the air. Laboratory observations (Clothier, 1966) prompted me to hypothesize that neotenes are capable of leaving the stream to disperse or to move around obstacles such as log jams, waterfalls, or small dams. On October 19 and November 3, 1969, two neotenes were taken while they were attempting to go around a small dam on Fall Creek, Lincoln County, Oregon. Both were out of the water, one being about ten feet and the other

about 15 feet from the stream. During these forays an efficient pulmonary system would be highly beneficial. Furthermore, neotenes were maintained in the respiration chamber for a three hour period during the total atmospheric experiments without showing any signs of distress. Thus, neotenes are believed to be primarily aquatic, but capable of leaving the water for short periods of time.

Recall that in the neotenic and transformed Dicamptodon there was an inverse relationship between temperature and percent of oxygen obtained from the water. This could be accounted for by a greater oxygen diffusion gradient between the dermal capillaries and the water. However, there was a direct relationship between temperature and the percent of oxygen uptake from the water for A. tigrinum (Whitford and Shermen, 1968). Thus, factors other than oxygen availability must be involved in the control of oxygen utilization in this species.

Conversely, there was a direct relationship between temperature and the percent of oxygen consumed from air in both life forms of D. ensatus, but an inverse relationship prevailed in this respect for neotenic and transformed A. tigrinum.

Thus, over the temperature ranges used in the experiments, the percent of oxygen extracted from the air increases in D. ensatus but decreases in A. tigrinum with higher temperatures. For some unknown reason the pulmonary system of A. tigrinum extracts a

smaller percent of oxygen from the air than water at higher temperatures than it does at lower temperatures.

Being able to extract a greater percent of oxygen from the air at higher temperatures would seem to have survival value for Dicamptodon. Many of the streams inhabited by Dicamptodon become excessively low in the summer and fall. Consequently the temperature rises and the oxygen content of the water decreases. Thus, an efficient pulmonary system would be most beneficial under these circumstances.

Branchial Respiration

The literature concerning oxygen uptake by the gills of urodeles is very sparse. Most workers either work with transformed animals or determine total oxygen consumption in the gilled forms. Guimond (1970), however, investigated the role that the gills play in aquatic respiration for Necturus maculosus and Siren lacertina. The gills of Necturus accounted for 50 to 60 percent of the total gas exchange at 5 °C, 15 °C, and 25 °C, while the gills of Siren contributed only five and two percent of the total oxygen uptake at 15 °C and 25 °C, respectively. At 15 °C the gills of the neotenous Dicamptodon ensatus accounted for 44 percent of the total oxygen consumption.

Czopek (1962) found that the branchial capillaries of Siren intermedia accounted for approximately two percent of all respiratory

capillaries. Although Guimond (1970) worked with Siren lacertina, his findings correlate nicely with the anatomical studies of Czopek. Unfortunately, no such comparison can be made for the Pacific Giant Salamander, for Czopek only investigated the transformed Dicamptodon.

Metabolism and Body Weight

It is generally agreed that small animals have a higher metabolic rate than large animals; that is, per unit of mass small organisms consume more oxygen than large ones. Metabolism is often expressed as a power function of body weight. Kleiber (1947), and Zeuten (1953) state that the relationship between body size and metabolism is comparable in homeotherms and poikilotherms. In fact, Hemmingsen (1950, in Prosser, 1961) concludes that metabolism in homeotherms, poikilotherms, and beech trees varies with the 0.73 power of weight.

The relationship between body size and metabolism has generally been investigated in terms of the so-called surface law. The "surface law" was first developed by Sarrus and Rameaux in 1837 (in Brody, 1945) and states that surface area is proportional to $W^{2/3}$, W being weight. Thus it follows that oxygen uptake is proportional to $W^{2/3}$. Many explanations have been offered to account for metabolism increasing by $W^{2/3}$. Krebs (1950) states that a decrease

in oxygen consumption occurs with size because as an organism increases in mass, cells with lower respiration rate, such as in connective tissue, become more prevalent. Moreover, Martin and Fuhrman (1955) report that the oxygen consumption of different tissues does not change with the same exponent of body weight as total metabolism. That is, tissues such as bone, fat, and connective tissue (having a low metabolic rate) are present in greater amounts in larger organisms. This results in a decline in metabolic rate with an increase in size of the animal. Zeuthen (1953), also points out that for reasons inherent in living matter, the metabolism grows less than the body and that organisms are always in danger of outgrowing their supplying mechanisms. In light of the explanations put forth, Von Bertallanffy (1957) concludes, "The explanation of the surface rule and the size dependence of metabolism in general thus remains rather unsatisfactory."

Values presented in the literature for the exponent b (in the equation metabolic rate = aW^b) are quite variable. Table 1 summarizes the values from various sources.

One can see that some of the values in Table 1 are close to the surface rule value of 0.67, while others are as low as 0.47 and as high as 0.94. Neotenous and transformed Dicamptodon, respiring aquatically, gave a b value of 0.87 and 0.68 respectively, while transformed adults exhibiting aerial respiration gave a b value of 0.39.

To test the possibility that metabolism in D. ensatus corresponds to the surface law, the following null hypothesis was tested: "The experimental value of b is not significantly different from the surface law value B, where B is 0.67." The test statistic is:

$$t = \frac{b-B}{S_b}$$

b = calculated exponent value

B = surface law exponent value

S_b = standard deviation of b

The 95 percent confidence level was chosen. Considering degrees of freedom, if one assumes the observations are from a normal population and the hypothesis is true, the distribution for t is n-2 and the values are 14(16-2) for the neotenic and 7 (9-2) for transformed animals respiring aquatically. The degrees of freedom for the transformed Dicamptodon respiring aerially are 9(11-2). The critical regions are 1.76 and 1.89 for neotenes and metamorphosed adults respectively which were respiring aquatically. The calculated t values for these animals were 0.60 and 0.58. Since $0.60 < 1.76$ and $0.58 < 1.89$, the hypothesis is accepted. For adults respiring aerially the calculated t value is -3.24. In this case a two tailed test is utilized. The critical region is -2.26 to +2.26. Since the t value is not within the critical region, the hypothesis is rejected. Thus,

the metabolic rate of neotenic and transformed Dicamptodon respiring aquatically is proportional to $W^{2/3}$. However, the metabolic rate of metamorphosed adults respiring aerially does not follow the surface law; the b value of 0.39 is lower than any I have seen in the literature.

Temperature Coefficients

The temperature coefficients of the unrestrained neotenic and transformed Dicamptodon respiring aerially were 1.82 and 2.71 respectively, while temperature coefficients of neotenic and metamorphosed individuals respiring aquatically were 1.45 and 2.10 respectively. Thus, temperature had a greater effect on the total oxygen consumption from the air than from the water in both metamorphosed and neotenic D. ensatus.

The above values fall within the range of values reported in the literature for amphibians. Whitford and Sherman (1968) report Q_{10} values of 1.43 and 2.00 for neotenic and transformed A. tigrinum respectively, between 15°C and 25°C. Neither their data nor mine agree with the generalization of Rao and Bullock (1954), who point out that it is natural to expect lower temperature coefficients in animals subjected to wide temperature fluctuations. The transformed urodeles of both species, being primarily terrestrial, would encounter greater temperature fluctuations, yet they have higher Q_{10} values

than the basically aquatic neotenes. The low Q_{10} values of the neotenic Dicamptodon may, however, have adaptive significance, for when they occasionally leave the stream for short periods of time (see page 30) their metabolism would be confined to a manageable increase.

Respiratory Quotients

Some indication of the type of metabolism may be ascertained from the respiratory quotient (R.Q. = CO_2/O_2), although finding certain values does not prove metablism of carbohydrate, protein, or fat. It is generally agreed that the R.Q. for carbohydrate is 1.0, for fat 0.70, and for protein 0.79.

Increase in R.Q. with an increase in temperature has been reported for several poikilotherms (Prosser and Brown, 1961). Hutchison, Whitford and Kohl (1968) reported this relationship in several anurans, while Guimond and Hutchison (1968) found a downward trend in R.Q. values with increasing temperature in Rana pipiens. The R.Q. of A. maculatum increased slightly with increasing temperature (Whitford and Hutchison, 1963). Furthermore, Whitford and Hutchison (1965) found that temperature had no effect on the R.Q. of representative individuals from three families of urodeles, Ambystomatidae, Salamandridae, and Plethodontidae.

The data of the present study showed that generally the R.Q.

of D. ensatus tended to increase slightly with increasing temperatues. Only in the restrained transformed animals did the R.Q. decrease slightly with increasing temperature. It appeared that under the experimental conditions of the present study, that D. ensatus were metabolising stored fat, as the average respiratory quotients for neotenic and transformed Dicamptodon were .68 and .65 respectively.

Breathing Rates

Temperature had a direct effect on breathing rate. Under aerial conditions, both neotenic and transformed Dicamptodon increased their buccal pumping rate with increasing temperature. Lung inspirations were negligible at lower temperature (5° C) in both life forms. However, as temperature increased (10° C-15° C), the number of lung inspirations remained almost constant in the neotene, but increased about three fold in the transformed individuals. At 10° C one-half of the salamanders exhibited lung inspiration, but at 15° C all of them used their lungs. That the pulmonary system in Dicamptodon becomes increasingly important in respiration at higher temperatures is not surprising, for this relationship is well documented in the literature for both anurans and urodeles. There is, however, a controversy concerning the role of the buccal mucosa in amphibian respiration. Mathes (1927), Vos (1936), Elkan (1955), and Foxon

(1964) believed that the buccal pumping of amphibians was olfactory in function. Noble (1925) concluded that they were respiratory in function. Czopek (1962), on the basis of cytological evidence, concluded that buccal pumping was olfactory in function, but he states that a final decision must wait until physiological investigations either reject or support his findings. Whitford and Hutchison (1963) presented evidence that the buccal mucosa plays an important role in amphibian respiration. Whitford and Hutchison (1965) have shown that in plethodontids, 15 to 24 percent of the oxygen consumed was taken through the buccopharyngeal mucosa at 15° C, and believe that 30 to 50 percent of the pulmonary oxygen uptake of the lunged forms could be accounted for by the buccopharyngeal cavity. Smith (1967) has shown that T. granulosa are capable of extracting oxygen from the water during buccal pumping.

In light of the above evidence, one cannot rule out the possibility that the buccopharyngeal mucosa participates in the sense of smell, but one must conclude that the buccopharyngeal mucosa also plays an important role in respiration.

It is believed that the increased buccal pumping rate and increased use of the lungs in Dicamptodon at higher temperatures is an important factor in their increase in total aerial oxygen uptake at higher temperatures.

Under aquatic conditions, the neotene increased the buccal

pumping rate with increasing temperature. The transformed Dicamptodon pumped water in and out of their buccal cavities in two ways, termed deep and shallow buccal pumping (see page 19). The deep buccal pumping rate remained about the same between 5 ° C and 10 ° C but it almost doubled at 15 ° C. The shallow buccal pumping rate decreased slightly between 5 ° C and 10 ° C and then increased about three fold at 15 ° C. Shallow buccal pumping was used by a greater number of the animals at 5 ° C than at 10 ° C or 15 ° C. Moreover, the data suggests that transformed Dicamptodon are capable of obtain- all their oxygen through the skin at 5 ° C and 10 ° C. This should result in a lower metabolic rate and would be advantageous to transformed individuals which are in underground waterways for extended periods of time. The increased rate of buccal pumping at 15 ° C would help compensate for the lesser diffusion gradient between water and skin at this temperature.

SUMMARY

The present study, conducted from September 1969 through July 1970, compared the aerial and aquatic respiration of transformed and neotenic D. ensatus. The relationship of animal weight and metabolism, and the relative importance of the cutaneous, pulmonary, and branchial respiratory mechanisms were investigated. The findings of the present study are summarized below.

Aerial Respiration

1. Total aerial oxygen uptake of the unrestrained neotene increased with temperature, and exhibited a significant difference between 5 ° C and 15 ° C.
2. Total aerial oxygen uptake of the unrestrained transformed Dicamptodon showed no significant difference between 5 ° C and 10 ° C, but the metabolic rate increased appreciably at 15 ° C.
3. Restraining the animals caused a significant increase in their metabolic rate. Moreover, the neotene appeared to shut down its cutaneous system with increasing temperature. This resulted in a decrease in total aerial oxygen consumption with an increase in temperature. The transformed Dicamptodon decreased its pulmonary oxygen uptake between 10 ° C and 15 ° C. Stress seemed to affect the cutaneous system of the neotene and

the pulmonary system of the metamorphosed adult. The pulmonary oxygen uptake of the restrained neotene increased linearly between 5 °C and 15 °C. The transformed adult maintained a constant oxygen uptake through the skin at the three experimental temperatures.

4. The neotene released an average of 70.7 percent of the total carbon dioxide from the skin. An average of 62.97 percent of the total oxygen was obtained through the skin.
5. The transformed adult released an average of 72.40 percent of the carbon dioxide through the skin, and an average of 65.90 percent of the total oxygen was obtained through the skin.

Aquatic Respiration

6. In transformed and neotenic Dicamptodon there was a significant difference in aquatic oxygen uptake between 5 °C and 10 °C, but not between 10 °C and 15 °C.

Comparison of Total Aerial and Aquatic Respiration in Neotenes and Total Aerial and Aquatic Respiration in Transformed Dicamptodon

7. The neotene obtained significantly more oxygen from the water than air at 5 °C and 15 °C.
8. There was no significant difference in the amount of oxygen obtained from air and water by transformed adult at the

three experimental temperatures.

Role of the Gills in Respiration

9. The gills accounted for 44 percent of the total aquatic oxygen uptake at 15 ° C.

Simultaneous Measurement of Aerial and Aquatic Respiration

10. The transformed adult respired exclusively by aquatic means at 5 ° C.
11. In both life forms, aquatic respiratory mechanisms became more important as the temperature decreased and aerial respiratory mechanisms became more important as the temperature increased.
12. The transformed Dicamptodon has a more efficient pulmonary system at temperatures above 5 ° C than that of the neotene.

Body Size and Metabolic Rate

13. The exponential value of b in the equation $M = aW^b$ was 0.87 and 0.68 for neotenic and transformed Dicamptodon respectively, which were respiring aquatically. These values do not differ significantly from that of the surface law $W^{2/3}$. The metabolic rate of metamorphosed adults respiring aerially does not

follow the surface law; the b value was 0.39. The above values were obtained at 15 °C.

Respiratory Quotients

14. Generally the R.Q. of D. ensatus tended to increase slightly with increasing temperature. It decreased slightly with increasing temperature in the restrained transformed adult.
15. The animals used in the present study appeared to be metabolizing their stored fat reserves.

Temperature Coefficients

16. Temperature had a greater effect on the total oxygen consumption from the air than from the water in both life forms.
17. The values obtained fall within the range of values reported in the literature for amphibians.

Ecological Significance

The findings of the present study correlate well with the ecology of Dicamptodon. Although transformed Dicamptodon are considered to be primarily terrestrial, it is known that they spend a great deal of time in the water. They have been collected in streams throughout the year and have a longer incubation period than any other salamander. Females and males have been found near nest sites in underground

waterways. Thus, one would expect the transformed adult to be quite flexible in its mode of respiration. It was found that metamorphosed Dicamptodon can extract as much oxygen from the water as from the air and that they are capable of respiring solely by aquatic means at low temperature.

Neotenes are basically aquatic but they occasionally take short forays on land to go around obstacles in the stream. They obtained significantly more oxygen from the water than from the air but are capable of living in the air. As the temperature increased, the cutaneous system appeared to be progressively shut down. This would aid in preventing desiccation. They also are capable of extracting a considerable amount of their oxygen through the pulmonary system.

Although the adult has a more efficient pulmonary system than the neotene, both life forms extracted a greater percentage of their oxygen from the air at higher temperature. This could have survival value for this species, for many of the streams inhabited by Dicamptodon become low and warm in the summer and fall. An efficient pulmonary system would be most beneficial under these conditions.

Figure 1.

Respirometer used to measure simultaneously cutaneous and pulmonary gas exchange in D. ensatus. T, plastic tube; R, rubber membrane; O_2, oxygen syringe; M, manometer; S, sodium hydroxide in beaker; F, front chamber; B, back chamber.

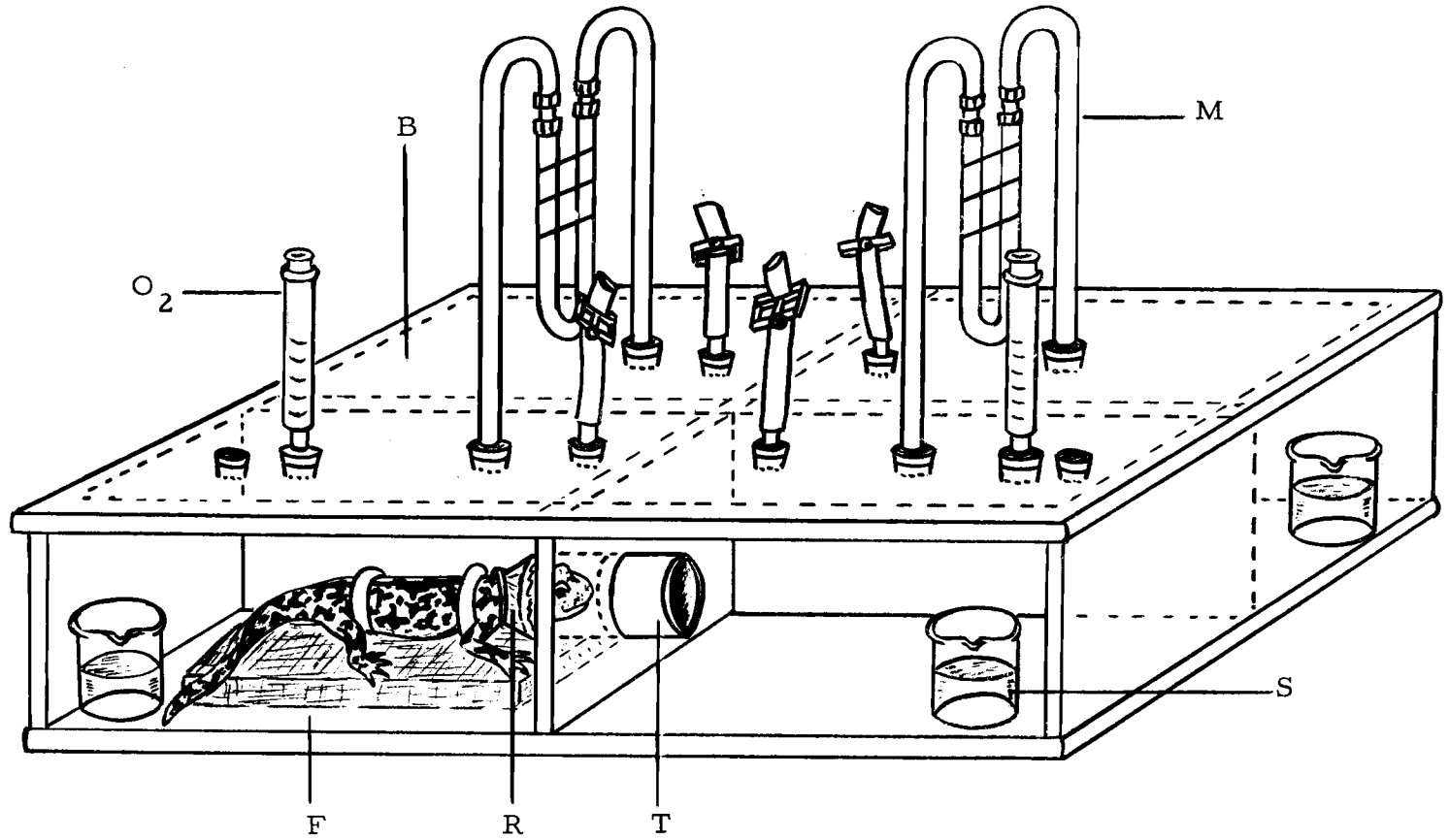

Figure 1.

Figure 2.

Total aerial oxygen uptake in unrestrained neotenic D. ensatus at 5 °C, 10 °C, and 15 °C. Numbers in parentheses; first number = number of determinations; second number = total number of hours of respiration measured. Vertical line represents range; thin horizontal line, mean; diagonally-lined rectangle on each side of mean, two standard errors. If SE's of two sets of data do not overlap, difference between means may be considered statistically significant at about the 5% level (Hubbs and Hubbs, 1953).*

Figure 3.

Total aerial gas exchange in unrestrained neotenic D. ensatus at 5 °C, 10 °C, and 15 °C.

*Hereafter, the data will be presented in this manner.

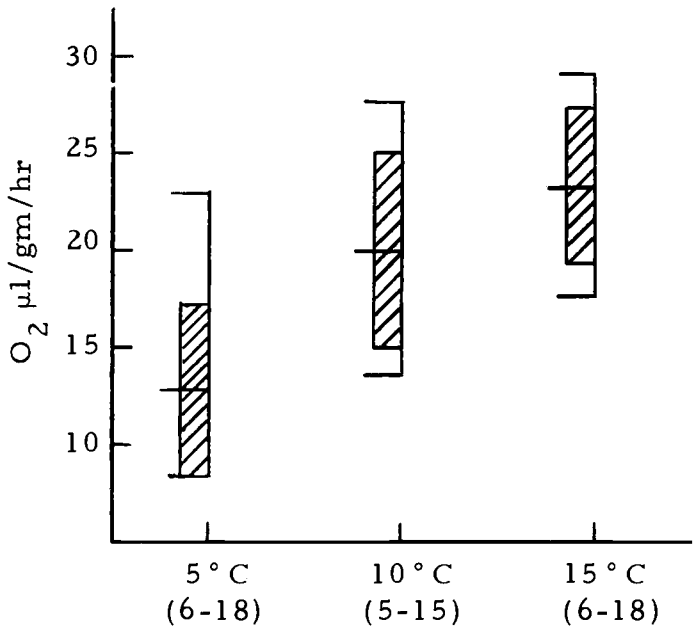

Figure 2

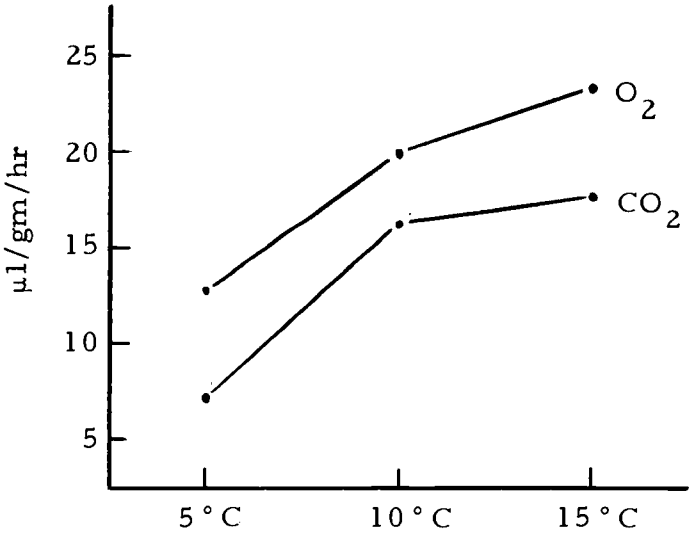

Figure 3

Figure 4.

Total aerial gas exchange in restrained transformed *D. ensatus* at 5° C, 10° C, and 15° C.

Figure 5.

Total aerial gas exchange in unrestrained transformed *D. ensatus* at 5° C, 10° C, and 15° C.

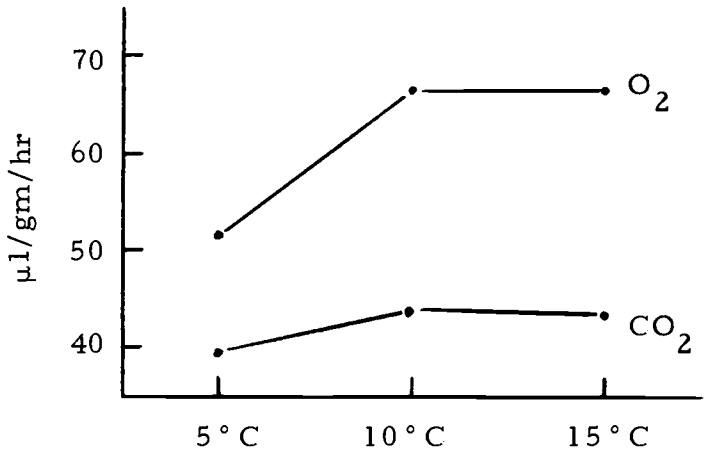

Figure 4

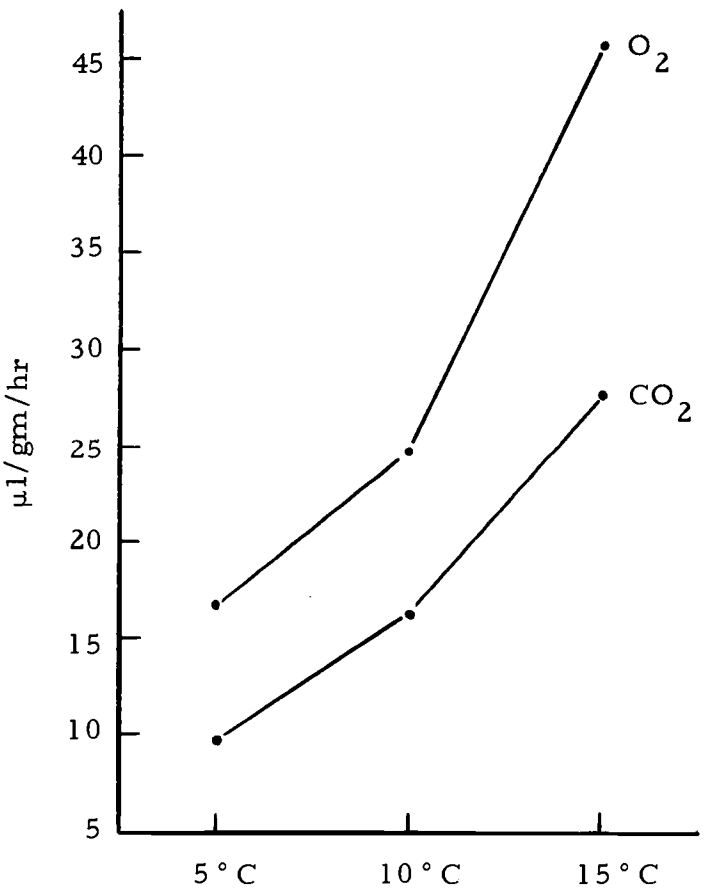

Figure 5

Figure 6.

Total aerial oxygen uptake in unrestrained transformed *D. ensatus* at 5 °C, 10 °C, and 15 °C.

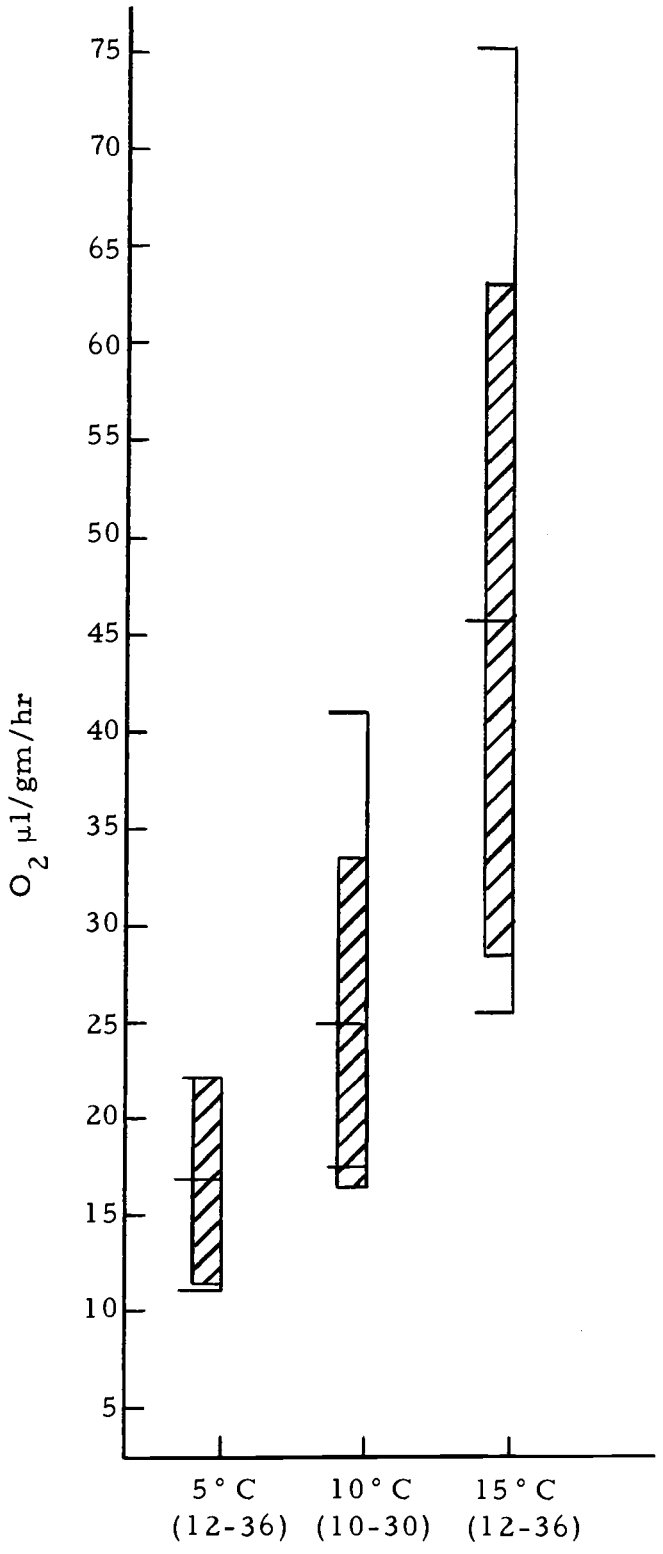
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
O_2 µl/gm/hr
5° C
(12-36)
10° C
(10-30)
15° C
(12-36)

Figure 6

Figure 7.

Cutaneous gas exchange in restrained transformed *D. ensatus* at 5 °C, 10 °C, and 15 °C.

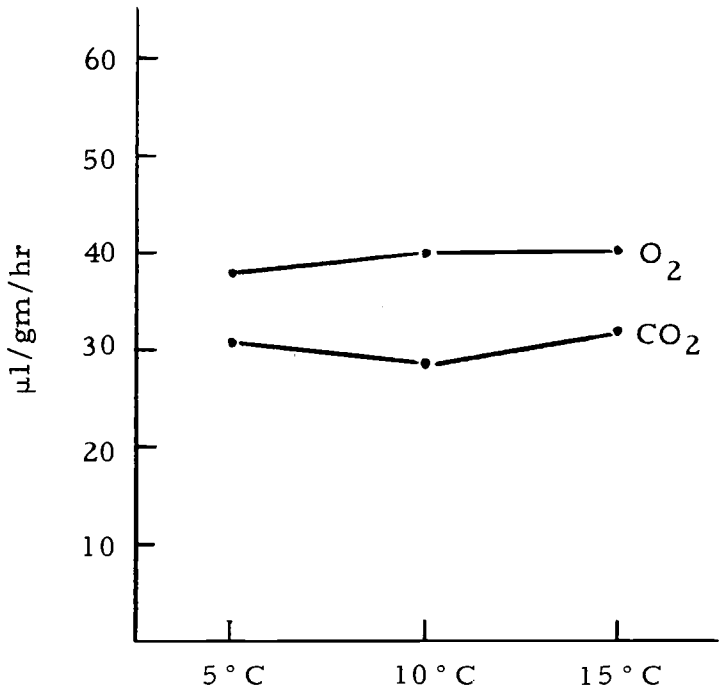

Figure 7

Figure 8.

Total oxygen uptake in restrained neotenic
D. ensatus at 5 °C, 10 °C, and 15 °C.

Figure 9.

Total gas exchange in restrained neotenic
D. ensatus at 5 °C, 10 °C, and 15 °C.

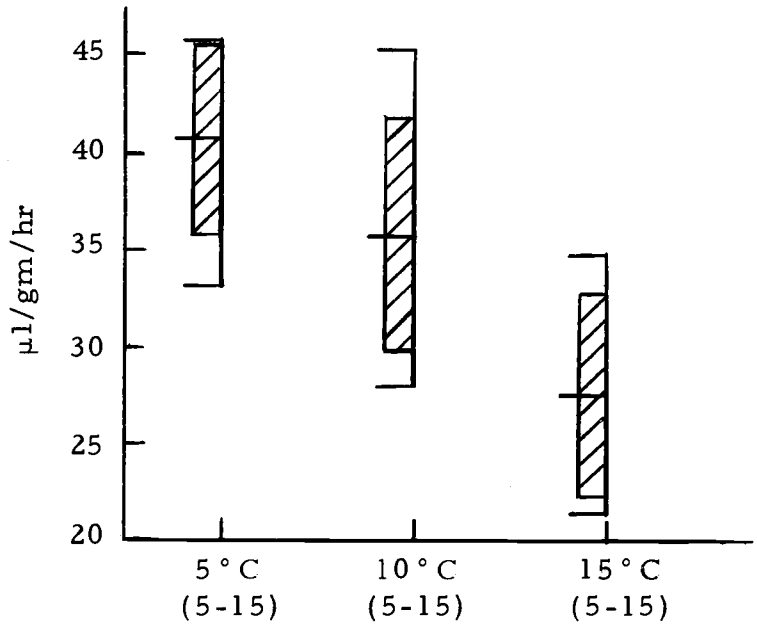

Figure 8

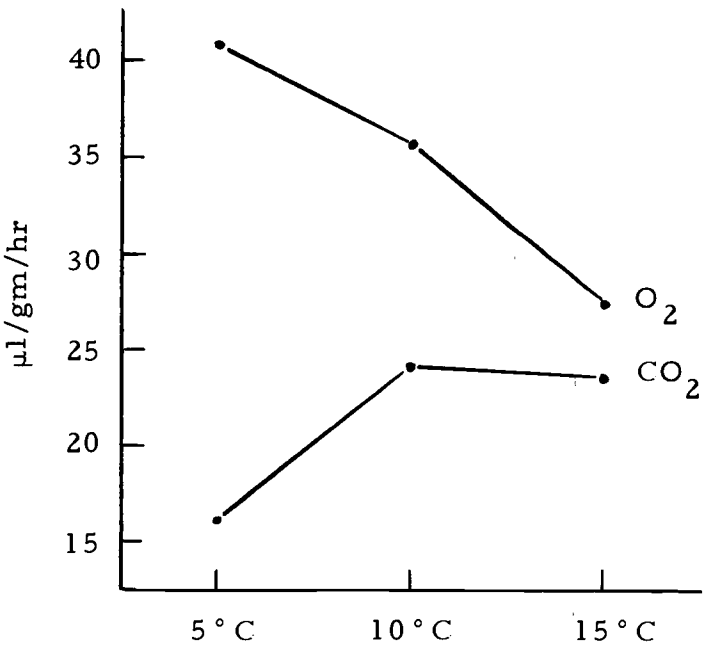

Figure 9

Figure 10.

Total oxygen uptake of restrained transformed *D. ensatus* at 5° C, 10° C, and 15° C.

Figure 11.

Total aquatic oxygen uptake of neotenic *D. ensatus* at 5° C, 10° C, and 15° C.

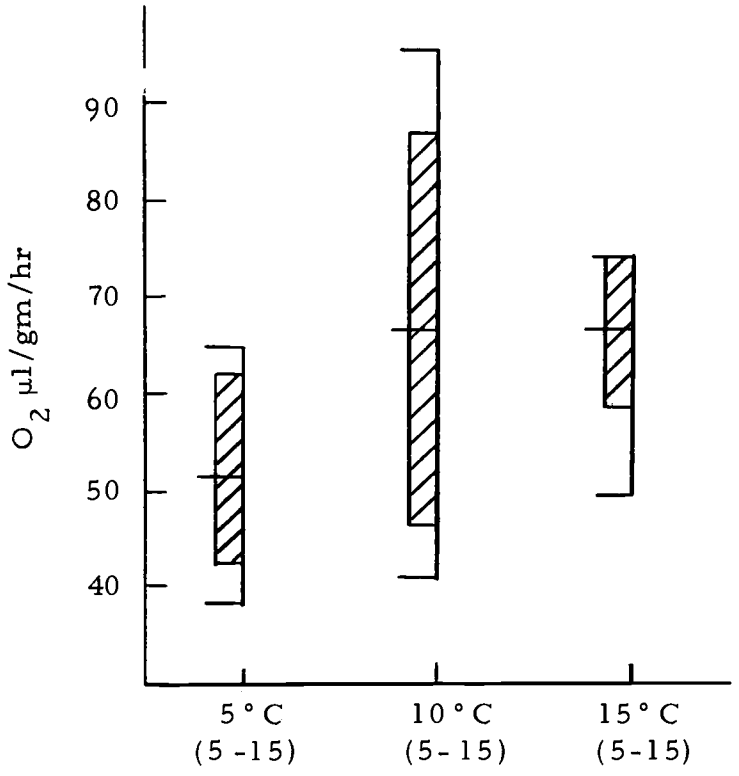

Figure 10

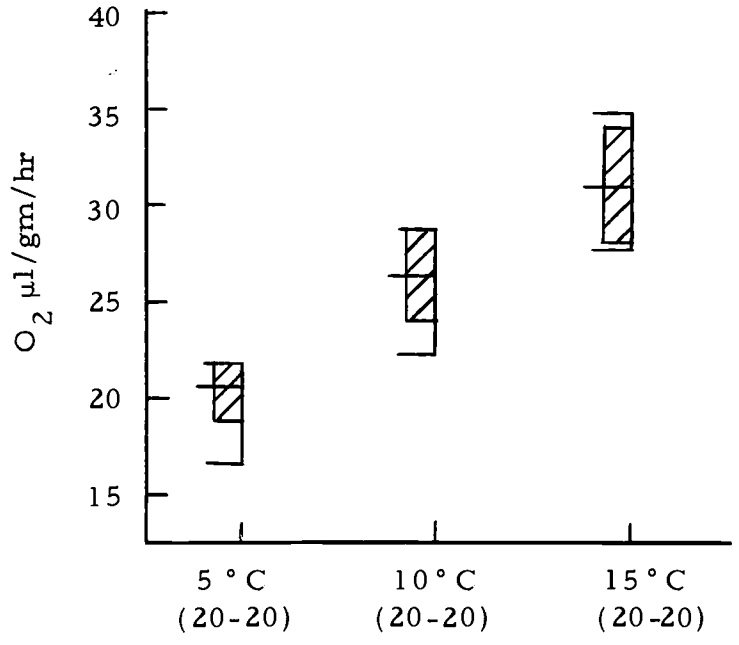

Figure 11

Figure 12.

Total aquatic oxygen uptake in transformed
D. ensatus at 5° C, 10° C, and 15° C.

Figure 13.

Pulmonary gas exchange in restrained transformed
D. ensatus at 5° C, 10° C, and 15° C.

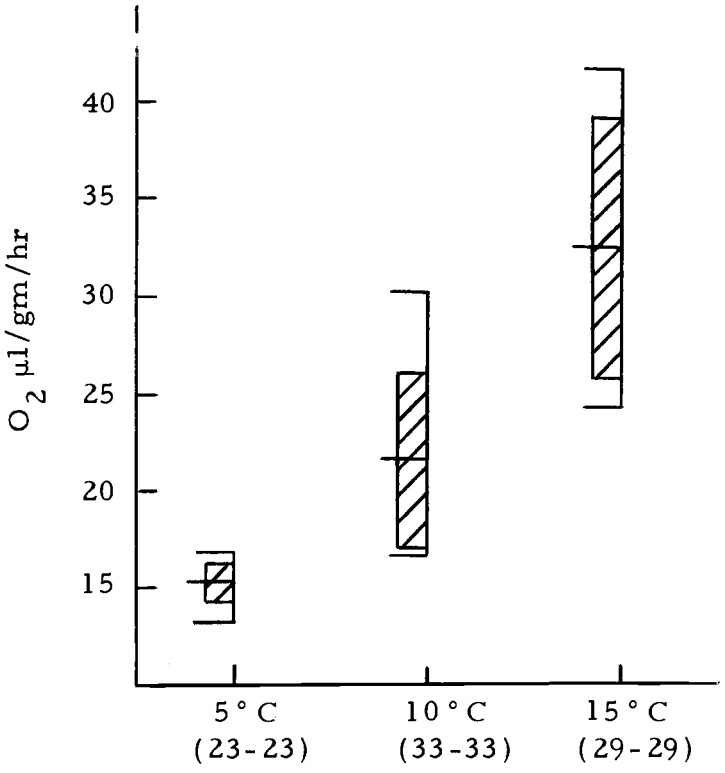

Figure 12

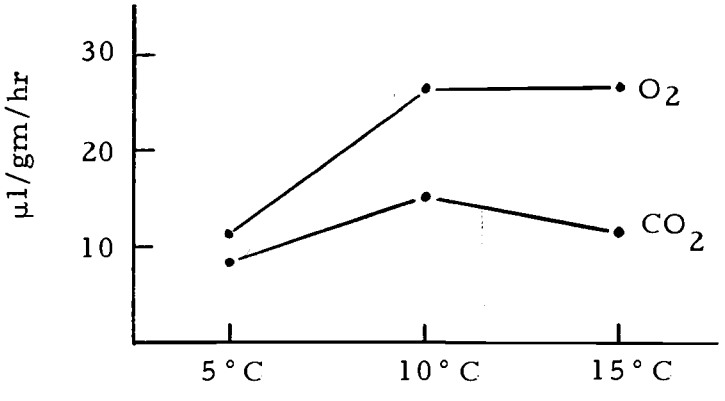

Figure 13

Figure 14.

Cutaneous oxygen uptake in restrained neotenic *D. ensatus* at 5°C, 10°C, and 15°C.

Figure 15.

Cutaneous gas exchange in restrained neotenic *D. ensatus* at 5°C, 10°C, and 15°C.

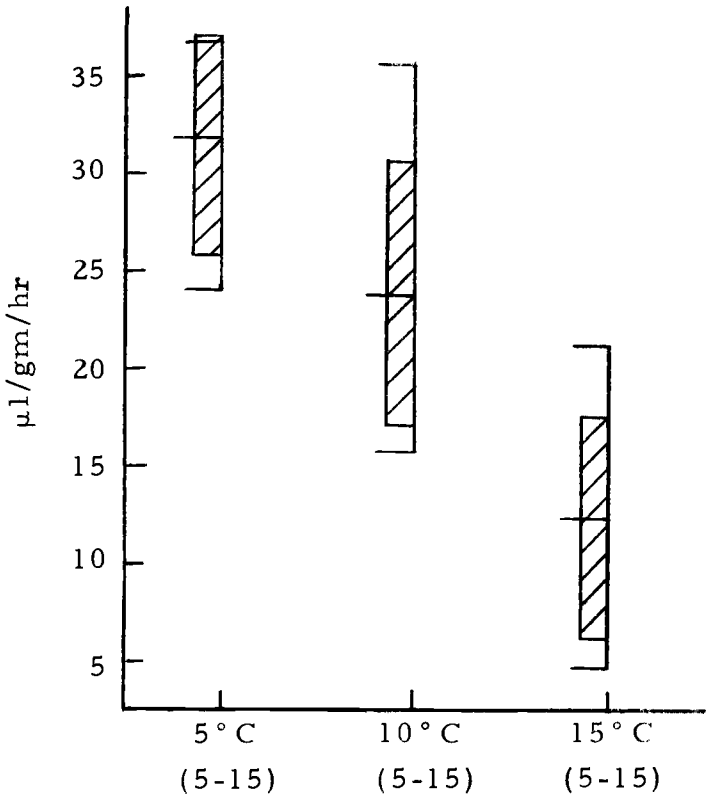

Figure 14

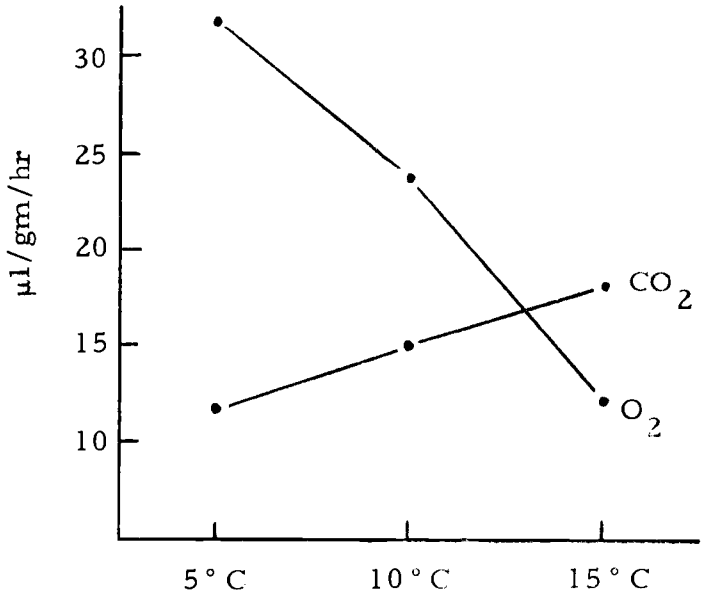

Figure 15

Figure 16.

Cutaneous oxygen uptake in restrained transformed *D. ensatus* at 5 °C, 10 °C, and 15 °C.

Figure 17.

Pulmonary oxygen uptake in restrained transformed *D. ensatus* at 5 °C, 10 °C, and 15 °C.

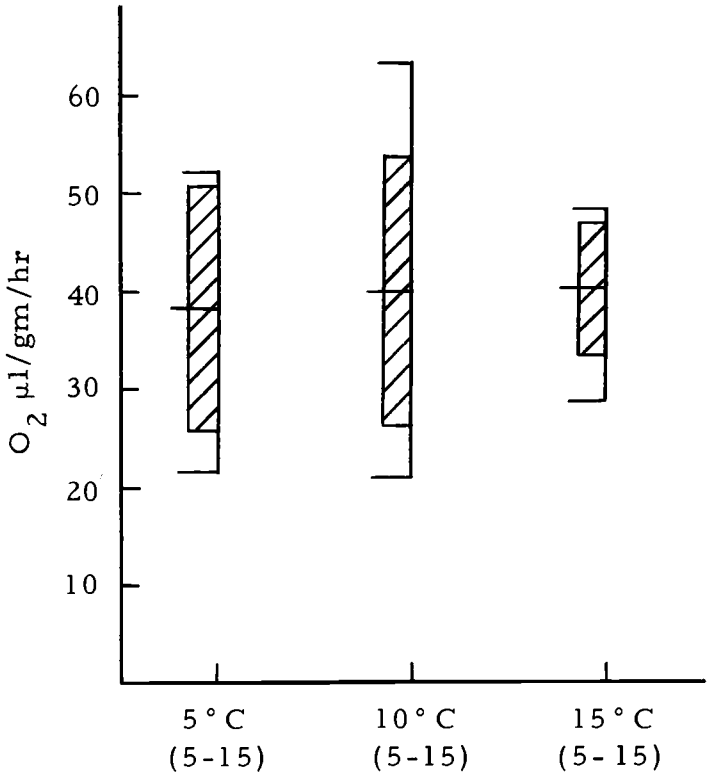

Figure 16

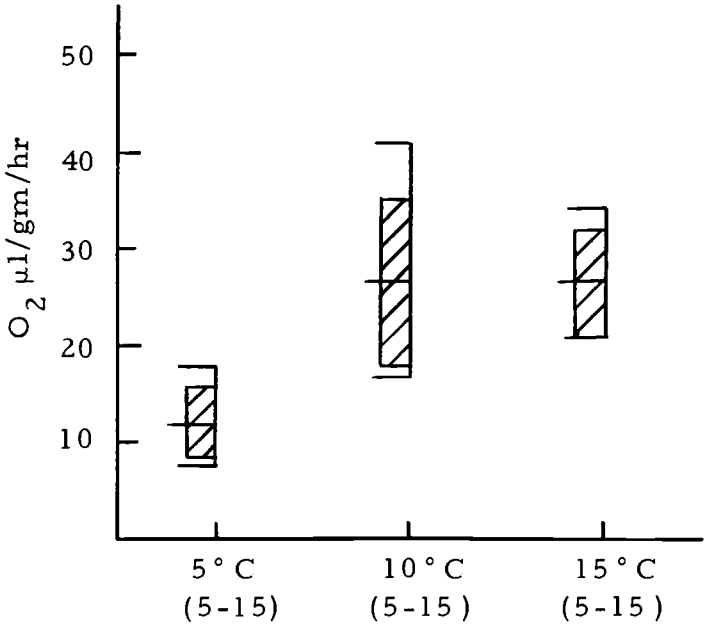

Figure 17

Figure 18.

Pulmonary oxygen uptake in restrained neotenic *D. ensatus* at 5 °C, 10 °C, and 15 °C.

Figure 19.

Pulmonary gas exchange in restrained neotenic *D. ensatus* at 5 °C, 10 °C, and 15 °C.

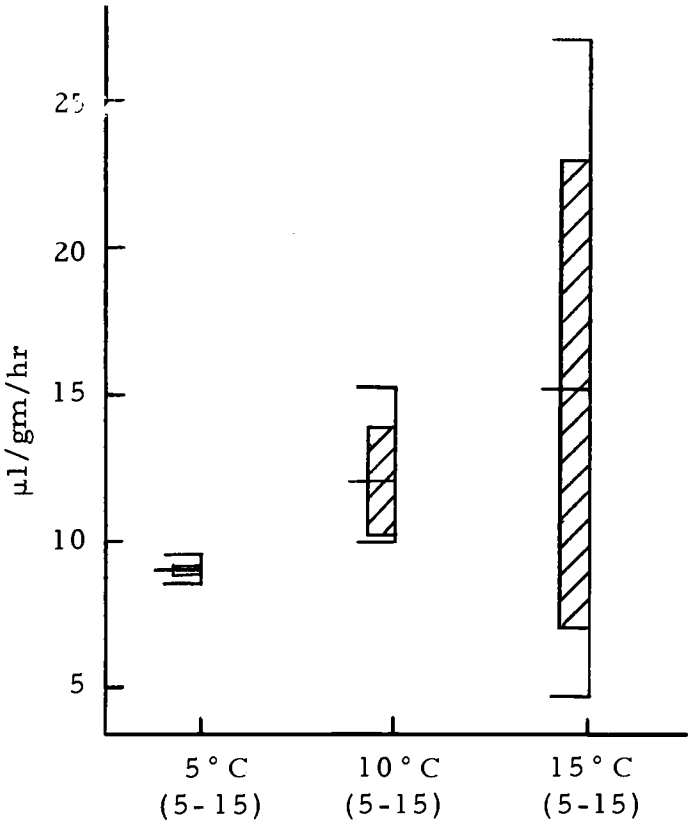

Figure 18

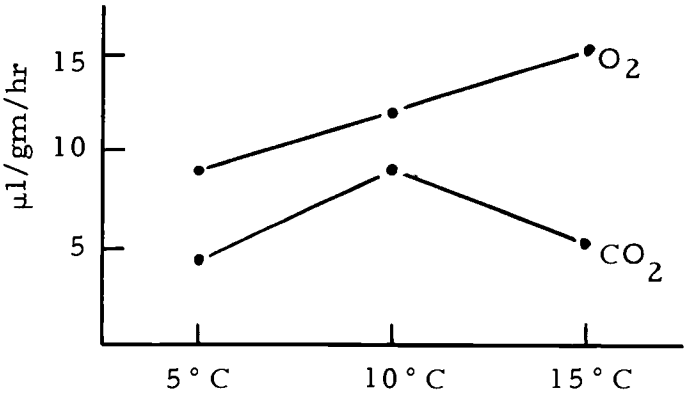

Figure 19

Figure 20.

Comparison of total oxygen uptake from air and water in transformed D. ensatus at 5° C, 10° C, and 15° C.

Figure 21.

Comparison of total oxygen uptake from air and water in neotenic D. ensatus at 5° C, 10° C, and 15° C.

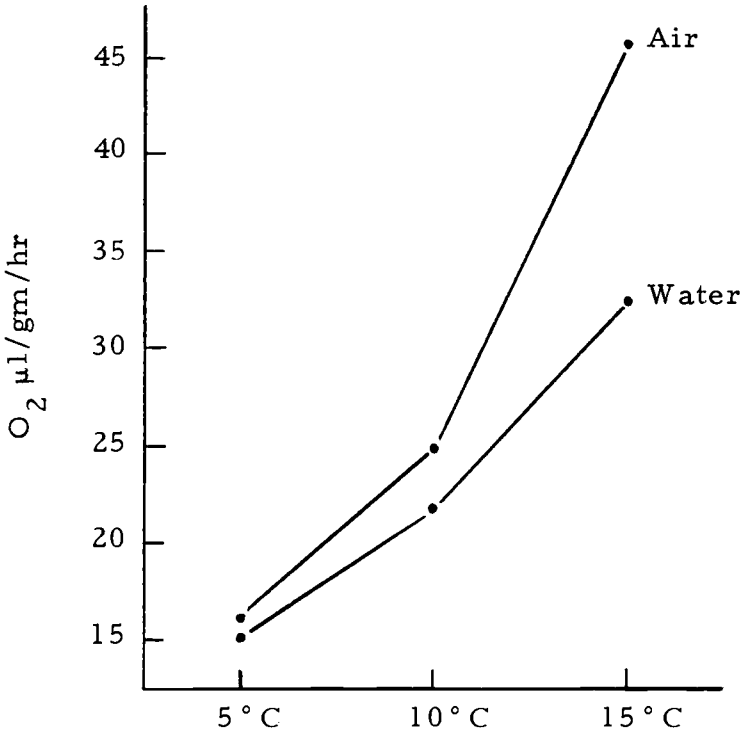

Figure 20

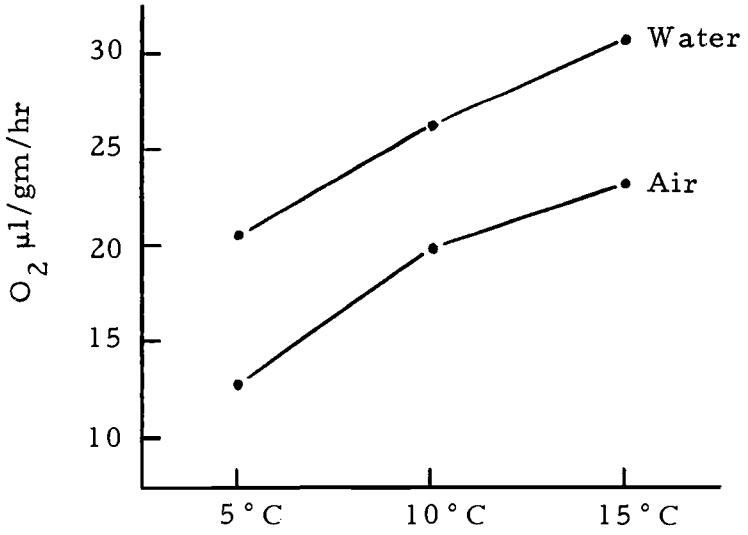

Figure 21

Figure 22.

Oxygen uptake from air and water and total oxygen consumption in neotenic and transformed D. ensatus at 5° C, 10° C, and 15° C. W, oxygen uptake from water; A, oxygen uptake from air; T, total oxygen uptake.

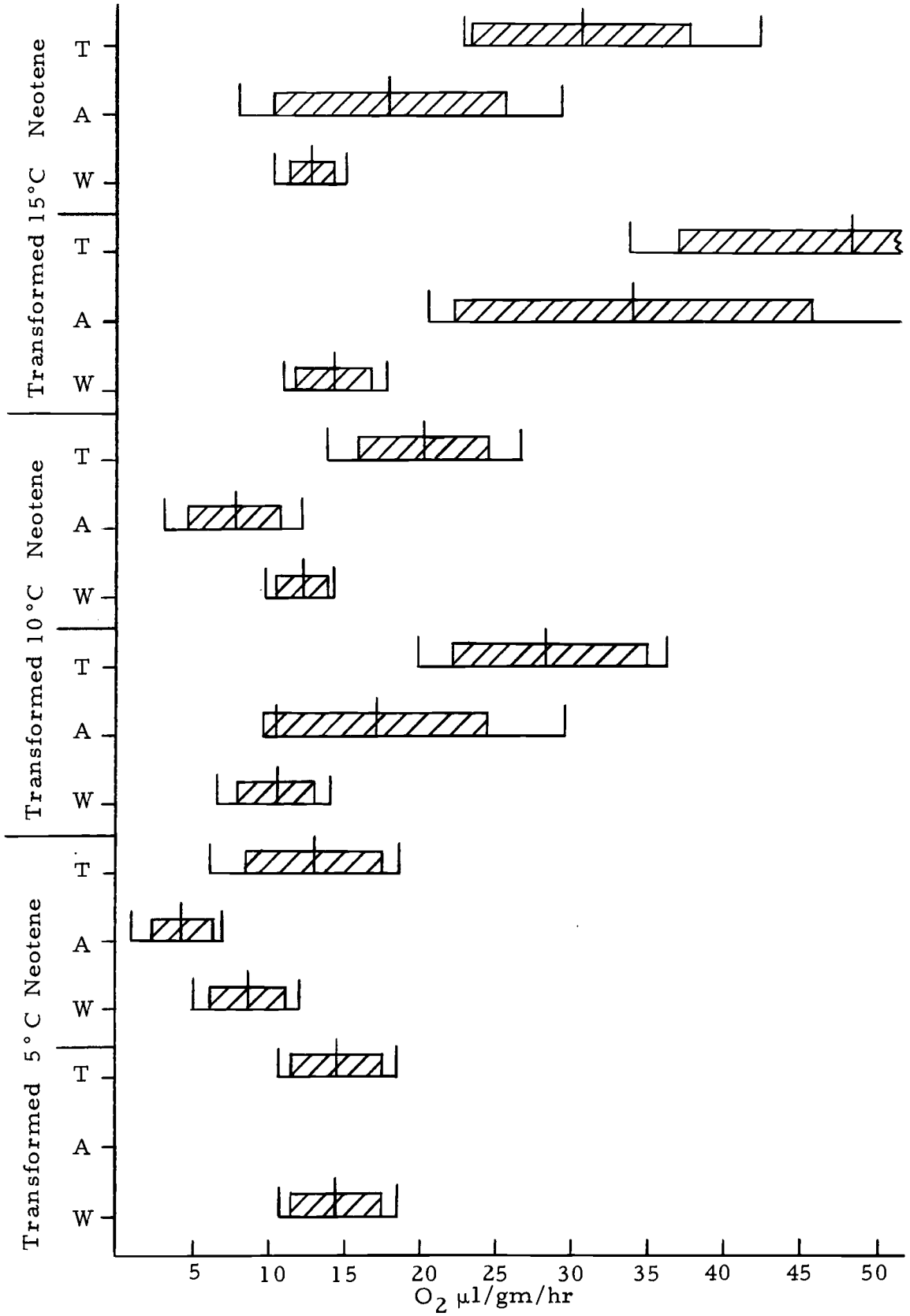
Transformed 15°C Neotene
T
A
W
T
A
W
Transformed 10°C Neotene
T
A
W
T
A
W
Transformed 5°C Neotene
T
A
W
T
A
W
5
10
15
20
25
30
35
40
45
50
O2 µl/gm/hr

Figure 22

Figure 23.

The relationship between body weight and oxygen uptake in transformed D. ensatus respiring aerially at 15 ° C.

Figure 24.

Total aquatic oxygen uptake in neotenic D. ensatus with and without gills at 15 ° C.

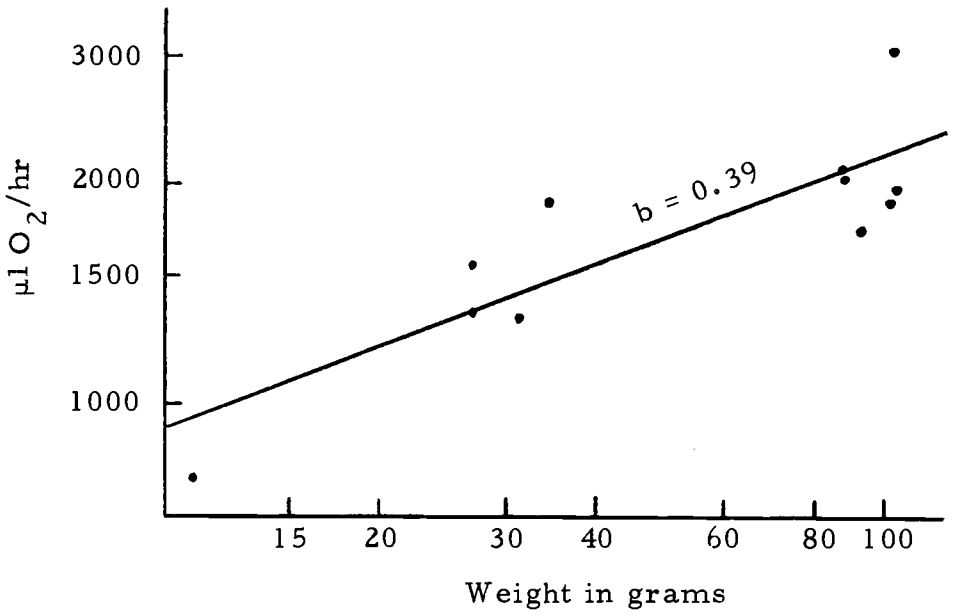

Figure 23

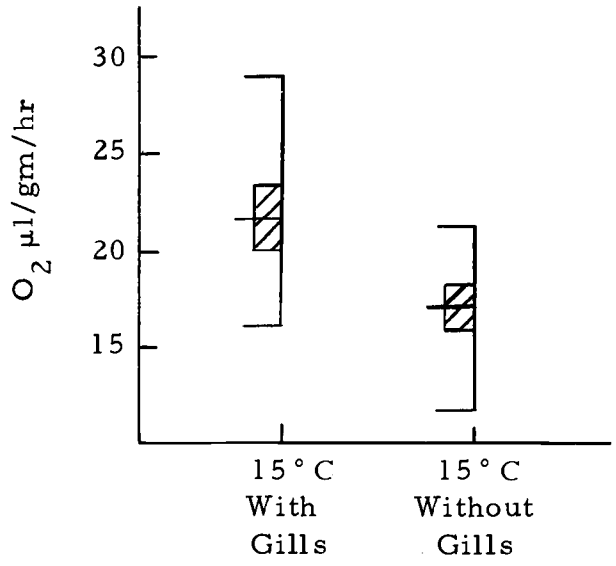

Figure 24

Figure 25.

The relationship between body weight and oxygen uptake in neotenic D. ensatus respiring aquatically at 15 °C.

Figure 26.

The relationship between body weight and oxygen uptake in transformed D. ensatus respiring aquatically at 15 °C.

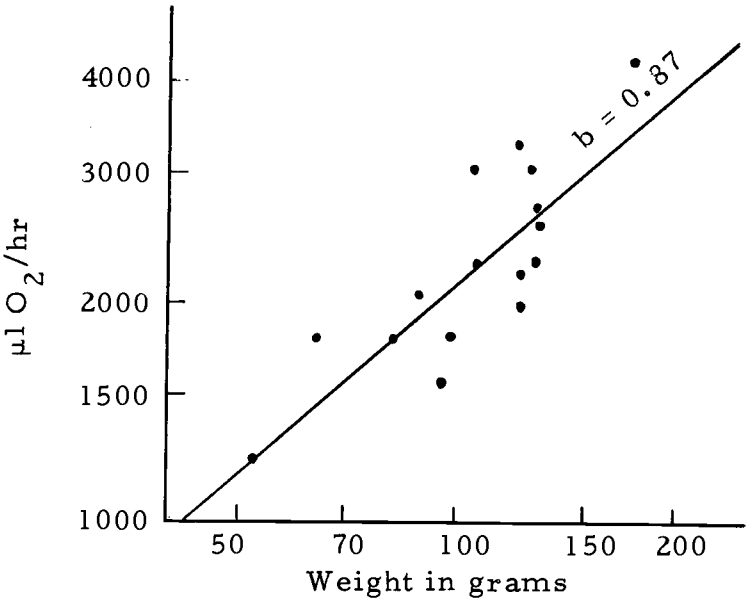

Figure 25

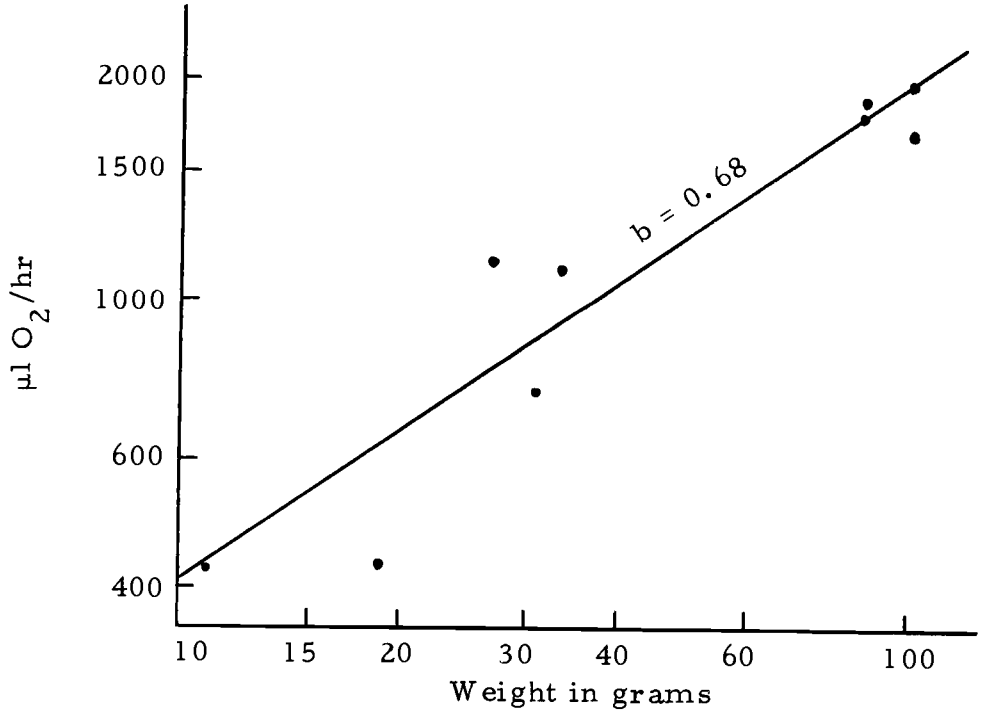

Figure 26

Table 1. Value of b in the equation $M = aW^b$ for various vertebrate groups.

Group	b*	Source
Fish	0.7-0.8	Zeuthen, 1949 (in Davison, 1955)
Fish	0.67	Rubner, 1924 (in Davison, 1955)
Frogs and Toads		
R. esculenta	0.67	Rubner, 1924 (in Davison, 1955)
Hylidae (25°C)	0.52	Hutchison et al., 1968
Ranidae (15°C)	0.64	"
Pelobatidae (15°C)	0.94	"
Bufonidae (5°C)	0.61	"
Tropical anurans	0.83-0.86	"
Salamanders		
E. nana (15°C)	0.68	Norris et al., 1963
E. neotenes (15°C)	0.92	"
E. pterophila	0.83	"
T. granulosa	0.70	Smith, 1967
Lunged forms	0.856	Whitford and Hutchison, 1967
Lungless forms	0.720	"
Reptiles		
Lizards	0.67	Zeuthen, 1949 (in Davison, 1955)
Reptiles	0.67	Benedict, 1938
Uta	0.47-0.64	Dawson and Bartholomew, 1956
Sceloporus	0.54-0.68	"
Homeotherms	0.73-0.75	Brody, 1945 Benedict, 1938 Kleiber, 1947

*b = ratio of change in metabolism with change in weight.

Table 2. Percent of total gas exchange through cutaneous and pulmonary system of neotenic and transformed Dicamptodon at 5 °C, 10 °C, and 15 °C.

	Neotenes			Transformed		
	5 °C	10 °C	15 °C	5 °C	10 °C	15 °C
Cutaneous O_2	77.7	66.5	44.7	77.1	60.2	60.2
Pulmonary O_2	22.3	33.5	55.3	22.9	39.8	39.8
Cutaneous CO_2	72.5	62.5	77.2	78.4	65.1	73.8
Pulmonary CO_2	27.5	37.5	22.8	21.6	34.9	26.2

Table 3. Percent of total oxygen obtained from water and air by neotenic and transformed Dicamptodon.

		Percent of O_2 from Water	Percent of O_2 from Air
15 °C	Transformed	29.6	70.4
	Neotene	41.8	58.2
10 °C	Transformed	38.3	61.7
	Neotene	61.3	38.7
5 °C	Transformed	100	0
	Neotene	66.4	33.6

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APPENDICES

APPENDIX I

ANIMALS OBTAINED

Experimental Temperature °C	Weight in Grams	Volume in mls.	Date Collected	Where Collected
NEOTENES				
15	105.92	103	28 Sept. 69	Lincoln Co., Ore.
15	121.49	117	5 Oct. 69	Lincoln Co., Ore.
15	124.01	118	5 Oct. 69	Lincoln Co., Ore.
15	125.35	117	28 Sept. 69	Lincoln Co., Ore.
15	129.90	127	28 Sept. 69	Lincoln Co., Ore.
15	133.18	130	28 Sept. 69	Lincoln Co., Ore.
10	52.81	50	27 Sept. 69	Lincoln Co., Ore.
10	83.30	81	19 Oct. 69	Lincoln Co., Ore.
10	98.86	96	28 Sept. 69	Lincoln Co., Ore.
10	129.01	125	19 Oct. 69	Lincoln Co., Ore.
10	131.04	120	28 Sept. 69	Lincoln Co., Ore.
10	145.02	140	28 Sept. 69	Lincoln Co., Ore.
5	65.85	64	22 Sept. 69	Lincoln Co., Ore.
5	89.44	86	28 Sept. 69	Lincoln Co., Ore.
5	107.26	104	3 Nov. 69	Lincoln Co., Ore.
5	127.67	119	5 Oct. 69	Lincoln Co., Ore.
5	130.86	123	3 Oct. 69	Benton Co., Ore.
5	141.66	140	5 Oct. 69	Lincoln Co., Ore.
5	175.27	166	29 Sept. 69	Benton Co., Ore.
TRANSFORMED DICAMPTODON				
15	11.12	10	29 Apr. 70	Benton Co., Ore.
15	19.36	18	28 Sept. 69	Lincoln Co., Ore.
15	20.97	20	31 May 70	Benton Co., Ore.
15	27.04	27	9 Sept. 69	Shoshone Co., Ida.
15	31.38	30	30 Oct. 69	Benton Co., Ore.
15	33.83	32	8 Sept. 69	Benewah Co., Ida.
15	86.71	80	30 Oct. 69	Benton Co., Ore.
15	88.47	85	26 Oct. 69	Benton Co., Ore.
15	101.15	85	17 Sept. 68	Benton Co., Ore.
15	102.43	95	26 Oct. 69	Benton Co., Ore.
10	9.89	10	29 July 69	Multnomah Co., Ore.
10	17.03	16	28 Sept. 69	Lincoln Co., Ore.
10	20.04	19	9 Sept. 69	Benewah Co., Ida.
10	28.63	27	27 Apr. 69	Benton Co., Ore.
10	29.09	27	29 July 69	Multnomah Co., Ore.
10	29.86	29	22 Aug. 68	Cowlitz Co., Wash.
5	17.08	17	28 Sept. 69	Lincoln Co., Ore.
5	22.43	22	22 Aug. 68	Cowlitz Co., Wash.
5	28.64	28	26 Oct. 69	Benton Co., Ore.
5	43.79	41	22 Aug. 68	Cowlitz Co., Wash.
5	59.61	56	22 Aug. 68	Cowlitz Co., Wash.

APPENDIX II

THE MICRO-WINKLER METHOD

The method described here is a modification of that of Fox and Wingfield (1938). The Winkler reaction may be run and the water sample measured in the same syringe. It is also convenient in that small, but manageable samples may be analyzed. Finally, with accurately graduated glassware and with sufficient precaution, results are obtainable which are as dependable as those of the standard Winkler Method (Welch, 1948).

The following is a summary of the Winkler reaction:

A. $2MnCl_2 + 4NaOH = 4NaCl + 2Mn(OH)_2$

B. $2Mn(OH)_2 + H_2O + O = 2Mn(OH)_3$

C. $2Mn(OH)_3 + 2KI + 6HCl = 2MnCl_2 + 2KCl + 6H_2O + I_2$

When manganous chloride is introduced in the presence of alkali, a manganous hydroxide precipitate is formed (A). The oxygen in the sample then oxidizes the manganous hydroxide to manganic hydroxide (B). The addition of acid results in the oxidation of the potassium iodide to iodine by the manganic ions. The amount of iodine released depends upon the extent of extent of manganic ion formed. The iodine is then titrated with sodium thiosulfate, using starch as an indicator. A loss of color results following the disappearance of the starch-iodine complex when iodine is reduced to

iodide. Two molecules of iodide are liberated by each molecule of oxygen:

$$I_2 + Na_2S_2O_3 = 2NaI + Na_2S_4O_6$$

Thus: $1/2\ O_2$ is equivalent to I_2.

Equipment

1. Reaction syringe - This was constructed from a 10 ml. glass syringe having a glass needle fitting. A capillary tube, drawn out to a fine tip, was connected to the needle fitting with a small piece of tygon tubing. The capillary tube should be fitted as closely as possible to the syringe, so that the space between them is minimal. The fine capillary tip prevents gases from diffusing in and out of the syringe.
2. Titration equipment - A syringe microburette (model SB2, Micro-Metric Instrument Co., Cleveland, Ohio) was used in conjunction with a 1 ml. syringe for titration of the sample. Determinations were made in a 30 ml. beaker. A magnestir was used to stir the solution. A 10 ml. microburette was used to standardize the sodium thiosulfate.

Reagents

1. Sodium thiosulfate - weight out 6.2 gm. of sodium thiosulfate and dissolve in 250 ml. of boiled and cooled distilled water.

Dilute this stock solution 25 ml/250 ml of boiled and cooled distilled water for standardization. Weigh out 0.8917 gm. KIO_3 and dissolve in 1 liter distilled water to make 0.025 normal solution for standardizing the sodium thiosulfate. Fill the 10 ml. burettte with diluted sodium thiosulfate. Pipette exactly 2 ml. of KIO_3 in a 30 ml. beaker and add 1 ml. of KI. Add 5 drops of concentrated H_3PO_4 and 2 drops of starch and titrate. Repeat three times and calculate the normality of the thiosulfate:

$$N_{KIO_3} \times V_{KIO_3} = N_{Na_2S_2O_3} \times V_{Na_2S_2O_3}$$

$$0.05 = N \times V$$

$$N = \frac{0.05}{V}$$

2. $MnCl_2$ - 100 gm./250 ml. distilled water.
3. Alkaline Iodide - 80 gm NaOH and 25 gm./250 ml. distilled water.
4. KI - 2.5 gm./250 ml. distilled water.
5. Starch - 2.5 gm./ 250 ml. distilled water. Heat slowly to 100° C. Cool and add 0.25 gm. salicylic acid.
6. H_3PO_4 - concentrated reagent.

Titration of Sample

Two steps must be performed very carefully. These are the volume measurement and the titration of the sample with sodium thiosulfate. The remaining reagents are added in excess of that required for the reaction.

1. Fill the dead space of the syringe and nozzle with $MnCl_2$, being careful to exclude all air bubbles.
2. Take in 5 ml. of the water sample to be tested for dissolved oxygen.
3. Draw in approximately two times the dead space volume of alkaline iodide.
4. Rotate the syringe five times to mix the manganous hydroxide thoroughly.
5. Lay the syringe down for three minutes to allow oxygen absorption.
6. Take in three to four times the dead space volume of phosphoric acid and rotate syringe until the precipitate is dissolved. The reaction has now ceased and exposure of the solution to the air is permitted.
7. Eject the solution into a 30 ml. beaker and wash the syringe with 2 ml. of distilled water. The wash is added to the titration vessel.

8. Add two drops of starch indicator and titrate the sample with the standardized sodium thiosulfate. The syringe microburette is used for titration.
9. Rinse the syringe with distilled water. When it is again rinsed with $MnCl_2$, the next sample may be taken.

Calculations

$$\text{ml. } O_2/L = \frac{n.A.5600}{V}$$

n = ml. sodium thiosulfate used in titration.

A. = normality of the thiosulfate

V. = volume of water sample titrated.

A correction was made for the dissolved oxygen in the manganous chloride and alkaline iodide. These reagents contain approximately 3.4 ml. O_2/L.

The dead space in the reaction syringe was 0.3 ml.

Thus: 0.3 ml. of $MnCl_2$ in sample

0.7 ml. of alkaline iodide in sample

1.0 ml. of reagents in sample.

There are 3400 µl of O_2 in one liter of reagent. Since the sample contained 1000 µl of reagent, there are 3.4 µl of O_2 in the sample.

APPENDIX III

COMPUTER PROGRAM *CLOTH

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      PROGRAM*CLOTH
      DIMENSION Z(15)
100   FORMAT(F4.4,15A4)
101   FORMAT(2F3.3,F5.2,F3.0,F6.2,F3.0)
102   FORMAT(1H-,F8.2,20H MICROL/GM/HR...LOG=,F10.6,
      10H...LOG WT=,

      1F10.6)
103   FORMAT(1H ,15A4)
      READ(30,100)T,Z
      XK=(T*5600.)/5.
      WRITE(61,103)Z
 1    READ(30,101)START,END,WT,ANVOL,CHBR,TIME
      IF(EOFCKF(30).EQ.1) GO TO 99
      START=START*XK*.001*CHBR
      END=(END*XK*.001)*(CHBR-ANVOL)
      X=((START-END)*1000.)/TIME*60/WT
      XL=ALOG10(X)
      WTL=ALOG10(WT)
      WRITE(61,102)X,XL,WTL
      GO TO 1
 99   END
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