THERMAL CONSIDERATION OF THE CHOROIDAL GLAND RETE MIRABILE IN THE RAINBOW TROUT (Salmo gairdneri)

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
DENNIS ANTHONY BARRACO
1975

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ABSTRACT

THERMAL CONSIDERATION OF THE CHOROIDAL GLAND RETE MIRABILE IN THE RAINBOW TROUT (*Salmo gairdneri*)

By

Dennis Anthony Barraco

The ability of the rainbow trout choroidal gland rete mirabile, a known oxygen concentrator, to function as a counter current heat exchanger was investigated. This was done with the supposition that proof of the existence of a heat exchanger would be demonstrated by 1) warmer than ambient temperatures at the surface of the retina and 2) a difference in the rates of thermal conductance through the eye between animals possessing intact retial circulation to those in which retial function had been abolished by bilateral pseudobranchectomy. Minute thermoresistive probes and appropriate Wheatstone bridge circuitry were constructed and utilized for the temperature measurements.

The results show that the mean eye (retinal) temperature was not statistically different from the mean ambient temperature. Also, the rates of ocular thermal conductance did not differ significantly between the intact and pseudobranchectomized animals. Hence, it was concluded that the vascular network in question was not an effective heat

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exchanger. A hypothesis, based upon the anatomical characteristics of the rete was presented as a possible explanation of the findings.

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By

Dennis Anthony Barraco

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Physiology

1975

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Dedicated to my Parents, whose emotional and financial support created at atmosphere in which the acquisition of knowledge became a desire and not a task.

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ACKNOWLEDGMENTS

The author wishes to express his thanks and appreciation to Dr. Hoffert for guidance and support throughout this study, aid in the preparation of this thesis, and for his invaluable electronic assistance.

I would also like to express my gratitude to Drs. Fromm and Pittman for their critical review of the manuscript and their suggestions for its improvement.

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The author is also indebted to the National Institute of Health for the support of this work through Grant No. 00009 from the National Eye Institute.

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INTRODUCTION

The chemical and thermal characteristics of an animal may differ markedly from that of its environment. The composition of the intracellular milieu differs from that of the extracellular fluids. Thus, an animal must rely on a means of efficient exchange to maintain a state of homeostasis. This often takes the form of simple diffusion of materials and energy across cell membranes. However, as the order of the animal kingdom becomes more complex the process of simple diffusion alone becomes inadequate to meet the many exchange needs of the organism.

This problem is occasionally solved by ingenious biological engineering in which exchange occurs between two counter flowing processes--a phenomenon known as counter current exchange. Scholander (1958) believes this phenomenon "not only vastly increases the efficiency of the exchange, but may also sometimes lend to the system new and unexpected features." He lists three basic interdependent properties of counter current exchange systems: 1) efficient diffusion exchange, 2) maintenance of a diffusion barrier in spite of flow, and 3) a capacity to maintain or sometimes build up large concentration gradients along the direction

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of flow. This phenomenon has been described as occurring in many situations with the kidney of mammals, swimbladder of deep sea fish, and the fins (and flukes) of whales being prominent examples. The latter two instances of counter current exchange systems are of particular interest to this study in that both rely on a specialized capillary network that apparently has evolved for the purpose of allowing an animal to possess a more efficient exchange process within its biological situation.

German anatomists of the 19th century observed arteries and veins in which blood flow was counter current. The vascular anomalies were termed "Wundernetze" (Scholander, 1957) which translates into English as "wonderful networks." Anatomists, however, most often refer to these structures by their Latin translation, "retia mirabile." These retia mirabile are responsible for some of the most striking examples of counter current exchange in nature, especially in relation to oxygen and heat concentration. For example, in the swimbladder rete of deep sea fish oxygen tensions 200-300 times that of ambient are built up and maintained (Scholander 1957, 1958). In fact, the efficiency of this exchange system is so great that Scholander (1954) calculated that in a single pass, a given concentration could be raised 3,000 times.

Similarly, a rete mirabile may also act to prevent heat loss. Many authors cite Claude Bernard (in 1876) as

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having been the first to postulate heat transfer between blood vessels after observing arrangements of closely allied arteries and veins in which flow was in opposite directions. However, it was some 70 years later before any real quantitative research bore out Bernard's hypothesis. Bazett et al. (1948) reported a continuous decrease of arterial and venous temperature in the arterial flow direction in the arm of man. Later, Scholander and Schevill (1955) demonstrated how such an exchange process could participate in an animal's adaptive response to climatic changes. In this study the authors showed that animals such as whales, possessing uninsulated highly vascularized fins and flukes, are constantly exposed to near freezing temperatures and still able to avoid excessive heat loss through these extremities by means of counter current heat exchange between arteries and veins.

More recently, Carey et al., 1971; Carey and Lawson, 1973; Carey, 1973 have indicated the importance of counter current heat exchange in the maintenance of high temperatures in the red muscle of tuna and other highly predatory marine fish. In these animals elaborate retia trap the metabolic heat of the red muscle in an effort to keep these tissues at an optimum level of activation.

Although the above models of counter current exchange are of great physiological significance, one that serves as the basis for this scientific inquiry, is a physiologic system in

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system in which a single rete mirabile appears to function not only in oxygen concentration but also as a heat exchanger. Linthicum and Carey (1972) report that the rete mirabile of the ocular choriodal gland in some marine teleosts not only has the capacity to maintain oxygen pressure above arterial levels but may also operate to maintain eye temperatures higher than the environmental temperature.

The fresh water teleost, Salmo gairdneri (rainbow trout), has been known to possess a very efficient counter current oxygen multiplier in its choroid rete (Fairbanks, Hoffert, and Fromm, 1969). However, little is known about this structure's capacity to simultaneously function as a heat exchanger. It was therefore the purpose of this investigation to determine if this rete likewise serves as a functional or quantitative counter current heat exchanger.

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LITERATURE REVIEW

The choroid rete (Figure 1) is a large horseshoe shaped body located behind the retina but within the eyeball of many fishes. It is composed of several thousand parallel arterial and venous capillaries closely intermingled such that arterial and venous flow is counter current. This vascular network supplies arterial blood to the capillary network underlying the retina called the choriocapillaris and in turn receives the venous outflow from these capillaries (Wittenberg and Haedrich, 1974). The choroid rete receives its blood supply from the ophthalmic artery which gains its supply from the pseudobranch. The pseudobranch is a modified (first spiracular) gill arch receiving oxygenated blood from the first efferent branchial gill artery. Wittenberg and Haedrich (1974) point out that the blood passing from the heart to the eye and back to the heart passes through five tandem sets of capillaries: the gill capillaries, the pseudobranchial capillaries, the afferent (arterial) capillaries of the choroid rete mirabile, the capillaries of the choriocapillaris underlying the retina and the efferent (venous) capillaries of the choroid rete (Figures 2 and 3).

Figure 1. Schematic representation of the choroidal gland and vascular supply to the eye of a teleostean fish. (From Barnett, 1951.)

a.c. = arterial capillaries of rete mirabile

ch. = choriocapillaris

c. scl. = sclera

o.a. = opthalmic artery

o.n. = optic nerve

v.c. = venous capillaries of rete mirabile

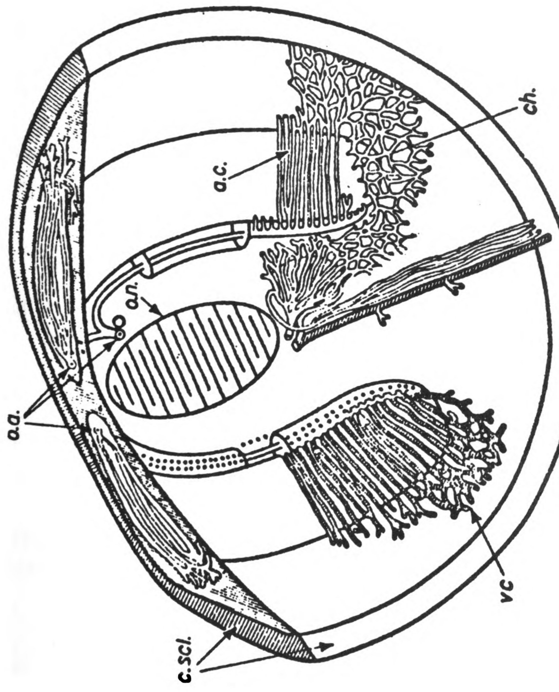

Figure 1

Figure 2. Arterial supply to the choroidal gland from the spiracular pseudobranch in teleostean fish. (From Francois and Neetens, 1962.)

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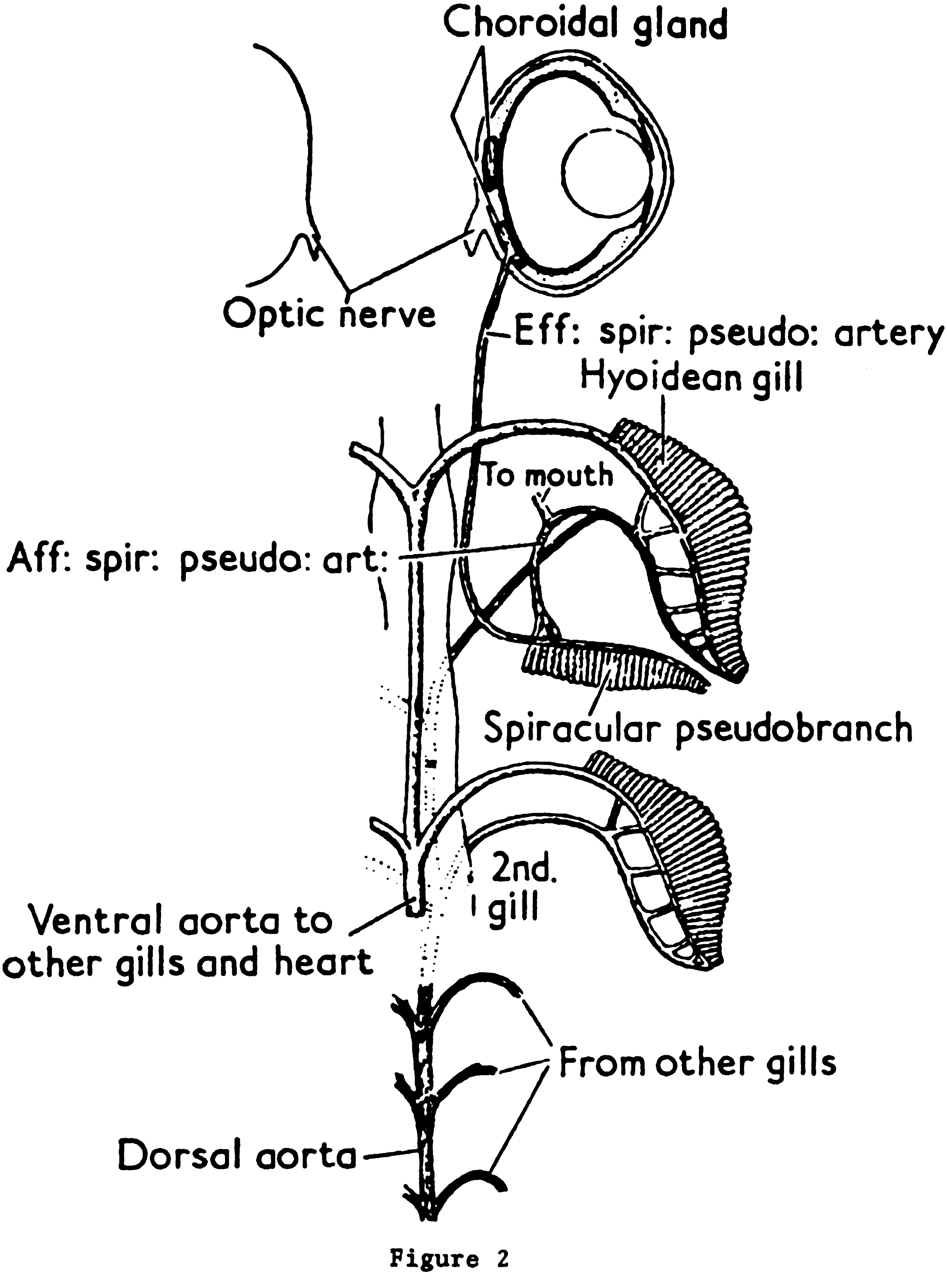
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Spiracular pseudobranch
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Ventral aorta to other gills and heart
From other gills
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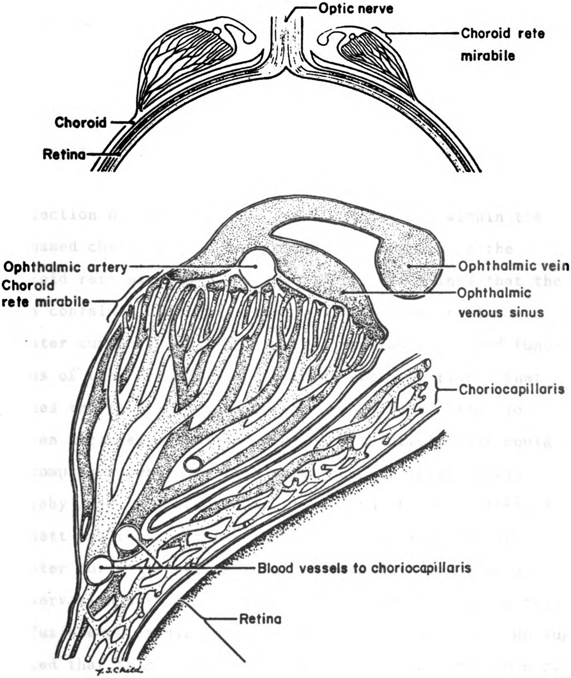
Figure 2

Figure 3. Semidiagrammatic representation of the choroid rete mirabile of the bluefish and its relation to the optic nerve and retina as seen in longitudinal section. (From Wittenberg and Wittenberg, 1974.)


Optic nerve
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Choroid
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Choroid rete mirabile
Ophthalmic vein
Ophthalmic venous sinus
Choriocapillaris
Blood vessels to choriocapillaris
Retina

Figure 3

Barnett (1951) in his paper on the structure and function of the teleostean choroidal gland cites the work of Albers (1806) as the first to recognize that the horseshoe-shaped body located in the choroidal layer of the teleost eye was a collection of blood vessels and not a muscle or gland as previously suggested. Later, in the middle of the 19th century further research, cited by Wittenberg and Wittenberg (1974), and performed by Muller (1839), Jones (1838), and Owen (1836) confirmed the fact that there is a collection of arterial and venous capillaries within the misnamed choroidal gland that came to be known as the choroid rete mirabile. Barnett (1951) determined that the rete contained different blood vessels in which flow was counter current. He rejected the previously assumed functions of the choroid rete as 1) an erectile tissue that pushes the retina forward for focusing, 2) a buffer to dampen arterial pulsations and, 3) a reservoir that could be compressed when the fish enters deeper water levels thereby injecting additional blood into the eye. Instead, Barnett put forth his own theory that, as a result of counter current flow, the choroid rete must function as a conservation system acting to minimize the loss of certain diffusible materials essential to retinal function. He suggested that this substance might be oxidized cytochrome c.

Evidence in support of Barnett's theory was reported ten years later when Wittenberg and Wittenberg (1962) demonstrated that the choroid rete of marine teleosts did minimize the loss of diffusible oxygen, essential to retinal function. The authors reported that the choroid rete analogous to the swimbladder rete, acts to create a large pressure of dissolved oxygen behind the retina, thus providing a gradient for diffusion in order to meet the vigorous oxygen demands of the avascular retina. Measurements of oxygen tension were made in the vitreous humor and were later found to directly parallel the extent to which the choroid rete itself was developed (Wittenberg and Wittenberg, 1974).

Recently, Linthicum and Carey (1972) have shown that some marine teleosts, in addition to maintaining core temperatures above ambient, are also able to maintain eye and brain temperatures 4-7°C above ambient. The authors partially attribute this finding to a counter current heat exchanger in the blood supply to the brain and eye which allows metabolic heat to accumulate in these organs. Since the choroid retia in the animals are quite prominent (Wittenberg and Wittenberg, 1974), it seems reasonable to assume that the phenomenon of heat concentration in the eye of these animals may be partially attributed to the presence of these retia. The possible relationship between these two observations--(1) that the oxygen tension in eye of marine teleosts appears to be directly related to the extent to which the choroid rete is developed, and (2) that these

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structures are also hypothesized to be at least in part responsible for a similar heat concentrating phenomenon--provided the motivating force of this study to determine whether an analogous situation existed in the eye of a freshwater teleost, the rainbow trout (Salmo gairdneri).

Fairbanks, Hoffert and Fromm (1969) have established that the choroid rete of the rainbow trout is capable of concentrating oxygen pressures up to 10-20 times that of arterial blood. This compares very favorably with the best ocular oxygen exchange systems of the marine teleosts (Wittenberg and Wittenberg, 1974). If one accepts the principle that the development of the choroid rete parallels its capacity to concentrate oxygen, one could conclude that the rainbow trout must possess a highly evolved counter current exchange system in its choroid rete mirabile. Indeed, Wittenberg and Wittenberg (1974) have classified the choroid rete of the rainbow trout as "large" in comparison to other marine and freshwater teleosts. Thus, if the choroid rete of the rainbow trout has been shown to exhibit a highly efficient exchange system for oxygen tensions, due to its advanced structural evolution, this retial system might also then display counter current heat exchange properties, manifested in the form of warmer than ambient eye temperatures.

The ability of the choroid rete to demonstrate heat concentration is of course dependent upon sufficient heat

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production by the retina. Baeyens, Hoffert and Fromm (1973) have published data that suggest that the rate of retinal metabolism is among the highest of all organs in the animal. More recently, Hoffert, Eldred and Fromm (1974) have measured retinal metabolism using direct calorimetric procedures and found that the average metabolic activity of isolated trout retinas was 3.334 cal hr^{-1} $retina^{-1}$. Both reports support the notion that the retina of the rainbow trout, may produce sufficient metabolic heat to result in significantly warmer than ambient eye temperatures if a counter current heat exchange system exists.

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RESEARCH RATIONALE

The objective of this study is to determine if the choroid rete mirabile of the rainbow trout, a freshwater teleost, functions as a counter current heat exchanger. Evaluation will be based upon the results obtained from the following procedures:

1. Direct, *in vivo*, eye temperature measurements made at the surface of the retina.
2. Comparison of the values for thermal conductance through the eye for the intact animal with that of an animal in which circulation through the choroid rete has been removed by bilateral pseudobranchectomy.

The rationale for these procedures is that if a counter current heat exchange does exist in the rainbow trout choroid rete the eye should exhibit a "trapping" of the metabolic heat produced by the retina. Thus, the greatest manifestation of this process would occur on the retinal surface. It seems appropriate to conclude that directly measuring temperature on the retinal surface would then provide quantitative determination of the heat concentrated by the counter current exchange system.

Similarly, if heat is actually concentrated in the eye, a thermal gradient dependent upon the difference between retinal and ambient temperatures, should be established. Ocular temperature gradients in animals with intact choroid retia should subsequently differ from those in which retial function has been removed by bilateral pseudobranchectomy, thus providing an additional method for determining if the choroid rete mirabile is acting to exchange heat.

MATERIALS AND METHODS

Transducer Selection

A temperature study such as this initially involves a choice between two thermoelectric transducers: those showing a resistance change (thermistors) and those producing a voltage (thermocouples). Each type has characteristic qualities that differentiates its use and applications in biological investigations. This study required that *in vivo* temperature measurements be made in the lightly sedated animal which was for the most part restrained but nevertheless capable of head and body movement. Initial priority was therefore given to an inherently rugged transducer. Thermistors exhibit this intrinsic quality. Further, unlike thermocouples, thermistors do not deteriorate but actually seem to improve with age and use (Karselis, 1973).

Secondly, a rapidly responding transducer was needed to accurately monitor the quickly changing water-bath and eye temperatures, so as to be able to discriminate between possible minute temperature differences critical to this study. Thermistors and thermocouples both possess fast response times and repeatability as assets but sensitivity is another matter. Over their respective temperature ranges thermocouples exhibit a voltage output increase of about 10 or 15 to 1 while thermoresistive transducers manifest a resistance change of 10 million to 1 (The 1973 Omega Temperature Measurement Handbook, Omega Engineering, Inc., Stamford, CT). It should be noted that thermocouples display excellent linearity over their temperature range and are the recommended transducer for most broad temperature range studies. Contrarily, thermistors do not show the same linearity but this lack of linearity was of no consequence for the temperature ranges (less than 10 degrees centigrade) used in this study. Thus, the great changes of resistance and voltage output per degree centigrade (i.e., system signal output) associated with thermoresistive transducers increased the ability of one to discern the otherwise insensible differences of temperature which were of paramount importance in this investigation.

Finally, the author originally intended to devise a temperature system that would possess portability (e.g., field studies). Therefore, a direct measurement of absolute temperature and lack of complex circuitry was essential. Thermistors provide a direct readout of absolute temperature without the necessary reference or cold junction compensation needed of thermocouples. Also, due to their high sensitivity, thermistors usually require less amplifier gain than thermocouples and thus the errors of amplifier instability are greatly reduced. In fact, thermistor variations

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with repeated readings are generally smaller than the overall accuracy of the measuring circuit.

In conclusion, thermoresistive transducer control systems are inherently sensitive, stable, fast acting, require simple circuitry, and as such provide one of the simplest and most versatile components available to electronic designers. These unique characteristics allowed for direct application to the many sensing, measurement, and control problems of this study that would have otherwise required elaborate and complex circuitry.

Construction of the Thermoresistive Transducers

Silicon thermistor beads, number 32A7, were purchased from Victory Engineering Corporation (Springfield, NJ). Specifications are listed in Appendix 1. The bead leads were spliced to the instrument leads, in this case 50 cm of 40 gauge enameled wire, after having carefully immobilized both sets of wire so as not to subject either set to strong pulls. Enamel was stripped off the ends of the 40 gauge wire, and as was the case with the thermistor bead leads, coated with flux. The actual splices were made by running a fine soldering tip bearing a drop of solder across the junctions of the respective wires. These junctions and the entire bead were subsequently insulated with a thin layer of "Insul-x", (Insul-x Products Corp., Yonkers, NY) and

left to dry 24 hours.

Next, the wires were pulled through approximately 20 cm of PE90 tubing with the thermistor bead being epoxied into the end of the tubing. The epoxy glue was allowed to set 24 hours.

Ultimately, the 40 gauge wire was soldered to 40 cm of stranded-braided cable utilizing the same procedure as above. This continuity of cable was fed into approximately 14 inches of PE350 tubing. Epoxy sealant was then used to attach the PE90 and 350 tubings as well as transfix the braided cable to the end of the PE350 tubing after slack had been provided to protect against sudden pulls. The braided cable was soldered to an eight contact socket and formation of a thermoresistive transducer was completed. Figure 4 diagrams this process.

Construction of a Bridge Circuit

The thermoresistive transducer may be used to measure temperature by placing the device in one leg of a Wheatstone bridge circuit. Figure 5 shows a modification of the commonly used Wheatstone bridge circuit (Heath, Adams, 1969) that was utilized in this study. This instrument allowed for separate calibration of six individual thermistor channels. Its main advantage was that this entire bridge circuit was contained in a metal sloping-panel cabinet

Figure 4. Schematic drawing detailing construction of the thermoresistive probes used in this study to directly measure eye temperatures and thermal conductance values. Specifications are contained in Appendix 1.

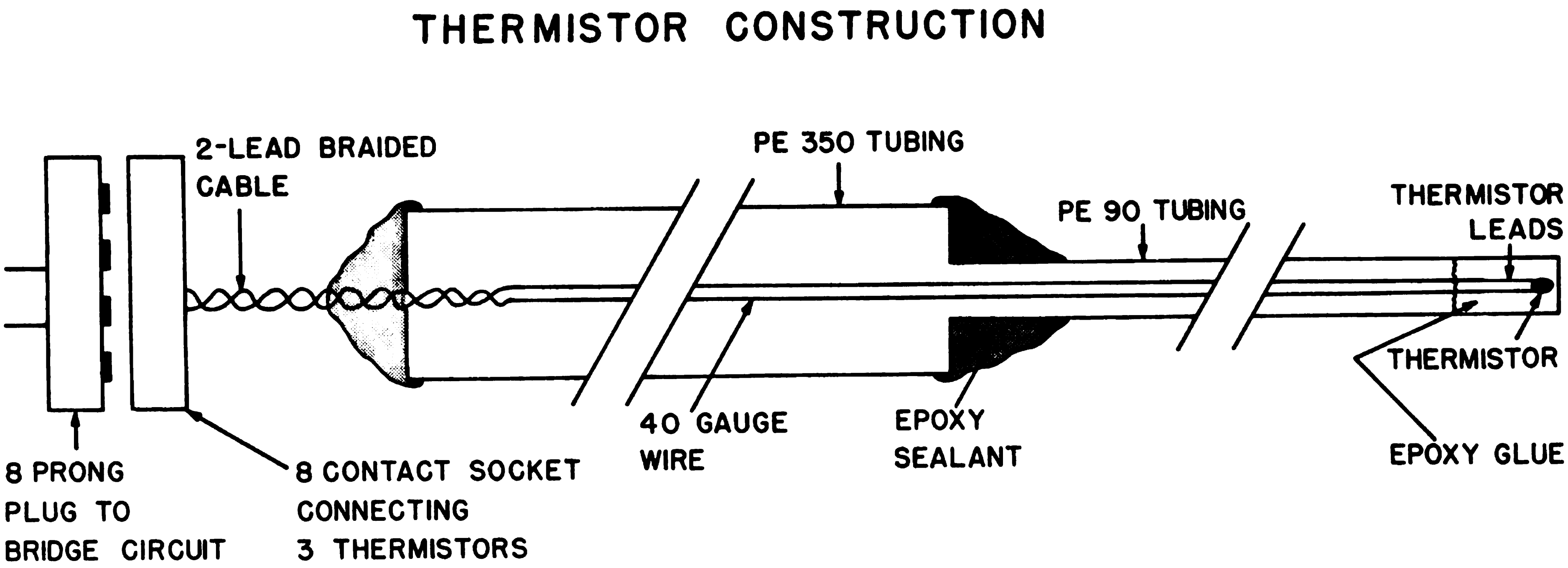
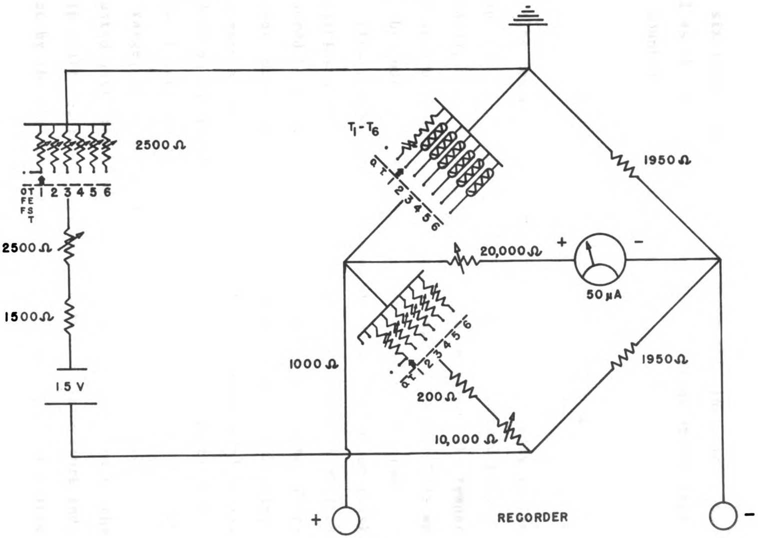

THERMISTOR CONSTRUCTION
2-LEAD BRAIDED CABLE
PE 350 TUBING
PE 90 TUBING
THERMISTOR LEADS
THERMISTOR
EPOXY GLUE
EPOXY SEALANT
40 GAUGE WIRE
8 PRONG PLUG TO BRIDGE CIRCUIT
8 CONTACT SOCKET CONNECTING 3 THERMISTORS

Figure 4

Figure 5. Wheatstone bridge circuitry utilized for thermoresistive temperature measurements. The thermistors are represented by T_1-T_6. The six 2500 Ω and 1000 Ω potentiometers in series function as the low and high range controls respectively. A 20,000 Ω potentiometer is used to control voltage through the microammeter which had a range of 0-50 μA. Similarly, a 10,000 Ω potentiometer and 200 Ω resistor are utilized to affect voltage through the 1000 Ω potentiometers, in effect acting as a course adjustment. In the test position the 2500 Ω potentiometer and 1500 Ω resistor in series with the low range control potentiometer may be substituted for a thermistor probe. The 2500 Ω potentiometer can be adjusted to a conveniently marked point on the recorder (e.g., corresponding to a temperature reading of say 8°C). During use, to compensate for a drop of battery voltage, the recorder reading may then be returned to this point by readjusting the 2500 Ω potentiometer.



WHEATSTONE BRIDGE

Figure 5

16.5 cm x 28 cm x 18.5 cm in size. The microammeter, stock number 701-0311 (Allied Electronics Corp., Fort Worth, TX), the six 1,000 Ω and 2,500 Ω potentiometers in series, as well as the single 2,500 Ω and 1,500 Ω potentiometer were all mounted on the front panel.

Calibration of Temperature Sensing Apparatus

The above circuitry was connected to a Moseley 10 inch strip chart recorder, Model 7100B (Hewlett Packard Co., Pasadena, CA). Full scale was set to represent the temperature span of 5-15°C with an input range of 20 mV. This was done by individually pre-setting each thermistor probe.

First, each transducer was placed into a vacuum flask containing water in the lower temperature range (5-8°C) as measured by a Scientific Apparatus Fractional Degree Thermometer, Model 9294-L16 (Arthur Thomas Co., Philadelphia, PA), with a range of -1 to +50°C, a tolerance of ±1°C, and graduation intervals of 0.1°C. The water was agitated by means of aeration to insure uniform temperature. The six low range, 2,500 Ω potentiometers in series were then adjusted until the thermistor readings, as recorded by the strip chart recorder, corresponded to the temperature indicated by the thermometer. Each probe was then placed into another vacuum flask containing water in the higher temperature range (12-15°C) and likewise appropriately set

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by the high control, or 1,000 Ω potentiometers in series. The temperature sensors were then shifted back and forth from vacuum flask to vacuum flask for minor adjustments of the potentiometers until each held its calibration. This procedure was conducted on the day of every experiment. However, it was found that from day to day the probes rarely needed recalibration and if at all it amounted to never more than a few tenths of a degree centigrade. In addition, the probes were checked for calibration after each experiment by placing all thermistors used that day in a common vacuum flask. All of these calibrations were performed in the incubator arrangement described below. Furthermore, it should be noted that before any of the thermoresistive transducers were employed the linearity of the circuitry was validated by placing the probes into a Haake Constant Temperature Circulator, Model FK (Haake Instruments, Inc., Rochelle Park, NJ), that was gradually heated and used to check several temperature points between 5 and 15°C. Specifications such as time constant, dissipation constant, bead size, tubing size, etc. of the thermoresistive probes are contained in Appendix 1.

Thermal Conductance Chamber

Calibration of the thermoresistive transducers and determinations of the heating and cooling rates for the different experimental preparations were made in a Fisher

Low Temperature Incubator, Model 82 (Fisher Scientific Co., Springfield, NJ). The temperature maintained within the incubator was 5°C (±0.5°C). The chamber was modified by placing a quarter inch thick sheet of clear acrylic plastic just inside the door. The plastic sheet covered the upper two-thirds of the incubator and contained two small hinged doors with plastic glove rings (fitted with sleeves) at the bottom. This allowed one to work within the chamber while at the same time minimizing possible temperature fluctuations.

The bottom one-third of the chamber held two large plastic pails each capable of holding approximately 12.5 liters of water. To insure uniform temperature both pails were mixed continuously by vigorous aeration. Two inch thick styrofoam insulation completely surrounded the "hot" pail. The top of the "cold" pail was left exposed. In addition, 15 cm of styrofoam was placed between the pails. The pail designated to hold "hot" water was kept at a temperature of approximately 13.0 ± 0.8°C by means of a 250 watt blade heater connected to a Cole-Parmer Temperature Regulator, Model 63 (Cole-Parmer Instrument Co., Chicago, IL). The other pail was designed to reflect the temperature of the interior of the chamber (5.0°C).

Two shelves were placed upon the metal incubator shelf level with the bottom of the plastic front. Upon one shelf was positioned two Cole-Parmer Oscillating Pumps, Model 7103-10, used to pump the respective pail contents.

The pumps' capacities were approximately 2,500 ml/min. The second shelf held the fish chamber made of 1/4 inch clear acrylic plastic and capable of holding approximately 800 ml of water. This shelf also held the eight prong plug to the Wheatstone Bridge Circuit located outside of the chamber. Figure 6 schematically represents the chamber arrangement.

Experimental Animals

Rainbow trout (*Salmo gairdneri*) 25-30 cm in length and weighing 150-200 g were obtained from Midwest Fish Farming Enterprises, Inc., Harrison, Michigan. The animals were transported to the Michigan State University, East Lansing, Michigan, in galvanized metal tanks lined with non-toxic paint and fitted with an agitator for aeration. At Michigan State University the fish were held in fiberglass tanks at 12±1.0°C, with a continuous flow of aerated water which was treated to remove chlorine and iron. The animals were exposed to light-dark periods of 16 and 8 hours, respectively.

Direct Eye Temperature Measurements

Direct eye temperature measurements required maximum temperature consistency and were therefore conducted in a constant temperature cold box. This cold box is also the site where the fish were kept just prior to experimentation. The basic characteristics of the compartment resemble those

Figure 6. Incubator arrangement that served as thermal conductance chamber. Refer to the text for details.

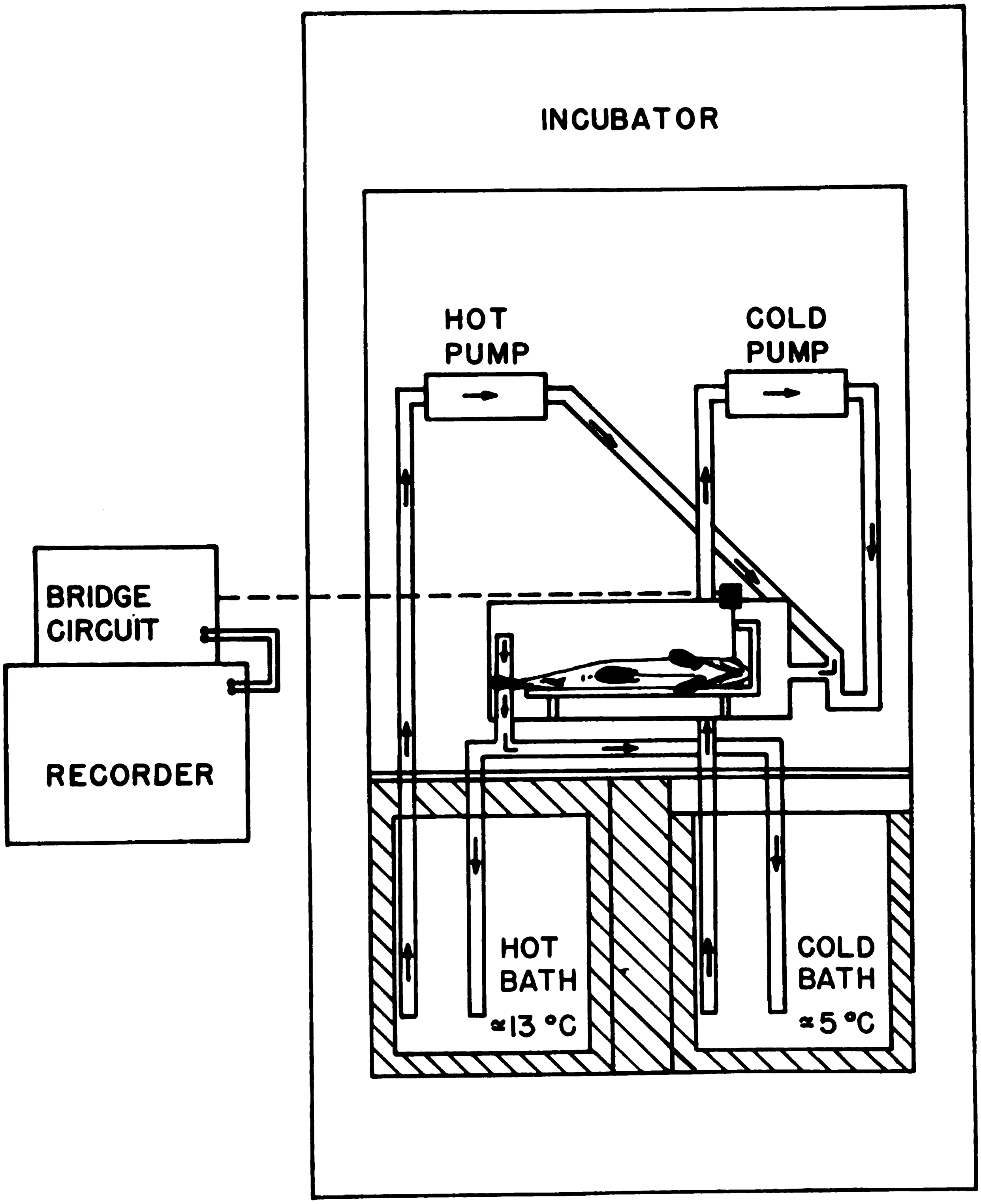
INCUBATOR
HOT PUMP
COLD PUMP
BRIDGE CIRCUIT
RECORDER
HOT BATH
≈13 °C
COLD BATH
≈5 °C

Figure 6

for the holding facilities, as described previously.

In this portion of the study the fish were individually removed from their holding tanks and placed into another similar tank of close proximity with aerated water containing MS-222, Tricaine Methanesulfonate (Ayerst Laboratories, New York, NY). When the animal appeared to reach stage 3 of anesthesia (Jolly, Mawdesley-Thomas, and Buche, 1972) it was brought to just below the surface of the water and secured by means of a firm grip with a damp cloth. At this point a 16 gauge stainless steel hypodermic needle was used to make a small incision in the cornea with a portion of the beveled edge. Then, in a procedure analogous to the one utilized by Linthicum and Carey (1972) to measure heat concentration in the tuna eye, a thermoresistive transducer was immediately inserted by hand into this opening and placed against the back of the eye presumably on the surface of the retina, as determined by resistance to further movement. This temperature was recorded and the procedure repeated for the other eye. A second thermistor, which had been placed into the tank prior to experimentation and secured at an approximate depth of 12 cm was used to record the water temperature for each corresponding animal. Eye and ambient temperature measurements were all recorded on the strip chart recorder at a paper speed 2 in/sec. The time for completion of the entire process, including removing the animal from its holding tank to the tank containing

MS-222, and measurement of the temperature in both eyes was approximately one minute. Mean ambient and eye temperatures were computed and the water temperature of the original holding tank was measured.

Thermal Conductance

For the determination of thermal conductance the fish was again sedated by placement into a pail of aerated water containing MS-222. When the animal appeared to reach stage 4 or 5 of anesthesia (Jolly et al., 1972) it was removed. The fish was then restrained sideways on an acrylic plastic bench by means of wires positioned across the animal. Caution was exercised with these restraints so as not to interfere with opercular movement. The head was immobilized so as to minimize the effects of violent movements. This was accomplished by placing a stainless steel 16 gauge hypodermic needle through the nasal passages which were positioned between two rubber stoppers. The cornea of the animal's eye was then pierced by a portion of the beveled edge of a 16 gauge needle. The thermoresistive transducer which had been attached to an acrylic post perpendicular to the bench was subsequently lowered into the eye and secured at the back of the eye on the surface of the retina. A second thermoresistive transducer was also attached to the acrylic post to measure water temperature. The bench, containing fish and respective temperature sensors, was then lowered into the 800 ml acrylic fish chamber contained within the incubator (Figure 6). The time for this procedure approximated 1-2 minutes.

Simultaneous with the entry of the fish into the holding chamber was initiation of the "hot" pump. When the eye temperature at the surface of the retina and the water temperature appeared to reach steady state, defined as an indistinguishable temperature difference (i.e., less than 0.01°C), and the fish appeared sufficiently recovered from the effects of the anesthesia (2-5 minutes), as determined by opercular movement, the "hot" pump was turned off and followed by immediate engagement of the "cold" pump and appropriate valve adjustments of the system (Figure 6). By means of switching periodically between the recordings of the two temperature transducers, the rates of cooling of both the water bath and retinal surface temperature were carefully monitored. When the two temperatures again seemed to approximate a steady state, the process was reversed and the heating rates recorded. The time for both processes approximated eight minutes.

Pseudobranchectomized Animals

The procedure of pseudobranchectomy consisted of bilaterally cauterizing the pseudobranch of the anesthetized fish and replacement of the animal to its holding tank for a period of 2-5 days. The animal was then subjected to the same procedure enumerated above for the intact animal. Evidence that the process of cauterization was successful was determined by the change in pigmentation of the fish (darker) and by the fact that the animals were more easily netted apparently due to a lack of awareness of any object in the animals' visual field.

Calculation of Thermal Conductance

Newton's law of cooling, which states that the change in heat of a body per unit time is proportional to the difference between its temperature and the ambient temperature, was used to compute the values for thermal conductance through the eye of the various experimental preparations described above. Newton's law may be mathematically stated as:

$$\frac{-dt}{dt} = \frac{C}{K} (T_B - T_A)$$

where C, thermal conductance, specifies the rate of heat change per degree centigrade difference between the body (or more specifically in this situation the eye) and ambient temperatures (Fry, 1967). Thermal conductance, C, is uncorrected for metabolism, but still may be used as an expression of thermal conductance (Bartholomew and Tucker, 1963).

Further, when log $(T_B - T_A)$ is plotted against time the value of C is given by the slope of the resulting curve multiplied by K/log e (Bartholomew and Tucker, 1963).

The specific heat of the tissue, K, in this case vitreous humor was assigned a value of 1.0 cal/gm. This was due to the fact that its composition is mostly water. A K value of 0.82 cal/gm is used by Bartholomew and Tucker (1963) and others in describing most other body tissues which apparently contain more solute. Therefore, log $(T_B - T_A)$ was plotted against time on one graph for all animals receiving a particular type of treatment (e.g., pseudobranchectomized-heating) and utilizing the Hewlett Packard-65 computer program, Exponential Curve Fit, a straight line was fitted through the points. This program computes the least squares fit of n pairs of data points $[(x_i, y_i), i = 1,2,...n]$ converting the exponential function of the form

$$y = ae^{bx}$$

into the linearized statement of the equation; $\ln y = \ln a + bx$. The only stipulation of the program is that y_i be greater than zero. Thus, the actual graphs show a plot of the absolute value of log $(T_B - T_A)$ against time, recorded in minutes.

Statistical Analysis

The Hewlett Packard-65 computer program, Paired t Statistic, was used in the Direct Eye Temperature measurements to determine if the mean eye temperature differed significantly from the corresponding mean ambient temperature ($P = 0.05$).

Comparison for a significant difference between thermal conductance values was performed by comparing the slopes of the respective regression lines. The operation of computing the thermal conductance values is simply a process of multiplying the slope of each thermal exchange rate curve by a constant, K/log e. Since the Hewlett Packard program, Exponential Curve Fit, converts to natural logarithms and it is assumed K equals 1.0 cal/gm, statistical comparison of the slopes of any two regression lines describing a thermal exchange would in effect also be the test for significance between thermal conductance values. The formula used to compare the slopes of two regression lines is given in Appendix 2.

RESULTS

Direct Eye Temperature Measurements

The data, contained in Table 1, documents that the paired observations, of eye to ambient temperature, are not significantly different from one another. In fact, the mean temperatures were found to be identical, i.e., 12.87°C.

A breakdown of the paired observations reveal 13 instances in which the eye temperature was higher than ambient (never more than 0.12°C), eight observations of equal eye and ambient temperatures, and nine cases where the eye exhibited a lower than ambient temperature (never greater than 0.17°C). The plausable explanation for this latter finding might be that the fish originally came from a holding tank with a water temperature lower than the tank in which the measurements were made. In the single measurement made of the original holding facility the water temperature was found to be 11.35°C.

Nonetheless, it is important to note that there was not one circumstance in all of the 30 eye measurements performed in which the eye exhibited temperatures 4-6°C above ambient as depicted in some predatory marine teleosts (linthicum and Carey, 1972) and as being indicative of the presence of

Table 1. Paired observations of ambient to eye temperatures of rainbow trout as measured underwater.

n	(T_B) eye temperature °C	(T_A) ambient temperature °C	T_B-T_A
1	12.95	12.95	0.00
2	12.95	12.95	0.00
3	12.85	12.80	+0.05
4	12.92	12.80	+0.07
5	12.91	12.91	0.00
6	12.95	12.91	+0.04
7	12.85	12.92	-0.07
8	12.88	12.92	-0.04
9	12.91	12.91	0.00
10	12.94	12.91	+0.03
11	12.95	12.91	+0.04
12	12.91	12.91	0.00
13	12.91	12.91	0.00
14	12.80	12.88	-0.08
15	12.83	12.88	-0.05
16	12.91	12.89	+0.02
17	12.92	12.89	+0.03
18	12.90	12.88	+0.02
19	12.90	12.88	+0.02
20	12.87	12.82	+0.05
21	12.87	12.82	+0.05
22	12.88	12.86	+0.02
23	12.89	12.86	+0.03
24	12.65	12.82	-0.17
25	12.72	12.82	-0.10
26	12.79	12.84	-0.05
27	12.82	12.85	-0.03
28	12.84	12.85	-0.01
29	12.81	12.81	0.00
30	12.81	12.81	0.00
$\bar{X}$	12.87±0.013(30)*	12.87±0.011(30)*	+0.003±0.00056 (30)*

*mean ± S.E.(n)

a functional counter current heat exchanger. Inconsequential bleeding of the iris did occur infrequently upon incision of the cornea.

Thermal Conductance

The results (Tables 2 through 7, Figures 7-12) demonstrate that complete blockage of circulation through the choroid rete mirabile by bilateral pseudobranchectomy had no effect upon the thermal exchange rates (cooling or heating) as reflected in the thermal conductance values.

As observed in the Direct Eye Temperature Measurements inconsequential bleeding of the iris did occur infrequently upon incision of the cornea. Also, heating of the "cold" water bath by the pump was noted during the cooling experiment. However, this generally averaged 0.2°C during an approximately 8°C (13-5°C) temperature change and as such was deemed insignificant to interpretation of the results. This problem was not encountered for the heating portion of the experiment because of thermostatic regulation of the "hot" water bath.

Table 2. Cooling curve points of nine intact animals as measured in a thermal conductance chamber for a temperature change of approximately 13-5°C.

x_i^1	y_i^2	x_i	y_i	x_i	y_i
1.0	4.25	1.0	3.35	8.0	0.15
2.0	2.80	2.0	2.15	9.0	0.10
3.0	1.44	3.0	1.11		
4.0	0.83	4.0	0.57	1.0	4.95
5.0	0.45	5.0	0.32	2.0	5.65
6.0	0.23	6.0	0.20	3.0	3.25
7.0	0.10	7.0	0.10	4.0	1.85
		8.0	0.07	5.0	1.05
1.0	5.70			6.0	0.60
2.0	4.85	1.0	4.37	7.0	0.30
3.0	1.20	2.0	2.69	8.0	0.25
4.0	0.60	3.0	1.53	9.0	0.15
5.0	0.35	4.0	0.88	10.0	0.10
6.0	0.20	5.0	0.51	11.0	0.10
7.0	0.10	6.0	0.30	12.0	0.05
8.0	0.05	7.0	0.18		
		8.0	0.12		
1.0	5.30	9.0	0.07		
2.0	3.30				
3.0	1.18	1.0	4.98		
4.0	0.83	2.0	2.69		
5.0	0.43	3.0	1.25		
6.0	0.24	4.0	0.58		
7.0	0.13	5.0	0.25		
8.0	0.06	6.0	0.10		
1.0	4.93	1.0	4.00		
2.0	2.75	2.0	2.20		
3.0	1.32	3.0	1.30		
4.0	0.69	4.0	0.80		
5.0	0.26	5.0	0.50		
6.0	0.12	6.0	0.35		
7.0	0.07	7.0	0.20		

[1] time in minutes

[2] absolute value of (T_B-T_A), where T_B and T_A represent eye and ambient temperature respectively in °C.

$n = 74$

$a = 6.269$

$b = -0.500$

$r^2 = 0.875$

$\Sigma x_i = 353.000$

$\Sigma x_i^2 = 2203.00$

$\Sigma \ln y_i = -40.762$

$\Sigma (\ln y_i)^2 = 170.864$

$\Sigma \ln y_i \cdot x_i = -454.146$

$S_{yx} = 0.508$

$t_{1/2} = 1.38$ min

Figure 7. Rate of a temperature change (13-5°C), as related to the absolute value of the difference between eye temperature, T_B, and ambient temperature, T_A, in nine rainbow trout. Choroid retial circulation has been left intact. The process was begun at a steady state ($T_B=T_A$) and as such the Y-intercept has no meaning but was plotted as determined by the magnitude of the slope. (T_B-T_A) values less than 0.1 have not been plotted but were utilized in computation of the regression statistics. Refer to Table 2 for statistical data.

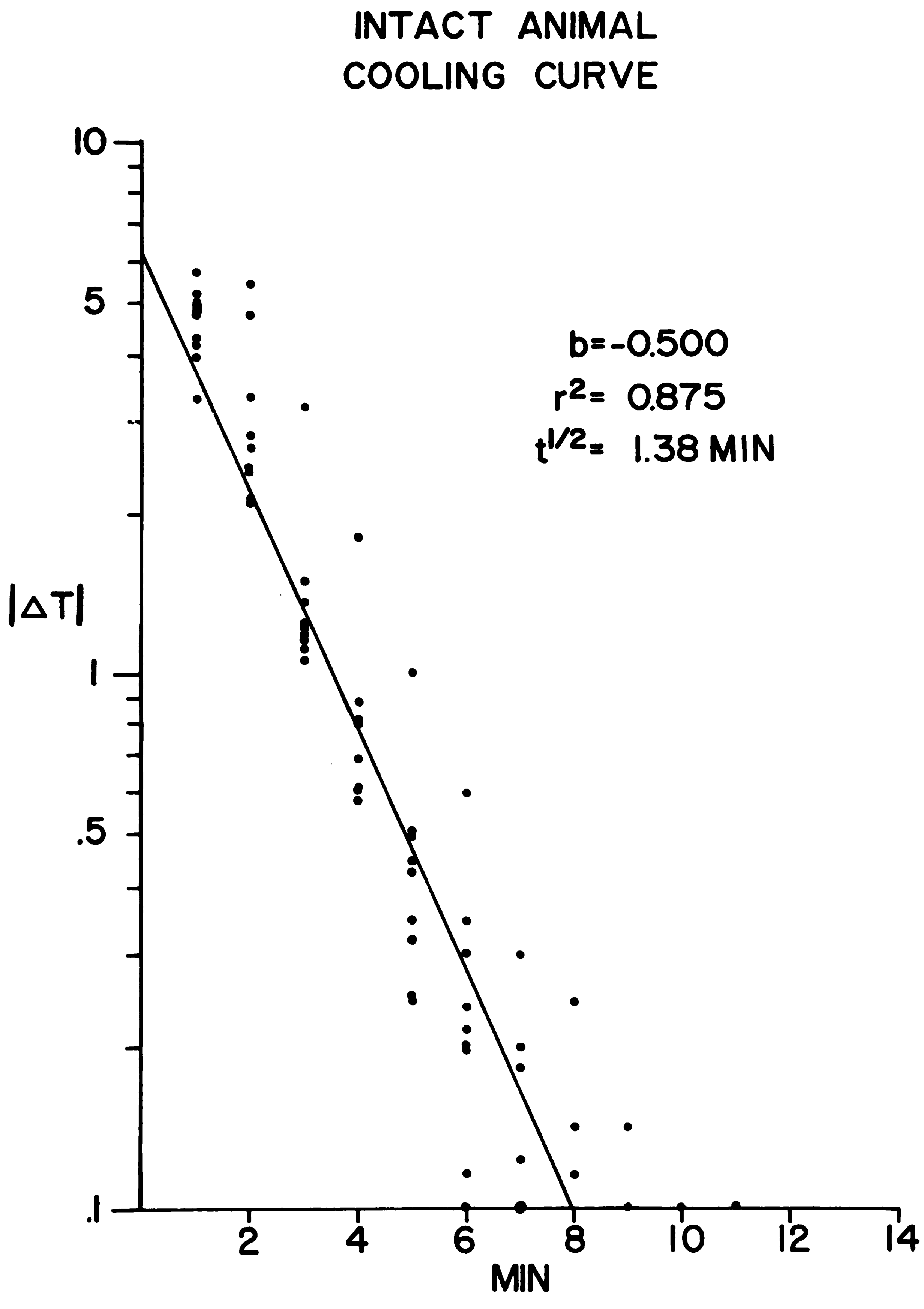
INTACT ANIMAL
COOLING CURVE
10
5
1
.5
.1
|ΔT|
b=-0.500
r2= 0.875
t1/2= 1.38 MIN
2
4
6
8
10
12
14
MIN

Figure 7

Table 3. Cooling curve points of nine bilaterally pseudobranchectomized animals as measured in a thermal conductance chamber for a temperature change of approximately 13-5°C.

x_i[1]	y_i[2]	x_i	y_i	x_i	y_i
1.0	5.79	1.0	6.00	1.0	5.88
2.0	2.99	2.0	4.38	2.0	3.83
3.0	1.88	3.0	2.77	3.0	2.23
4.0	0.97	4.0	1.68	4.0	1.35
5.0	0.56	5.0	0.96	5.0	0.84
6.0	0.30	6.0	0.55	6.0	0.51
7.0	0.19	7.0	0.30	7.0	0.31
8.0	0.11	8.0	0.15	8.0	0.21
9.0	0.07	9.0	0.07	9.0	0.14
				10.0	0.09
1.0	6.35	1.0	4.40		
2.0	5.22	2.0	3.05	1.0	6.71
3.0	3.35	3.0	1.97	2.0	6.05
4.0	2.08	4.0	1.28	3.0	4.20
5.0	1.17	5.0	0.72	4.0	2.78
6.0	0.66	6.0	0.43	5.0	1.87
7.0	0.33	7.0	0.27	6.0	1.26
8.0	0.17	8.0	0.17	7.0	0.85
9.0	0.08	9.0	0.12	8.0	0.59
10.0	0.02	10.0	0.08	9.0	0.42
				10.0	0.29
1.0	6.13	1.0	4.85	11.0	0.22
2.0	4.48	2.0	4.36	12.0	0.15
3.0	2.98	3.0	2.92		
4.0	2.00	4.0	1.79	1.0	5.00
5.0	1.29	5.0	1.09	2.0	3.50
6.0	0.83	6.0	0.65	3.0	2.18
7.0	0.64	7.0	0.39	4.0	1.30
8.0	0.35	8.0	0.25	5.0	0.76
9.0	0.21	9.0	0.16	6.0	0.45
10.0	0.14	10.0	0.10	7.0	0.25
11.0	0.07	11.0	0.07	8.0	0.13
				9.0	0.08

[1] time in minutes

[2] absolute value of (T_B-T_A), where T_B and T_A represent eye and ambient temperature respectively in °C.

$n = 91$

$a = 9.372$

$b_2 = -0.457$

$r = 0.897$

$\Sigma x_i = 510.000$

$\Sigma x_i^2 = 3672.00$

$\Sigma \ln y_i = -29.289$

$\Sigma (\ln y_i)^2 = 198.655$

$\Sigma \ln y_i \cdot x_i = -535.792$

$Syx = 0.466$

$t_{½} = 1.52$ min

Figure 8. Rate of a temperature change (13-5°C), as related to the absolute value of the difference between eye temperature, T_B, and ambient temperature, T_A, in nine rainbow trout. Choroidal retial circulation has been eliminated by bilateral pseudobranchectomy. The process was begun at a steady state ($T_B = T_A$) and as such the Y-intercept has no meaning but was plotted as determined by the magnitude of the slope. ($T_B - T_A$) values less than 0.1 have not been plotted but were utilized in computation of the regression statistics. Refer to Table 3 for statistical data.

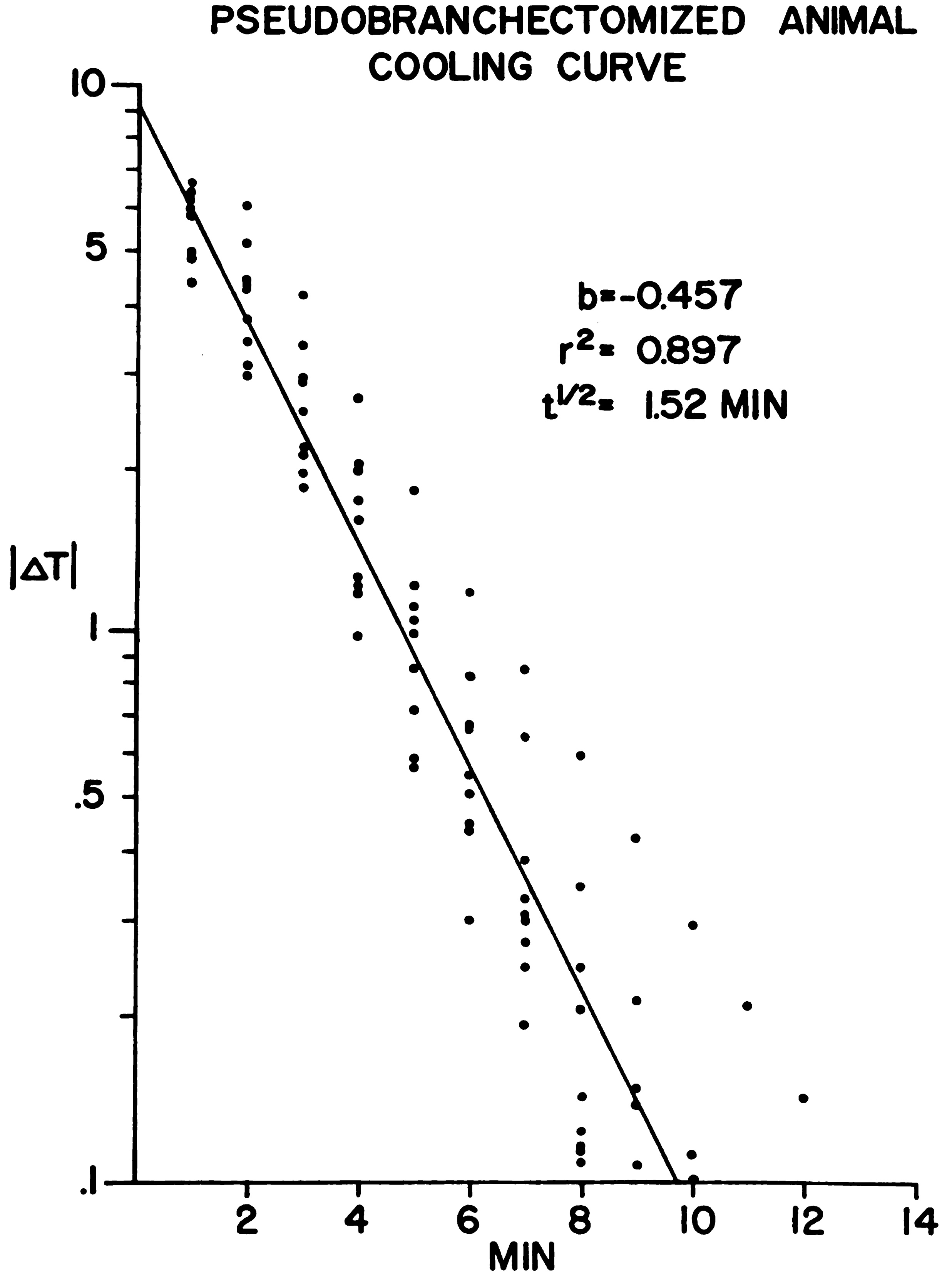
PSEUDOBRANCHECTOMIZED ANIMAL COOLING CURVE
b=-0.457
r^2= 0.897
$t^{1/2}$= 1.52 MIN
|ΔT|
10
5
1
.5
.1
2
4
6
8
10
12
14
MIN

Figure 8

Figure 9. Comparison of the rates of a temperature change (13-5°C), as related to the absolute value of the difference between eye temperature, T_B, and ambient temperature, T_A, in rainbow trout. Choroid retial circulation has been left intact and conversely removed by bilateral pseudobranchectomy. The process was begun at a steady state ($T_B = T_A$) and as such the Y-intercepts have no meaning but were plotted as determined by the magnitude of the slopes. The slopes are not significantly different.

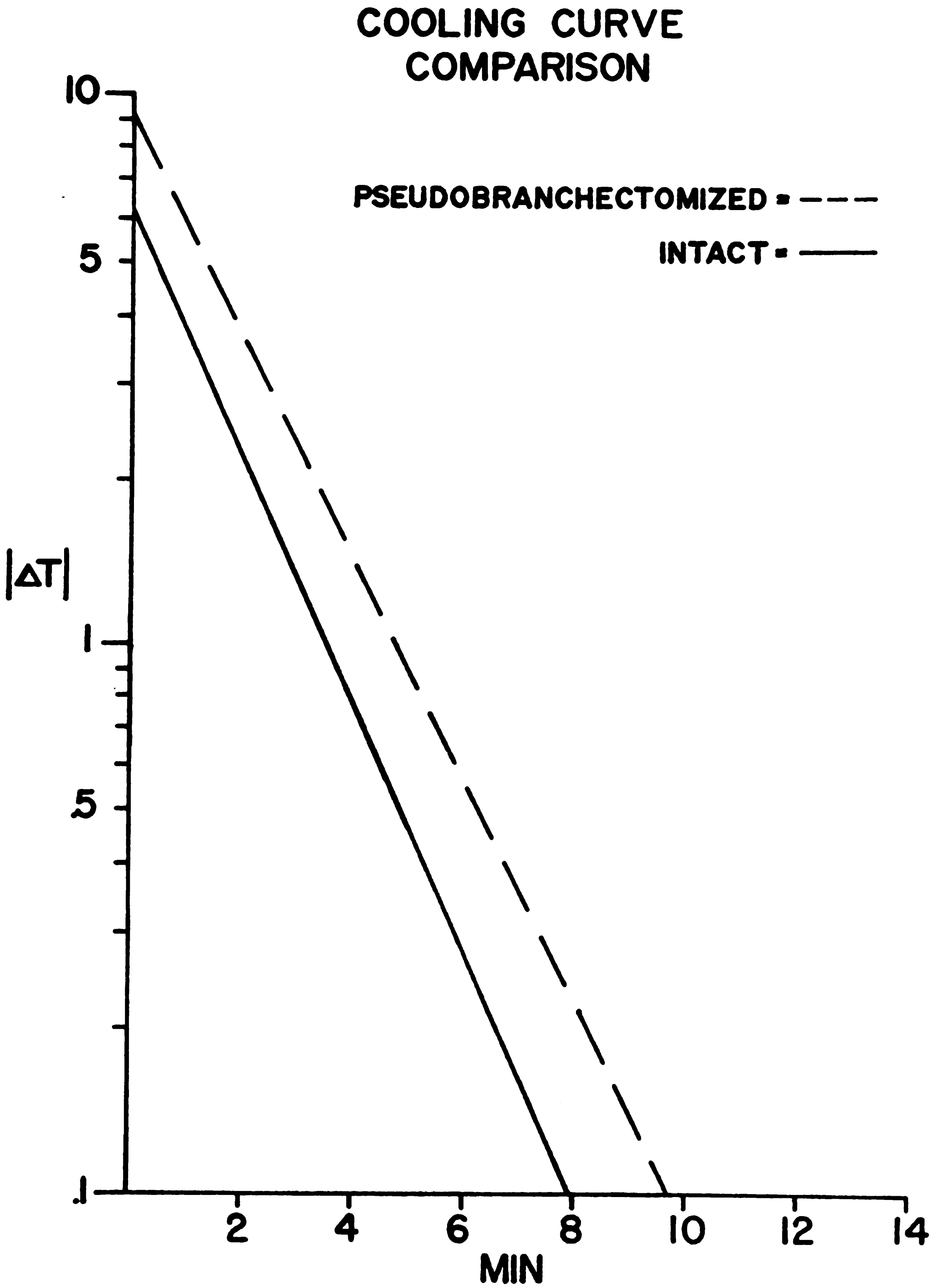
COOLING CURVE
COMPARISON
PSEUDOBRANCHECTOMIZED = - - -
INTACT = ———
|ΔT|
10
5
1
.5
.1
2
4
6
8
10
12
14
MIN

Figure 9

Table 4. Heating curve points of nine intact animals as measured in a thermal conductance chamber for a temperature change of approximately 5-13°C.

x_i[1]	y_i[2]	x_i	y_i	x_i	y_i
1.0	2.59	1.0	4.42	1.0	5.70
2.0	1.30	2.0	3.25	2.0	3.80
3.0	0.68	3.0	2.07	3.0	2.00
4.0	0.31	4.0	1.17	4.0	0.90
5.0	0.13	5.0	0.72	5.0	0.40
6.0	0.05	6.0	0.30	6.0	0.20
		7.0	0.15	7.0	0.10
1.0	3.95	8.0	0.06		
2.0	2.18				
3.0	1.03	1.0	4.25		
4.0	0.45	2.0	2.77		
5.0	0.16	3.0	1.47		
6.0	0.05	4.0	0.72		
		5.0	0.37		
1.0	4.32	6.0	0.19		
2.0	2.92	7.0	0.11		
3.0	1.30	8.0	0.05		
4.0	0.65	9.0	0.03		
5.0	0.25				
6.0	0.08	1.0	3.50		
		2.0	5.80		
1.0	4.08	3.0	4.30		
2.0	2.45	4.0	2.80		
3.0	1.35	5.0	1.55		
4.0	0.67	6.0	0.80		
		7.0	0.35		
1.0	6.20	8.0	0.15		
2.0	2.80	9.0	0.05		
3.0	1.40				
4.0	0.65				
5.0	0.25				
6.0	0.15				
7.0	0.05				

[1] time in minutes

[2] absolute value of (T_B-T_A), where T_B and T_A represent eye and ambient temperature respectively in °C.

$n = 62$
$a = 8.702$
$b = -0.622$
$r^2 = 0.843$
$\Sigma x_i = 255.000$

$\Sigma x_i^2 = 1357.00$
$\Sigma \ln y_i = -24.616$
$\Sigma (\ln y_i)^2 = 151.456$
$\Sigma \ln y_i \cdot x_i = -293.128$
$Syx = 0.610$
$t½ = 1.11$ min

Figure 10. Rate of a temperature change (5-13°C), as related to the absolute value of the difference between eye temperature, T_B, and ambient temperature, T_A, in nine rainbow trout. Choroid retial circulation has been left intact. The process was begun at a steady state ($T_B=T_A$) and as such the y-intercept has no meaning but was plotted as determined by the magnitude of the slope. (T_B-T_A) values less than 0.1 have not been plotted but were utilized in computation of the regression statistics. Refer to Table 4 for statistical data.

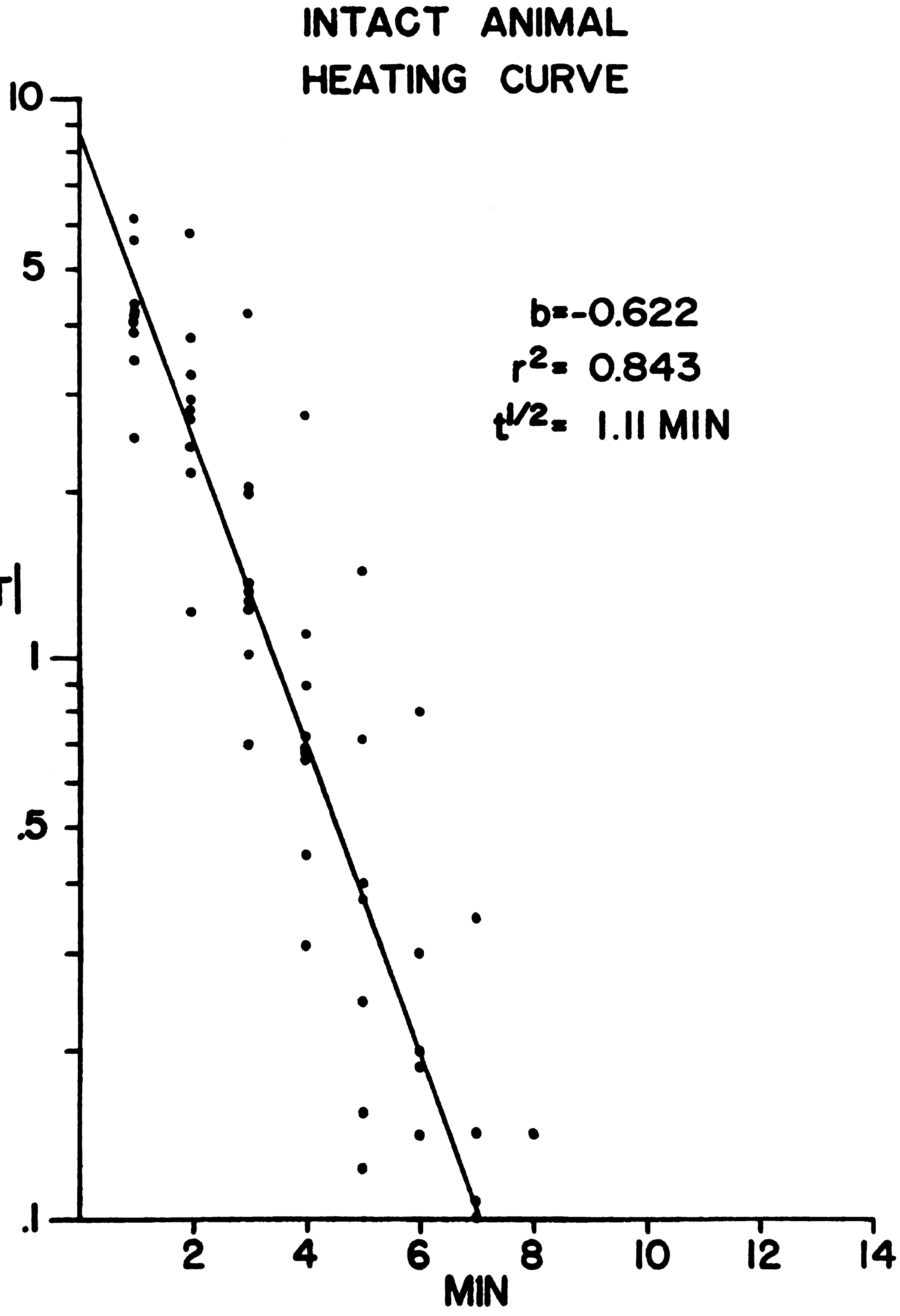
INTACT ANIMAL
HEATING CURVE
b=-0.622
r^2= 0.843
$t^{1/2}$= 1.11 MIN
|ΔT|
10
5
1
.5
.1
2
4
6
8
10
12
14
MIN

Figure 10

Table 5. Heating curve points of nine bilaterally pseudobranchectomized animals as measured in a thermal conductance chamber for a temperature change of approximately 5-13°C.

x_i[1]	y_i[2]	x_i	y_i	x_i	y_i
1.0	6.35	6.0	0.40	1.0	4.85
2.0	4.22	7.0	0.22	2.0	3.65
3.0	2.40	8.0	0.12	3.0	2.42
4.0	1.41	9.0	0.05	4.0	1.45
5.0	0.71			5.0	0.80
6.0	0.30	1.0	4.70	6.0	0.43
7.0	0.10	2.0	2.62	7.0	0.22
		3.0	1.39	8.0	0.10
1.0	4.62	4.0	0.71		
2.0	2.93	5.0	0.34	1.0	4.98
3.0	1.85	6.0	0.16	2.0	2.87
4.0	0.98	7.0	0.05	3.0	1.71
5.0	0.52	8.0	0.02	4.0	0.91
6.0	0.26			5.0	0.47
7.0	0.12	1.0	3.55	6.0	0.24
8.0	0.04	2.0	2.40	7.0	0.11
		3.0	1.37	8.0	0.04
1.0	5.07	4.0	0.73		
2.0	3.33	5.0	0.37		
3.0	2.15	6.0	0.18		
4.0	1.40	7.0	0.08		
5.0	0.83				
6.0	0.48	1.0	6.12		
7.0	0.27	2.0	4.97		
8.0	0.14	3.0	3.45		
9.0	0.05	4.0	2.34		
		5.0	1.43		
1.0	5.38	6.0	0.93		
2.0	3.77	7.0	0.54		
3.0	2.35	8.0	0.31		
4.0	1.33	9.0	0.16		
5.0	0.75	10.0	0.09		

[1] time in minutes

[2] absolute value of (T_B-T_A), where T_B and T_A represent eye and ambient temperatures respectively in °C.

$n = 74$

$a = 10.281$

$b = -0.576$

$r^2 = 0.882$

$\Sigma x_i = 345.000$

$\Sigma x_i^2 = 2051.000$

$\Sigma \ln y_i = -26.344$

$\Sigma(\ln y_i)^2 = 175.865$

$\Sigma \ln y_i \cdot x_i = 377.818$

$Sxy = 0.522$

$t_{1/2} = 1.20$ min

Figure 11. Rate of a temperature change (5-13°C), as related to the absolute value of the difference between eye temperature, T_B, and ambient temperature, T_A, in nine rainbow trout. Choroid retial circulation has been eliminated by pseudobranchectomy. The process was begun at a steady state ($T_B=T_A$) and as such the y-intercept has no meaning but was plotted as determined by the magnitude of the slope. ($T_B=T_A$) values less than 0.1 have not been plotted but were utilized in computation of the regression statistics. Refer to Table 5 for statistical data.

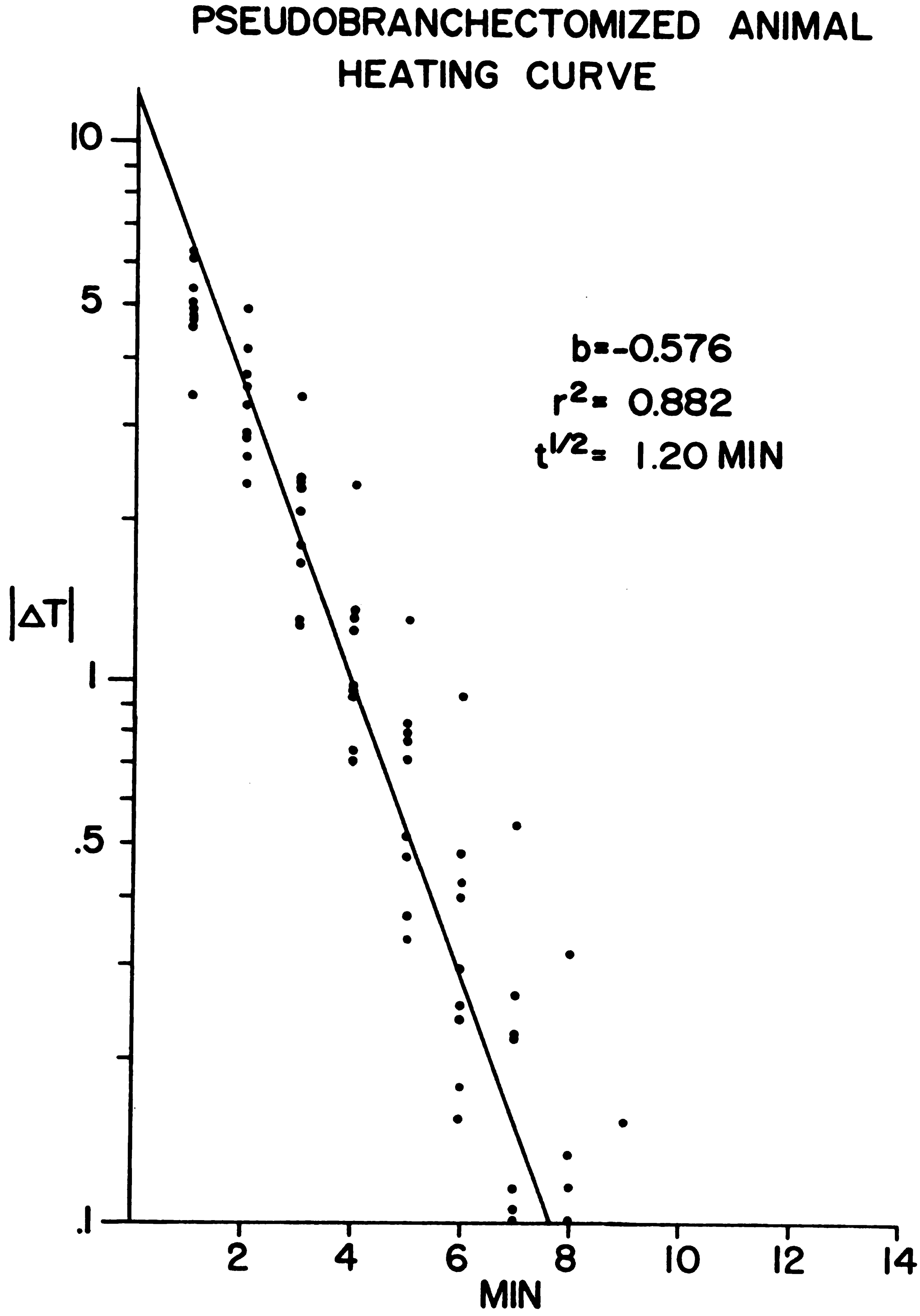
PSEUDOBRANCHECTOMIZED ANIMAL
HEATING CURVE
b=-0.576
r^2= 0.882
$t^{1/2}$= 1.20 MIN
|ΔT|
10
5
1
.5
.1
2
4
6
8
10
12
14
MIN

Figure 11

Figure 12. Comparison of the rates of a temperature change (5-13°C), as related to the absolute value of the difference between eye temperature, T_B, and ambient temperature, T_A, in rainbow trout. Choroid retial circulation has been left intact and conversely removed by bilateral pseudobranchectomy. The process was begun at a steady state ($T_B = T_A$) and as such the y-intercepts have no meaning but were plotted as determined by the magnitude of the slopes. The slopes are not significantly different.

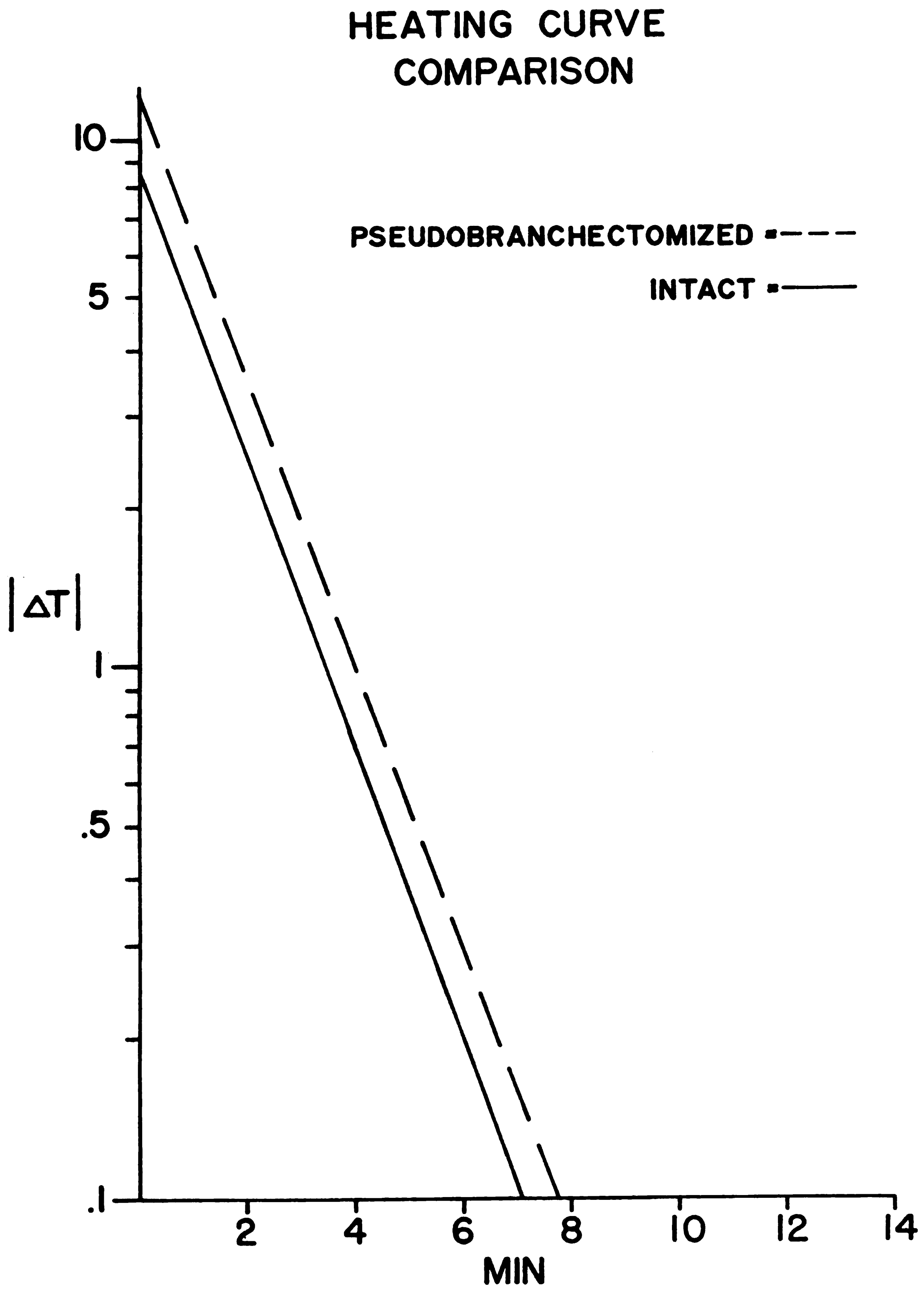
HEATING CURVE
COMPARISON
PSEUDOBRANCHECTOMIZED
INTACT
|ΔT|
10
5
1
.5
.1
2
4
6
8
10
12
14
MIN

Figure 12

Table 6. Effect of bilateral pseudobranchectomy on the rate of thermal exchange through the eye of the rainbow trout for a temperature change of approximately 13-5°C. The rates are not significantly different.

	Intact (nine animals)	Pseudobranchectomized (nine animals)
Thermal Conductance (cal gm^{-1} min^{-1} °C)	0.500±0.0002(74)*	0.457±0.0001(91)*
$t_{1/2}$ (min)	1.38	1.52

*mean ± S.E. (n)

Table 7. Effect of bilateral pseudobranchectomy on the rate of thermal exchange through the eye of the rainbow trout for a temperature change of approximately 5-13°C. The rates are not significantly different.

	Intact (nine animals)	Pseudobranchectomized (nine animals)
Thermal Conductance (cal gm^{-1} min^{-1} °C)	0.622±0.0004(62)*	0.576±0.002(74)*
$t_{1/2}$ (min)	1.11	1.20

*mean ± S.E. (n)

DISCUSSION

The results of the investigation show the mean retinal and ambient temperatures to be statistically equal. Similarly, the values of thermal conductance for experiments of heating and cooling are not significantly different between animals with intact retial function to those in which retial circulation had been eliminated by pseudobranchectomy. It can be deduced from the data compiled that there is not sufficient reason to postulate that the choroid rete mirabile of the rainbow trout is acting to concentrate heat. Therefore, the rete must not be functioning quantitatively as a counter current heat exchanger.

One explanation for the lack of positive results could be inaccurate temperature measurements. In internal measurements there is an important relationship between the size of the electrode being used and the area one wishes to evaluate. The electrode must be small enough to insure measurements are being taken of the internal milieu and not the external environment. It is believed that in the present study measurements had actually reflected retinal surface and not ambient temperature, for apparent reasons.

First, Figure 13 shows a plot of a typical thermal exchange experiment (in this case heating in the intact animal). The graph shows that eye and ambient temperatures differ by as much as six degrees centigrade and that the two temperatures do not reach a steady state for approximately eight minutes. Second, evidence of district temperature measurements can be inferred by evaluating the relationship between electrode to eye size. The diameter of the temperature measuring thermistor bead is 0.03 cm (Appendix 1). Recently, Hoffert (unpublished) has determined that the distance from the retinal surface to the cornea of the eye approximates 0.96 cm, or approximately 32 times the distance covered by the thermistor bead. If one assumes thermal conductance along the entire thermoresistive probe is insignificant because of its plastic composition, and that self heating of the probe is negligible as calculated (Appendix 1), then it is improbable that the thermoresistive probe with its minute thermistor bead is depicting anything but retinal surface temperature. Thus, in view of the above arguments, it is concluded that the apparent lack of data in support of the rainbow trout choroid rete functioning as a counter current heat exchanger was not due to inaccuracy in temperature measurements. However, when a stereotaxic instrument is developed which will secure against head and eye movement of fish it is recommended that eye temperatures be measured with the

Figure 13. Typical thermal exchange graph. This plot simultaneously shows the rates of ambient (●——●) and eye (Δ——Δ) temperature change of a rather instantaneous bath water alteration of approximately 5.5-12.5°C in the rainbow trout possessing intact choroid retial circulation.

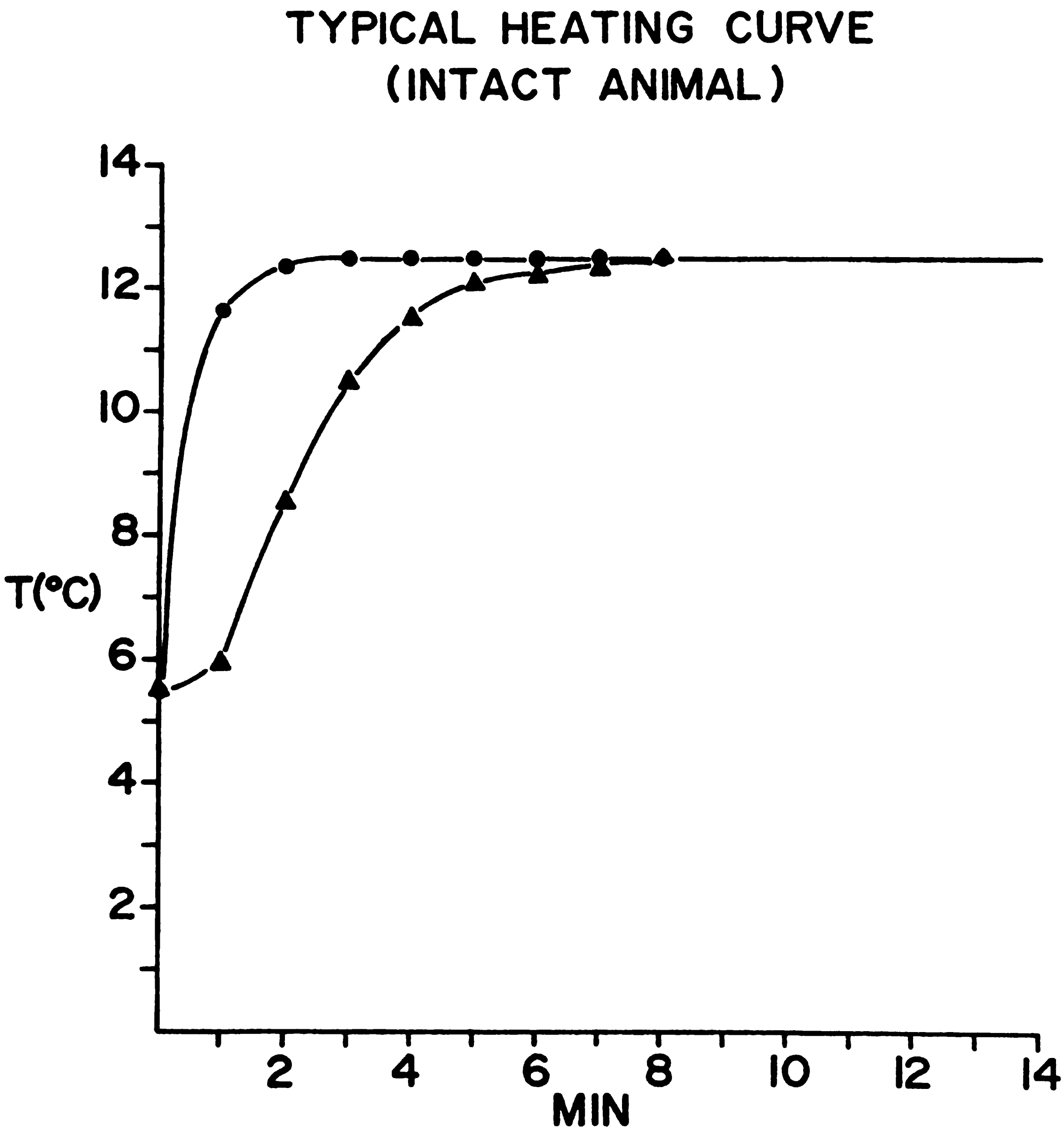
TYPICAL HEATING CURVE
(INTACT ANIMAL)
14
12
10
8
6
4
2
T(°C)
2
4
6
8
10
12
14
MIN

Figure 13

smaller, but more fragile, thermocouple probes. This could insure obtaining a more detailed temperature profile of the rainbow trout eye. It would also more accurately establish the gradients of temperature present in the eye and substantiate or refute the accuracy of the temperature measurements conducted in this study.

Why then does the tuna eye exhibit temperatures 4-6°C warmer than ambient when the trout eye does not? A plausible explanation may be contained in examination of the different anatomical situations surrounding each choroid rete mirabile. First, Linthicum and Carey (1972) noted that the internal carotid rete, which shows one of the greatest heat accumulations in the tuna (15°C above ambient as measured near the prootic bone) has vascular channels leading to the vicinity of the sclera of the eye. The authors indicate that this vasculature breaks up into a network of vessels, unnamed, but differentiated from the choroid rete. Contrarily, the rainbow trout is not known nor would be expected to possess a retial carotid circulation that concentrates heat and channels warmer than ambient blood to the vicinity of the trout eye. Secondly, it has been found (Stevens and Fry, 1971; Linthicum and Carey, 1972) that tuna possess brain temperatures 4-7°C above ambient (noting that the brains are positioned in close proximity to the eyes). Again, this is not believed to be the case with rainbow trout. Stevens and Fry (1970) reported that the freshwater teleost, Tilapia mossambica, has a mean brain temperature 0.45°C above ambient. Similar results would be anticipated in trout. Thus, the major portion of the tuna eye unlike the trout is encompassed by a blood supply originally 15°C warmer than ambient, and a nervous tissue mass 4-7°C warmer than ambient.

Subsequently, if a large portion of the eye is surrounded by warmer than ambient tissues and fluid then the thermal gradient from the metabolically active retina through the posterior section of the eye would be greatly decreased, thus reducing or preventing loss of thermal energy (heat). This was observed by Linthicum and Carey (1972) who recorded strong temperature gradients within the eye. The authors found that the posterior half of the eye was in some cases 10°C warmer than the anterior half. Therefore, the thermal flux of energy would have but one way to flow; through the front of the eye. This assumes that the brain warms the eye (and not the antithetic condition of the eye warming the brain) due to its larger highly metabolic mass. The consideration that heat flow is unidirectional through the eye presents another important aspect when comparing the anatomical situations of the tuna and trout eye, that of relative eye size.

The equation for thermal conductivity (not to be confused with thermal conductance) may be employed to consider the importance of eye size diameter in heat transfer.

The formula states:

$$\frac{\Delta Q}{\Delta t} = kA\,\frac{T_2 - T_1}{L}$$

where ΔQ equals heat transferred, Δt change in time, k thermal conductivity, A cross-sectional area, T_2 and T_1 respective temperatures, and L length (Halliday, and Resnick, 1966). It can be ascertained from this equation that the amount of heat transferred is dependent upon the length of transfer. An increase in the magnitude of L decreases the effective thermal flux. Analogously, if retinal metabolism (heat) is being produced in separate eyes of diameter, D, and 5D, respectively, the movement of thermal energy away from the eye of diameter D is greater than the eye of diameter 5D. This slower thermal diffusion would manifest itself in a greater build-up of heat at the retina. If it is correctly assumed that the tuna eye is of a larger diameter than the trout eye then it is conceivable that the larger tuna eye could exhibit a warmer temperature simply on this basis.

Similarly, this rationale may be applied to an area change, assuming the eye is a spherical body. As the diameter increases the surface area per unit mass decreases. In the present situation the tuna eye would have less surface area per unit mass available for heat transfer (presuming retinal metabolism is proportional per unit mass). This again, could cause the larger eye to exhibit a warmer temperature than the smaller eye. This contention is corroborated in work collected by Linthicum and Carey (1972). The authors observed that the average eye temperature of a tuna weighing 400-900 lbs, was 2°C higher than tuna weighting 15-25 lbs. Ergo, eye size in conjunction with the other differences in the anatomical situations enumerated above doubtlessly contributes to the dissimilar eye temperatures observable in the tuna and rainbow trout.

Linthicum and Carey (1972) attributed the warmer than ambient tuna eye temperatures to the warm carotid circulation probably acting in conjunction with the choroid rete mirabile, which would be functioning as a counter current heat exchanger. The authors do not mention what part elevated brain temperatures or eye size might play in this phenomenon. Further, their reasoning that the tuna choroid rete mirabile is acting to concentrate heat may be inaccurate because the choroid rete mirabile of the tuna eye like the choroid rete mirabile of the rainbow trout eye is in all probability structurally unable to function as an effective heat exchanger.

There is a correlation of importance between vessel size and its counter current exchange function. For instance, gas exchangers must be of capillary size, less than one-hundredth of a millimeter (Schmidt-Nielsen, 1972). This smaller size has the effect of increasing the number of vessels, for a given area, therefore increasing surface area, an essential requirement if diffusion of a relatively slower dispersing molecular species, like oxygen, is to occur. Conversely, heat exchangers contain vascular channels of millimeter size or greater. Stevens, Lam, and Kendall (1974) found the cross-sectional area of peripheral heat exchanger vessels in tuna to range from 1000-5000 μm^2 compared to vasculature of an oxygen exchanger like the swimbladder where vessel size approximates 72 μm^2. These larger vessels allow for greater blood flow, which facilitates the transfer of heat per unit time. This is due to the fact that the blood does not possess specific heat carrying molecules like exists for oxygen (i.e., hemoglobin), but is limited to the thermal carrying capacity of blood which is dictated by thermodynamic laws (the specific heat of blood). Coupled with the fact that heat or thermal energy diffuses much quicker than a molecular species such as oxygen, it becomes apparent that for a vessel to effectively transfer heat it must contain a sufficient quantity of blood.

It appears most probable that this is why the rainbow trout choroid rete mirabile is unable to show heat concentration. Its vessels are too small and as such do not contain the volume of blood necessary to function as a counter current heat exchanger. The best evidence in support of this is the fact that the trout choroid rete is a very efficient counter current exchanger for oxygen (Fairbanks, Hoffert, and Fromm, 1969). The retial vessels must then be

of capillary size which unquestionably reduces the structures' effectiveness to exchange thermal energy.

The same reasoning may be applied to the tuna eye. The tuna is a predacious animal and thus must depend upon acute vision. This implies high retinal metabolism that for the most part (i.e., 60%) is oxygen dependent. Therefore, as in the trout eye a highly developed counter current exchange system for oxygen must be present. Inherent in this argument then that the tuna choroid rete functions to concentrate oxygen would be the unlikely existence of a simultaneous heat exchanger based upon the reasons enumerated above.

Hence, the choroid rete mirabile is an anatomical arrangement of vessels which seems to have evolved as a means of concentrating oxygen in the eyes of many teleosts by means of counter current flow in capillary size vessels. It is due to the minute dimensions of these vessels that suggests that this structure might not function quantitatively to concentrate heat and thus act simultaneously as a counter current heat exchanger.

This discussion has many times stressed the word "quantitatively" in reference to defining the presence or absence of a counter current heat exchanger. It is felt that there can be little doubt that at least "qualitatively" there must be some exchange of heat between choroid retial vessels in trout or tuna. That is, when arteries and veins lie in close proximity and exhibit a counter flow of blood it is assumed that some exchange of heat occurs. However, this heat transfer is insufficient to result in measurable or quantifiable amounts.

Future research evaluating and comparing the anatomical relationships of the tuna and trout eye is needed. This could take the form of 1) measuring the relative content of the highly insulatory fat and bone surrounding each ocular orbit and 2) in the case of rainbow trout, brain temperature measurements to supplement existing data on other teleosts (Stevens and Fry, 1970). More importantly, study is needed in histological examination of the choroid retia themselves. Especially in the realm of actual measurements of the size of the retial vessels, and postulations as to how much blood each rete is capable of holding per unit time. This would be an approach to evaluate the possible role any rete mirabile might play in counter current heat exchange.

CONCLUSIONS

The results of this study show that the rainbow trout choroid rete mirabile is unable to function quantitatively as a counter current heat exchanger. This conclusion was based on the facts that 1) the trout eye does not show a concentration of heat and 2) that no significantly different values of thermal conductance through the eye were obtained from animals with intact choroid retial circulation and from those in which retial function had been removed by bilateral pseudobranchectomy.

It is suggested that the choroid rete vessels are small enough to efficiently concentrate oxygen, but antithetically not large enough to carry the amount of blood needed to allow the rete to act as a current heat exchanger.

It is further postulated that the tuna eye, which is known to exhibit warmer than ambient temperature, does not, in all probability, rely on a counter current heat exchange of its choroid rete mirabile to account for this phenomenon, as some authors have suggested. Rather, unlike the rainbow trout eye, the tuna eye is situated anatomically so that it may gain heat from 1) the warm (15°C above ambient) blood of the carotid rete and 2) the warmer (4-7°C) than ambient brain. These circumstances permit thermal flux in only one direction, i.e., out the front of the eye. In addition, the tuna eye is of such a diameter (assumed to be greater than the trout) that heat transfer is slow and as such this also contributes to the warmer than ambient temperature in the eye and thus to the impression that a counter current heat exchanger is present.

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APPENDICES

APPENDIX 1

Thermoresistive Transducer Specifications

Bead diameter	0.03 cm
PE 90 tubing	
inside diameter	0.09 cm
outside diameter	0.13 cm
PE 350 tubing	
inside diameter	0.32 cm
outside diameter	0.40 cm
Dissipation Constant	0.10 mw./°C*
Time Constant	1.00 sec* 0.09 sec#
Zero-Power Resistance R_o @ 25°C	2,000 ± 25%*
Temperature Coefficient @ 25°C	-3.40%/°C*
Ratio--R_o @ 0°C/R_o @ 50°C	5.60*
Ratio--R_o @ 25°C/R_o @ 125°C	14.60*
Self Heating	2.97×10^{-5} -- 4.94×10^{-5} watts[†] 4.21×10^{-4} -- 7.09×10^{-4} cal[†]

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[*] supplied by manufacturer, measured in still air @ 25°C.

[#] measured by author using bridge circuitry and thermal conductance chamber (described in Material and Methods) for a temperature change of 7.2-10.4°C.

[†] measured at 12°C and accounting for a possible resistance change of 60%.

APPENDIX 2

Equation for Comparison of the Slopes of Two Regression Lines

$$\frac{b_i - b_2}{\sqrt{(ss_E^{1} + ss_E^{2}) \,/\, (n_1 + n_2 - 4)\ (1/ss_x^{1} + 1/ss_x^{2})}}$$

where:

$$ss_x^{0} = \Sigma x_i^{2} - \frac{(\Sigma x_i)^{2}}{n}$$

$$ss_E = (S_{y \cdot x})^{2} \cdot (n); \quad S_{y \cdot x} = \sqrt{\frac{\Sigma y_i^{2} - a_o \Sigma \ln y_i - a_1 \Sigma x \ln y_i}{n - 2}}$$

= standard error estimate of y on x

$a_1 = b =$ slope

$a_o = \bar{y} - a_1 \bar{x}$