An Abstract of the Dissertation of

James Whitton Buell for the degree of Doctor of Philosophy
in the Department of Biology
to be taken June 1973
Title: Argon-Nitrogen Ratios in the Swimbladder Gas of Physostomous Fishes with Particular Reference to the Rainbow Trout, *Salmo gairdnerii*.

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Robert W. Morris

Argon-nitrogen ratios in the swimbladder gas of physostomous fishes have generally been found to be lower than the value for air, whereas the ratios for physoclistous fishes have been found to be higher than the value for air. The purpose of the present study is to account for the low ratios in physostomous fishes.

Argon-nitrogen ratios are presented for several species of physostomous and physoclistous fishes from samples taken in the field; ratios are lower than the value for air in physostomes, higher in physoclists. Submersion experiments correlate depth histories of *Salmo gairdnerii* with changes in the argon-nitrogen ratios in their swimbladder gas. Changes (decreases) in swimbladder volume are also correlated with time in fish restricted from the surface. A model is developed which indicates the degree to which argon-nitrogen ratios tend to approach a predicted equilibrium state. Permeabilities of argon and nitrogen in swimbladder membrane are determined and the role of relative permeabilities in the changing of argon-nitrogen ratios

observed in submersion experiments is established. The experimentally determined relative permeabilities of argon and nitrogen are compared with values computed from the molecular radii and solubilities of the two gases.

A general model is presented which accounts for changes in argon-nitrogen ratios in the swimbladder gas of physostomes on the basis of the physical properties of these gases. The conclusions of previous workers are discussed in the light of this model.

ARGON-NITROGEN RATIOS IN THE SWIMBLADDER GAS OF PHYSOSTOMOUS FISHES WITH PARTICULAR REFERENCE TO THE RAINBOW TROUT, SALMO GAIRDNERII

by

JAMES WHITTON BUELL

A DISSERTATION

Presented to the Department of Biology
and the Graduate School of the University of Oregon
in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy

June 1973

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ACKNOWLEDGEMENTS

I gratefully acknowledge my advisor, Dr. Robert W. Morris, and the members of my doctoral committee whose guidance and extraordinary patience were indispensable to the completion of this work. Thanks to the departmental secretaries and accounting staff who put up with far more than they should have in these many years.

This work was supported in large measure by a Public Health Service Training Grant (# 5T01 GM 336 07) to J. W. B.

I regard with great gratitude those various taverns in and around Eugene where I could occasionally (?) venture to either lose or regain my sanity, depending upon the circumstances surrounding the moment; and those fast friends who helped.

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INTRODUCTION

It was pointed out by Denton (1961) in his review of buoyancy in fish and cephalopods, that aquatic and marine animals which enjoy neutral buoyancy are at a distinct advantage over those which do not. The most widespread adaptation for attaining neutral buoyancy among the bony fishes is a gas-filled swimbladder. This organ, probably derived from an accessory respiratory organ which arose during early Actinopterygian evolution (Goodrich, 1930; Westoll, 1944), is used as a hydrostatic organ in fishes residing in waters ranging in depth from the surface to more than 1000 meters. Alexander (1959a,b,c,d) showed that for twenty-two species of fishes, the swimbladders (*in situ*) obeyed Boyle's law very well, indicating that the hydrostatic pressure of the gas within the swimbladder necessarily matches that of the environment, which increases on the order of one atmosphere for every ten meters of depth.

On the basis of the structure of the organ, fishes having swimbladders are generally placed into one of two groups: Physostomes, in which the pneumatic duct between the swimbladder and the gut remains open, and physoclists, in which this duct degenerates during larval stages into a ligament, thus isolating the swimbladder from the gut. Physostomes may fill their swimbladders by gulping air, but physoclists must fill their swimbladders using only gases dissolved in the water in which they live; since these gases are available at a total concentration of one atmosphere regardless of depth, the secretion of gas is frequently accomplished against very high pressure gradients. Equilibrium partial pressures of gases do increase with depth, but only on the order of 14% for every 100 atmospheres of hydrostatic pressure (Enns et al., 1965). Therefore, as depth increases there is an ever increasing gradient between the gas present in the swimbladder and the gases dissolved in the water outside. Thus the gas-filled swimbladder presents two problems: First, to deposit the gas, and second, to keep it there against pressure gradients that would favor the diffusion of gases through the tissues and back into the water.

During the past two decades, the mechanism utilized by physoclists to secrete gas into the swimbladder has received considerable attention. The high point of this attention was reached with a definitive paper by Kuhn, et al. (1963). Some modifications have been made by subsequent authors, but the general model presented by Kuhn is still considered substantially correct. According to current theory, gas secretion is accomplished by means of countercurrent multiplication of oxygen unloaded from hemoglobin by lactic acid deposited at the closed end of a rete, which is closely associated with the swimbladder itself. The increased solute concentration elevates the partial pressures of other gases present in the blood (N_2, CO_2, A), and these contribute to the secreted gas. Due to its abundant supply from hemoglobin, oxygen is the predominant gas which is secreted.

Physostomous fishes, however, have received relatively little attention. Composition of the swimbladder gas in these fishes indicates that a very different mechanism exists for the deposition and maintenance of gas in the swimbladder. Work by several authors (Scholander, 1951; Copeland, 1952; Fange, 1953; Saunders, 1953; Scholander and van Dam, 1956; Tait, 1956; Wittenberg, 1958; Kuhn and Kuhn, 1961; Steen, 1963; Sundnes, 1963) indicates very high concentrations of nitrogen. Scholander and van Dam (1953), Scholander (1954, 1956), Wittenberg (1957), Tait (1956), Sundnes _et al_. (1958), and Douglas (1967) all point to the importance of measuring the argon-nitrogen ratio (usually expressed $100A/N_2$) in swimbladder gas in order to determine whether or not nitrogen is treated as an inert gas or is involved in some active secretion process. This position is reiterated by Denton (1961) and Jones (1957) in their review articles. Their reasoning was that if the argon-nitrogen ratios observed in swimbladder gas were the same as the value for air, nitrogen could be expected to be behaving as an inert gas. If the ratio for the swimbladder gas was low, it could be assumed that nitrogen was being actively secreted by a chemical process. Data in the literature on argon-nitrogen ratios in swimbladder gas are few and extremely variable, especially for physostomous fishes. No statistical treatment was performed by any of the authors. Scholander (1956), Tait (1956), and Sundnes _et al_. (1958) all claim that the ratios they observed were the same as in air. Sundnes _et al_. presented no data at all in their paper. Tait based his conclusions on one measurement ($100A/N_2 = 1.02$ as compared with 1.196 in air), and a t-test applied to the data presented by Scholander shows, despite a large variance, that there is a difference between the values for swimbladder gas and that for air ($P < 0.01$). The trend seems to be that the argon-nitrogen ratios decrease with increased depth of capture, but data are insufficient to warrant any firm conclusions in this regard. In no case were data taken on the length of time a fish had resided at the depth of capture.

Several authors (Jacobs, 1934; Saunders, 1953; Tait, 1959; Brawn, 1962; Harvey, et al., 1968; Tait, 1970) have found that a number of physostomes including some of the isospondylids are incapable of gas secretion, but have swimbladder gas composition falling into the general physostome pattern.

The purpose of this study is to examine the argon-nitrogen ratios in the swimbladder gas of isospondylid fishes, to seek an explanation for whatever departures these ratios show from the condition in air, and to evaluate the contention that low argon-nitrogen ratios can be used as an indicator for active nitrogen secretion. In addition, the results of previous workers in this area will be reevaluated.

MATERIALS AND METHODS

SUBJECTS

During the course of this study swimbladder gas samples were taken from a number of species. Fishes used in the survey included representatives of both physostomes and physoclists. The methods of capture varied from species to species.

Coho salmon (*Oncorhynchus kisutch*) were taken in waters just outside Coos Bay, Oregon, by hook and line from a fishing boat. All were mature adults weighing from 7 to 12 pounds. All were in good condition. Swimbladder gas samples were taken immediately upon capture.

Smelt (*Hypomesus pretiosus*) were seined in shallow water (10-15 feet) near the mouth of Coos Bay with a 250 x 20 ft. seine with 1/2 inch mesh. Individuals in good condition were transferred to fiberglass holding tanks where they were allowed to "settle down" for 3-5 days. Only those individuals which appeared to adjust well to their new environment were sampled.

Herring (*Clupea pallasii*) were obtained from commercial live-bait suppliers who had seined them in Salmon Harbor, Oregon. The individuals had been held in large live cars for up to three days before they were obtained. All individuals sampled were mature adults in good condition.

Shad (*Alosa sapidissima*) were caught along with smelt and herring as described above. All individuals sampled were between

10 and 14 inches in fork length and in good condition. They were placed in fiberglass holding tanks for up to 12 days before sampling.

Tom cod (Microgadus proximus) and perch (Brachyistius brevipinnis) were caught in shallow water (10-15 feet) by hook and line from docks in Coos Bay near Charleston, Oregon. The tom cod were sampled immediately upon capture; the perch were placed in fiberglass holding tanks for 5 to 7 days before sampling.

Black rockfish (Sebastes melanops) were caught by hook and line at a depth of 30 to 50 feet in water off Simpson's Reef just south of Coos Bay, Oregon. Mature adults were sampled immediately upon capture.

Juvenile chinook salmon (Oncorhynchus tshawytscha) were obtained from the Oregon Fish Commission hatchery at Oakridge, Oregon. Individuals were just entering the smolt stage and were in good condition. Samples were taken 6 to 10 days after the individuals were transferred to concrete holding tanks (with a maximum depth of 4 feet) at the University of Oregon.

Rainbow trout (Salmo gairdnerii) were obtained from the Oregon Game Commission hatchery at Leaburg, Oregon. Individuals measured from 8 to 11 inches in fork length. Samples were taken 3 days to 5 weeks after the individuals were transferred to concrete holding tanks at the University of Oregon.

Rainbow trout from the Leaburg hatchery were also used in submersion and swimbladder volume experiments and swimbladder membranes from these fish were used in the permeability experiments.

With the exception of those individuals used in the submersion experiments at Smith reservoir, all individuals were held in cubical concrete tanks, 4 feet square, with a constant flow of dechlorinated municipal water, at a temperature ranging between 10 and 18°C. They were fed live earthworms (Lumbricus sp.) and commercial trout food. All fish used in experiments appeared healthy and active.

SAMPLING TECHNIQUES

All samples of swimbladder gas from all experiments and all gas samples for permeability experiments were taken in disposable 5 cc. glass syringes (Bekton Dickenson: "Hypak") fitted with 3/4 inch #27 needles and tripple-sealed rubber-tipped plungers. These syringes proved to be exceptionally gas-tight. The dead space in the syringes was filled with saturated aqueous lithium chloride to prevent contamination of the sample. Once the gas samples were drawn into the syringes, the plunger was partially depressed, and the needle quickly withdrawn from the swimbladder (or diffusion chamber) and inserted into a small rubber stopper, and the plunger depressed further, thus creating a positive pressure inside the syringe and preventing any contamination of the sample with air. The size of the samples ranged from 1 to 5 cm^3 of gas, but was usually about 4 cm^3. Syringes containing samples were iced whenever possible in the field and stored in a refrigerator in the laboratory until analyzed. Analysis was usually made within 48 hours of the time of sampling.

ANALYSIS

Gas samples were analyzed for the percent content of argon, nitrogen, and oxygen. The apparatus used for analysis is represented in Fig. 1. A Fisher Gas Partitioner was used as the core of the apparatus. This small chromatograph was fitted with a 78 inch long 1/8 inch i.d. copper column packed with 42/60 screen 13X type molecular seive. Helium was used as the carrier gas. Best results were achieved using a carrier gas flow rate of 80 cm^3 per minute produced by a pressure above the column of approximately 12 p.s.i.

Since the retention times of argon and oxygen in the chromatograph column are the same under these conditions, each gas sample had to be divided into two aliquots and analyzed separately.
One aliquot was injected into a receiving tube filled with a 1:10 mixture of 30% pyrogallic acid and 25% potassium hydroxide (Fig. 1). The sample was shaken vigorously for not less than 30 seconds to remove all of the oxygen present. About 0.5 cm^3 of the remaining gas was analyzed for argon and nitrogen. The second aliquot was analyzed without treatment for argon-plus-oxygen and nitrogen. At least one air standard was run immediately before each sample was analyzed. In the case of permeability experiments, the starting gas mixture (49.7% argon, 50.3% nitrogen) was used as the standard. Examples of strip-chart records for standards and swimbladder gas samples are given in Fig. 2. The percent composition of the samples and the argon-nitrogen ratios ($100A/N_2$) were calculated according

FIGURE 1

Apparatus used for gas analysis. The portion of the sample to be analyzed for argon and nitrogen is injected through the serum cap in the side of the receiving tube for sample loop A and stripped of its oxygen content by shaking it vigorously with a mixture of pyrogallol and KOH. The portion to be analyzed for argon-plus-oxygen and nitrogen is injected into the receiving tube for sample loop B which is filled with saturated aqueous lithium chloride. When the sample has been introduced into the receiving tube the 4-way stopcock is turned to the "bypass" position (as in sample loop A), allowing 0.5 ml. of the gas sample to be manipulated into the loop as shown by the small arrow. With the sample in the loop and the 3-way stopcock closed, the 4-way stopcock is turned to the "open" position (as in sample loop B), allowing carrier gas to enter the loop and take the sample to the chromatograph. The sample loop is flushed and excess sample is expelled through the "exhaust."

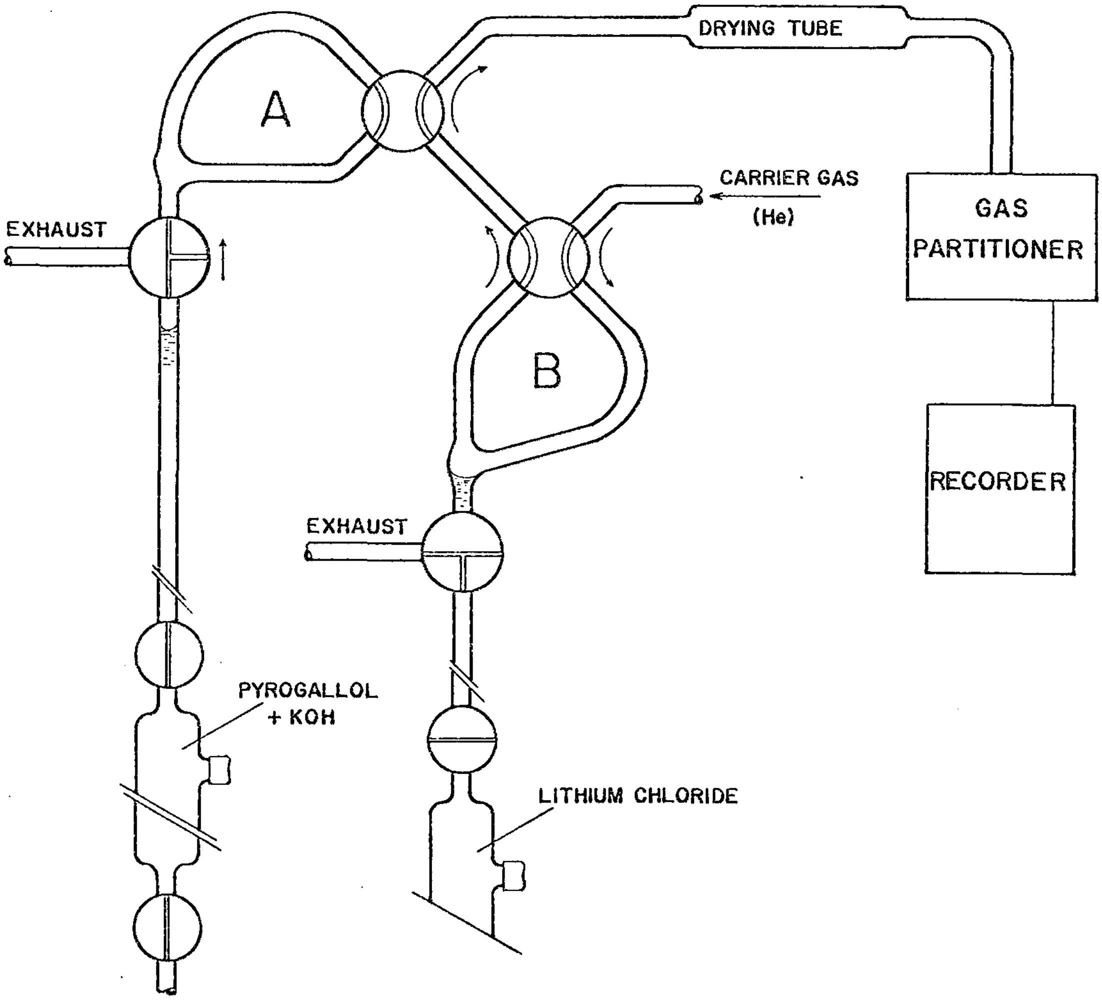

DRYING TUBE
A
CARRIER GAS
(He)
GAS PARTITIONER
EXHAUST
B
RECORDER
EXHAUST
PYROGALLOL + KOH
LITHIUM CHLORIDE

FIGURE 2

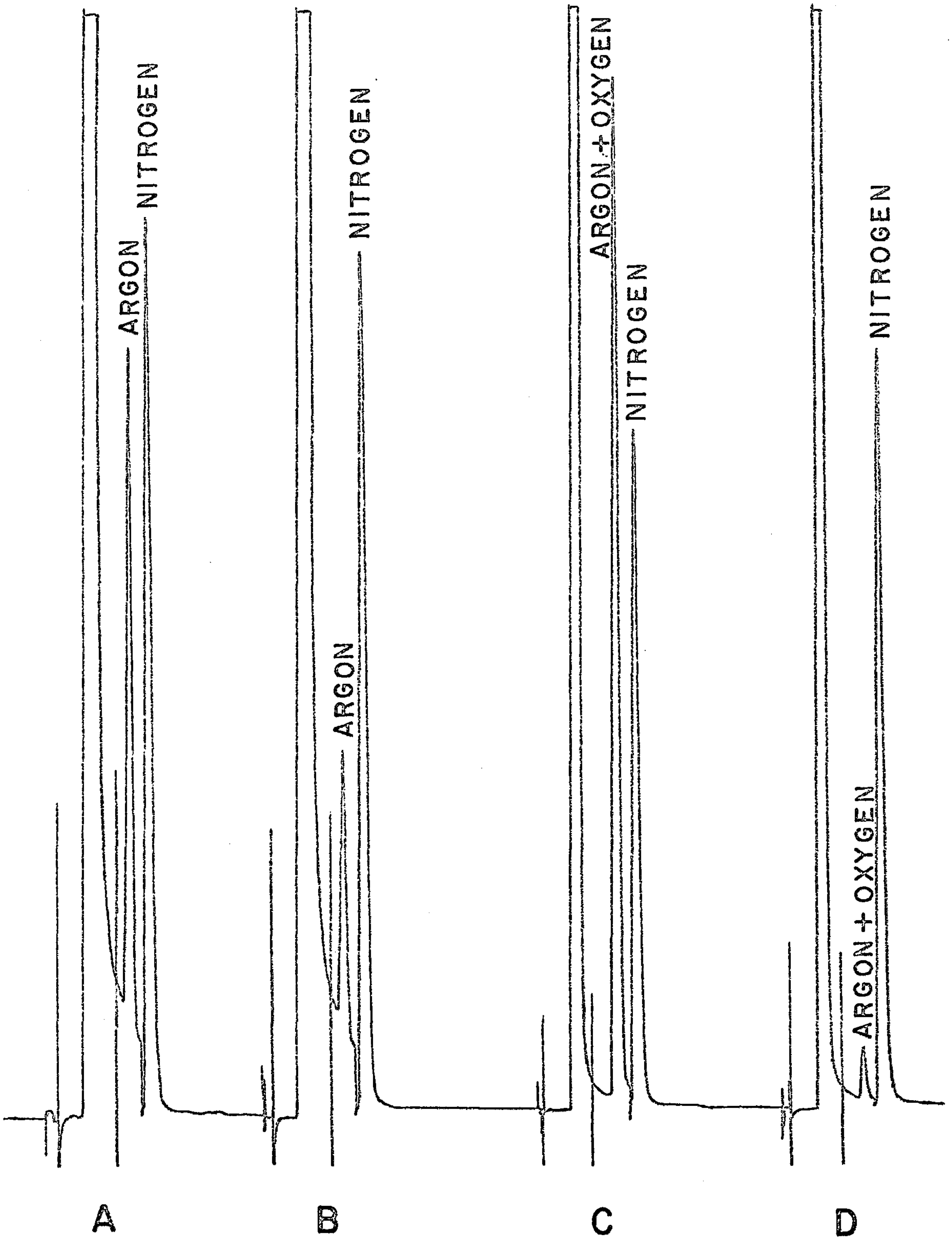
Examples of strip-chart records for standards and swimbladder gas samples. The sensitivity setting for the argon peaks was 50 times that for the nitrogen peaks; for the argon-plus-oxygen peaks 5 times that for the nitrogen peaks.

A. Air standard for argon and nitrogen analysis.

B. Argon and nitrogen analysis record for swimbladder gas of rainbow trout submerged for 10 days at 32 feet.

C. Air standard for argon-plus-oxygen and nitrogen analysis.

D. Argon-plus-oxygen and nitrogen analysis record for swimbladder gas of rainbow trout submerged for 10 days at 32 feet.


ARGON
NITROGEN
A
ARGON
NITROGEN
B
ARGON + OXYGEN
NITROGEN
C
ARGON + OXYGEN
NITROGEN
D

to the computational method outlined in Appendix A.

SUBMERSION EXPERIMENTS

Experiments involving the submersion of fish to a known depth (pressure) for a prescribed period of time before sampling in order to monitor the effects of depth history on swimbladder gas composition were carried out in two groups. Those where the fish were held within one foot of the surface but prevented access to it were carried out in the laboratory. Individuals between 7 and 9 inches in fork length were placed in 15 gallon glass aquaria. The fish were allowed to adjust to their new environment for one or two days, after which a plastic grille was lowered to cut off access to the surface. Time was measured from the initiation of restriction from the surface.

The second group of submersion experiments involved fish held at depths of 32 and 64 feet (2 and 3 atmospheres total hydrostatic pressure), and was carried out at Smith Reservoir (altitude: 2,605 ft.) on the McKenzie River above Blue River, Oregon. Fish used in these experiments, measuring 9 to 11 inches in fork length, were transported directly from the Leaburg hatchery to the reservoir, a distance of about 25 miles. At the reservoir, groups of 15 to 25 fish were placed in live cars made from 55 gallon enamel lined drums with both ends cut out and covered with 1/2 inch mesh nylon netting. The live cars were held at their prescribed depths by 3/8 inch plastic rope attached to large spherical plastic buoys (Fig. 3). The fish were not allowed access to the surface before sampling. Syringes containing the samples were iced and transported to the laboratory for analysis.

SWIMBLADDER VOLUME EXPERIMENTS

In order to determine if rainbow trout are able to retain swimbladder gas even under the most favorable conditions, the swimbladder volumes of those fish "submerged" in the laboratory to depths not greater than 1 foot were determined for each individual before restriction from the surface and after the prescribed restriction period. The apparatus used is a modification of the types used by Kanwisher and Ebeling (1957) and Brawn (1962), and is illustrated in Fig. 4. The device is composed of a steel pressure chamber which is completely filled with water and into which the fish is placed, a graduated capillary manometer, and a micrometer syringe. The entire system is filled with water, except for a small air bubble in the capillary. Once the fish is sealed in the pressure chamber, the small bubble is compressed to 90% of its original volume by adding a measured volume of water to the closed system from the micrometer syringe. According to Boyle's law, after the subtraction of a blank reading, the volume added by the micrometer syringe is equal to 10% of the swimbladder volume. This small amount of compression was used to reduce any error that could

FIGURE 3

Suspension system for live cars used in the submersion experiments.

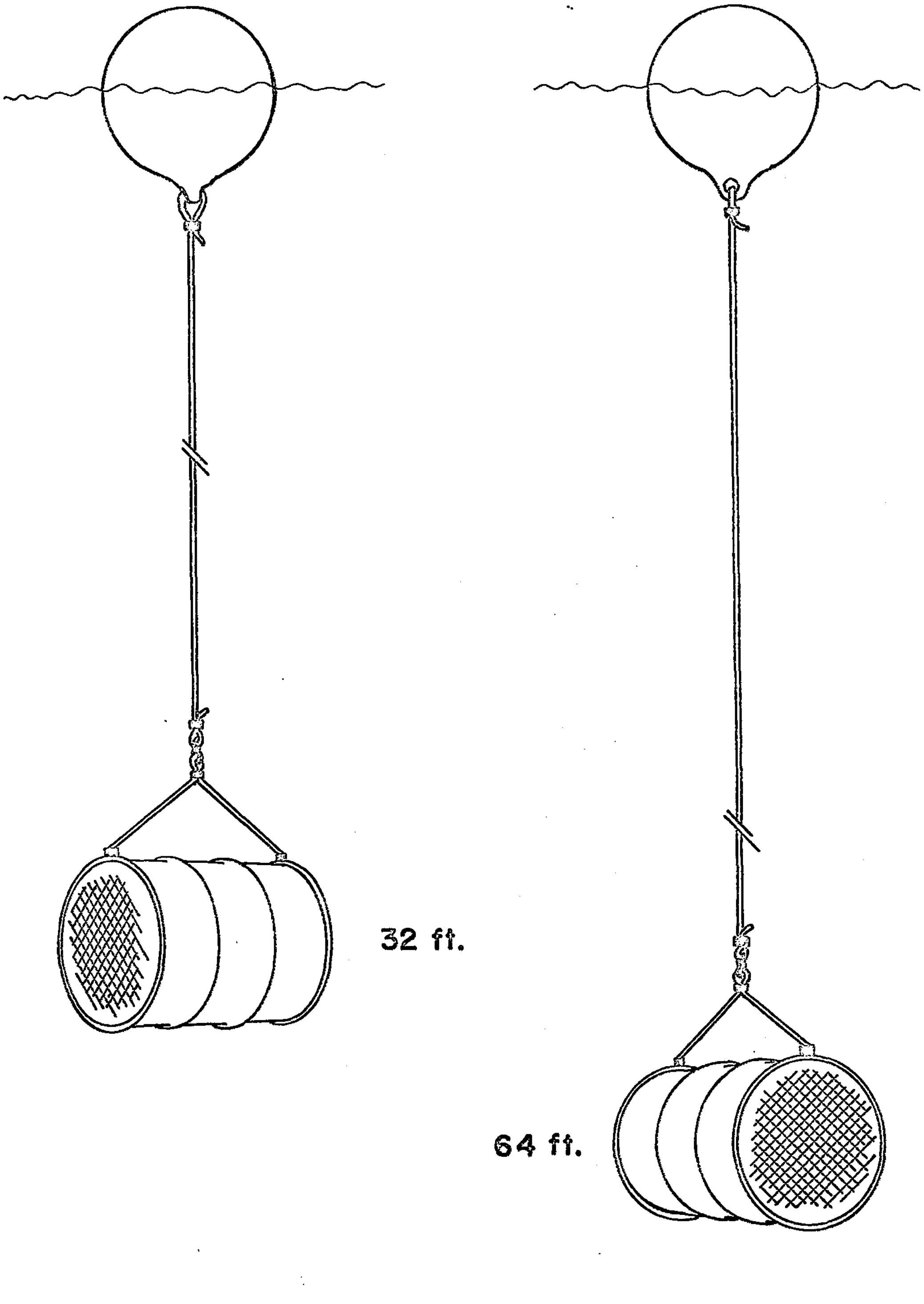
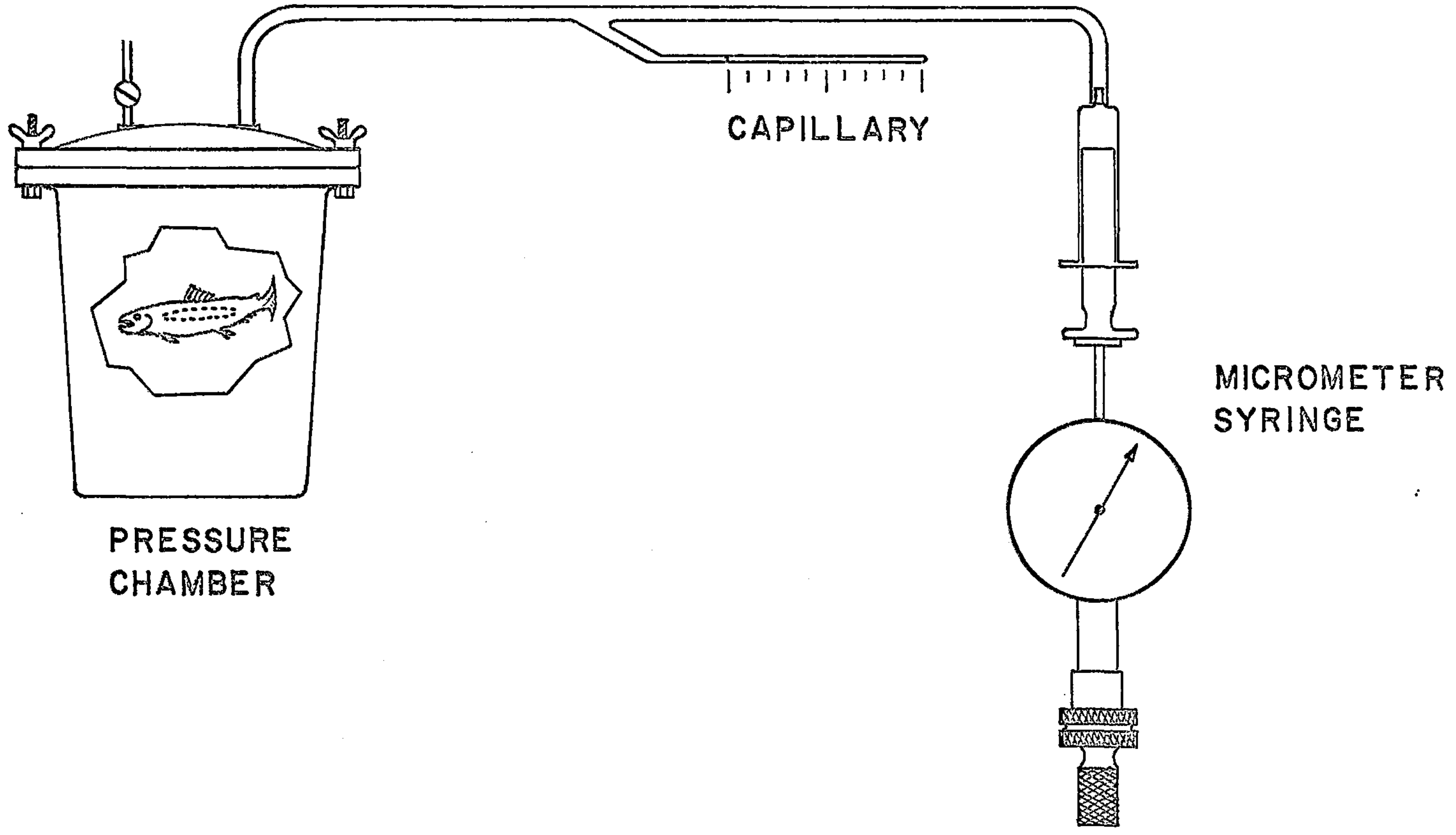

32 ft.
64 ft.

FIGURE 4

Apparatus for determining swimbladder volume. As the pressure of the closed system is increased with the micrometer syringe, the swimbladder and the bubble in the capillary decrease in volume proportionately.


CAPILLARY
MICROMETER SYRINGE
PRESSURE CHAMBER

be introduced by tissue resistance of the fish to the reduction of the volume of its swimbladder. Computational formulae are given in Appendix A.

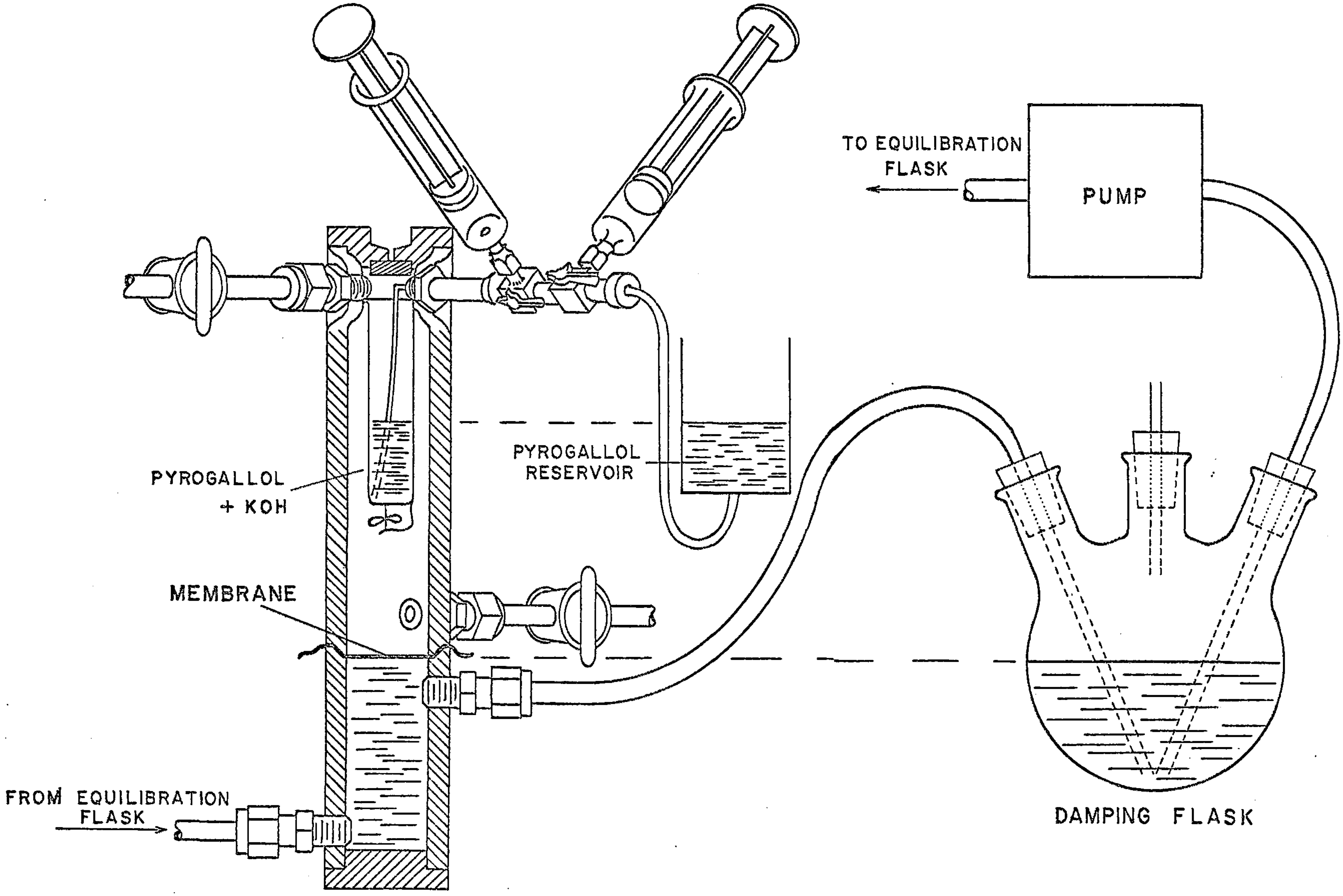
PERMEABILITY EXPERIMENTS

Experiments intended to establish the relative permeabilities of argon and nitrogen in swimbladder membrane and dialysis membrane were performed using the apparatus shown in Fig. 5. The experiments were designed to yield raw data indicating the rate of change of a volume of a known mixture of the two gases (49.7% argon and 50.3% nitrogen) and the ratio of the two gases at the termination of the experiment. Data were also taken on the temperature of the system (maintained at 10±1°C.), the initial volume occupied by the gas mixture (54.0 cm^3), the surface area of the membrane involved in the diffusion process (5.07 cm^2), the barometric pressure, and the wet weight of the membrane. From these data the permeability coefficients of argon and nitrogen could be calculated according to the computational method outlined in Appendix A.

The membrane was placed in the experimental apparatus so that it formed a boundary between the two halves of a 1 inch i.d. cylindrical ucite chamber. Care was taken not to stretch the membrane thereby changing its physical properties. The bottom half of the chamber was perfused (25-50 cm^3 per minute) with Salmo Ringer's (Δ0.58°C., pH=7.2) (Lockwood, 1961) which was saturated with a

FIGURE 5

Apparatus for measuring the permeability of argon and nitrogen in swimbladder and dialysis membranes. The two syringes mounted near the top of the chamber are for providing the initial charge of pyrogallol and KOH to the absorbent cup. Other features of the apparatus are explained in the text.


TO EQUILIBRATION FLASK
PUMP
PYROGALLOL + KOH
PYROGALLOL RESERVOIR
MEMBRANE
FROM EQUILIBRATION FLASK
DAMPING FLASK

mixture of O_2 and just enough CO_2 to maintain a pH of 7.2 (about 0.7% CO_2). The perfusing Ringer's acted as sink into which gases diffusing through the membrane flowed and by which they were carried out of the system. It was recycled by passing it through a re-equilibration flask where any recently acquired gases were blown off by the O_2-CO_2 mixture with which the solution was being re-equilibrated. The gas mixture was adjusted by a pair of capillary-bypass manometers, one controlling the flow rate of pure O_2 and the other the flow rate of a mixture of 95% O_2 and 5% CO_2. In order to prevent any contamination of the re-equilibrated saline by air, it flowed by gravity from the re-equilibration chamber to the diffusion chamber through all-glass tubing and 1/4 inch "Swagelok" fittings equipped with teflon crimping rings. Circulation was maintained by a peristaltic pump (Harvard Apparatus Corp., Model 1201) located downstream from the diffusion chamber. Between the pump and the diffusion chamber was a leveling and damping vessel which damped out any oscillations in flow rate induced by the peristaltic pump and kept the level of the Ringer's within the chamber from falling or rising and stretching the membrane.

At the beginning of an experiment, the top half of the chamber was flushed for not less than 5 minutes with a mixture of approximately equal parts of argon and nitrogen (49.7% argon and 50.3% nitrogen) and then sealed. A 10 cm^3 calibrated cup suspended in the top half of the chamber was supplied with a measured amount of alkaline pyrogallol (1.2 ml. 75% KOH and 0.4 ml. 30% pyrogallol) to absorb all the O_2 and CO_2 that diffused up through the membrane from the Ringer's below. As argon and nitrogen diffused out of the top half of the chamber the change in the pressure of the gas was compensated for by the addition of more of the 30% pyrogallol solution to the absorbent cup from a leveled reservoir outside the chamber. The increase in volume of the liquid in the cup was a direct measure of the total volume of the gas which had diffused out of the top half of the chamber. The gas in the chamber was constantly mexed by a small propeller attached to a magnetic stirring flea mounted on the bottom of the O_2-CO_2 absorbent cup. The absorbent was also stirred by a magnetic flea in the cup. The fleas were driven by a rotating magnet located just outside the chamber. The entire system was maintained at 10 ± 1°C.

During the course of an experiment readings of the change in gas volume were taken at least every 3 hours over a period of 24 to 72 hours for each replicate run. If the membrane developed a leak, data for the run were collected only for the readings up to but not including the reading before the leak was discovered.

At the end of an experiment, a gas sample was taken from a sampling port at the top of the chamber and analyzed in the same manner as swimbladder samples.

RESULTS

Data collected during the course of this study have yielded results which fall roughly into three categories. First, a survey was made of the argon-nitrogen ratios in ten species of fish, including representatives of both physostomes and physoclists, which showed that the ratios differed from that in air, but in different directions; ratios were lower than that in air for physostomes, higher in physoclists. Second, submersion experiments were performed in which depth histories of rainbow trout (Salmo gairdnerii) were correlated with changes in the argon-nitrogen ratios in their swimbladder gas. A corollary to these experiments involved correlating changes (decreases) in swimbladder volume with time in fish restricted from the surface. As a part of this subsection, a mathematical model is developed which indicates the degree to which argon-nitrogen ratios tend to approach a predicted equilibrium state. Third, permeability experiments were performed which determined the relative permeabilities of argon and nitrogen in swimbladder membrane, and established the role of relative permeabilities in the changing argon-nitrogen ratios observed in the submersion experiments. As a part of this subsection, the experimentally determined relative permeabilities of argon and nitrogen are compared with values computed from the molecular radii of the two gases and their solubilities. Changes in argon-nitrogen ratios were observed in vitro and correlated with time by allowing a known mixture of argon and nitrogen to diffuse through the isolated swimbladder membrane.

SURVEY

The general difference between physostomous fishes and physoclistous fishes with respect to the composition of swimbladder gas is most evident in the far greater abundance of "inert" or "incombustible" gas in the physostomes. A relatively small body of literature concerns itself with the separation of the incombustible gases into argon and nitrogen and the reporting of their relative concentrations (Wittenberg, 1957; Douglas, MS, 1967; Tait, 1956; Sundnes, et al., 1958; Scholander, 1966). All of these authors felt that the study of argon-nitrogen ratios would provide some insight into the problem of explaining swimbladder gas composition in physostomes. Differences between physostomes and physoclists with respect to argon-nitrogen ratios in swimbladder gas are more difficult to assess, however, on the basis of information given in the literature. Wittenberg (1957) found the argon-nitrogen ratios for newly secreted gas from three marine physoclists (Opsanus tau, Prionotus carolinus, and Stenotomus versicolor) and an eel, a functional physoclist (Anguilla rostrata) were considerably greater than the ratio in air. Douglas (MS, 1967) reported the argon-nitrogen ratios of a wide variety of marine physoclists captured at depths ranging from the surface to 3500 meters. Representatives from the first 100 meters displayed argon-nitrogen ratios equal to or (usually) somewhat greater than that in air. At greater depths, this trend appears at first to be reversed, but analysis of the data indicates that within a single species, the argon-nitrogen ratio increases with depth. This depth related feature of Douglas' data will be elaborated upon in the discussion section.

All other argon-nitrogen ratios reported in the literature are for physostomes. The overwhelming trend among the physostomes is for the argon-nitrogen ratios to be less than in air (Tait, 1956; Sundnes, et al., 1958; Scholander, 1956), despite the claims by Scholander and Tait that their data do not show such a trend. The pattern that begins to emerge is that, in general, physostomous fishes exhibit argon-nitrogen ratios in their swimbladder gases which are less than that in air, and physoclistous fishes, at least in the first 100 meters from the surface, have ratios which are greater than that in air.

In order to expand somewhat the breadth of data on the argon-nitrogen ratios in the swimbladder gases of different species, samples were taken from a variety of marine and freshwater fishes, both physostomous and physoclistous. A summary of the data taken in this survey is given in Table I and the data are represented, along with those from Scholander (1965) and Tait (1956) in Fig. 6. It should be pointed out that although Scholander's data were all taken from fish caught at the same depth, they are extremely variable. It is suggested that this variability is due to variable depth histories of the individuals comprising Scholander's sample.

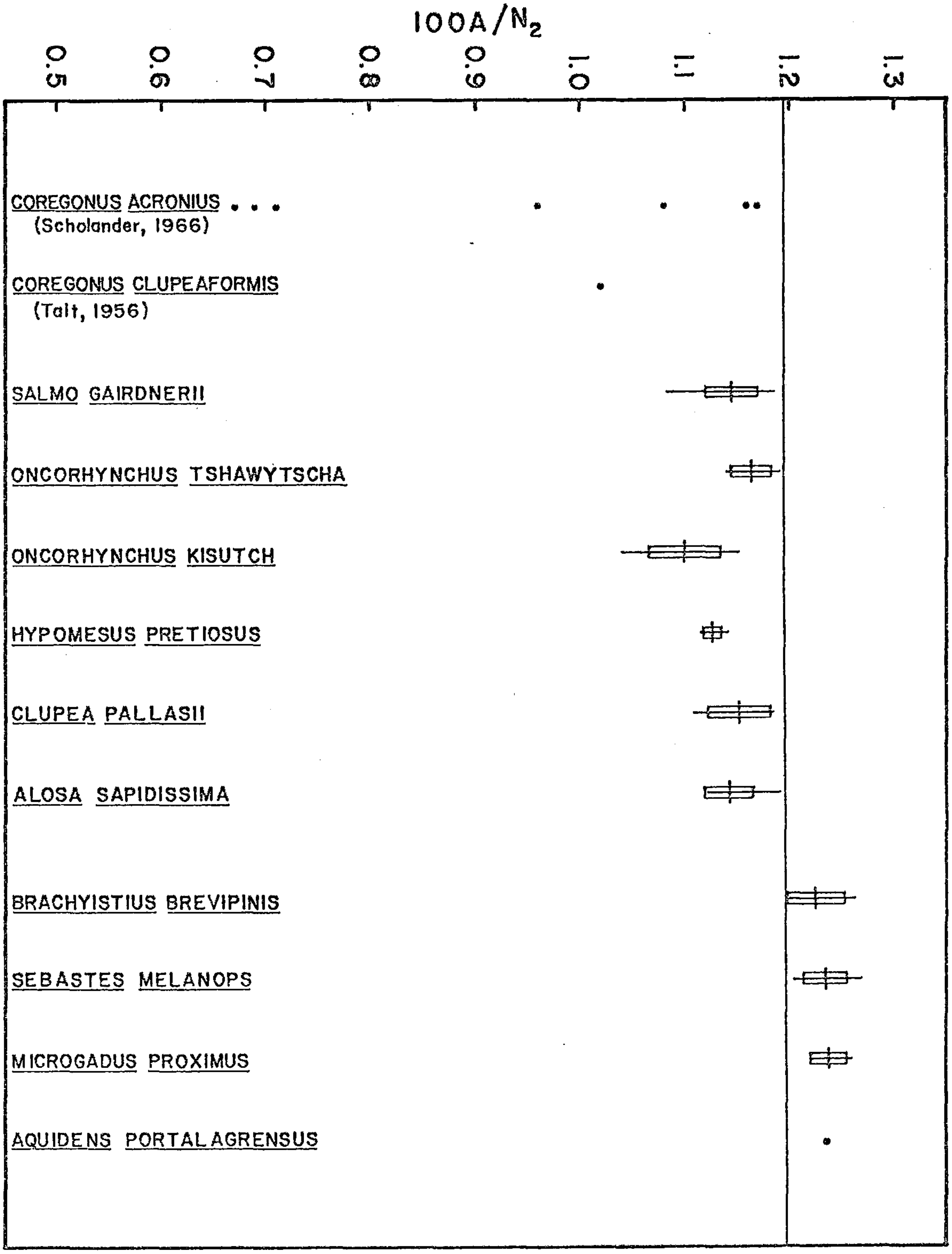
TABLE I

Summary of Survey Experiments ((n): sample size, S: standard deviation)

SPECIES	$100A/N_2$	(n)	s	$P_{\overline{y} \geq \mu_o}$	$P_{\overline{y} \leq \mu_o}$
Oncorhynchus kisutch	1.101	(15)	0.034	$P<<0.001$	
Hypomesus pretiosus	1.127	(9)	0.009	$P<<0.001$	
Clupea pallasii	1.153	(15)	0.030	$P<0.001$	
Alosa sapidissima	1.143	(7)	0.023	$P<0.001$	
Oncorhynchus tshawytscha	1.165	(6)	0.019	$0.005<P<0.01$	
Salmo gairnderii	1.143	(22)	0.025	$P<<0.001$	
Microgadus proximus	1.237	(4)	0.017		$0.005<P<0.01$
Brachyistius brevipinis	1.125	(6)	0.028		$0.01<P<0.05$
Sebastes melanops	1.234	(7)	0.021		$0.001<P<0.005$
Corregonus acronius (Scholander)	0.920	(7)	0.226	$0.005<P<0.01$	

FIGURE 6

Summary of data for survey of argon-nitrogen ratios in the swimbladder gases of various fishes. The horizontal line represents the value for air ($100A/N_2 = 1.196$). Means, ranges, and standard deviations are represented. Data from Scholander (1966) are plotted discretely because of the comparatively large variance.


100A/N_2
0.5
0.6
0.7
0.8
0.9
1.0
1.1
1.2
1.3
CORGONUS ACRONIUS
(Scholander, 1966)
COREGONUS CLUPEAFORMIS
(Tait, 1956)
SALMO GAIRDNERII
ONCORHYNCHUS TSHAWYTSCHA
ONCORHYNCHUS KISUTCH
HYPOMESUS PRETIOSUS
CLUPEA PALLASII
ALOSA SAPIDISSIMA
BRACHYISTIUS BREVIPINIS
SEBASTES MELANOPS
MICROGADUS PROXIMUS
AQUIDENS PORTALAGRENSUS

In the statistical testing of the groups of argon-nitrogen ratios in swimbladder gas determined for each species, a special t-test (Sokal and Rohlf, 1969) for judging deviations of sample means from a prescribed standard was used. The test statistic is

$$t_s = \frac{\overline{Y}-\mu_o}{s_{\overline{y}}}$$

where $\overline{Y}$ is the sample mean, μ_o is the standard (argon-nitrogen ratio in air), and $s_{\overline{y}}$ is the standard error of the sample mean (not the standard deviation of the individual sample points, because the deviation being tested is that of a sample mean around a parametric mean, μ_o). The tests are one-tailed; the alternate hypothesis in the care of physostomous fishes is that the argon-nitrogen ratios in swimbladder gas are less than that in air (H_a: $\overline{Y}<\mu_o$), and in the case of physoclistous fishes that the ratios are greater than in air (H_a: $\overline{Y}>\mu_o$). The probabilities given in Table I are for type I error (rejecting a true null hypothesis). The tests show that all physostomous fishes sampled had argon-nitrogen ratios in their swimbladder gas which were less than that in air, and all physoclistous fishes sampled had ratios greater than that in air.

SUBMERSION EXPERIMENTS

The literature on the composition of gas in the swimbladders of physoclistous fishes indicates that the pressure of "incombustible" gas, presumed in many cases to be pure nitrogen, is very high and increases linearly with depth (Saunders, 1953; Scholander and Van Dam, 1953; Scholander, 1956; Tait, 1956; Sundnes, et al., 1958; Sundnes, et al., 1969). The percentage of the incombustible fraction generally shows a slight increase with depth from about 90% to 99% of the total, but the data from all sources considered together are quite variable and a statement of a general trend is not supportable by the data. A somewhat smaller body of literature concerns itself with the separation of the incombustible gases into argon and nitrogen and the reporting of their relative concentrations (Scholander, 1956; Tait, 1956; Sundnes, et al., 1958). All of these authors felt that the study of argon-nitrogen ratios would provide some insight into the problem of explaining swimbladder gas composition in physostomes. The trend throughout this literature is that the argon-nitrogen ratios for all species of physostomes show a marked decrease with the depth of capture. It is very interesting that Scholander (1954, 1956), asserts that the argon-nitrogen ratios of swimbladder gas in the whitefish Coregonus acronius, captured at a depth of 60-80 meters, are the same as that for air. Statistical treatment of Scholander's data indicates, however, that the ratios are very different from that in air ($0.005<P<0.01$). This curious interpretation of the data will be dealt with in the discussion section of this work. It is sufficient to note at this juncture that the argon-nitrogen ratios in the swimbladders of physostomes appear to be inversely related to depth.

One weakness of all investigations on the composition of swimbladder gas in physostomes is that in no case was the depth history of the sampled individuals known. If it can be assumed that swimbladder gas composition is related to depth, fishes with habits involving periodic vertical migrations, whether to feed, avoid predators, or for any other reason may exhibit argon-nitrogen ratios not in steady-state with respect to the depth of capture. An understanding of the process involved in any change in composition resulting from a change in depth must necessarily include some knowledge of depth history. By the same token changes in composition of swimbladder gas should be inducable in individuals by experimentally altering the depth at which they reside. Composition would be expected to change as a function of time until some steady-state is reached which would be characteristic of the new depth. It is this compositional steady-state and the rate at which it is approached that are of interest in elucidating the mechanism of change.

Jacobs (1934) found that the salmonids *Hucho* and *Salmo* were unable to augment experimentally reduced swimbladder volumes when denied access to the surface. Representatives of Esocidae and Cyprinidae, on the other hand, could refill their swimbladders, but only very slowly. Gee (1968) found that the longnose dace Rhinichthys cataractae, a cyprinid, fit the Jacobs pattern, and could refill its swimbladder over a period of several days. Saunders (1953) asserted that, in general, physostomes of the order Ostariophysi, including the catostomids and cyprinids, are able to fill their swimbladders utilizing dissolved gases, but that at least some of the members of the more primitive order Isospondyli, including the salmonids and osmerids, are unable to do so. There is indirect evidence presented by Saunders that the bloater Leucicthys hoyi (an isospondylid) can secrete gas, but the rate, if such secretion does occur, is unknown. Brawn (1962) reported that neither the Atlantic herring (Clupea harengus) nor the Pacific herring (C. pallasii) are able to secrete gas. Harvey, Hoar, and Bothern (1968) found that kokanee and sockeye salmon (Oncorhynchus nerka) "like other salmonids" are "incapable of utilizing dissolved gases for swimbladder inflation." Tait (MS, 1959) compared the ability of various salmonids to retain gas when prevented from refilling their swimbladders at the surface. He found that salmonids that normally inhabit deep water such as the lake trout (Salvelinus namaycush) retain gas well, but shallow water representatives such as the brook trout (S. Fontinalis) lose gas rapidly. In another paper (1970) Tait states that a S. namaycush x S. fontinalis hybrid displayed gas loss characteristics midway between those of the two parent species. In none of Tait's experiments were the fish able to maintain neutral buoyancy when restrinced from the surface.

It is important to note that almost all of these physostomes, whether they can utilize dissolved gases to fill their swimbladders or not, display much the same relationship between depth and gas composition. This suggests that the curious composition of swimbladder gas in these fishes with respect to low argon-nitrogen ratios may not depend so much upon how gases get into the swimbladder as how they are lost from it.

A group of experiments was performed to determine the effects of hydrostatic pressure and time (depth history) on swimbladder gas composition. The objectives were to monitor changes, if any, in the percentages of various gases (A, N_2, and O_2) in the swimbladder as well as changes in argon-nitrogen ratios, and to determine whether or not gas secretion was taking place. Groups of rainbow trout (Salmo gairdnerii) (n=12-26) were submerged in live cars to depths of 32 and 64 feet, corresponding to total hydrostatic pressures of 2 and 3 atmospheres respectively. Groups were held at their respective depths for 2, 4, 6, 8, and 10 days, after which sampling was performed. Individuals were sampled only once, then released. Another set of experiments was performed in the laboratory, where individuals were held at a depth of less than 1 foot, but denied any access to the surface. Sampling was performed after 4, 6, 8, 10, and 12 days. In this latter set of experiments swimbladder volumes were determined for each fish before restriction from the surface commenced and just prior to sampling of the swimbladder gas. Results for swimbladder volume determinations are given in Table II. In every case the volumes decreased over time, despite the shallow depth at which the fish were kept. The percent changes (decreases) from the original values were computed and these data were treated by analysis of variance for standard regression. The results of this analysis are given in Table III and the data are plotted in Fig. 7. All individuals were observed carefully during periods of restriction from the surface. All were close to neutral buoyancy at the start of each experiment and swam placidly in mid-water, with no apparent effort or uneasiness. After a few days, however, as the fish became negatively buoyant, they became agitated, trying to break through the plastic grille which kept them from the surface. Those kept from the surface for more than 6-8 days appeared to spend most of their time resting on the bottom of the tank and, except for occasional struggles to gain access to the surface, only ascended with considerable effort into mid-water during feeding. Animals which became negatively buoyant were obviously distressed and most certainly would have filled their swimbladders by any means possible had they been able to do so.

A summary of the data gathered on the effects of depth history on the percentages of A, N_2, and O_2 and on the argon-nitrogen ratios in swimbladders of fish subjected to submersion experiments is given in Table IV. It can be seen from an inspection of the data that there is a general decrease in the proportion of O_2 with time

TABLE II

Summary of Swimbladder Volume Experiments
(V_o: initial volume, V_t: volume at the end of the test period)

4-day			6-day			8-day		
V_o	V_t	% change	V_o	V_t	% change	V_o	V_t	% change
6.25	4.01	35.84	6.17	4.32	29.98	6.30	3.25	48.41
5.97	3.93	33.84	5.44	3.27	39.89	5.72	2.98	47.90
5.72	3.84	32.87	5.82	3.36	42.27	5.99	2.86	52.25
5.83	4.33	25.73	6.23	3.42	45.10	6.61	3.53	46.60
6.10	4.12	32.46	5.90	3.14	46.78	6.26	2.99	52.24

10-day			12-day		
V_o	V_t	% change	V_o	V_t	% change
5.95	2.27	61.85	7.17	2.28	68.20
5.80	2.27	61.03	6.97	2.09	70.01
6.03	2.10	65.17	6.71	2.34	65.13
6.14	2.65	56.84	5.84	1.98	66.10
6.27	2.21	64.75			

TABLE III

Anova table and standard regression for percent changes in swimbladder volumes as a function of time: single classification anova for unequal sample size; more than one value of Y per value of X. Estimation of variance components indicates a significant effect of time on swimbladder volumes in fish restricted from the surface ($P<<0.001$), that differences among groups may be accounted for by linear regression ($P<0.001$), and that deviations from regression are probably not significant ($0.25<P<0.50$).

Source of Var.	df	SS	MS	F_s	$P_{s_1^2=s_2^2}$
$\bar{Y}-\bar{\bar{Y}}$ Among Groups	4	39.3120	9.8280	61.46	$P<<0.001$
$\hat{Y}-\bar{Y}$ Linear Reg.	1	38.9104	38.9104	290.59	$P<0.001$
$\bar{Y}-\hat{Y}$ Dev. from Reg.	3	0.4016	0.1339	0.837	$.25<P<.50$
$Y-\bar{Y}$ Within Groups	20	3.1970	0.1599		
$Y-\bar{\bar{Y}}$ Total	24	24.5090			

Regression equation: $\hat{Y} = 13.537 + 4.607X$

FIGURE 7

Percent change (decrease) in swimbladder volume plotted against time for rainbow trout restricted from the surface. The least squares regression equation for the line in the figure is: $\hat{Y} = 13.537 + 4.607X$.

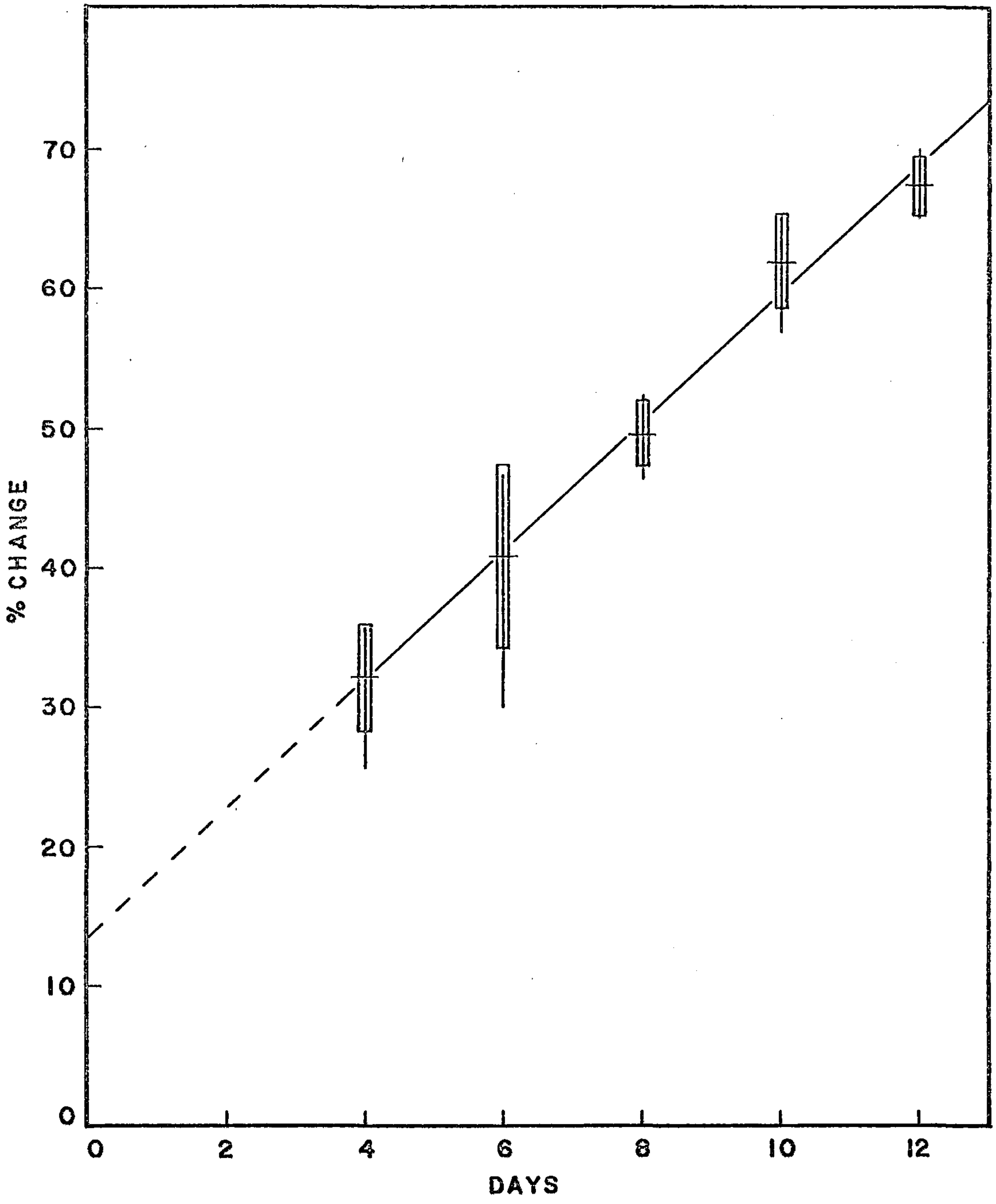

70
60
50
40
30
20
10
0
% CHANGE
0
2
4
6
8
10
12
DAYS

TABLE IV

Summary of Submersion Experiments
(all entries are means of sample size (n))

Atm.	days	0	2	4	6	8	10	12
1	%A	1.01		1.01	1.00	0.98	0.99	0.98
	%N_2	88.83		96.30	96.71	95.20	97.23	96.03
	%O_2	11.05		2.66	2.29	3.82	1.67	2.99
	100A/N_2	1.1430		1.0529	1.0386	1.0274	1.0191	1.0209
	(n)	(22)		(5)	(5)	(6)	(5)	(4)
2	%A		0.87	0.83	0.72	0.71	0.68	
	%N_2		95.61	97.17	97.43	98.31	98.52	
	%O_2		3.52	2.00	2.18	0.98	0.80	
	100A/N_2		0.9109	0.8530	0.7479	0.7277	0.6880	
	(n)		(14)	(15)	(26)	(12)	(19)	
3	%A		0.80	0.67	0.56	0.54	0.52	
	%N_2		95.48	97.84	98.60	99.19	98.94	
	%O_2		3.77	1.55	0.84	0.28	0.55	
	100A/N_2		0.8411	0.6877	0.5687	0.5403	0.5272	
	(n)		(15)	(25)	(26)	(13)	(18)	

and depth. This relation will be elaborated on in the discussion section.

Argon-nitrogen ratios can be seen to decrease with time, the magnitude of the rate of decrease being a function of depth. When plotted, the data appear to indicate a hyperbola, and the following argument is made in defense of the position that the function describing the data is truly hyperbolic. There is a conceivable case in which a single species of gas in a system like the one under investigation accounts for 100% of the gas present. In such a case, if an infinitesimal amount of this gas were replaced with another, the ratio of the first gas to the second would drop precipitously from infinity to some new value. If the process of replacement was continued the first derivative (rate constant) of the function describing the change in the ratio would change from minus infinity to zero as the system approached the limit where the ratio of the first gas to the second is zero. Thus, the function describing the change in the ratio with time can be seen to be an hyperbola which is assymtotic at t_o and $gas_1/gas_2 = 0$, assuming that the process proceeds in an orderly way and no new conditions arise which would inflect the function.

The process under investigation appears to be a special case of the general one outlined above. The differences are that the argon-nitrogen ratio is approximately 0.0115 instead of infinity at t_o, that the tendency of the ratio to change is a function of the differences in chemical potentials of the gases inside and outside the swimbladder, and that the rate of change is a function of the relative permeabilities of the gases in question. These provisions do not change the general form of the function, and it can be estimated by the following polynomial:

$$f(x) = a_o + \frac{a_1}{t+c} + \frac{a_2}{(t+c)^2} + \ldots + \frac{a_k}{(t+c)^2},$$

where $f(x)$ is $100A/N_2$ at time t, $a_o \ldots a_k$ are coefficients of regression, and c is a constant equal to the distance of the vertical asymptote from the ordinate at t_o.

If $\frac{1}{t+c}$ is designated as x' and $100A/N_2$ as Y, the polynomial becomes a standard polynomial of the form

$$y = a_o + a_1x' + a_2x'^2 + a_3x'^3 + \ldots + a_kx'^k.$$

Since c cannot be set a priori or determined empirically in a direct way, it must be estimated from available information; that is, values of y ($100A/N_2$) in the region t_o to $t=10$. This can be done by taking a range of values for c based on an estimate of its value, and choosing that value which yields the least residual squares when the data are fitted to a polynomial regression equation.

Inasmuch as polynomial regression is a special case of linear regression, the theory of minimizing residual squares with the addition of successive terms to the polynomial in order to find the appropriate number of terms needed for the "best fit" assumes linearity of the x' component. In this instance, x' in the polynomial is taken to equal 1/x+c. Since c is an estimated independent variable, x' is nonlinear in c. Strictly speaking, therefore, the theory does not apply to the case in question. It can be argued, however, that successive approximation technique or interpolation to obtain the estimate of c will affect the confidence in the regression equation only slightly, and subtracting one or two degrees of freedom should expand the confidence interval to an appropriate degree. The total number of y's (109 and 119) is large enough in this case to presume an accurate fit despite any error involved in estimating c.

Bearing in mind the above considerations, all abscissal values (time) for submersion experiments were transformed by adding a variety of constants (c's) in turn and taking the reciprocals of these sums as independent variables for use in a standard polynomial regression computer program*. The program performed analysis of variance and provided regression equations for all values of c. Although a polynomial regression procedure was used, it was determined that there was in no case a significant improvement of fit when polynomials of greater than first order were tried ($P_{\text{type II}} < 0.01$).

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*BMD05R - Polynomial Regression; Health Sciences Computing Facility, UCLA.

Anova tables and regression equations incorporating those values of c which yielded the least residual squares in the successive approximation procedure are given in Tables V, VI, and VII, representing bodies of data corresponding to the three depths used in the submersion experiments. The Y-intercepts for the transformed regression equations represent horizontal asymptotes, the equilibrium condition, for the change in argon-nitrogen ratios with respect to time. The transformed data for depths of 32 and 64 feet are plotted with their regression lines in Fig. 8. The retransformed data for all three depths are plotted with regression lines in Figs. 9, 10, and 11 (0, 32, 64 feet, respectively). All regressions are highly significant ($P<<0.001$).

As may be expected, there is a relation between the argon-nitrogen ratio equilibrium condition and depth. The horizontal asymptotes are plotted against depth in Fig. 12.

The relations which have become evident from the submersion experiments lend themselves to a general mathematical description. This description is intended to show specifically whether or not there is a tendency, under the conditions encountered in the submersion experiments, for the argon-nitrogen ratios to approach an equilibrium state which can be predicted on the basis of the physical properties of the two gases. The model assumes that fish with swimbladders are mere bubbles (with very thick "walls"), but since it is the equilibrium condition and not the rate at which it is reached that is of

TABLE V

Analysis of variance and regression analysis of argon-nitrogen ratios for fish submerged 0 ft. The analyses show a highly significant regression ($P \ll 0.001$) and good correlation ($r = 0.80739$) between the argon-nitrogen ratios and time.

$c = 3.05$

Source of Var.	df	SS	MS	F_s	$P_{s_1^2=s_2^2}$
$\hat{Y}-\bar{\bar{Y}}$ Due to Regression	1	0.14763	0.14763	84.27	$P \ll 0.001$
$\bar{Y}-Y$ Dev. from Reg.	45	0.07684	0.00175		
$Y-\bar{\bar{Y}}$ Total	46	0.22647			

Regression coefficient: 0.48249

Standard error of regression coefficient: 0.05249

Correlation coefficient: 0.80739

Regression equation: $\hat{Y} = 0.98473 + 0.48249X'$

Retransformed regression equation: $\hat{Y} = 0.98473 + \dfrac{0.48249}{X + 3.05}$

TABLE VI

Analysis of variance and regression analysis of argon-nitrogen ratios for fish submerged 32 ft. The analyses show a highly significant regression ($P<<0.001$) and excellent correlation ($r = 0.95881$) between the argon-nitrogen ratios and time.

$c = 3.70$

Source of Var.	df	SS	MS	F_s	$P_{s_1^2=s_2^2}$
$\hat{Y}-\bar{\bar{Y}}$ Due to Regression	1	2.86697	2.86697	1207.9	$P<<0.001$
$\bar{Y}-Y$ Dev. from Reg.	106	0.25160	0.00237		
$Y-\bar{\bar{Y}}$ Total	107	3.11857			

Regression coefficient: 2.36806

Standard error of regression coefficient: 0.06812

Correlation coefficient: 0.95881

Regression equation: $\hat{Y} = 0.51861 + 2.36806X'$

Retransformed regression equation: $\hat{Y} = 0.51861 + \frac{2.36806}{X + 3.70}$

TABLE VII

Analysis of variance and regression analysis of argon-nitrogen ratios for fish submerged 64 ft. The analysis shows a highly significant regression ($P<<0.001$) and excellent correlation ($r = 0.96588$) between the argon-nitrogen ratios and time.

$c = 3.40$

Source of Var.	df	SS	MS	F_s	$P_{s_1^2=s_2^2}$
$\hat{Y}-\bar{\bar{Y}}$ Due to Regression	1	5.81939	5.81939	1627.3	$P<<0.001$
$\bar{Y}-Y$ Dev. from Reg.	117	0.41841	0.00358		
$Y-\bar{\bar{Y}}$ Total	118	6.23780			

Regression coefficient: 2.91839

Standard error of regression coefficient: 0.07238

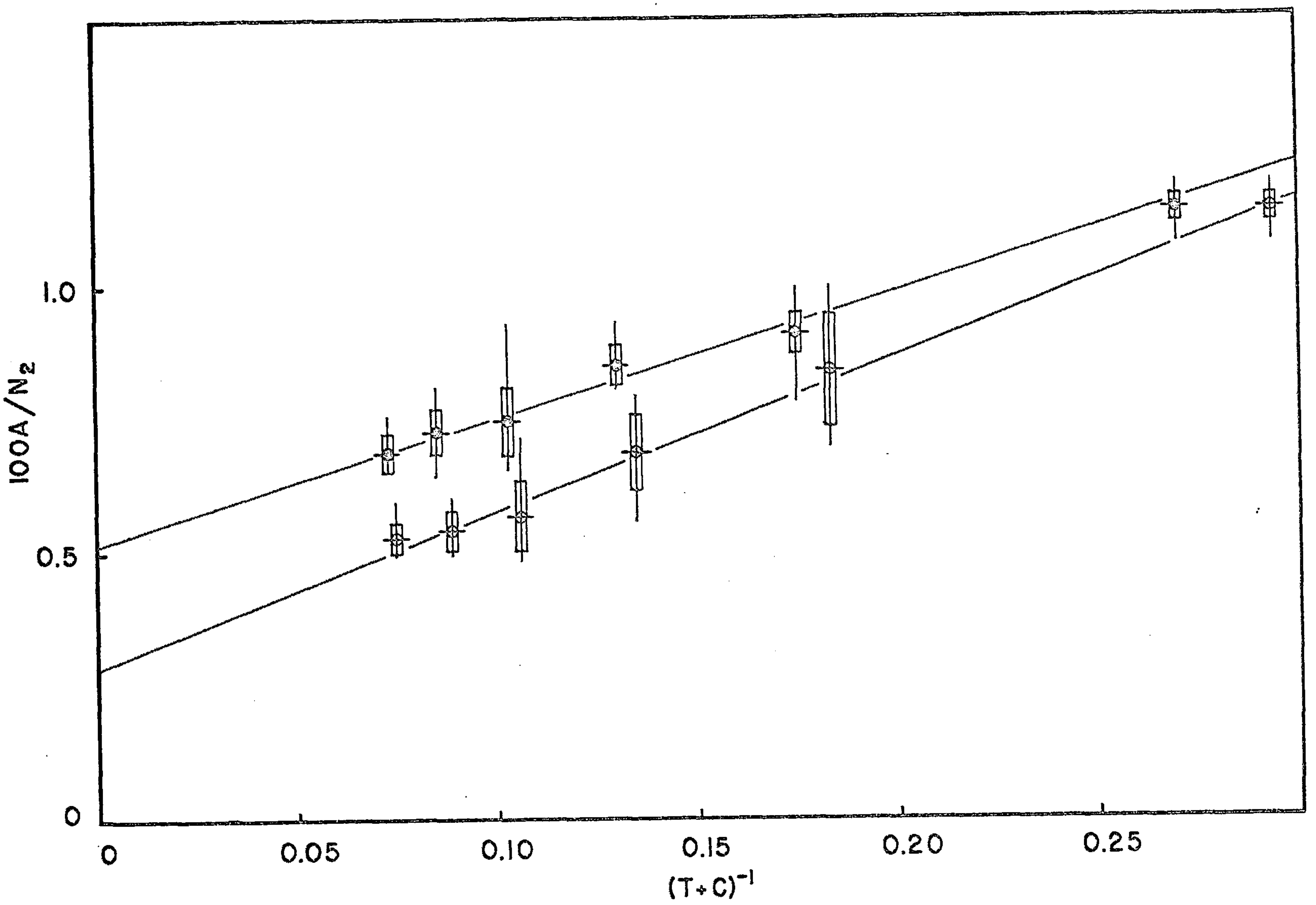
Correlation coefficient: 0.96588

Regression equation: $\hat{Y} = 0.28660 + 2.91839X'$

Retransformed regression equation: $\hat{Y} = 0.28660 + \dfrac{2.91839}{X + 3.40}$

FIGURE 8

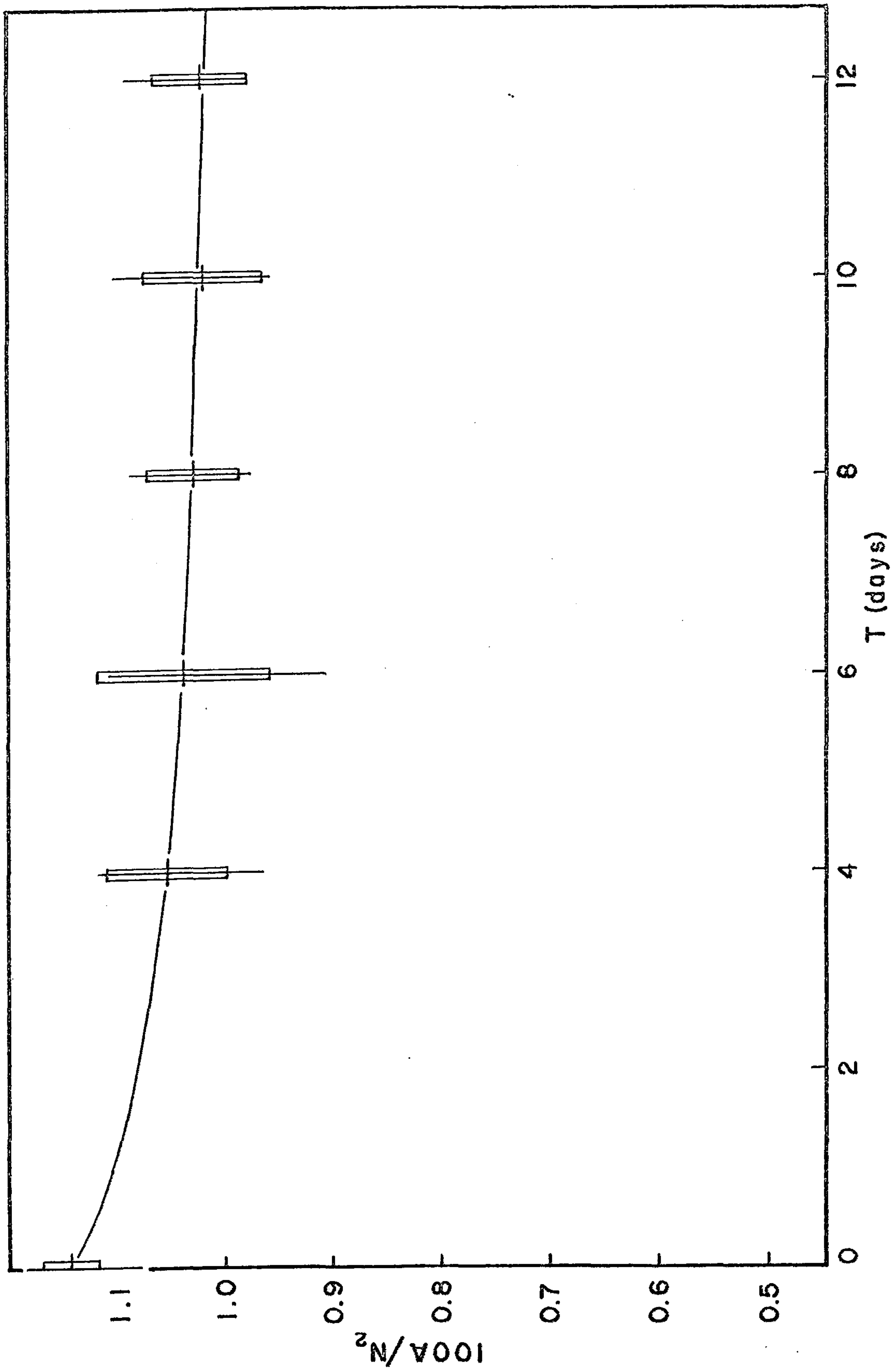
Transformed data (means, ranges, and standard deviations) and least squares regression (1^{st} order) for argon-nitrogen ratios in swim-bladder gas of rainbow trout submerged 32 feet (closed circles) and 64 feet (open circles) plotted against time. The regression equation for the transformed data for the 32 foot series is $\hat{Y} = 0.51861 + 2.36806X'$; that for the transformed data for the 64 foot series is $\hat{Y} = 0.28660 + 2.91839X'$.



1.0
0.5
0
100A/N_2
0
0.05
0.10
0.15
0.20
0.25
$(T+C)^{-1}$

FIGURE 9

Argon-nitrogen ratios in swimbladder gas of rainbow trout submerged less than one foot plotted against time. Means, ranges, and standard deviations of groups of data are represented, along with the least squares hyperbolic regression line. The regression equation is $\hat{Y} = 0.98473 + \frac{0.48249}{X+3.05}$.



1.1
1.0
0.9
0.8
0.7
0.6
0.5
100A/N$_2$
0
2
4
6
8
10
12
T (days)

FIGURE 10

Argon-nitrogen ratios in swimbladder gas of rainbow trout submerged 32 feet plotted against time. Means, ranges, and standard deviations of groups of data are represented, along with the least squares hyperbolic regression line. The regression equation is

$$\hat{Y} = 0.51861 + \frac{2.36806}{X+3.70} .$$

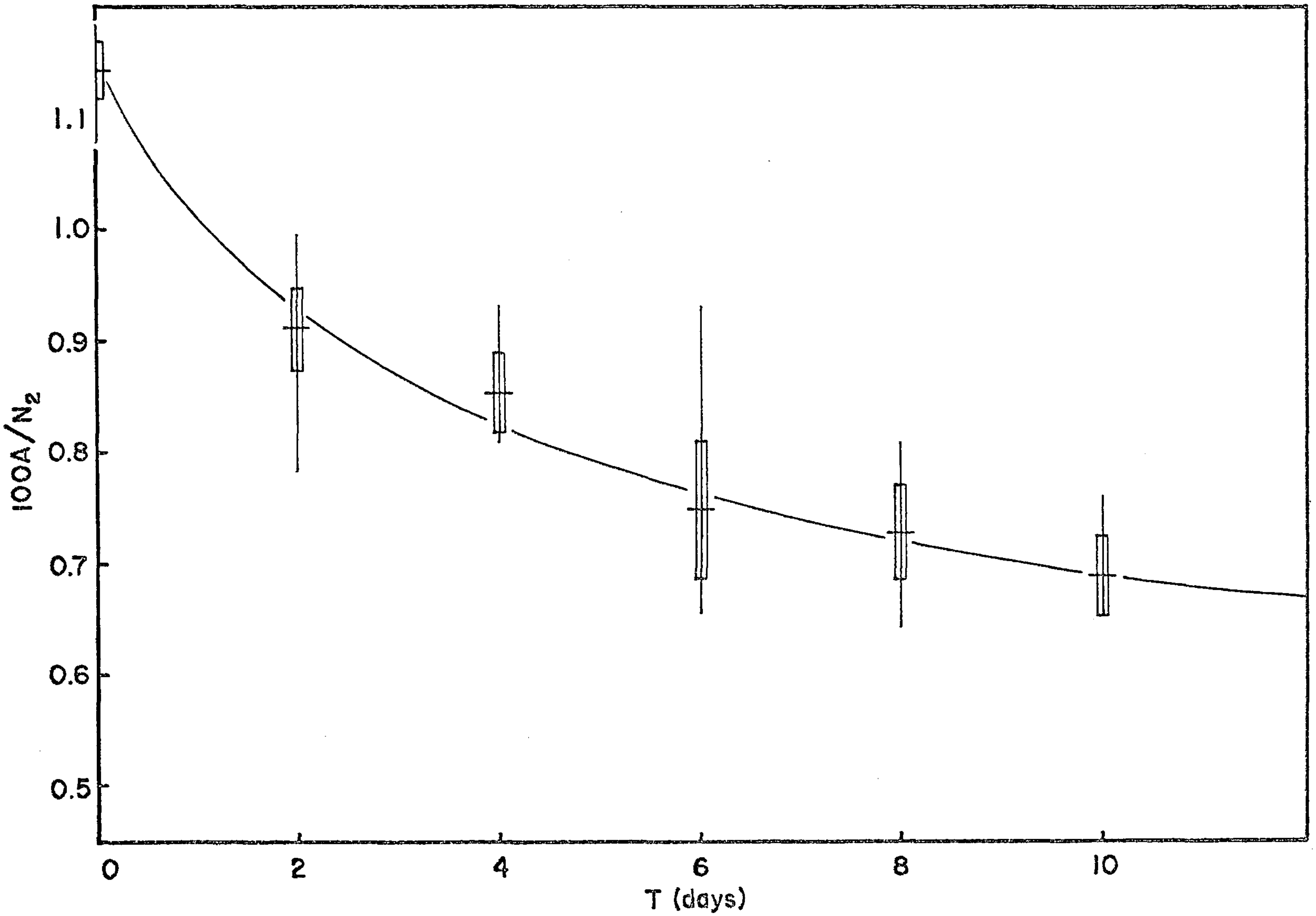

1.1
1.0
0.9
0.8
0.7
0.6
0.5
100A/N_2
0
2
4
6
8
10
T (days)

FIGURE 11

Argon-nitrogen ratios in swimbladder gas of rainbow trout submerged 64 feet plotted against time. Means, ranges, and standard deviations of groups of data are represented, along with the least squares hyperbolic regression line. The regression equation is

$$Y = 0.28660 + \frac{2.91839}{X+3.40} .$$

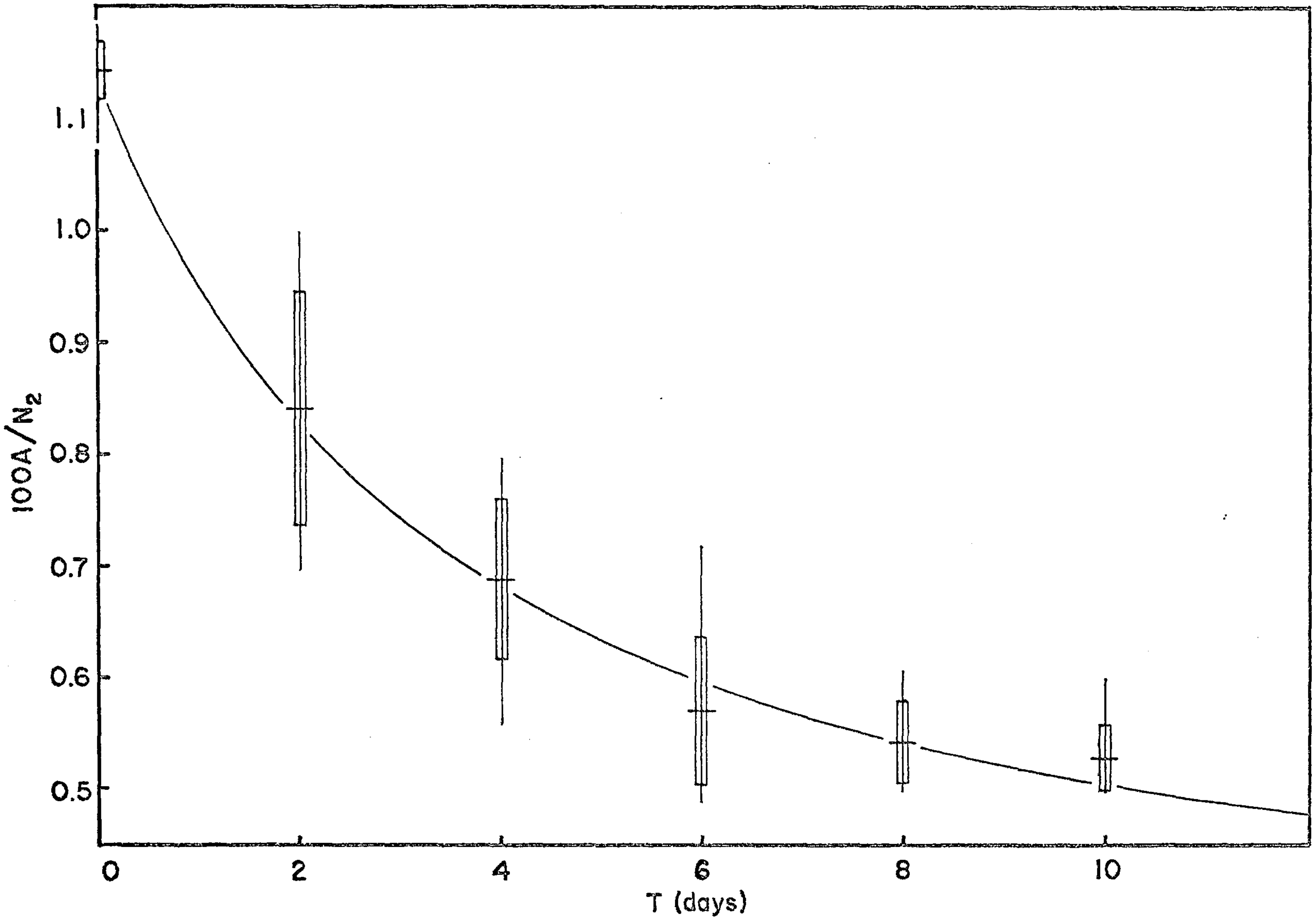
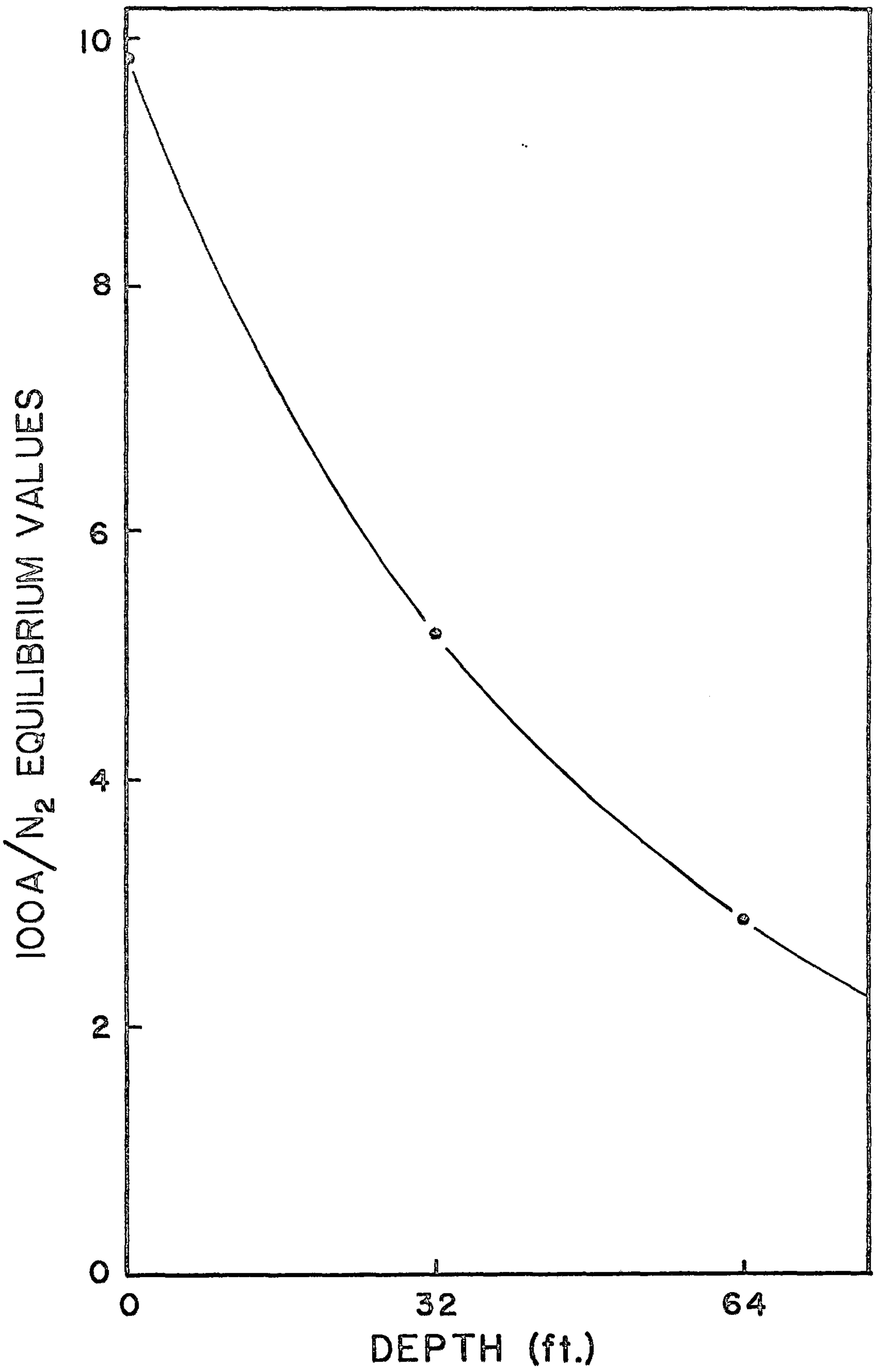

1.1
1.0
0.9
0.8
0.7
0.6
0.5
100A/N_2
0
2
4
6
8
10
T (days)

FIGURE 12

Horizontal asymptotes (equilibrium values) for changes in argon-nitrogen ratios in the swimbladder gas of rainbow trout plotted against submersion depth. The curve is not a least squares fit, but should give an indication of the nature of the relation.



100A/N$_2$ EQUILIBRIUM VALUES
10
8
6
4
2
0
0
32
64
DEPTH (ft.)

interest here, the assumption may be made without invalidating the model. A second assumption is that no gas secretion is taking place. Secretion (to make up for loss of gas due to diffusion) would obviously lead to an argon-nitrogen steady-state quite different from that predicted by a model taking into account only passive loss, and would depend to a large extent on the argon-nitrogen ratio of newly secreted gas as well as whether or not the secretion rate was keeping up with the rate of gas loss.

The symbols to be used are defined as follows:

x : mole fraction of a gas
n : number of moles of a gas
t : time
T : absolute temperature
R : gas constant (0.082 l. atm. mol^{-1} °K^{-1})
p : hydrostatic pressure (mm. Hg)
p_o: partial pressure of a gas outside the "bubble"
r : "bubble" radius (cm)
δ : proportionality constant (see text)
α : solubility coefficient of a gas
D : diffusion coefficient of a gas
$\overline{P}$: permeability coefficient; the product of α and D
d : thickness of the diffusion barrier ("bubble wall")

The number of mols of a gas in a spherical bubble is given as

$$n = \frac{4}{3RT}\pi r^3 p. \tag{1}$$

From Fick's first law of diffusion,

$$dn/dt = -\delta 4\pi r^2 (p-p_o) \tag{2}$$

where the proportionality constant δ is equal to $\frac{D\alpha}{d}$. Wyman, et al., (1952) showed that the values for α and D change in opposite directions with changes in temperature in such a way that their product, $\overline{P}$, remains roughly constant over the range of biological temperatures.

In a mixture of gases, equation (1) can be rewritten for any one gas as

$$n_1 = \frac{4\pi r^3}{3RT} x_1 p \tag{3a}$$

and equation (2) for the same gas as

$$-dn_1/dt = \delta_1 4\pi r^2 (x_1 p - p_{1_o}) \ . \tag{4a}$$

For the second gas in a mixture of two gases, equations (1) and (2) may be rewritten as

$$n_2 = \frac{4\pi r^3}{3RT}(1-x_1)p \tag{3b}$$

and

$$-dn_2/dt = \delta_2 4\pi r^2 [(1-x_1)p - p_{2_o}] \ . \tag{4b}$$

If the subscript "1" is allowed to stand for argon and the subscript "2" for nitrogen, equations (3a, b) and (4a, b) may be combined, after taking account of the fact that $\delta_1 \approx 2\delta_2$ and bearing in mind that n, x, and r are all functions of t, to obtain in terms of dx_1 and dt:

$$dx_1 = \frac{3RT\delta_2}{rp}[x_1^2 p - x_1(p+p_{2_o}+2p_{1_o}) + 2p_{1_o}]dt \quad . \qquad (5)$$

It can be seen that there exists a value of x_1 such that $dx_1/dt = 0$, $r \neq 0$. In this case it is the quantity enclosed in brackets in equation (5) which diminishes to zero as equilibrium is approached, and which may serve as an index in comparing the relative "closeness" of several samples to equilibrium. The sign of this quantity indicates whether the mol fraction of argon (x_1) is increasing (+) or decreasing (-) with time. When the data (means) from the submersion experiments are treated with the quantity enclosed in brackets in equation (5) (designated λ), it becomes apparent that, given enough time, dx_1/dt will approach zero, but will never become positive, which would be the case if nitrogen were being selected for in an active way. Values of λ computed from means of groups of data from submersion experiments for depths of 32 and 64 feet are given in Table VIII and are plotted in Fig. 13. All computations were based on barometric pressures in Eugene, Oregon and then corrected for the altitude at which the experiments took place (2605 ft.). The average barometric pressure at that altitude was found to be 688 mm. Hg.

TABLE VIII

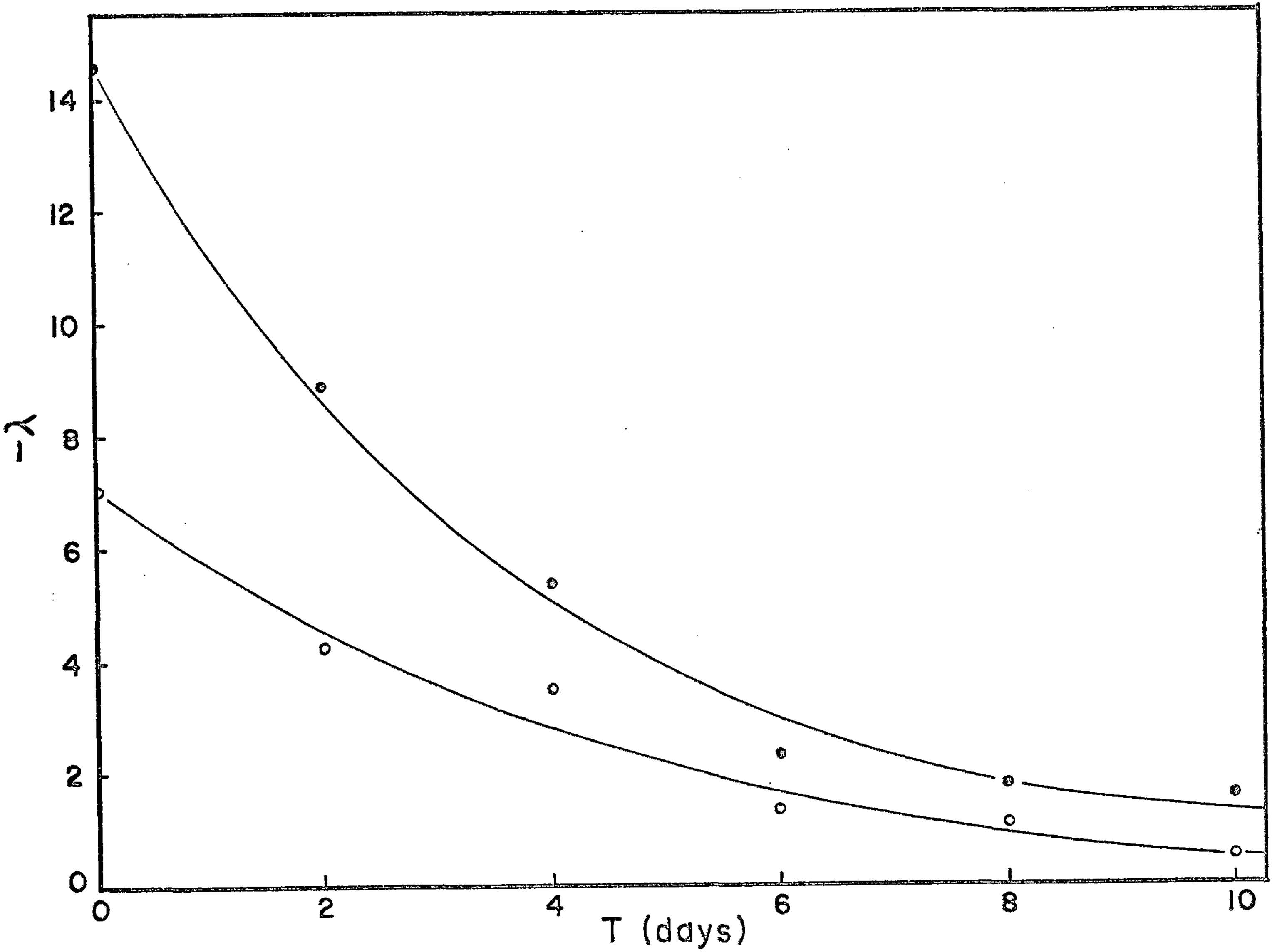
λ-Values From Means of Groups of Submersion Data

$$\lambda = x_1^2 p - x_1(p + p_{2_o} + 2p_{1_o}) + 2p_{1_o}$$

	32 ft.		64 ft.	
t (days)	$x_1(\mathrm{x}10^2)$	$-\lambda$	$x_1(\mathrm{x}10^2)$	$-\lambda$
0	1.01	7.04	1.01	14.62
2	0.87	4.27	0.80	8.91
4	0.83	3.49	0.67	5.37
6	0.72	1.31	0.56	2.36
8	0.71	1.11	0.54	1.81
10	0.68	0.51	0.52	1.57

FIGURE 13

Values of λ ($\lambda = x_1^2 p - x_1(p+p_{2_o}+2p_{1_o}) + 2p_{1_o}$) computed from means of groups of data from submersion experiments for depths of 32 feet (open circles) and 64 feet (closed circles).


$-\lambda$
14
12
10
8
6
4
2
0
0
2
4
6
8
10
T (days)

PERMEABILITY EXPERIMENTS

In order to establish the possible role of differential permeabilities of argon and nitrogen in swimbladder membrane in the changing ratios of these gases observed in field experiments, a series of experiments was performed using the isolated membrane. It was reasoned that if argon was passing out of the swimbladder faster than nitrogen, the argon-nitrogen ratio would decrease with time. Evidence that the permeability of argon might exceed that of nitrogen in swimbladder membrane exists in the literature.

Enns et al. (1967), in considering the loss of inert gases from the swimbladders of physoclistous fishes concluded that solubility alone determines the rates at which gases are lost. There was no confirmation from field or experimental data given. In the light of other work, their reasoning seems incomplete at best. Wyman et al. (1952) invoked Fick's first law of diffusion and confirmed experimental observations by calculating that the rate of solution of a bubble in sea water is proportional to the product of the diffusion coefficient and the solubility of the diffusing gas. They also showed that the change in composition of a bubble of a binary gas mixture over time as it dissolves will favor the gas with the lesser product of solubility and diffusion coefficients. Van Lieu and Hlastala (1969) performed a similar investigation on small bubbles in blood and came to the same conclusions.

Piiper, Canfield and Rahn (1962) studied the absorption rates of inert gases from subcutaneous gas pockets in rats, and found that the ratio of absorption rates for argon and nitrogen ($\dot{V}_A/\dot{V}_{N_2}$, where $\dot{V}$ is the rate of decrease of the volume of a gas) was 1.90. They reasoned that for a diffusion limited case the ratio of absorption rates should be equal to the ratio of the "permeation coefficients" (the phrase coined by Dittmer and Grebe, 1958). These coefficients are defined in exactly the same way the term "permeability" is for this work as the product of the solubility of a gas (α) and its diffusion coefficient (D). Tucker and Tenny (1966) refined the work of Piiper, Canfield and Rahn and predicted a ratio of 2.10. Their own experimental results showed a ratio of only 1.80. Piiper, Humphrey and Rahn (1962), in a limited theoretical treatment of the gas content in pressurized subcutaneous gas pockets and swimbladders, arrived at an equation giving the ratio of absorption rates of two gases in terms of the product of the ratio of their "permeation coefficients" and the ratio of the differences of their partial pressures inside and outside the gas pocket. Van Lieu _et al_. (1968) generalized these conclusions for both exit and entry of gas vis-a-vis a gas pocket.

Piiper (1970) in a more rigorous theoretical treatment of absorption of inert gases from gas pockets in animal tissues showed that the ratio of rates of absorption of two inert gases in binary mixture is determined by the ratio of partial pressure differences and by a factor depending on the physical properties of the gases. In a diffusion limited case these physical properties were again asserted to be the solubility of the gas in tissues and its diffusion coefficient. The author incorrectly cited Piiper, Canfield and Rahn (1962), using for empirical verification of his model a ratio of absorption rates uncorrected for partial pressures, but nonetheless managed to come to close agreement with the wrong figure. Fortunately, the error was a relatively small one. The theoretical value given for the ratio of absorption rates ($\dot{V}_A/\dot{V}_{N_2}$) for a diffusion limited system was 1.92. It should be pointed out that the values used by Piiper (1970), Piiper, Canfield and Rahn (1962), and Piiper, Humphrey and Rahn (1962) for the solubilities of argon and nitrogen are those for distilled water. The ratio of solubilities of these gases in animal tissues can be quite different from that in water, depending on the nature of the tissue. In addition, the values for diffusion coefficients and those for solubility coefficients used by these authors in their calculations were taken for different temperatures.

In the present study a somewhat different approach was taken. A basic weakness of all the models developed by the authors cited above was that each was constructed from data of principally the same nature as those used to verify the model itself. In other words, a model describing the change in volume of a bubble can be constructed from relations which were developed by watching bubbles dissolve. It would seem that a more valid approach would be to construct a model from data of an entirely different nature than those used to verify the model. In this way if the model and the data compare favorably, one may have a great deal more confidence in the validity of any explanation offered by the model.

Fick's first law of diffusion states that the rate of transfer of a substance S (dQ_s/dt) in direction x is proportional to the concentration gradient ($-dC_s/dx$), and the area ($dy \cdot dz$) through which the transfer is taking place. The equation for transfer is therefore:

$$dQ_s/dt = D_s(dy)(dz)(-dC_s/dx), \tag{1}$$

where D_s is an appropriate proportionality constant. This proportionality constant is the coefficient of diffusion of S. Solving for D_s, equation (1) becomes

$$D_s = \frac{dQ_s/dt}{(dy)(dz)(-dC_s/dx)} \tag{2}$$

D_s varies with the absolute temperature, the molecular weight of S, and with the degree of solvent-solute interaction. When considering the diffusion of gases, it is convenient to express concentration gradients in terms of partial pressures, quantities in terms of volumes at standard temperature and pressure (STP), and concentrations as volumes at STP per volume of solution. The Bunsen solubility coefficient α expresses solubility in terms of the volume of gas (V_x) dissolved in a volume of liquid (V_{liq}) and the pressure of the gas (P_x):

$$\alpha = (V_{x(STP)}/V_{liq})/P_x. \qquad (3)$$

Equation (1) may now be rewritten, replacing the expression for area (dy·dz) with the symbol A and applying equation (3), as:

$$dQ_s/dt = A\ D_s\alpha_s(-dP_s/dx) \qquad (4)$$

or

$$dQ_s/dt = (\text{permeability coefficient})A(-dP_s/dx) \qquad (5)$$

where the permeability coefficient is defined as:

$$\overline{P} = \alpha_s D_s. \qquad (6)$$

The product of the solubility coefficient and the diffusion coefficient has also been termed the "diffusion constant" for a gas in a liquid or solid. The term "permeability coefficient" was chosen here to avoid any confusion with "diffusion coefficient."

The Stokes-Einstein relation describes the diffusion coefficients of gases in terms of their molecular radii and viscosities:

$$D = \frac{RT}{N_o} \cdot \frac{1}{6\pi\eta r} \qquad (7)$$

where D is the diffusion coefficient, R is the gas constant, T is the absolute temperature, η is the viscosity, N_o is Avagadro's number, and r is the molecular radius. Since it was judged impossible to measure the viscosity of either swimbladder membrane or dialysis membrane directly, individual determinations of how closely the data follow the Stokes-Einstein relation are also impossible. A relative determination can be made, however, by combining the Stokes-Einstein relation with equation (6) derived from Fick's first law and comparing the ratios of the experimentally determined permeabilities of nitrogen and argon in one membrane to a predicted theoretical ratio. In this case, viscosities cancel, along with R, T, N_o, and 6π, and one is left with:

$$\frac{\overline{P}_1}{\overline{P}_2} = \frac{r_2 \cdot \alpha_1}{r_1 \cdot \alpha_2} \tag{8}$$

This equation states that as long as two gases are diffusing through the same membrane or into the same liquid, their relative permeabilities, $\overline{P}_{1,2}$, will depend only on their radii and solubilities. Measurements of the radii of nitrogen and argon appear together in several places in the literature (McClellan and Harnsberger, 1967; el Nadi, 1951; Loeb, 1931; CRC Handbook, 1970). The solubilities of argon and nitrogen in swimbladder membrane at 10°C. were calculated from freezing point depressions given by Busnel (1942) and found to be $\alpha_A = 0.0318$ and $\alpha_{N_2} = 0.0160$. Studies by Krieger, et al. (1967) show clearly that the permeability coefficients are concentration independent, allowing application of $\overline{P}$'s determined at one gas concentration to systems where the gas concentration is considerably different. With this information, theoretical ratios of permeabilities for these two gases (argon and nitrogen) may be calculated. These theoretical ratios are given in Table IX.

TABLE IX

r_{N_2}/r_A	METHOD	PREDICTED P_A/P_{N_2}
1.1049	viscosity[1]	2.6311
1.2314	gas isotherms[1]	2.9323
1.2343	Sutherland's const.[3]	2.9392
1.2343	heat conductivity[2]	2.9392
1.0946	Van der Waals[1]	2.6066
1.0953	$b = RT_k/15P_k$ (Wohl)[1]	2.6082
1.0938	viscosity[2]	2.6047
1.0714	Van der Waals[2]	2.5513
1.0417	absorption[4]	2.4806

[1] Loeb, 1931

[2] CRC Handbook, 51st ed., 1970

[3] el Nadi, 1951

[4] McClellan, 1967

Experiments were performed which were designed to measure the permeabilities of argon and nitrogen across swimbladder membrane and dialysis membrane. A known volume of gas (54.0 cm^3) with a known starting composition (49.7% argon, 50.3% nitrogen) was allowed to diffuse across a membrane of known thickness (Tables X and XI) and a known surface area (5.07 cm^2) into a "sink" on the other side. From changes in the volume occupied by the gas and changes in its composition (argon-nitrogen ratio), the permeabilities of these gases were determined. The results of these experiments are given in Tables X and XI and plotted in Fig. 14.

The permeability coefficients computed for argon ($\overline{P}_A$) and for nitrogen ($\overline{P}_{N_2}$) were tested against each other to see if there was a difference in means. A special Model I single classification analysis of variance for two groups was used (Sokal and Rohlf, 1969). The tests showed through estimation of variance components that in both swimbladder membrane and dialysis membrane there is a highly significant difference between the permeability coefficients ($P<<0.001$). The tests are summarized in Tables XII and XIII.

In comparing the results of the diffusion experiments involving both swimbladder membrane and dialysis membrane to the predicted ratio of permeabilities calculated from the Stokes-Einstein relation and Fick's first law, the values for the ratios of permeabilities calculated from the radii of A and N_2 found in the literature and given in Table IX were treated as a population, as were the results

TABLE X

Permeabilities for Argon and Nitrogen Through Swimbladder Membrane

RUN	HRS.	$\overline{P}_A$ ($\times 10^{-7}$ cm^{2}sec^{-1})	$\overline{P}_{N_2}$ ($\times 10^{-7}$ cm^{2}sec^{-1})	$\overline{P}_A/\overline{P}_{N_2}$	Membrane Thickness ($\times 10^{-2}$ cm)
1	24	0.899	0.334	2.69	0.658
2	24	0.989	0.362	2.73	0.601
3	24	0.807	0.522	1.55	0.611
4	18	0.994	0.350	2.84	0.771
5	24	0.816	0.204	2.40	0.620
6	36	0.806	0.405	1.99	0.780
7	24	1.119	0.358	3.13	0.750
8	24	1.186	0.344	3.45	0.771
9	48	0.887	0.367	2.42	0.733
10	48	1.012	0.351	2.88	0.968
11	48	1.054	0.290	3.63	0.752
12	72	1.208	0.406	2.98	1.015
13	72	1.320	0.439	3.10	0.959
14	48	1.039	0.348	2.99	0.714
15	48	1.130	0.378	2.99	1.030
16	48	1.108	0.350	3.17	0.906
	mean:	1.023	0.363	2.80	
	s.d.	0.1545	0.0671	0.520	

TABLE XI

Permeabilities for Argon and Nitrogen Through Dialysis Membrane

RUN	HRS.	$\overline{P}_A$	$\overline{P}_{N_2}$	$\overline{P}_A/\overline{P}_{N_2}$	Membrane Thickness (x 10^{-2} cm)
		(x 10^{-7} cm^2 sec^{-1})			
1	24	1.075	0.445	2.42	0.541
2	24	1.280	0.431	2.97	0.541
3	24	1.344	0.422	3.18	0.541
4	24	1.081	0.417	2.59	0.541
5	24	0.980	0.411	2.38	0.541
6	48	1.227	0.432	2.84	0.541
7	48	1.158	0.463	2.50	0.541
8	48	1.125	0.455	2.47	0.541
9	72	1.151	0.517	2.23	0.541
10	72	1.121	0.445	2.52	0.541
	mean:	1.154	0.444	2.61	
	s.d.:	0.1058	0.0308	0.295	

FIGURE 14

Permeability coefficients computed for argon (open circles) and nitrogen (closed circles) in swimbladder and dialysis membranes. Means, ranges, and standard deviations are represented.

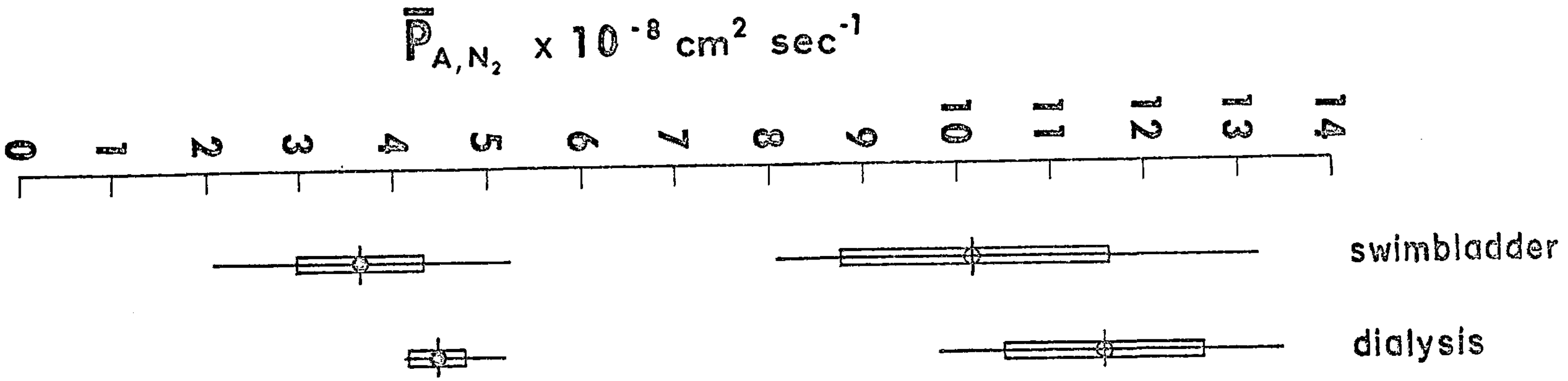

$\bar{P}_{A,N_2} \times 10^{-8}\ cm^2\ sec^{-1}$
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
swimbladder
dialysis

TABLE XII

Anova table for significance of difference between permeability coefficients of argon and nitrogen in swimbladder membrane. Special Model I single classification anova for two groups. Estimation of the variance components shows that there is a significant difference between permeability coefficients ($P \ll 0.001$).

Source of Var.	df	SS	MS	F_s	$P_{s_1^2=s_2^2}$
$\bar{Y}-\bar{\bar{Y}}$ Between groups	1	3.48876	3.48876	250.3	$P \ll 0.001$
$Y-\bar{Y}$ Within groups	30	0.41824	0.01394		
$Y-\bar{\bar{Y}}$ Total	31	3.90700			

TABLE XIII

Anova table for significance of difference between permeability coefficients of argon and nitrogen in dialysis membrane: special Model I single classification anova for two groups. Estimation of the variance components shows that there is a significant difference between permeability coefficients ($P<<0.001$).

Source of Var.	df	SS	MS	F_s	$P_{s_1^2=s_2^2}$
$\bar{Y}-\bar{\bar{Y}}$ Between groups	1	2.52334	2.52334	414.3	$P<<0.001$
$Y-\bar{Y}$ Within groups	18	0.10963	0.00609		
$Y-\bar{\bar{Y}}$ Total	19	2.63297			

from replicate diffusion experiments. Each group of permeability ratios thus obtained was tested against the others for a difference in means. Since each group was found to be heteroscedastic with respect to the other two ($P_{s_1^2=s_2^2} < 0.1$, see Table XIV), a special t-test for samples whose variances are assumed to be unequal was used (Sokal and Rohlf, 1969). The results of these tests, given in Table XV, indicate that the ratios of permeabilities of argon and nitrogen in swimbladder and dialysis membranes do not differ significantly from values predicted by calculations based on the molecular radii and solubilities of these gases. The data are plotted in Fig. 15.

Values for the permeabilities of both argon and nitrogen through a number of artificial polymer membranes appear together in the literature (Paul and DiBenedetto, 1965; Michaels, 1961; Barton, 1960; Schulz, 1956). The ratios of these values ($\overline{P}_A/\overline{P}_{N_2}$) are given in Table XVI. It may be seen that the ratios compare well with those predicted from molecular radii and solubilities.

Besides having importance in the calculation of permeability coefficients, the change in the composition of the argon-nitrogen gas mixture above the membrane in the diffusion chamber becomes important when it is compared qualitatively to the changes in swimbladder gas composition observed in field experiments. The diffusion experiments were terminated at various times so that their durations ranged from 18 to 72 hours. Data on the argon-nitrogen ratios at the

TABLE XIV

F-tests for heteroscedasticity of groups of unequal size. The test shows that each group of permeability ratios (predicted, for swimbladder membrane, and for dialysis membrane) is heteroscedastic with respect to the other two.

$\overline{P}_A/\overline{P}_{N_2}$	n	$\overline{Y}$	s^2	F_s	$P_{s_1^2=s_2^2}$
Swimbladder	16	2.803	0.2700	8.0119	$.001<P<.005$
Predicted	9	2.699	0.0337	3.1106	$.025<P<.05$
Dialysis	10	2.610	0.0868	2.5757	$.05<P<.10$

TABLE XV

T-test for equality of ratios of permeabilities of argon and nitrogen in swimbladder and dialysis membranes to predicted ratios: Special t-test for equality of means of heteroscedastic samples of unequal size. The tests show that the ratios in swimbladder and dialysis membranes do not significantly differ from predicted ratios.

$$t'_{\alpha} = \frac{t_{\alpha[\nu_1]} \frac{s_1^2}{n_1} + t_{\alpha[\nu_2]} \frac{s_2^2}{n_2}}{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}$$

Tested Groups	mean	t'_s	$P_{\mu_1=\mu_2}$
Swimbladder membrane	2.803	0.7242	$0.4<P<0.5$
Predicted	2.699		
Dialysis membrane	2.610	0.2526	$0.9<P<0.9$
Predicted	2.699		

FIGURE 15

Ratios of permeability coefficients for argon and nitrogen ($\overline{P}_A/\overline{P}_{N_2}$) predicted from the molecular radii and solubilities of these gases, and observed ratios in swimbladder and dialysis membranes. Means, ranges, and standard deviations are represented.

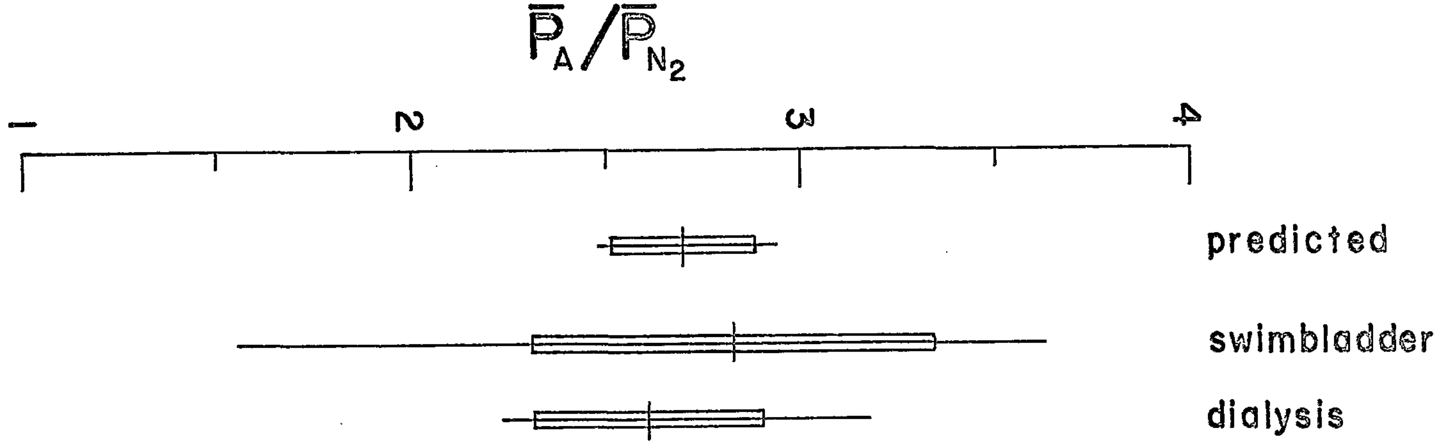

$\bar{P}_A/\bar{P}_{N_2}$
1
2
3
4
predicted
swimbladder
dialysis

TABLE XVI

Permeability Ratios ($\overline{P}_A/\overline{P}_{N_2}$) for Various Membranes

MEMBRANE	$\overline{P}_A/\overline{P}_{N_2}$	n	REFERENCE
EPR[1]-3806	2.553	5	Paul & DiBenedetto (1965)
EPR[1]-3480	2.677	3	"
EPR[1]-3460	2.289	2	"
EPR[1]-3418	2.622	4	"
Cis-4(cis-poly-butadiene)	2.298	6	"
Ameripol[2]-1502	2.503	5	"
Hycar[2]-2001	2.548	4	"
Polyethylene	2.75	1	Barton (1960)
Block polystyrol	2.652	1	Schulz (1956)
Hemipolystyrol	2.712	1	"
Amorphous polyethylene	2.811	1	Michaels (1961)
Hydrogenated polybutadiene	2.763	1	"

[1] Ethylene-propalene co-polymer

[2] Styrene-butadiene co-polymer

end of the experiments can therefore be regressed on time in much the same way as those for the submersion experiments, and an equation for the rate of change derived. Any comparison between these data and those for submersion experiments, however, must be strictly qualitative. The reasons for this restriction will be discussed more fully in the discussion section, but they basically spring from the fact that rates of change and equilibrium conditions, although analogous in the two cases, differ considerably in their quantitative aspects.

These experiments were exceptional in that the starting composition of the gas mixture (A/N_2 at $t=0$) was known precisely, the gas having been obtained as a certified mixture from the supplier. Furthermore, this same mixture was used as a standard for calibration during analysis of the samples collected at the end of each experiment. It may be said, therefore, that the starting points of these experiments were known with such precision and accuracy that variation was not measurable. Standard regression analysis of these data would not reflect the *a priori* knowledge that the regression line, if there is one, would pass through a fixed Y-intercept. It was therefore necessary to develop a technique for performing analysis of variance which satisfies the condition of prior knowledge of a fixed starting point. The technique, given in Appendix B, forces the regression line through the origin, requiring that the data be transformed to fit this condition. The actual and transformed data for argon-nitrogen ratios at the termination of permeability experiments for swimbladder membrane are given in Table XVII; those for dialysis membrane are given in Table XVIII. The fixed ordinate-intercept in both cases is $A/N_2 = 0.9881$.

For purposes of comparison, analyses of variance for standard regression and fixed ordinate-intercept regression were performed for both sets of experiments. Table XIX summarizes the analysis of variance for standard regression of argon-nitrogen ratios as a function of time for permeability experiments involving swimbladder membrane. The tests show that the ratio changes with time ($P<0.001$) and that the change may be accounted for by linear regression ($0.001<P<0.005$). Table XX summarizes analysis of variance for fixed ordinate-intercept regression for the same data. These tests give a considerably stronger indication of the effect of time on the argon-nitrogen ratio. The probabilities of a Type I error in assuming a change in argon-nitrogen ratios over time and that the change may be accounted for by linear regression have become $P\ll0.001$ in both instances. The data are plotted in Fig. 16 with the fixed ordinate-intercept regression line (solid line) and its 95% confidence limits (broken lines). It should be pointed out that no translation of a fixed point regression line is possible. For this reason the only standard error and confidence limits which are appropriate apply to the estimated regression coefficient $\hat{\beta}$. The methods for computation of these statistics are given in Appendix B.

TABLE XVII

Argon-nitrogen ratios (Y) for permeability experiments with swimbladder membrane, and transformed data (Y') for use in calculation of fixed ordinate-intercept regression. X=hours; fixed ordinate-intercept=0.9881 (starting A/N_2).

n	X=18	X=24	X=36	X=48	X=72
1	Y = 0.9387	0.9200	0.9259	0.8817	0.8016
2		0.9054		0.8737	0.7713
3		0.9543		0.8718	
4		0.9342		0.8293	
5		0.9080		0.8352	
6		0.9033		0.8734	
7		0.8857		0.8570	

Transformation: $Y_i' = Y_i - 0.9881$

n	X=18	X=24	X=36	X=48	X=72
1	Y' = -0.0494	-0.0681	-0.0622	-0.1064	-0.1865
2		-0.0827		-0.1144	-0.2168
3		-0.0338		-0.1163	
4		-0.0539		-0.1588	
5		-0.0801		-0.1529	
6		-0.0848		-0.1147	
7		-0.1024		-0.1311	

TABLE XVIII

Argon-nitrogen ratios (Y) for permeability experiments with dialysis membrane, and transformed data (Y') for use in calculation of fixed ordinate-intercept regression. X=hours; fixed ordinate-intercept= 0.9881 (starting A/N_2).

n	X=24	X=48	X=72
1	Y = 0.895	0.756	0.701
2	0.864	0.783	0.689
3	0.853	0.790	
4	0.891		
5	0.904		

Transformation: $Y_i' = Y_i - 0.9881$

n	X=24	X=48	X=72
1	Y' = -0.093	-0.232	-0.287
2	-0.124	-0.205	-0.299
3	-0.135	-0.198	
4	-0.097		
5	-0.084		

TABLE XX

Anova table and fixed ordinate-intercept regression for argon-nitrogen ratios as a function of time (hours) for permeability experiments with swimbladder membrane: Single classification anova for unequal sample size; more than one value of Y per value of X. Estimation of variance components indicates a significant effect of time on argon-nitrogen ratios ($P<<0.001$), that differences among groups may be accounted for by linear regression ($P<<0.001$), and that deviations from regression are probably not significant ($0.25<P<0.50$).

Source of Var.	df	SS	MS	F_s	$P_{s_1^2=s_2^2}$
$\bar{Y}'-\bar{\bar{Y}}'$ Among Groups	5	0.238512	0.047702	102.15	$P<<0.001$
$\hat{Y}-\bar{Y}$ Linear Reg.	1	0.236776	0.236776	545.57	$P<<0.001$
$\bar{Y}-\hat{Y}$ Dev. from Reg.	4	0.001736	0.000434	0.929	$.25<P<.50$
$Y'-\bar{Y}'$ Within Groups	13	0.006071	0.000467		
$Y'-\bar{\bar{Y}}'$ Total	18	0.244538			

(Transformation: $Y' = Y - 0.9881$)

Regression equation: $\hat{Y} = 0.9881 - 0.002714X$

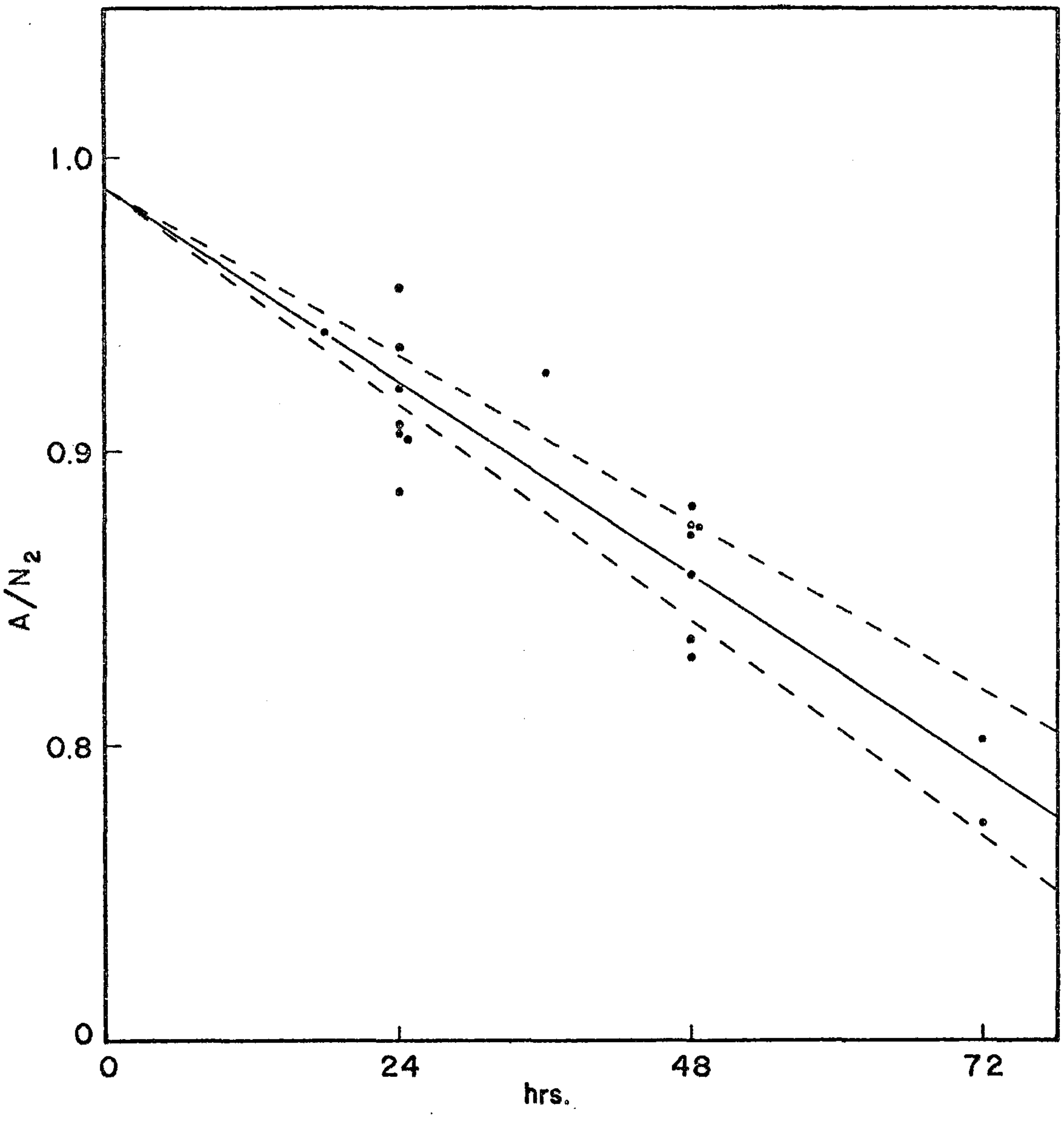
Standard error and confidence limits (95%) for b:

$S_b = 0.000116$

$L_1 = -0.003036$; $L_2 = -0.002392$

FIGURE 16

Argon-nitrogen ratios plotted against time for permeability experiments involving swimbladder membrane. The regression line (solid line) was computed using fixed ordinate-intercept regression analysis. The broken line represents 95% confidence limits around the estimated regression coefficient, $\hat{\beta}$.



1.0
0.9
0.8
0
A/N$_2$
0
24
48
72
hrs.

Analysis of variance for standard regression and fixed ordinate-intercept regression for experiments involving dialysis membrane are given in Tables XXI and XXII respectively. The results of these tests are comparable to those obtained for experiments involving swimbladder membrane. The data are plotted in Fig. 17.

In none of these analyses were the deviations from regression sufficiently large to allow pooling of the sum of squares of deviations from regression with the sum of squares within groups when testing for the presence of linear regression ($0.25<P<0.50$ in all cases).

Although "fixed point regression" appeared appropriate from purely theoretical grounds, it seemed advisable that a test be made of the null hypothesis that there is no improvement in fit when using a two-parameter line (standard regression) in place of a one parameter line (fixed point regression). The pairs of regressions for both swimbladder membrane and dialysis membrane were tested using the method given in Appendix B. The $F_{1-\alpha}$ statistics show that the probabilities of making Type II errors in using fixed point regression and assuming no increase in residual squares are $0.1<P<0.2$ for swimbladder membrane (Table XXIII), and $0.25<P<0.50$ for dialysis membrane (Table XXIV).

These probabilities are somewhat misleading in that to be completely correct a sample of a size equal to the number of experimental data points with a mean value of $\overline{Y}=0$ and variance equal to that introduced by gas sample analysis techniques would have to be

TABLE XXI

Anova table and standard regression for argon-nitrogen ratios as a function of time (hours) for permeability experiments with dialysis membrane: Single classification anova for unequal sample size; more than one value of Y per value of X. Estimation of variance components indicates a significant effect of time on argon-nitrogen ratios ($P<0.001$), that differences among groups may be accounted for by linear regression ($0.05<P<0.10$), and that deviations from regression are probably not significant ($0.25<P<0.50$).

Source of Var.	df	SS	MS	F_s	P $s_1^2=s_2^2$
$\bar{Y}-\bar{\bar{Y}}$ Among Groups	2	0.055272	0.027636	74.01	$P<.001$
$\hat{Y}-\bar{Y}$ Linear Reg.	1	0.054996	0.054996	158.27	$.05<P<.10$
$\bar{Y}-\hat{Y}$ Dev. from Reg.	1	0.000276	0.000276	0.95	$.25<P<.50$
$Y-\bar{Y}$ Within Groups	7	0.002614	0.0002904		
$Y-\bar{\bar{Y}}$ Total	9	0.057886			

Regression equation: $Y = 0.9740 - 0.003956X$

TABLE XXII

Anova table and fixed ordinate-intercept regression for argon-nitrogen ratios as a function of time (hours) for permeability experiments with dialysis membrane: Single classification anova for unequal sample size; more than one value of Y per value of X. Estimation of variance components indicates a significant effect of time on argon-nitrogen ratios ($P<<0.001$), that differences among groups may be accounted for by linear regression ($P<<0.001$), and that deviations from regression are probably not significant ($0.25<P<0.50$).

Source of Var.	df	SS	MS	F_s	P $s_1^2=s_2^2$
$\bar{Y}'-\bar{\bar{Y}}'$ Among Groups	3	0.362924	0.120975	322.60	$P<<0.001$
$\hat{Y}-\bar{Y}$ Linear Reg.	1	0.362306	0.362306	1172.51	$P<<0.001$
$\bar{Y}-\hat{Y}$ Dev. from Reg.	2	0.000618	0.000309	0.82	$.25<P<.50$
$Y'-\bar{Y}'$ Within Groups	7	0.002614			
$Y'-\bar{\bar{Y}}'$ Total	10	0.365538			

(Transformation: $Y' = Y - 0.9881$)

Regression equation: $\hat{Y} = 0.9881 - 0.004239X$

Standard error and confidence limits (95%) for b:

$s_b = 0.000124$

$L_1 = -0.004773$; $L_2 = -0.003705$

FIGURE 17

Argon-nitrogen ratios plotted against time for permeability experiments involving dialysis membrane. The regression line (solid line) was computed using fixed ordinate-intercept regression analysis. The broken line represents 95% confidence limits around the estimated regression coefficient, $\hat{\beta}$.

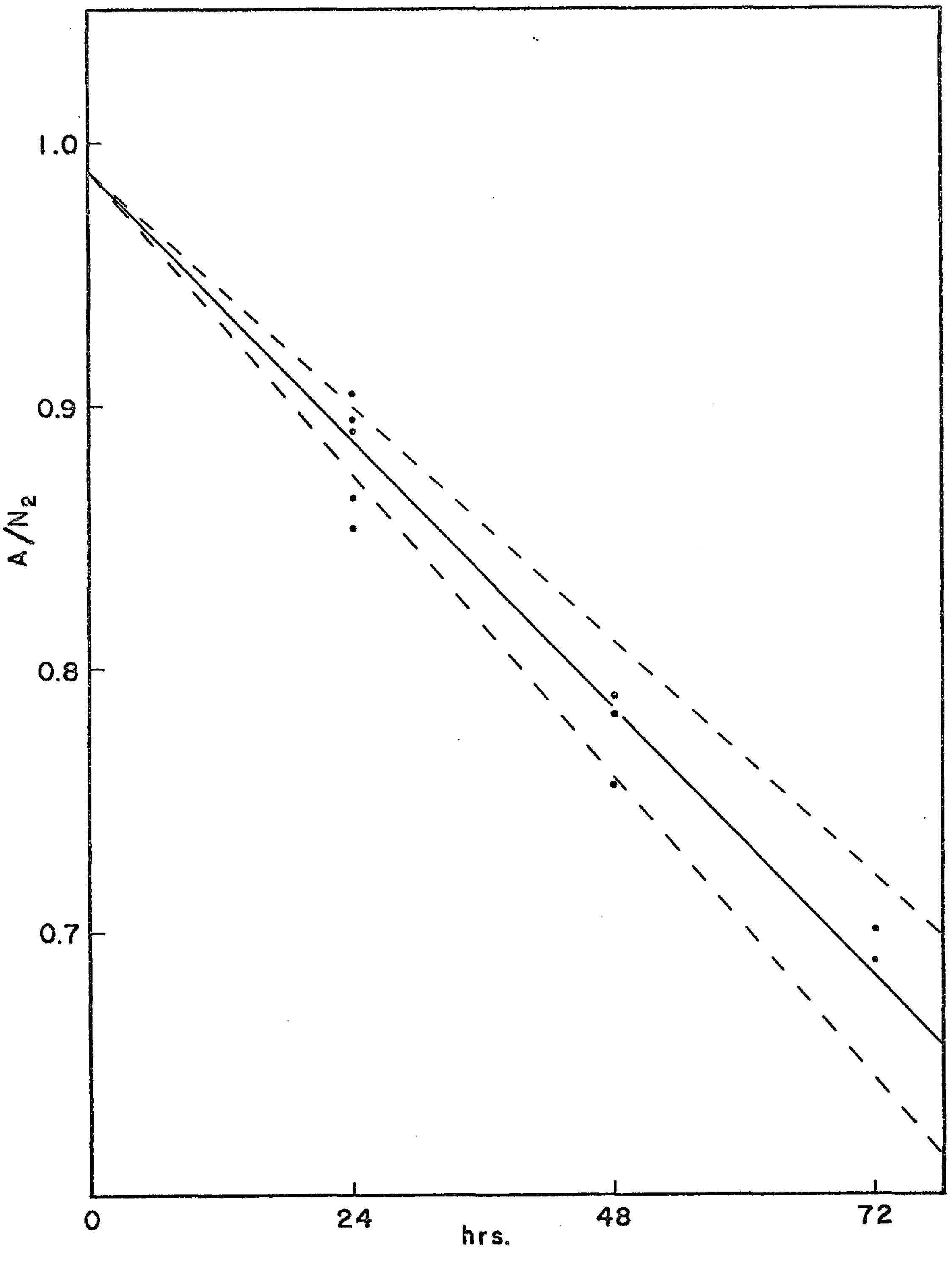

1.0
0.9
0.8
0.7
A/N_2
0
24
48
72
hrs.

TABLE XXIII

F-test for improvement of fit with a 2-parameter line regressing permeability ratios of argon and nitrogen on time for swimbladder membrane. The test shows that the probability of a type II error in using fixed ordinate-intercept regression and assuming no increase in residual squares is $0.1<P<0.2$.

Source of Variation	df	SS	MS	F_s	$P_{type\ II}$
$\bar{Y}-\hat{Y}$ Dev. from Reg. $\hat{Y}=bX$	4	0.001736			
$\bar{Y}-\hat{Y}$ Dev. from Reg. $\hat{Y}=a+bX$	3	0.001695	0.000565	0.073	$0.1<P<0.2$
difference	1	0.000041	0.000041		

TABLE XXIV

F-test for improvement of fit with a 2-parameter line regressing permeability ratios of argon and nitrogen on time for dialysis membrane. The test shows that the probability of a type II error in using fixed ordinate-intercept regression and assuming no increase in residual squares is $0.25<P<0.50$.

Source of Variation	df	SS	MS	F_s	$P_{\text{type II}}$
$\bar{Y}-\hat{Y}$ Dev. from Reg. $\hat{Y}=bX$	2	0.000618			
$\bar{Y}-\hat{Y}$ Dev. from Reg. $\hat{Y}=a+bX$	1	0.000276	0.000276	1.239	$.25<P<.50$
difference	1	0.000342	0.000342		

introduced at t=0 for the standard regression analysis. Such a procedure could not be entirely justified, however, since the added data would not be real.

What is clear from the tests is that in the case of swimbladder membrane the fixed ordinate-intercept regression line is justified with a small loss of predictive precision and in the case of dialysis membrane some predictive precision is sacrificed for greater accuracy. In the latter case, visual inspection of the data (Fig. 17) reveals a possible curvilinearity (also suggested by the test for improvement of fit). There is no doubt that both of the functions describing the change in argon-nitrogen ratios over time are curves with horizontal asymptotes at some equilibrium condition, $A/N_2>0$. The data obtained in these experiments, however, fall on a rather linear portion of the curve. Since data near the equilibrium condition are not at hand, only linear regression may be used to describe the data.

In summary, the results of the permeability experiments show that the permeability coefficients of argon and nitrogen are different, that for argon being the larger, and that this difference can lead to a faster transfer of argon across both swimbladder and dialysis membranes. A faster transfer of argon will in turn result in a significant and orderly reduction in the argon-nitrogen ratio. Under the conditions in this study, the reduction is approximately linear over a period of 72 hours.

DISCUSSION

The observation that argon-nitrogen ratios in the swimbladder gas of physoclists are greater than the value for air and that those of physostomes are less than the value for air suggests, in conjunction with the considerably greater abundance of oxygen in the swimbladders of physoclists, that the mechanisms underlying the deposition and retention of gas in the swimbladders of these two groups of fishes are different. Since the secretory mechanism for physoclists is known, an explanation can be put forward. Argon, the more soluble of the two gases is affected to a greater degree by the addition of a solute than is nitrogen; i.e., the solubility of argon is reduced more than that of nitrogen. Argon therefore tends to be multiplied to a greater extent proportionately than nitrogen. Thus a physoclist which is actively secreting would exhibit an elevated argon-nitrogen ratio in its swimbladder gas. It is suggested that Douglas (MS, 1967) and Wittenberg (1957), among others, are incorrect in supposing that the higher solubility (and hence, they suppose, the higher permeability coefficient) accounts for higher argon-nitrogen ratios. The necessary condition is a relatively greater multiplication of argon. A higher permeability coefficient of argon through the tissues, on the other hand, or a greater rate of diffusion through blood would not be in only one direction. Increased "back diffusion" in the blood within the rete capillaries would cancel the increased "forward" movement of gas into the swimbladder. High permeability through the walls of the capillaries along the length of the rete would tend to allow argon to escape from the multiplier faster than nitrogen. The process cited by Douglas and Wittenberg as an explanation for high argon-nitrogen ratios in physoclists would, working alone, tend to produce an effect opposite to that which they observed.

Douglas (MS, 1967) collected a great quantity of data on argon-nitrogen ratios of marine fishes ranging in depth from the surface to over 3500 meters. There is a depth-related feature in these data which he did not adequately deal with. Since he performed no statistical operations on the data, Douglas' own conclusions are rather subjective. Figures displaying argon-nitrogen ratios in the swimbladders of a large variety of fishes are presented, but representatives of different groups of fishes are not separated. The figures indicate a downward trend in argon-nitrogen ratios with increased depth, and, if all fishes are considered together, the relation is valid. It would seem much more prudent, however, to analyze data somewhat more critically. Douglas' data on Coryphaenoides acrolepis, a physoclist caught between 969 and 2377 meters, when regressed on depth, show that, despite a relatively large variance component probably due to varying depth histories of his specimens, argon-nitrogen ratios increase with depth ($P<0.001$, see Table XXV).

The observation by Douglas that physoclists have argon-nitrogen ratios greater than air in relatively shallow waters (less than 100 meters in depth) but are somewhat less than air in

TABLE XXV

Anova table and regression for the data of Douglas (MS, 1967) on argon-nitrogen ratios in the swimbladders of *Coryphaenoides acrolepis* as a function of depth: Single classification anova for unequal sample size; more than one value of Y per value of X. Estimation of variance components indicates a significant effect of depth on argon-nitrogen ratios ($P<0.001$), that differences among groups may be accounted for by linear regression ($P<0.001$), and that deviations from regression are not significant ($0.005<P<0.01$).

Source of Var.	df	SS	MS	F_s	$P_{s_1^2=s_2^2}$
$\bar{Y}-\bar{\bar{Y}}$ Among Groups	20	0.91343	0.04567	4.604	$P<0.001$
$\hat{Y}-\bar{Y}$ Linear Reg.	1	0.41543	0.41543	26.377	$P<0.001$
$\bar{Y}-\hat{Y}$ Dev. from Reg.	19	0.49800	0.02621	2.602	$.005<P<.01$
$Y-\bar{Y}$ Within Groups	34	0.33727	0.00992		
$Y-\bar{\bar{Y}}$ Total	54	1.25070			

physoclists residing at great depths is most likely due to the rapid leaking of argon out of the swimbladder, more than compensating for a faster argon secretion rate. The increasing argon-nitrogen ratios with depth within species tends to corroborate the idea that secretion of argon is faster than nitrogen even though observed argon-nitrogen ratios are less than the value for air. Douglas contends that it is the difference in solubility coefficients (α's) of argon and nitrogen with increasing salinity that is responsible for the slight preference for argon secretion. He reasons that an increasing ratio of solubility coefficients of argon and nitrogen with increasing salinity (α_A/α_{N_2} = K·salinity) is a factor leading to higher argon-nitrogen ratios often found in physoclist swimbladder gas. He missed the vital point that it is the rate of change of the individual solubility coefficients as salinity changes which is important, not the absolute values at a given salinity. The relation of interest is that the decrease in α_A with increasing salinity is always greater than the corresponding decrease in α_{N_2}. Douglas relation does not imply this and, taken by itself, tends to indicate lower, not higher, argon-nitrogen ratios in newly secreted gas.

All physoclists sampled in this investigation were shallow water fishes and all had argon-nitrogen ratios greater than the value for air; all physostomes sampled had argon-nitrogen ratios less than the value for air. These data fit the trends displayed by those of previous workers. It is worth noting that Scholander (1954, 1956), despite data to the contrary, reported that the argon-nitrogen ratios he measured for the swimbladder gas of Coregonus acronius are the same as the value for air. His data were highly variable and, as pointed out in the results section, this condition was probably due to a large variation in the depth histories of his specimens. Whatever the reason, Scholander's data, although they show a trend, are too few to support any critical analysis of the relations among time, depth, and argon-nitrogen ratios. The data on argon-nitrogen ratios presented here for the series of submersion experiments were obtained by closely controlling the depth histories of the experimental subjects and are therefore much less variable than those of any previous investigator. From these data the relations among time, depth, and argon-nitrogen ratios can be illucidated.

Data were also taken on the changes in the percentages of oxygen, argon, and nitrogen. An inspection of these data (Table IV) shows that there is a general decrease in the proportion of O_2 with time and depth. This represents a general contradiction to the slight increase reported in the literature for Coregonus lavaretus (Sundnes, et al., 1958; Sundnes, et al., 1969). Those data are few and highly variable, however, and data for other physostomes, particularly the Isospondyli, indicate a slight decrease in $\%O_2$ with depth (Saunders, 1953; Tait, 1956; Sundnes, et al., 1958; Sundnes, et al., 1969). For those physostomes which are capable of gas secretion (e.g., the Cyprinids) the $\%O_2$ decrease with depth is less obvious, a condition which is probably due to an O_2 concentration in freshly secreted gas which is greater than that in the equilibrium condition. As depth increases, the tendency for O_2 to be lost to the tissues is greater, accounting for the downward trend.

The swimbladder volume experiments, performed as a corollary to the submersion experiments, indicated that Salmo gairdnerii cannot secrete gas into the swimbladder even under the most ideal conditions. It was abundantly clear from the behavior of fish restricted from the surface, but residing at a depth of less than one foot, that as swimbladder volume decreased, gas would have been secreted if it was at all possible.

The decreases in argon-nitrogen ratios in swimbladder gas over time, which occur at a rate which is a function of the depth at which the fish is held, can only be the result of the loss of gas from the swimbladder. Thus, for Salmo gairdnerii, the low argon-nitrogen ratios in swimbladder gas are not a result of the deposition of gas into the swimbladder, but of the loss of gas from it. As will be made clear in the discussion of permeability coefficients, the greater tendency for gas to leak out of the swimbladder (the greater the rate of volume decrease as a function of time), the greater the tendency for the argon-nitrogen ratio to decline to a value representing an equilibrium state. This phenomenon is clearly evident in the results of the submersion experiments (see Figs. 9, 10, and 11). The initial rates of decrease of the argon-nitrogen ratios are greater with increased depth, and the equilibrium levels, plotted in

Fig. 12, are also depth dependent. The method for determining the equilibrium values for argon-nitrogen ratios in swimbladder gas for a given depth is explained in the results section. The reason for depth dependence of the argon-nitrogen ratio equilibrium values should become apparent in the discussion of the differing permeabilities of argon and nitrogen through membranes.

The idea that argon-nitrogen ratios in the swimbladders of fish subjected to submersion experiments declined to a value which could be predicted on the basis of the physical properties of the two gases was tested by the formulation of a mathematical description of the presumed process. Because of certain simplifying assumptions made in the model, it deals only with the tendency of the ratios to approach a specific equilibrium, and not with the rate at which that equilibrium is approached. Application of data from submersion experiments to the model yields a series of λ-values which, as explained in the results section, are an indication of both the closeness of the argon-nitrogen ratios to a predicted equilibrium condition and the direction in which the process will proceed in order that an equilibrium may be reached. When one considers all of the possible sources of variation in the model such as the estimates of average barometric and hydrostatic pressures, estimates of the partial pressures of each gas in the water at a given depth, approximations of the proportionality constants ($\delta_{1,2}$) from values of diffusion and solubility coefficients found in the literature, the approximation that $\delta_1 \approx 2\delta_2$, and errors in estimation of the mole fractions of the two gases inside the swimbladder due to sampling error, the λ-values obtained agree remarkably well with the hypothesis that the predicted equilibrium ($\lambda=0$) was being approached (see Fig. 13). Any exogenous factors not taken into account by the model such as vertical migration or gas secretion would undoubtedly have yielded vastly different results. For example, if nitrogen were being secreted selectively, the λ-value would be expected to become positive, a tendency which is not evident in the data.

Permeability experiments were performed to independently confirm an assumption in the model which was used to compute λ-values, specifically that $\overline{P}_A > \overline{P}_{N_2}$. As noted in the results section, previous authors have constructed models of diffusion and permeability phenomena in animal tissues from information gleaned initially from experiments of much the same nature as those used to verify the model. In order to avoid this somewhat circular reasoning, experimental data were compared to values of $\overline{P}_A/\overline{P}_{N_2}$ predicted on the basis of molecular radii of the diffusing gas molecules, the well established solubilities of these gases in distilled water, and the effect of freezing point depression on solubilities of gases in water. None of the parameters used in the construction of the model was determined originally from the diffusion of a gas through a liquid. It was felt that in this way confirmation of the model would be as independent of the model itself as possible. The results of the permeability experiments, performed with excised swimbladder membrane and dialysis membrane confirm the assumption that the permeabilities of argon through swimbladder and dialysis membrane is between two and three times as great as those of nitrogen through the same membranes. The data also compare remarkably well with the ratios of permeabilities ($\overline{P}_A/\overline{P}_{N_2}$) predicted on the basis of molecular radii and solubilities. Thus the assumption that $\overline{P}_A$ is greater than $\overline{P}_{N_2}$ is confirmed and with it the model used to compute λ-values from the submersion data. These results, together with the ratios of permeabilities of argon and nitrogen through a variety of synthetic membranes found in the literature (see Table XVI) attest to the generality of the phenomenon.

Although the primary reason for conducting the permeability experiments was to determine permeability coefficients of argon and nitrogen and to confirm the assumption regarding their relative magnitude, data on the change with time in the composition of the gas in the diffusion chamber can also be treated in much the same way as data obtained from the submersion experiments. Argon-nitrogen ratios for gas in the diffusion chamber were regressed on time as shown in Fig. 16. The equation derived for the rate of change of argon-nitrogen ratios is purely descriptive of the data and cannot be extrapolated in its present form beyond the range of the data it describes. The reason for this limitation is that the process leading to changes in gas composition in the diffusion chamber is the same as that leading to change in gas composition in the swimbladder. As explained in the results section, this relation is hyperbolic, with the horizontal asymptote at some equilibrium value $100A/N_2>0$. The argon-nitrogen ratios from the permeability experiments, when regressed on time, fall in such a linear part of the relation that deviations from a straight-line regression equation are not significant. It should be pointed out that the starting argon-nitrogen ratio in the diffusion chamber was approximately 1, whereas that in the swimbladders of <u>Salmo gairdnerii</u> was on the order of $1x10^{-2}$. Despite this difference the data are heuristic in that they, like those from the submersion experiments, demonstrate a decrease in argon-nitrogen ratios with time.

It was one of the purposes of this work to evaluate the position that argon-nitrogen ratios in swimbladder gas significantly lower than the value for air tend to indicate the active deposition of nitrogen into the swimbladder. Scholander (1956), Tait (1956), Saunders <u>et al</u>. (1958), and Douglas (MS, 1967) all claim that there is no active secretion of nitrogen solely on the basis of the similarity of argon-nitrogen ratios measured in swimbladder gas to the value for air. The results of this work show that the observation of low argon-nitrogen ratios in swimbladder gas is not a sufficient condition to suspect active nitrogen secretion. The low ratios are due instead to a greater permeability coefficient for argon, allowing it to escape more easily to the environment, leaving nitrogen behind in an ever increasing proportion until an equilibrium is reached. Physostomes that secrete gas actually show argon-nitrogen ratios in their swimbladders which are somewhat higher than those which have no secretory organ.

APPENDIX A

COMPUTATIONAL FORMULAE

ARGON-NITROGEN RATIOS:

$$100A/N_2 = 1.196\ \left(\frac{\text{standard } N_2}{\text{standard } A}\right)\left(\frac{\text{sample } A}{\text{sample } N_2}\right)$$

where "standards" and "samples" are values proportional to volumes of gas detected by the gas chromatograph.

ARGON, NITROGEN, AND OXYGEN PERCENTAGES:

$$\%N_2 = \frac{100}{1 + \left(\frac{O_2+A}{N_2}\right)}$$

$$\%A = 100A/N_2(\%N_2)$$

$$\%O_2 = 100 - (\%N_2 + \%A)$$

where

$$\frac{O_2+A}{N_2} = 0.2802\ \left(\frac{\text{standard } N_2}{\text{standard } O_2+A}\right)\ \left(\frac{\text{sample } O_2+A}{\text{sample } N_2}\right)$$

SWIMBLADDER VOLUMES:

$$\text{Volume} = \left(\frac{100V_c}{V_c - V_{c_o}}\right)\ (V_x - V_b)$$

where

V_{c_o} = cappillary volume before compression

V_c = cappillary volume after compression

V_b = "blank" volume injected to produce compression

V_x = volume injected during swimbladder compression

PERMEABILITY COEFFICIENTS:

$$\overline{P}_A = \left(\frac{\Delta V_A}{t}\right) (d)\ (A)^{-1}\ (P_{A(t/2)})^{-1}\ (760 \text{ mm Hg})$$

$$\overline{P}_{N_2} = \left(\frac{\Delta V_{N_2}}{t}\right) (d)\ (A)^{-1}\ (P_{A(t/2)})^{-1}\ (760 \text{ mm Hg})$$

where

$$P_{A(t/2)} = P\left(\frac{\left(\frac{\%A}{100}\right)_i + \frac{(A/N_2)_t}{1 + (A/N_2)_t}}{2}\right)$$

$$P_{N_2(t/2)} = P\left(1 - \frac{\left(\frac{\%A}{100}\right)_i + \frac{(A/N_2)_t}{1 + (A/N_2)_t}}{2}\right)$$

and

t = duration of experiment

V_i = initial gas volume

V_t = terminal gas volume

$V_{A(i)}$ = initial volume of argon

$V_{N_2(i)}$ = initial volume of nitrogen

d = membrane thickness

A = surface area of membrane

P = barometric pressure

$(A/N_2)_i$ = initial argon-nitrogen ratio

$(A/N_2)_t$ = terminal argon-nitrogen ratio

$$V_{N_2(t)} = \frac{V_t}{1 + (A/N_2)_t}$$

$$V_{A(t)} = V_t - V_{N_2(t)}$$

$$\Delta V_A = V_{A(i)} - V_{A(t)}$$

$$\Delta V_{N_2} = V_{N_2(i)} - V_{N_2(t)}$$

APPENDIX B

A COMPUTATION METHOD FOR REGRESSION THROUGH THE ORIGIN; MORE THAN ONE VALUE OF Y PER VALUE OF X

Experiments are frequently designed in such a way that the starting point of a process is known absolutely, or with such great precision and accuracy that variation is not measurable. For example, the change in height of a plant may be measured over a period of time. Obviously, at $t=0$, Δh is known to be exactly zero. In such cases it appears appropriate for the true regression equation to be

$$\eta = \beta X.$$

Since the regression line must pass through the origin, the regression coefficient estimator b is calculated directly from observed Y's and experimentally determined X's rather than from deviations of these values. Assuming the values of Y are distributed randomly around η ($\mu Y=\beta X$) with a variance σ^2, and that the observations are independent, the estimated regression equation is

$$\hat{Y} = bX.$$

The sum of squares of deviations between the observed values Y_i and the predicted values $\hat{Y}_i$ is

$$R = \Sigma(Y_i-\hat{Y}_i)^2 = \Sigma(Y_i-bX_i)^2.$$

Differentiating with respect to b and setting equal to zero to minimize the sum of squares R gives

$$\frac{dR}{db} = -2\Sigma(Y_i-bX_i)X_i = 0,$$

which yields the equation

$$b = \frac{\Sigma X_i Y_i}{\Sigma X_i{}^2}.$$

When computing regression statistics using more than one value of Y for each value of X, that is when the data are collected in groups with respect to the X variate, analysis of variance may be used to full advantage. The "among groups" sum of squares is used in calculating both the presence and significance of the regression and the significance of the deviations from the regression. When the technique of forcing the line through a fixed point (in this case the origin) is used, careful consideration must be given to how the fixed point should be weighted when calculating the sum of squares among groups, and the sum of squares of deviations from the line. In most cases where fixed-point regression is appropriate, each data point represents the extent to which a certain parameter has "progressed" from a known fixed starting point. The sum of squares among groups should reflect the *a priori* knowledge that the regression line, if there is one, will pass through a fixed point. Because the null hypothesis being tested is that the mean square of the deviations among groups is equal to the mean square of deviations within groups, the fixed point is, in fact, assumed to be the parametric mean, μ, of the population of group means. Therefore the sum of squares among groups is actually the sum of squares of group means, weighted for sample size, from the parametric mean μ. In this case, the fixed point is at the origin and μ=0. The sum of squares among groups may be expressed simply as

$$\sum^{a} \frac{(\Sigma^{n_i} Y_i)^2}{n_i} .$$

A new formula for the sum of squares within groups must also be developed. It has been shown that

$$b = \frac{\Sigma X_i Y_i}{\Sigma X_i^2} ,$$

and that

$$\Sigma(Y_i - \overline{Y})^2 = \Sigma(Y_i - bXi)^2 .$$

It is also true that

$$Y_i = (Y_i - \overline{Y}) + \overline{Y} = (Y_i - \overline{Y}) + bX_i .$$

Squaring and summing over i yields

$$\Sigma Y_i^2 = \Sigma(Y_i-\overline{Y})^2 + \frac{(\Sigma X_i Y_i)^2}{\Sigma X_i^2}$$

Rearranging,

$$\Sigma(Y_i-\overline{Y})^2 = \Sigma Y_i^2 - \frac{(\Sigma X_i Y_i)^2}{\Sigma X_i^2} .$$

Since for any one group of data X is a constant,

$$\overset{n_i}{\Sigma} (Y_i-\overline{Y})^2 = \overset{n_i}{\Sigma} Y_i^2 - \frac{(X_i \overset{n_i}{\Sigma} Y_i)^2}{n_i X^2}$$

$$= \overset{n_i}{\Sigma} Y_i^2 - \frac{(\overset{n_i}{\Sigma} Y_i)^2}{n_i} .$$

Summing over all groups,

$$SS_{within} = \sum^{a} \overset{n_i}{\Sigma} Y_i^2 - \frac{(\overset{n_i}{\Sigma} Y_i)^2}{n_i}$$

$$= \sum^{a} \frac{n_i \overset{n_i}{\Sigma} Y_i^2 - (\overset{n_i}{\Sigma} Y_i)^2}{n_i}$$

As previously stated, the calculated value of $b_{y.x}$ in fixed-point regression is based on observed values of Y and experimentally determined values of X rather than the deviations of these values from an estimated one. It is therefore appropriate to calculate the explained sum of squares due to regression in terms of b. The simplified expression is

$$b_{y.x}(\overset{a}{\Sigma}X(\overset{n_i}{\Sigma}Y_i)),$$

which may also be written

$$\frac{(\Sigma X_iY_i)^2}{\Sigma X_i^{\,2}} = \frac{(\overset{a}{\Sigma}X(\overset{n_i}{\Sigma}Y_i))^2}{\overset{a}{\Sigma}n_iX^2} .$$

The degrees of freedom of some of the statistics in fixed-point regression are also affected. Because this technique takes advantage of specific information not normally considered in linear regression, namely knowledge of a fixed starting point, more confidence may be had in values for sum of squares among groups (d.f. = (a+1)-1=a) and sum of squares of deviations from regression (d.f. = (a+1)-2=a-1). This naturally increases the total degrees of freedom by one $(\text{d.f.} = (\overset{a}{\Sigma}n_i+1)-1=\overset{a}{\Sigma}n_i)$. The degrees of freedom of the sum of squares within groups is not changed because the value is not affected by the assumption of a fixed point, and its computational formula is not weighted.

Since the mean square of the deviations from regression is used to calculate confidence limits around b, the t-statistic used has the same degrees of freedom, a-1. For 95% confidence limits, $L_{1,2} = b \pm t_{0.05[a-1]}S_b$, where S_b is also computed from determined values of X rather than from deviations.

COMPUTATIONAL PROCEDURE:

Basic computations made for each group:

$$\overset{n_i}{\Sigma}\, Y, \quad \overset{n_i}{\Sigma}\, Y^2, \quad (\overset{n_i}{\Sigma}\, Y)^2, \quad \overline{Y}, \quad n$$

ANOVA computations:

① $$SS_{among} = \sum^{a} \frac{(\overset{n_i}{\Sigma}\, Y)^2}{n_i}$$

② $$SS_{within} = \sum^{a} \left(\frac{n_i \overset{n_i}{\Sigma}\, Y_i^2 - (\overset{n_i}{\Sigma}\, Y_i)^2}{n_i} \right)$$

③ SS_{total} = quantity ① + quantity ②

④ $\overset{a}{\Sigma} n_i X^2$

⑤ $\overset{a}{\Sigma} n_i X\overline{Y} = \overset{a}{\Sigma} X(\overset{n_i}{\Sigma}\, Y_i)$

⑥ Regression coefficient = $b_{y.x} = \frac{\Sigma X_i Y_i}{\Sigma X_i^2}$ = $\frac{\text{quantity ⑤}}{\text{quantity ④}}$

⑦ Explained sum of squares = $\Sigma \hat{y}^2 = \frac{(\Sigma X_i Y_i)^2}{\Sigma X_i^2}$ = $\frac{(\text{quantity ⑤})^2}{\text{quantity ④}}$

⑧ Unexplained sum of squares = $\Sigma d^2_{y.x} = SS_{among} - \Sigma \hat{y}^2$

= quantity ① - quantity ⑦

Sample ANOVA Table (with regression):

Source of variation	d.f.	SS	MS	F_s
$\bar{Y}-\bar{\bar{Y}}$ among groups	a	SS_a:①	①/a	MS_a/MS_w
$\hat{Y}-\bar{\bar{Y}}$ linear reg.	1	$SS_{\hat{y}}$:⑦	⑦/1	$MS_{\hat{y}}/MS_{y\cdot x}$
$\bar{Y}-\hat{Y}$ dev. from reg.	a-1	$SS_{y\cdot x}$:⑧	⑧/a-1	$MS_{y\cdot x}/MS_w$
$Y-\bar{Y}$ within groups	$\overset{a}{\Sigma} n_i-a$	SS_w:②	②/$\overset{a}{\Sigma} n_i-a$	
$Y-\bar{\bar{Y}}$ total	$\overset{a}{\Sigma} n_i$	SS_t:③		

It is recommended that a test be made of the null hypothesis that there is no improvement in fit when using the two-parameter line (ordinary linear regression) in place of the one-parameter line (fixed-point regression). The $F_{1-\alpha}$ statistic is the mean square of the difference between the sums of squares of deviations from regression of the one-parameter line and the two-parameter line divided by the mean square of the deviations from regression of the two-parameter line. In tabular form:

Source of variation	d.f.	SS	MS	F_s
$\bar{Y}-\hat{Y}$ dev. from reg. $\hat{Y}=bX$	a-1	$SS_{y\cdot x_{(1)}}$		
$\bar{Y}-\hat{Y}$ dev. from reg. $\hat{Y}=a+bX$	a-2	$SS_{y\cdot x_{(2)}}$	$SS_{y\cdot x_{(2)}}{}^{a-2}$	$MS_{dif}/MS_{y\cdot x(2)}$
	1	SS_{dif}	$SS_{dif}/1$	

Standard error and confidence limits:

No translation of a fixed-point regression function is possible. For this reason the only standard error and confidence limits which are appropriate apply to the regression coefficient.

Calculations:

Standard error: $S_b = \sqrt{\dfrac{S_{y\cdot x}{}^2}{\Sigma X^2}} = \sqrt{\dfrac{MS_{y\cdot x}}{\textcircled{4}}}$

95% confidence limits:

$$L_1 = b - t_{0.5[a-1]} S_b$$

$$L_2 = b + t_{0.5[a-1]} S_b$$

F-test for difference between two regression coefficients:

$$F_{s[1,a_1+a_2-2]} = \frac{(b_1-b_2)^2}{\dfrac{\overset{a}{\Sigma} n_i X_1^2 + \overset{a}{\Sigma} n_i X_2^2}{(\overset{a}{\Sigma} n_i X_1^2)(\overset{a}{\Sigma} n_i X_2^2)} \quad (\overline{S}_{y\cdot x}{}^2)}$$

$$\text{where } \overline{S}_{y\cdot x}{}^2 = \frac{\overset{a}{\Sigma} d_{y\cdot x_1^2} + \overset{a}{\Sigma} d_{y\cdot x_2^2}}{a_1 + a_2 - 2}$$

$$= \frac{(b_1-b_2)^2}{\left(\dfrac{\textcircled{4}_1 + \textcircled{4}_2}{\textcircled{4}_1 \textcircled{4}_2}\right) \left(\dfrac{\textcircled{8}_1 + \textcircled{8}_2}{a_1 + a_2 - 2}\right)}$$

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