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GAS TENSIONS IN TISSUES OF TROUT AND CARP EXPOSED TO DIURNAL CHANGES IN OXYGEN TENSION OF THE WATER*

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INTRODUCTION

When an artificial gas pocket is formed by injection of air into a tissue space the gases equilibrate with the tissue-blood environment and assume a state of constant composition which is maintained until all the gas is eventually resorbed. In man (Rahn, 1957) and in rats (Van Liew, 1968) evidence has been presented that the O_2 and CO_2 tensions of gas in such pockets are virtually the same as those of the venous blood draining these tissues provided that air or gases of lower O_2 concentrations are breathed (see also review by Piiper, 1965). Since the techniques required for sampling and analysis of the equilibrated gas are relatively simple, this method provides a convenient approach for the determination of gas tensions of the blood-tissue environment of unanaesthetized, unrestrained animals.

To interpret the tissue gas tensions in fish it is important to relate these to the normal gas tensions of arterial and mixed venous blood in unrestrained, free-swimming animals. These have recently become available for the rainbow trout (Stevens & Randall, 1967) and for the carp (Garey, 1967), the two species chosen for this study.

Experiments were performed on free-swimming fish at the New York State Fish Hatchery at Caledonia, N.Y. The waters which supply this station come from a slowly moving spring-fed stream with considerable photosynthetic activity which imposes a large diurnal fluctuation in the O_2 content of the water. During the experiments in the summer the O_2 tensions of the water rose as high as 230 torr in the late afternoon and fell to pre-dawn values as low as 50. Such large changes might be expected to influence the tissue O_2 tensions. For this reason tissue gas-tension analyses were performed over long enough periods to establish a positive correlation with the changes in water O_2 tension.

METHODS

Two experiments were carried out during the summer and one in the winter on fish which were maintained in the raceways of the fish hatchery. The rainbow trout (*Salmo gairdneri*) were 2 years old, weighing about 500 g., while the carp (*Cyprinus*

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carpio) weighed between 2–3 kg. Water temperatures fluctuated a few degrees about a mean of 10 or 11° C.

Tissue gas analysis. Two days prior to our observations fish were taken from the raceway and air was injected into their coelomic cavities using a syringe and a no. 20 needle. The amount of air introduced depended upon the size of the fish and ranged from 20 to 40 ml. Previous studies had shown that after 24–36 hr. gas composition is constant, indicating a state of near-equilibrium between the O_2 and CO_2 partial pressures of the gas pocket and that of the surrounding tissue.

Periodically thereafter 5 ml. gas samples were withdrawn and analysed in duplicate for CO_2 and O_2 using the Scholander 0·5 c.c. Gas Analyzer. The fractional composition of each gas was then expressed as the partial pressure. Thus,

$$P_{O_2} = F_{O_2} \times (P_B - P_{H_2O}),$$

where F_{O_2} is the fractional O_2 composition, P_B is the barometric pressure and P_{H_2O} is the vapour tension of water at the particular temperature.

Water O_2 tension. An O_2-electrode (Instrumentation Laboratory, Inc., or Radiometer) was utilized to measure the ambient water P_{O_2}. Temperature equilibration between the water and electrode was ensured by the continuous circulation of water from the raceway through the electrode housing. Calibrations were checked before each sequence of measurements by setting the zero O_2 point with boiled water or nitrogen gas and the high O_2 point with air-saturated water obtained from a submerged flask in which air was constantly bubbled through a sintered disk. Thus calibration samples and the electrode were maintained at the ambient water temperature.

Water samples for analysis were drawn into 5 ml. glass syringes after the syringes had been carefully filled and emptied several times to avoid release of gas bubbles, particularly during the afternoon when the waters become supersaturated with O_2.

Oxygen-dissociation curves of blood. The oxygen content of carp and trout blood was established at various O_2 tensions with CO_2 tensions maintained at 2, 5 and 8 torr. The equilibrations were made on the Astrup microtonometer at 10° C. and the gas and blood-gas contents were determined by the micro-Van Slyke technique.

RESULTS

Cyclic changes in water O_2 tension. The large diurnal fluctuations in water P_{O_2} for given days in June 1964, July 1966 and February 1965 are shown in Figs. 1–3 and are clearly related to the day and night period. However, the degree of supersaturation and undersaturation of O_2 depends upon many other factors including the degree of cloudiness. The amplitude of the O_2 cycle observed in February is smaller even though the water temperature was only a few degrees lower.

O_2 and CO_2 tensions intissues. Mean values of the composition of the equilibrated gas pockets for the trout and carp are given in Table 1 and their O_2 tensions are plotted in Figs. 1–3 with the simultaneously observed water O_2 values. It will be noted in Figs. 1 and 3 that for the trout there is a parallelism between the O_2 tensions of water and tissue although the latter appears to lag by approximately 4 hr. The O_2 tensions in the carp (Fig. 1) do not show any cyclic changes, and furthermore their values are distinctly less than those for the trout.

Oxygen-dissociation curves of blood. These were established at 10° C. in both species at CO_2 tensions of 2, 5 and 8 torr. Fig. 4 shows the dissociation curves for both species at the CO_2 tension closest to the CO_2 values that were observed in the gas pocket. The average gas-pocket values (see Table 2) are also indicated on the graph.

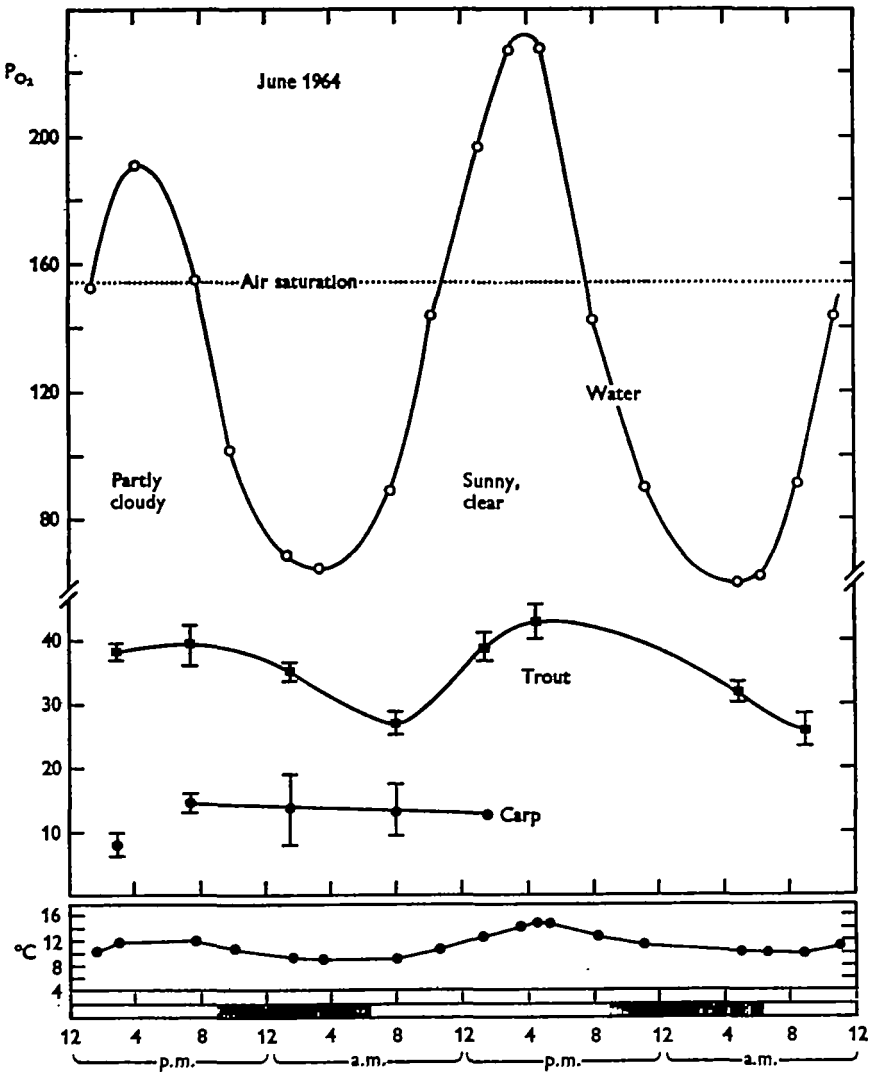

Fig. 1. Diurnal changes in O_2 tensions of water and tissues of trout and carp during the month of June. Ordinate, mm. Hg.; abscissa, 4 hr. intervals; shaded area represents night.

DISCUSSION

Water oxygen tensions. Diurnal changes in O_2 tension due to aquatic photosynthesis and respiration of natural waters are well appreciated, but the amplitude of the variations above and below the normal air saturation level depends upon many more factors than the degree of solar radiation. One might mention here only the physical effects

of the changes in water temperature during the day and night which contribute in a minor but predictable manner to this fluctuation. For example, a rise from 10–15° C. decreases the O_2 solubility enough to increase the tension by about 10% provided there is no change in O_2 content. A similar decrease in O_2 tension would be expected during the night upon cooling. The hatchery personnel have observed that on frequent

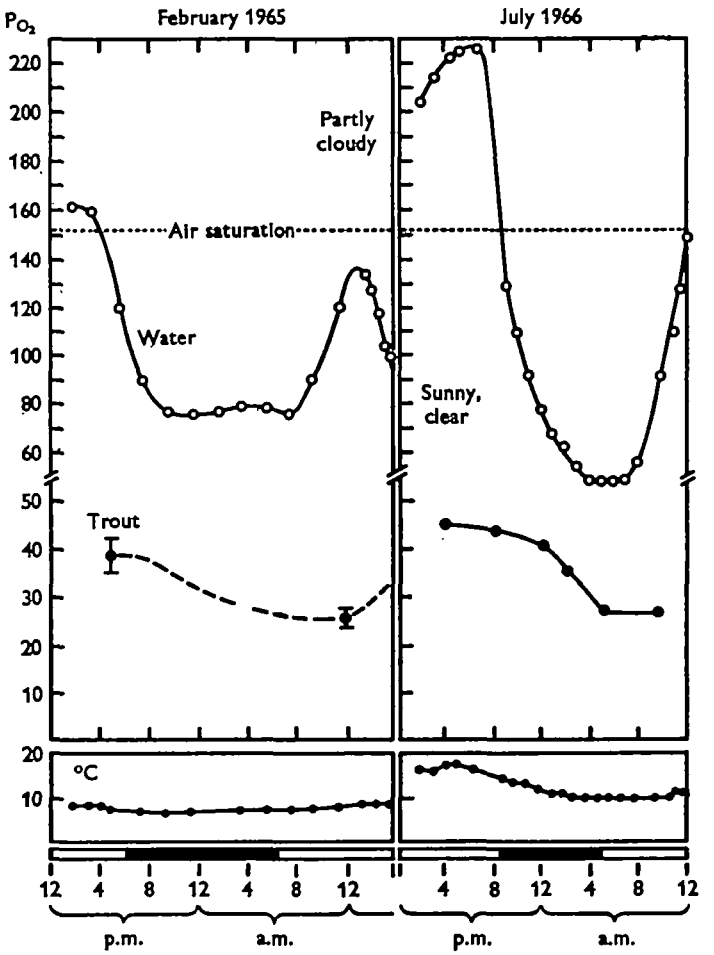

Fig. 2 Fig. 3

Fig. 2. Diurnal changes in O_2 tensions of water and tissues of trout during the month of February.
Fig. 3. Diurnal changes in O_2 tensions of water and tissues of trout during the month of July.

occasions the pre-dawn O_2 tensions during the summer months fall to such low values that asphyxia and distress symptoms are seen in the rainbow trout. Presumably these occur when the O_2 tensions fall below 50 torr.

Gas tensions in tissues and blood. The steady-state O_2 and CO_2 tensions found in gas pockets of mammals closely reflect the partial pressures of gases in the surrounding tissues and the venous blood draining these tissues (Rahn, 1957; Van Liew, 1968). Relatively little information is available from similar comparisons in cold-blooded

Table 1. *Average value and standard error of the tissue CO_2 and O_2 tension of trout and carp during the diurnal changes of the water O_2 tensions*

Day	Time	Water P_{O_2}	Temp. (° C.)	Trout n	Trout P_{CO_2}	Trout P_{O_2}	Carp n	Carp P_{CO_2}	Carp P_{O_2}
June 1964									
25	3 p.m.	152	12	7	6·8 (0·7)	38·6 (1·3)	7	6·4 (0·5)	8·2 (1·7)
	8 p.m.	159	10	8	6·7 (0·2)	39·6 (2·8)	4	6·1 (0·3)	14·6 (1·0)
26	2 a.m.	68	9	7	6·3 (0·7)	35·0 (1·3)	4	5·2 (0·5)	13·8 (5·3)
	8 a.m.	88	9	7	5·4 (0·3)	27·0 (1·7)	4	4·8 (0·5)	13·1 (3·8)
	2 p.m.	195	13	6	5·9 (0·4)	38·8 (2·0)	2	5·2 —	12·7 —
	5 p.m.	225	15	5	8·9 (0·7)	42·6 (2·5)	—	—	—
27	5 a.m.	58	10	8	6·3 (0·5)	31·4 (1·2)	—	—	—
	9 a.m.	89	10	8	6·9 (0·4)	25·5 (2·3)	—	—	—
February 1965									
9	5 p.m.	121	8	5	5·9 (0·4)	38·8 (3·0)	—	—	—
10	12 noon	121	8	4	5·7 (0·6)	25·7 (1·2)	—	—	—
July 1966									
13	4 p.m.	221	17	3	—	43 —	—	—	—
	8 p.m.	160	14	3	—	43 —	—	—	—
	12 midnight	80	12	3	—	41 —	—	—	—
14	2 a.m.	56	11	3	—	36 —	—	—	—
	5 a.m.	49	10	3	—	28 —	—	—	—
	9 a.m.	100	10	3	—	27 —	—	—	—

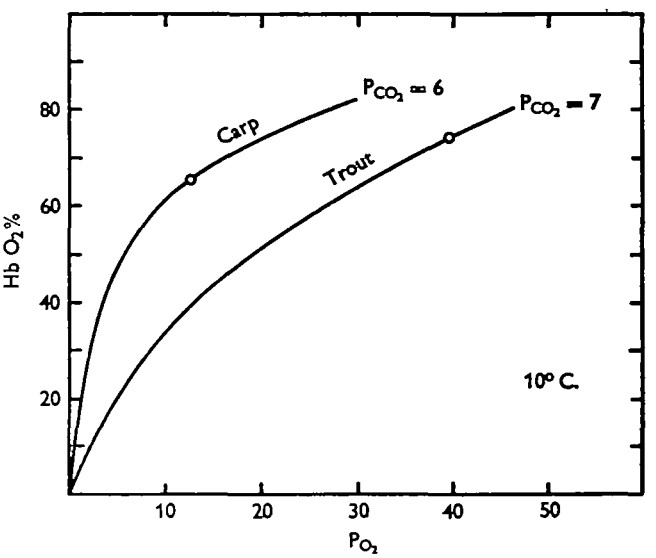

Fig. 4. O_2 dissociation curves for carp and trout at CO_2 tensions of 6 and 7 torr, respectively. Each circle, therefore, represents the O_2 and CO_2 tensions of the tissues as well as the venous blood draining the pocket if a near equilibrium between these is assumed.

vertebrates. Recently Torre (1967) established in intra-abdominal and subcutaneous gas pockets of frogs (*Rana catesbiana*) that at body temperatures from 5 to 25° C. the CO_2 tensions were consistently higher than the arterial blood values by 4–6 mm. Hg., while Bondi (1967) found a similar relationship between the CO_2 tensions of the intra-abdominal gas pocket and the mixed venous blood in the turtle.

For a comparison of our tissue gas tensions with those of arterial and venous blood we may rely upon the recent observations of Stevens & Randall (1967), Holeton & Randall (1967) on the rainbow trout, and those of Garey (1967) on the carp. These blood values were all obtained by implanted catheters in free-swimming animals and at temperatures comparable to those of our studies. Table 2 shows the average gas tensions in blood and their variance for the trout and carp when the water O_2 tensions were 134 and 108 torr, respectively. The trout values were obtained at temperatures between 4 and 8° C. and the carp values at 10° C. These blood values are compared with our tissue gas tensions and represent the mean value of the individual analyses when the water O_2 tensions were above 90 torr.

Table 2. *A comparison of gas tensions of tissue and blood in the trout and the carp*

(The blood values for the trout were taken from the data of Stevens & Randall (1967) and for the carp from Garey (1967). The latter data were selected when the water O_2 tensions were greater than 90. The tissue values were averaged from the individual values of Table 1. In the case of the trout, only those were taken which reflect water O_2 tension above 90.)

	O_2 tension (mm. Hg.)						CO_2 tension (mm. Hg.)					
	Trout			Carp			Trout			Carp		
	O_2	S.E.	n	O_2	S.E.	n	CO_2	S.E.	n	CO_2	S.E.	n
Water	134	—	—	108	2·1	30						
Arterial	85	4·7	13	33	3·8	30						
Tissue	40	0·8	48	13	1·2	25	6·7	0·2	38	5·7	0·2	25
Mixed venous	19	1·4	9	3	0·3	33	5·7	0·2	6	3·6	0·7	14
Arterial							2·3	0·1	7	3·1	0·2	20

It will be noted that in both species the tissue O_2 tensions lie between the arterial and mixed venous value while the CO_2 tensions are higher than the mixed venous value. This is in contrast to the mammalian gas pocket where the simultaneous O_2 and CO_2 values deviate from the mixed venous or arterial values in such a manner that they reflect a gas exchange ratio of the pocket tissue between 0·8 and 1·0 (Rahn, 1957). In such a case a pocket CO_2 tension higher than the mixed venous value is associated with a pocket O_2 tension appropriately lower than the mixed venous O_2. For the O_2 tensions of gas pockets in our fish we would have predicted, therefore, a CO_2 tension between arterial and mixed venous blood. A possible explanation for the high P_{CO_2} value which we observed is that the tissue is releasing lactic acid or other acid metabolites into the blood. Such a mechanism was suggested by Van Liew (1968) to explain his finding of exceptionally high P_{CO_2} in gas pockets in rats when P_{O_2} was unusually low. In our fish the acid might be a normal metabolic product of the particular tissue around the gas pocket or it might be a localized tissue reaction to the presence of a foreign body, i.e. the gas pocket.

The diurnal changes in tissue O_2. It is clear from Figs. 1 and 3 that the tissue O_2 tensions in the trout follow the diurnal fluctuations in water O_2 tension, but there is a lag of several hours between the gas-pocket and the water. This is best explained by a relatively small perfusion surrounding the large gas-pocket volume. Any change in the blood gas tension would be slow to be reflected in the pocket composition.

An analysis of the cyclic changes of the tissue O_2 tension in trout is presented in Fig. 5. Here we have indicated the predicted O_2 tensions of arterial and mixed venous blood which might be expected during the diurnal changes in water O_2 tensions as described in Fig. 1. The blood values during the changes in water O_2 were taken from the data recently presented by Holeton & Randall (1967). Using implanted catheters they determined the gas tensions of arterial and mixed venous blood of trout subjected to a continuously decreasing water O_2 tension. These values are here translated into the fluctuating water O_2 values of Fig. 1 and interpolated for water O_2 values above 160 torr (dotted lines). Upon this background we have re-plotted the tissue

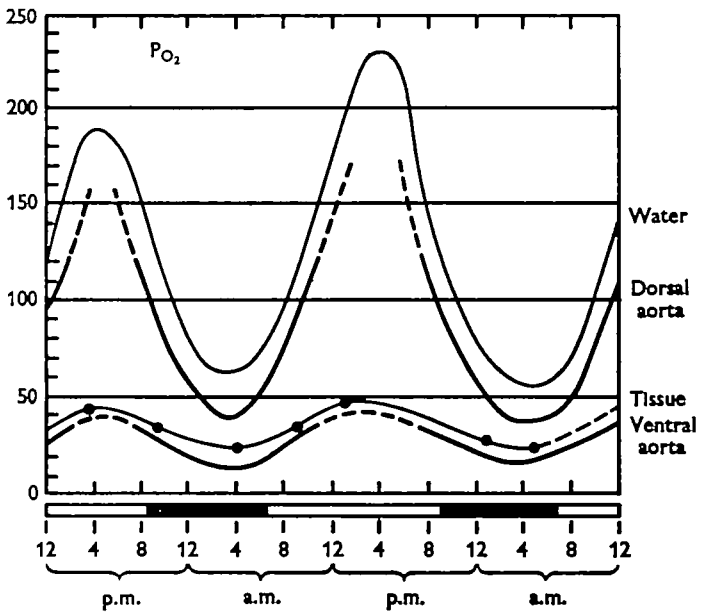

Fig. 5. Predicted changes in O_2 tensions of blood and tissue of the trout as a consequence of the diurnal change in water O_2 observed in Fig. 1. The dorsal aorta and ventral aorta values were taken from the data of Holeton & Randall (1967). The tissue O_2 values of Fig. 1 were re-plotted by introducing a 4 hr. lag (see text). This figure emphasizes the effectiveness of the O_2 and CO_2 dissociation curves in reducing fluctuations in O_2 values for tissues and mixed venous blood in spite of the large fluctuations in environmental and arterial O_2.

values of Fig. 1 or Table 1 except for the fact that we compensated for a 4 hr. lag in response time. This 4 hr. compensation, as shown in Fig. 4, provides an excellent fit by bringing out the parallelism of the tissue values with the mixed venous values. These might be expected to parallel the latter even more closely were it not for the relatively large ratio of gas volume to tissue perfusion which not only introduces the time lag but must also reduce the amplitude of the tissue O_2 cycle.

The lag time also explains the tissue P_{O_2} differences depicted for the two observations made in the month of February (Fig. 2 and Table 1). At the time of sampling at 5 p.m. and the following noon the water O_2 tensions were identical. Yet the afternoon sample had an average P_{O_2} of 38 and the noon sample a value of 26. If one considers an approximate 4 hr. lag, the former reflects the previous high water O_2 tensions while the latter still reflects the low water O_2 value of the previous night.

On this basis we have interpolated the expected values (dotted line) between the two observations.

Diurnal changes in tissue CO_2 tensions. Holeton & Randall (1967) observed a large increase in ventilation volume with the decrease in water P_{O_2}. Since the metabolic rate was not changed appreciably this would have lowered the arterial and mixed venous P_{CO_2} provided the water P_{CO_2} remained essentially zero. One would, therefore, predict that a similar increase in ventilation with the fall of the ambient O_2 tension would affect the CO_2 tensions in our gas pockets. That this actually happened can be seen from the values in Table 1. Although the changes are not very large the lowest tissue CO_2 values are associated with the lowest water O_2 values and are most probably due to the increased ventilation.

The tissue gas tension in the carp. The data on the carp are based on observation during one 24 hr. period. The O_2 tensions are not only appreciably lower than in the trout, but also appear to remain constant in spite of the large fluctuations in the water. The most probable answer is to be found in the O_2 dissociation curve, which, when compared with that of the trout, is shifted far to the left (Fig. 4). At the normal arterial CO_2 tension (Table 2) the haemoglobin is essentially saturated at an O_2 tension of 20 torr, and Garey (1967) has shown that fluctuations in the water P_{O_2} between 130 and 50 torr do not appear to alter the arterial P_{O_2}. One may conclude that the whole O_2 transport mechanism is little affected by changes in water O_2 tensions of the magnitude which we observed. In contrast, with trout, it is quite clear from the experiments of Holeton & Randall (1967) that the ventilation and the arterial P_{O_2} respond quite promptly to any changes in water O_2 below the air-saturation level.

Oxygen dissociation curves and tissue gas tensions. The difference in the tissue O_2 tension between the carp and trout is best appreciated when we plot their average values (Table 2) on their corresponding O_2 dissociation curves (Fig. 4). By doing this we assume that the gas-pocket tensions are in near equilibrium with those of the venous blood draining the pockets. By interpolation between the O_2 dissociation curves established for a P_{CO_2} of 5 and 8 torr the tissue points were plotted on a $P_{CO_2} = 6$ dissociation curve for the carp and a $P_{CO_2} = 7$ dissociation curve for the trout. This analysis suggests that the degree of O_2 unsaturation (*c.* 60–70%) of the venous blood leaving the tissue is similar in both species, but that the O_2 tension difference is the result of the large displacement between the two curves.

REFERENCES

BONDI, K. R. (1967). The effects of temperature on the acid-base balance of turtles. Thesis, State University of New York at Buffalo, Buffalo, N.Y.

GAREY, W. F. (1967). Gas exchange, cardiac output and blood pressure in free swimming carp (*Cyprinus carpio*). Dissertation, State University of New York at Buffalo, Buffalo, N.Y.

HOLETON, G. F. & RANDALL, D. J. (1967). The effect of hypoxia upon the partial pressure of gases in the blood and water afferent and efferent to the gills of rainbow trout. *J. Exp. Biol.* **46**, 317–27.

PIIPER, J. (1965). Physiological equilibria of gas cavities in the body. In *Handbook of Physiology, Sec.* 3. *Respiration.* Vol. II, pp. 1205–18. Ed. W. O. Fenn and H. Rahn. American Physiological Society, Washington, D.C.

RAHN, H. (1957). Gasometric method for measurement of tissue oxygen tension. *Fedn Proc.* **16**, 685–8.

STEVENS, E. D. & RANDALL, D. J. (1967). Changes in gas concentrations in blood and water during moderate swimming activity in rainbow trout. *J. Exp. Biol.* **46**, 329–37.

TORRE, C. M. (1967). The effect of temperature on carbon dioxide clearance in frogs. Thesis, State University of New York at Buffalo, Buffalo, N.Y.

VAN LIEW, H. D. (1968). Oxygen and carbon dioxide tensions in tissue and blood of normal and acidotic rats. *J. Appl. Physiol.* **25**, 575–80.