

AERIAL AND AQUATIC RESPIRATION IN AXOLOTL AND TRANSFORMED *AMBYSTOMA TIGRINUM*

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Studies dealing with the role of the skin, lungs, and buccopharyngeal cavity in respiration in salamanders (Whitford and Hutchison, 1963, 1965, and 1966) have been confined to an analysis of gas exchange in an air environment. In the semidesert and desert areas of the Southwest, populations of the tiger salamander, *Ambystoma tigrinum*, spend much of their life in semi-permanent or permanent bodies of water. Consequently, an evaluation of the role of the various respiratory surfaces in this species must be conducted in an aquatic environment to determine the role of the skin, lungs, and gills in aquatic respiration when the animals are able to obtain atmospheric oxygen by surfacing.

In New Mexico some populations are composed of both transformed and neotenic adults (axolotls). The factors leading to the failure of metamorphosis are not known nor is the adaptive significance of neoteny in these populations understood. Studies of gas exchange in both life forms of adult *A. tigrinum* could aid in an interpretation of ecological and physiological adaptations to pond environments.

MATERIALS AND METHODS

Collections of axolotl and transformed adult *A. tigrinum* were made at several cattle tanks on the Corralitos Ranch and Joranada Experimental Ranch, Dona Ana Co., New Mexico. The animals were acclimated in constant temperature chambers at 15 C or 25 C, 14 hr light (centered at noon MST), and 10 hr dark photoperiod for a minimum of 10 days prior to the experiments. The axolotls were maintained in aerated aquaria and the transformed salamanders were housed in finger bowls on moist paper towels.

Aerial and aquatic respiration were measured separately and simultaneously in a respirometer made from a desiccator. The bottom portion of the desiccator contained a magnetic stirring bar and Beckman oxygen electrode separated from the rest of the chamber by a wire screen. The desiccator was filled with 1600 ml of aged tap water and placed in a water bath at the desired temperature. The water was aerated by an air stone for 30 min before an animal was placed in the respirometer. The oxygen analyzer was calibrated, a salamander placed in the respirometer and the air-water interface covered with a layer of mineral oil. The desiccator cover containing a syringe and manometer was then sealed on the desiccator. A container of KOH to absorb carbon dioxide was attached to the lid. A flask with an air volume equal to that of the

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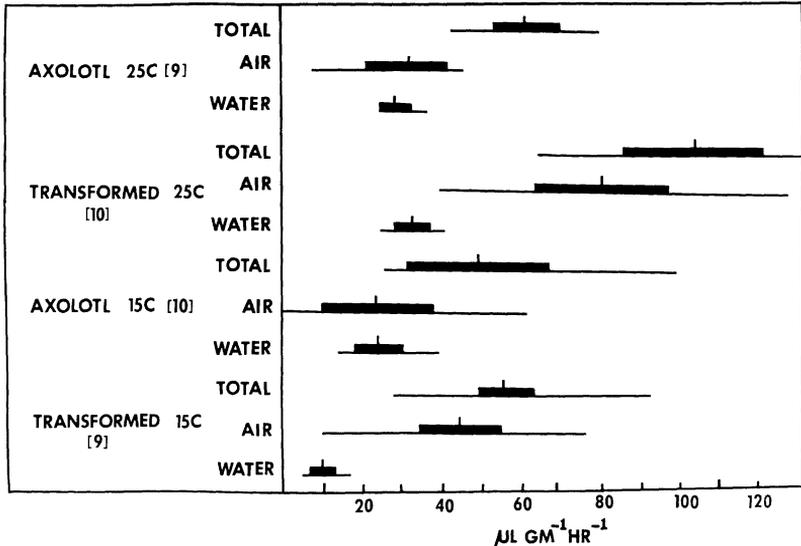


FIG. 1.—Oxygen uptake from air and water and total oxygen consumption in axolotl and transformed *Ambystoma tigrinum* at 15 C and 25 C. The vertical lines represent the mean; the horizontal lines, the range and the black bar on either side of the mean equals the 95% confidence interval of the mean. The numbers in brackets indicate the number of salamanders in each sample.

air phase of the respirometer attached to the manometer corrected for minor temperature and barometric pressure changes.

The respirometer and water bath were placed on a "Magnestir" unit. The spinning of the magnetic stirring bar in the respirometer produced sufficient movement of water past the electrode for accurate dissolved oxygen determinations. The animal was able to surface freely to gulp air. Oxygen removed from the air phase was indicated by movement of the manometer liquid. Oxygen introduced into the air phase by the syringe compensated for movement of the manometer. Preliminary experiments showed that the mineral oil layer effectively prevented diffusion of oxygen between the gas phase and air phase. The oil layer also prevented diffusion of oxygen between air and water as the animal surfaced. The oil did not adhere to the skin or gill filaments of the animals after they surfaced. The salamanders were closely observed during the experiments and the frequency and duration of surfacing was recorded for each animal. All gas volumes were corrected to STP.

RESULTS

The data on aerial, aquatic, and total oxygen consumption in *A. tigrinum* are summarized in Fig. 1. There was no significant difference in total oxygen consumption of axolotl and transformed

TABLE 1.—Frequency and duration of surfacing in axolotl and transformed *Ambystoma tigrinum* related to oxygen uptake from the water at 15 C and 25 C.

Group of salamanders	Temperature	Per cent oxygen from the water		Number of times surfaced per hour		Minutes spent at surface per hour	
		\bar{x}	range	\bar{x}	range	\bar{x}	range
Transformed	15	22.7	9.3–47.8	1.6	1.0–3.5	55.5	31.5–60.0
Axolotl	15	50.6	19.5–100.0	17.2	1.5–3.2	4.6	0.03–22.1
Transformed	25	29.3	22.2–38.8	7.0	1.0–11.0	30.0	0.16–60.0
Axolotl	25	59.4	41.3–81.3	11.2	8.0–13.0	0.24	0.12–0.72

salamanders at 15 C. At 25 C total oxygen consumption of the transformed *A. tigrinum* was significantly higher than that of the axolotls. At 15 C the transformed animals obtained an average of 22.7% of their total oxygen requirements from the water as compared with 50.6% from the water by axolotls. At 25 C the axolotls obtained 59.4% of their oxygen from the water and the transformed animals obtained only 29.3% from the water. There was considerable variation in the per cent of the oxygen requirement obtained from the water by axolotls at 15 C. Four of the axolotls studied at 15 C obtained more than 75% of their oxygen from the water. The total oxygen consumption of these four animals was less than $30 \mu\text{l gm}^{-1} \text{hr}^{-1}$, significantly lower than the metabolism of the other axolotls at 15 C. One animal which did not surface had the lowest total oxygen consumption of the axolotls at 15 C.

There was no correlation between oxygen consumption and weight in axolotl and transformed *A. tigrinum* at 15 C ($r = .10$ and $.24$, respectively). However, there was a correlation between the total oxygen consumption and per cent oxygen obtained from the water ($r = .76$, $p < .01$). Individuals obtaining a higher per cent of their oxygen from the water had lower total oxygen consumption. At 25 C oxygen consumption in axolotl and transformed *A. tigrinum* was related to body size as expressed by the general equation $M = KW^{.85}$ given by Whitford and Hutchison (1967).

Data on duration and frequency of surfacing are summarized in Table 1. Although the transformed salamanders surfaced fewer times, they remained at the surface for extended periods. Several transformed animals swam at the surface with their head protruding through the oil layer for the duration of the experiment at both 15 C and 25 C. The axolotls tended to swim to the surface, gulp air, and slowly sink back to the bottom of the respirometer. There was greater variability in the frequency and duration of surfacing in axolotl and transformed salamanders at 15 C than at 25 C.

Temperature had a greater effect on the oxygen uptake from the air than from the water in both transformed and neotenic *A. tigrinum*, as indicated by the higher Q_{10} values. There was also a

TABLE 2.— Q_{10} values of aerial, aquatic, and total oxygen consumption between 15 C and 25 C in axolotl and transformed *Ambystoma tigrinum*.

Oxygen consumption	Axolotl	Transformed
Aerial	1.72	2.69
Aquatic	1.14	1.79
Total	1.43	2.00

marked difference in the effect of temperature on the rate of increase in total oxygen consumption in axolotl and transformed animals (Table 2).

DISCUSSION

The per cent total oxygen obtained via the skin in transformed *A. tigrinum* in water was lower than that of *A. tigrinum* in air, i.e., less than 30% in water as compared with more than 50% in air (Whitford and Hutchison, 1965). Transformed *A. tigrinum* had a lower total oxygen consumption at 15 C in water when compared with animals in air but at 25 C there was no difference in total oxygen consumption between the transformed *A. tigrinum* in this study and those studied by Whitford and Hutchison (1965). The reduction in the oxygen percentage obtained via the skin by animals in water is undoubtedly related to a lower oxygen diffusion gradient between the environment and dermal capillaries. Since oxygen uptake through the skin is largely dependent on the oxygen diffusion gradient between the skin and water, the availability of oxygen could limit oxygen consumption at 15 C. This might account for the differences in total oxygen consumption in *A. tigrinum* in water and in air. At 25 C there was no difference in metabolism in transformed salamanders in air and water indicating that factors other than oxygen availability must be involved in control of oxygen consumption.

The absence of a correlation between weight and oxygen consumption at 15 C is apparently related to the great variation in activity in both neotenic and non-neotenic animals. This is further supported by the correlation between oxygen percentage obtained from the water and metabolic rate. Animals most active in obtaining oxygen from the air had the highest total oxygen consumption. The data for *A. tigrinum* at 15 C also indicate that neotenes are capable of obtaining 75% of their total oxygen requirements from the water when exhibiting minimal activity.

Marked increases in oxygen consumption at 15 C have been reported for many species of temperate zone amphibians (Whitford and Hutchison, 1963, 1965, and 1967; Hutchison, Whitford, and Kohl, 1968; Whitford, 1968). There has been no satisfactory explanation for this marked increase in oxygen consumption at 15 C.

Data from this and previous studies indicate that 15 C may represent an optimal temperature for activity in temperate zone amphibians. This might result in higher levels of activity and consequently higher measures of standard oxygen consumption when studies are made on animals capable of movement and where activity cannot be controlled.

The difference in oxygen consumption in neotenes and non-neotenes was directly related to difference in activity. Transformed salamanders actively swam at the surface for extended periods accounting for the higher total oxygen consumption. Axolotls in water obtained about the same percentage of their total oxygen requirement from the water as non-neotenic *A. tigrinum* obtained through the skin in air (Whitford and Hutchison, 1965). Consequently the gills compensated for the reduction in oxygen tension in water as compared with air. Gills increased oxygen uptake from the water and allowed a reduction in activity necessary to supply the remainder of the animals oxygen requirement. The resulting reduced energy requirements of axolotls may provide selective advantage for *A. tigrinum* populations in desert areas.

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