Oxygen Consumption of Whole Animal and Tissues in Temperature Acclimated Amphibians. (25549)

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(Introduced by Howard M. Klitgaard)
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It has been established that in many poikilotherms there is a difference in metabolism in members of a species from warm and cold environments. Animals obtained from the cold having a higher rate of oxygen consumption than those from a warmer environment, when both are measured at an intermediate temperature. It may be reasonable to assume that in the intact organism total body oxygen consumption is the sum of the rates of the various tissues. Therefore, a study of tissue respiratory metabolism from cold and warm acclimated animals of the same species should indicate the relative contribution of each tissue to changes of oxygen uptake noted in the intact organism. Freeman(1) found increased respiratory metabolism in brain brei but not in skeletal muscle of goldfish (*Carassius auratus*). A similar finding was reported by Suhrman(2). Roberts(3) working with muscle slices from the crab found increased metabolism in tissue from cold acclimated animals. Many such diverse reports on the compensation for temperature in metabolism of poikilotherms have been excellently reviewed by Bullock(4). The present report is concerned with acclimation of frogs (*Rana pipiens*) and newts (*Triturus viridescens*) with a study of selected frog tissues to learn which tissues most nearly parallel the whole animal response to the changed environmental temperature. It is hoped that such information will provide a tissue which can be analyzed more extensively as to the mechanism involved in the change of metabolism noted during acclimation.

Methods. Adult *Triturus viridescens* and *Rana pipiens*, obtained from field collectors in Tenn. and Wisc., respectively, were maintained at room temperature (19-21°C) for one week before beginning of an experiment. The frogs were divided into 2 groups and placed in constant temperature incubators, one group at 5°C and the other at 25°C. Triturus treated in a similar manner, were maintained at 15°C and 30°C. None of the animals were fed during these acclimation experiments. Total body oxygen consumption was measured in a constant volume respirometer using the Warburg technic. The flasks used were specially designed with a volume of about 100 ml and a protected side arm for the CO_2 absorbant. Animals were not anesthetized and the position of an animal was noted at beginning of a determination period. If it had changed position those readings were not used. No difference in oxygen consumption could be detected between animals in which the shaker was employed and in those determinations in which it was not used. Tissue metabolism was measured manometrically in the Warburg according to the method of Umbreit *et al.*(5). Slices of liver, cardiac muscle (ventricle), sartorious muscle and strips of intact stomach wall were removed from animals selected at random from a particular acclimated group. The slices were placed in the flasks with chilled Mine's solution buffered with phosphate at pH 7.4(6). An additional sample of each tissue was placed in a weighing bottle for drying at 100°C for determination of dry weight. A series of *Triturus viridescens* was thyroidectomized according to the method of Adams(7). Respiratory metabolism was studied periodically during the pre- and post-thyroidectomy stages. The athyroid condition was verified by histological sections after termination of experiments.

Results. Total body respiratory metabolism of frogs is presented in Fig. 1. The higher rate of oxygen consumption for the 5°C acclimated animals was highly significant ($p = < .001$) when determined at 10°C, significant when measured at 20°C ($p = < .03$), but at 30°C oxygen uptake of both groups was practically identical.

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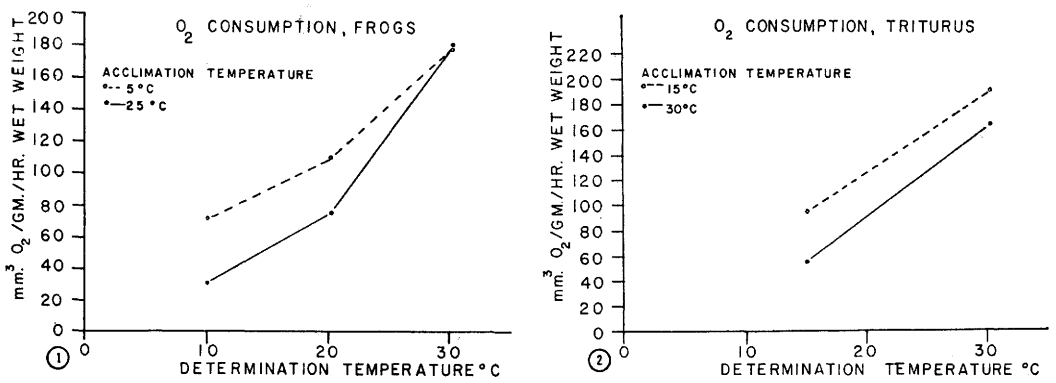

FIG. 1. Total body oxygen consumption of frogs acclimated at 5°C and 25°C. Each point is mean from 18 animals selected randomly from a population kept at specified temperature.

FIG. 2. Total body oxygen consumption of Triturus acclimated at 15°C and 30°C. Each point is mean from 12 determinations.

Triturus is also capable of making a metabolic adjustment in response to cold acclimation (Fig. 2); however, in Triturus there was a difference in the 2 acclimated groups ($p = < .01$) when O_2 uptake was measured at 30°C as well as at 15°C. In thyroidectomized Triturus whose respiratory metabolism was measured both before and after thyroidectomy, there was no difference in oxygen uptake between the 2 conditions. Some of these thyroidectomized animals were kept up to 60 days after operation. From the present experiments, it was concluded that the thyroid is not a major factor in regulation of oxidative metabolism in adults of *Triturus viridescens*.

The metabolism of the tissues studied is summarized in Fig. 3. Oxygen uptake of liver slices obtained from 5°C acclimated frogs was higher than that from the 25°C group. The difference noted, however, was not significant even after 20 days of acclimation. The respiratory metabolism of skeletal muscle and stomach increased with time in the cold acclimated animals but decreased progressively in the group adjusting to the warmer temperature. The difference in oxygen consumption for these 2 tissues was highly significant ($p = .006$, muscle; $p = .002$, stomach) after 10 days. The general shape of the curves for these 2 tissues suggests that the changes in metabolic rate were the result of a gradual and cumulative process. Cardiac muscle (ventricle) responded in an opposite manner from the other tissues studied. The tissue from the 5°C group had a lower oxygen consumption than tissue obtained from the animals acclimated to 25°C. The significance of the differences ($p = .048$) noted was not as great as for the previous tissues but clearly indicates a change quite different from skeletal muscle. The metabolism of heart tissue reflected more closely the physical activity of the organ at the designated acclimation temperature.

In this study, oxygen consumption was expressed in terms of wet weight of tissue. Wet/dry weight ratios were determined at beginning and at end of the experimental acclimation period. From Table I, it will be noted that these wet/dry ratios were not different regardless of the experimental condition under which the animals were maintained. This finding upheld the validity of expressing oxygen consumption in reference to wet weight. In addition, these data rule out the possibility that the differences in metabolic rate noted in this study were due to changes of tissue water content.

Discussion. This study has demonstrated increased respiratory metabolism in frogs and Triturus acclimated to cold (5°C) as compared to animals kept at 25°C when the measurement was made at a common temperature. Fromm(8), in his study of seasonal variations of metabolism in frogs, reported decreases in oxygen consumption with refrigerated (4°C) frogs determined at 22-25°C. The reason for such diverse results is not clear, however, the present investigation agrees with the report

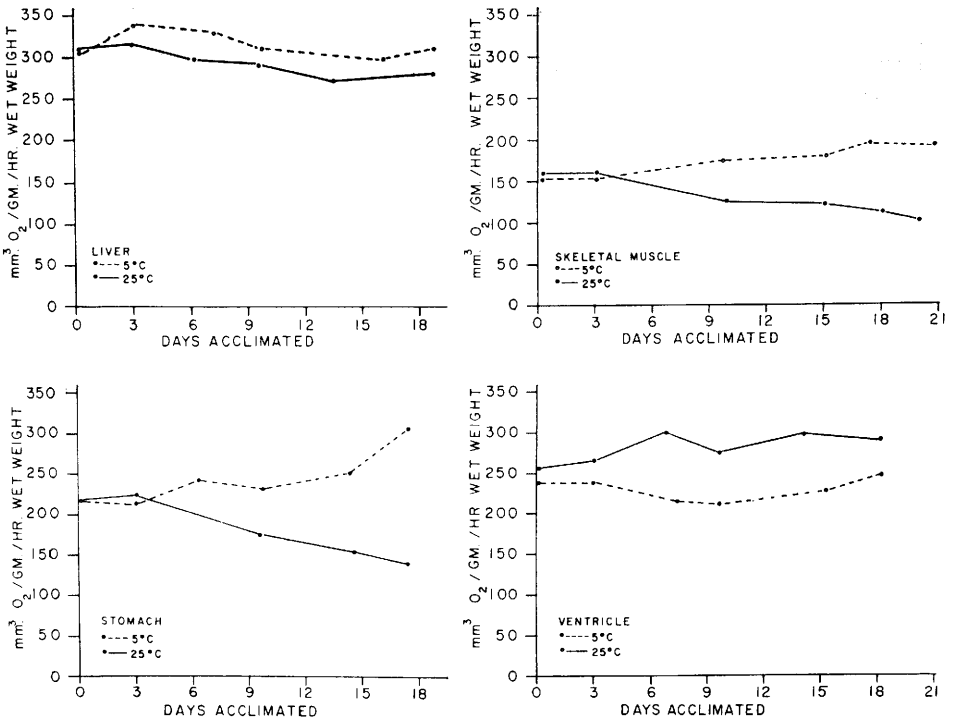

FIG. 3. Oxygen consumption of tissue slices from liver, skeletal muscle, cardiac muscle (ventricle) and stomach taken from frogs acclimated at 5°C and 25°C. Determinations of tissue slices were made at 25°C. Each point is mean from 8 determinations except for stomach at 18 days which is mean from 4 determinations and ventricle 25°C at 7 days which is mean from 5 measurements.

of Stangenberg(9) and her values for oxygen consumption agree closely with those in this experiment.

Tissue metabolism showed diverse changes with acclimation to temperature. It could reasonably be expected, that skeletal muscle, since it represents such a large portion of the body mass, would follow the pattern of changes exhibited by whole animal metabolism. This is, indeed, what was found in our study and is in agreement with Stangenberg (9). Goldfish skeletal muscle(1) demonstrates no change but this may be a species difference. It is interesting to note that ventricular muscle had changes of metabolism which would more closely parallel the physical activity at the acclimation temperature than the respiratory changes noted in the whole animal due to acclimatization. This unexpected finding cannot be clarified from the present data, but deserves fuller investigation. It may possibly be due to the phenomenon associated with measurements made on isolated tissues. It will be noted that all isolated tissues had a higher O_2 uptake per gram than did the whole animal at the same temperature.

The causal mechanisms in the changes of respiratory metabolism reported here cannot be clarified from data at hand. Changes in

TABLE I. Wet/Dry Weight Ratios and % Water in Tissues of *Rana pipiens*.

	Brain		Heart		Liver		Skeletal muscle	
	Wet/Dry	% H_2O	Wet/Dry	% H_2O	Wet/Dry	% H_2O	Wet/Dry	% H_2O
Normal*	5.85	(82.90)	5.88	(82.75)	4.11	(75.68)	5.45	(81.62)
5°C	5.88	(82.98)	5.80	(82.76)	4.16	(75.96)	5.47	(81.70)
25°C	5.80	(82.45)	5.70	(82.36)	3.89	(73.94)	5.30	(81.05)

* Normal tissue samples taken from animals kept at 20°C. Samples were taken at same time the groups were placed at acclimation temperatures. Each value is mean of 8 samples.

water content of the tissue were not a factor in the present investigation since no change was detected with acclimation. Stangenberg (9) reports a significant increase in samples of muscle tissue taken from animals kept at 6°C. These had a higher water content than those at 20°C. If water is a factor, does this mean that metabolism could be increased by artificially increasing water content of a given tissue?

Summary. Total body metabolism of *Rana pipiens* and *Triturus viridescens* was determined in animals acclimated to cold and warm environments. Those animals adjusted to cold had a higher oxygen uptake when measurements were made at an intermediate temperature. Thyroidectomy had no effect on respiratory metabolism of Triturus. Tissue metabolism of skeletal muscle and stomach had higher oxygen consumption when taken from cold acclimated (5°C) animals than when obtained from warm adjusted (25°C). Liver showed no significant changes. Ventricle responded just the opposite from skeletal muscle and stomach. The wet/dry weight ratios of all 4 tissues did not change during acclimation.

1. Freeman, J. A., *Biol. Bull.*, 1950, v99, 416.
2. Suhrman, R., *Biol. Zentralbl.*, 1955, v74, 432.
3. Roberts, J. L., *Proc. XIXth int. Physiol. Congr.*, 1953, p706.
4. Bullock, T. H., *Biol. Rev.*, 1955, v30, 311.
5. Umbreit, W. W., Burris, R. H., and Stauffer, S. F., *Manometric Techniques and Tissue Metaoblism*, 1951, Burgess, Minneapolis.
6. Rogers, C. G., Textbook of Comparative Physiology 1938, McGraw Hill, N. Y.
7. Adams, E. A., Kuder, A., Richards, L., *J. Exp. Zool.*, 1932, v63, 1.
8. Fromm, P. O., *J. Cell. Comp. Physiol.*, 1955, v45, 343.
9. Stangenberg, G., *Arch. ges. Physiol.*, 1955, v260, 320.

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CO_2 Retention in Anesthetized Dogs after Inhibition of Carbonic Anhydrase.* (25550)

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Berliner and Orloff(1) pointed out that an immediate effect of inhibiting the carbonic anhydrase of the blood "would be expected to be a diminution of the elimination of CO_2 and the accumulation of CO_2 in the body until a new steady state was reached." Such a steady state would require that elimination of CO_2 equaled its metabolic production. Measurements of CO_2 output 30 minutes or more after administration of carbonic anhydrase inhibitors revealed no such diminution(2,3). Mithoefer(4) made frequent measurements prior to 30 minutes, but found CO_2 output to be below the control measurement only in a dog in which ventilation was held constant. In the experiments reported here, particular attention was paid to the changes in CO_2 output occurring during the first 30 minutes after injection of a carbonic anhydrase inhibitor.

Methods. Dogs anesthetized with sodium pentobarbital were intubated with a tracheal cannula having an inflatable cuff so that an airtight seal was made within the trachea. Expired gas was collected in a Douglas bag from a respiratory valve attached to the cannula. Analyses of CO_2 and O_2 in duplicate samples of expired gas were made with a thermal conductivity type of CO_2 analyzer (Gow-Mac Gas Master) and a Beckman Model C oxygen analyzer. The volume of gas remaining in the Douglas bag was measured by a dry test meter, and corrected for the vol-

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