A comparison of whole animal and summated tissue respiratory rates of the abalone, *Haliotis cracherodii*

by

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INTRODUCTION

This study was undertaken to determine respiratory rates for the abalone, Haliotis cracherodii, to measure respiratory rates of its component tissues, and, finally, to compare summated tissue respiratory rates with those of the intact organism. Several features of the organism recommend this species for such an investigation, and, as will be suggested below, information so obtained will relate to several questions of interest.

Molluscs exhibit a great diversity of physiological systems and have successfully adapted to a wide variety of habitats. Respiratory systems are among the notable examples of diversity in this phylum, and molluscs are classified partially on the basis of the type of respiratory system they possess.

The members of one class of Mollusca, the Gastropoda, undergo torsion during development. This 180° turn of the visceral mass results in placing the primitive gills, or ctenidia, and the mantle cavity anteriorly, near the head. A further result is the positioning of the anal and nephridial openings alongside the gills. Several methods have evolved which prevent the fouling of the inhalent respiratory current by this close association. These methods entail reduction of organs and rearrangement of water flow.

In the subclass Prosobranchia, members of the orders Mesogastropoda and Neogastropoda have achieved separation of waste products from the incurrent water circulation by elimination of the right gill, retention of only one filament of the remaining gill, and displacement of the gill

structure to the right side of the mantle cavity. Water enters from the left anterior, flows over the gill, and exits on the right side, removing waste from the anal opening, which has been relocated at the right mantle edge.

Only in the order Archeogastropoda is the primitive gill structure retained. Water enters anteriorly, is directed over the gills, and passes out through shell perforations. In Haliotis, water enters anteriorly and ventrally, is swept up and over the gills by ciliary currents, and exits dorsally through a series of holes above the left side of the mantle cavity. The most posterior openings also carry out waste from the anus and kidneys (Crofts, 1929).

Respiratory rates have been determined for a number of molluscan species (Ghiretti, 1966; Nicol, 1960; Altman and Dittmer, 1971). At the outset of this study, rates for only one Haliotid, the small European species Haliotis tuberculata, could be found in the literature (Montuori, 1913). It was of interest therefore to determine the respiratory rate for H. cracherodii, a representative of the Archeogastropoda, in which torsion has occurred but both ctenidia have been retained.

Recently Visser (1972) has determined respiratory rates for H. corrugata. This species, while found from the intertidal area out to 180 feet depths, is usually located in coves and bays, at depths of 20-80 feet. H. cracherodii, by contrast, may be found from near high tide to depths of 20 feet, with the majority being located intertidally (Cox, 1962). These differential preferences in location raise the question of whether there is an accompanying difference in oxygen consumption.

Comparison of tissue respiratory rates with those of the intact animal is an important consideration. Conventionally, results of physiological studies using cells and tissues are extrapolated to apply to the whole animal. As stated by Kerkut and Laverack (1957), this is a useful hypothesis, but not one that has been widely tested.

Most information relating to this hypothesis has come through investigations of another question. An inverse relationship between size and unit respiratory rate, known as the $W^{2/3}$ rule, is known to apply to homeothermic organisms of a wide range of sizes. In an attempt to determine whether this rule can be extended to the component tissues of the organism, several investigators, beginning with Barcroft (1908), compared respiratory rates of whole animals of various sizes with respiratory rates of selected tissues from the same animals.

Martin and Fuhrman (1955) reviewed this work, and, as a more logical approach, suggested comparison of whole animal respiratory rates with tissue rates should include a summation of rates for all tissues of the organism. They then applied this technique to the dog and mouse. Kerkut and Laverack (1957) have extended its application to the study of the gastropod, Helix pomatia, a pulmonate snail.

In each of the above mentioned investigations choice of a suspending medium in which to measure tissue respiration was a problem. Krebs (1950) has commented upon the importance of a suitable medium in producing accurate and reliable measurements of tissue respiratory rates. Physiological solutions developed and used by Krebs have been employed by other workers including Kerkut and Laverack.

The choice of Helix was based on the suitability to such a study of tissues normally bathed in a blood sinus and not provided a high pressure blood supply. This same criterion of suitability applies to Haliotis. Additional features answer the need for an acceptable medium and make Haliotis an almost ideal system in which to study tissue respiration. The animal, covered by a single shell, is easily removed for dissection. The blood, which normally bathes most of the tissues, can readily be removed in sufficient quantity to be used, unaltered except for centrifugation, as the medium for the tissues. Since no clotting mechanism exists and hemocyanin is contained in the plasma, the blood is used in essentially the form in which it existed in the intact organism.

REVIEW OF THE LITERATURE

Early investigators of invertebrate respiration were interested only in determining rates of respiratory exchange for intact experimental animals. Such measurements provided an overall view of oxygen consumption among the various species of molluscs, as well as for other invertebrates. These reports made apparent that molluscs exhibit highly variable metabolic rates. See for example Vernon (1895), Thunberg (1905), Parnas (1910), and Montuori (1913).

As information accumulated, investigators began to draw generalizations concerning the effects of age, size, and physiological condition of the specimen in producing variable rates. These relationships were reviewed by Krogh (1916). Some years later Bruce (1926) pointed out that very little had been published on fluctuations of metabolic activity associated with sexual and seasonal cycles. Such long-period fluctuations were acknowledged to exist but had not been quantitatively evaluated. His work on mussels tested the effects of state of reproductive maturity and season, as well as those of temperature and oxygen pressure, on respiratory rates. He was able to show a seasonal respiration cycle for mussels and established that seasonal changes in respiratory rates are in part associated with the state of sexual maturity of the animal.

Most subsequent studies of whole animal respiration have been concerned with the effect of one or more physical or physiological factors or the effects of long term cycles. The result has been that normal or standard values for the respiratory rates of molluscs usually appear in the literature only as base values for the evaluation of these factors.

Various methods of measurement have been developed and several conventions for reporting oxygen consumption have been adopted. Taken together, these facts make it difficult to assemble a meaningful list of comparative respiratory rates for molluscs. Lists of respiratory rates for some representative molluscs are found in Ghiretti (1966) and Nicol (1960).

Thorough reviews of the literature concerning the effects of various physicochemical and physiological factors on molluscan respiration are presented by Ghiretti (1966) and Nicol (1960). Interactions between these effects are extremely complex, and most of these factors will be considered only briefly here.

Concerning the effect of temperature upon respiration, Nicol (1960) cautions that while Q_{10} values of 2 to 3 are usual, much higher values may be found for some species at low temperatures. Q_{10} for a given species must be determined for the temperature range of concern. In general, oxygen consumption increases with increasing temperature up to the point that temperature produces adverse effects. The specific response of the organism, however, will depend on the temperature to which it is acclimated. Animals acclimated to higher temperatures will begin to increase oxygen consumption, in response to temperature increases, at higher temperature levels than animals acclimated at lower temperatures. This has been demonstrated for species of intertidal gastropods (Sandison, 1968), fresh-water gastropods (Daniels and Armitage, 1969), and bivalves (Read, 1962).

Molluscs show a variety of responses to changes in oxygen partial pressure. The phylum contains both regulators and conformers. Different responses are shown even between closely related species. This is

illustrated by two species of Ancylidae (Berg, 1952). Oxygen consumption of a stream species, Acroloxus fluviatilis remains approximately constant over a range of oxygen partial pressures, while that of A. lacustris falls drastically as oxygen partial pressure diminishes. That effects of lowered oxygen tension may be long lasting was shown by van Dam (1935) in his observations of oxygen utilization rates in lamellibranchs. For example, an animal may have a high respiratory rate for some time to repay oxygen debt incurred during a low tide.

The respiratory response of molluscs to changes in salinity appears to be closely related to osmoregulatory activity. According to Lang (1968), ability to increase respiratory rate with decreasing salinity may be an adaptive feature acquired through induction of enzyme systems.

Response, through change in metabolic rate, to any of these external factors may be dependent upon season, age, state of sexual maturity, nutritional state, or activity of the organism. Similarly, each of these physiological factors may be shown to have a direct effect of its own upon respiratory rate. Activity and sexual maturity produce increases in rate; age or the starved condition a decrease in rate. Cyclical adjustments to changing seasons may also be observed.

A final physiological factor may be considered, this one having figured prominently in research on respiratory physiology of both tissues and whole animals. A huge body of literature has demonstrated the relationship between size and oxygen consumption. Zeuthan (1953) and Hemmingsen (1960) have summarized this information for a large number of organisms ranging in size from bacteria to large mammals. These reviews

indicate oxygen uptake is an exponential function of body weight and is expressed by the equation

$$\frac{dO_2}{dt} = aW^b$$

where the power of the body weight is 0.751 ± 0.015 (Hemmingsen, 1960). Numerous investigations have shown this fundamental relationship between metabolism and body size to be valid for molluscs, but with considerable variation being noted in the value of b. Newell and Pye (1971) have recently reviewed this relationship for molluscs considering the effects of additional, superimposed factors such as salinity, shore level, temperature and season.

Attempts to extend this concept of size-related oxygen consumption to the tissue level has generated much conflicting information on tissue respiration and the relationship between total animal respiratory rates and tissue respiratory rates. Several approaches have been used to determine whether the dependence of metabolism on size is based on cellular or organismic mechanisms. As will be seen, certain trends of thought have developed, but no conclusive answer or explanation has been reached.

Weymouth, Field, and Kleiber (1942), relying largely on Kleiber's measurements of rat, rabbit, and sheep tissue (Kleiber, 1941), concluded that respiration of excised tissues parallels that of the intact animal and therefore indicates that controlling mechanisms are intrinsic and, if of central origin, must be long lasting, since they are persistent in the <u>in vitro</u> situation.

Comparing the respiratory quotients of five tissues from nine

mammalian species, Krebs (1950) found no strict parallelism between tissue respiratory quotients and basal heat production of intact animals. He noted that, in general, tissues of larger species exhibited metabolic rates somewhat lower than homologous tissues of smaller species but attributed most of the decrease in basal metabolic rate in larger animals to the increasing preponderance of connective tissue and muscle in these organisms.

In relating their measurements of rat tissue respiration to body size of the whole animal, von Bertalanffy and Pirozynski (1953) saw a definite and significant correlation between rate of tissue respiration and body size for only one tissue, the diaphragm. Their interpretation was that the decrease in whole animal metabolic rate with increase in size depended on regulative factors lying in the organism as a whole.

All three of these investigations, resulting in widely different findings and conclusions, were based on measurements of tissue oxygen consumption. Another approach to understanding the relationship between tissue metabolism and total metabolism has been to measure both whole animal respiratory rates and tissue respiratory rates for the same animal. The respiratory rate for each tissue is determined. The product of the rate and the weight of the represented organ is obtained. The sum of the products for all the organs measured is taken. That sum is then compared with the previously determined oxygen consumption for the whole animal.

Barcroft (1908) performed blood flow and blood gas analysis on a single dog and found that summated organ respiration accounted for 80% of the metabolic rate of the dog. He suggested the resting metabolism in the dog represented the arithmetic sum of tissue respiration. Barcroft's

assumptions, though based on meager evidence, have tended to be supported by later workers.

However, first attempts at similar measurements did not produce supporting evidence for Barcroft's work. Reports by Terroine and Roche (1925) and Grafe, Reinwein, and Singer (1925) both showed that resting metabolism of animals varied inversely with body size while respiration of homologous tissues *in vitro* did not. Grafe, Reinwein, and Singer (1925) estimated from *in vitro* measurements that summated tissue respiration of the rat was 1.9 times that of the resting metabolism. They assumed that in larger animals an "organismic factor" must limit tissue respiration *in vivo* but not *in vitro*.

Field, Belding, and Martin (1939) challenged the assumptions of size and activity used by Grafe, *et al.* in estimating summated tissue respiration. Field and co-workers used a large number of measurements, a more homogeneous group of animals (rats), and what they considered to be more realistic allowances for activity and for the respiratory contributions of supportive tissues. They calculated summated tissue respiration to be approximately 65% of the resting metabolic rate of the intact animal. The summed tissue respiration being less than whole animal respiration was taken as evidence that the level of tissue respiration, whether measured *in situ* or *in vitro*, is determined in part by somatic factors. It was their assumption that such factors probably affect respiration *in situ*, and at least the initial level of respiration *in vitro*, to about the same extent.

Martin and Fuhrman (1941), using the dog, found summated tissue

respiration to equal 79% of resting metabolism. By their calculations, summated tissue respiration, with allowances for minimal activity, represents approximately 89% of resting metabolism in both the rat and the dog.

In a similar study using more refined techniques, Martin and Fuhrman (1955) found summated tissue respiration, when based on one hour average respiratory rates, represented approximately 88% of the metabolic rate of the whole dog and 61% of the metabolic rate of the mouse. If the summations were based on rates extrapolated to the time the tissue measurement was begun the percentages were 72 and 105 for mouse and dog respectively.

As indicated above, Martin and Fuhrman and their co-workers have done much of the published work regarding comparison of total animal and tissue respiration. Theirs are the only studies which compare the summed respiration of all body tissues with respiration of the whole mammal. Conclusions reached by Martin and Fuhrman therefore represent the present interpretation of research relating total animal respiration to tissue respiration in mammals. They conclude that studies of tissues in vitro do not give evidence of a regular progression of oxygen consumption values according to an exponential value of the body weight, and that changing connective tissue content and changing tissue proportions between animals of different sizes account for differences in total animal metabolic rates.

Two studies made to determine the relationship between whole animal respiratory rates and tissue respiratory rates of invertebrates have been used to support the postulated role of basic cellular metabolic differences in regulation of total animal respiration of animals of differing sizes.

Weymouth, et al. (1944) have shown that total oxygen consumption and weight specific oxygen consumption of the intact crab, Pugettia producta, and tissue respiration of the crab mid-gut gland all decrease with increasing body weight. These parallel decreases in whole animal and tissue respiration were taken as an indication of intracellular control of respiration in the intact animal. The authors speculate that the central control of respiration may be through genetic regulation of the development of enzyme levels.

In a study of respiratory physiology of nine species of marine crabs, Vernberg (1956) found that active species had higher respiratory rates than similar sized, less active species. The fact that oxygen consumption of isolated sections of gill tissue of these species followed a similar relationship was taken as evidence for cellular metabolic differences determining respiratory rates.

It must be noted that these speculations are based on comparison of total respiratory rates with respiratory rates of only one component tissue in each instance. A more useful comparison has been made by Kerkut and Laverack (1957) using the pulmonate snail Helix pomatia. In this case, respiratory rates for all tissues were summed for comparison with total animal respiration.

Measurements of Helix respiration show the average rate of respiration of the whole animal is usually lower than that of the isolated tissues. Since the animal, respiring at its maximum rate, can attain the rate of respiration of its isolated tissues, Kerkut and Laverack entertain the possibility that there may be some type of control of respiration in the intact animal which brings its normal range to about 60% of the

possible maximum rate.

To recapitulate the present status of research in relating tissue respiration to respiration of the whole animal: In mammalian systems tested whole animal respiration exceeds the sum of tissue respiration. It is concluded that in these systems no direct relationship exists between body size and tissue respiratory rates. Increasing total animal respiratory rates with decreasing body size are not thought to be determined by changes in tissue respiratory rates, but by changes in proportions of supportive tissue to body weight. In invertebrate systems summed tissue respiratory rates exceed those of the whole animal. For those systems it is suggested that decreasing total animal respiratory rates with increasing size may result from central control of tissue respiratory rates.

As in the case of comparing whole animal respiration rates of various molluscs, it is difficult to formulate a list of comparative respiratory rates for molluscan tissues. In evaluating the extent of cyanide sensitive enzymes in tissue respiration of invertebrates, Robbi (1949) has produced a list of respiratory rates for some tissues of the squid and the black oyster. A similar list for the octopus was provided by Ghiretti-Magaldi, Giuditta, and Ghiretti (1958) in their evaluation of the effects of oxygen tension on tissue respiration. Finally, Kerkut and Laverack (1957) list respiratory rates for all tissues of a pulmonate snail. Most other molluscan tissue respiratory rates in the literature represent control values in experiments testing the effect of one or more intrinsic or extrinsic factors upon oxygen consumption of one or more tissues.

Among the tissues most studied are hepatopancreas, heart, gill, and muscle. That a variety of factors do affect tissue rates can be seen from the following representative studies.

Baldwin (1938) reported the average respiratory rate of 2.93 μl/mg per hour for Helix hepatopancreas could be increased by addition of galactose to the medium. Later, Rees (1953) found essentially no difference in respiratory rates for hepatopancreas from active and hibernating Helix, in spite of differences in enzyme content of the organs.

Herold and co-workers have investigated the effects of various factors on the heart tissue of snails (Helix). Increasing partial pressures of oxygen had the effect of increasing heart rhythm and oxygen consumption (Herold, Joly, and Nicolet, 1965), and this seemed to be related to the extent to which the suspending medium was oxygenated (Ripplinger and Herold, 1967). Increasing intraventricular pressure (Herold, 1966) and ion augmentation of the medium (Herold and Bey, 1966) had similar stimulatory effects.

According to Pritchard, Huston, and Martin (1963), octopus heart tissue can withstand anoxia for periods up to 48 hours before showing oxygen consumptions lower than control tissue samples. Navez, et al. (1941) showed that disruption of heart tissue of Venus drastically affected its respiratory rate. Cutting reduced the rate by 10% and mincing reduced it by 50%.

Gill tissue of the freshwater mussel Dreissensia was reported by Wernstedt (1944) to have a respiratory rate of 18.7 μl/mg nitrogen per hour which remained unaltered for 24 hours. Oxygen consumption of isolated gill tissue of the marine mussel Mytilus varies with salinity, reaching a

maximum at a salinity corresponding to that of the water to which the whole animal has been adapted (Lang, 1968). Hopkins (1946) has found higher respiratory rates for gill tissue of young clams than older clams during the summer season, the higher rate being associated with a greater ciliary activity for gills from young clams.

A similar but non-seasonal decrease in oxygen with increasing age was found for muscle tissue from specimens of Pecten irradians and Venus mercenaria (Hopkins, 1930). Closing muscle exhibited a greater respiratory rate than non-striated adductor muscle from five species of clam.

Age differences were also found in tissues of Portuguese oysters. The average rate of oxygen consumption of four tissues from 30 month old oysters was 75% that of the same tissues from oysters 10 to 15 months old (Chapeau, 1932).

Comparison of respiratory rates of tissues presents the same problem of lack of uniformity in presentation of data encountered in comparing whole animal rates. Rates may be based on wet or dry tissue weights or on an animal weight specific basis. Alternatively, rates may be expressed on a nitrogen weight basis or stated on a volume basis. Also contributing to the difficulty of making comparisons are the lack of uniformity in testing conditions used by various investigators and the failure, in some instances, to report all of the conditions of experimentation. In some cases the conditions under which the tests are conducted do not represent physiological conditions for the intact organism, and this fact must be taken into account in comparing tissue rates from various species.

If in vitro measurements are to be used validly in drawing conclusions about the in vivo situation, every effort must be made to approximate

physiological conditions. One of the most perplexing problems is the provision for an appropriate medium for the tissues. Ideally the medium should consist of the same fluid to which the cells are normally exposed. In mammalian systems disruption of the circulation alters the condition of the blood. The use of whole blood is precluded because of its coagulation properties. Measures to prevent the clotting mechanism introduce further variables. Blood serum differs considerably from whole blood, and, as stated by Krebs (1950), serum is not necessarily the best medium in which to measure tissue respiration. The serum may rapidly become depleted of substrates for cell respiration. At the same time, metabolic products may accumulate to toxic levels. If physiological solutions are to be formulated, careful attention must be given to ion contents and ratios (Krebs, 1950). It has been shown, furthermore, that not all tissues respond equally well to the same solutions (Krebs, 1950; Kerkut and Laverack, 1957).

It is obvious that, in the case of mammalian tissues, oxygen supplies to the tissues do not diffuse as readily in vitro as they did when the tissue was provided oxygen through a system of capillaries permeating the tissue. It is difficult to assess to what degree diffusion in vitro duplicates physiological conditions for tissues normally serviced by an open circulatory system. Since availability of oxygen to respiring cells will affect the rate of respiration, tissue sample size and surface area available for diffusion of respiratory gases is an important consideration in preparation of specimens. Lang (1968) has also shown that the rate at which samples and medium are shaken is a significantly important factor to be controlled. His work showed that, at apparently optimum conditions,

increasing the shaking rate by 50% produced an increased tissue respiratory rate.

When tissue respiratory rates and sums of these individual rates are compared to the respiratory rate of the intact animal several more problems become apparent. It is uncertain to what extent *in vitro* measurements reflect the *in vivo* situation. Tissues often maintain active respiration *in vitro* for several hours. However, it is not known how these rates compare with those of the tissues at the time they were removed from the animal. Martin and Fuhrman (1955) have suggested the initial rates may be ascertained from extrapolation of the curve of respiratory rates of isolated tissue during the time measured. Kerkut and Laverack (1957) challenged the use of this method for obtaining initial rates for mammalian tissues since the mammalian tissues typically exhibit a sharp decrease in respiratory rate soon after isolation and more gradual decreases during longer periods of isolation. Such an estimation may be more valid for molluscan tissues, which maintain more nearly constant rates throughout the isolation period. Even if extrapolation to time zero is assumed to provide a valid estimate of the *in situ* rates of most of the tissue, it can not be expected that it would indicate respiratory rates of tissues such as muscle which, it would be assumed, have a much higher rate when active. Feng (1932) has shown a higher rate of oxygen consumption for skeletal muscle under stretch, and Bülbring (1953) has shown smooth muscle also increases oxygen consumption when under tension.

Oxygen consumption data yield maximum useful information only when collected under carefully regulated experimental conditions, approximating

as closely as possible the physiological situation, and when reported in a manner which readily lends them to comparison with similar data from other investigators.

MATERIALS AND METHODS

Handling of Animals

Abalone used in this study were obtained from Pacific Bio Marine Supply Company of Venice, California and were shipped to Iowa via air freight. En route the animals were packed in plastic bags of cold sea water in styrofoam ice boxes. On arrival in Ames, Iowa, the animals were transferred to sea water tanks where they were allowed to cling to the tank sides. Artificial sea water recirculating in the tanks was made from a salt mixture obtained from Aquarium Systems of Eastlake, Ohio, and adjusted to a density of 1.025. Temperature in the tanks was maintained at 16 to 18°C. Animals were allowed to remain undisturbed in the tanks for at least one week prior to use. Though they were kept for as long as three to six months in the tanks, no attempt was made to feed the animals, so they must be considered to have been in a starved condition.

Measurement of Whole Animal Respiration

Animals in this phase of the study are numbered. Numbers 1 to 10 were obtained in June, 1971; numbers 11 to 15 in August, 1971; numbers 21 to 27 in November, 1971; animal 31 was obtained in May, 1972.

For measurement of whole animal respiration a respirometer chamber was constructed of 1/4 inch plexi-glass. The chamber measured 7 1/2 inches long by 5 1/2 inches wide by 5 inches deep and had a maximum capacity of 3,360 ml. Chamber sides and plexi-glass lid were fitted

for screw closure. A water tight seal was achieved by insertion of a 1/8 inch Neoprene gasket.

Plexi-glass tubes in two corners of the chamber allowed water to be introduced into and removed from the chamber. These tubes were approximately equal in length to the depth of the container and were perforated by a series of holes along their lengths so that water flow was distributed through the chamber. To each of the Plexi-glass tubes in the lid was attached flexible Tygon tubing. The Tygon tubing in turn was fitted with stoppered T-tubes through which intermittent measurements of the oxygen content of the water could be made.

Apparatus for the measurement of oxygen consumption by the animal within the chamber was arranged in one of two ways:

The first method employed gravity flow and had the advantage of allowing gradual changes of oxygen partial pressure in the water furnished to the animal. In this system, water from a five gallon bottle on a high shelf was allowed to flow through a one gallon bottle submerged in the constant temperature bath with the respirometer. Water then flowed through a short glass cooling coil on its way into the chamber. Excurrent flow rate was regulated by means of a screw clamp.

In the second system, circulation of water through the respirometer chamber was accomplished by a tubing pump. This method offered the advantage of furnishing water from a constantly well aerated supply over an unlimited time period, since the water could be re-circulated. Here, air was bubbled through sea water in an open five gallon aquarium submerged, along with the respirometer, in a large constant temperature

chamber. Water was pumped from the aeration tank through the respirometer chamber and back, through the pump, to the aeration tank.

In either test system the animal was allowed a 30 minute equilibration period. Following this, measurement of the oxygen content of incurrent and excurrent flow was made at 15 minute intervals using a Beckman Model 160 oxygen analyzer and Beckman macro oxygen electrodes. Oxygen consumption was calculated from the change in oxygen partial pressure and the rate of flow through the chamber. Since the solubility coefficient (α) of oxygen in water at 20°C and 760 mm Hg is 0.031, the following equation was used

$$\text{Oxygen Consumption } m\ell/g \text{ per hr} = \frac{\frac{\text{mm Hg } O_2}{760} \times 1000 \times \alpha \times \ell/\text{hr flow rate}}{\text{soft body wet weight in grams}}$$

Routinely the test period lasted two hours. When possible each animal was measured five times. Animals being used for comparison with the respiration rates of their own tissues were each tested once for a period of four or eight hours.

Measurement of Tissue Respiration

Respiratory rates of selected tissues of the abalone were determined by the direct Warburg method (Umbreit, Burris, and Stauffer, 1964). Measurements were made at 20 $\pm$ 0.1°C. The suspending medium for the tissue was blood from the abalone, and the gas phase was air. The thermobarometer contained blood. Flasks were shaken at a rate of 60 cycles per minute.

Blood to be used as the medium was obtained by a deep slice into the center of the foot to drain the hemocoel. Blood was allowed to run out into a beaker, the volume was measured, and then the blood was centrifuged in cold tubes for ten minutes at 900 x G. From the centrifuge tubes it was decanted into a flask for pipetting into the Warburg flasks.

After blood had been drained from the abalone, each organ to be used was dissected out and placed on a glass plate for further dissection. Tissue samples of suitable size were removed, rinsed in sea water, and gently blotted for transfer to tared weighing vessels. Weighed samples were placed in the blood in the Warburg vessels. The vessels were attached to the manometers and situated in the constant temperature bath for a 15 minute equilibration period before they were sealed. The blood and tissues were kept chilled through each of these stages, and the dissection and transfer of tissues to the constant temperature bath was completed within a maximum of 45 minutes.

Some tissues, such as heart, gill, kidney, red muscle, and gonad, of filamentous, reticulate, or membranous form, could be used as obtained. Others required some manipulation to assure adequate diffusion. Thin sections of mantle were taken and subdivided by razor cuts. Very thin slices of foot muscle were shaved from the central cut in the foot. Each tissue was washed and blotted to remove mucus, and gonads were thoroughly washed to remove eggs or sperm. Except where it is indicated that several samples of the same tissue were taken for comparison,

tissue specimens were obtained from the same position from each animal measured. All samples weighed less than one gram and most weighed from 0.2 to 0.5 grams.

Comparison of Whole Animal Respiration and Tissue Respiration

The last phase of the study compared the oxygen consumption of the whole animal with the oxygen consumption of its component tissues. The respiratory rates of each of five animals were measured as described above. Subsequently, respiratory rates for representative tissues of each of the animals were determined. Using the weight of each organ and the respiratory rate of its component tissue, a summed tissue respiratory rate was calculated for each animal and a balance sheet for total and tissue respiratory rates was constructed for each.

RESULTS

Whole Animal Respiration

Whole animal respiratory rates were measured for 23 specimens. Respiratory rates are reported as μl oxygen/gram soft body wet weight per hour. The average over the whole two hour period is represented. Originally it was intended that each animal should be measured five times for a period of two hours and, finally, once for a more extended period of four to eight hours. Only six animals survived to complete the series of six measurements or more. Others died or were sacrificed previous to the completion of the test series.

Table 1 shows the results of these measurements for all 23 animals. Although the overall average respiratory rate is 16.31, inspection of the table shows a range of rates from 1.83 μl/g per hour for number 3 to 74.88 μl/g per hour for number 26. Similarly, individual animals usually presented a range of rates, with the average rate not necessarily falling mid-way in the range.

The wide range of respiratory rates for individual animals must be considered in any analysis of the effects of variables upon respiration rate. However, some relationships can be noted.

Observation of the data indicated that animals of similar size seemed to have similar average respiratory rates and that there tended to be an inverse relationship between respiratory rate and size. Regression analysis showed that the relationship between respiratory rate and soft body wet weight is linear, with an average slope of -0.19.

Table 1. Total animal respiratory rates for all animals measured

Animal number	Number of measurements	Average oxygen consumption (μl/g per hr)	Range
1	2	32.94	5.78 - 60.09
2	1	32.51	
3	6	7.06	1.86 - 17.09
4	6	7.23	1.95 - 12.31
5	1	30.12	
6	4	13.94	9.43 - 22.78
7	6	11.02	5.23 - 25.87
8	7	7.63	2.82 - 13.66
9	6	8.04	4.44 - 15.12
10	3	9.10	2.86 - 15.71
11	6	12.72	3.74 - 20.57
12	1	15.25	
13	1	19.67	
14	1	4.15	
15	3	6.61	2.59 - 13.05
21	2	25.27	17.49 - 33.05
22	1	8.22	
23	1	8.50	
24	1	36.82	
25	1	19.95	
26	3	39.09	9.01 - 74.88
27	3	10.01	5.00 - 17.01
31	1	9.38	

The correlation coefficient, significant at the 0.01 level, is 0.5. The regression of respiratory rate on body weight is shown graphically in Figure 1.

A comparison of respiratory rates for male and female abalone (Table 2) would appear to indicate a higher average rate for females. A Student's t test of the differences between the means for males and females, including all measurements, shows the means to be significantly different at the 0.05 level. It should be noted, however, that of the 23 animals measured, only four were males. Further, of these four males, two were among the four largest animals measured and, as has just been shown, larger animals tend to have lower rates.

When all measurements are used in computing the overall average respiratory rates, 12 of the 23 animals are each represented by less than three measurements. In view of the wide range of rates shown by a single animal, probably averages of three or more measurements would better represent the respiration of a single animal. A t test of the difference between mean respiratory rates for males and females, using only rates that represent an average of three or more measurements, shows the difference not to be significant. Since only two males survived to be measured three or more times the mean for males is based on an undesirably small sample size.

When season of the year is considered as a variable, as in Figure 2, no seasonal pattern of respiratory rates can be detected. This fact becomes even more apparent when multiple measurements for a single animal are compared as in the graphs of Figures 3 through 8. Minimum

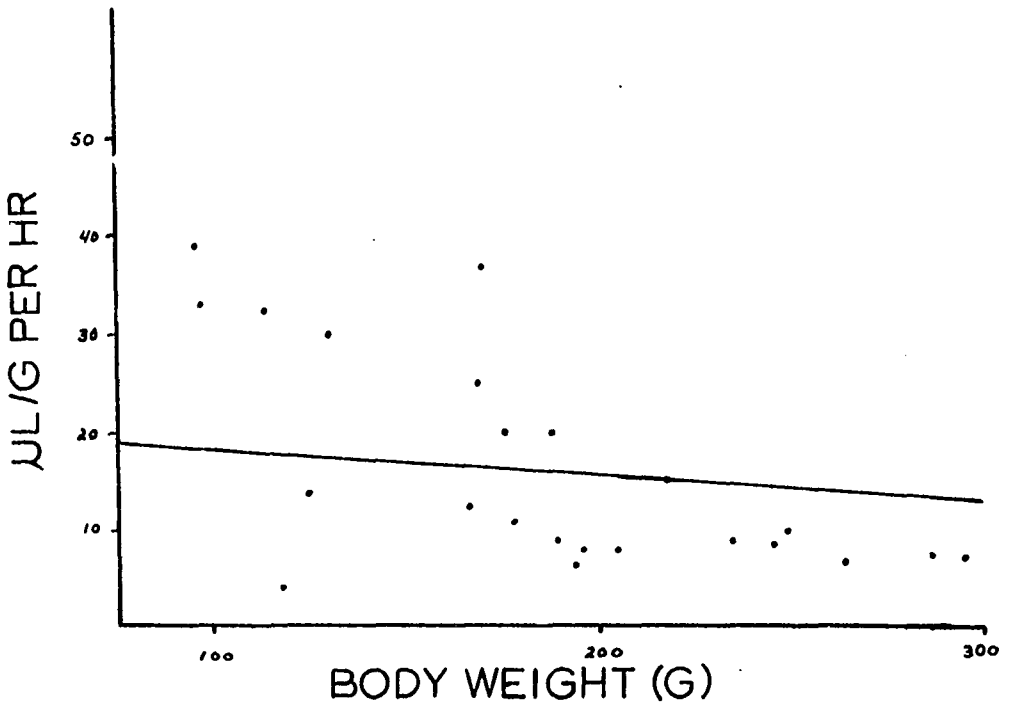

Figure 1. Respiratory rate as a function of body weight

Table 2. Comparison of total animal respiratory rates of male and female specimens

Female				Male			
Animal	No. of measures	Wt. (g)	Avg. Resp. Rate (µl/g per hr)	Animal	No. of measures	Wt. (g)	Avg. Resp. Rate (µl/g per hr)
3	6	263.1	7.06	11	6	163.7	12.72
4	6	294.5	7.23	27	3	249.0	10.01
6	4	124.7	13.94	14	1	118.0	4.15
7	6	178.0	11.02	23	1	246.1	8.50
8	7	186.5	7.63				
9	6	196.1	8.04		Average	194.2	8.85
10	3	189.4	9.01				
15	3	194.2	6.61				
26	3	96.5	39.09				
1	2	98.1	32.94				
2	1	114.5	32.51				
5	1	130.6	30.12				
12	1	218.4	15.25				
13	1	190.5	19.67				
21	2	169.4	25.27				
22	1	205.2	8.22				
24	1	169.9	36.82				
25	1	176.2	19.95				
31	1	234.8	9.38				
	Average	180.6	17.88				

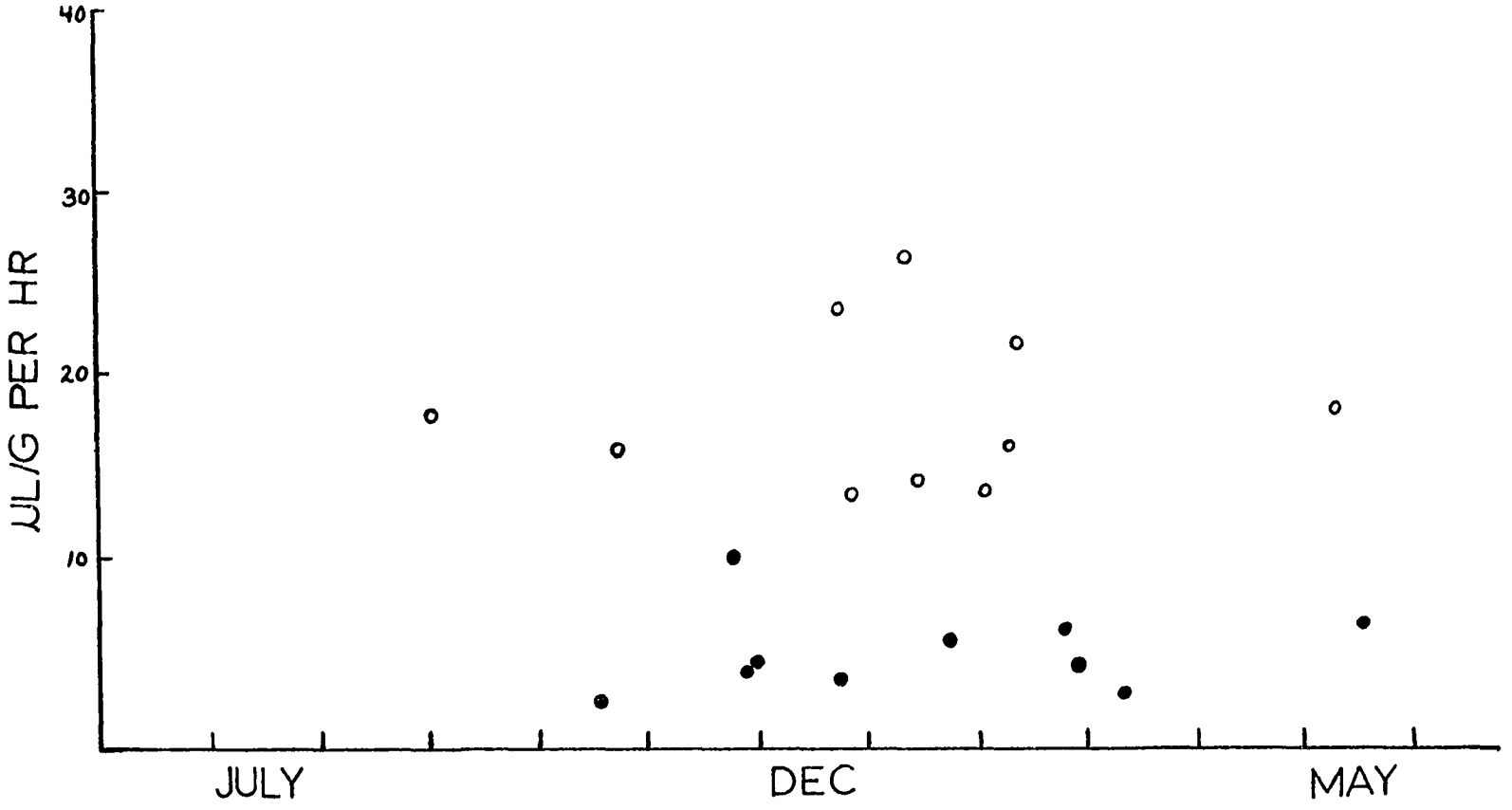

Figure 2. Scatter diagram of maximum (o) and minimum (●) respiratory rates shown by 10 animals measured three or more times

Figure 3. Respiratory rates of animal No. 3 during six two hour test periods and one four hour test period

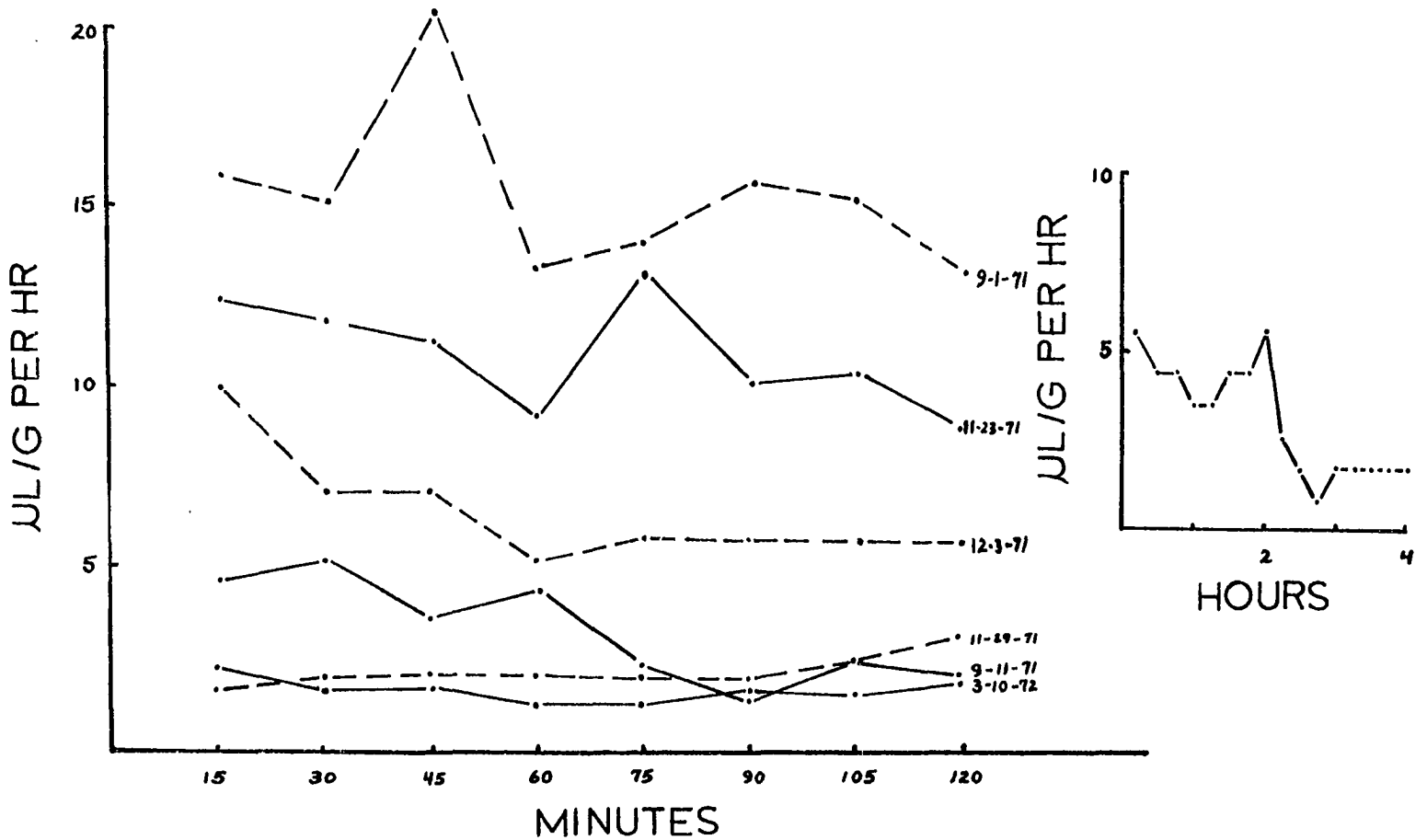
20
15
10
5
JUL/G PER HR
9-1-71
11-23-71
12-3-71
11-29-71
9-11-71
3-10-72
15
30
45
60
75
90
105
120
MINUTES
10
5
JUL/G PER HR
2
4
HOURS

Figure 4. Respiratory rates of animal No. 4 during six two hour test periods and one eight hour test period

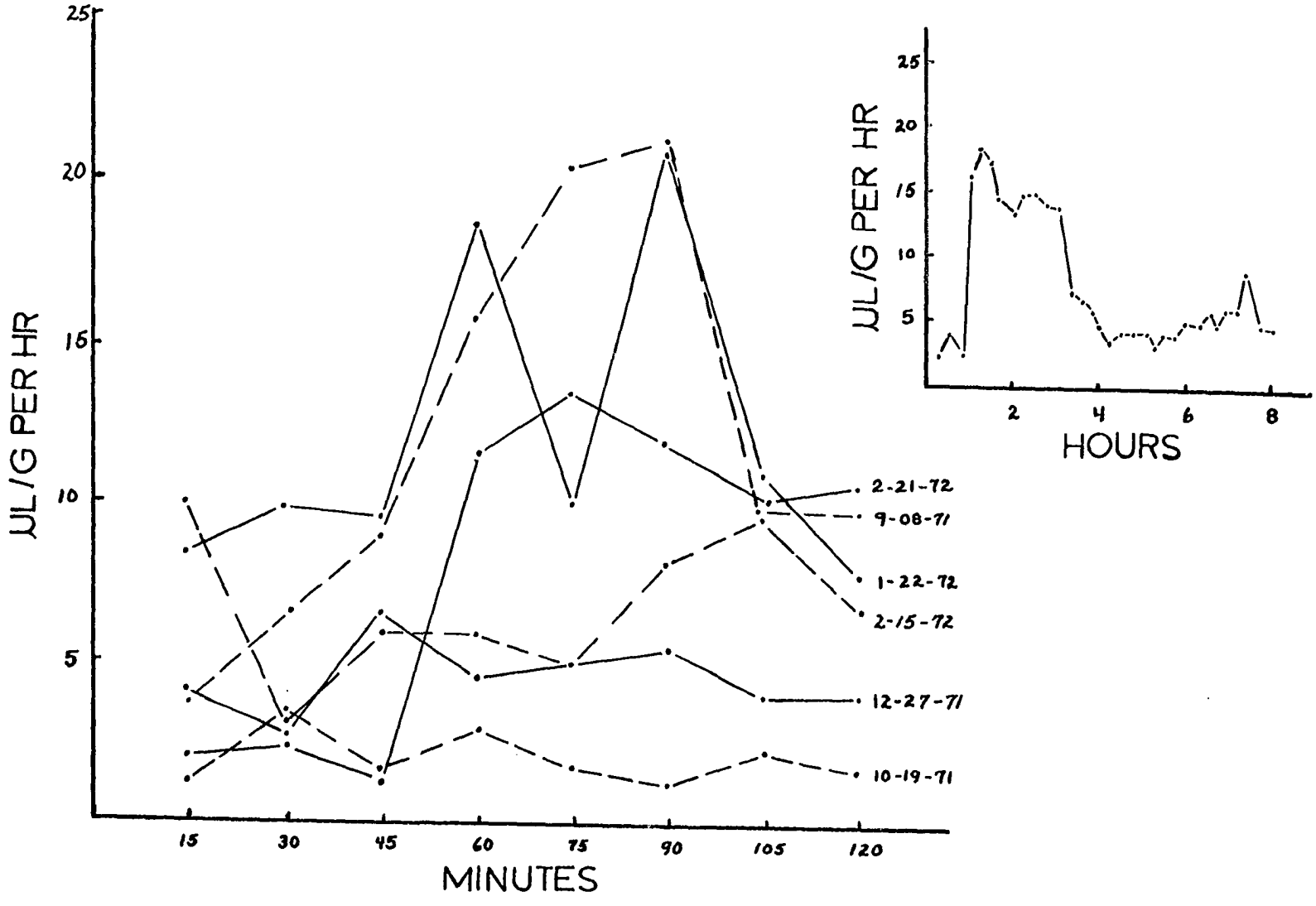
25
20
15
10
5
UL/G PER HR
15
30
45
60
75
90
105
120
MINUTES
2-21-72
9-08-71
1-22-72
2-15-72
12-27-71
10-19-71
25
20
15
10
5
UL/G PER HR
2
4
6
8
HOURS

Figure 5. Respiratory rates of animal No. 8 during six two hour test periods and one four hour test period

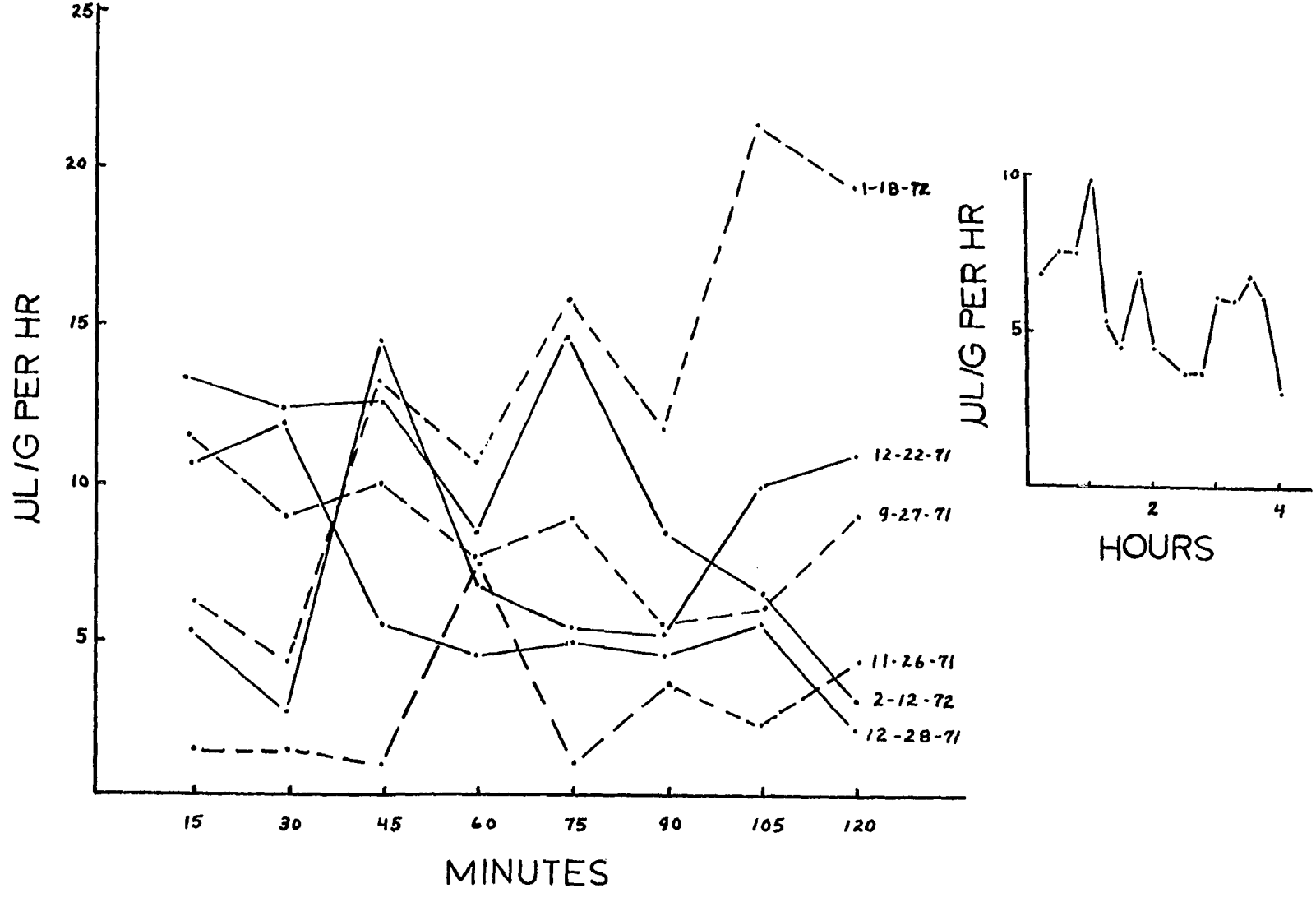
25
20
15
10
5
JUL/G PER HR
1-18-72
12-22-71
9-27-71
11-26-71
2-12-72
12-28-71
15
30
45
60
75
90
105
120
MINUTES
10
5
JUL/G PER HR
2
4
HOURS

Figure 6. Respiratory rates of animal No. 9 during six two hour test periods and one four hour test period

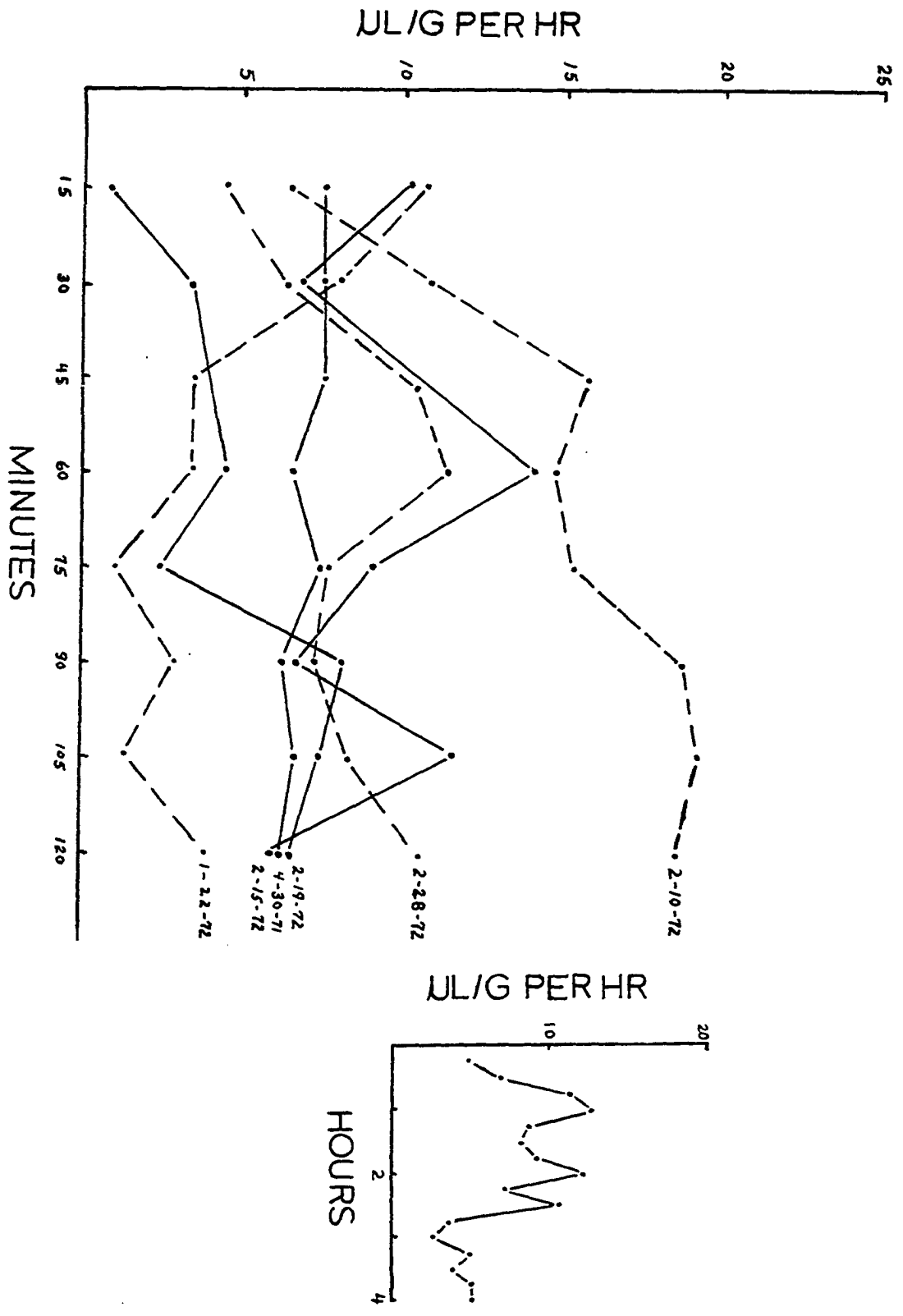
UL/G PER HR
5
10
15
20
25
MINUTES
15
30
45
60
75
90
105
120
2-10-72
2-28-72
2-19-72
4-30-71
2-15-72
1-22-72
UL/G PER HR
10
20
HOURS
2
4

Figure 7. Respiratory rates of animal No. 11 during six two hour test periods and one four hour test period

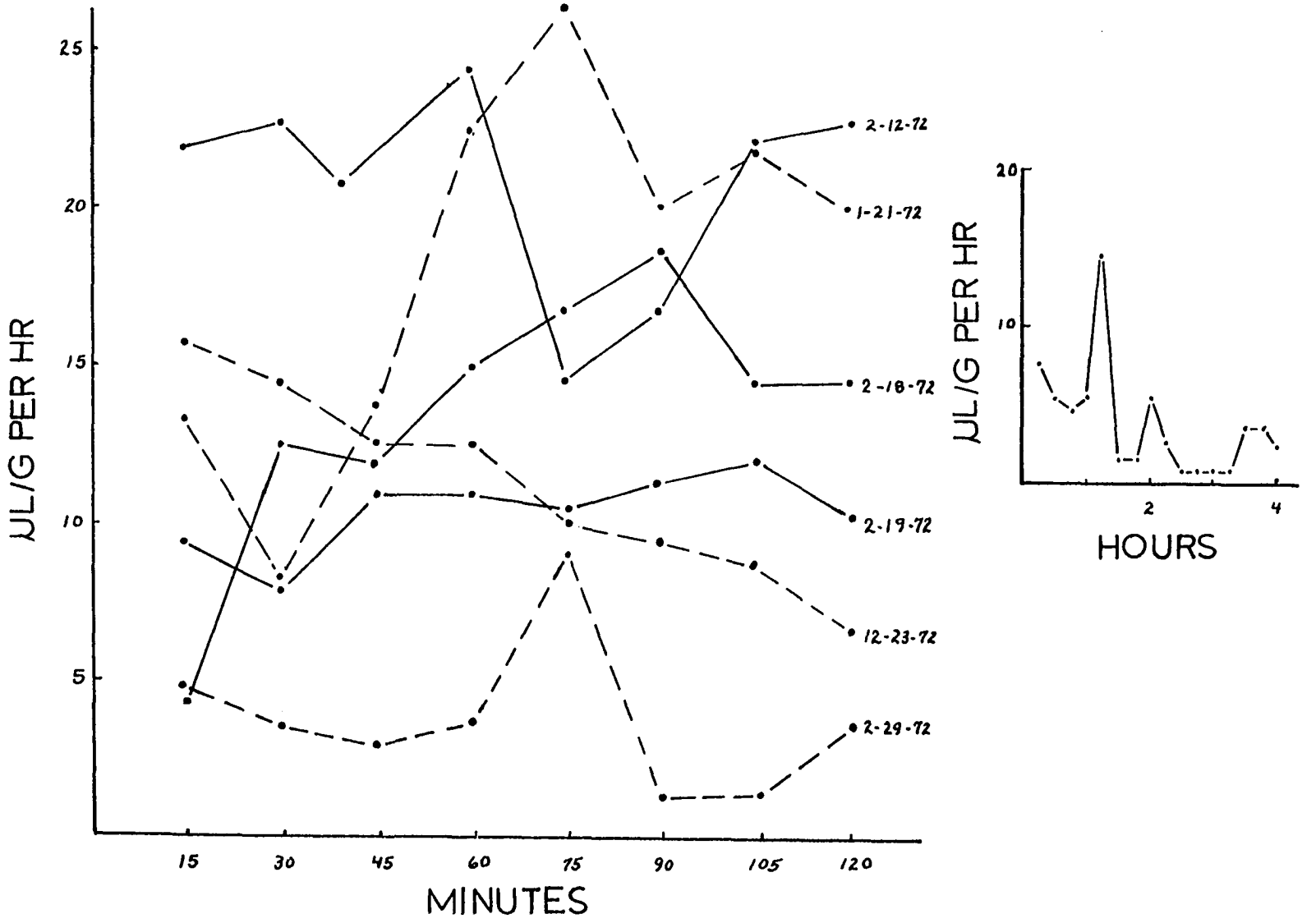
25
20
15
10
5
JUL/G PER HR
2-12-72
1-21-72
2-18-72
2-19-72
12-23-72
2-29-72
15
30
45
60
75
90
105
120
MINUTES
20
10
JUL/G PER HR
2
4
HOURS

Figure 8. Respiratory rates for animal No. 27 during three two hour test periods and one four hour test period

UL/G PER HR
5
10
15
20
25
15
30
45
60
75
90
105
120
MINUTES
2-29-72
2-19-72
2-18-72
UL/G PER HR
10
20
2
4
HOURS

and maximum rates seem to occur independent of season and without any ordered relationship to previous or subsequent rates. Consistent trends toward increased or decreased rates are not shown.

Similarly, no correlation can be shown between average respiratory rates and period of time the animals have been held under laboratory conditions. Some animals showed the highest average rates during the earliest test sessions; others during the latest.

Originally it had been intended to measure the effects of varying partial pressure of oxygen upon the respiratory rates of the abalone. Measurements of respiratory rate made in the gravity flow system allowed for gradual decreases in the oxygen content of the water. Measurements made using this system showed that rates varied widely, irrespective of the changes in oxygen content. That the fluctuations were not dependent on changes in oxygen partial pressure was confirmed by the measurements made in the pumped flow system, where water of a constant high content of oxygen was furnished to the animal. Because of the wide variation in rates already observed under these conditions, no attempt was made to measure the effect of changes in temperature. Rather, all measurements were made at 20°C, a temperature within the range of habitat conditions for Haliotis cracherodii.

Tissue Respiration

In the initial phase of this study 22 animals were used for measurements of tissue respiratory rates. Rate determinations were done on nine tissue samples from each animal. Additional measurements were

made using tissues from the six animals used for comparison of whole animal and tissue respiratory rates. Measurements were made at the same temperature used for measuring whole animal respiration. Use of blood as the suspending medium and air in the gas phase was thought to provide the closest approximation to the physiological conditions of the tissues.

Table 3 summarizes the tissue respiratory rates for selected tissues of Haliotis. Figures are given on both a wet and a dry weight basis for readings taken at 15 minute intervals over a period of two hours. Tissue respiratory rates vary from 132.1 μl/g wet weight per hour for crop tissue to 38.6 μl/g wet weight per hour for tissue of the male gonad. No consistent relationship is seen between the wet weight and dry weight respiratory rates, presumably because of differing water content of different tissues. However, for many of the tissues, the dry weight respiratory rate is greater than the wet weight respiratory rate by a factor of approximately five.

On a wet weight basis a marked difference is shown between average respiratory rates for the right and left kidneys. Differences in average respiratory rate for tissue of male and female gonads is shown on both a wet weight and a dry weight basis. A t test revealed the difference between mean respiratory rates for gonad tissues was significant at the 0.05 level.

Listed in Table 4 is the average percent composition of soft body wet weight represented by individual organs. Extensive draining of the hemocoel previous to dissection tends to produce reduction of

Table 3. Average respiratory rates of selected tissues on a sample wet weight and a sample dry weight basis

Tissue	2 hr average oxygen consumption µl/g wet wt. per hr ± S D	n	2 hr average oxygen consumption µl/g dry wt. per hr ± S D	n
Gill	69.60 ± 34.10	13	362.09 ± 162.08	13
Mantle	54.35 ± 19.51	15	466.91 ± 272.00	15
Muscle	37.42 ± 14.55	12	194.68 ± 85.89	12
Heart	99.02 ± 27.88	11	604.89 ± 163.78	11
Gonad (F)	51.32 ± 21.38	11	237.09 ± 140.26	7
Gonad (M)	38.56 ± 12.16	7	174.90 ± 36.30	7
Radula	75.55 ± 41.53	7	268.69 ± 70.11	7
Hepatopancreas	63.02 ± 14.68	16	303.10 ± 126.64	16
Crop	132.11 ± 35.26	16	679.95 ± 234.40	16
Kidney (R)	110.28 ± 29.57	5	538.46 ± 170.90	5
Kidney (L)	84.42 ± 77.70	4	546.22 ± 307.20	4
Osphradium	49.71 ± 17.38	4	276.36 ± 82.08	4
Red muscle	80.87 ± 24.34	7	480.11 ± 79.51	7
Salivary gland	91.86 ± 36.81	3	294.21 ± 119.12	3

Table 4. Percent of total soft body wet weight represented by individual organs

Organ	Organ weight Average (g)	(Range)	Percent ± S D	n
Heart	0.28	(0.22 - 0.36)	0.10 ± 0.0	7
Gill	4.18	(2.45 - 5.36)	1.88 ± 0.52	13
Muscle	143.44	(101.27 - 185.85)	64.89 ± 8.28	8
Mantle	9.34	(5.40 - 12.30)	4.23 ± 0.81	16
Gonad (F)	13.48	(7.94 - 16.92)	5.74 ± 1.55	5
Gonad (M)	11.25	(3.05 - 19.93)	4.91 ± 2.31	8
Hepatopancreas	8.19	(2.29 - 12.29)	3.46 ± 1.32	8
Crop	0.45	(0.34 - 0.55)	0.23 ± 0.01	5
Radula	0.48	(0.37 - 0.58)	0.23 ± 0.06	7
Osphradium	0.91	(0.53 - 1.38)	0.47 ± 0.14	6
Red muscle	0.43	(0.21 - 0.75)	0.21 ± 0.10	8
Odontophore	0.65	(0.52 - 0.75)	0.30 ± 0.08	5
Salivary glands	0.32	(0.22 - 0.47)	0.17 ± 0.07	6
Kidneys	1.27	(1.01 - 1.90)	0.56 ± 0.11	6

organ size, particularly in large, soft organs. Likewise, the starved condition of the animals used over extended time periods resulted in size decrease and change in appearance of some organs. For these reasons, organs from the animals used in the study comparing whole animal with summed tissue respiratory rates were not included in computation of these composition percentages.

Comparison of Whole Animal Respiration and Tissue Respiration

Table 5 summarizes the results of the study in which the respiratory rate of five animals was measured at least six times and then the average rates for each of these was compared with the combined rates of the component tissues. Figures 3 through 8 graphically show the results of measurements of whole animal respiration for each of six animals. Tables 6 through 11 show balance sheets constructed for each specimen. A balance sheet has been drawn for animal number 27, results of whole animal respiration measurements are shown, and data on this animal have been included in the summary sheet, although only three whole animal measurements were made on this individual.

Each balance sheet shows the final wet weight of the component organs and the average respiratory rate of tissue samples from these organs. From these figures an average hourly oxygen consumption for the represented organs has been calculated. The sum of these consumption values, presumably representing the total oxygen consumption of the tissues, has been compared with the average oxygen consumption of the intact animal.

Table 5. Comparison of whole animal and summated tissue respiration at 20°C for six specimens of Haliotis cracherodii

Animal	Oxygen Consumption: Whole animal µl/hr (Range)	Summated tissue µl/hr	$\frac{\text{Whole animal}}{\text{summated tissue}} \times 100$
3	1,865 (490 - 4,496)	5,468	34.12
4	2,180 (576 - 3,688)	6,221	35.04
8	1,422 (527 - 2,548)	5,238	27.15
9	1,577 (870 - 2,965)	2,689	58.65
11	2,082 (612 - 3,367)	4,933	42.21
27*	2,521 (1,249 - 4,316)	5,095	49.48

*Values for whole animal respiration of animal No. 27 based on only three measurements; all others on six measurements.

Table 6. Balance sheet for animal No. 3

Tissue	Wet Weight (g)	Average Oxygen Consumption µl/g per hr	Average µl/hr
Foot	93.39	40.17	3,751.48
Mantle	9.86	73.13	721.06
Hepatopancreas	5.15	77.03	396.71
Gills	2.80	75.70	211.96
Gonad	1.68	76.54	128.58
Digestive tract	4.51		
Osphradium	0.84		
Kidneys	1.12	131.31	147.07
Odontophore	0.52		
Radula	1.17		
Crop	0.51	87.62	44.69
Red muscle	0.54	99.63	53.80
Heart	0.28	45.50	12.74
Totals	122.37		5,468.09

Whole animal respiration / Summed tissue respiration $= \frac{1,865.55}{5,468.09^{*}} = 34.12\%$

*Represents 94.25% of total tissue weight.

Table 7. Balance sheet for animal No. 4

Tissue	Wet Weight (g)	Average Oxygen Consumption µl/g per hr	Average µl/hr
Foot	119.24	37.06	4,419.03
Mantle	10.53	86.68	912.74
Hepatopancreas	4.93	60.71	299.30
Gills	2.80	65.31	182.87
Gonad	2.57	68.52	176.10
Digestive tract	1.65		
Osphradium	0.95		
Kidneys	0.93	150.42	139.89
Odontophore	0.75		
Radula	0.60		
Crop	0.41	169.77	69.61
Red muscle	0.17	124.53	21.17
Totals	145.53		6,220.71

$$\frac{\text{Whole animal respiration}}{\text{Summed tissue respiration}} = \frac{2,179.80}{6,220.71^{*}} = 35.04\%$$

*Represents 97.28% of total tissue weight.

Table 8. Balance sheet for animal No. 8

Tissue	Wet Weight (g)	Average Oxygen Consumption µl/g per hr	Average µl/hr
Foot	69.85	56.63	3,955.61
Mantle	7.62	61.54	468.93
Hepatopancreas	3.83		225.74
Gills	2.08		184.72
Gonad	2.43		181.16
Digestive tract	1.52		
Osphradium	0.82		
Kidneys	0.80		159.69
Odontophore	0.73		
Radula	0.50		
Crop	0.34	112.90	38.39
Red muscle	0.22	107.27	23.60
Heart	0.27		
Totals	91.01		5,237.84

Whole animal respiration / Summed tissue respiration $= \frac{1,422.24}{5,237.84^{*}} = 27.15\%$

*Represents 95.78% of total tissue weight.

Table 9. Balance sheet for animal No. 9

Tissue	Wet Weight (g)	Average Oxygen Consumption µl/g per hr	Average µl/hr
Foot	78.98	21.14	1,669.64
Mantle	5.94	66.50	395.01
Hepatopancreas	1.20	94.43	113.32
Gills	1.77	103.42	183.05
Gonad	1.84	78.65	144.72
Digestive tract	1.76		
Osphradium	0.53		
Kidneys	0.73	116.78	85.24
Odontophore	0.71		
Radula	0.65		
Crop	0.43	146.95	63.19
Red muscle	0.75	45.80	34.35
Heart	0.11		
Totals	95.40		2,688.52

Whole animal respiration / Summed tissue respiration $= \frac{1,576.85}{2,688.52^{*}} = 58.65\%$

*Represents 96.06% of total tissue weight.

Table 8. Balance sheet for animal No. 8

Tissue	Wet Weight (g)	Average Oxygen Consumption µl/g per hr	Average µl/hr
Foot	69.85	56.63	3,955.61
Mantle	7.62	61.54	468.93
Hepatopancreas	3.83	58.94	225.74
Gills	2.08	88 81	184.72
Gonad	2.43	74.55	181.16
Digestive tract	1.52		
Osphradium	0.82		
Kidneys	0.80	199.61	159.69
Odontophore	0.73		
Radula	0.50		
Crop	0.34	112.90	38.39
Red muscle	0.22	107.27	23.60
Heart	0.27		
Totals	91.01		5,237.84

Whole animal respiration / Summed tissue respiration $= \frac{1,422.24}{5,237.84^{*}} = 27.15\%$

*Represents 95.78% of total tissue weight.

Table 9. Balance sheet for animal No. 9

Tissue	Wet Weight (g)	Average Oxygen Consumption µl/g per hr	Average µl/hr
Foot	78.98	21.14	1,669.64
Mantle	5.94	66.50	395.01
Hepatopancreas	1.20	94.43	113.32
Gills	1.77	103.42	183.05
Gonad	1.84	78.65	144.72
Digestive tract	1.76		
Osphradium	0.53		
Kidneys	0.73	116.78	85.24
Odontophore	0.71		
Radula	0.65		
Crop	0.43	146.95	63.19
Red muscle	0.75	45.80	34.35
Heart	0.11		
Totals	95.40		2,688.52

Whole animal respiration / Summed tissue respiration $= \frac{1{,}576.85}{2{,}688.52^{*}} = 58.65\%$

*Represents 96.06% of total tissue weight.

Table 10. Balance sheet for animal No. 11

Tissue	Wet Weight (g)	Average Oxygen Consumption µl/g per hr	Average µl/hr
Foot	55.03	74.40	4,094.23
Mantle	6.31	40.01	252.46
Hepatopancreas	2.24	91.53	205.03
Gills	1.58		
Gonad	1.90	67.13	127.55
Digestive tract	1.55		
Osphradium	0.37		
Kidneys	1.63	92.72	151.13
Odontophore	0.56		
Radula	0.38		
Crop	0.55	119.89	65.94
Red muscle	0.48	54.22	26.03
Heart	0.15	69.30	10.40
Totals	72.73		4,932.77

$$\frac{\text{Whole animal respiration}}{\text{Summed tissue respiration}} = \frac{2,082.33}{4,932.77^{*}} = 42.21\%$$

*Represents 93.90% of total tissue weight.

Table 11. Balance sheet for animal No. 27

Tissue	Wet Weight (g)	Average Oxygen Consumption µl/g per hr	Average µl/hr
Foot	117.80	22.90	2,697.62
Mantle	12.31	92.10	1,133.75
Hepatopancreas	8.33	44.23	368.44
Gills	4.57	26.77	122.34
Gonad	12.95	35.65	461.67
Digestive tract	6.80		
Osphradium	0.75		
Kidneys	1.70	103.15	175.36
Odontophore	0.65		
Radula	0.50		
Crop	0.75	104.72	78.54
Red muscle	0.46	79.31	36.48
Heart	0.17	121.38	20.64
Totals	167.74		5,094.84

Whole animal respiration / Summed tissue respiration $= \frac{2,520.85}{5,094.84^{*}} = 49.48\%$

*Represents 94.81% of total tissue weight.

DISCUSSION

Molluscs are known to exhibit highly variable respiratory rates. Results of the measurement of 23 Haliotis cracherodii specimens have shown that Haliotis is typical of other molluscs in this respect. Weinland (1919) has reported as much as a 13 fold change in the respiratory rate of a single specimen of the clam Anodonta. On the average, ranges for a single abalone in this study showed a maximum of a 5 fold increase, however, maximum values for some animals were 8 to 10 times the minimum values.

The average respiratory rate for animals measured in this study was 16.31 μl/g per hour. This is a reasonable figure for a relatively large and sedentary poikilotherm (refer to Zeuthan, 1953). It also seems to compare well with the rates for various other molluscs (Ghiretti, 1966). Montuori's figure for H. tuberculata (Montuori, 1913) is higher (24 to 87 μl/g per hour); however, the considerably smaller size and the higher temperature of measurement (24° C) for H. tuberculata may account for the large difference between the two species of Haliotis. The average rate of 13.82 μl/g per hour for H. corrugata (Visser, 1972) is also in good agreement, especially if, again, the larger size of H. corrugata and the lower temperature (12° C) at which it was measured are considered. Respiratory rates of H. cracherodii are less than half the rates for a variety of poikilothermic vertebrates and very much less than that of mammals. For example, the

respiratory rate of the cow is on the order of 124 µl/g wet weight per hour and that of the long tailed shrew 10,600 µl/g per hour (Prosser and Brown, 1961).

As indicated in the review of the literature, numerous factors have been shown to affect the rate of oxygen consumption of molluscs. The effects of several of these factors upon the respiratory rates of Haliotis have been examined.

Regression analysis shows that respiratory rates of Haliotis are an inverse function of body size. It is assumed that increased oxygen consumption with decreased size is not a reflection of physiological changes produced by the starved condition of the animals. Ghiretti (1966) has stated that molluscs may either show a decreased oxygen consumption or no change in response to starvation. Observations during dissection of abalone definitely showed changes in the size and appearance of organs, presumably resulting from the starved condition of the animal. However, respiratory rates for individual abalone held for several months without feeding do not suggest any apparent relationship between respiratory rate and nutritional state.

Additional investigations of the effects of variables upon respiration in this species should involve larger numbers of animals of both sexes. Ideally, the animals used would not be exposed to laboratory conditions for long periods of time. However, measurement of respiratory rate at regular intervals, during both the breeding and nonbreeding season, would be of interest in view of the fact that the present study has shown a significant difference in average respiratory rates

between the two sexes but no seasonal changes in the respiratory rate. It also remains of interest to determine if the apparent difference between respiratory rates for males and females is real or merely reflects size differences between the sexes.

Respiratory rates of isolated tissues from H. cracherodii also exhibit considerable variation. This variation is not unexpected for molluscan tissue. Baldwin (1938) reported that wide variation in respiratory rates for hepatopancreas tissue from different snails necessitated his taking all samples for a given test from a single snail. Moreover, Kerkut and Laverack (1957) have shown multiple samples from a single Helix liver produced an average respiratory rate having a large standard error. Thus, tissues from molluscan organs may show a large variation either between organs from different specimens or within the same organ. This proved to be the case with Haliotis tissue, where respiratory rates of organs from different animals routinely showed variations of from 20 to 50% and multiple samples from the same organ, even from adjacent sections, showed wide variation.

Ranks of individual tissues vary, depending upon whether tissue respiratory rates are based on a wet weight or dry weight basis. In either case, crop, kidney, and heart tissue are among those showing highest respiratory rates, and foot muscle and gonads show the lowest rates. Considering the fact that both skeletal muscle (Feng, 1932) and smooth muscle (Bülbring, 1953) have shown higher oxygen consumptions under tension, it is curious to note that while abalone foot muscle

consistently shows a low respiratory rate, the rate for red muscle from the odontophore exhibits a high rate.

A difference between males and females in average whole animal respiratory rates has been discussed. Differences in respiratory rate of male and female gonads were examined. Rates for female gonadal tissue were approximately one and one half times those for tissue from male gonads, on either a wet weight or a dry weight basis. A t test showed the difference between the means of the wet weight respiratory rates to be significant at the 0.05 level.

A tendency to reduction in size of the right gill, kidney, and auricle among archeogastropods suggests the question of whether any difference in respiratory rate can be noted between right and left organs. Respiratory rates of samples from right and left gills showed no more variation between sides than that shown by multiple samples from the same side. Comparison of respiratory rates for right and left kidneys was also of interest. In Haliotis, circulation within the smaller, left kidney differs from circulation through the right kidney. All deoxygenated blood passes through the right kidney before going to the ctenidia. The left kidney receives only oxygenated blood and only a small quantity that has not already been cleared of wastes by passage through the right kidney. Crofts (1929) has described histological and physiological differences between the two renal organs. Differences in respiratory rate would not be surprising. However, the apparent difference between average respiratory rates for the two kidneys when rate is figured on a wet weight basis disappears when rates are calculated

on a dry weight basis. If any differences exist between respiratory rates of tissues from contralateral organs of Haliotis, data from this study have been insufficient to reveal them.

It is interesting to compare tissue respiratory rates among the few molluscs for which rates for several tissues are available. Table 12 presents a comparison of respiratory rates of isolated tissues of Helix pomatia (Kerkut and Laverack, 1957), Octopus vulgaris (Ghiretti-Magaldi, Giuditta, and Ghiretti, 1958) and Haliotis cracherodii. All rates are given on a dry weight basis.

Only in general trends is any consistency shown for tissue rates from the three molluscan species. As was the case for Haliotis, snail and octopus muscles tend to have relatively low respiratory rates, while heart, gill, and glandular tissues tend to have higher rates. Mantle tissues from octopus and abalone have strikingly similar rates. Otherwise, tissue rates for the octopus are two to four times higher than those for the abalone. Likewise, tissues of the snail have much higher rates than those of the octopus, except for the kidney, the rates for which are similar.

Several factors may account for the wide differences in tissue respiratory rates demonstrated by these three species of molluscs. Measurements on Helix and Octopus were made at 28° and 24° respectively, or eight and four degrees higher than the temperature used for Haliotis. Snail tissue was measured in a physiological solution and octopus tissue in sea water, while the medium for abalone tissue was blood from the source specimen. Thus, experimental conditions are not parallel for

Table 12. Comparison of tissue respiratory rates for tissues from three species of molluscs; comparative conditions of measurement discussed in text

Tissue	Species		
	Helix pomatia	*Octopus vulgaris*	*Haliotis cracherodii*
	µl/g per hr	µl/g per hr	µl/g per hr
Liver	2.78	0.67	0.30
Midgut	2.56		0.68
Mantle	1.76	0.42	0.47
Kidney	2.24	2.07	0.54
Foot	0.67		0.19
Gill		1.64	0.36
Heart		1.57	0.61
Salivary gland		0.83	0.29

the three studies. Assuming a Q_{10} of 2, temperature alone can account for much of the increase in rates from abalone to octopus to snail. The octopus, a cephalopod, is a much more active animal than either the abalone or the snail, and it might be assumed that its tissues would be enzymatically capable of producing high respiratory rates *in vitro*. Although both the snail and the abalone are classified as gastropods and thus are more closely related, they differ considerably in size. The smaller of the two, *Helix*, shows a higher whole animal respiratory rate (20 to 80 µl/g wet weight per hour) according to Ghiretti (1966). The respiratory rate of *Haliotis cracherodii*, as stated, averages 16.3 µl/g wet weight per hour. If, as suggested by Weymouth *et al.* (1944) and Vernberg (1956), individual tissue respiratory rates parallel size related respiratory rate increases of the whole animal, then larger rates would be expected for individual tissues of the snail.

Variations in experimental and physiological conditions may be cited to account for some of the difference noted between tissue respiratory rates of several molluscs. Another difference between respiratory rates in *Helix* and *Octopus* deserves further attention. Kerkut and Laverack (1957) have reported that changing the gas phase from air to oxygen has no effect upon the respiratory rates of isolated tissues of *Helix*. Ghiretti-Magaldi, Giuditta, and Ghiretti (1958) found that introduction of oxygen caused a doubling of respiratory rate in most tissues from *Octopus*. It will be of interest to determine the effect of increasing partial pressures of oxygen upon the respiratory rates of *Haliotis* tissue.

As discussed in the review of the literature, studies comparing respiratory rates of whole mammals and their component tissues have shown that summed tissue rates are less than rates for the whole animal. When allowance is made for *in vivo* activity of the tissues, the estimated sums may approximate the rate of respiration of the whole animal (Martin and Fuhrman, 1955).

In the only such comparison of total animal respiratory rates with tissue respiratory rates using invertebrate animals Kerkut and Laverack (1957) have found the reverse situation. Summed tissue respiration exceeded that of the whole animal. For *Helix pomatia* the average rate of respiration of the whole animal represented approximately 60% of the possible maximum tissue respiratory rate.

Results presented here for respiration of *Haliotis cracherodii* agree in substance with the finding for *Helix pomatia*. Averages for six specimens indicate that the total animal is respiring at 27 to 58% of the calculated potential for the tissue. In only one instance, that of animal number nine respiring at its maximum measured rate, was the whole animal found to respire at a rate in excess of the possible maximum rate calculated for summed tissue respiration.

Kerkut and Laverack have offered three suggestions to account for the opposing relationships between whole animal and tissue respiration observed for mammalian and molluscan systems. They point out that the *in vitro* condition is more nearly like the *in situ* situation for molluscan tissues, which are normally bathed in a hemocoel. Mammalian tissues

receive oxygen supplies through a closed circulatory system which is disrupted on isolation of the tissue.

The second suggestion relates to the immediate effects produced by this disruption. Mammalian tissues show a marked decrease in respiratory rate during the early stages of isolation and more gradual decreases during the longer periods of isolation. It is suggested that summed tissue respiration of mammalian tissues might represent a greater percentage of whole animal respiration if based on respiratory rates existing at the time of isolation.

Finally, assuming that differences between the molluscan and mammalian systems are real, the possibility of a central control mechanism regulating tissue respiratory rates is discussed. The authors stated their intention to investigate the possible existence of hormonal control of respiration in Helix.

Investigations of the relationship between total and tissue respiration in other species of molluscs would be useful in determining whether the relationships observed in Helix and Haliotis are examples of a general phenomenon. Further investigation of the situation in Haliotis is suggested. Even though it was attempted in the present study to maintain physiological conditions during in vitro measurements, it is still uncertain whether the in vivo situation was reproduced. Effects of such factors as the partial pressure of oxygen and the presence or absence of metabolites are unknown. Thus, although this study suggests the

operation of some type of central inhibition, further studies should investigate the influence of these factors upon tissue respiratory rates.

SUMMARY

1) Total animal respiratory rates for Haliotis cracherodii were measured using a flow through respirometer.

2) Average respiratory rate for 23 specimens was 16.31 μl O_2/g wet weight per hour.

3) It was found that whole animal respiratory rates are highly variable, considering either comparison of several specimens or successive measurements of the same specimen. The data indicated an inverse relationship between size and respiratory rate. Regression analysis revealed the relationship is linear with a slope of -0.19.

4) The present data did not suggest any relationship between respiratory rate and either season or length of time animals were held under laboratory conditions.

5) Tissue respiratory rates were determined by the direct Warburg method.

6) Respiratory rates of isolated tissues were also highly variable. Rates ranged from 37.42 μl/g wet weight per hour for foot muscle to 132.11/g per hour for crop tissue.

7) A significant difference was found between the rates for tissue from male and female gonads; significant differences were not detected between tissues of contralateral organs within individual specimens.

8) Comparison of the summed tissue respiratory rates with whole animal respiratory rates indicate the whole animal respired at 27 to 58% of the maximum possible respiratory rate of the tissues. This situation is comparable to that found for the only other gastropod on which a similar

investigation has been reported, but differs from the mammalian situation, where whole animal respiration exceeds that of the isolated tissues. Factors which may account for this difference between mammalian and molluscan systems are discussed.

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