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VALIDATION OF THE DOUBLY-LABELED WATER
($H^3H^{18}O$) METHOD FOR MEASURING WATER FLUX AND
ENERGY METABOLISM IN TENEBRIONID BEETLES
Paul D. Cooper

**VALIDATION OF THE DOUBLY-LABELED WATER ($\text{H}^3\text{H}^{18}\text{O}$)
METHOD FOR MEASURING WATER FLUX AND
ENERGY METABOLISM IN TENEBRIONID BEETLES**

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FOOTNOTES

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ABSTRACT

Cooper, Paul D. Validation of the doubly-labeled water ($\text{H}^3\text{H}^{18}\text{O}$) method for measuring water flux and energy metabolism in tenebrionid beetles.

Doubly-labeled water ($\text{H}^3\text{H}^{18}\text{O}$) has been used to determine water flux and energy metabolism in a variety of vertebrates. This study examines the applicability of this technique to arthropods. The theory of the technique depends upon the assumption that doubly-labeled water introduced into the animal's body water equilibrates with water and carbon dioxide by the action of carbonic anhydrase. Tritium (^3H) is lost from the animal only with water while oxygen-18 is lost with both water and carbon dioxide. The difference between the rates of loss of the two isotopes is proportional to CO_2 loss rate. Validation of the use of tritiated water for measuring water flux was accomplished by comparing gravimetric measurements of water gain with flux rates determined by loss of tritiated water. At room humidity, an overestimate for influx calculated from labeled water calculations was found, averaging 12 mg H_2O (g.d^{-1}). Comparison of CO_2 loss rate determined isotopically with rates of CO_2 loss determined by standard metabolic rates also yielded overestimates for the isotopic technique, overestimates ranging between 20 and 30%. The relevance of this for studies using labeled water for studying water fluxes and energy metabolism of free-ranging arthropods is discussed.

Running head: Water balance in tenebrionid beetles

SUMMARY

1. Tritiated water estimates of total body water in tenebrionids overestimated gravimetrically determined values.
2. Determination of water influx using tritiated water overestimates gravimetrically measured influx by 44% when tenebrionid beetles are fed on relatively dry food and kept at room humidity.
3. Calculation of rates of carbon dioxide production from changes in concentration of doubly-labeled water yielded values greater than rates of carbon dioxide production measured using gas chromatograph or oxygen analyzer, overestimates ranging between 20 and 30%.
4. Although isotopic measurements of water influx and energy metabolism yield rates which are overestimates of rates measured by standard laboratory techniques, the doubly-labeled water technique can still be used in field studies of water and energy balance of arthropods provided that laboratory validation studies are performed.

INTRODUCTION

Water and energy requirements of free-ranging animals have long been of interest to physiological ecologists. Previously, animals were studied under laboratory conditions and the results were extrapolated to the field. Doubly-labeled water ($\text{H}^3\text{H}^{18}\text{O}$) has been shown to measure water turnover rates and CO_2 production in captive vertebrates with errors less than 10% (reviewed by Nagy, 1975, 1980; Nagy and Costa, 1980), but recent studies have questioned the accuracy of this technique for arthropods (King and Hadley, 1979; Yokata, 1979). The present study assesses the validity of using $\text{H}^3\text{H}^{18}\text{O}$ for measurement of water input and loss and energy metabolism in two species of desert arthropods, Eleodes armata and Cryptoglossa verrucosa.

Theory of Doubly-labeled Water Technique

Lifson and McClintock (1966) discussed the theory and conditions of which the doubly-labeled water method functions. The doubly-labeled water is added to the body fluids, and the isotopes become distributed in both water and dissolved carbon dioxide in the presence of carbonic anhydrase, as shown by equation 1:

The oxygen isotope is then lost from the body with water and carbon dioxide, while the hydrogen isotope is lost only with water. Water efflux is proportional to the change in total amount of hydrogen isotope. Since oxygen is lost with water and carbon dioxide, the decrease in the oxygen isotope with time represents the rate of both water and CO_2 loss. The difference between the hydrogen and oxygen isotope loss rates is thus a measure of CO_2 loss.

The water loss and CO_2 production rates can be calculated using equations developed by Lifson and McClintock (1966). Assuming that body water content changes linearly with time and that the rate of water loss is constant during the measurement period, the rate of water loss is calculated by equation 2 using measured isotopic values,

$$r_{\text{H}_2\text{O}} = \frac{\Delta N}{\Delta t} \frac{(K_{2\text{H}^*} - K_N)}{K_N} \quad (2)$$

where $r_{\text{H}_2\text{O}}$ is the rate of water loss, ΔN is the change in body water in moles, Δt is the length of time of the measurement, $K_{2\text{H}^*}$ is the apparent fractional turnover rate of the hydrogen isotope and K_N is equal to $\frac{\Delta \ln N}{\Delta t}$. If carbon dioxide is produced at a constant rate, the rate of CO_2 production can be calculated using equation 3:

$$r_{\text{CO}_2} = \frac{\Delta N}{2\Delta t} \frac{(K_{\text{O}^*} - K_{2\text{H}^*})}{K_N} \quad (3)$$

where K_{O^*} is the apparent fractional turnover rate of the oxygen isotope, and 2 is in the denominator to balance the equation, as there are two moles of oxygen atoms per mole of carbon dioxide.

Nagy (1975) expanded and included appropriate conversion factors in the above equations so that rate of water loss and metabolic rate were expressed in milliliters of water and carbon dioxide per unit weight per time. He also modified an equation from Lifson and McClintock to determine water input rate, using loss rate and weight changes, as shown in equation 4:

$$\frac{\text{ml H}_2\text{O influx}}{\text{kg day}} = \frac{\text{ml H}_2\text{O efflux}}{\text{kg day}} + \frac{2000(W_2 - W_1)}{t (M_1 + M_2)} \quad (4)$$

where $W_2 - W_1$ is change in total body water between the beginning and end of the experimental period, while $M_1 + M_2$ is included to determine mean weight during the time of measurement.

Methods and Materials

The present experimental protocol consists of two parts. The first part examines the accuracy of using tritiated water to calculate the rate of water input. The second part compares carbon dioxide production rates calculated from changes in doubly-labeled water and metabolic rates determined from standard laboratory methods.

Experimental Animals

Eleodes armata and Cryptoglossa verrucosa, two desert tenebrionid beetles, were captured by can trap near the Desert Research Station of the Barstow Unified School District in Hinkley, California. The beetles were maintained at room temperature in the laboratory and fed a diet of carrots and blood meal-flour mixture (3:7 w/w), and water was provided ad libitum.

Total Body Water

Seven E. armata and seven C. verrucosa were weighed and injected with 10 μ l tritiated water (0.2 μ Ci). After allowing three hours for fluid equilibration (Bohm and Hadley, 1977), samples of hemolymph were withdrawn from the heart, using 10 μ l micropipettes. The hemolymph samples were then distilled (Wood et al., 1975) to get pure water samples, and 2 μ l aliquots were added to scintillation fluid (toluene, Triton X-100, POP) and counted for radioactivity (Beckman LS 230). Total body water was calculated from dilution of tritiated water (Foy and Schnieden, 1960).

Following the removal of the hemolymph sample, animals were placed in 50 ml beakers and set in a drying oven (60°C). After three days, animals were removed and reweighed. The difference between initial and final weighings was assumed to be total body water.

Tritium Validation

Eight E. armata were injected through the intersegmental membrane between the prothorax and elytra with 10 μ l tritiated water (0.2 μ Ci). After four hours, a hemolymph sample was withdrawn by puncturing the heart with an insect pin and allowing the fluid to fill a 10 μ l capillary tube. Samples taken at the end of the experimental period were handled in the same way. Hemolymph samples were processed as above.

The experiment lasted ten days. Animals were maintained in plastic cups at 30°C and room humidity (avg. 0.3-0.5). Beetles were allowed either to feed on blood meal-flour mixture or to drink on alternate days. Feeding periods lasted two hours, while water was available for only one hour. The amount of food ingested was determined by weighing the cup with food before and after the feeding period. Drinking was measured by weighing the animal before and after exposure to water. Feces were collected daily and dried to determine the total dry mass feces produced. Metabolic water production was calculated from total energy assimilated (energy ingested - energy egested), assuming $RQ = 1.0$ for carbohydrate diet ($RQ = .955$ by diet analysis, and metabolic water may be overestimated by 13% as a result). Rates for drinking, feeding, and metabolic water rate were calculated by dividing total values by time and average body weight.

Water input rates using isotopic data were determined using the equation of Lifson and McClintock (1966) for linearly changing body weight as modified by Nagy (1975). Total body water at the beginning was estimated by tritiated water dilution method (Foy and Schnieden, 1960), while final body water was determined gravimetrically by drying the animal at 60° for three days.

Validation of Doubly-labeled Water
Method for Determining Metabolic Rate

Animals were injected through the intersegmental membrane with 5-10 μ l doubly-labeled water ($\text{H}^3\text{H}^{18}\text{O}$) (0.2 $\mu\text{Ci}^3\text{H}$ total, 49.4 atoms % excess ^{18}O). Following equilibration (4-6 hours), hemolymph samples were taken by heart puncture, and the animals were then placed in either sealed Plexiglas metabolism chambers (d 17.63 cm x 12.7 cm) or small glass desiccator jars (2-3 l) and not fed during the course of the experiment. Periodically (2-5 days depending on size of chamber), gas samples were removed and injected into either a gas chromatograph (Beckman GC 240, Por Q, or silica gel column) to determine amount of CO_2 produced, or an O_2 analyzer (Beckman E2), with CO_2 production being calculated from oxygen consumption and respiratory quotient. Gas chromatograph samples were quantified by comparison to CO_2 standards run simultaneously ranging from 0.5 to 5.0%, although carbon dioxide concentrations inside the metabolic chambers rarely went over 2%. Respiratory quotients for the oxygen measurements were determined using the method of Vleck (1978) in which two gas samples were injected into the O_2 analyzer, one with both CO_2 and H_2O absorbed using Ascarite and drierite, and the second sample only had H_2O removed.

Experiments were run at room temperature and humidity (range 22-26°C and 30-50% r.h.) or at 28°C with humidity either 0 or 90%. In the latter experiments temperature was controlled using a BOD incubator, while humidity was maintained constant with either drierite or a 10% w/v KOH solution.

At the conclusion of each experiment, a final hemolymph sample was taken and the animal killed and dried to constant weight (60°C) to determine final total body water.

Hemolymph samples were micro-distilled to obtain pure water (Wood et al., 1975). Part of the sample was used to count tritium content as described before, while 6-7 μ l were used to determine the ^{18}O content by proton activation (Wood et al., 1975). The CO_2 production rate was calculated from the tritium and ^{18}O values using the Lifson- McCintock (1966) equation for linearly changing body weight as modified by Nagy (1975).

RESULTS

Total Body Water

Tritiated water dilution in E. armata yielded total body water of $70.4 \pm 4.3\%$ fresh weight compared to gravimetric measurement of $58.7 \pm 3.3\%$. The values for E. verrucosa were $63.6 \pm 2.8\%$ by tritium dilution and $52.6 \pm 3.4\%$ by drying animals. Tritium dilution body water contents were subsequently corrected when estimating initial body water in further $\text{H}^3\text{H}^{18}\text{O}$ studies.

Tritium Validation

Tritium estimates for water input rates were higher than rates measured gravimetrically (Figure 1). The mean isotopic measurement

averaged $12 \text{ mg (g.day)}^{-1}$ higher than the gravimetric determination, the difference was statistically significant ($t = 2.43$, $df = 16$, $p < .01$).

Doubly-labeled Method for Determining Metabolic Rate

Regression analysis showed that metabolic rates calculated from doubly-labeled water data were consistently higher than laboratory gas measurements (Figure 2). The $\text{H}^3\text{H}^{18}\text{O}$ overestimate ranged between 20 and 30% $[(\text{measured rate} - ^{18}\text{O rate}/\text{measured rate} \times 100)]$ under all conditions except for measurements made on animals kept at high humidity. Because of large variations, no statistical differences were present in some of the experimental groups (Table 1).

DISCUSSION

Theory of $\text{H}^3\text{H}^{18}\text{O}$ Technique

Assumptions inherent in calculating water flux using isotopes were discussed by Lifson and McClintock (1966) and have been further examined by Nagy (1980) and Nagy and Costa (1980). The six assumptions are: 1) body water volume remains constant during the measurement period, 2) rates of water influx and efflux are constant, 3) the isotopes labels only the H_2O in the body, 4) the isotope leaves the body only as H_2O , 5) the specific activity of isotope in water lost from the animal is the same as the body water, and 6) labeled or unlabeled water in the environment does not enter the animal via respiratory or skin surfaces.

Previous studies on arthropod water fluxes used mathematics which also included certain of these assumptions (Wharton and Devine, 1968; Knulle and Devine, 1972; Arlian and Wharton, 1974; Buscarlet et al., 1978); however, Lifson and McClintock allowed for changing total body water, while the other studies did not. If equations assuming constant body

water are used when total body water is changing, significant errors in calculated water flux rates can occur (Nagy and Costa, 1989). Unlabeled water vapor entering animals also yields erroneous flux rates, unless the only major source of input is vapor influx. Since vapor influx accounts for most of the water gain in arthropods absorbing water from subsaturated atmospheres, Wharton and his co-workers were able to determine water input rates for several mites and ticks. However, as arthropods gained weight above and lost weight below critical equilibrium activity (CEA), total body water did not remain constant. While recognizing this problem (Devine and Wharton, 1973), their model used to determine rates of water gain and loss assumed constant body water. Arlian and Wharton (1974) attempted to correct for errors in flux rates occurring below CEA by assuming a two-component system, one uptake route being passive, the other active, but they still failed to account for the changing body water.

Buscarlet et al. (1978) and models of Wharton and his associates are simply special cases of the Lifson-McClintock model, which assumes that body water is constant during the experimental period if body water at the beginning equals body water at the end of the experiment. As differences usually are found between total body water measured at the beginning and that determined at the end of the experiment, models assuming changing body water content are more appropriate (Nagy and Costa, 1980), as permitted by Lifson and McClintock's mathematics.

Certain assumptions similar to those used for measuring water fluxes with isotopes must also be incorporated into measurements of metabolism with the doubly-labeled water ($\text{H}^3\text{H}^{18}\text{O}$) technique. Assumptions 2-5 are

expanded to include isotopic oxygen occurring in both H_2O and CO_2 . If total body water changes with time, then the model of Lifson and McClintock can accommodate this variation to some extent. Buscarlet et al. (1978) again present a model which is a special case (i.e., requires constant total body water) of the more comprehensive mathematics presented by Lifson and McClintock. Since total body water usually changes, the latter model is again chosen for its wider applications.

Total Body Water

Tritiated water dilution has been used to estimate total body water (TBW) in many animals (reviewed by Nagy and Costa, 1980). The error between measured and isotopically calculated total body water varies greatly, as shown in Table 2. With arthropods, total body water is overestimated by approximately 10% presumably because of hydrogen binding to tissues, and thus tritium dilution space includes both water and some tissues. The errors observed in arthropods tend to be higher than errors observed in other phyla. In a theoretical treatment, Culebras et al. (1977) attempted to determine the overestimate in rats as a result of freely-exchanging hydrogens in proteins, carbohydrates, and fats in the body. Perhaps arthropods have a higher proportion of exchangeable hydrogens than other taxa. King and Hadley (1979) demonstrated that scorpions have two components determining tritium turnover, the first being more rapid and representing tissue-bound tritium and water loss, and the second representing water loss only. The former component lasted approximately five days and accounted for about 8% of the tritium, thus estimation of body water before this would be erroneous. Studies with beetles do not indicate this long period before equilibration (Bohm and Hadley, 1977).

Tritium Validation

Disagreement between isotopic measurement of influx and gravimetric determinations is expected, as the assumption concerning no unlabeled water entering through integument and respiratory surfaces is violated at all absolute humidities above zero. Arlian and Wharton (1974) called this avenue of input, passive influx in their two-compartment model for vapor uptake in the mite, Dermatophagoides. Nagy and Costa (1980) examined this phenomenon in kangaroo rats and showed that the difference between gravimetric and isotopic measures of influx increased with absolute humidity. I found that influx in starved E. armata and C. verrucosa increased with vapor activity (Cooper, 1981) and the magnitude of the influx overestimate falls within the range expected for animals maintained between a_v 0.3 and 0.5, normal room conditions.

Errors in influx rates as a result of vapor input via integument and respiratory surfaces have been considered in other papers examining water balance of arthropods using isotopes (Bohm and Hadley, 1977; Arlian, 1979; King and Hadley, 1979; Yokota, 1979). Besides the present work, however, the only attempt to validate the tritiated water technique in arthropods was the study of Buscarlet et al. (1978). They reported no difference between isotopic and gravimetric measurements of influx in locusts (38.6 vs 37.7 $\mu\text{l/g.d}$), despite the animals being maintained at room humidity. Perhaps the error induced by use of their steady-state equation for animals whose total body water was changing counteracted the error resulting from vapor input, although the exact balancing of errors was unlikely. The locusts were eating very moist food (7.13 mg $\text{H}_2\text{O/mg}$ dry mass food), thus total body water would probably remain constant

during the experiment, permitting the use of steady-state equations.

It is unclear, then, why no overestimate for vapor was observed.

For comparison, Table 3 contains summaries of H^3HO validation studies in mammals, reptiles, and arthropods. The greatest errors occur at high absolute humidities in the kangaroo rat, the lizard (Uta), the tortoise (Gopherus), and the beetle (E. armata). Nagy and Costa (1980) provided some insight into the reasons for variation, some of which included differences in H^3HO/H_2O fractionation ratio (proportion of tritiated water which evaporates at a different rate relative to normal water) between animals; the formation of boundary layers reducing water vapor input; and size, as small animals have high surface area to volume ratios, and a relatively large cutaneous unlabeled water vapor input may occur. The smallest animals, Uta and E. armata, do exhibit the largest input errors, and the size theory may account for most of the observed discrepancy between isotopic and gravimetric measurements of water gain in these animals.

From my research, tritiated water can be used to calculate water input rates in free-ranging animals, but these rates will include passive, unlabeled water vapor absorption. To calculate the other components of water gain, some controls for determining only passive vapor influx must be run in free-ranging experimental animals. Therefore, before using H^3HO to measure water flux in arthropods, validation experiments must be conducted to determine the magnitude of vapor input.

Measurement of CO_2 Production Using $H^3H^{18}O$

The doubly-labeled water ($H^2H^{18}O$ or $H^3H^{18}O$) technique for measuring CO_2 production has been demonstrated to be relatively accurate in mammals,

birds, and reptiles with errors ranging between an overestimate of 3.0% for gophers (Gettinger, personal communication) to an underestimate of 7.4% for the lizard, Uta (Nagy, 1980). In arthropods, however, the errors are usually much greater (Table 4), the exceptions being locusts and the measurement of metabolism in a closed system for the scorpion, Paruroctonus mesaensis. In addition, the errors are usually overestimates, the study with locusts again differing.

The consistency of magnitude and direction of errors found with doubly-labeled water measurements of metabolism in arthropods indicates that one of the basic assumptions may not be valid. Nagy (1980) demonstrated that whether steady-state, linearly changing, or exponentially changing total body water equations were used to determine metabolism, only slight differences in calculated rates of carbon dioxide production occurred. Further, his analysis suggested that only uptake of unlabeled CO_2 resulted in an overestimate of metabolism, and the magnitude of this overestimate was inversely related to absolute humidity. As my experiments were all conducted in closed systems, CO_2 and water vapor present should have been in equilibrium with ^{18}O concentrations in the body fluid. This situation may result in an underestimate rather than an overestimate, as the concentration in body fluids decreases with time, and the atmosphere then has a relatively greater concentration of ^{18}O . Nagy (1980) showed that in such conditions, isotope recycling causes an underestimate of metabolism.

The error occurring at high humidity suggests that vapor exchange may affect metabolic rate calculations. This would only occur if H^3HO and H_2^{18}O were lost at different rates, and Yokota (1979) calculated that

H_2^{18}O has a higher rate of vaporization than H^3HO . If cuticle is the major barrier to diffusion, the difference among the heats of vaporization for H_2O , H^3HO , and H_2^{18}O would be small relative to the energy required to evaporate across the cuticle, and little fractionation would occur. However, the energy for vaporization will influence vapor fluxes across moist respiratory surfaces. If water exchange is large enough across these surfaces, H_2^{18}O may diffuse outward at a faster rate than H^3HO , and the difference between tritium and ^{18}O biological decay rates would be exaggerated. Rates of water loss via tracheae can be quite large when spiracles remain open (Cooper, 1981) lending support to this explanation, but vapor exchange through spiracles can be greatly reduced under dehydrating conditions (Edney, 1977; Yokota, 1979). This spiracular response should reduce the error derived from doubly-labeled water data, since the difference in the rates of water lost in its different forms would decrease as the proportion of total water lost from the spiracles decreased. This was not observed in dry air experiments, but as these animals were initially well-hydrated, spiracular water loss may have been only slightly restricted. Future experiments may test this hypothesis using arthropods with previously reduced water content.

Comparison with other arthropod validation studies indicates that only with Locusta migratoria was no error observed between measured and $\text{H}^3\text{H}^{18}\text{O}$ calculated metabolic rates (Buscarlet et al., 1978). This study differed from other validations, as metabolism was not measured continuously, but only for two two-hour periods during the week-long experiment. As CO_2 production rate calculated from doubly-labeled water data is actually an average daily metabolic rate integrated over the length of the

experimental period, extrapolation of metabolic rate from short measurement intervals may be in error. In addition, the locusts were introduced into special containers for infrared gas analysis, and this manipulation may have increased metabolic rate by inducing activity.

Because of the consistent overestimate of carbon dioxide production rates with $\text{H}^3\text{H}^{18}\text{O}$, a correction factor of 0.89 was determined from the mean ratio of measured CO_2 production rate to isotopic calculated CO_2 production rate in all my experiments. Multiplying isotopic rates by this factor gave rates which were not significantly different ($p > 0.05$) from measured rates in all groups except the high humidity study.

An overestimate in metabolism at high humidity was also reported by Nagy and Costa (1980) in kangaroo rats, but Gettinger (personal communication) found no error with gophers. Obviously, this presents some problems for studying animals which may live in environments where humidity may be high, such as burrows. In field studies with E. armata and C. verrucosa (Cooper, 1981), metabolic rates were calculated from $\text{H}^3\text{H}^{18}\text{O}$ data following adjustment by the 0.89 factor. Beetles were often in burrows, thus this slight correction of metabolic rate may not be completely adequate. Future studies on validation may consider calculating correction factors as functions of humidity and using this relation for adjusting field CO_2 production rates.

In summary, correction for the overestimate may permit the $\text{H}^3\text{H}^{18}\text{O}$ technique to be used for field studies on arthropod energetics, assuming that laboratory studies are valid for field conditions. I suggest that future validation studies should be performed in case the observed error in metabolism is not consistent among arthropod groups.

LITERATURE CITED

- Arlian, L. G. 1979. Significance of passive sorption of atmospheric water vapor and feeding in water balance of the rice weevil, Sitophilus oryzae. *Comparative Biochemistry and Physiology* 62A: 725-733.
- Arlian, L. G. and G. W. Wharton. 1974. Kinetics of active and passive components of water exchange between the air and a mite, Dermatophagoides farinae. *Journal of Insect Physiology* 20:1063-1077.
- Bohm, B. C. and N. F. Hadley. 1977. Tritium determined water flux in the free-roaming desert tenebrionid beetle, Eleodes armata. *Ecology* 58:407-414.
- Buscarlet, L. A. and J. Proux. 1975. Etude a l'aide de l'eau tritiee du renouvellement de l'eau corporelle chez Locusta migratoria migratorioides. *Comptes Rendus Hebdomadaires des Seances de l'Academie des Sciences, Series D* 281:1409-1412.
- Buscarlet, L. A., J. Proux, and R. Gerster. 1978. Utilisation du double marquage $HT^{18}O$ dans une etude de bilan metabolique chez Locusta migratoria migratorioides. *Journal of Insect Physiology* 24:225-232.
- Cooper, P. D. 1981. Water balance in two desert tenebrionid beetles, Eleodes armata and Cryptoglossa verrucosa. Ph.D. thesis, University of California, Los Angeles, Los Angeles, California. 220 pp.
- Culebras, J. M., G. F. Fitzpatrick, M. F. Brennan, C. M. Boyden, and F. D. Moore. 1977. Total body water and exchangeable hydrogen. II. A review of comparative data from animals based on isotope dilution and desiccation, with a report of new data from the rat. *American Journal of Physiology* 232:R60-R65.

- Culebras, J. M. and F. M. Moore. 1977. Total body water and exchangeable hydrogen. I. Theoretical calculation of nonaqueous exchangeable hydrogen in man. *American Journal of Physiology* 232:R54-R59.
- Devine, T. L. and G. W. Wharton. 1973. Kinetics of water exchange between a mite, Laelaps echidnina, and the surrounding air. *Journal of Insect Physiology* 19:243-254.
- Edney, E. B. 1977. Water balance in land arthropods. *Zoophysiology and Ecology*, Vol. 9. Springer-Verlag: Berlin, Heidelberg, New York. 282 pp.
- Foy, J. M. and H. Schnieden. 1960. Estimation of total body water (virtual tritium space) in the rat, cat rabbit, guinea pig and man, of of the biological half-life of tritium in man. *Journal of Physiology* 154:169-176.
- Green, B. and J. D. Dunsmore. 1978. Turnover of tritiated water and ^{22}Na in captive rabbits (Oryctolagus cuniculus). *Journal of Mammalogy* 59:12-17.
- Holleman, D. F. and R. A. Dieterich. 1975. An evaluation of the tritiated water method for estimating body water in small rodents. *Canadian Journal of Zoology* 53:1376-1378.
- King, W. W. and N. F. Hadley. 1979. Water flux and metabolic rates of free-roaming scorpions using the doubly labeled water technique. *Physiological Zoology* 52:176-189.
- Knulle, W. and T. L. Devine. 1972. Evidence for active and passive components of sorption of atmospheric water vapor by larvae of the tick Dermacentor variabilis. *Journal of Insect Physiology* 18:1653-1664.

- Lifson, N. and R. McClintock. 1966. Theory of use of the turnover rates of body water for measuring energy and material balance. *Journal of Theoretical Biology* 12:46-74.
- Nagy, K. A. 1972. Water and electrolyte budgets of a free-living desert lizard, Sauromalus obesus. *Journal of Comparative Physiology* 79: 39-62.
- Nagy, K. A. 1975. Water and energy budgets of free-living animals: measurement using isotopically labeled water. In: *Environmental Physiology of Desert Organisms*. Hadley, N. F. (ed.). Stroudsburg, PA., Dowden, Hutchinson and Ross, Inc., pp. 227-245.
- Nagy, K. A. 1980. CO₂ production in animals: analysis of potential errors in the doubly labeled water method. *American Journal of Physiology* 238:R466-R473.
- Nagy, K. A. and D. P. Costa. 1980. Water flux in animals: analysis of potential errors in the tritiated method. *American Journal of Physiology* 238:R454-R456.
- Nagy, K. A., R. S. Seymour, A. K. Lee, and R. Braithwaite. 1978. Energy and water budgets in free-living Antechinus stuartii (Marsupialia: Dasyuridae). *Journal of Mammalogy* 59:60-68.
- Nagy, K. A., V. H. Shoemaker, and W. R. Costa. 1976. Water, electrolyte and nitrogen budgets of jackrabbits (Lepus californicus) in the Mojave Desert. *Physiological Zoology* 49:351-363.
- Siri, W. F. and J. Evers. 1962. Tritium exchange in biological systems. In: *Tritium in the Physical and Biological Sciences*, Vol. 2. Symposium of the International Atomic Energy Agency, pp. 71-80.

- Smith, B. S. W. and A. R. Sykes. 1974. The effect of route of dosing and method of estimation of tritiated water space on the determination of total body water and the prediction of body fat in sheep. *Journal of Agricultural Science* 82:105-112.
- Vleck, D. 1978. The energetics of activity and growth. Ph.D. Thesis, University of California, Los Angeles, pp. 146.
- Wharton, G. W. and T. L. Devine. 1968. Exchange of water between a mite, Laelaps echidnii, and the surrounding air under equilibrium. *Journal of Insect Physiology* 14:1303-1318.
- Wood, R. A., K. A. Nagy, N. S. MacDonald, S. T. Wakakuwa, R. J. Beckman, and H. Kaaz. 1975. Determination of oxygen-18 in water contained in biological samples by charged particle activation. *Analytical Chemistry* 47:646-650.
- Yokota, S. D. 1979. Water, energy and nitrogen metabolism in the desert scorpion Paruroctonus mesaensis. Ph.D. Thesis, University of California, Riverside, pp. 316.

Table 1 Comparison of CO₂ loss rate by O₂ analyzer or gas chromatograph with rate calculated by doubly-labeled water data for E. armata and C. verrucosa.

Arthropod	(n)	Temp.	R.H.	Method	CO ₂ Prob. Measured ml CO ₂ g ⁻¹ h ⁻¹	O-18 Rate Calculated ml CO ₂ g ⁻¹ h ⁻¹	% Error (Avg.)
<u>E. armata</u>	(12)	25 ⁰	room	gas chromatog.	.449 ± .139	.505 ± .182 ^{NS}	+20.5
<u>E. armata</u>	(5)	25 ⁰	room	O ₂ analyzer	.398 ± .068	.474 ± .064 ¹	+20.5
<u>E. armata</u>	(7)	28 ⁰	dry air	O ₂ analyzer	.457 ± .085	.515 ± .096 ¹	+26.9
<u>E. armata</u>	(7)	28 ⁰	90%	O ₂ analyzer	.233 ± .074	.406 ± .220 ¹	+67.2
<u>C. verrucosa</u>	(8)	25 ⁰	room	gas chromatog.	.224 ± .057	.282 ± .088 ^{NS}	+28.7

Table 2 Comparison of total body water measured gravimetrically and by tritium in various arthropods.

Taxa	Weight g	Percent Error with Tritium	Reference
Arthropods			
Beetles			
<u>C. verrucosa</u>	0.75	+11.0	present study present study Bohm and Hadley (1977)
<u>E. armata</u>	1.0	+11.7	
		+ 8.6	
Scorpions			
<u>Hadrurus</u> <u>arizonensis</u>	6.0	+ 6.0	King and Hadley (1979)
Locusts			
<u>Locusta</u>	1.6	+ 8.8	Buscariet and Proux (1975)
Reptiles			
<u>Anole (Anolis)</u>	1.2	+ 4.0	Nagy and Costa (1980) Nagy and Costa (1980)
<u>Chuckawalla</u>	167.0	+ 3.9	
Mammals			
Rodents			
Mouse			
<u>Calomys</u>	18	+ 5.2	Holleman and Dieterich (1975)
<u>Peromyscus</u>	19	+ 2.3	
<u>Mus</u>	20?	+ 3.7	Holleman and Dieterich (1975) Siri and Evers (1962) Nagy et al. (1978)
<u>Antechinus</u>	26	+ 6.2	
Vole			
<u>Microtus</u>	28	+ 0.1	Holleman and Dieterich (1975)
Rat			
<u>Dipodomys</u>	44	+ 2.1	Nagy and Costa (1980) Culebras and Moore (1977)
<u>Rattus</u>	227	+ 1.7	
Rabbit			
<u>Oryctolagus</u>	1800	+ 3.1	Green and Dunsmore (1978)
Sheep			
<u>Ovis</u>	41000	+ 3.0	Smith and Sykes (1974)

Table 3 Summary of H^3HO validation studies in animals. Adapted from Nagy and Costa (1980).

Animal	M (g)	Absolute Humidity	Error in H^3HO Method		Reference
		mg H_2O (l air) $^{-1}$	ml (kg day) $^{-1}$	%	
Mammals:					
Kangaroo rat (<u>Dipodomys</u>)	35	19.8	+26.8	+44.2	Nagy and Costa (1980)
Squirrel (<u>Ammospermophilus</u>)	96	8-12	+1.1	+0.5	Karasov (personal communication)
Gopher (<u>Thomomys</u>)	125	18	-3.0	-3.2	Gettinger (1981)
Jackrabbit (<u>Lepus</u>)	1800	6-12	+1.7	+1.1	Nagy et al. (1976)
Rabbit (<u>Oryctolagus</u>)	1800	20	+2.0	+3.6	Green and Dunsmore (1978)
Monkey (<u>Alouatta</u>)	5600	20	-3.0	-4.0	Nagy and Milton (from Nagy and Costa, 1980)
Reptiles:					
Lizard (<u>Uta</u>)	3	12	+6.1	+29.1	Nagy and Costa (1980)
Lizard (<u>Sauromalus</u>)	167	4	-0.3	-2.0	Nagy (1972)
Tortoise (<u>Gopherus</u>)	520	12	+1.3	+11.5	Nagy and Costa (1980)
Arthropods:					
Beetle (<u>Eleodes</u>)	1	12	+12.0	+44.0	present study

Table 4 Comparison of validation experiments using doubly-labeled water ($\text{H}^3\text{H}^{18}\text{O}$) method for measuring metabolism in arthropods.

Arthropod	(n)	Temp.	R.H.	Method	CO_2 Prob. Measured $\text{ml CO}_2 \text{ g}^{-1} \text{h}^{-1}$	0-18 Rate Calculated $\text{ml CO}_2 \text{ g}^{-1} \text{h}^{-1}$	% Error (Avg.)
<u>E. armata</u>	(12)	25 ⁰	room	gas chromatog.	.449 ± .139	.505 ± .182 ^{NS}	+20.5
<u>E. armata</u>	(5)	25 ⁰	room	O ₂ analyzer	.398 ± .068	.474 ± .064 ¹	+20.5
<u>E. armata</u>	(7)	28 ⁰	dry air	O ₂ analyzer	.457 ± .085	.515 ± .096 ¹	+25.9
<u>E. armata</u>	(7)	28 ⁰	90%	O ₂ analyzer	.233 ± .074	.406 ± .220 ¹	+67.2
<u>C. verrucosa</u>	(8)	25 ⁰	room	gas chromatog.	.224 ± .057	.282 ± .088 ^{NS}	+28.7
<u>Hadrurus</u> <u>arizonensis</u> ²	(5)	room	room	gas chromatog.	.321 ± .044	.434 ± .069 ¹	+36.5
<u>Parurectonus</u> ³ <u>mesaensis</u>	(4)	room	unk	manometric (open)	.031 ± .004	.051 ± .005	+61.0
<u>P. mesaensis</u> ³	(5)	room	unk	manometric (closed)	.043 ± .007	.045 ± .013	+5.6
<u>Locusta</u> <u>migratoria</u> ⁴	(9)	28 ⁰ day 18 ⁰ night	unk	infrared gas analyzer	41.7 ± 8.5 (mg CO ₂ /h)	40.5 ± 11.7 (mg CO ₂ /h)	-2.9

¹Significant difference between two methods ($p < .05$).

²King and Hadley (1979).

³Yokota (1979).

⁴Buscarlet (1979).

FIGURE CAPTIONS

Figure 1 Regression of water influx measured with tritiated water plotted against gravimetric measurement of water influx in E. armata.

$$Y = .8765X + 15.316, r^2 = .924, df = 6.$$

Figure 2 Regression of metabolic rate measured with doubly-labeled water plotted against metabolic rate determined using either gas chromatograph or oxygen analyzer. All values of experiments plotted. $Y = 0.907X + .116, r^2 = 0.64, df = 29.$

FIGURE 1

FIGURE 2