

SHORT COMMUNICATION
URINE CLEARANCE RATES AND APPARENT
PERMEABILITY OF *GAMMARUS DUEBENI*
EXPOSED TO VARYING CONDITIONS

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Recent investigations indicate that some species of Crustacea have the ability to alter their apparent permeability to water in order to reduce the osmotic stress placed on the animal (Smith, 1967, 1970, 1976; Bolt, 1982; Lockwood, Bolt & Dawson, 1982). The apparent permeability to water of the amphipod *Gammarus duebeni* (Lilljeborg) varies dramatically when the animal is subjected to varying salinity regimes (Lockwood, Inman & Courtenay 1973; Dawson, 1982; Bolt, 1982).

It has been proposed that *G. duebeni* reduces osmotic stress by changing its permeability to water as the osmotic gradient between the haemolymph and the external medium varies (Bolt, 1983). This conclusion was based upon results obtained using THO as a radioactive marker to measure water exchange rates. However, until such changes in apparent permeability to THO can be confirmed by a different experimental approach, the possibility remains that these changes are an artefact of the THO method (Smith, 1967).

In an attempt to answer this question, Bolt (1983) demonstrated that *G. duebeni* exhibited large and sometimes rapid changes in apparent permeability in conditions of changing salinity whilst *Chaetogammarus marinus* (Leach) and *Gammarus locusta* (Linnaeus) did not. This result partly confirms that the change in apparent permeability in *G. duebeni* is not due to experimental error but it still does not eliminate the possibility that unstirred layers (Nernst, 1904; Motais, Isaia, Rankin & Maetz, 1969) or other artefacts could be present in *G. duebeni* and not in *G. locusta* or *C. marinus*. To eliminate successfully the term 'apparent' from the definition of this phenomenon, permeability changes must be demonstrated by another technique.

THO experiments measure the dynamic fluxes of water into and out of the animal, and such exchanges of water in the presence of an osmotic gradient cause a bulk flow in the direction of the osmotic gradient. If this bulk flow is inwards, there must either be an increase of internal pressure or an equal flow of excreted fluid out of the animal. It is assumed that in *G. duebeni* the inward bulk flow is matched by an outward flow of urine. Thus, theoretically, measurement of urine flow and osmotic gradient should permit calculation of the half-time of exchange of water for

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the animal. This technique is only practicable if the bulk flow of water is into the animal and the animal is urinating. Thus it may only be used when the osmotic concentration of the haemolymph of the animal is greater than that of the external medium. In recent studies on the crab *Holthuisana transversa*, Greenaway (1981) investigated three glomerular filtration rate (GFR) markers commonly used in higher vertebrates, ^{14}C polyethylene glycol 4000, ^3H inulin and ^{51}Cr EDTA, and concluded that ^{51}Cr EDTA is likely to provide the most reliable estimate of filtration rates. For this reason, ^{51}Cr EDTA was selected for the present study.

^{51}Cr EDTA was obtained from the Radiochemical Centre, Amersham, Bucks. The preparation contained 3–7 mg ^{51}Cr EDTA ml^{-1} and 0.9–2.4 mg sodium edetate BPC ml^{-1} . The specific activity was 1–2 mCi mg^{-1} Cr, and to obtain the required activity per unit volume it was necessary to concentrate the material 10:1 by freeze drying.

Animals were injected through the cuticle of the lateral surface using a fine drawn-out Pasteur pipette and oral pressure. The animals were put in a counting tube, which was placed in a 'well-type' scintillation counter attached to a Panax Reigate counter. The salinity in the counting chamber was determined by controlling the salinity in the inflow reservoir. Water was pumped through the chamber in order to flush away the excreted urine.

A continuous signal was taken from the rate meter of the Panax Reigate counter and fed *via* a land line to an analogue filter (kemo) and then to an RCA COSMAC CDP 18S020 microprocessor for data logging. The microprocessor was programmed to sample repeatedly and digitize the signal from the Panax Reigate. These values were scanned over 60-s periods and stored in memory. The microprocessor was then used to calculate the urine loss as a percentage loss of body fluid per unit time using the following equations.

Percentage loss of body fluid per unit time =

$$\frac{2}{\Delta t n} \frac{C_{i+n} - C_i}{C_{i+n} + C_i} \times 100, \quad (1)$$

where Δt is the time between points, C_i is counts at time t , n is 1 if adjacent values are used and n is 2 if alternate values are used. Osmotic concentration of the haemolymph and medium were calculated from the sodium concentrations using the relationship $1 \text{ osmol} = 500 \text{ mmol l}^{-1} \text{ Na}$.

$$M_m \text{ or } M_a = \frac{55.56}{55.56\lambda}, \quad (2)$$

when λ is 0–1.0 osmol, blood or medium, M_m is molar concentration of medium and M_a is molar concentration of haemolymph.

Using the net flow calculations from Lockwood & Inman (1973), the theoretical half-time of exchange of water ($t_{1/2\text{calc}}$) can be calculated.

$$\frac{\left(\frac{M_m - M_a}{M_m}\right) 100 \ln^2}{O_s} = t_{1/2\text{calc}}, \quad (3)$$

where O_s is percentage loss of body fluid per minute (cf. equation 1).

The calculated theoretical half-time of exchange of water ($t_{1/2\text{calc}}$) can be compared directly with the half-time of exchange as found by tritiated water ($t_{1/2}\text{THO}$) as both values refer to half-times of exchange of haemolymph volume. However, absolute values of both methods cannot be compared with confidence.

The half-time of exchange of water using THO was measured using the technique described in Bolt, Dawson, Inman & Lockwood (1980). The microprocessor system used to control the external medium is described in Lockwood *et al.* (1981).

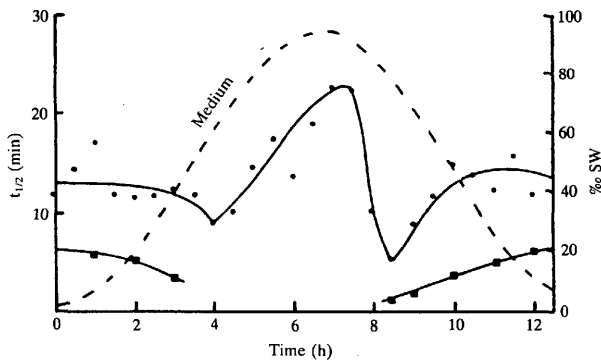


Fig. 1. Comparison of the half-time of the exchange of water determined using THO with the $t_{1/2}$ calculated from urine clearance rates in *Gammarus duebeni*. Animals are exposed to a 12 h 25 min salinity cycle from 2–30‰ sea water (SW); ●, $t_{1/2}\text{THO}$ (from Bolt, 1983), $N=5$; ■, $t_{1/2\text{calc}}$, $N=4$. Each point represents a mean of N separate measurements on N individuals. Lines fitted by eye.

G. duebeni were acclimated to a cycling salinity regime (2‰ SW to 32‰ SW over a 12 h 25 min time period at 15°C for four cycles) and then injected with ^{51}Cr EDTA. The radioactivity of the animals was monitored and the half-time of exchange of water for *G. duebeni* ($t_{1/2\text{calc}}$) was calculated during those parts of the salinity cycle when the animal was hypertonic to the external medium (Fig. 1). Unfortunately, when the animal is hypotonic to the medium there is no urine flow and $t_{1/2\text{calc}}$ cannot be obtained. Even so, the pattern of change was clear and consistent (Fig. 1).

In order to compare further the $t_{1/2\text{calc}}$ and the $t_{1/2}\text{THO}$, another period of significant change in apparent permeability is needed when the animal's haemolymph is hypertonic to the medium. In further experiments, *G. duebeni* were

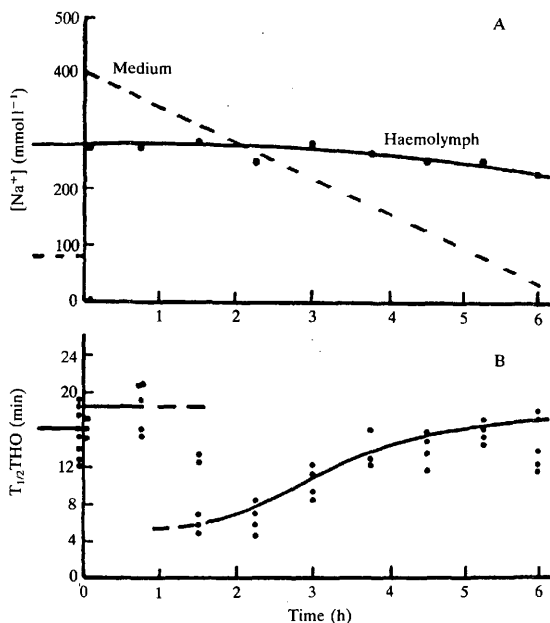


Fig. 2. (A) Haemolymph and medium sodium concentration in *Gammarus duebeni* exposed to varying salinities between 2‰ and 30‰. Each point represents a group of five animals. (B) Half-time of exchange of THO ($t_{1/2} \text{ THO}$) in *G. duebeni* exposed to varying salinities, between 2‰ and 30‰ SW. Points represent individual animals. Lines fitted by eye.

acclimated to 2‰ SW for 48 h and transferred to 100‰ SW. The salinity was then reduced to 2‰ SW in a linear fashion over 6 h using a second microprocessor. The sodium concentration of the haemolymph and medium and the apparent permeability using THO were measured. The results showed a significant change in $t_{1/2} \text{ THO}$ while the animals were hypertonic to the medium (Fig. 2). This treatment was repeated with animals injected with $^{51}\text{Cr EDTA}$, with which the urine flow was measured, and $t_{1/2} \text{ calc}$ found (Fig. 3). Results of a control experiment using *G. duebeni* acclimated to 2‰ SW, injected with $^{51}\text{Cr EDTA}$ and transferred to 2‰ SW are illustrated in Fig. 3. These show that absolute values of $t_{1/2} \text{ calc}$ obtained from this technique should not be used as there is a steady drift of $t_{1/2} \text{ calc}$ in an animal at equilibrium with the external medium, demonstrating a change in clearance rates of the tracer as the time from injection increases.

Animals in the experimental regime showed a consistent change in $t_{1/2} \text{ calc}$, exhibiting a larger change in $t_{1/2} \text{ calc}$ than the change shown by $t_{1/2} \text{ THO}$. However,

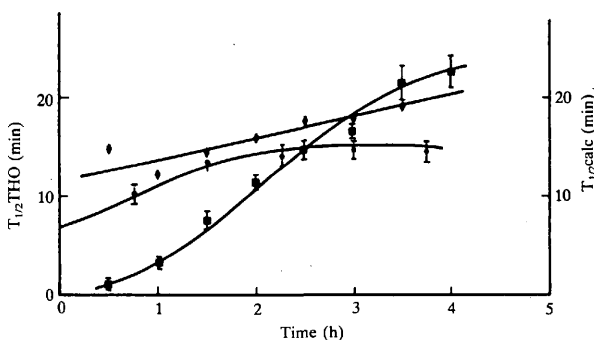


Fig. 3. Comparison of the half-time of exchange ($t_{1/2}$) of water using THO with the $t_{1/2}$ calculated from urine clearance rates in *Gammarus duebeni* exposed to varying salinities between 2‰ and 30‰ SW. ● = $t_{1/2}\text{THO}$ (group of five animals), ■ = $t_{1/2}\text{calc}$ (group of five animals) and ◆ = $t_{1/2}\text{calc}$ control (groups of four animals). Vertical bars represent \pm S.E.M. Lines fitted by eye.

if the slope of the 2‰ control is taken into account, this change would be reduced to a similar magnitude as that of the change in $t_{1/2}\text{THO}$.

The technique of estimating the half-time of exchange of water using urine flow as an indicator of bulk flow involves many assumptions. This technique assumes that the excretion of ^{51}Cr EDTA is effectively measuring the urine flow, and that the ^{51}Cr EDTA urine to blood (U/B) ratio is constant at unity throughout the experiment. It is also assumed that ^{51}Cr EDTA is lost only through urination.

Reigel *et al.* (1974) compared radioactive markers and concluded that the loss of ^{51}Cr EDTA *via* routes other than the urine was relatively small.

Lockwood & Inman (1973) have shown that the U/B ratio varies in *G. duebeni* from 1.05 in 100‰ SW to 1.15 in 2‰ SW. Although these variations in U/B ratio would affect the $t_{1/2}\text{calc}$, the magnitude of the observed changes in $t_{1/2}\text{calc}$ could not be explained by such small changes in the U/B ratio.

Using ^{51}Cr EDTA to calculate $t_{1/2}$ as a complimentary method to the use of tritiated water as a direct marker strongly suggests that the 'apparent' permeability to water observed in *G. duebeni* is real. This conclusion is also supported by experiments showing different responses to tritiated water of *G. duebeni*, *G. locusta* and *Chaetogammarus marinus* (Bolt, 1983), where only *G. duebeni* exhibits radical changes in apparent permeability. It seems highly unlikely that changes in $t_{1/2}\text{THO}$ were artefacts of the experimental technique in *G. duebeni* but not in *G. locusta* and *C. marinus*.

It is, therefore, concluded that the present and earlier results taken together obviate the necessity for the term 'apparent' when discussing permeability changes measured using THO as a marker in the amphipod crustaceans studied.

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