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SHORT COMMUNICATION

USE OF Tc-99m-PERTECHNETATE TO FOLLOW LIQUID WATER UPTAKE BY *PORCELLIO SCABER*

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Terrestrial isopods are, apart from the amphipods, the only group of crustaceans living on land. They have evolved some functional and anatomical adaptations to manage water and ion exchange between themselves and their environment, and these are different from those in all other crustaceans. In higher terrestrial isopods, liquid water is taken in by mouth (Hoese, 1981) and water vapour is absorbed in the pleoventral space (Wright and Machin, 1990; Wright and O'Donnell, 1992). Spencer and Edney (1954) also showed that water from wet filter paper can be taken up by the animals' rear appendages (uropods).

Studies of water gain have been based on gravimetric measurements (Spencer and Edney, 1954; Mayes and Holdich, 1975; Coenen-Staß, 1981), the use of tritiated water (Mayes and Holdich, 1975) and on the use of coloured water solutions (Spencer and Edney, 1954; Hoese, 1981). However, owing to the different experimental techniques, various experimental conditions and different pretreatments of the animals (desiccated or starved), the role of the uropods in liquid water uptake by the terrestrial isopod *Porcellio scaber* Latreille (Isopoda: Crustacea) is still uncertain. The uptake sites of liquid water have not been precisely determined.

The aim of our work was to investigate liquid water uptake sites in well-hydrated animals. In the present study, a Tc-99m-pertechnetate water solution was used, and experiments were also performed with an aqueous dye (Fuchsin) solution under similar conditions. Blocking experiments were performed by occluding those parts of the animal's body that are supposed to be involved in liquid water uptake (the mouth, the anus together with the uropods, or both sites).

The use of dye solutions is a conventional test in biology, but the application of the radioactive isotope Tc-99m, which was used for this purpose for the first time in the present study, needs to be described in some detail. Since 1957, Tc-99m has been widely accepted for nuclear medicine diagnostic procedures. This choice is largely based on its physical properties, including the absence of beta decay, a half-life of 6h and a 140keV

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photon, which provide good penetration and easy detection (McElvany and Hopkins, 1989). Technetium is an artificial element. Its chemical form after production in nuclear reactors or from fission molybdenum generators is normally $Na^{99m}TcO_4$ (sodium pertechnetate). $NaTcO_4$ is highly soluble in water, where 7+ is the most stable of the technetium oxidation states. TcO_4^- cannot be evaporated even at reduced pressure or on heating up to 40°C (Novak and Fajgelj, 1983). $^{99m}TcO_4^-$ is very similar to iodide in size (tetrahedral ion, $V=4\times10^{-23}\,cm^3$) and hence in its biodistribution. For imaging specific body organs, ^{99m}Tc is chemically bound to different organic or inorganic molecules before application. In some nuclear medicine studies, ^{99m}Tc is also used as pertechnetate (Rosenthall, 1967).

Tc-99m-pertechnetate stock solution was obtained from an Ultratechnecow fission molybdenum/technetium generator (Mallincrodt), diluted with distilled water and mixed. The specific activity of the working solution was around $1MBq\,ml^{-1}$, representing $5\times10^{-9}\,mol\,l^{-1}$ Tc-99m-pertechnetate solution. The Tc-99m decay mode (isomeric transition) leads to Tc-99 ($t_{1/2}=2.1\times10^5$ years), so the pertechnetate concentration effectively does not change during the experiment. However, our results were corrected for Tc-99m decay during the experiment.

Filter paper discs (diameter 5cm) were placed in Petri dishes and moistened in the centre with 400 μl of Tc-99m-pertechnetate solution or 0.5% acid Fuchsin (pH7.0) aqueous solution. Groups of four or five animals with a blocked mouth, blocked uropods (immobilized uropods and blocked anus), or both parts blocked, and a group of intact animals were placed on these filter paper discs for 4h. The blockage was performed with epoxy paste. After the test period, the animals were separately transferred to polyethylene vials for measurement of total body activity. Animals were then dissected. In decapitated animals with the last pleonite segment cut off, the gut and four digestive gland tubes (hepatopancreas) were pulled out without touching the outer surface of the animal to avoid any possible radioactive contamination. Each separate tissue was then transferred to a polyethylene vial, 1ml distilled water was added and the contents were homogenized to obtain similar measuring geometry. The intensity of the Tc-99m gamma line (140keV) was measured on an ORTEC well-type pure Ge coaxial detector, connected to a Canberra 90 multichannel analyzer system. The percentages of Tc-99m activity measured in the gut of *P. scaber* are shown in Fig. 1. In the case of the exposure to Fuchsin solution, after dissection the animals were mounted in glycerol and the distribution of the dye was examined by light microscopy. The appearance of dye inside the animal's digestive system is described in Table 1. The presence of dye or Tc-99m activity in the digestive system (gut, digestive glands) was taken as proof of liquid water uptake by the animal.

During exposure of the animal on the moist substratum and to water-vapour-saturated air, water gain could occur in several ways. These are water vapour absorption, cutaneous absorption and liquid water uptake by the mouthparts and/or by the uropods. The only two possibilities which allow the observation of liquid water uptake separately from water vapour absorption are the use of coloured water or of an aqueous solution of a radioactive isotope other than tritium. As mentioned above, the pertechnetate ion is not volatile, so that water vapour absorption by the animal cannot be detected even if it takes place. Our experiments, in which dead or totally blocked animals were exposed on the

same substratum, did not show any evidence (activity) of ^{99m}Tc inside the body (gut, hepatopancreas). This is proof that Tc-99m-pertechnetate does not penetrate through the cuticle and that it can enter the body only by the active process of liquid water uptake. It also shows that blockage with epoxy paste was successful. The use of ^{99m}Tc-pertechnetate allowed us to follow liquid water uptake specifically by mouth parts or

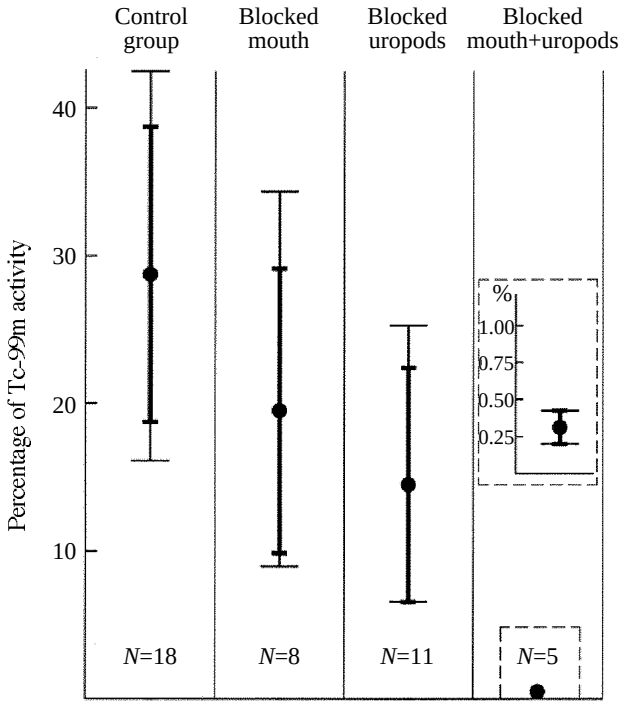

Fig. 1. Histogram showing the percentage of ^{99m}Tc activity measured in the gut of *Porcellio scaber* exposed on filter paper discs moistened with 400 μl of ^{99m}Tc-pertechnetate solution for different groups of animals. Results are calculated as a percentage of the total activity of the animal: $\%G = 100G/T$ (where G is activity of the gut, T is total activity and N is number of animals). Bold lines are mean ± S.D. and normal lines show the maximum and minimum values measured.

Table 1. *The distribution of dye solution inside the body of animals exposed to 400 μl of fuchsin solution on filter paper discs in Petri dishes for 4 h*

Group of animals	Appearance of dye inside the digestive system	
	Gut*	Hepatopancreas
Intact animals	Yes	Yes
Blocked mouth	Yes	No
Blocked uropods†	Yes	Yes
Blocked mouth+uropods	No	No

*Anterior chamber, papillate region and rectum; †immobilised uropods and blocked anus.

uropods. Using a ^{99m}Tc solution, much smaller amounts of liquid water taken up by the animal can be detected than by the use of coloured water, and the amounts are smaller than those that can be determined by gravimetric measurements. Expressed in practical terms, this means that an activity of up to 30000counts per 500s was measured in the gut, when the animal was exposed on a substratum moistened with 400 μl of ^{99m}Tc-pertechnetate solution. When animals were exposed at different moisture levels of the substratum (100, 200 or 300 μl of water), ^{99m}Tc activity was also detected inside the body of the animals (5–8 times lower than on exposure to 400 μl; in real numbers this represents up to 4000counts per 500s), while there was no evidence of colour inside the body under these conditions.

For animals exposed to 400 μl of ^{99m}Tc solution, the uptake of activity corresponds to a water mass taken up of about 0.05mg. However, it should be pointed out that, owing to probable differences in the metabolism and internal distribution of pertechnetate and water, the ^{99m}Tc activity distribution may not be a good indicator of the subsequent dynamics of water in the animal, though it is a valid indicator of uptake by mouthparts and uropods.

In our experiment, in contrast to previous similar studies, well-hydrated animals were used which were free of any hydration stress before the exposure, so they did not need to increase their water content during the test. Further, no behavioural or visible damage to the animals' tissue was observed as a result of exposure to ^{99m}Tc gamma rays (the absorbed dose rate is relatively low at between 1.2 and 3.8mSvh^{-1}) or to epoxy paste.

From our results, it is evident that well-hydrated *P. scaber* exposed to a moistened substratum and saturated air can take up liquid water both by mouth and through the uropods. The experimental data provided by the new technique directly support the results obtained by Spencer and Edney (1954) and their conclusion that the uropods are involved in liquid water uptake by *P. scaber.*

Our future interest will be focused on the dynamics of liquid water uptake by *P. scaber* under different experimental conditions and on morphological studies of the pleon, which will together clarify the actual role of the uropods in liquid water uptake by *P. scaber*.

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References

COENEN-STAß, D. (1981). Some aspects of the water balance of two desert woodlice *Hemilepistus afghanicus* and *Hemilepistus raeumuri* (Oniscidea, Isopoda, Crustacea). *Comp. Biochem. Physiol.* **71**, 405–419.

HOESE, B. (1981). Morphologie und Function des Wasserlaitungssystems der terrestrischen Isopoden (Crustacea, Isopoda, Oniscoidea). *Zoomorphologie* **98**, 135–167.

MAYES, K. R. AND HOLDICH, D. M. (1975). Water exchange between woodlice and moist environment, with particular reference to *Oniscus asellus. Comp. Biochem. Physiol.* **51**A, 295–300.

MCELVANY, K. D. AND HOPKINS, K. T. (1989). Radiochemistry and radiopharmacology. In *Nuclear Medicine Technology and Techniques.* St Louis: The C.V. Mosby Company.

NOVAK, J. AND FAJGELJ, A.(1983). Tc-99m extraction generator. *Vestn. Slov. Kem. Drus.* **30**, 1–7.

ROSENTHALL, L. M. D. (1967). Detection of altered cerebral arterial blood flow using technetium-99m pertechnetate and gamma-ray scintillation camera. *Radiology* **88**, 713–718.

SPENCER, J. O. AND EDNEY, E. B.(1954). The absorption of water by woodlice. *J. exp. Biol.* **31**, 491–496.

WRIGHT, J. C. AND MACHIN, J. (1990). Water vapor absorption in terrestrial isopods. *J. exp. Biol.* **154**, 13–30.

WRIGHT, J. C. AND O'DONNELL, M. J. (1992). Osmolarity and electrolite composition of pleon fluid in *Porcellio scaber* (Crustacea, Isopoda, Oniscidea): implications for water vapour absorption. *J. exp. Biol.* **164**, 189–203.