carriers (+/mdg) or actually normal (+/+) and not mutants (mdg/mdg). Carriers are indistinguishable from their normal littermates. Thus, the results in this study either represent characteristics of normal fetal liver or reflect the genetic influence of muscular dysgenesis.

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Demonstration of vitamin D_3 metabolism in *Mytilus edulis*[1]

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Summary. Radiolabeled vitamin D_3 was converted into several polar metabolites upon incubation with tissue homogenates from the common mussel *Mytilus edulis*. Chromatographic analysis indicated that the metabolites have chromatographic mobilities different from those of known standards. The results suggest that vitamin D_3 is metabolized in mussels via pathways that differ from the vertebrate systems.
Key words. Vitamin D_3 metabolism; marine mussel; chromatographic analysis.

While the role of vitamin D_3 as a regulator of calcium and phosphorus metabolism is now well understood in vertebrates[3,4], it has not been established whether it has any function in invertebrates, especially in the species with shells or other calcified structures. There are several groups of invertebrates which may obtain vitamin D_3 from their diet or synthesize it from endogenous 7-dehydrocholesterol, and it is interesting to study whether they can utilize it.

Because the metabolic activation of vitamin D_3, first to 25-hydroxyvitamin D_3 ($25(OH)D_3$) and then to 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), is required for the expression of its activity in vertebrates[3,4], the demonstration of such reactions in invertebrates would be an indication of biological significance. We wanted to study the possible presence of metabolic pathways for vitamin D_3 in the common mussel, *Mytilus edulis*.

Materials and methods. M. eduli, collected near Blåbergsholm, The Baltic Sea, were maintained in aerated glass tanks in sea water at +4 °C. A 40% (w/v) homogenate of the mussels (without shells and feet) was prepared in 15 mM Tris-acetate buffer containing 190 mM sucrose and 1.9 mM magnesium acetate, pH 7.7. Into incubation flasks were pipetted 2 ml homogenate, 1 ml cofactor solution (7.5 mM glucose-6-phosphate, 1.5 mM NADP, 25 mM sodium succinate and 0.5 unit glucose-6-phosphate dehydrogenase) in Tris-acetate buffer, and the substrate,

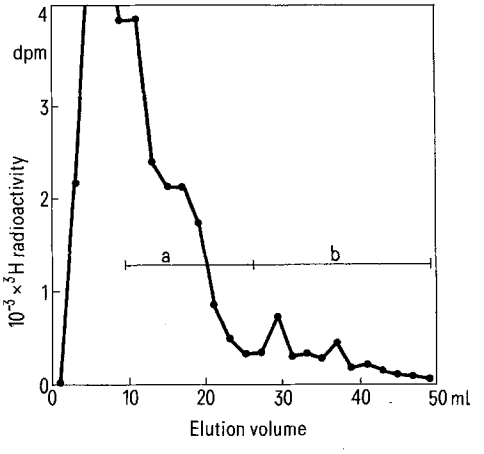

Figure 1. Sephedex LH-20 column chromatographic profile of extracts of mussel homogenates incubated with [^{3}H] vitamin D_3; standard vitamin D_3 eluted between 3–10 ml. 2-ml fractions were collected, and 50 µl of each was taken for the determination of radioactivity. Fractions between 10–26 ml (a) were combined for the HPLC separation of peaks MI and MII, and those between 26–56 (b) for the more polar peaks.

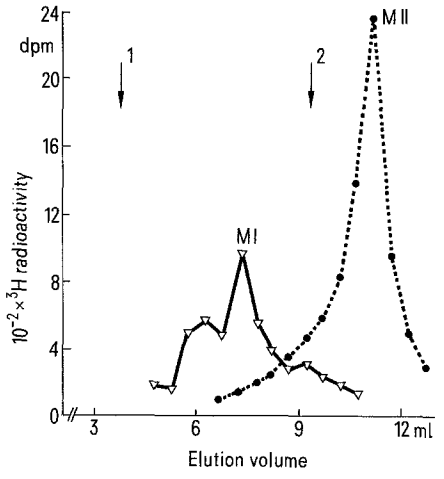

Figure 2. HPLC profile of metabolite peaks MI (▽—▽) and MII (●---●) on a Zorbax-Sil column eluted with hexane-propan-2-ol (24:1). Initial separation of the two peaks was performed on a Nucleosil column using the same solvent. Arrows show the elution positions of vitamin D_3 (1) and 25(OH)D_3 (2).

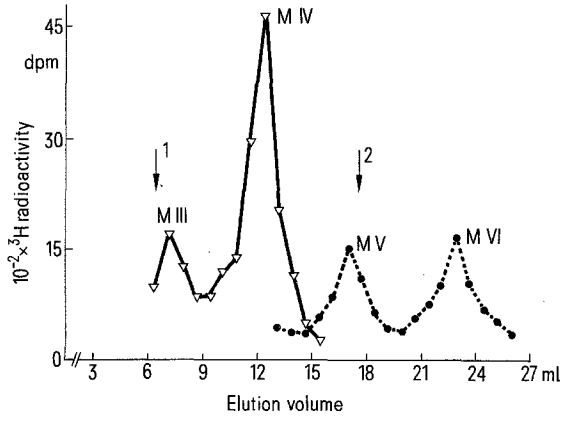

Figure 3. HPLC profile of metabolite peaks MIII and MIV (▽—▽), and MV and MVI (●---●) on a Zorbax-Sil column eluted with hexane-propan-2-ol (9:1). The peaks were initially separated on a Nucleosil column followed by the combining of two peaks for further analysis. The elution positions of 24,25$(OH)_2D_3$ (1) and 1,25$(OH)_2D_3$ (2) are indicated by arrows.

[1α,2α – ^{3}H] vitamin D_3 (16 Ci/mmol; Amersham International; purified by high-performance liquid chromatography (HPLC) before use), was added in 50 µl ethanol. After 3 h at +25 °C with constant shaking, lipids were extracted with dichloromethane. The solvent was evaporated under nitrogen, and the residue was initially chromatographed on a 1 × 19 cm column of Sephadex LH-20 in hexane-chloroform-methanol-water (9:1:1:0.015) followed by further characterization on HPLC as described in Results.

Results and discussion. Chromatography of the lipid residues from the incubations on Sephadex LH-20 indicated the presence of radiolabeled compounds more polar than [^{3}H] vitamin D_3 (fig. 1). From control incubations, where the incubation was stopped immediately, only unreacted substrate was recovered. Further chromatography of the material that eluted between 10–26 ml from the Sephadex column on HPLC with a Nucleosil 50–7 silicic acid column eluted with hexane-propan-2-ol (24:1) divided the radioactivity into two peaks that were reanalyzed using a more efficient Zorbax-Sil column and the same solvent. Again, two peaks (MI and MII) were observed (fig. 2). MI eluted between standard vitamin D_3 and 25(OH)D_3 and MII was more polar than 25(OH)D_3.

The more polar material, eluting between 26–56 ml from the Sephadex column, was also analyzed using the same HPLC columns in succession; the solvent was changed to hexane-propan-2-ol (9:1). The radioactivity was separated into four peaks (fig. 3): MIII, MIV and MV eluted between standard 24,25-dihydroxyvitamin D_3 (24,25$(OH)_2D_3$) and 1,25$(OH)_2D_3$, and peak MVI after 1,25$(OH)_2D_3$.

Only occasional studies have investigated the metabolism or the role of vitamin D_3 in invertebrates. The early study of Wagge[5] identified vitamin D_3 as an essential nutrient for the land snail *Helix aspersa*. This was followed by the more recent finding that two species of land snails, *Theba pisana* and *Hiersolyma levantina* are capable of metabolizing [^{3}H] vitamin D_3 to several polar products[6]. One of them was identified as [^{3}H] 25(OH)D_3, but not by very rigorous chromatography. Two other metabolites remained unidentified.

Working with the sea urchin, *Psammechinus miliaris*, Hobbs and Pennock[7] showed the conversion of [^{3}H] vitamin D_3 to polar products. Again, a putative [^{3}H] 25(OH)D_3 was detected, but no chromatograms were given, and the chromatographic identification was very premature. In these previous studies[6,7] 1,25$(OH)_2D_3$ was not detected.

On the basis of the chromatographic data of the present study (figs 2 and 3), we conclude that no [^{3}H] 25(OH)D_3 was formed by *M. edulis* tissue in vitro. In fact, none of the [^{3}H] peaks exhibited similar chromatographic mobilities compared to those of major vitamin D_3 metabolites in vertebrates. Therefore, the metabolic pathways present in *M. edulis* do not appear to proceed via 25(OH)D_3, and the enzyme systems that catalyzed the conversion reactions in the present study may not be specific for vitamin D_3. It cannot be stated that the mussel vitamin D_3 metabolites are products of activation: their modifications may be related to inactivation or secretion.

Our results confirm the earlier findings that invertebrates are capable of vitamin D_3 metabolism[6,7]. The reactions observed here probably occur also in vivo: phytoplankton, the principal diet of *M. edulis*, contains 7-dehydrocholesterol, the precursor of vitamin D_3[8], and in shallow water it may be exposed to sufficient solar irradiation to cause the photochemical synthesis of vitamin D_3[9]. Further studies are required to determine the identity and function of mussel vitamin D_3 metabolites.

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