pounds. This mainly applies to the amidinohydrazones 3f and 3g. But also for the less specific chinazolinones, perimidines and the remaining amidinohydrazones the IC_{50}-values for ACE are always clearly smaller in relation to those of CPA and CPB.

We have presented here new types of ACE inhibiting compounds. These substances differ very clearly from all well-known ACE inhibitors. The ACE inhibition of the presented compounds has to be evaluated in relation to the IC_{50} of captopril and other well-known ACE inhibitors measured under the same conditions (see table 4). Until now these new classes of ACE inhibitory compounds are of course not able to complete or replace the most active ACE inhibitors. We expect, however, a further increase in the activity of these compounds by a systematic modification of the side chains of our compounds in accordance with the conceptions of the active ACE center as proposed by Cushman[1] and Petrillo[3].

Table 4. ACE inhibitory activities of captopril, enalapril and HOE-498

Comp.	IC_{50} (10^{-6} moles/l)
Captopril	0.03
Enalapril (maleat)	4.0 (ester; prodrug)
HOE-498	1.7 (ester; prodrug)
HOE-498	0.0012 (free acid)

Immediately after the submission of the manuscript, K.-C. Liu and L.-Y. Hsu published an article about 'Synthesis and antihypertensive activity of some quinazolinone derivates' (Archs Pharm. *318* (1985) 502). The mechanism of the hypotensive action is not described, but all these antihypertensive compounds are structurally closely related to our substances. These studies, consequently, support the aim of our work.

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Ultrastructural localization of catalase and D-amino acid oxidase in 'normal' fetal mouse liver

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Summary. In the hepatocytes of 'normal' fetal mice from mothers which were carriers of muscular dysgenesis, catalase and D-amino acid oxidase (DAAO) positive as well as negative peroxisomes were observed. DAAO reaction product was occasionally localized in patches around cell membranes and DAAO-positive peroxisomes were frequently observed near mitochondria.
Key words. Ultrastructure; catalase; D-amino acid oxidase; fetal mouse liver; hepatocytes; peroxisomes; muscular dysgenesis.

Peroxisomes (microbodies) are now recognized as respiratory cellular organelles. Enzymes present in peroxisomes (peroxisomal enzymes) are not synthesized in peroxisomes but in the cytosol. Peroxisomal proteins in rat liver are synthesized on membrane bound polysomes[2] or on free polysomes[3], released into the cytosol and transported to peroxisomes[2,3]. Cytosolic catalase has been localized in liver parenchyma[4] but its absence from the Golgi apparatus and rough endoplasmic reticulum suggests that the channeling of newly synthesized catalase from the rough endoplasmic reticulum to the peroxisome is unlikely[5]. No connections were demonstrated between peroxisomes and endoplasmic reticulum by electron microscopic cytochemistry[6]. Biogenesis of peroxisomes is still not well understood.

Peroxisomes contain the marker enzyme catalase and one or more oxidases. Some of these enzymes have been demonstrated by cytochemical techniques in peroxisomes but not at the sites of their synthesis or in their pathways to the peroxisomes. Since peroxisomes are formed during tissue development and differentiation, as has been shown by various studies[7], an embryonic stage may show peroxisomal enzymes at the sites of synthesis or en route to peroxisomes. With this assumption, the present study of only two of the cytochemically demonstrable enzymes, catalase and DAAO, was undertaken in 'normal' fetal mouse liver. Another objective was to learn whether or not heterogeneous populations of hepatic peroxisomes with respect to catalase and DAAO do exist, at least during development. Catalase plays a protective role against peroxide toxicity by degrading H_2O_2 while oxidases generate H_2O_2[8]. Thus, H_2O_2 generation and degradation occur in the peroxisomes. DAAO catalyzes the oxidation of D-d-amino acids[9] and its role in metabolizing substrates such as biogenic amine and glyoxylate is beginning to unfold[10].

Material and methods. The liver from a 'normal' mouse embryo (fetus of 19 days gestation obtained from the mother carrier of muscular dysgenesis) was dissected out under anesthesia, cut into pieces and fixed in cold 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4. For catalase cytochemistry, the liver tissue was fixed for 2 h, washed in 0.1 M cacodylate buffer and incubated for 20–60 min at 37 °C in Novikoff's medium[11] slightly modified and used at pH 9.7. The incubation medium contained 0.020 g 3,3'-diaminobenzidine (DAB) tetrahydrochloride (Sigma Chemical Co.), 9.8 ml 0.05 M 1,2-propanediol buffer at pH 9.0, 0.2 ml 1% hydrogen peroxide. The tissue was subse-

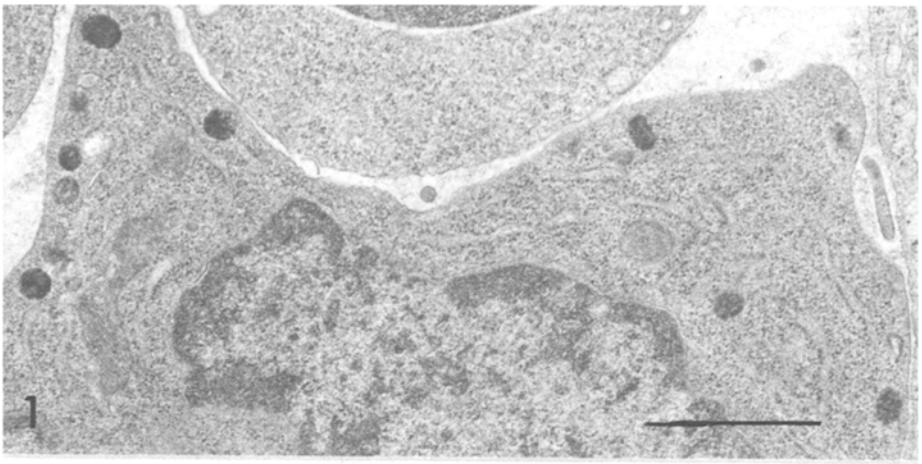

Figure 1. Hepatocyte showing spherical peroxisomes with catalase reactions of various intensity. Note that mitochondria are larger in size than the peroxisomes. Bar 1000 nm.

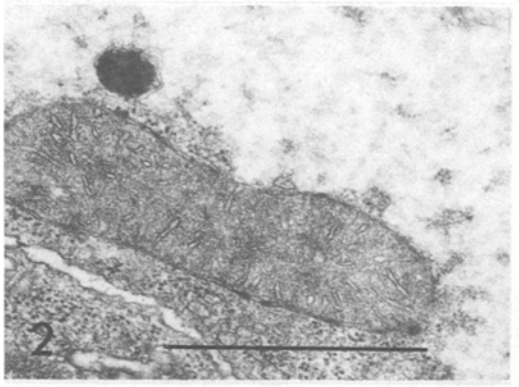

Figure 2. DAAO-positive peroxisome seen in close proximity to the mitochondrion in the hepatocyte cytoplasm. The peroxisome appears to be at the stage of acquiring a limiting membrane. Bar 1000 nm.

Figure 3. Note the reaction product for DAAO in patches in the narrow pericellular space between adjoining hepatocytes. No DAAO-positive peroxisomes are identified in the cytoplasm of this hepatocyte. Bar 1000 nm.

quently washed in 0.1 M cacodylate buffer, pH 7.4. Catalase oxidizes DAB to form a homogeneous reaction product. For DAAO cytochemistry, the liver was fixed for only 30 min, washed in 0.1 M cacodylate buffer at pH 7.4, and preincubated for 30 min at 25 °C in the presoak[12] (0.084 g 3-amino-1,2,4-triazole (AT, Sigma), 10 ml 0.1 M Tris-maleate buffer at pH 7.4–7.5, 0.012 g cerous chloride). Final incubation for 0.5–2 h was done in the medium[12] containing 0.084 g AT, 10 ml 0.1 M Tris-maleate buffer at pH 7.4–7.5, 0.012 g cerous chloride and 0.057 g D-proline (Sigma). The tissue was then washed in a slightly acidic 0.1 M cacodylate buffer (pH 6.0) to remove cerium hydroxide precipitate. Oxidases generate H_2O_2 which is then trapped by cerium ions from the medium to form an insoluble reaction product. After reaction, the tissue was postfixed in 1% osmium tetroxide, dehydrated in graded ethanol and propylene oxide, and embedded in araldite mixture. Ultrathin sections were cut, stained with 2% uranyl acetate and lead citrate, and examined under an HU-11 (Hitachi) electron microscope.

Results and discussion. Hepatocyte differentiation occurs during early fetal development. Catalase-positive and negative peroxisomes with an average diameter of 200 nm (n = 9) were seen in 7 out of 100 profiles of hepatocytes (fig. 4). The nucleoids and matrical plates of peroxisomes are usually obscured in cytochemical preparations by the homogeneous reaction product. However, some non-reactive peroxisomes show matrical plates (fig. 4). Matrical plates with varying matrical density have been demonstrated in hepatic peroxisomes in morphological preparations in a condition like vitamin E deficiency[13–15].

For DAAO cytochemistry, the tissue was fixed for a short time (30 min) as the enzyme is known to be extremely sensitive to the fixative glutaraldehyde. DAAO-positive and negative peroxisomes with an average diameter of 160 nm (n = 4) were observed

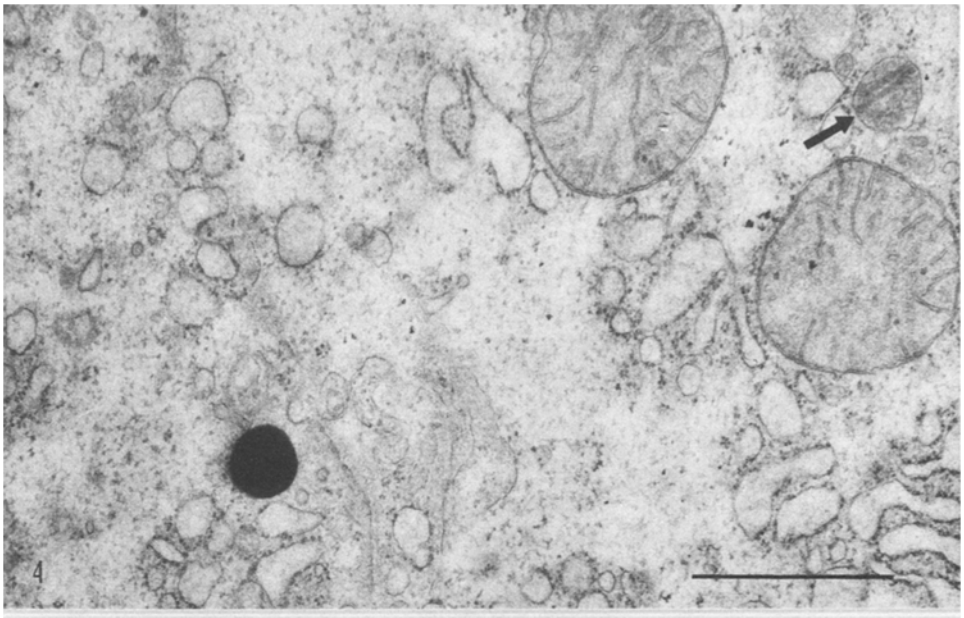

Figure 4. Intensely positive peroxisome for catalase activity seen in the cytoplasm of a hepatocyte. Note another peroxisome (arrow) which is catalase-negative with single delimiting membrane and matrical plates. Bar 1000 nm.

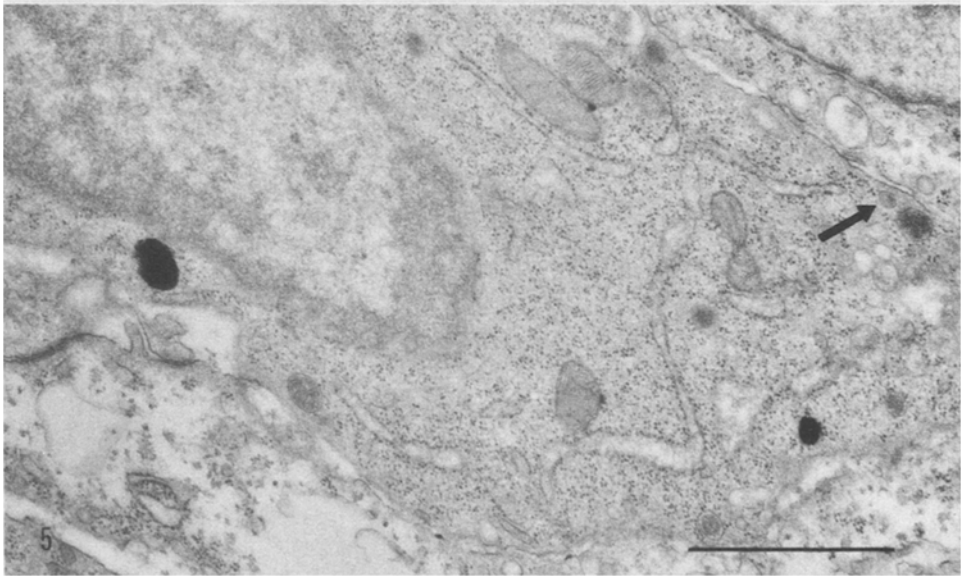

Figure 5. Two intensely DAAO-positive peroxisomes seen in the hepatocyte. Note one (arrow) of the few DAAO-negative peroxisomes contains nucleoid body. Bar 1000 nm.

in 3 out of 100 profiles of hepatocytes (fig. 5). Occurrence of DAAO-positive peroxisomes close to mitochondria (fig. 2) suggests the possibility of a mitochondrial origin of DAAO and its transport to the peroxisomes during development. These peroxisomes were also found in the process of acquiring their single delimiting membranes. DAAO-positive peroxisomes seem to have a different pattern of distribution from that of catalase-positive peroxisomes. The reaction product of DAAO activity was occasionally visualized in patches between cell membranes of hepatocytes at adjoining sites (fig. 3). DAAO is an induced enzyme[16] and therefore under certain conditions it may show up elsewhere, for instance on the cell membranes. DAAO on the cell membranes may be in fact an NADH oxidase such as is seen on the surface of human polymorphonuclear leucocytes as a result of H_2O_2 production. The results, however, give no clue as to the synthetic sites or the pathways. Measurements indicate that DAAO-containing peroxisomes are often smaller in size than catalase-containing peroxisomes.

It seems possible that catalase-dominant peroxisomes may be poor in DAAO while DAAO-dominant peroxisomes may be deficient in catalase. This means that catalase-containing peroxisomes may not be positive for DAAO in DAAO preparations as are DAAO-containing peroxisomes in catalase preparations. This suggests that the heterogeneous populations of hepatic peroxisomes with respect to catalase and DAAO exist during development. Total loss of enzyme activity in some peroxisomes only, due to factors such as fixation and dehydration, does not seem to be possible. The significant heterogeneity among peroxisomes with respect to catalase-rich and oxidase-poor organelles has been suggested on the basis of differences in sedimentation behavior[18] and shown cytochemically in several cell types in the nervous system[12]. Similar heterogeneity among peroxisomes may exist for other peroxisomal enzymes as well.

Muscular dysgenesis (mdg/mdg) is a recessive autosomal lethal in the mouse. The mutation causes developmental arrest and degeneration of skeletal muscle[19,20]. Our 'normal' fetal mice were

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,

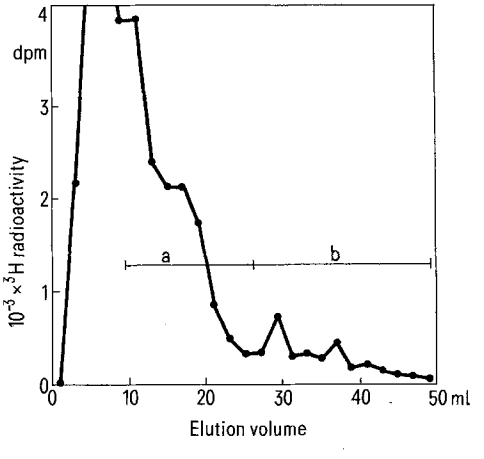

Figure 1. Sephedex LH-20 column chromatographic profile of extracts of mussel homogenates incubated with [^{3}H] vitamin D_3; standard vitamin D_3 eluted between 3–10 ml. 2-ml fractions were collected, and 50 µl of each was taken for the determination of radioactivity. Fractions between 10–26 ml (a)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