Subcellular Fractionation of the Liver from Northern Pike, *Esox lucius**

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Since many environmental pollutants sooner or later reach our rivers, lakes and seas, it is of importance to investigate the metabolism of xenobiotics by aquatic organisms. Although the earliest reports indicated an absence of drug-metabolizing enzymes in fish, it is now quite clear on the basis of many different studies that a wide variety of fish exhibit patterns of drug metabolism which are qualitatively similar to those seen in mammals.

We have begun to characterize the pathways of xenobiotic metabolism in the liver of the Northern pike.[1] This fish is found in fresh and brackish water throughout the northern hemisphere, is a top predator, and does not migrate but resides in a relatively small area. In addition, Northern pike in certain regions is known to exhibit high tumor frequency, *e.g.*, 21 % of this fish in certain waters have lymphosarcomas.

Our studies have shown that the liver of the Northern pike exhibits NADPH-cytochrome *c* reductase, cytochrome P-450, benzo[*a*]-pyrene monooxygenase, epoxide hydratase, and glutathione *S*-transferase activities that are between 13 and 305 % of the corresponding activities in rat liver.[1] In order to further investigate the metabolism of xenobiotics in this tissue, as well as to determine the possible relationship of this metabolism to tumorigeneses, it was desirable to obtain relatively pure preparations containing fragments of the endoplasmic reticulum. In addition, comparison of the subcellular fractionation pattern obtained with pike liver to that observed with mammalian liver was expected to support the basic similarly of these tissues, but could also reveal differences. Finally, comparison of the subcellular distributions of different drug-metabolizing enzymes should give hints concerning physiological interactions between these various systems in pike liver. To our knowledge only one other fish, the rainbow trout,[2] has been the object of such a study to date.

Fish were caught in the Stockholm archipelago and kept in running cold water. Livers were homogenized in 0.25 M sucrose using 4 up-and-down strokes of a Potter-Elvehjelm homogenizer at 440 RPM and the homogenate subsequently adjusted to contain 1 g liver/5 ml. The "nuclear" pellet was collected by centrifugation for 10 min at 600 g_{av}, the "mitochondrial" pellet by centrifugation for 15 min at 7100 g_{av}, and the "microsomal" pellet and supernatant by centrifugation for 150 min at 105 000 g_{av}. DNA (a presumptive marker for the nucleus),[3] cytochrome oxidase (mitochondria),[4] *p*-nitrophenyl-mannosidase (Golgi),[5] AMPase (plasma membrane),[6] catalase (peroxisomes),[7] β-glycerophosphatase (lysosomes),[8] lactate dehydrogenase (cytosol),[9] NADPH-cytochrome *c* reductase (endoplasmic reticulum),[10] cytochrome P-450,[11] benzpyrene monooxygenase,[12] epoxide hydratase,[13] glutathione *S*-transferase[14] and protein[15] were all determined on freshly-prepared subfractions using published procesures.

Fig. 1 shows the distribution of the various markers upon subcellular fractionation of pike liver. In general, this pattern is seen to resemble closely the corresponding pattern obtained with rat liver,[16] as well as with the liver from rainbow trout.[2] Some differences are, however, apparent. The "micro-

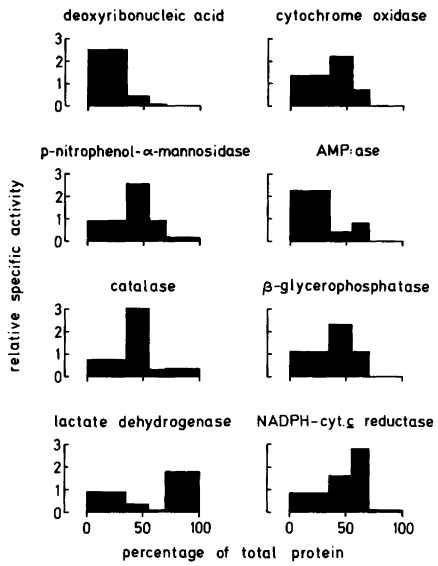

Fig. 1. Distribution of presumptive marker enzymes after subcellular fractionation of pike liver. The different fractions are, from left to right, the "nuclear", "mitochondrial", "microsomal" and supernatant fractions. Relative specific acticity = the specific activity of the fraction/the specific activity of the combined fractions.

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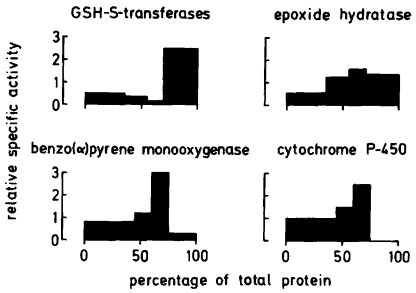

Fig. 2. Distribution of some drug-metabolizing systems after subcellular fractionation of pike liver. See legend to Fig. 1

somal" pellet from pike liver is more contaminated with mitochondria and less contaminated with Golgi than are rat liver microsomes. At the same time, trout liver microsomes are much more heavily contaminated with lysosomes and plasma membrane than is the corresponding fraction from pike.

The "microsomal" fraction from pike liver contains about 30–35 % of the total liver NADPH-cytochrome *c* reductase activity, a figure which is somewhat lower than that found for rat liver microsomes.[16] The relatively largest contamination of pike liver microsomes seems to arise from the plasma membrane, the Golgi, and lysosomes, which is also the case for rat liver.[16] If electron microscopy, which we are currently performing, supports these conclusions, a relatively well-defined microsomal fraction will be available for studies of drug-metabolizing systems in pike liver.

Fig. 2 shows the distribution of cytochrome P-450, benzpyrene monooxygenase activity, epoxide hydratase and glutathione *S*-transferase activity upon subcellular fractionation of pike liver. There are two very surprising aspects of this pattern. In the first place 35–40 % of the total epoxide hydratase activity measured with styrene oxide as substrate is recovered in the soluble fraction. The possibility that this activity is associated with small microsomes that do not pellet under the conditions used is rendered unlikely by the use of a long centrifugation time (150 min, see above) and by the observation that only very small amounts of NADPH-cytochrome *c* reductase and cytochrome P-450 are recovered in the soluble fraction. This latter observation also speaks against general solubilization of components of the endoplastic reticulum during homogenization and/or subfractionation. It is now becoming clear that mammalian liver also contains soluble epoxide hydratase activity,[17] but not with styrene oxide as substrate.[18]

The second surprising aspect of these studies is the very high level of glutathione *S*-transferase activity present in pike liver. This level is more than 3 times the corresponding level in rat liver. Most of the pike liver glutathione *S*-transferase activity is recovered in the soluble fraction (Fig. 2), but the small amount of activity recovered in the "microsomal" fraction may reflect the presence of an enzyme which truly belongs to the endoplasmic reticulum.[19]

As in rat liver, the cytochrome P-450 system and at least part of the epoxide hydratase seem to be localized on the same organelle in pike liver; while glutathione *S*-transferase activity and the rest of the epoxide hydratase appear to be soluble. These localizations may be of physiological significance with regard to interactions between the various drug-metabolizing systems.

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