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Esterase

XIX. Biochemical and Ultrahistochemical Investigations of the Non Specific Esterase in Nuclei from Mouse Liver

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Summary. The present investigations reveal that the non specific esterase from mouse liver nuclei is exclusively located in the perinuclear cistern and that the nuclear chromatin does not contain any esterase as a rule. However, esterase is associated with lipid droplets which are seldomly found within nuclei. Two different esterases are demonstrated with the employed substrates in the nuclear envelope. It is exemplified that the esterase, found in isolated nuclei, can neither be understood as qualitative nor as quantitative equivalent of the esterase demonstrated ultrahistochemically at nuclear membranes.

Introduction

There are several biochemical investigations regarding the contents of esterase (E. C. 3.1.1.x; x = 1, 2 and others) in mouse and rat liver nuclei (Underhay *et al.*, 1956; Markert and Hunter, 1959; Carruthers and Baumler, 1961; Shibko and Tappel, 1964; Barrow and Holt, 1971; Ljungquist and Augustinsson, 1971). Histochemical techniques also indicated poor esterase activity at the periphery of nuclei in rat liver (Gössner, 1958). Electron microscopic enzyme demonstrations later revealed, that the esterase in nuclei is exclusively associated with their membranes (Bell and Barnet, 1965; Kawashima, 1970; v. Deimling and Madreiter, 1972; v. Deimling *et al.*, 1973). Biochemical investigations of esterase in isolated nuclear membranes were performed by Pokrovskii *et al.* (1970).

In order to supplement and critically evaluate the afore mentioned findings we focussed our investigations regarding the subcellular location of the esterase on two questions:

1. How to assure, that no intranuclear esterase exists? Theoretically it is possible, that esterase is present even in the chromatin. Yet the demonstration fails because the substrate is completely seized and hydrolyzed by the nuclear membranes and does not reach the interiour of the nuclei.

2. Are the biochemical data to be understood as a quantitative and qualitative equivalent of the ultrahistochemical demonstration of esterase at nuclear membranes? Traditionally the same substrates are not used for histochemistry and quantitative determinations of esterase. Consequently a clear correlation of the results is not possible without reservation.

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In the present paper we submit new results from the comparison of biochemical and ultrahistochemical investigations of esterase in mouse liver nuclei. The investigations were performed on intact liver tissue as well as on isolated liver nuclei. As universal substrate we applied quinoline derivates which are suitable for quantitative determinations as well as for ultrahistochemical demonstration of the esterase. Our studies are supplemented by electrophoretic analyses on polyacrylamide gels.

Materials and Methods

Animals. Adult, male NMRI mice from our own breeding, housed in macrolon cages, had free access to Altromin R and water. They were killed by fracturing of the neck. The vena cava inferior in those animals not used for ultrahistochemical enzyme demonstration was opened and the inner organs perfused from the left ventricle with 2.0 ml cold (10° C) 0.9% NaCl solution.

Homogenate. For quantitative determinations and electrophoresis the tissue homogenates used for the isolation of liver nuclei was employed.

Isolation of Nucleic from Liver. In accordance with the scheme outlined by Kartenbeck *et al.* (1973) in hypertonic sucrose. For each experiment the livers of 14 animals were pooled.

Isolation of Microsomes. As described by Ljungquist (1971). The microsomal fractions were prepared each from one liver which was not subjected to the isolation of nuclei.

Enzyme Assays. Esterase activities were measured against 3 different substrates: p-nitrophenyl propionate (pNPP) as described by Huggins and Lapides (1947) in the modification of Staeudinger *et al.* (1973), O-acetyl-8-hydroxiquinoline (Ch-O-2) and S-acetyl-8-mercaptoquinoline (Ch-S-2) according to v. Deimling *et al.* (1973). For enzyme activity determinations the microsomal and nuclear fraction was resuspended 1/10 (w/v) in 0.2% Triton X-100, rehomogenized and centrifuged for 1 hour at 100000 g. The tissue homogenate was diluted with 2.0% Triton X-100 in a relation of 9/10 (v/v), rehomogenized and centrifuged as described above. Measurements were performed on the supernatants.

Protein Measurement. Method of Lowry, modified by Rieder (1966).

Acrylamide Disc Electrophoresis. Our procedure followed the analytical standard method described by Maurer (1968). 50 mU (substrate: pNPP) esterase (100000 g supernatant liquid of cell particle fractions, homogenized in aqueous Triton X-100) were applied to each gel (7.0% separation gel, 80 mm in length and 5 mm ⌀; gel buffer: 75.6 mM Tris, 12 mM Cl^-, pH 8.9; electrode buffer: 4.95 mM Tris, 37.4 mM glycine, pH 8.3; 10° C). The enzyme reaction for esterase was studied with α-naphthyl acetate and fast red TR in 0.3 M phosphate buffer, pH 6.5.

Electron Microscopical Enzyme Demonstration. 1 mm^3 cubes of liver tissue were fixed for 3 hours in 2% glutaraldehyde in 0.1 M Na-cacodylate buffer, pH 7.35 and then left for 2 hours in 10% dimethylsulfoxide. Cryostate sections, 30 µm thick, were incubated (25 min at 9° C) and treated as described by v. Deimling and Madreiter (1972). The nuclear fractions were rehomogenized in 2% glutaraldehyde and resedimented. After a fixation time of 20 min, the pellets were chopped into small pieces, washed in cacodylate buffer and incubated (13 min at 9° C). Incubation solution:

24 ml cacodylate buffer, 0.1 M, pH 7.35;
23 ml destilled water;
3 ml solution of complexed bismuth nitrate;
0.3 mMol substrate (56 mg Ch-O-2, 61 mg Ch-S-2).

Secondary fixation with osmium tetroxide, embedding in Epon, staining of ultrathin sections with uranyl acetate and lead citrate. Sections were studied with a Zeiss EM 9 S-2.

Results

Demonstration of Esterase in Fixed Liver Tissue

With Ch-O-2 as substrate low esterase activity is demonstrated in all cisterns of the smooth endoplasmic reticulum (SER), marked by finely granulated reaction

product (RP) which is diffusely distributed throughout their interiors (Fig. 1). Likewise RP can be found between the inner and outer mitochondrial membranes. The RP which is distributed in such a way superposes the electron micrographs, at low magnifications, with a greyish dust and does not allow one to distinguish fine structural details. Beyond this, short segments of SER-cisterns are quite tightly filled with RP. The perinuclear cistern (PC) shows the same enzyme distribution pattern as the SER: beside the diffusely in their interior distributed, finely granulated RP, short segments are tightly packed with RP. In addition a strong reaction of esterase, found at the periphery and weaker in the interiors of all lipid droplets is striking. Liver cells, which have stored big amounts of fat, sometimes contain small lipid droplets in the chromatin. Those lipid droplets are normally surrounded by a rim of RP, whereby esterase is not found elsewhere in the chromatin. Seldomly, but more frequent in some hepatocytes a strong esterase activity is observed in the matrices of a few mitochondria. In the extracellular space esterase is often found also in the space of Dissee, in bile capillaries and between neighbouring hepatocytes, but without any recognizable regularity. In addition esterase activity is found on erythrocyte-membranes and in the sinusoidal lumen without relation to cellular structures. The enzyme distribution pattern in liver cells is quite different from the above if Ch-S-2 is used for esterase demonstration (Fig. 2). In this case the reaction takes place in the cisterns of the SER which are (with short interruptions) generally filled with RP. In the same way the PC is quite tightly packed with RP, some short segments being without reaction. Only a low esterase activity can be shown at the periphery of lipid droplets. In the extracellular space esterase reaction is merely found on erythrocyte membranes and free in the sinusoidal lumen.

Demonstration of Esterase in Liver-nuclear Fractions

Fractionated nuclei are in dense arrangement and their membranes are mostly uninjured buth with occasional protrusions. Here and there one finds destroyed nuclei or fragments of chromatin without membraneous envelope. The demonstration of esterase with Ch-O-2 in isolated nuclei from liver reveals an enzyme distribution which is different from the pattern seen in nuclei in intact liver tissue (Figs. 3, 5). Local accumulations of RP are hardly detected in the nuclear envelope. Instead, the nuclear membranes are studded with finely granulated RP. Even in fractionated liver nuclei a few can be found which do contain lipid droplets surrounded by esterase activity (Fig. 5). Between the nuclei, but predominantly in contact with nuclear membranes, clodes of RP are located which have no discernable relation to microsomal material. Microsomal membranes however are studded with tiny grains of RP as a rule. Sporadically RP is found in nuclei with ruptured membranes or in chromatin fragments (Fig. 7). No esterase activity could be demonstrated within intact nuclei.

Also with Ch-S-2 as substrate it is evident that the envelope of fractionated nuclei does not contain the same quantity of RP as the PC does in compact liver tissue (Figs. 4, 6). Only here and there short sections with accumulations of RP are seen. Neither the nuclear membranes nor the microsomal contaminations of the fractions reveal any esterase activity, which is, however, found in solitary ac-

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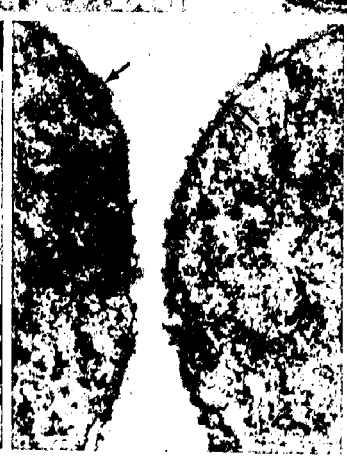

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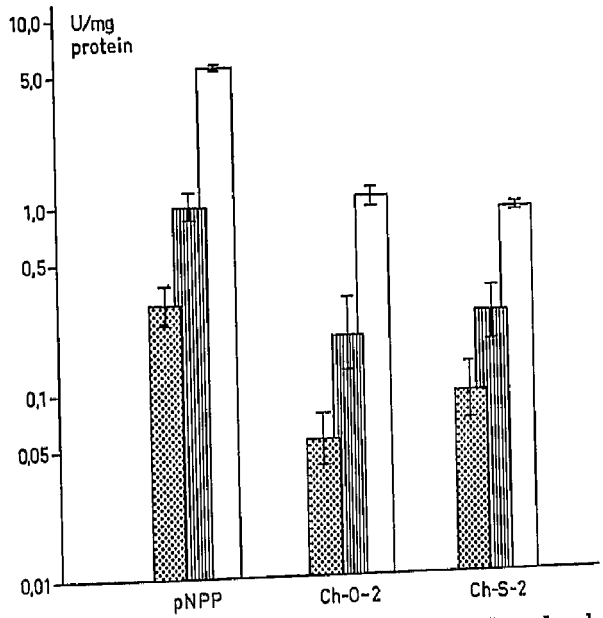

Fig. 8. Specific activities (U/mg protein) of isolated nuclei (dotted columns), homogenate (hatched columns) and microsomes (white columns) from liver with three different substrates. Averages from 2 experiments

cumulations in the medium inbetween the nuclei but often in contact with nuclear membranes. Even with Ch-S-2 no esterase activity can be demonstrated in nuclei with intact membranes.

Assays of Esterase Activity

Fig. 8 shows the specific activities of esterase from liver homogenate and isolated nuclei from liver. Since the ultrahistochemical demonstration of esterase has revealed the same enzyme distribution pattern of PC and SER, the specific

Fig. 1. Liver, demonstration of esterase with Ch-O-2. Reaction product (*RP*) within cisternae of smooth endoplasmic reticulum (*SER*) and within the perinuclear cistern (*PC*) diffusely distributed and finely granulated (↗) and with occasional accumulations (↗↗). Esterase activity further at lipid droplets (*LP*) and within the matrices of a few mitochondria (*M*). 6300×

Fig. 2. Liver, demonstration of esterase with Ch-S-2. SER and PC are tightly filled with RP (↗↗). Short sections are devoid of RP (↗). Little reaction at LP. ×6300

Fig. 3. Isolated nuclei from liver, demonstration of esterase with Ch-O-2. Reaction of esterase diffusely and finely granulated on nuclear membranes (↗). Clodes of RP between nuclei and imposed on nuclear membranes (↗↗). ×2500

Fig. 4. Isolated nuclei from liver, demonstration of esterase with Ch-S-2. Accumulations of RP within short sections of the PC (↗↗). Nuclear membranes devoid of esterase activity (↗). ×2500

Fig. 5. As Fig. 3. Nuclear membranes studded with finely granulated RP (↗). Clodes of RP between nuclei and imposed on their membranes (↗↗). Lipid droplet (*LP*), surrounded by esterase activity within chromatin of a nucleus. ×9500

Fig. 6. As Fig. 4. Short sections of the PC filled with RP (↗↗). Nuclear membranes without reaction (↗). ×9500

Fig. 7. As Fig. 3 and 5. Esterase activity within chromatin of a fragmented nucleus (↗). ×4500

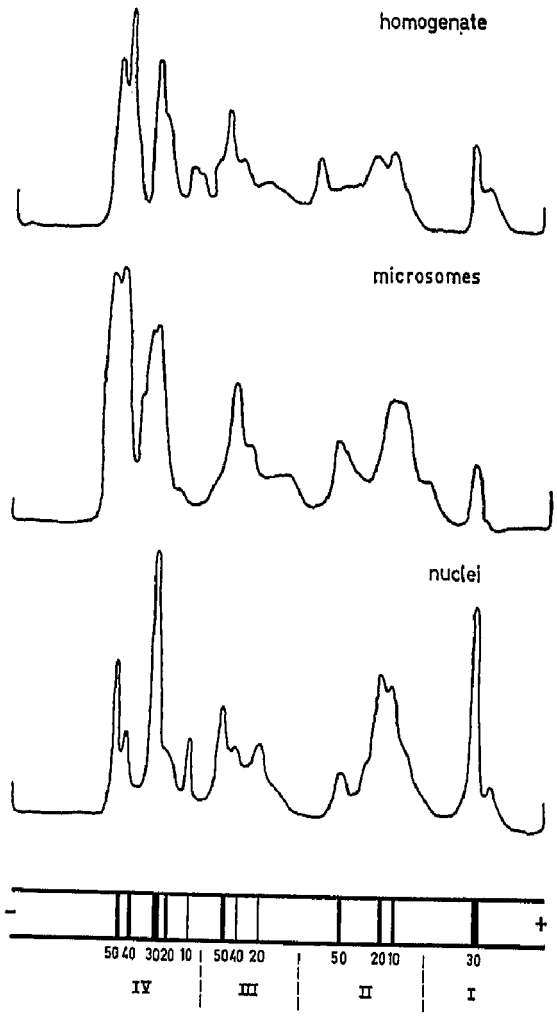

Fig. 9a—d. Separation of esterase from cell particle fractions of liver by analytical disc electrophoresis, densitograms. Schematic demonstration of the banding pattern with nomenclature of components

activities of liver microsomes were also presented for comparison. Determinations of enzyme activities were performed with pNPP as classical substrate as well as with those substrates used for the electron microscopical enzyme demonstration, Ch-O-2 and Ch-S-2. There is no significant difference between the specific activities of the tissue homogenate against both substrates; the same holds for the liver microsomes. On the other hand, the specific activity of isolated nuclei from liver is 1.7—fold higher with Ch-S-2 than with Ch-O-2.

Disc Electrophoresis

The densitograms of esterase from liver homogenate, -microsomes and -nuclei, separated by disc electrophoresis are shown in Fig. 9. All quantitatively important components of the homogenate are represented in the microsomal as well as in the nuclear fraction. Differences between same bands in different fractions are small. However, the esterase component IV_{30} is more strongly represented in nuclei than in the homogenate or in microsomes.

Discussion

It was demonstrated by v. Deimling *et al.* (1973) that two different qualities of kidney esterase in mice can be distinguished with Ch-O-2 and Ch-S-2 as substrates. Similarly, the esterases in mouse liver, demonstrated with the same substrates, differ in their cellular localisation and subcellular distribution. The esterase, demonstrated with Ch-O-2 occurs with low activity in all cisterns of the SER, including the perinuclear cistern but with local accumulations. Moreover Ch-O-2 is hydrolysed at all lipid droplets and in the matrices of several mitochondria. If Ch-S-2 is used for the demonstration of esterase instead of Ch-O-2 more reaction product, indicating higher esterase activity, is found throughout the SER and the PC, but with local interruptions. Some Ch-S-2 is hydrolysed at lipid droplets but none in mitochondria. According to this we conclude that the perinuclear cistern of nuclei from liver contains at least two different esterases which differ from each other in their compartimental distribution and their substrate specifity. We can state that the chromatin of nuclei from hepatocytes does not contain any esterase (with the employed substrates). Stating this we can be sure that during incubation sufficient substrate reaches the chromatin of intact nuclei as the reaction for esterase at lipid droplets in nuclei from liver cells with fatty changes has prooved (Fig. 5). Non specific esterase has a strong tendency to attach to cell particles, especially to microsomes, if cells are mechanically injured. According to this we attribute the RP which is found in destroyed nuclei to esterase deriving from other cell compartments which had been adsorbed to chromatin during the fractionation procedure.

The electron microscopical demonstration of esterase in isolated nuclei from liver further reveals that during cell particle fractionation a considerable amount of esterase is lost from the PC. This holds especially for the esterase demonstrated with Ch-S-2. In compact liver tissue the PC is quite tightly filled with RP if this substrate is employed, whereas in the PC of isolated nuclei RP is found only here and there. On the other hand RP is found in the nuclear fraction between the nuclei, independent from microsomal contaminations, the origin of which remains unclear. These findings are in accordance with our earlier reported observations (Böcking *et al.*, 1974) regarding the change of location of kidney esterase from the mitochondrial matrix to the surrounding cytoplasm, especially to microsomes, following mechanical injury of the cell. There we characterized the esterase as a predominately soluble enzyme. The esterase found within the PC also seems to be predominately soluble and to diffuse into the surrounding medium during the preparatory procedure. An attachment of esterase to membraneous material could also be observed in fractionated nuclei with Ch-O-2 as substrate. Esterase activities assayed in isolated nuclei from liver must therefore be interpreted with caution. In the present investigations isolated nuclei from liver reveal a 1.7-fold higher esterase activity with Ch-S-2 than with Ch-O-2. Yet the comparison of the ultrahistochemical demonstrations of esterase led one to expect a greater difference between the activities. The validity of the quantitative data from the nuclear fractions is further reduced by the finding of an unspecific adsorption to membraneous material. Therefore we regard the determination of esterase activity in isolated nuclei an unsuitable method for calculating the percentage of nuclear

from the total liver esterase. For the same reasons the validity of the electrophoretical analysis of esterase from the three cell particle fractions is limited. The similarity of the banding pattern obtained from the three cell particle fractions under analysis is suspicious of a non specific isolation of the esterase which is in situ associated with only one population of organelles. Rather it is our opinion that during cell particle fractionation a change of location of esterase occurs. The esterase, which is primarily and predominantely soluble, escapes from its original cell compartment and is unspecifically adsorbed to membraneous material in particular (compare Böcking *et al.*, 1974).

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