

Intracellular Localization of Esterase in Rat Liver. BY ELUNED UNDERHAY, S. J. HOLT, H. BEAUFAY,* AND C. DE DUVE. (*From the Courtauld Institute of Biochemistry, Middlesex Hospital Medical School, London, and the Laboratory of Physiological Chemistry, University of Louvain, Belgium.*)†

It has been shown by means of a histochemical staining technique, considered to conform to sound basic principles, that esterase activity is mainly associated with microscopic structures around the bile canaliculi of formol-calcium-fixed rat liver (1). A comparable, but less precise, localization is given by entirely independent histochemical techniques (1, 2). It has also been observed that acid phosphatase activity may be similarly localized (3) and this is particularly noticeable with the formol-calcium-fixed tissue, when the enzyme once again appears to be associated with intracellular particles in the vicinity of the bile canaliculi (4). This suggests that the two enzymes may be associated with the same intracellular structures. However, the enzymes behave very differently in liver dispersions fractionated by differential centrifugation. Whereas the microsomes contain most of the esterase activity as tested against a variety of substrates (5-9), acid phosphatase has been found to belong to an intermediate group of cytoplasmic particles, comparable in size to the smaller mitochondria (7, 10-13). A number of other acid hydrolases appear to be associated with these particles, which have been termed lysosomes for this reason (11). Preliminary electron microscope investigations (14) have shown that they may be identical with the dense peribiliary bodies described by Rouiller (15) and by Palade and Siekevitz (16).

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Thus, the results furnished by the two techniques tend to be mutually confirmatory for acid phosphatase, but not for esterase. In the face of this discrepancy, we have reinvestigated the distribution of esterase amongst separated rat liver fractions, taking the following additional precautions: (a) The fractionation was performed according to the scheme of de Duve *et al.* (11), which involves the isolation of an intermediary "light mitochondrial fraction" characterized by a high concentration of acid phosphatase and of other lysosomal enzymes, as of dense peribiliary bodies; (b) The substrates used for the esterase determinations included indoxyl acetate and α -naphthyl acetate, which are closely related to those used in the histochemical processes; (c) The fractions were also analyzed for glucose-6-phosphatase, an enzyme known to belong exclusively to the microsomes (17, 11). The results of these experiments are given in Table I.

The distributions found for esterase with indoxyl and α -naphthyl acetates are practically identical and differ from that of glucose-6-phosphatase only by the presence of a greater amount of activity in the final supernatant. Cholinesterase activity was also determined and shows a similar distribution, but the nuclei appear to be proportionately more active toward acetylcholine than toward the other esters. It should be mentioned, however, that only a minimal contribution to the total hydrolysis of the non-choline esters can be ascribed to cholinesterases under the conditions of

the histochemical tests and manometric assays, in view of the enormously greater activity of the non-specific esterases which also hydrolyze these substrates. Of greatest importance, however, is the fact, established with all three substrates, that the mitochondrial fractions

those obtained previously by other authors and suggest that esterase is a true microsomal enzyme and does not originate from lysosomes or from dense peribiliary bodies. Microsomes are believed to be derived from the endoplasmic reticulum (16) and one interpretation

TABLE I

Component	Absolute values per gm. liver	Percentage distribution						No. of experiments
		N	M	L	P	S	Recovery	
Nitrogen.....	32.4 mg.	16.4	16.5	6.6	19.4	36.7	95.6	3
Glucose-6-phosphatase..	19.6 μ moles/min.	6.3	2.4	8.6	72.2	4.4	93.9	2
Esterase:								
Indoxyl acetate.....	117 μ moles/min.	6.3	2.6	4.2	67.3	11.4	91.8	3
α -Naphthyl acetate...	135 " "	5.5	2.7	4.3	66.2	9.0	87.7	2
Acetylcholine.....	1.7 " "	18.6	4.0	5.0	62.0	19.1	108.7	3

N, nuclear fraction; M, heavy mitochondrial fraction; L, light mitochondrial fraction; P, microsomal fraction; S, final supernatant.

Glucose-6-phosphatase determined by the method of de Duve *et al.* (11).

Esterase was determined by a manometric technique at pH 7.4 and 37° using a substrate concentration of $6 \times 10^{-3}M$.

do not contain more esterase than can be accounted for by their contamination with microsomes, as estimated by glucose-6-phosphatase activity. In order to reconcile these results with the hypothesis that esterase is associated with lysosomes in the intact cell, one must assume that the enzyme is quantitatively detached from its support when the tissue is dispersed and is then largely taken up by the microsomes as the result of an adsorption process. Experiments designed to verify this possibility were, however, entirely negative. Washed microsomal (or mitochondrial) fractions, when suspended in a solution of soluble esterase, previously extracted from rat liver microsomes by a modification of the procedure of Burch (18), did not increase in activity.

The results described above confirm

to be given for the histochemical findings is that the components of this reticulum which are present around the bile canaliculi are particularly rich in esterase. On the other hand, electron micrographs of liver microsome fractions (16) indicate that these are not morphologically homogeneous, but appear to include material such as that derived from agglomerations of other membranous structures known to occur in the vicinity of the bile canaliculi of parenchymatous liver cells (16, 19). The material present in the microsomal fractions may thus be enzymically heterogeneous, although not appearing so when fractionated by current methods of differential centrifugation. A similar situation obtains in the case of the alkaline phosphatases of kidney and intestine, which have been localized in specific microscopic cell structures and

are also recovered in the microsomal fraction (17). Finally, the effect of fixation on the enzymic integrity of the liver cell must be taken into account in interpreting the histochemical results. Although similar staining patterns for esterase are obtained with entirely different methods of fixation, a histochemical and biochemical study of this aspect is being undertaken in an attempt to evaluate the effects of fixation processes.

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