activity, respectively (about 1 μg of enzyme per ml) 0.05% albumin reduces this inhibition to 74%, 32%, and 60%. TAPPEL AND DILLARD[15] have claimed that the effect of cholesterol (and retinol) on β-glucuronidase is due to disaggregation of the enzyme molecule into inactive units. This conclusion was also drawn on the basis of the experiments with albumin, known as "activator-associator", which partially prevents the inhibition.

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1 W H FISHMAN, S S GOLDMAN AND R DELELLIS, *Nature*, 213 (1967) 457
2 D ROMEO, N STAGNI, G L SOTTOCASA, M C PUGLIARELLO, B DE BERNARD AND F VITTUR, *Biochim Biophys Acta*, 130 (1966) 64
3 G A LEVVY AND J CONCHIE, in G J DUTTON, *Glucuronic Acid*, Academic Press, New York, 1966, p 301
4 B U MUSA, R P DOE AND U S SEAL, *J Biol Chem*, 240 (1965) 2811
5 B V PLAPP AND R D COLE, *Arch. Biochem Biophys*, 116 (1966) 193
6 E BUDDECKE AND O HOEFELE, *Z Physiol Chem*, 347 (1966) 173
7 J W WADDELL, *J Lab Clin Med*, 48 (1956) 311
8 A G GORNALL, C J BARDAWILL AND M M DAVID, *J Biol Chem*, 177 (1949) 751
9 B J DAVIS, *Ann N Y Acad Sci*, 121 (1964) 404
10 D. E WILLIAMS AND R A REISFELD, *Ann N Y Acad Sci*, 121 (1964) 373
11 W M MITCHELL, *Biochim Biophys Acta*, 147 (1967) 171
12 H N FERNLEY, *Biochem J*, 82 (1962) 500
13 R. G MARTIN AND B N AMES, *J Biol Chem*, 236 (1961) 1372
14 J L WEBB, *Enzyme and Metabolic Inhibitors*, Vol II–III, Academic Press, New York, 1966
15 A L TAPPEL AND C J. DILLARD, *J Biol Chem*, 242 (1967) 2463

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The use of naphthyl esters as substrates in esterase determinations

One of the most frequently used substrates for esterase determinations is α-naphthyl acetate, particularly useful in histochemical identification and in detecting esterase activity after resolution by electrophoresis or chromatography (gel, paper, *etc*)[1–3]. Recently, a study on the isoenzyme status of esterases in certain vertebrate tissues was based on the use of this ester and some related α- and β-naphthyl derivatives[4,5]

The choice of substrate in assaying esterase activity depends on several factors, the most important ones being the technique used and the specificity of the activity measured As long as a purified preparation is known to contain only one active enzyme, the generalization may be made that any ester can be used as substrate in assaying the activity of this particular enzyme Because of the multiple forms of esterases

usually present in crude preparations, the main problem in assaying the various activities individually in such material is to ascertain whether or not an ester used is actually split by one or by several enzymes Furthermore, when the ester used is a nonspecific substrate for several forms of esterases, it must be stressed that one substrate rarely has the same affinity for various esterase forms Esterases may also be present which do not split the substrate used

Since it is not possible at the present time to measure the molarity of esterase active sites or the relative number of active protein molecules present, the specific

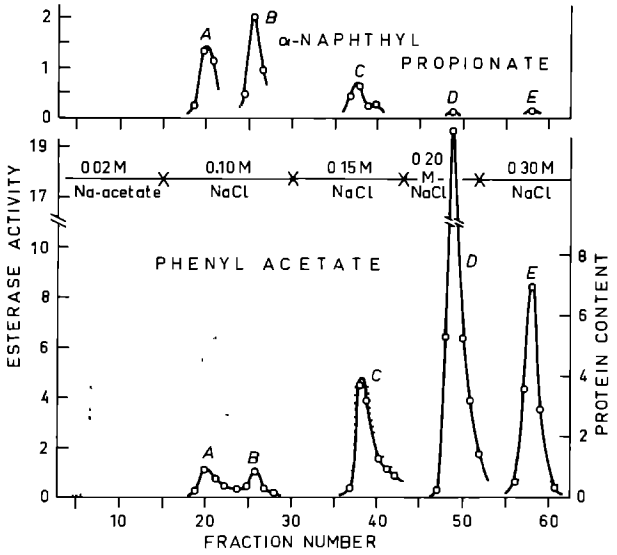

Fig 1 Fractionation of rat plasma by column chromatography on DEAE-cellulose The sample (4.5 ml plasma) was applied to a column (2 cm × 12 cm) of Whatman DE-52 in 0 02 M sodium acetate, pH 5 5 Elution was carried out by a concentration of NaCl increased stepwise as indicated on the graph Eluate samples of 6 ml were analysed for esterase activity with phenyl acetate and α-naphthyl propionate (top graph), and for protein (dotted line) in an Uvicord (at 254 mμ) The protein content of Peak fractions A–E was determined by the Lowry method Esterase activity is expressed in μmoles · min^{-1} ml^{-1} and protein content in mg ml^{-1}

activity with the best possible substrate for each esterase component might be regarded as the most practical index However, in comparisons between species or tissues, or similar studies, the relative specific activities towards a particular substrate can be a useful measure provided it is stated what activities are measured and what types of esterase are not detected There is, unfortunately, no universal ester known which can be used to measure all esterases present in, for instance, blood plasma

In our preparative and comparative studies on these enzymes various substrates in combination with selective esterase inhibitors have been employed. Naphthyl acetates, as well as other esters of α- and β-naphthol, were found to be hydrolysed very differently by various types of these enzymes Arylesterases (EC 3.1 1 2) either do not split these esters at all or at a very low rate[6,7]. Phenyl acetate or its *p*-nitro derivative is the substrate of choice for these enzymes, as are the choline esters for cholinesterases (EC 3 1.1 7 and 3 1 1 8) A recent study on the esterases present in rat plasma

will demonstrate, as an example, the shortcomings of the use of a naphthyl ester in selective esterase determination

Rat plasma was fractionated on a DEAE-cellulose column using NaCl of increasing concentration for elution (details in Fig 1) The fractions were analysed for esterase activity (manometric technique) with phenyl acetate (and propionate) and naphthyl propionate (and acetate). The activity thus determined was found in 5 peaks (A–E), the specific activities of which are listed in Table I The various peak fractions were also analysed by starch-gel electrophoresis, and esterase activity was detected in the gel with a histochemical method using α-naphthyl propionate (or acetate) as substrate[8] In addition, the sensitivity of each esterase peak fraction to

TABLE I

PROPERTIES OF RAT PLASMA ESTERASES SEPARATED ON A DEAE-CELLULOSE COLUMN

Peak fractions A–E from the experiment shown in Fig 1 were tested for esterase activity (μmoles of phenyl acetate or α-naphthyl propionate hydrolyzed per min per mg protein, Warburg technique), the activity in Fig 1 is expressed per ml of fraction The sensitivity of esterase activity was tested against mipafox (bis-monoisopropylamino fluorophosphine oxide), $HgCl_2$ and $La(NO_3)_3$ with phenyl acetate as substrate, inhibition is expressed as per cent of control Fractions were analysed by starch-gel electrophoresis (α-naphthyl propionate as substrate)

Peak fraction	*A*	*B*	*C*	*D*	*E*
Substrate hydrolysis (μmoles)					
Phenyl acetate	0 19	0 75	0 54	29 0	27 1
Inhibition (%)					
Mipafox, 10 0 μM	88	100	0	0	0
Hg^{2+}, 1 0 μM	0	0	94 5	93 5	96 5
La^{3+}, 3 0 μM	0	0	52 5	55 5	55 5
Esterase active bands on gel electropherograms, protein region	Albumin	Albumin and β-globulin	Trace only in albumin	Not visible	Not visible
Type of esterase present	Carboxyl-esterase	Carboxyl-esterase and cholin-esterase	Aryl-esterase and trace of cholin-esterase	Aryl-esterase	Aryl-esterase

selective esterase inhibitors was studied The results of the latter experiments are summarised in Table I

The results indicate that peak fractions A and B contain all carboxylesterase activity and B also contains cholinestarase, according to the definitions proposed previously for these enzymes[9]. The carboxylesterase(s) hydrolysed α-naphthyl propionate (and also phenyl propionate) at a much higher rate than did other esterases in rat plasma Arylesterases, present in Peaks D and E, split phenyl acetate at a much higher rate than did the carboxyl esterases, and α-naphthyl propionate (and acetate) at a rate which was hardly detected with the manometric technique. Arylesterases (of Fractions D and E) were not detectable with the histochemical technique used in the starch gel after electrophoretic resolution. The results obtained with selective inhibitors (an organophosphate ester and Hg^{2+}) correspond to those obtained with

selective substrates and indicate a complete separation of the carboxylesterases from the arylesterases Peak fraction C also contains some of the arylesterases and a trace of cholinesterases

The results presented show that the use of α-naphthyl esters will not give a picture of all esterases present It may not be valid to state that a tissue is characterised by a high or low content of a specific esterase when activity has been assayed with this type of substrate only.

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1 C L MARKERT AND R L HUNTER, *J Histochem Cytochem*, 7 (1967) 42
2 J PAUL AND P FOTRELL, *Biochem J*, 78 (1961) 418.
3 D J ECOBICHON AND W KALOW, *Can J Biochem, Physiol*, 43 (1965) 73
4 R S HOLMES AND C J MASTERS, *Biochim Biophys Acta*, 132 (1967) 379
5 R S HOLMES AND C J MASTERS, *Biochim Biophys Acta*, 146 (1967) 138
6 K-B AUGUSTINSSON AND G EKEDAHL, *Acta Chem Scand*, 16 (1962) 240
7 W PILZ, *Z Klin. Chem Klin Biochem*, 5 (1967) 1
8 K-B AUGUSTINSSON AND B HENRICSON, *Biochim Biophys Acta*, 124 (1966) 323
9 K-B AUGUSTINSSON, *Ann N Y Acad Sci*, 94 (1961) 844

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Chymotrypsin esterase activity in the presence of oleic and taurocholic acid

In a recent study we observed that sodium oleate inhibited the esterase activity of chymotrypsin (EC 3 4 4 5) on a specific substrate, *N*-acetyl-L-tyrosine ethyl ester (ATEE)[1]. MOSOLOV[2] had noted previously a similar effect on trypsin by the salts of capric, palmitic and stearic acids Differential spectra of the enzyme in 0 045 M caprate and 8 M urea were almost identical and there was an increase in levorotation of the enzyme in the presence of lipid These observations led MOSOLOV[2] to speculate that lipids altered the tertiary structure of trypsin by interference with normal hydrophobic bonding

The assay of fecal chymotrypsin activity is of practical value in diagnosing pancreatic malfunction[3]. The presence of steatorrhea, however, reduces the accuracy of the method[1,4]. The present study was undertaken to investigate further the nature of the enzyme–lipid interaction and the effect of conjugated bile salts on this process

50 μg/ml chymotrypsin (Worthington Biochemicals) was incubated for 24 h at 25° in aqueous systems containing 0 45 mM Ca^{2+} with varying proportions of sodium oleate and sodium taurocholate (Nutritional Biochemicals) The composition of the sodium taurocholate was 73.6% taurocholic acid and 26.4% other conjugated bile acids Similar systems were also run without Ca^{2+} 1 0 ml of this incubation system was diluted with 3 0 ml of 0 5 mM Tris buffer containing 5 mM Ca^{2+} and 0 5 M NaCl immediately preceding assay. The reaction was initiated with 1.0 ml 0.125 M ATEE in 50% methanol and activity assessed at 25° using a Metrohm Combititrator and

Abbreviation ATEE, *N*-acetyl-L-tyrosine ethyl ester