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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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

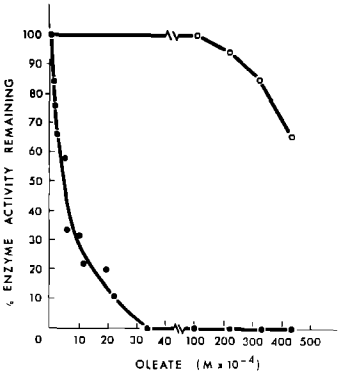

Fig 1 The effect of oleate on the esterase activity of chymotrypsin with and without added bile salt. All systems incubated for 24 h Sodium taurocholate concn was 500 10^{-4} M ○—○, taurocholate, ●—●, no bile salt All points on graph represent mean values for triplicate runs agreeing within 10%

automatic titration with 0 0223 M NaOH as the titrant. The reaction was allowed to proceed for 2 min at pH 7 8 Activities of test systems were expressed as percent of control systems (devoid of added lipid and bile salt).

Sodium oleate caused a marked reduction in the esterolytic activity of chymotrypsin Ca^{2+} did not influence this effect Inclusion of sodium taurocholate ($500 \cdot 10^{-4}$ M) virtually prevented the inhibitory process from taking place except at very high lipid concentrations (Fig 1). At reduced bile salt and lipid concentrations and a mole ratio of bile salt to lipid of 1.5:1, a marked protective effect against lipid on the esterase activity of chymotrypsin was observed (Table I) The inhibitory process was a slow one, so that at a sodium oleate concentration of $2.6 \cdot 10^{-4}$ M, 64% enzyme activity remained at 16 h and 44% residual activity was apparent after 40 h of incubation. It can be seen from the reciprocal plot illustrated in Fig 2 that the inhibitory effect of sodium oleate (Hormel standard exceeding 99% purity) on the esterase activity of chymotrypsin on ATEE was noncompetitive[5]. Furthermore, the effect of sodium oleate could not be altered by dilution of the incubation system.

When bile salt (final concentration 3 3 10^{-4} M) was added to enzyme systems

TABLE I

THE EFFECT OF INCREASING BILE SALT CONCENTRATION ON ENZYME ACTIVITY IN THE PRESENCE OF $2\,9 \cdot 10^{-4}$ M OLEATE

Sodium taurocholate concn × 10^4 *(M)*	*Percent enzyme activity at 24 h*
22 1	95
13 3	94
4 4	92
2 2	54
0 44	45
0 36	43
0 18	42
0 04	42

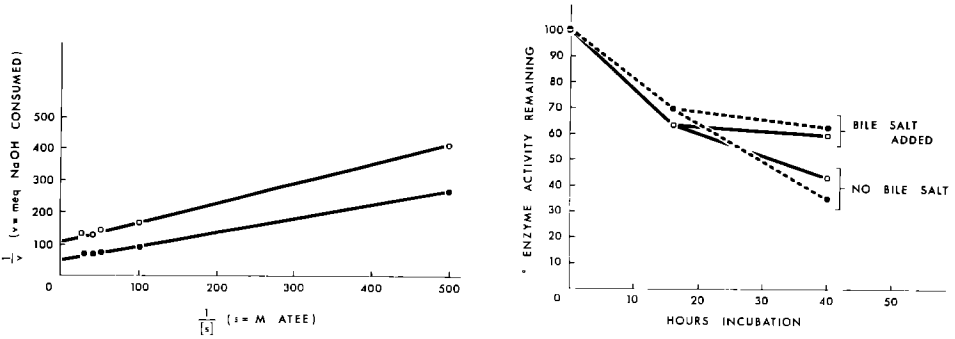

Fig 2 Lineweaver–Burk plot illustrating inhibition kinetics of oleate on chymotrypsin to be noncompetitive in nature Oleic acid concn was 5 10^{-4} M and that of chymotrypsin 2 6 10^{-6} mM (based on an approximated enzyme mol wt of 25 000) ○—○, oleate, ●—●, control All points on graph represent mean values for triplicate runs agreeing within 10%

Fig 3 The role of bile salt in preventing chymotrypsin inactivation by sodium oleate (———) and methyl oleate (— — —) Sodium oleate concn was 2 6 10^{-4} M, methyl oleate concn was 1 10^{-4} M and sodium taurocholate concn was 3 3 10^{-4} M, chymotrypsin was 50 μg/ml Bile salt added after 16 h of incubation

which had been incubated previously for 16 h in the presence of sodium oleate (Hormel standard) or methyl oleate and allowed to incubate for an additional 24 h, further inactivation of enzyme by lipid was prevented Methyl oleate, mole for mole, exerted a greater inhibitory effect on chymotrypsin than sodium oleate (Fig. 3)

While the beneficial effect of bile salt might conceivably be *via* an enhancing effect on the enzyme directly, the work of LIPPEL AND OLSON[6] would mitigate against this These workers failed to attribute any such effect of bile salts on nonlipolytic digestive enzymes The well established detergent properties of bile salts might serve to isolate lipid from enzyme Supporting this is the observation that inclusion of bile salt in incubation systems virtually eliminated the inhibitory effects of oleic acid; furthermore, addition of the bile salt after 16 h of incubation prevented further inhibition from taking place. The behavior of chymotrypsin in the presence of lipid and bile salt suggests that the inhibition of esterase activity effected by lipid is one of hydrophobic interaction between enzyme and lipid The observations utilizing methyl oleate, a nonionic analogue of oleic acid, add support to this interpretation

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