CHAPTER 5

THE PIGEON BREAST MUSCLE LIPASE

The results of the investigations undertaken to elucidate the properties of the pigeon breast muscle lipase are dealt with in this chapter. The purpose of this study was to compare the properties of this enzyme with those of the pancreatic lipase. The properties of the pigeon pancreatic lipase have already been presented(Chapter 4).

Material and Methods

As already mentioned this enzyme was studied in the same manner and under similar conditions as the pigeon pancreatic lipase. An ether defatted dry powder of the pigeon breast muscle prepared in the manner described in chapter 4 was used as the enzyme material. 500 mg. of this powder was extracted in 5 ml. water in a small mortar in cold for 1 hour, centrifuged at about 2500 r.p.m. and the supernatant used as the enzyme solution. 1 ml. of the solution contained 13 - 14 mg. of protein, estimated colorimetrically according to the micro-Kjeldahl method (Hawk et al, 1954). The nitroprusside reaction and the lead-blackening test for sulphur gave negative results with this solution. The presence of large amount of iron in this sol - ution was indicated by the fact that BAL formed an insoluble blue green precipitate, which, when ashed was found to contain iron in large quantities (Ref. chapter 4)

Lipase activity was manometrically determined according to the method described in chapter 4. In studying the effect of various substances the methods employed and the chemicals used were the same as for the pancreatic lipase. The control flask (without any addition) produced on the average 11 - 12 μ moles of CO_2 equivalent to the amount of butyric acid liberated.

Results

Effect of Various Substances

Inorganic salts: (Figs. 1 and 2) $MgCl_2$ at low concentrations slightly activated the enzyme. NaCl, KCl, $CaCl_2$ and NH_4Cl were inhibitory.

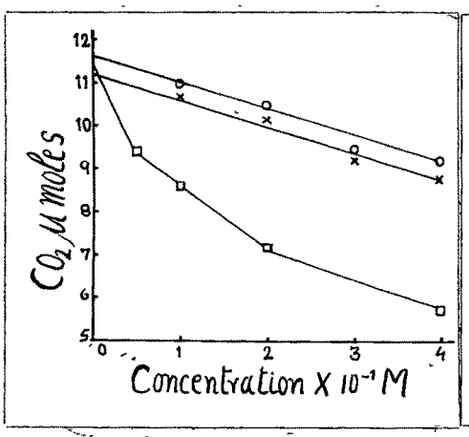


Fig. 1
Effect of inorganic salts on the pigeon breast muscle lipase. x, NaCl; O, KCl; □, NH_4Cl.

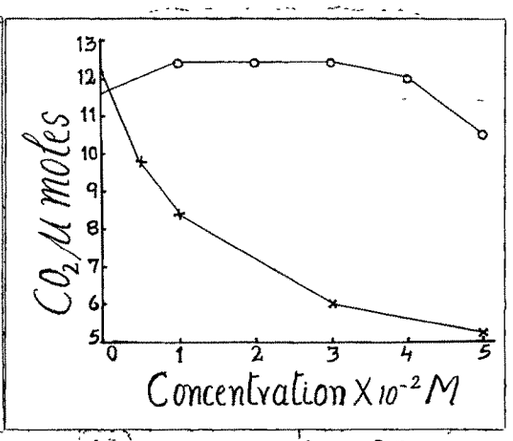


Fig. 2
Effect of inorganic salts on the pigeon breast muscle lipase. x, $CaCl_2$; O, $MgCl_2$.

Intermediate metabolites: (Fig. 3) At a concentration of 0.0025 M, all the substances except citrate activated the enzyme to a limited extent. Highest activation was obtained in the case of α ketoglutarate at a concentration of 0.01 M.

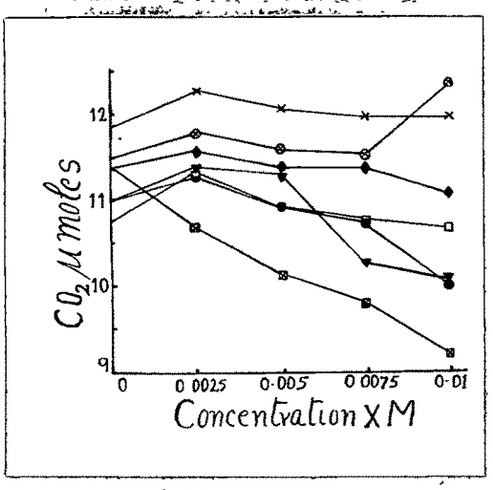


Fig.3.Effect of intermediate metabolites on the pigeon breast muscle lipase.x ,pyruvate; ●,succinate; ▼ ,fumarate;□,malate; ♦,lactate; ⊗ ,∝ketoglutarate; ⊠, citrate.

Amino acids: (Fig.4) Both the amino acids tested, viz. histidine and L-methionine, were inhibitory. At a concentration of 0.01 M the inhibition by methionine was only 4% and by histidine about 25%.

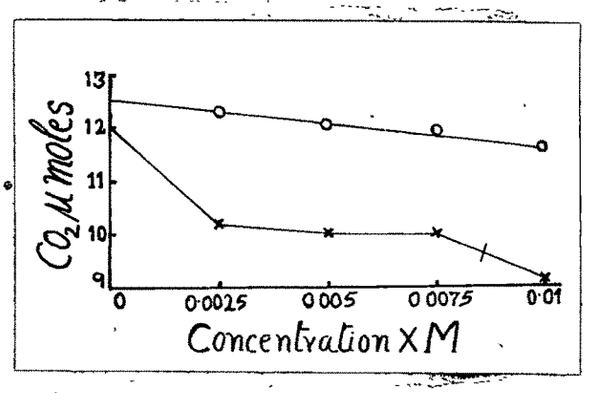


Fig.4.Effect of amino acids on the pigeon breast muscle lipase. x, histidine; 0,L-methionine.

ATP: (Fig.5) Slightly activated the muscle lipase. There was no inhibition upto a concentration of 10^{-3}M.

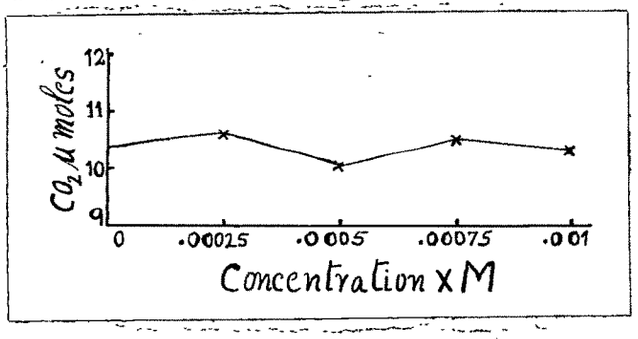


Fig.5. Effect of ATP on the pigeon breast muscle lipase.

Heparin: (Fig.6) Inhibited the enzyme about 12% at a concentration of 40 μg/ml.

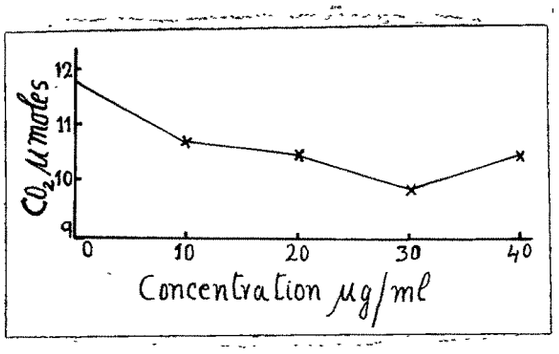


Fig.6.Effect of heparin on the pigeon breast muscle lipase.

Urethane: (Fig.7) Slightly activated the enzyme at a concentration of 0.2%. At higher concentrations the enzyme was inhibited, the inhibition being about 12% at a concentration of 1%.

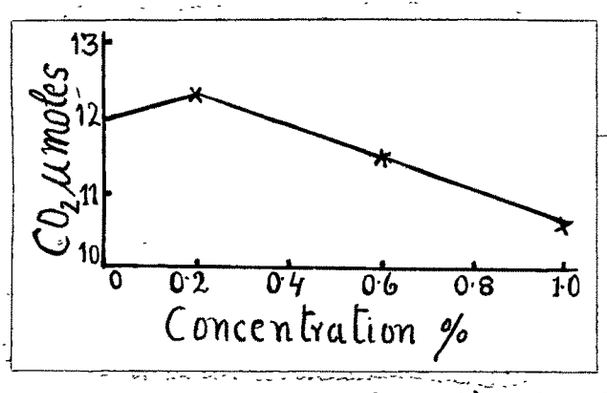


Fig.7.Effect of urethane on the pigeon breast muscle lipase.

Sodium taurocholate: (Fig.8) Was found to inhibit the enzyme to the extent of about 50% at a concentration of 0.01 M

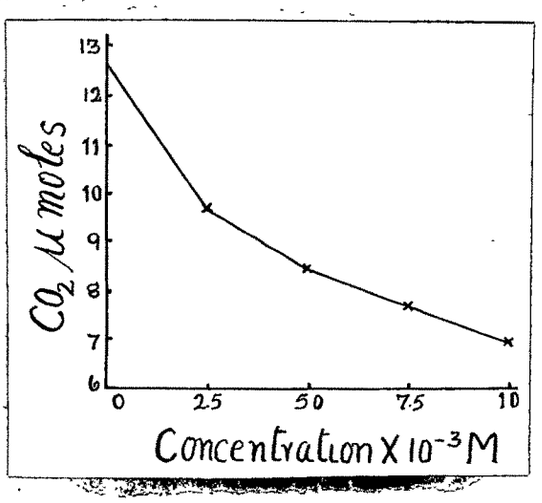


Fig.8.Effect of sodium taurocholate on the pigeon breast muscle lipase.

Metal chelating agent: 8-hydroxyquinoline inhibited the enzyme about 4.5% at the concentration used for the pancreatic lipase.

Thiol reagents: Alkylating agent- Iodoacetate . The enzyme was not affected much by this reagent at concentrations upto 10^{-2}M (Fig.9).

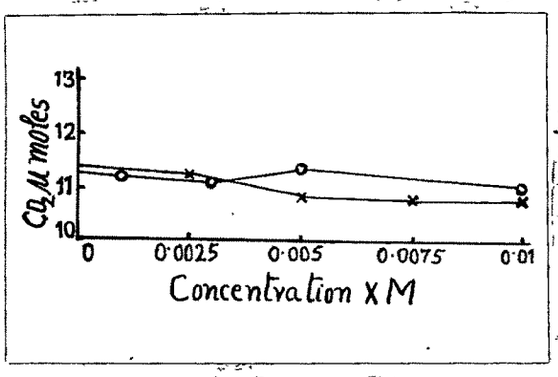
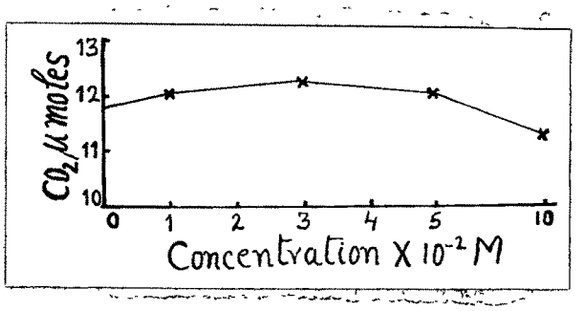


Fig.9.Effect of iodoacetate and pot.ferricyanide on the pigeon breast muscle lipase. x, iodoacetate; O, pot.ferricyanide.

Oxidizing agent: Potassium ferricyanide at concentrations upto 10^{-2}M was without any action on this enzyme (Fig.9).

Reducing agents: Monothiols - Thioglycollate slightly activated the enzyme at concentrations below 0.05 M . At higher concentrations it inhibited the enzyme. Only 4% inhibition was noticed at a concentration of 0.1M (Fig.10).

Fig.10.Effect of thioglycollate on the pigeon breast muscle lipase.



Glutathione and cysteine: Both these substances were inhibitory. Glutathione was more effective as an inhibitor. At a concentration of 0.05 M, the enzyme was inhibited 90% by glutathione and about 5o% by cysteine (Fig.11).

At lower concentrations, viz. 0.001 to 0.003 M, glutathione still inhibited the enzyme, but cysteine activated it slightly at a concentration of 0.003 M (Fig. 12)

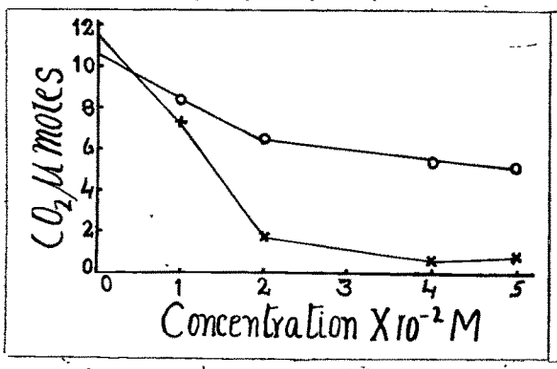


Fig. 11

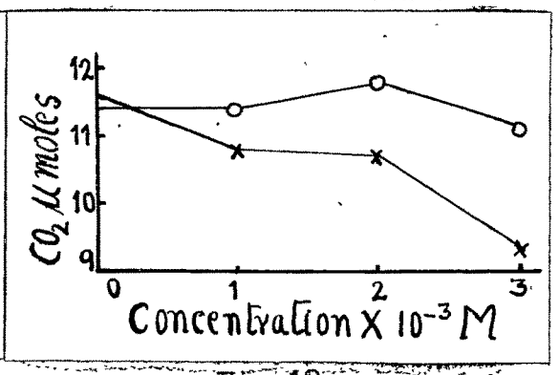


Fig. 12

Figs. 11 and 12. Effect of glutathione and cysteine on the pigeon breast muscle lipase. x, glutathione; O, cysteine.

Dithiol: At all concentrations BAL inhibited the enzyme. The inhibition was directly related to the concentration of the substance added (Fig. 13).

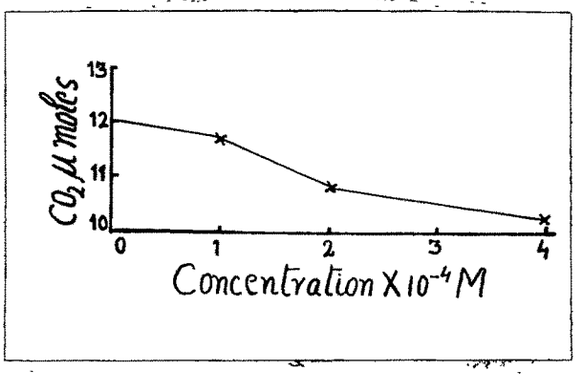


Fig. 13. Effect of BAL on the pigeon breast muscle lipase.

Mercaptide forming substances: PCMB at all concentrations was inhibitory. At a concentration of 0.0025 M, the inhibition was about 75%. (Fig. 14)

Mercuric chloride: At the same concentrations as that of PCMB, $HgCl_2$ inhibited the enzyme more effectively (Fig. 14). Even at very low concentrations of $HgCl_2$, the enzyme was considerably inhibited.

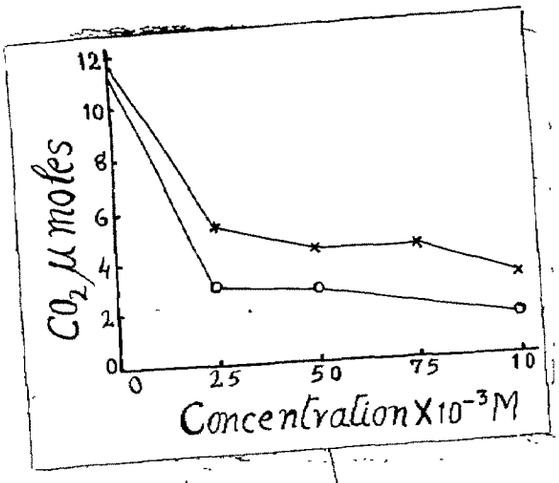


Fig. 14. Effect of PCMB and $HgCl_2$ on the pigeon breast muscle lipase.
x, PCMB; O, $HgCl_2$.

Effect of preincubating the enzyme first with $HgCl_2$ and then with BAL. Conditions of the experiment same as for the pancreas (Chapter 4). The results are given in tables 1 and 2.

Activity, µl CO_2 liberated		
Control, without addition (1)	Enzyme + $HgCl_2$, 10^{-5}M (2)	Enzyme + $HgCl_2$, 10^{-5}M + BAL, 10^{-4}M (3)
285	271	246

Table 1. Effect of preincubating pigeon breast muscle lipase with $HgCl_2$ and BAL

It can be seen that the enzyme is slightly inactivated by $HgCl_2$. The addition of BAL brings about a further inhibition of the enzyme by 9%.

Activity, µl CO_2 liberated		
Control, without addition (1)	Enzyme + $HgCl_2$, 10^{-4}M (2)	Enzyme + $HgCl_2$, 10^{-4}M + BAL, 10^{-3}M (3)
257	200	250

Table 2. Effect of preincubating pigeon breast muscle lipase with $HgCl_2$ and BAL.

At this concentration the muscle lipase was inhibited 22% and the inhibition was almost completely reversed by BAL.

Effect of preincubating the enzyme first with BAL and and then with $HgCl_2$. Conditions of the experiment were the same as for the pancreatic lipase. The results are given in table 3.

Activity, μl CO_2 liberated		
Control, without addition (1)	Enzyme + BAL, 2.5×10^{-4}M (2)	Enzyme + BAL, 2.5×10^{-4}M + $HgCl_2$, 2.4×10^{-4}M (3)
223	175	170

Table 3. Effect of preincubating the pigeon breast muscle lipase with BAL and $HgCl_2$.

22% of the activity of the enzyme was inhibited by BAL. This inhibition was not reversed by $HgCl_2$.

Discussion

The studies on enzymes hydrolyzing fats and esterases have recently been extensively reviewed by Ammon and Jaarma (1950). It is known that lipases from different sources differ from each other with regard to their solubility, specificity and kinetic properties. Even though there is a certain degree of overlapping of substrate specificity between the nonspecific esterases and lipase, it is generally agreed that the enzymes hydrolysing tributyrin is a true lipase (Desnuelle, 1951). From the results

presented in this chapter, it is clear that the muscle enzyme hydrolyses tributyrin rapidly. In a titrimetric system also the enzyme readily hydrolyses tributyrin, but the hydrolysis of olive oil is very slow (Chapter 1) . The rate of hydrolysis of olive oil by the pigeon pancreatic lipase is likewise very slow compared to tributyrin. However, in a manometric system, olive oil is not hydrolysed by any of these enzymes, an observation also made by Martin and his colleagues (Martin and Peers, 1953), using oat lipase. The nonhydrolysis of olive oil in such a system, according to these authors, is due to the predominance of water in the system.

The purpose of the present study was to compare the properties of the muscle enzyme with those of the pancreatic lipase. The studies on the latter enzyme is reported in chapter 4. It was suggested that each of these enzymes is adapted for maximal activity in the physiological environ - ment in which it acts, on the grounds that the pancreatic enzyme is activated by sodium taurocholate and inhibited by intermediate metabolites. The studies on muscle lipase also support this assumption. This enzyme is inhibited by sodium taurocholate and is activated by intermediate metabolites. It should be remembered that the pancreatic lipase acts in an environment rich in bile salts which are promoters of digestion. The muscle lipase on the other hand is intracellular and the metabolic pool constitutes its physiological environment. The exact mode of

activation of lipase by bile salts is not understood. The present study shows that the activation is not due to the emulsification of the substrate by reducing its surface tension , as is commonly believed. The fact that ATP does not inhibit the muscle lipase upto a concentration of 10^{-3}M , further supports my assumptions. It should be noted here that at this same concentration the pancreatic lipase is inhibited about 56% by ATP. Heparin inhibits the muscle enzyme and the pancreatic enzyme. The enzyme does not require any cation for its activity. The possibility that the enzyme requires cations for its activity and the required cations in sufficient amounts is present in the muscle enzyme preparation is not excluded. At any rate added cations have only an inhibitory effect except in the case of Mg, which shows a slight activation at low concentrations.

The negative results obtained with the nitroprusside reaction and the lead-blackening test indicate that the enzyme does not contain either -SH groups or -S-S- groups in its protein. The pancreatic enzyme also gave the same results. In this context it is interesting to note the behaviour of this enzyme in the presence of sulphydryl reagents. Both, potassium ferricyanide and iodoacetate inhibited the pancreatic enzyme. The inhibition by the former is not due to the oxidation of the -SH group and the inhibition by the latter is explained as due to combination with reactive NH_2 groups (Chapter 4). These chemicals, however, do not appreciably inhibit the muscle lipase, at concentr - ations used for the pancreatic lipase. This may be due to

the fact that the protein content of the muscle enzyme preparation is about 20 times higher than that of the pancreatic enzyme preparation and there is competition between the enzyme protein and the non-enzymic protein for the inhibitor. An alternative suggestion is that it may be due to interaction with other substances present in the enzyme solution.

The muscle lipase apparently differs from the pancreatic lipase in that the latter is activated by $HgCl_2$ at very low concentrations , while the former is not. This may not be a real difference, if, the activation by $HgCl_2$ is due to the binding of some inhibitory substance present in the enzyme. PCMB, just like $HgCl_2$, inhibits the muscle enzyme at all concentrations. This inhibition undoubtedly is due to the binding of reactive NH_2 groups of the protein, because there are no -SH groups in the enzyme with which these substances form mercaptides. It is known that the mercaptide forming substances can also combine with the NH_2 groups of proteins (Barron, 1951). It was already pointed out that Little and Caldwell (1943) have shown that freely reactive NH_2 groups are required for the activity of the pancreatic lipase.

It has been shown that the pigeon pancreatic lipase is a metallo-protein or an enzyme requiring metal for its activity and the reactivity of the enzyme with reducing substances such as monothiols and dithiol is due to reaction with the metal part of the enzyme and not with any -SH or -S-S- groups. Thiols combine with metals forming mercaptides of varying degrees of reversibility. The inhibition of metal containing enzymes by BAL is believed to be due to this reaction (see chapter 4).

The muscle enzyme preparation contains large amount of iron. This iron might be mostly from the myoglobin and other soluble substances present in the muscle. However, when the metal was precipitated by BAL , the enzyme was inactivated, showing that some metal is essential for the enzyme activity. Th insoluble blue green precipitate formed with BAL is a BAL - iron compound (Barron et al, 1947; Webb and van Heyningen, 1947). The essential metal may also be one like Mg which can form soluble metal complexes with BAL. The reactivation by BAL, of the enzyme inhibited by $HgCl_2$ may be due to the removal of the inhibitory effect by combining with this metal for which BAL has a greater affinity. However , unlike the pancreatic lipase the inhibition of the enzyme by BAL is not reversed by the addition of $HgCl_2$. The inhibition of the muscle lipase by 8-hydroxyquinoline though to a lesser degree also suggests that it is a metal containing enzyme. The failure of the chemical in inhibiting the enzyme may be explained as due to the extremely low concentration of the chemical used and the very high concentration of metals present in the enzyme solution. Higher concentrations of this reagent could not be tested because of its very low solubility in water. Monothiols like cysteine can also form complexes with metal ions (Warburg and Sakuma, 1923). The inhibition of the muscle lipase, can be therefore explained as due to formation of complexes with metal ions required for its activity. The

slight activation of the enzyme by cysteine and thioglycollate at low concentrations may be due to the binding of inhibitory metal ions present in the enzyme solution. Where as this enzyme is activated by low concentrations of cysteine, the pancreatic lipase is activated by low concentrations of glutathione. The ability of these substances to combine with different metals vary considerably (Barron et al, 1947; Webb and van Heyningen, 1947).

In conclusion it may be said that the muscle lipase in all essential features resembles the pancreatic lipase. Both appear to be metallo-protein or enzyme requiring metal ions and reactive NH_2 groups for activity and are devoid of reactive -SH or -S-S- groups. No requirement of added cations could be demonstrated. Heparin is inhibitory to both the enzymes. The differences in the reactivity of these enzymes in the presence of various substances is due to interference with inhibitory or activating substances present in the crude enzyme preparation.