CHAPTER 6

THE RAT HEART LIPASE

The presence of a lipase in the heart muscle of vertebrates was reported in an earlier chapter. The concentration of lipase in the heart muscle of different vertebrates is related to the basal metabolism of the animal and is supposed to play a role in the energy metabolism of the heart by breaking down fat into fatty acids and glycerol, which in turn are effigiently oxidized by the enzyme system present in this muscle, a role similar to the one played by the skeletal muscle lipase such as the pigeon breast muscle lipase. It was thought therefore that the heart muscle lipase should resemble the skeletal muscle lipase with regard to specificity, kinetic and other properties. A detailed study was undertaken to find out how far this enzyme from these two different sources compare with each other on the one hand and the pancreatic lipase on the other. The rat heart was chosen as a convenient material for this study.

Material and Methods

In the studies on the pigeon pancreatic lipase and the pigeon breast muscle lipase, it was found that an ether defatted dry powder of these tissues was superior to the acetone powder as an enzyme material. Treatment of these tissues with acetone definitely destroys a large amount of lipase. An acetone powder of the breast muscle was found

to be completely inactive. A similar observation was also made by Korn and Quigley (1955) in their studies on the lipolytic activity of the rat heart muscle. Rats were decapitated and the heart removed and placed on filter paper. The hearts were split open and all the blood removed by means of filter paper. They were then cut into small pieces, dried and a powder prepared according to the method mentioned earlier.

200 mg. of this powder was extracted in 5 ml. water in cold for 1 hour, centrifuged at about 3000 r.p.m. for 5 min. and the clear supernatant used as the enzyme solution. 1 ml. of this solution contained 6 -7 mg. protein. The nitroprusside reaction for -SH and the lead-blackening test for sulphur gave negative results with this enzyme solution. An insoluble blue green precipitate was obtained when this solution was incubated with BAL. This precipitate is a BAL - iron compound because it is known that BAL can form such a compound with iron (Chapter 4). The precipitate was ashed and the ash taken up in 3 N HCl was found to contain a large amount of iron.

The method of determination of lipase activity was exactly similar to the one used for the study of the pancreatic and breast muscle lipase of the pigeon.

Results

Effect of Various Substances

Inorganic salts: (Figs. 1 and 2) All the substances tested

were inhibitory.

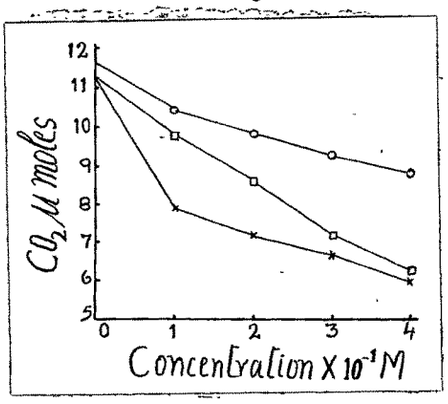


Fig.1. Effect of inorganic salts on the rat heart lipase. x, NaCl; O, KCl; □, NH_4Cl.

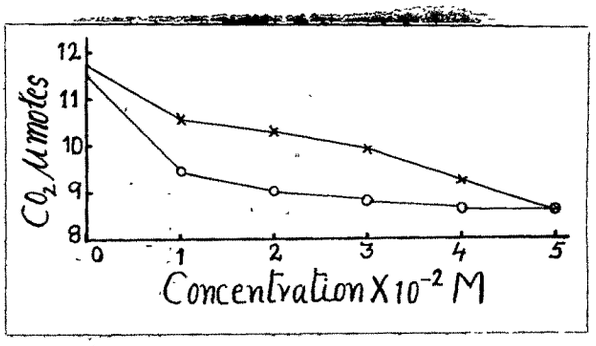


Fig.2. Effect of inorganic salts on the rat heart lipase. x, $CaCl_2$; O, $MgCl_2$.

Intermediate metabolites: (Fig.3) At a concentration of 0.0025 M all the substances activated the enzyme to a limited extent. At higher concentrations the enzyme was further activated by succinate and fumarate, while the others inhibited the enzyme.

Fig.3. Effect of intermediate metabolites on the rat heart lipase. x, pyruvate; □, malate; O, succinate; ▼, fumarate; ⊗, α ketoglutarate; ⊠, citrate; ♦, lactate.

Amino acids: (Fig.4) Both histidine and L-methionine inhibited the enzyme. Inhibition was only slight.

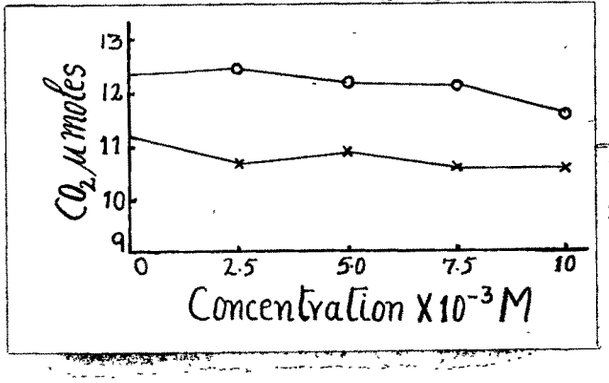


Fig.4. Effect of amino acids on the rat heart lipase x, histidine; O, L-methionine.

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

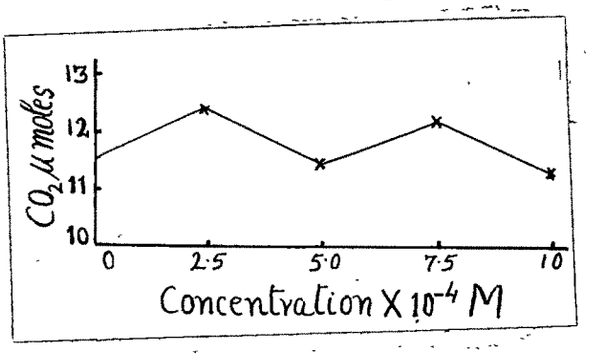
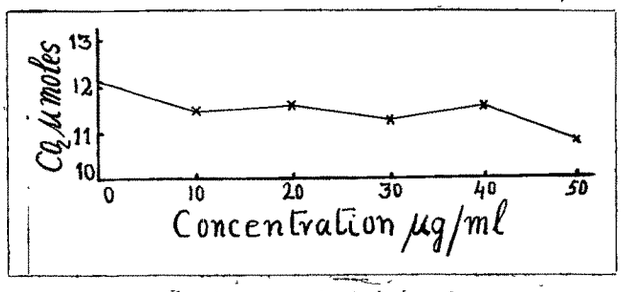


Fig.5.Effect of ATP on rat heart lipase.

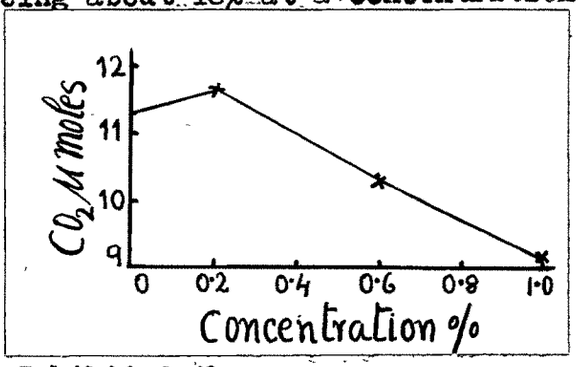
Heparin: (Fig.6) Was inhibitory.

Fig.6.Effect of heparin on the rat heart lipase.



Urethane: (Fig.7) The enzyme was slightly activated in a 0.2% solution . High concentration of the substance was inhibitory, the inhibition being about 18% at a concentration of 1%.

Fig.7.Effect of urethane on the rat heart lipase.



Sodium taurocholate: (Fig.8) Inhibited the enzyme.

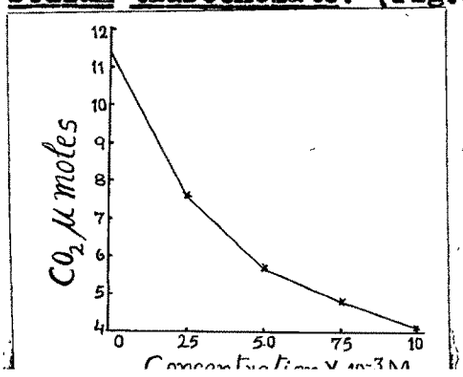


Fig.8.Effect of sodium taurocholate on the rat heart lipase.

Metal chelating agent: 8-hydroxyquinoline. When 1.5 ml. of the enzyme was preincubated at 37°C for 30 min. with 1.5 ml. of a saturated solution of this chemical, the enzyme was inhibited 3.3%.

Thiol reagents: Alkylating agent. Iodoacetate did not affect the enzyme much. But the tendency was to inhibit (Fig.9).

Oxidizing agent: Potassium ferricyanide slightly inhibited this enzyme (Fig.9).

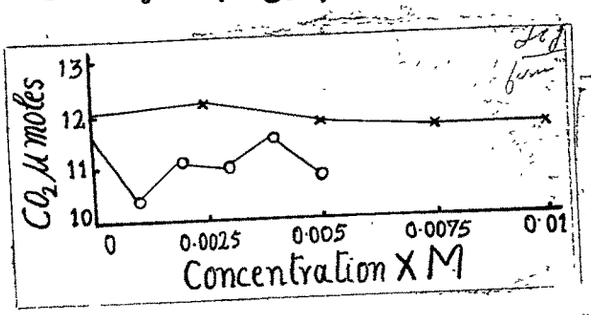
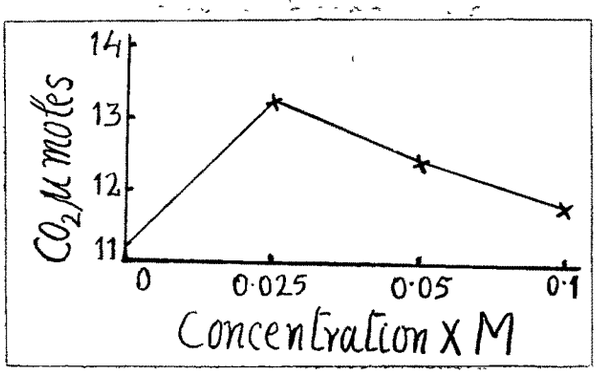


Fig.9.Effect of iodoacetate and pot.ferricyanide on the rat heart lipase. x,iodoacetate; 0,pot. ferricyanide

Reducing agents: Monothiols. Thioglycollate activated the enzyme about 18% at a concentration of 0.025 M. Higher concentrations of the substance were inhibitory (Fig.10).

Fig.10.Effect of thioglyco - llate on the rat heart lipase.



Glutathione and cysteine: Both these substances inhibited the enzyme at high concentrations. Whereas the inhibition by glutathione was almost complete at a concentration of 5×10^{-2}M, cysteine inhibited this enzyme only about 20%

at this concentration. (Fig.11) At lower concentrations viz. 0.001 - 0.005 M, both cysteine and glutathione slightly activated the enzyme (Fig.12).

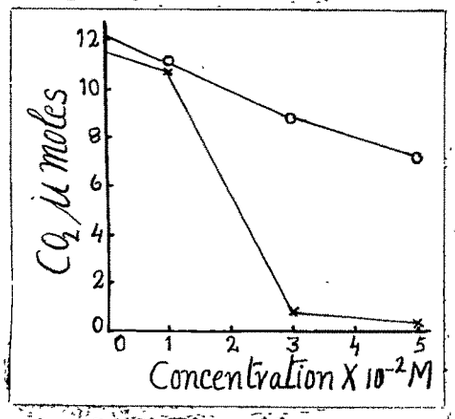


Fig. 11

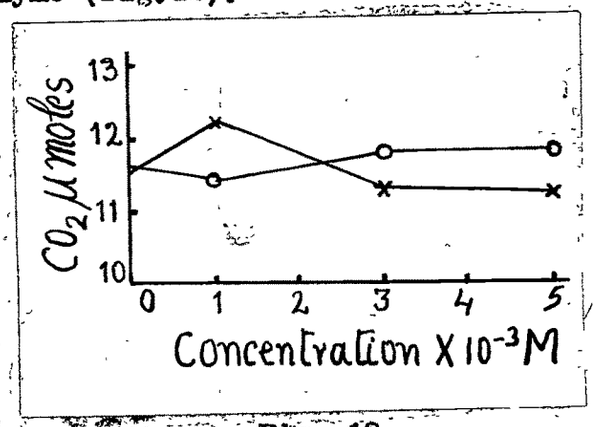


Fig. 12

Figs. 11 and 12. Effect of glutathione and cysteine on the rat heart lipase. x, glutathione; 0, cysteine.

Dithiol: At concentrations between 0.0001 and 0.0004 M, BAL activated the heart lipase. At higher concentrations, however, the effect was inhibitory (Fig.13). It was found that when the enzyme solution was precipitated with BAL, the supernatant after centrifugation showed decreased activity.

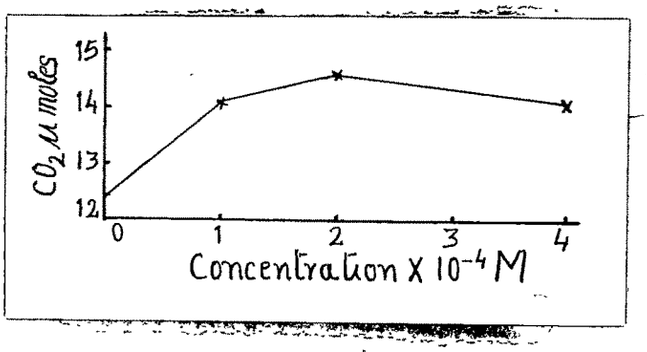


Fig.13.Effect of BAL on the rat heart lipase.

Mercaptide forming substances: PCMB completely inhibited the enzyme at a concentration of 0.0025 M (Fig.14).

Mercuric chloride: Was also highly inhibitory. About 90% inhibition was obtained at a concentration of 10^{-2}M (Fig. 14).

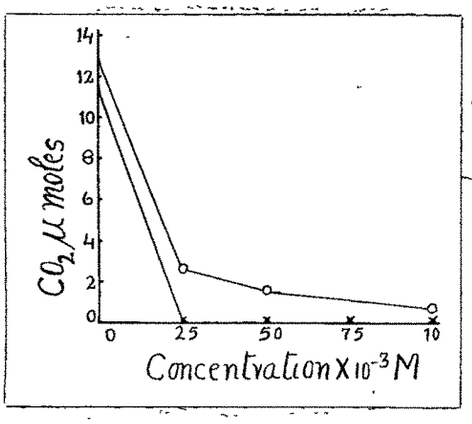


Fig. 14. Effect of PCMB and $HgCl_2$ on the rat heart lipase. x, PCMB; O, $HgCl_2$.

Effect of preincubating the enzyme successively with $HgCl_2$ and BAL. Conditions of the experiment were the same as for pigeon pancreatic and breast muscle lipase. The results are given in tables 1 and 2.

Activity, µl CO_2 evolved		
Control, without addition (1)	Enzyme + $HgCl_2$, 10^{-5}M (2)	Enzyme + $HgCl_2$, 10^{-5} + BAL, 10^{-4}M (3)
225	221	274

Table 1. Effect of preincubating rat heart lipase with $HgCl_2$ and BAL.

The enzyme was not appreciably affected by $HgCl_2$ at this concentration. A 24% activation of the enzyme was brought about by the addition of BAL.

At higher concentrations of the substances, the heart lipase was inhibited about 25% by $HgCl_2$ and the inhibition was completely reversed by the addition of BAL (Table 2).

Activity, μl CO_2 evolved		
Control, without addition (1)	Enzyme + $HgCl_2$, 10^{-4}M (2)	Enzyme + $HgCl_2$, 10^{-4}M + BAL, 10^{-3}M (3)
184	138	190

Table 2. Effect of preincubating rat heart lipase with $HgCl_2$ and BAL.

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

Activity, μl CO_2 evolved		
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)
180	253	179

Table 3. Effect of preincubating rat heart lipase with BAL and $HgCl_2$.

The enzyme was activated about 40% by BAL and the activating effect was completely removed by $HgCl_2$.

Discussion

The purpose of the present study, as already stated, was to find out if the heart enzyme is a true lipase and it could be put under the same class as belong the pancreatic lipase and the pigeon breast muscle lipase.

In the earlier chapter, comparison was drawn between the substrate specificity and other properties of the, pancreatic lipase and the breast muscle lipase of pigeon and concluded that they are essentially similar except for certain minor differences in their behaviour in the presence of certain chemicals, which is largely due to the presence or absence of activating or inhibiting substances in the extract. The influence of bile salts and intermediate metabolites on these enzymes was thought to be on account of what may be called the adaptability of these enzymes to their physiological environment. The pancreatic lipase was activated by sodium taurocholate and inhibited by ATP and intermediate metabolites, while the breast muscle enzyme was inhibited by the bile salt and activated or uninhibited by ATP and intermediate metabolites at low concentrations. The rat heart lipase is also similarly inhibited by sodium taurocholate and activated by intermediate metabolites and unaffected by ATP. In this respect the heart enzyme resembles the pigeon breast muscle lipase.

Heparin inhibits the heart lipase. Inhibition by heparin was also observed in the case of the pigeon pancreatic and breast muscle lipase. Added inorganic salts greatly inhibited the heart enzyme just as they inhibited the pancreatic and breast muscle lipase. It is not concluded therefore that the enzyme does not require the cations for activity. It is possible that the undialysed crude extract used in this study contains cations in sufficient quantities if required for the enzyme activity. However, added cations

are inhibitory and not required for enzyme activity.

This enzyme like the pigeon pancreatic and breast muscle lipase does not contain -SH or -S-S-groups in its protein, because the nitroprusside reaction and the lead - blackening test gave negative results with this enzyme solution. The behaviour of this enzyme in the presence of sulphydryl reagents also is very similar to that of the pigeon breast muscle lipase. Whereas the pigeon pancreatic lipase is inhibited by iodoacetate and pot. ferricyanide, the rat heart lipase like the pigeon breast muscle lipase is not inhibited to any great extent..This may be due to the relatively high concentration of the protein in the enzyme solution. The protein concentration of this enzyme is 10 times that of the pancreatic enzyme solution and one half that of the pigeon breast muscle enzyme solution.

Both PCMB and $HgCl_2$ inhibit the rat heart lipase just as they inhibit the pigeon breast muscle lipase. Inhibition by these substances are probably due to combination with the reactive NH_2 groups which are necessary for its activity, and not due to combination with -SH groups by forming mercaptides, because no such groups are present in the enzyme solution. Little and Caldwell (1943) have shown that reactive NH_2 groups are essential for the activity of pancreatic lipase. The heart lipase apparantly differs from the pancreatic enzyme in that the latter is activated by small quantities of $HgCl_2$ while the former is not. A possible explanation is that the activation of the pancreatic

lipase by $HgCl_2$ may be due to combination with some inhibitory substance present in the crude extract.

It has been suggested that the pancreatic lipase is a metallo-protein or a metal requiring enzyme and the reactivity of reducing substances is due to combination with the metal part of the enzyme and not with any -SH or -S-S- groups. It is also known that 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. (Chapter 4). The heart enzyme preparation contains large quantities of iron. Ions of other metals may also be present in the solution. It was observed that the iron content of the heart enzyme solution is much higher than that of the pigeon breast muscle solution. The inhibition of this enzyme by low concentrations of $HgCl_2$ was only slight. But addition of small amounts of BAL to this enzyme brings about an activation which may be due to the removal of some toxic metallic ions by combining with them. Higher concentrations of $HgCl_2$ is toxic and the reversal of the inhibition by BAL is due to its combination with Hg for which BAL has a greater affinity. The same explanation holds good for the activation of this enzyme by BAL and the removal of the activation by the addition of $HgCl_2$. It was also noted that when the enzyme solution was precipitated by the addition of large quantities of BAL and centrifuged, the lipolytic activity of the supernatant was considerably reduced. It is therefore believed

that the rat heart lipase like the pigeon pancreatic and the breast muscle lipase requires metal for its activity. 8- hydroxyquinoline also inhibits the enzyme to a limited extent. The inability of the chemical to inhibit the enzyme completely is due to the extremely low concentration used and the abundance of metal present in the enzyme which may compete for the inhibitor. Monothiols like cysteine are known to form metal complexes. The inhibition of the heart lipase by cysteine and glutathione may be said to be due to combination with the metal ions required for its activity. The activation of the enzyme by these substances at low concentrations and by thioglycollate can be explained as due to combination with inhibitory metal ions present in the enzyme solution. The variations in the ability of these substances to combine with different metal ions will account for the variations in the behaviour of these enzymes in the presence of these substances.

It should be concluded that the rat heart lipase is identical with the pigeon pancreatic and breast muscle lipase with regard to its specificity and other properties. Like the other lipase this enzyme also appears to be a metallo-proteon or, one which requires metallic ions and reactive NH_2 groups for activity and is without -SH or -S-S- groups in its molecule. Added cations as well as heparin are inhibitory. The difference in the activity of this enzyme in the presence of various substances is due to interference with inhibitory or activating substances present in the

crude undialysed enzyme preparation.

Mention should be made in this connection of the work by Korn and Quigley (1955) on the lipoprotein lipase of rat heart and adipose tissue. These workers observed that serum lipoproteins and not simple triglycerides are the major substrates for tissue lipases. It is doubtful whether this statement is correct. This lipoprotein lipase according to these authors is inactive in the absence of added cations and heparin. They also showed that aqueous extracts of the fresh adipose tissue do not require heparin or cation for its activity. But aqueous extracts of an acetone powder of the same material is shown to be activated by heparin and cations. I have seen that aqueous extracts of the fresh heart muscle as well as the ether defatted powder of the tissue readily hydrolyses simple triglycerides without any added cations or heparin and these substances on the contrary are inhibitory to this enzyme. Fawcett (1951) expressed doubt as to whether the adipose tissue contains a true lipase or an esterase after a histochemical study of the enzyme , using the saturated "Tweens" as substrates. Recently George and Eapen (1957) using "Tween 80" and tributyrin as substrates in histochemical and quantitative methods respectively showed the presence of a high concentration of true lipase in the adipose tissue of the pigeon. It is fairly well established therefore that these tissues contain a true lipase, which in all essential features is identical to the pancreatic lipase. It is also

possible that the"lipoprotein lipase" of Korn and Quigley and the lipase I have been studying are one and the same, because the former can catalyse the hydrolysis of simple triglycerides and chylomicrones. Carlson and Wadstrom(1957) expressed the opinion that the action of the clearing factor on chylomicrones is a simple lipolysis. The chemical changes occurring during the clearing of chylomicrones in vitro can then be described as a simple hydrolysis of triglycerides to partial glycerides, fatty acids and glycerol, quite similar to the action of pancreatic lipase upon fat emulsion. It is also known that fat containing long chain fatty acids are not effectively hydrolysed even by pancreatic lipase in a medium predominated by water. The aqueous extract of the "lipoprotein lipase" of the above authors also hydrolyses coconut oil to a certain extent. The inability, of the aqueous extract of the acetone powder to catalyse the reactions may be due to the denaturing of the enzyme by acetone. This inactivation of the enzyme may be reversed by other proteins in the presence of heparin and cations. This is indicated by the fact that in the case of the aqueous extract of the rat adipose tissue, heparin and cation requirement could not be shown and the requirement of these substances was easily demonstrable in the case of the aqueous extract of an acetone powder of the tissue(Korn and Quigley,1955). From the data presented from my experiments it is clear that aqueous extracts of the ether defatted powder is active by itself and is not

dependent on any added substance.

Iselin and Schuler (1957) also reported that heparin has no effect when added to clear extracts from fresh or lyophilized hearts or to soluble enzyme preparations partially purified by fractionation with ammonium sulphate and they suggested that heparin acts as an agent promoting the liberation of the enzyme from tissue cells. This suggestion may not be correct, for, Korn and Quigley could demonstrate activation of the enzyme, by heparin , in aqueous extracts of the acetone powder of the rat adipose tissue. Overbeek (1957) reviewing the studies on the fat splitting enzymes in blood remarked that lipoproteins are attacked by ordinary lipases, which makes it hard to prove that certain preparations actually contain lipoprotein lipase and not just lipase.

Very recently Nachlas and Blackburn (1958) studied the activity of urinary lipase and compared it with pancreatic lipase using chromogenic fatty acid esters as substrates, in a colorimetric system. They concluded that the two enzymes are similar and that the urine contains an anti lipase which prevents the detection of the enzyme by ordinary methods and that the anti lipase is a dialysable substance and suggested that similar antilipases may be present in the serum also, which masks the action of the serum lipase. This is in agreement with my suggestion that the muscle lipase and the pancreatic lipase are essentially the same and the differences in the behaviour of the enzymes

in the presence of added substances may be due to the presence or absence of inhibitory or activating substances in the undialysed crude enzyme preparations used. It is also interesting to note from their data that the efficiency of the pancreatic and urinary lipases decreases as the length of the carbon chain of the fatty acid in the ester used as substrate increases, an observation I also have made in the case of the muscle and the pancreatic lipases.

It may be concluded that the rat heart and the adipose tissue contain a true lipase which can hydrolyse triglycerides and the apparent differences exhibited by these enzymes may not be real and may be due to the differences in the method of assay and also in the method of preparing the enzyme solutions.