THE RAT HEART LIPASE*

by

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ABSTRACT

The properties of rat heart lipase are compared with those of the pigeon pancreatic and breast muscle lipase. In almost all respects this enzyme very much resembles the pigeon breast muscle lipase. The activity of this enzyme is not dependent on any added cations or heparin. It is claimed that this enzyme and the "lipoprotein lipase" of literature are one and the same and is identical to the pigeon pancreatic and breast muscle lipase.

Introduction

The presence of a lipase in the heart muscle of vertebrates was reported in an earlier paper[1]. 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 efficiently 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 these enzymes 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[2] and the pigeon breast muscle lipase[3], 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[4] 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 described earlier[2,3].

200 mg. of this powder was extracted in 5 ml. distilled water in cold for 1 hour, centrifuged at about 3000 r.p.m. for 5 min. and the clear supernatant

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used as the enzyme solution. 1 ml. of this solution contained 6-7 mg. protein. Protein was estimated according to the micro-Kjeldahl method[5]. 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 (2 : 3 : dimercaptopropanol). This precipitate is a BAL-iron compound, because it is known that BAL can form such a compound with iron[6]. The precipitate was ashed and the ash taken up in 3 N HCl was found to contain a large amount of iron.

Lipolytic activity of the extract was determined manometrically in a bicarbonate-CO_2 buffer system of *pH* 7·4 at 37°C[7]. An emulsion of tributyrin (4% v/v) in 0·0148 *M* $NaHCO_3$ prepared by shaking in a conical flask with 1 drop of "Tween 80" (Atlas powder Co. U.S.A.) was used as the substrate. Each reaction flask contained 1·5 ml. 0·025 *M* $NaHCO_3$, 0·5 ml. of the substance under test (the various chemicals used) in concentrations to give the final concentration as noted below, and 0·5 ml. enzyme in the main chamber and 0·5 ml. substrate in the side arm in a total volume of 3 ml. This gives a final concentration of 0·0148 *M* $NaHCO_3$ with a *pH* of 7·4 in the flask[8]. The test solutions were introduced into the reaction flask before the addition of the enzyme, except in cases where it is otherwise stated. The flasks and manometers were gassed for 3 min. with a mixture of 95% N_2 and 5% CO_2. After equilibration for 10 min. in the constant temperature water bath, the substrate was tipped in and again allowed to equilibrate for another 3 min. This period is sufficient to ensure complete mixing of the contents of the flask. The readings were taken at regular intervals for 1 hour.

For each experiment a control was run in which 0·5 ml. distilled water was added in place of the solutions under test. Auto-hydrolysis was found to be nil. The readings given are after correction for the thermo-barometer. The controls produced on the average 11-13 μ moles of CO_2 equivalent to the amount of butyric acid liberated.

Results

Effect of various substances

Inorganic salts (Figs. 1 and 2)—All the substances tested were inhibitory.

Metabolic intermediates (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 fumerate, while the others inhibited the enzyme.

Amino acids (Fig. 4)—Both histidine and methionine inhibited the enzyme. The inhibition was only slight.

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

Heparin (Fig. 6) was inhibitory.

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%.

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

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 aqueous 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 the enzyme (Fig. 9).

Reducing agents—Monothiols. Thioglycollate activated the enzyme about 18% at a concentration of 0·025 M. Higher concentrations of the enzyme were inhibitory (Fig, 10).

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—0·005M, both cysteine and glutathione slightly activated the enzyme (Fig. 12).

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.

Mercaptide forming substances—PCMB (p-chloromercuribenzoate) completely inhibited the enzyme at a concentration of 0·0025M (Fig. 14).

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

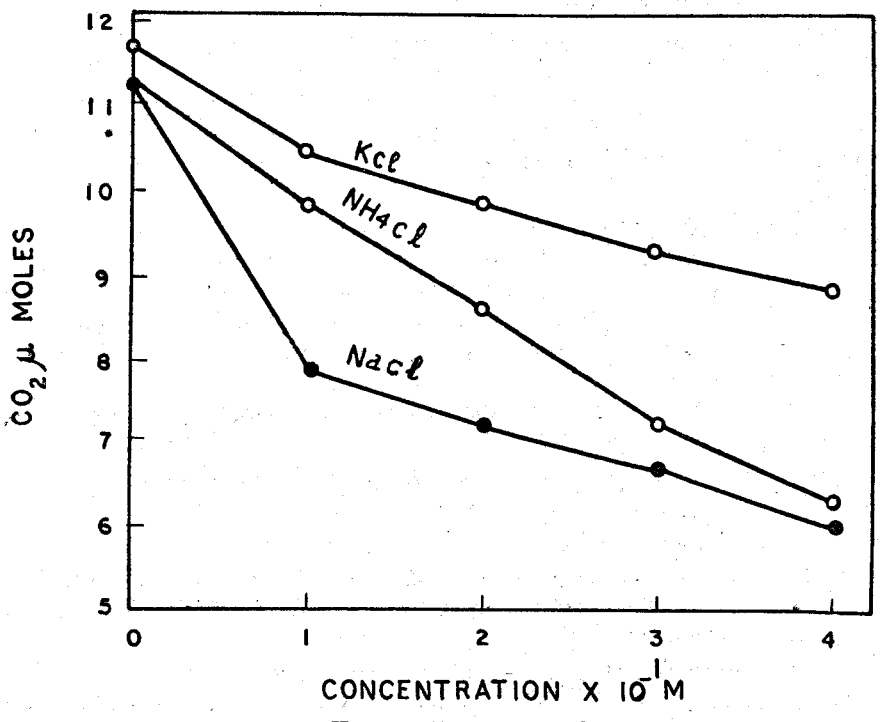

FIG. 1—Inorganic salts.

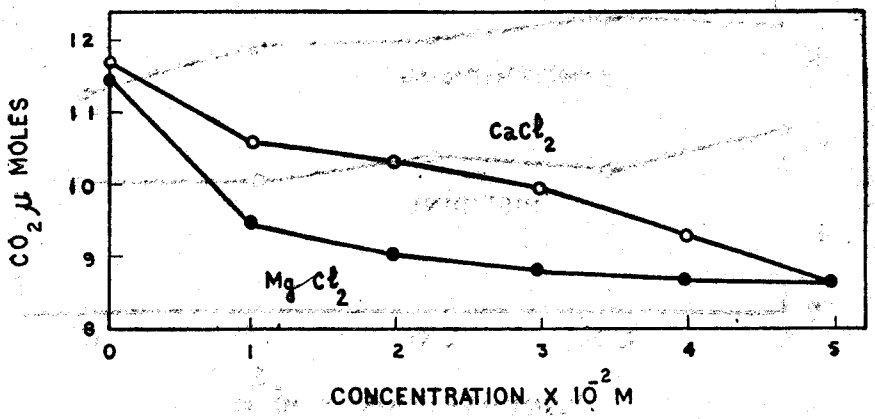

FIG 2—Inorganic salts.

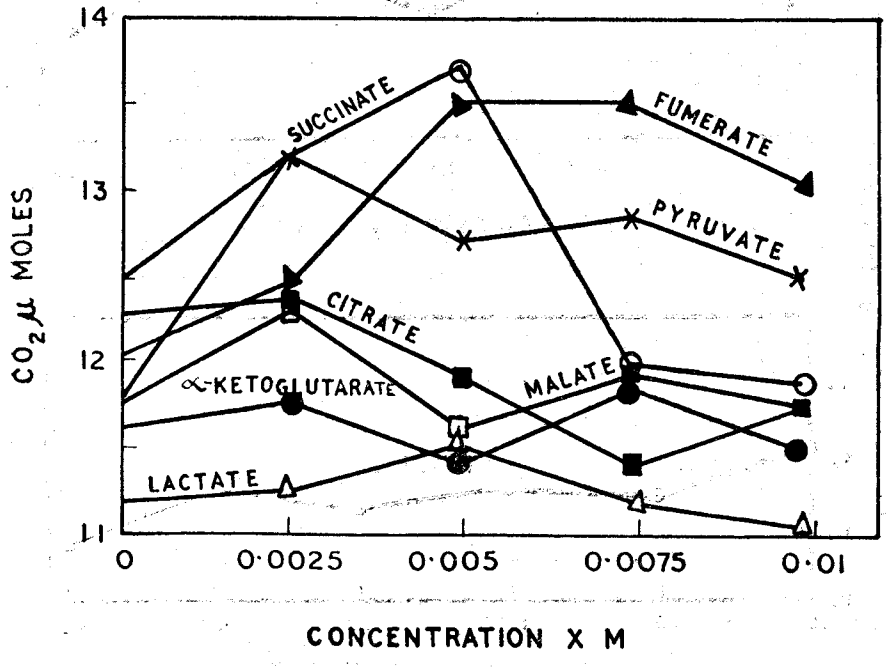

FIG 3—Metabolic intermediates.

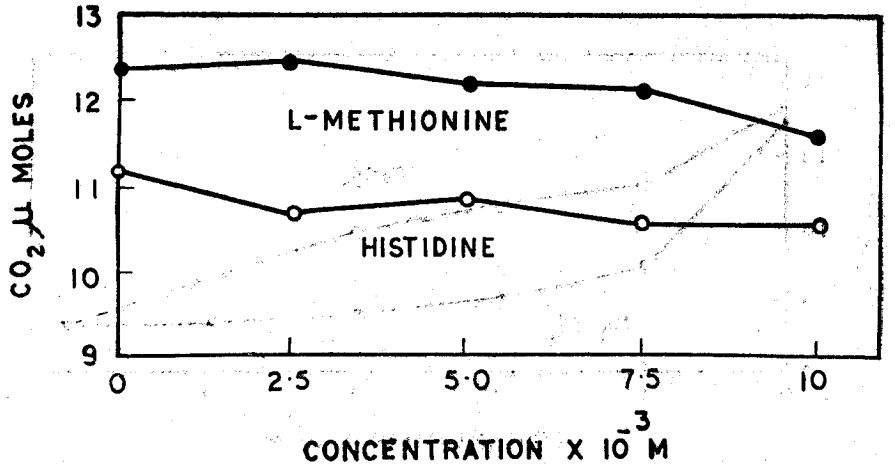

FIG 4—Amino acids.

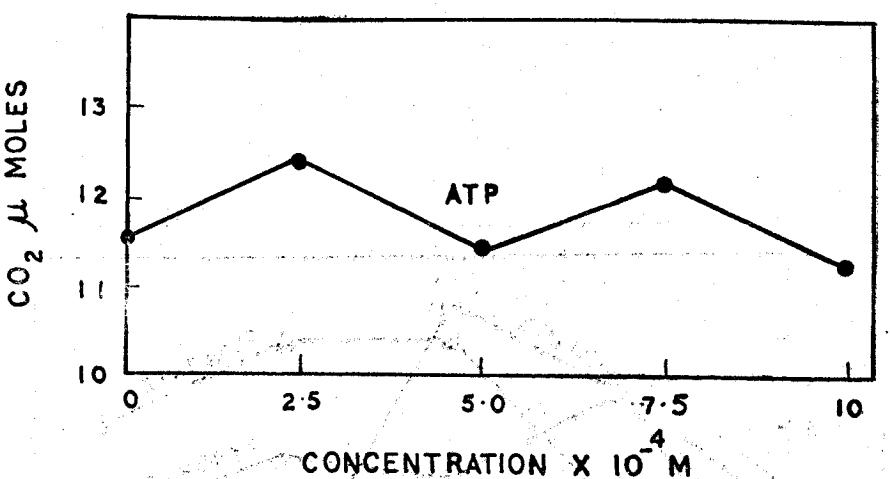

FIG 5—ATP.

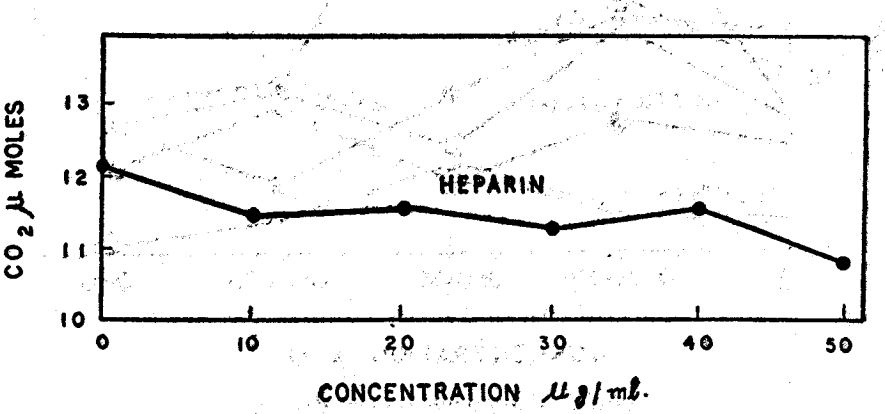

FIG 6—Heparin.

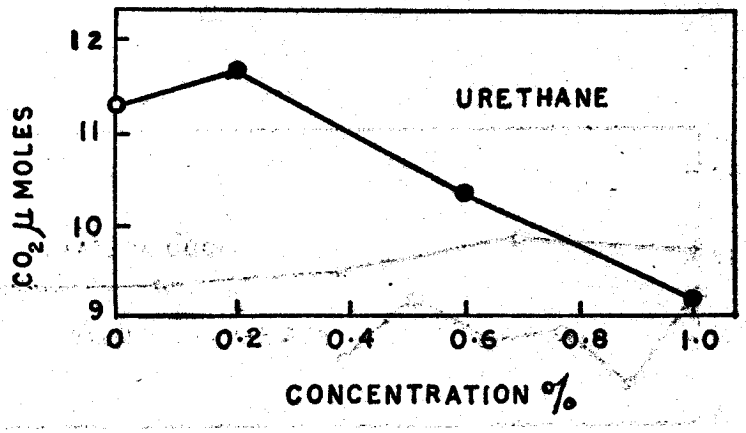

FIG 7—Ure-thane.

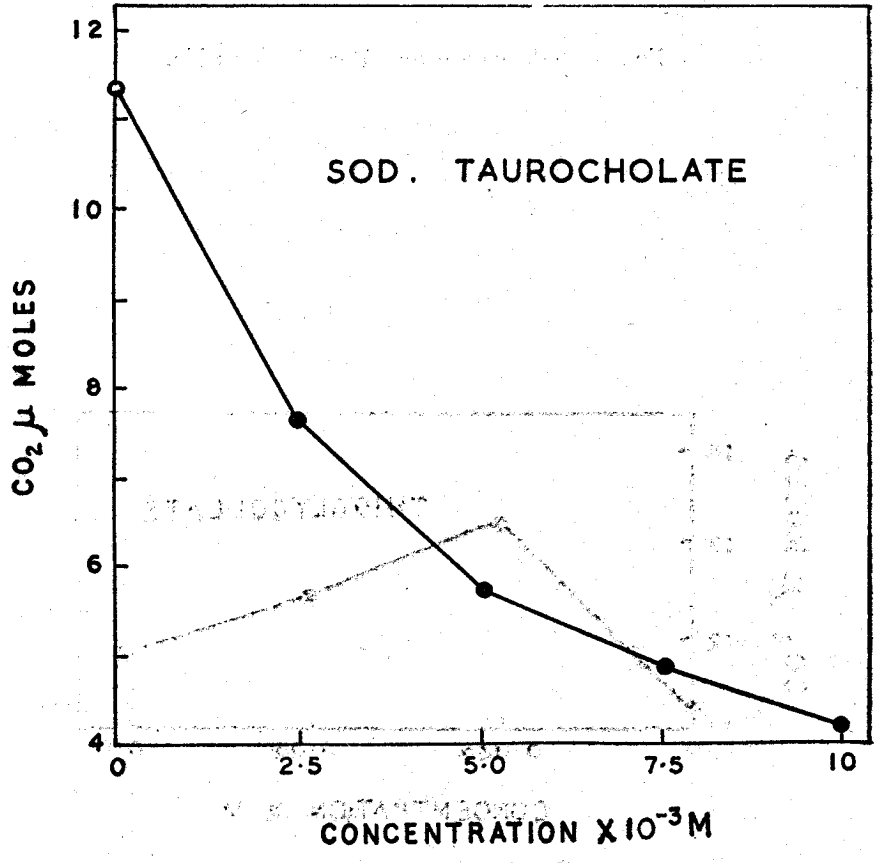

FIG 8—Sodium taurocholate.

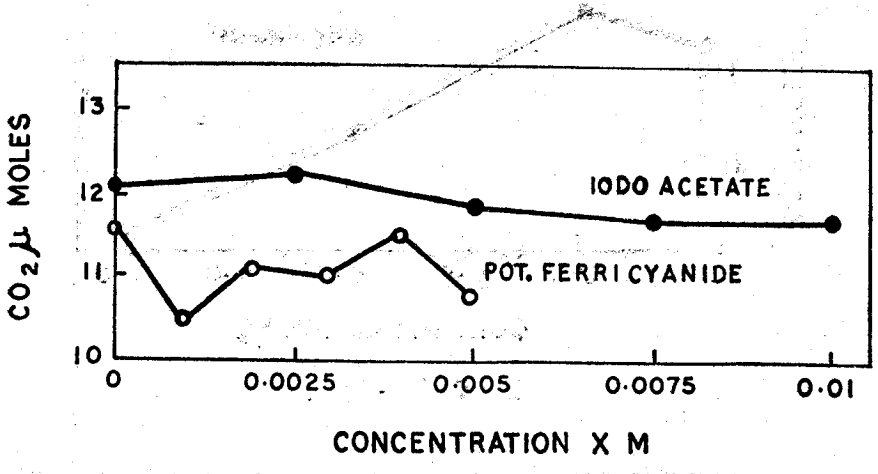

FIG 9—Iodoacetate and Pot. ferricyanide.

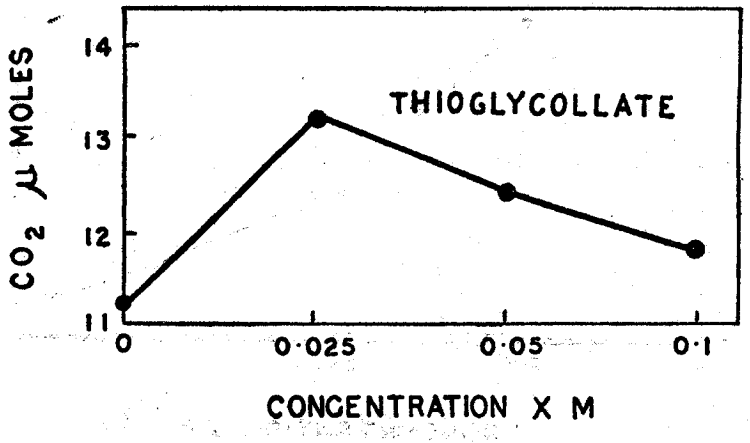

FIG 10—Thioglycollate.

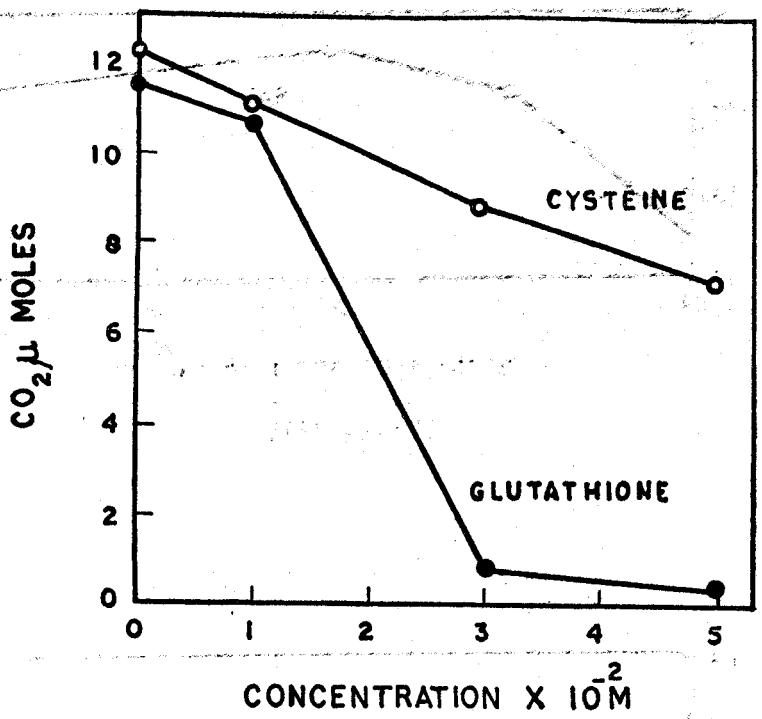

FIG 11—Glutathione and Cysteine.

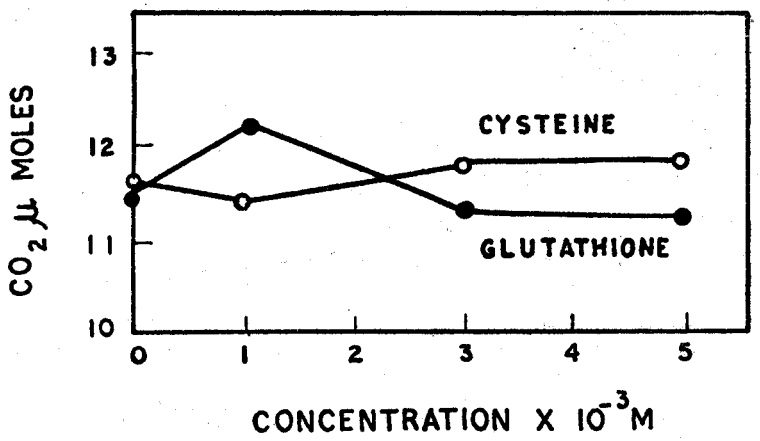

FIG 12—Glutathione and Cysteine.

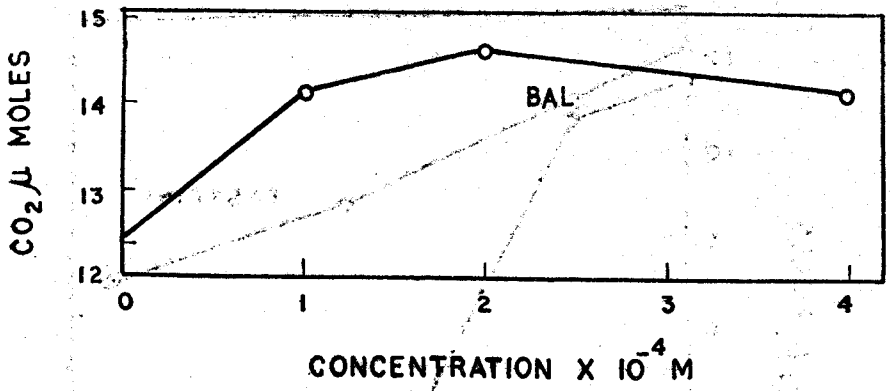

FIG 13—BAL.

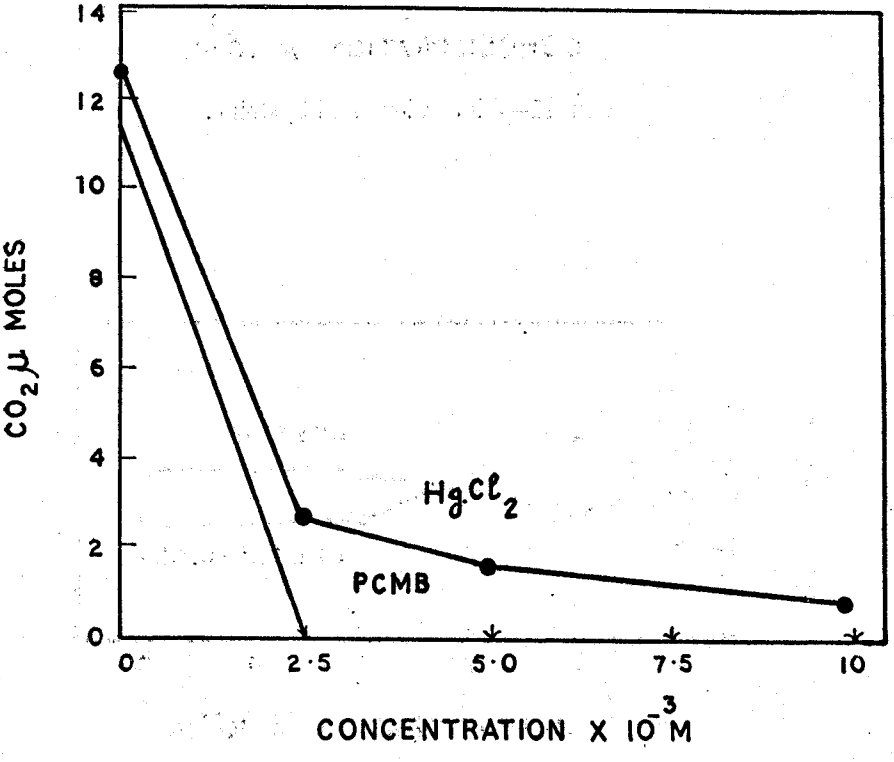

FIG 14—PCMB and $HgCl_2$

Effect of preincubating the enzyme successively with $HgCl_2$ and BAL. To 1·5 ml. of the enzyme solution was added 0·5 ml. $2·4\times10^{-4}$M $HgCl_2$ and incubated for 15 min. at 37°C. A control was also similarly incubated side by side with 0·5 ml. H_2O added to 1·5 ml. of the enzyme (tube 1). To 1 ml. of the former was added 1 ml. H_2O (tube 2) and to the remaining 1 ml. 6×10^{-4}M BAL (tube 3). To tube 1 was added 2 ml. H_2O so as to make the concentration of the enzyme the same in all the three tubes. All the tubes were again incubated for 15 min. and 1 ml. each was added to the Warburg flasks in duplicate and the activity of the enzyme determined as usual. The results are given in table 1. Flask 2 had a final concentration of 10^{-5}M $HgCl_2$ and flask 3 contained 10^{-5}M $HgCl_2$ and 10^{-4}M BAL.

TABLE 1

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

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

The enzyme was not appreciably affected by $HgCl_2$ at this concentration. A 24% activation 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).

TABLE 2

Effect of preincubating rat heart lipase with $HgCl_2$ and BAL

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

Effect of preincubating rat heart lipase first with BAL and then with $HgCl_2$— 1·5 ml. of the extract was incubated for 15 min. with 0·5 ml. 6×10^{-3}M BAL at 37°C. A similarly incubated enzyme to which 0·5 ml. H_2O was added was used as control (tube 1). To one ml. of the former was added 1 ml. H_2O (tube 2) and to the remaining 0·6 ml. $2·4\times10^{-4}$M Hg Cl_2 and 0·4 ml. H_2O (tube 3). 2 ml. H_2O was added to tube 1 and the tubes incubated for a further period of 15 min. 1 ml. each of these preparations was added to the flasks and the activity noted.

The final concentration of BAL and $HgCl_2$ in the flasks was $2 \cdot 5 \times 10^{-4}$ M and $2 \cdot 4 \times 10^{-4}$M respectively. (table 3).

TABLE 3

Effect of pre-incubating rat heart lipase with BAL and $HgCl_2$

Activity, μl CO_2 evolved		
Control, without addition	Enzyme + BAL, $2 \cdot 5 \times 10^{-4}$M	Enzyme+BAL, $2 \cdot 5 \times 10^{-4}$M $+HgCl_2$, $2 \cdot 4 \times 10^{-4}$ M
(1)	(2)	(3)
180	253	179

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 belongs the pigeon pancreatic lipase and the pigeon breast muscle lipase. In two earlier papers[2,3] comparison was drawn between the substrate specificity and other properties of the pancreatic lipase and 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 and inhibiting substances in the extract. The influence of bile salt and metabolic intermediates 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 metabolic intermediates, while the breast muscle enzyme was inhibited by the bile salt and activated or uninhibited by ATP and metabolic intermediates at low concentrations. The rat heart lipase is also similarly inhibited by sodium taurocholate and activated by metabolic intermediates 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

is also 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 is probably due to combination with the reactive NH_2 groups which are necessary for its activity and cannot be due to combination with —SH groups by forming mercaptides, because no such groups are present in the enzyme solution. Little and Caldwell[9] have shown that reactive NH_2 group are essential for the activity of pancreatic lipase. The heart lipase apparently differs from the pancreatic enzyme in that the later 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 substances 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.[10] The heart enzyme preparation contains large quantitites 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 the 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 $HgCl_2$ are 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 breast muscle lipase requires metal for is activity. 8-hydroxyquinoline also inhibits the enzyme to a limited extent. The inability of this chemical to inhibit the enzyme completely may be due to the low concentration used and the abundance of metal present in the enzyme solution which may compete for the inhibitor. Monothols 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 the 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 metalloprotein 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 [4] 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 tissues do not require heparin or cation for its activity. But aqueous extracts of an acetone powder of the same material are 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 hydrolyse simple triglycerides without any added cations or heparin and these substances on the contrary are inhibitory to this enzyme. Fawcett[11] 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 the substrates. Recently George and Eapen[12] 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 studied are one and the same, because the former can catalyse the hydrolysis of simple triglycerides and chylomicrones. Carlson and Wadstrom[13] 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 also known that fat containing long chain fatty acids are not effectively hydrolysed even by pancreatic lipase in a medium predominated by water[14]. 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.[4] 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[15] also reported that heparin has no effect when added to clear extracts from fresh or lyophilised hearts or to soluble enzyme preparation 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[16] 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[17] 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 anti-lipases may be present in the serum also which mask the action of the serum lipase. This is in conformity with the 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[3]. 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.

It may be concluded that the rat heart contains a true lipase which can hydrolyse triglycerides and the deviations from earlier reports regarding its properties may be due to differences in the method of assay and also in the method of preparing the enzyme solution.

Acknowledgement

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