## Chapter 5

# PROPERTIES OF INSECT FAT BODY LIPASE

## II. SOME BIOCHEMICAL PROPERTIES

The general occurrence of lipase activity in orthopteran fat body has been mentioned in chapter 2. The kinetic properties and substrate specificity of insect fat body lipase have also been discussed (chapter 4). Biochemical properties of lipases obtained from different organs have been studied by various workers (Nachlas and Seligman,1949; Ovarbeck and Van Der Vies, 1955; Korn, 1955 a b; Mattson and Beck, 1956; Hollet and Meng, 1956; George and Scaria, 1959; Wood, 1959; Wills, 1960; George and Eapen, 1960; Lynn and Perryman, 1960; DiNella et al, 1960; Bhakthan, 1961; George and Iype, 1962). It is known from these studies that the biochemical properties of lipases when compared to the properties of the mammalian pancreatic lipase vary in different animals.

Comparatively few studies have been carried out on insect lipases. Studies on lipases of cockroach mid-gut (Schlottke, 1937), of Bombyx larvae (Yamafugi and Yonozawa, 1935), of Gallaria (Mankiewiez, 1949) and house-fly larvae (Baker and Pavetsky, 1958) have been made. Since the insect fat body lipase differed in its kinetic properties and substrate

specificity from most other lipases, a biochemical study of the insect fat body lipase was undertaken.

## MATERIALS AND METHODS

A 2.5 or 5% fat body homogenate prepared as described in the preceeding chapter was used in the enzyme study. Lipase activity was determined manometrically using the conventional Wargburg apparatus, in a bicarbonate-carbondioxide buffer system at pH 7.0. Substrate used was 0.02 M tributyrin in 0.0058 M sodium bicarbonate buffer Each Warburg flask contained 1.5 ml of 0.01 M sodium bicarbonate buffer, 0.5 ml of enzyme solution and 0.5 ml of the test solution in concentrations mentioned in the text, in the main chamber and 0.5 ml of 0.02 M tributyrin in 0.0058 M sodium bicarbonate buffer and emulsified with a drop of tween 80, in the side arm. The enzyme solution was introduced last in the flask and no test material was allowed to remain in contact with the enzyme solution for more than 20 minutes before the commencement of the reaction. The experiment was carried out in a constant temperature water bath at 37°C and the results are expressed as µl $CO_2$ evolved during the initial 30 minutes.

## RESULTS

1. The effect of netallic and other ions on the activity of the fat body lipase.

Chlorides of Ca, Mg, $NH_4$, K, Na, Co, Ba, Cu and Hg were used. Excepting Cu and Hg, the salts were used at concentrations of 0.05, 0.1, 0.2, and 0.3 M. Cu and Hg were used at concentrations of 0.02, 0.002 0.0002 and 0.00002 M. The rsults are shown in Table 1 and 2.

TABLE. 1.

Table showing the effect of metallic and other ions on the activity of the insect fat body lipase.

Results expressed as % of control.

| Substance | Concentration (M) | | | |
|---|---|---|---|---|
| | 0.05 | 0.1 | 0.2 | 0.3 |
| $CaCl_2$ | 110,5 | 103.5 | 99.0 | 97.0 |
| $MgCl_2$ | 105.5 | 103.5 | 93.0 | 81.0 |
| NaCl | 100.5 | 98.0 | 93.0 | 90.0 |
| KCl | 98.0 | 93.0 | 89.5 | 83.0 |
| $BaCl_2$ | 96.5 | 93.0 | 87.5 | 82.0 |
| $NH_4Cl$ | 89.0 | 71.5 | 67.5 | 63.0 |
| $CoCl_2$ | 81.0 | 76.5 | 62.5 | 60.0 |

2. Effect of metal complexing agents on the activity of the fat body lipase.

The effects of Versene regular, oxlate, succinate, aspartate and citrate were studied at various molar concen-

TABLE 2.

Table showing the effect of Cu and Hg on the activity of the fat body lipase.

Results expressed as % of control.

| Substance | Concentration(M) | | | |
|---|---|---|---|---|
| | 0.00002 | 0.0002 | 0.002 | 0.02 |
| $CuCl_2$ | 48.0 | 33.5 | 18.5 | 5.5 |
| $HgCl_2$ | 45.0 | 15.0 | 11.5 | 0.0 |

trations and the results have been shown in figure 1 and 2.

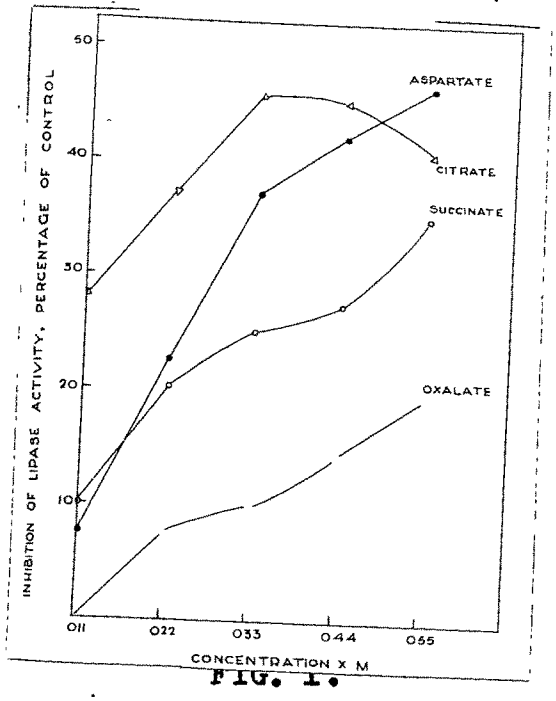


FIG. 1.

Graph showing the effect of oxlate, succinate, aspartate and citrate on the activity of the fat body lipase.

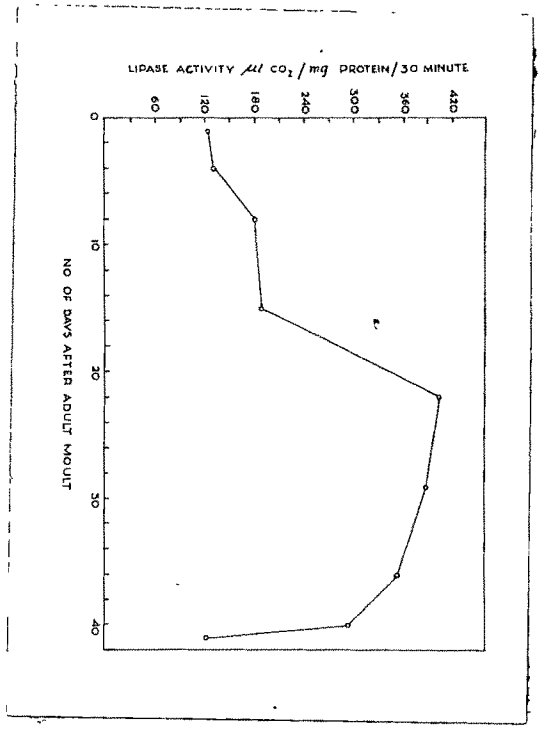


FIG. 2.

Graph showing the effect of Versene regular on the activity of the fat body lipase.

At a concentration of 0.011, M, oxalate did not inhibit the enzyme activity, aspartate and succinate inhibited slightly and citrate inhibited about 30%. Versene regular inhibited the enzyme activity completely between 0.01 and 0.005 M concentration while at 0.001 M concentration the inhibition was very slight.

## Restoration of lipase activity by metal ions inhibited by Versene regular

The reactivation of the enzyme activity inhibited by Versene regular was studied by adding ions of Ca, Ba, Mg and Co. Each reaction flask contained 1.5 ml

of sodium bicarbonate buffer, 0.25 ml of enzyme solution, 0.25 ml of 0.005 M Versene and 0.25 ml of distilled water in the main chamber and 0.5 ml of substrate and 0.25 ml of 0.01 M metal chloride in the side arm. In control experiments metal ion and Versene were omitted. The results are shown in Table 3. Versene was in contact with the enzyme solution for about 20 minutes before the commencement of the experiment.

TABLE 3.

Table showing the effect of metal ions(0.01 M) on the activity of the fat body lipase inactivated by 0.005 M versene.

| Conditions | Lipase activity (%) |
|---|---|
| Control | 100.0 |
| Versene | 0.0 |
| Versene + Ba | 94.0 |
| Versene + Ca | 74.5 |
| Versene + Mg | 67.5 |
| Versene + Co | 64.0 |

It is seen from the table that Barium was the most effective metal in restoring the lipase activity inactivated by Versene. Ca, Mg and Co also reactivated the enzyme activity but to a lesser extent.

### 3. The effect of -SH reagents on the activity of the insect fat body lipase.

A study of the effect of a variety of typical -SH reagents on the activity of the fat body lipase was undertaken The enzyme was found to be inhibited by some members of this groups of -SH enzyme inhibitors but was quite resistent to some others (Table 4; fig. 3). The effect of l-cystein was also tried on the enzyme inhibition by -SH reagents (table 5).

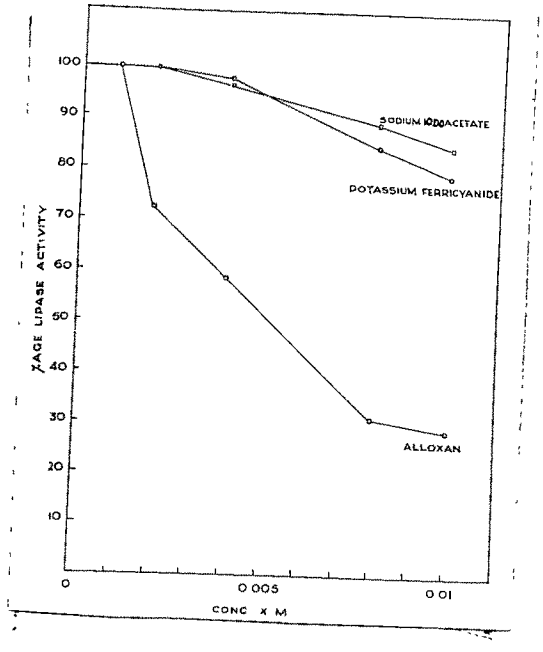


FIG. 3

Graph showing the effect of some sulphydryl reagents on the activity of the insect fat body lipase.

TABLE 4

Table showing the effect of some sulphhydryl reagents on the activity of the insect fat body lipase.

Results expressed as % of control.

| Reagents | Concentration (M) | Inhibition (%) |
|---|---|---|
| Iodine | 0.00001 | 100.0 |
| p-chloromercuribenzoate | 0.003 | 88.0 |
| | 0.03 | 96.0 |
| Alloxan | 0.001 | 0.0 |
| | 0.002 | 27.5 |
| | 0.004 | 40.5 |
| | 0.008 | 69.0 |
| | 0.01 | 71.0 |
| K-Ferricyanide | 0.002 | 0.0 |
| | 0.008 | 15.0 |
| | 0.01 | 20.0 |
| Na-Iodoacetate | 0.002 | 0.0 |
| | 0.008 | 11.0 |
| | 0.01 | 15.0 |
| N-ethylmaleimide | 0.01 | 35.5 |
| | 0.05 | 41.5 |
| | 0.1 | 81.5 |

4. The effect of surface active agent.

Sodium taurocholate at three different molar concentrations was used. It inhibited the enzyme at all the concentrations tried. At a concentration of 0.01 M, 46% inhibition was obtained. At concentrations of 0.005 and 0.0025 M, the activity of the enzyme was 71.5% and 82.5% of the control respectively.

TABLE 5.

Table showing the reversal by l-cystein of inhibition of lipase activity by some -SH inhibitors.

| -SH inhibitor | Concentration (M) | %activity in the presence of inhibitor | %activity in the presence of inhibitor + twice M concentration of l-cystein. |
|---|---|---|---|
| p-Chloromercuri-benzoate | 0.03 | 4.0 | 82.0 |
| Alloxan | 0.01 | 29.0 | 35.0 |
| N-ethylmaleimide | 0.01 | 64.6 | 81.5 |

5. The effects of miscellaneous substances.

The effect of many substances used in the study of lipases were studied on the activity of the insect fat body lipase and has been shown in table 6.

Hydrolysis of vitamin A palmitate by the fat body lipase

The action of the insect fat body lipase on vitamin A palmitate was studied as in the case of tributyrin. An unemulsified solution of vitamin A palmitate at a concentration of 4 mg/ml was used as substrate. The rate of hydrolysis was 8.5 ul $CO_2$/mg protein/ initial 30 minutes.

TABLE 6

Table showing the effect of certain substances on the activity of the insect fat body lipase.

| Substance | Concentration | Inhibition (%) |
|---|---|---|
| Gum arabic | 1.0% | 2.0 |
| | 0.5% | 2.0 |
| | 0.25% | 0.0 |
| Protamine sulphate | 0.02% | 44.5 |
| | 0.01% | 38.0 |
| | 0.005% | 35.0 |
| Heparin | 40 µg/ml | 21.5 |
| | 30 µg/ml | 8.0 |
| | 10 µg/ml | 7.0 |
| Fluoride | 0.05 M | 13.5 |
| | 0.1 M | 22.0 |
| | 0.2 M | 44.0 |
| Quinine hydrochloride | 0.01 M | 89.0 |
| Bovine serum albumin | 0.7% | 4.5 |
| | 0.35% | 4.5 |
| Adrenalin | 80 µg/ml | 91.5 |
| | 20 µg/ml | 16.0 |
| | 0.5 µg/ml | 0.0 |
| Vitamin A palmitate | 4 µg/ml | 45.0 |
| | 2 µg/ml | 27.0 |

# DISCUSSION

The classification of esterases on the basis of biochemical investigations, is still partly a controversial issue but certain well established facts are known by which lipases can be distinguished from nonspecific esterases and the lipoprotein lipase. However, the differences mentioned are always relative rather than absolute.

Of the metal ions studied only $Ca^{++}$ and $Mg^{++}$ slightly activated the lipase activity at concentrations 0.05 and 0.1 M. Activation of lipase by calcium has been observed by many workers (Willstatter and Memmen, 1924; Bamann and Laeverenz, 1934; Desnuelle et al, 1951; DiNella et al, 1960). It was further suggested by these workers that the main function of calcium was to remove as insoluble soaps, the fatty acids formed by hydrolysis of fat. Though this may be true of higher fatty acids, this mechanism cannot account for the activation of the hydrolysis of esters of lower fatty acids. The activation of lipase by calcium is also dependent on other factors like the nature of the emulsion of the substrate, the method of assay and the concentration of the bicarbonate buffer (Wills, 1960). Moreover, in the manometric method, the fatty acids liberated combine with the bicarbonate buffer to liberate $CO_2$. Wills, (1960) proposed two more roles to calcium, a direct activation resulting from concentration at the fat/water

interface and a stabilizing effect on the enzyme which was not observed by earlier workers and which is quite distinct from its activating effect on triglyceride hydrolysis.

The probable role of $Ca^{++}$ as a stabilizing metal of the fat body lipase was tested in a different way from that of Wills (1960). In the study of the stability of the homogenate solution of the fat body, the enzyme activity was measured in the fat body homogenate solution stored at 37°C for a period of 18, 36 and 72 hours duration with added $CaCl_2$ (0.01 M) and without it. It was found that the deterioration of the enzyme activity was not prevented with the added $CaCl_2$ furnishing no evidence as to the stabilizing property of the calcium.

Magnessium is known to activate pigeon heart muscle lipase but not sheep heart muscle lipase which has been related to the higher magnessium content of the pigeon blood (George and Iype, 1962).

$Cu^{++}$ and $Hg^{++}$ inhibited the fat body lipase considerably at all concentrations tried. Complete inhibition of the enzyme activity was obtained with $Hg^{++}$ at 0.02 M concentration. It is known that the heavy metals like Zn, Cu and Hg inhibit enzyme activity by combining with the reactive -SH groups. Lipases from the pigeon

breast muscle (George and Scaria, 1959 b), pigeon adipose tissue (George and Eapen, 1960), pigeon and sheep heart muscle (George and Iype, 1962) and insect flight muscle (Bhakthan, 1961) are all known to be inhibited by heavy metals.

The protein component of a number of enzymes contain -SH groups which are freely reacting or sluggish. It has been previously suggested that lipases possesse -SH groups (Weinstein and Wynne, 1936; Singer and Barron 1945). It has been proposed that they are essential constituents of the active centre (Scott, 1953). But george and Scaria (1959 b) in a study of the pigeon breast muscle lipase, attributed the inhibition of the enzyme activity by p-chloromercuribenzoate as due to the binding of reactive $-NH_2$ groups. Amoung the various -SH reagents tried, iodine, alloxan, p-chloromercuribenzoate and N-ethyl maleimide were highly inhibitory while K-ferrycyanide and Na-iodoacetate did not inhibit the enzyme to any appreciable extent. As the inhibition caused here was due to reagents which react with -SH groups, the effect of l-cysteine was tried on this lipase inhibition. It was noted that the inhibition caused by p-chloromercuribenzoate could be reversed to a great extent but not that caused by alloxan and N-ethylmaleimide. It is known that in general mercaptide forming substances combine reversibly with the sulphhydryl groups (Dixon and Webb, 1958). The lesser sensitivity

to agents such as iodoacetate or ferricyanide is not necessarily an indication of the absence of -SH groups. Barron (1951) points out that one reason for this may be insufficient number of -SH groups in close proximity to form -S-S linkage and another may be the nearby presence of strong electronegative polar groups which could interfere with the oxidation process. Wills (1960) however, suggested that lipase definitely possesses -SH groups but that these groups are situated adjacent to, but not in, the active site. This seems also true for the insect fat body lipase.

The fat body lipase was found to be inhibited to different levels by the metal complexing agents tried. The inhibition by Versene was very strong and this inhibition could be reversed by the addition of $Ba^{++}$ or $Ca^{++}$ to a greater extent than by the addition of $Mg^{++}$ or $Co^{++}$. This may suggest the possible existence of a metal stabilizer in the enzyme protein.

It has been known since long that the addition of bile salts increases the rate of hydrolysis of fat by pancreatic lipase. Bile salts are also surface active agents and therefore, emulsifying agents. There activating effect could simply be due to emulsification of the fatty substrate.

Pigeon pancreatic lipase was actvated by sodium

taurocholate (Scaria, 1958) while pigeon breast muscle lipase (George and Scaria, 1959 b), pigeon adipose tissue lipase (George and Eapen, 1960), pigeon and sheep heart muscle lipases (George and Iype, 1962) and insect flight muscle lipase (Bhakthan, 1961) were all inhibited. Surface active agents are known to cause denaturation of enzyme protein (Cowgill, 1955). These agents in dilut solutions display anomalies in physicochemical properties forming micelles or aggregates arising out of association of molecules of surface active agents and only at these concentrations they exert their profound effect on proteins (McBrain, 1950). Inhibition of the fat body lipase by sodium taurocholate may be due to rearrangement of the enzyme protein or change in the thermodynamic environment of the reaction mixture caused by the micelle structure or both.

Gum arabic had no effect on the insect fat body lipase. George and Iype (1962) obtained activation of pigeon and sheep heart muscle lipases by gum arabic

Protamine sulphate was inhibitory to the insect fat body lipase which inhibited 35% of the activity at 0.005% concentration. At this concentration it had no effect on the pigeon heart muscle lipase but inhibited sheep heart muscle lipase (George and Iype, 1962). Protamine sulphate is a known inhibitor for the lipoprotein lipase (Engelberg, 1956; Hollet and Meng, 1956) though it slightly inhibits pancreatic lipase (Engelberg, 1956 ). However, Rizak (1961) found no action on adipose tissue lipase by protamine sulphate.

Heparin had no effect on pig adipose tissue lipase (Lynn and Perryman, 1960). Heparin which is an inhibitor of lipoprotein lipase (Korn, 1955 a, b), slightly inhibited the insect fat body lipase.

Lipoprotein lipase is not affected by fluoride (Cherkes and Gordon, 1959). Fluoride inhibited the fat body lipase at higher concentrations. Rizak (1961) reported similar results for adipose tissue lipase. Fluoride did not inhibit intestinal or pancreatic lipase activity (DiNella et al, 1960) or pig adipose tissue lipase (Lynn and Perryman, 1960). The descrepancy has been explained by DiNella et al (1960) as due to partial inhibition by fluoride, the stimulatory effect of protein on pancreatic lipase. They observed a 25% inhibition by $2.5 \times 10^{-3}$ M fluoride when the pancreatic lipase was tested in the presence of 1% serum albumin or aqueous crude homogenate.

Bovine serum albumin had negligible effect on the insect fat body lipase.

Quinine has been employed to distinguish lipases which are inhibited by this substance from esterases which are unaffected (Rona and Takata, 1922) though Møyer et al (1955 b), found that it inhibited both lipases and esterases. Quinine was highly inhibitory to insect fat body lipase at a concentration of 0.01M.

The inhibition caused by vitamin A seems to be competitive inhibition because it was found that the enzyme solution hydrolysed vitamin A palmitate to some extent, when used as substrate.

From the study of the properties of lipases from different sources, it is known that these enzymes are adapted for maximal activity in their respective physiological environment and that their specificity would depend to a large measure on their environment. Nevertheless, lipases from various sources show some common properties which make them distinguishable from nonspecific esterases and the lipoprotein lipase. Until recently biochemical information for distinguishing these enzymes, has been inadequate. A great advance was made by Webb (1948) who suggested that the so called organophosphorus compounds could be used to distinguish lipases from esterases. Aldridge (1953 a,b; 1954) , Mounter and Wittakar (1953) and Møyer and his associates (1955 a,b) extended the idea further so that now we can distinguish at least three types of nonspecific esterases as well as a lipase of the pancreatic type.

Lipoprotein lipase is characterised by properties which make its recognition possible. Its pH optimum is 8.5 (Korn and Quingley, 1957). It is inhibited by low levels of protamine sulphate, ethylenediaminetetraacetate, pyrophosphate E-600, diisopropylflurophosphate, synthetic detergents, bile salts, heavy metals and by salts of ionic strength greater than 0.1 M (Korn, 1958; Hollenberg, 1959; cherkes and Gordon, 1959).

In conclusion, therefore,it may be said that the insect fat body lipase is definitely different from lipoprotein lipase since its pH optimum is 7.0 and it is not inhibited by NaCl to any extent nor activated by heparin. Fluoride inhibited the enzyme. The other biochemical properties observed may well relate insect fat body lipase to vertebrate pancreatic lipase.