Introduction

In recent years considerable interest has been shown in the study of the structure and physiology of the adipose tissue and much information on this neglected tissue is now made available. It is now realized that this tissue is not merely for storage of fat but is a highly organized system where fat is synthesized and metabolized. However, most of the studies have been carried out on laboratory mammals with the result that our present knowledge of this tissue cannot be regarded as universal for the adipose tissue in general and is, therefore, highly inadequate. On the other hand, it is possible that a similar tissue in lower animals such as invertebrates would present a simpler structural and metabolic pattern than that of the higher forms. It, therefore, becomes logical to believe that a study of this tissue in the lower forms such as insects would be rewarding in understanding the structure and physiology of this tissue. The present study on the insect fat body was therefore carried out.

In the insect body there is no comparable organ like the liver and the adipose tissue of vertebrates or the hepatopancreas of crustaceans and some other invertebrates. It has therefore, often been suggested, that the fat body of insects may be functionally similar to the liver and adipose tissue of vertebrates. However, this tissue in insects is unique and may be considered to be metabolically very active concerned with synthesis, storage, oxidation and release of fat and other metabolites.

Though insect fat body is known to store protein and carbohydrate, fat forms the chief reserve especially in Orthoptera. Thus the orthopteran fat body is an ideal material for the study of fat metabolism. This tissue in Orthoptera is also a convenient tissue for biochemical as well as histological and histochemical work. It can be readily dissected and obtained free of other tissues (excepting some tracheoles). It can be spread in thin sheets of one or two cell layer thickness on a slide saving the time and labour for the processing and sectioning. Since most of the constituents of the fat body are supposed to be labile on homogenization or sectioning, this method of using whole fat body sheets proved very advantageous.

For a proper understanding and accurate evaluation of the functions of any tissue or organ the intimate study of its structure is a prerequisite. By surveying the commonly occuring Orthoptera, it was concluded that in general there are two types of fat body, white and yellow and some intermediate types in colour shades. A histological and histochemical study was, therefore, undertaken of the yellow fat body of Poicelocera picta and compared with the white type of Periplaneta americana.

The fat body of insects has been shown to contain various metabolite enzymes and the overall operation of the Krebs' cycle in this tissue is known (Clements, 1959). However, the virtual absence of the Krebs' cycle enzymes from the larvae of *Phormia* (McGinnis *et al.*, 1956) and the occurrence of a truncated cycle by the apparent absence of some of the Krebs' cycle enzymes eg. succinic dehydrogenase in *Schistocerca* (Kilby and Neville, 1957) were reported. So an attempt was made to demonstrate succinic dehydrogenase by using a modified histochemical method and whole fat body sheets in the fat body of *Schistocerca gregaria* and some grasshoppers.

Insects show major differences in the amount of lipid they contain (which may vary from 1- 50% of their fresh weight), the degree of unsaturation of the lipids and the content of phospholipids, cholesterol etc. But there has been only limited fundamental biochemical work on lipid metabolism in insects, most studies being done in the field of fat digestion and sterol metabolism. It may be mentioned here that fat metabolism during sustained muscular activity has been a major field of work in our laboratories and it has been shown that fat formed the chief source of energy during flight in birds (George and Jyoti, 1957), bats (George and Jyoti, 1955) and insects (George *et al.*1958). Energy metabolism during rest and activity has been investigated in some insects. Most of the insects studied have been found to use fat (Zebe and

McShan, 1957; Krogh and Weis-Fogh, 1951; Weis-Fogh, 1952; Beall, 1948) while some are shown to transform cabohydrate into fat and use fat for the energy metabolism. The fatty acids in the blood or the flight muscles may prove inadequate at times of intense activity so that we can assign a major role to the fat body in reinforcing the muscles with metabolites.

Recently, some studies on the adipose tissue with special reference to such of the animals like birds which indulge in sustained muscular activity, have been published from our laboratories (Eapen, 1959). Migratory birds are known to accumulate large amounts of fat in their adipose tissue and liver (McGreal and Farner, 1956; Odum and Perkinson, 1951; Odum and Connel, 1956; Naik, 1963) Insects are also known to store fat in their fat body prior to migration (Beall, 1948; Weis-Fogh, 1952). Realizing the importance of fat as fuel for sustained muscular activity a study of an enzyme system responsible for the esterification as well as degradation of fat in the fat body was thought necessary. The lipase activity in the fat body of a few orthopteran insects, which included one migratory form, a few non-migratory forms but which idulge in sustained flight and the rest poor fliers, was therefore studied.

Apart from being a source of energy reserve for sustained flight the fat body of insects also serves as a source of energy during embryogenesis, metamorphosis, gonadal development, hibernation and starvation. The fat reserve and lipase activity of the fat body in two insects were studied, in one with reference to starvation and in the other with reference to ovarian development. Periplaneta americana can withstand prolonged starvation so that the changes in the fat could be followed with considerable accuracy while in Poicelocera picta which is a seasonal insect, the ovarian development could be easily studied.

During ovarian development, protein-yolk deposition is one of the major events and calls upon the insect to release great amount of energy material from its reserve. Considering the central role of ribonucleic acid in protein synthesis, the probable role of the fat body in supplying the protein material to the developing oocyte was investigated by studying histochemically the ribonucleic acid content of the fat body during the period of ovarian development and vitellogenesis.

In recent years many neurosecretory centres in the insect body have been shown to control a variety of metabolic activities. Hormonal control of fat metabolism is known in many insects. Since changes in lipid content lipase activity and ribonucleic acid content were observed during oocyte development, a few observations were made on the median neurosecretory cells of the pars intercerebralis of the brain of Poicelocera picta during ovarian development.

The main interest in the present study has been restricted to fat metabolism in the orthopteran fat body with particular reference to the probable role of the fat synthesizing and degrading enzyme lipase.

The normal functioning of the process of β-oxidation (Nelsen, 1958) and synthesis of lipids (Clement, 1959; Zebe and McShan, 1959; Tietz, 1961) are reported in the insect fat body. A lipase responsible for the degradation of triglycerides to fatty acids and glycerol was reported by George and Eapen (1959 a, b) in the locust fat body. However, in a study of fat transport in locust Tietz (1962) found that glycerides could be released from the fat body into the surrounding medium i.e, haemolymph which was an active process inhibited by fluoride and cyanide. Even if triglyceride release takes place from the fat body as noted by Tietz (1962), the involvement of lipase cannot be ruled out and this has been discussed at length in chapter 6.

Lipases from different sources have been studied by many investigators and from our laboratories too lipases from various animal tissues like pigeon pancreas (George and Scaria, 1959a; Scaria, 1958) pigeon breast muscle (George and Scaria, 1959 b) pigeon and sheep heart muscle (George and Iype, 1962), pigeon adipose tissue (George and Eapen, 1960) and insect flight muscle (Bhaktan,1961), have been carried out. These studies have shown that lipases from different sources differ considerably as to their kinetic as well as biochemical properties and each is best suited to act in its physiological environment. Therefore, a detailed study of the insect fat body lipase regarding its kinetic properties,substrate specificity and certain biochemical properties was undertaken. Insect fat body showed such properties which distinguished it from other lipases as well as from the lipoprotein lipase of mammals.

For the study of lipase the fat body from Periplaneta americana was used. The lipase activity in this tissue was consistent and within a convenient range and hence the choice of this tissue.

The main bulk of the work in this thesis has been concentrated in an attempt to establish the physiological role of lipase in fat metabolism in the fat body of insects under normal conditions as well as under stresses of starvation and reproduction and the dynamic state of the lipids of the insect fat body.

The results obtained in the present investigation have been discussed in the respective chapters. Many of the results obtained may seem inconclusive and the paucity of the available knowledge of insect biochemistry has made them difficult to explain. However, efforts have been made wherever possible to compare the results with those of vertebrate animals.

The following preliminary report has been published in joint authorship with Professor, J.C.George, my guiding teacher.

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