THESIS.

FATTY BODIES OF THE LARVA OF

HELIOPHILA PHRAGMITIDICOLA.—GUEN.

FOR THE DEGREE OF

B. S.

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Fatty Bodies of Larva of Heliophila phragmitidicola, Gnen.

The earliest mention of fatty bodies in the literature consulted by me is by Kirby and Spence 1826 (1#) In their treatise on Entomol-ogy they say "that in the larval state of insects there is a many lobed mass occupying the entire body cavity wrapping and protecting the internal organs. It is accumulated as a store of nutriment for growth and development during the pupa state".

The next mention I have seen was by M.Th.Lacordaire in 1839 (2). He gives the following points in regard to the fatty tissue of insects.

1st. "That they are a peculiar substance whose structure and functions appear to be identical with the fat of vertebrates.

2nd. They appear to surround certain organs for protection, forming a kind of cushion between the neighboring organs, especially around the dorsal vessel and digestive canal.

3rd. It serves as nutrition and plays a part exactly resembling that of the fat of mammals subject to hibernation.

4th. They have an excretory function resembling that of the liver of higher animals".

Practically the same views were presented by Burmeister (3) at about the same time.

These and other writers for the most part discussed the gross

#. Numbers refer to bibliography

anatomy and physiology of these organs, and but little was done in the line of histological examination or the study of their embryological development until comparatively recent times.

A number of authors might be mentioned, as Packard (6) and Comstock (11), who have spoken briefly on the subject; but they have merely quoted the results of the investigations of other men and have not themselves worked out any new facts.

In recent times (1886) Wielowiejski (10) has made some histological studies on Phryganeid larvae with special reference to the blood tissue (Blutgewebe) . Under this term he includes the following structures:- (1) "the blood corpuscles. (2) the fat body proper (3) the pericardial fat-body. (4) the oenocytes, of which he distinguishes three varieties in some insects. He first fully described the oenocytes and named them from their wine yellow color He pointed out that these oenocytes are not infrequently the largest cells in the body except the ova, that they are arranged in metameric clusters in the trachigerous abdominal segments, and that they are more or less intimately associated with the blood and fat body. They sometimes occur in the thoracic region and extend over the sternal region. The seperate cells of the cluster are usually independent of one another but in rare instances fuse in pairs or to form small clusters". He did not maintain a common origin of the various kinds of blood tissue.

Graber, however, (13) in his study of embryology of Stenobothrus, Hydrophilus and other forms, did not hesitate to conclude that the different forms of blood tissue are genetically related. the conclusions at which he arrives are stated very briefly by Wheeler (14) as follows;

1. "The oenocytes are derived from the ectoderm.

2. They are metamorphosed into the fat body.

3. The blood corpuscles arise from the fat body (and also from the oenocytes ?) Ergo the fat body and the blood are ectodermal structures."

Wheeler (12) says "Beside lining the mesenteron, the corpus adiposum, represented during the embryonic life of Doryphora by a number of granular cells which constantly increase in size up to the time of hatching, probably originates from the entoderm. I have observed in several cases that before the two posterior bands of entoderm have reached the middle of the embryo, a number of granular and somewhat larger cells are to be found mingled with the cells of the bands. I conclude that these cells are of entodermic origin because when first seen they are associated with the entoderm cells and resemble them more closely than they resemble the adjacent mesodermic elements. At first small, these fat cells gradually but constantly increase in size, their cytoplasm and nuclei increasing in about the same ratio. They wander about in the body cavity, but finally attach themselves to the ectodermic body walls, especially in the posterior two-thirds of the embryo on each side of the heart. They remain more or less globular or oval, the side in contact with the wall hollowing out a concavity in the cells of the ectoderm. The granulation of the cytoplasm which first distinguishes the fat cells from the true entodermic elements becomes coarser with the increase in volume. In the embryo ready to hatch the adipose cells have acquired gigantic dimensions."

In a later article (14) he discusses the various views presented by different writers and from his own observations on the embryology of Blatta, Xiphidium, Dytisicus and several other species is led to a conclusion directly opposite to that advanced by Graber as to the origin of the fat body. He says; "Graber maintains that the fat body, at least in part, arises from these oenocyte clusters. But a section through a young Blatta embryo shows most conclusively that this is not the case. The oenocytes may be seen still forming a part of the ectoderm from which they have differentiated while the fat body is simply a thickened portion of the inner coelomic wall. The thickening is largely due to an accumul tion of fat vacuoles in the cytoplasm of the mesoderm cells. Were Graber correct in his assumption we ought either to find no adipose tissue in the embryo outside of the eight trachigerous abdominal segments or be able to show that the oenocytes migrate into the head, thorax and terminal abdominal segments and there form the fat body since fat tissue is developed in all these regions of the body. But although some of the oenocytes do later on migrate into the metathorax and perhaps even into the mesothorax they never occur in the head. Moreover, long before any migration takes place, thickenings of the coelomic wall are found giving rise to the fat body in the thorax, gnathitic segments of the abdomen. Furthermore the oenocytes, so far as I have been able to observe, are always perfectly distinct from the fat body, never contain fat-vacuoles, and never divide after they are once differentiated from the ectoderm during embryonic life. It follows then that the fat body is not derived from the oenocytes, that it is not of ectodermal but of mesodermal origin as claimed by the majority of authors".

In his study of the contagious diseases of insects Forbes (8) has found that the fatty bodies are the first parts to be attacked by certain of these diseases and that they are speedily destroyed.

From the foregoing articles we may draw the following summary statement of knowledge and opinion concerning the origin, structure, and uses of the fatty bodies.

The fatty bodies are of mesodermic origin since they are formed from the ceolomic cavity by inlargement of it and the transforming of the cells into fat cells in which are to be found fat vacuoles and are not formed from the oenocytes which are entodermal because if they were it would be necessary to account for their appearance in the head, thorax, and abdominal segments before they migrate at all as fatty tissue is formed early in all regions. And they never have been known to migrate into the head.

In structure they are many lobed organs which completely enclose the other organs, they are composed of various shaped cells of two kinds, the fatty bodies proper and the pericardial fatty bodies. The former seldom if ever have more than one nucleus to each cell and the cytoplasm being more compact. The latter have two or more nuclei and the cytoplasm is less compact.

They are related to the blood tissue in function and hence are classes with them and sometime they are said to have a common origin.

Their functions are as follows;- 1. The function nearly identical with fat of vertebrates. 2. It serves as a store of nutriment for transformations and hibernation. 3. They have an excretory function resembling that of the liver of higher animals. 4. Form a protection for some of the organs, forming a kind of cushion between neighboring organs especially the dorsal and digestive canal.

My observations are based on specimens of _Heliophila phragmitidicola_ collected in the spring of 1892 at Champaign, Ill. Before giving an account of the fatty bodies themselves, I shall make some statements regarding the methods employed in killing and fixing and give a general description of the internal anatomy of the specimens under examination.

Heliophila (Leucania) phragmitiditicola hibernates as a larva and can be found in the spring nearly full grown.

Internal Anatomy.

Upon opening the body of a larva by mediam dorsal incision, the first organs seen are the fatty bodies (Fig.I.C.) completely enveloping the other organs. Holding them in place are the tracheae which commence in the trunks from the stigmata and extend in all directions, dividing and subdividing into minute branches the extremities of which apparently end in the fatty bodies. In the dorsal medial line of the body, completely surrounded by fatty tissue, is the cylindrical heart extending the entire length of the body. The reproductive glands are found in the eighth segment on the dorsal side of the body above the heart. The fatty bodies also completely surround the alimentary canal (Fig.I.A.) which fills nearly the entire body cavity. It is divided in the following divisions;- the mouth cavity, the oesophagus, extending through the first three segments and continually increasing in diameter, the stomach which is a little greater in diameter and extends to the tenth segment, and beyond this the intestine, which is very much smaller than the stomach and which ends with the anus. At the beginning of the intestines the Malpighian vessels (Fig.I.L.) appear as long slender freely floating tubes. The silk glands (Fig.I.S.) are found on the ventral side of the bodyand they extend the entire length of it. The glands commence in front as very fine tubes and continue thus to the fourth segment, where they increase to about three times their original size and continue to the eighth segment. They then return upon themselves and again reach the fourth segment, where they again return upon themselves, becoming smaller and finally reaching the posterior end of the body.

The nerve cord (Fig.I.N.) is situated on the ventral side of the body. It is of a pinkish hue and is composed of eleven ganglia two situated in the head which supply the nerves to the sense organs and eight in the body between the first and eleventh segments. The ganglia are connected by nervous matter.

Killing.

On Jan 28th, Feb 22nd, April 5th some larvae were stupefied by the fumes of cyanide of potassium. Water was put into a test tube filling it about a quarter full, and into this were dropped the larvae, and the water was then boiled. The larvae kept for dissection were left in water, the others transferred to 50% alcohol for a half hour, then into 70% alcohol one hour, then into 95% alcohol indefinitely.

On Feb 25th, March 29th and April 5th some larvae were killed by pouring boiling corrosive sublimate over them and washing them with water for five minutes Those used in staining were put thr through alcohol as above, but those for dissection were kept in water.

On April 5th some larvae were killed with 70% alcohol and dissected in alcohol of the same strength.

Staining and Mounting Slides.

The larvae were taken from 90% alcohol and put into 70% alcohol for half an hour, then into borax carmine or cochineal. Once they were put into the stain whole and left in staining medium for one week. At other times they were divided into halves and left for three days, or else until penetrated, when they assumed a dark red color. If stained with borax carmine, they were put into acidulated alcohol until they assumed a bright red color, which required about three days. They were then transferred to 95% alcohol for an hour. The larvae stained with cochineal were put from the stain into 95% alcohol. The remainder of the process was the same for both, and was as follows; into alcohol and chloroform 24 hours, then into paraffine 19 hours, then into paraffine 50 deg.C. on water bath for two hours. They were then mounted in small paper boxes filled with warm paraffine, which was colled rapidly until hard.

Sections were then cut with the microtome. The sections were fastened to the slides with collodion fixative and the paraffine dissolved out in turpentine. They were then mounted in Canada Balsam. The borax stained larvae which were divided into halves gave the best results.

Fatty Bodies.

Filling the large body cavity are the fatty bodies in the form of large lobes of fat cells, which are of two kinds, the fatty body proper (Fig.I.F.) and the pericardial fatty body (Fig.I.P.). These fatty bodies completely inclose the viscera, being especially abundant around the pericardial sinus and above the nervous system. They are kept in place by numerous branches of the tracheae, which, according to Macloskie, (5) "end in the fat cells". They vary from white to yellow in color, and are nearly transparent. They serve as a store of nutriment laid up especially for transformation. It has been noticed that the fatty bodies become very abundant before the transformations and materially lessen during them, and it has further been noticed that the larvae which hibernate become well supplied with fat. I found that the first larvae in the spring had comparetively little fat, but the amount increased materially in a very short time.

Again Macloskie (5) says "that the terminal cells of thetracheae are stellate and are abundant at the fatty bodies which by a process of slow combustion cause luminosity".

Microscopic.

The fatty bodies (Fig.I.F)as seen through the microscope are lobe shaped. The lobes surround the alimentary canal, and there is one lobe beneath the nerve cord. In a section taken from about the second segment of the body, the lobes form nearly one continuous band around the alimentary canal. In a section from near the center of the body, there were parts of twobands on the dorsal and ventral sides of the alimentary canal; and in a section taken from about the tenth segment of the body the fatty bodies are distributed about as in the central portion. The lobes vary in length from a little less than .05 m.m. - .7 m.m. according to the number of cells in each lobe and the amount of fat in each cell. They are about .04 m.m. - .06 m.m. in breadth.

The cells are all very nearly alike in size, shape and general appearance. They are from .04 m.m. - .06 m.M. in length; .01 m.M. in breadth; and from .01 m.m. - .015 m.m. in thickness. The cell wall is very thin and the cytoplasm is granular. In some cells a part of the cytoplasm has been transformed into a fat globule which in a very few instances have broken down the entire cell with its nucleus. The nucleus of each cell was deeply stained and easily recognizable, and was about .005 m.m. in diameter.

The pericardial fatty-bodies (Fig.I.P.) were only distinguishable in about the seventh to the tenth segments inclusive. They form a sheath around the pericardial sinus, send out arms from the dorsal and ventral sides which are about .3 m.m. in length and .02 m.m. in breadth. The cells of this tissue are very irregular in shape and the cytoplasm is granular. There are two or three nuclei to each cell which are about .005 - .01 m.m. in diameter.

Above the nerve cord is a lobe of fat which is about .06 m.m. in length and .02 m.m. in breadth, the nuclei are situated near the ends.

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Fig I.

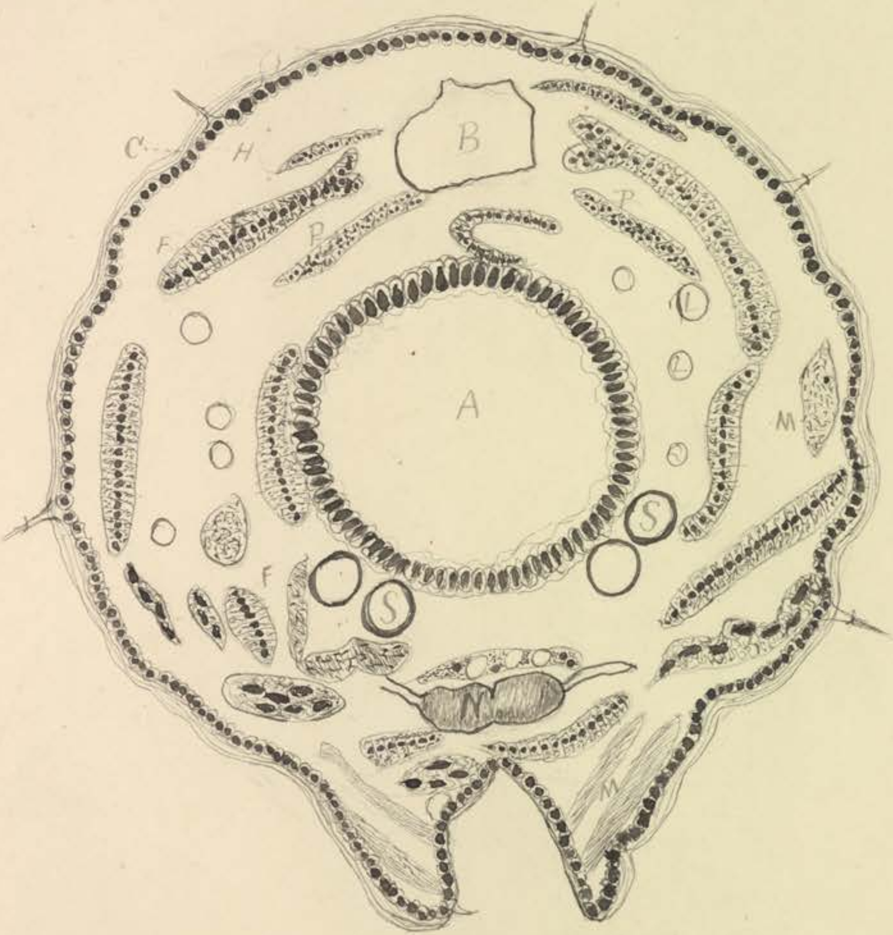

Fig II

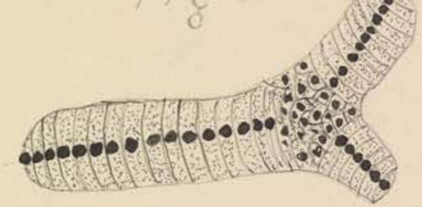

Fig III

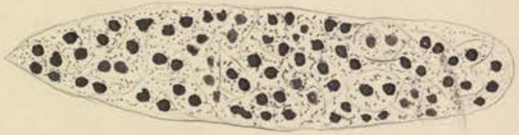

Fig. I. Cross section of larva.

A. Alimentary canal.

B. Heart.

C. Cuticle.

H. Hypodermis.

F. Fatty Bodies proper.

P. Pericardial fatty bodies.

L. Malpighian vessels.

M. Mussel tissue.

N. Nerve cord.

S. Silk Glands.

Fig. II. Fatty bodies proper cut so as to show it from different angles.

Fig. III. Cross section of Pericardial fatty bodies.