HISTOLOGICAL AND PHARMACOLOGICAL STUDIES
ON THE HOUSE CRICKET HEART

by

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment for the degree of Master of Science.

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July, 1970

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Suggested short title:

HEART OF HOUSE CRICKET

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Entomology
1970
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ABSTRACT

The histology of the heart and associated organs of the house cricket, Acheta domesticus (L.), was studied using vital stains and sections examined with the light microscope. The heart system consists of a simple tubular dorsal vessel with ostial valves, symmetrically arranged alary muscles, a well-developed diaphragm membrane, numerous mononucleate or multinucleate pericardial cells and two to three pairs of phagocytic organs. The heart is well innervated by two lateral cardiac nerves and twelve pairs of segmental nerves.

Pharmacologically, the heart was studied with the semi-isolated preparation. Both 'young' and 'old' adult hearts were studied. The young heart tended to be accelerated by acetylcholine, dopamine, serotonin, arecoline and noradrenalin, but was unaffected by nicotine. The action of acetylcholine
on the young heart was potentiated by eserine and by curare, but was antagonized by atropine. The old heart behaved differently from the young heart in response to certain drugs, and generally was less sensitive to all drugs.
L'histologie du coeur et des organes associés du grillon domestique *Acheta domesticus* (L), fut étudiée à l'aide de teintures vitales et de sections examinées au microscope. Le coeur consiste en un simple vaisseau dorsal tubulaire pourvu de valves ostiales, de muscles alaires disposés symétriquement, d'un diaphragme membraneux bien développé, de nombreuses cellules péricardiales mononucléées ou multinucléées et de deux à trois paires d'organes phagocytiques. Le coeur est bien innervé par deux nerfs cardiaques latéraux et douze paires de nerfs segmentés.

Pharmacologiquement, le coeur fut étudié au moyen de préparation semi-isolée. L'étude porta sur des coeurs adultes 'jeunes' et 'vieux'. Le coeur jeune tendait à être accéléré par l'acétylcholine, la dopamine, la sérotonine, l'arécoline...
et la noradrénaline mais n'était pas affecté par la nicotine.
L'action de l'acétylcholine sur le jeune cœur fut amplifiée
par l'ésérine et par le curare, mais fut antagonisée par
l'atropine. Le vieux cœur se comporta différemment du
jeune cœur en réponse à certaines drogues, et fut en général
moins sensible à toutes les drogues.
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ACKNOWLEDGEMENTS

I wish to express my deepest gratitude to my supervisor, Professor J.E. McFarlane, for his enthusiasm, encouragement, guidance and instruction during the research and invaluable suggestions in preparation of this thesis.

Thanks are also due to Mrs. J. Dubreuil for rearing the insects which were used in this work.

Special appreciation is due to Dr. V.R. Vickery for providing necessary apparatus for taking pictures, and also for correcting all the names of the various insects. Appreciation is also extended to Miss H.C. Lim for helping to take the pictures.

Some other friends who kindly contributed their time to translate some literature are sincerely acknowledged.

Financial assistance provided by the National Research Council is gratefully appreciated.

Finally, no words can really describe my appreciation to my lovely parents for their moral support all the time, across the miles.
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I. INTRODUCTION

As in other animals, the circulatory system plays a very important role in the life of the insect. Harvey, Swammerdan and Malpighi, among other early workers, found an interesting small pulsating dorsal vessel in the insect more than three hundred years ago. Much work on the heart then followed, but most investigations were concentrated on morphology. It was found that this dorsal vessel and its associated organs are similar in all insects but with a considerable variation in different species or even in different individuals.

A few workers began to study the pharmacology of the insect heart at the beginning of this century. It was already known that drugs affect the heartbeat of vertebrates in many different ways. The assumption was made that insect tissues are fundamentally similar to vertebrate tissues in their response to drugs, and the interpretation of the results of pharmacological studies therefore had this vertebrate bias. In fact, many results do not easily fit this assumption.

Recently, McFarlane (1967) studied the rate of heartbeat in semi-isolated preparations of the adult house cricket, *Acheta domesticus* (L.) (Gryllidae; Orthoptera), and found that
the rate increased with age up to six weeks followed by a decline during the last few weeks of life. The effect of drugs also varied with age: acetylcholine, serotonin and dopamine tended to accelerate young hearts and to inhibit old hearts.

The present work with the house cricket was undertaken to provide information on the structure of the heart and associated organs as a basis for the interpretation of McFarlane's pharmacological studies. A beginning was made with the light microscope, although electron microscopy may provide the final answers to certain problems. In addition, further pharmacological work with a number of the classical drugs was carried out in order that a clearer picture of the age changes might be obtained.
II. REVIEW OF LITERATURE

A. THE STRUCTURE OF THE DORSAL VESSEL AND ASSOCIATED ORGANS

The structure of the dorsal vessel of insects and its associated organs has been studied for a long time. Generally speaking, the structure follows more or less the same general pattern, but there is a variation from group to group, and even from individual to individual.
1. The Dorsal Vessel

The dorsal vessel is located at the dorsal side of the insect. It is a cylindrical tube lying immediately beneath the tergites, between the dorsal longitudinal muscles, and extends from the posterior end of the abdomen to the head, where it accompanies the oesophagus beneath the brain (Wigglesworth, 1965).

The part lying on the abdomen is usually enlarged and more or less divided into chambered dilations and is called the heart. The part lying in the thorax and the head is called the aorta, and it is usually straight and without chambers. The term 'chamber' is used to indicate a region of the dorsal vessel between successive sets of ostia (Straus-Durckheim, 1828, cited from Jones, 1964). But the above distinction is not applicable in many insects, e.g. the cockroach, Periplaneta americana (L.), in which the chambered heart can extend throughout the abdomen into the thorax (McIndoo, 1945). On the other hand, the unchambered part can extend down to the abdomen, e.g. the Agrionid dragon-fly, where it is reduced to a single chambered heart with a single pair of ostia in the whole abdomen (Wigglesworth, 1965).

Gerould (1938) stated that, except for the Hymenoptera,
the 'chambered' heart is characteristic of aquatic insects and the 'non-chambered' heart is characteristic of terrestrial insects, the differences being associated with the differences in respiration. But the aorta can pulsate as well as the heart as has been proved for numerous insects (Barnhart, 1961; Wigglesworth, 1965; McCann, 1969).

Due to the difficulty in differentiating clearly between the heart and the aorta, Newport (1836-1839) preferred to call the abdominal portion of the dorsal vessel the 'heart', the thoracic portion of the dorsal vessel the 'aorta', and the cephalic portion of the dorsal vessel the 'cephalic aorta' (cited from Jones, 1964).

From these considerations, the present writer does not see any point or necessity to make a distinction between the heart and aorta. But from the embryonic point of view, these two parts are not the same. Johannsen and Butt (1941) pointed out that the anterior part develops from the dorsal antennal coelomic sacs, and the posterior part is derived from two separated sets of cardioblasts which unite along the dorsal midline at the time of blastokinesis.

Snodgrass (1935) stated that "the walls of the heart consist of muscle tissue immediately derived from the cardio-
blasts, or heart forming cells of the embryonic mesoderm, which are converted into semicircular or circular striated muscle fibers that compose the heart tube. The muscles may consist of interlacing striated fibrillae embedded in sarcoplasm, as in Aeschna (Zawarzin, 1911), or longitudinal and circular myofibrillae, as in Nepa (Hamilton, 1931), or circular myofibrillae only, as in the mosquito, Anopheles quadrimaculatus Say (Jones, 1954). There is often a reticulum of connective tissue cells called the 'advantitia' ('pericardium') outside the outer membrane as in Sitophilus oryzae (Murray and Tiegs, 1935). In the Peloridiidae, the very thin dorsal vessel wall appears structureless, there being no sign of the striations of muscle fibers; it is lined with a loose and extremely delicate membrane (Pendergrast, 1962). The aortic part of the vessel in Drosophila melanogaster (Meig.) is without a muscular wall (Miller, 1965).

Typically, the posterior end of the dorsal vessel is closed, but in Japyx, it is open (Grassi, 1887).

The dorsal vessel may be in contact with the body wall directly, as in Phaenoserphus viator (Eastham, 1929), or by numerous radiating filaments, as in the honeybee Apis mellifera L. (Snodgrass, 1956). It is suspended in the body cavity
mainly by many fine alary muscle fibers and connective tissue fibers (Wigglesworth, 1965).

The main type of dorsal vessel found in the insect is a simple straight tube, but in many insects there are some modifications, e.g. segmental swellings, diverticula or other special structures (Jones, 1964). For example, the cockroach has four pairs of segmental vessels in the abdomen and two diverticula in the thorax (Nutting, 1951). In some bees, there is an arch with a series of loops just behind the ascending portion of the dorsal vessel (Wille, 1958). Generally speaking, the modifications occur in the abdomen more often than in the thorax. In certain cases, the thoracic part has a specialized configuration also, such as two vertical aortic loops in the thorax of the Odonata (Whedon, 1938); these were thought to have the function of production and distribution of hormones in spite of the obvious pulsatic function.

2. The Ostial Valves

The ostia only appear in the heart chambers, and are slit-like openings, one on each side of the chamber (Snodgrass, 1935). Their margins are prolonged inwards to form lip-like valves, which are called the ostial valves (Wigglesworth, 1965).
Occasionally, valves may be developed from folds of the inner wall of the heart and isolated from the ostia, as in *Chironomus dorsalis* (Meig.) (Popovici-Baznosanu, 1905). In *Tipula selene* (Wettinger, 1927), there are a pair of well-developed valves just anterior to the ostia in each heart chamber.

The position of the valves is usually lateral, but in some cases, they are more nearly dorsal, as in the *Pseudophyl-linae* (Nutting, 1951).

The valves are closed during systole, when the internal blood pressure brings the lips together. At diastole the lips are forced apart when the blood is aspirated from the pericardial sinus (Nutting, 1951).

The innervation of the ostial valve has been studied in *Carausius morosus* Brunner (Opoczyńska-Sembratowa, 1936).

In addition to the above ostia, which Nutting (1951) preferred to call 'incurrent ostia', there are some ventral opened 'excurrent ostia' in saltatorial Orthoptera. Nutting observed that there are no internal valves inside these ostia, but an excurrent flow of blood is assured by the papilla of cells surrounding each opening.
Usually the insect heart is closed at the caudal end and therefore without ostia. But posterior ostia can be seen in some insects, such as in Corethra, Ceromasia, and others (Wigglesworth, 1965).

3. The Dorsal Diaphragm

The dorsal diaphragm (pericardial septum) lies immediately below the dorsal vessel (Wigglesworth, 1965) and consists of diaphragm muscles (alary muscles) and the surrounding delicate connective tissue membrane (DuPorte, 1959). A more detailed definition is "The membranous and muscular sheets of the laterodorsal parts of the body wall, separating the dorsal sinus (pericardial sinus) from the perivisceral sinus" (Snodgrass, 1935). The degree of development in both the membranous and the muscular portions of the diaphragm varies with different insects (Snodgrass, 1935).

a. The diaphragm membrane

The diaphragm membrane is a delicate, transparent, amorphous or serrate-edged membrane. In the space above this membrane is contained the heart, alary muscles, pericardial cells, fat body and trachea.
The membrane can be fenestrated, as in the cockroach (Brocher, 1931), in which the blood passes through the membrane to the pericardial sinus. Or it may be imperforate, as in the grasshopper (Wigglesworth, 1965) in which case the blood must flow around the posterior border.

Many insects have a well-developed diaphragm membrane, e.g. roaches and the non-flying tettigonioids (Nutting, 1951), the membrane extending far from the heart. Other insects have only a poorly developed diaphragm membrane, e.g. Drosophila (Miller, 1965), where the membrane occupies only the area containing the alary muscles. In certain insects such as Diapheromera corilleae Rehn and Hebard (Nutting, 1951) or in Peloridiidae (Pendergrast, 1962), the continuous connective sheet is absent and is replaced chiefly by strands of pericardial cells. In Paratenodera sinensis (Sauss) or Taeniopoda eques (Burm.), there is no diaphragm membrane, and it is largely replaced by thin, fenestrated sheets of fat body, loosely bound by connective tissue (Nutting, 1951). Some Heteroptera such as Dysdercus fasciatus (Stål), Euoplops scapha (F.) and Phonocutus nigrofasciatus Stål also lack this diaphragm membrane (Hinks, 1966). On the other hand, many Orthoptera have been found to contain double diaphragm membranes, such as Grylloblatta c. campodeiformis E.M. Walker, Stenopelmatus fuscus Haldeman, Liparascelis sp., Neoconocephalus ensiger (Harris),
and *Microcentrum rhombifolium* (Sauss.) (Nutting, 1951). But the secondary diaphragm is limited to the abdominal segments bearing ostia, and never appears in the thoracic part. It also does not extend very far from the heart.

Normally, the diaphragm membrane is well developed in the abdomen (Snodgrass, 1935), but in some cases, a vestige of the diaphragm membrane may extend into the thorax (Nutting, 1951).

Generally, the attachments of this membrane to the tergite always join with the attachments of the alary muscles, except in *G. campodeiformis*, in which the dorsal diaphragm membrane is attached to the segmental tracheal trunks and the alary muscle is attached to the front of the tergite (Nutting, 1951).

In sections, it is easy to see that this membrane is a cellular layer, one cell thick (Nutting, 1951). Obviously, Nelson's (1924) suggestion that the dorsal diaphragm membrane may be in the nature of a basement membrane of the pericardial cell itself is incorrect.

Finally, it should be mentioned that this dorsal diaphragm is free from the body wall except at the points of attachment.

b. The alary muscles

The alary muscles (aliform muscles) derive their name from
the fact that they form the wings of the heart (Miall and Hammond, 1900). Typically, they occur in a fan-shaped triangular form (Snodgrass, 1935). But in some insects, such as Dissosteira carolina (L.), the muscle fibers are all approximately transversely parallel and arise serially along the laterodorsal parts of the body wall (Snodgrass, 1935).

The muscle fibers usually reach to the edge of the heart but some of the fibers end halfway along the diaphragm (Snodgrass, 1935). Some abnormal arch-shaped alary muscles, with both ends attached to the heart wall, are found in the silkworm Bombyx mori L. (Ke, 1932).

The alary muscle retains an obvious metameric arrangement (Hinks, 1966), and is generally present only in the body segments containing a chamber of the heart (Snodgrass, 1935), and is therefore usually limited to the abdomen. As mentioned in the section on the dorsal vessel, the chambered heart can extend into the thorax and the unchambered part can continue down to the abdomen. Therefore the alary muscle may occur in the thorax or may not occur in the abdomen.

The number of alary muscles is variable according to the number of heart chambers. Usually, there is one pair for each chamber arranged symmetrically on each side of the heart, but
in many cases, there are more than one pair (Hinks, 1966) arranged in no apparent order (Jones, 1954).

Contrary to the impression given by their name, the alary muscles are really composed of true muscle fibers and elastic connective tissue fibers rather than muscle only (Nutting, 1951). The degree of the connective tissue varies with different insects. For example, the Orthopteroid alary muscle appears to have a much higher degree of connective tissue than *Aeschna* (Zawarzin, 1911).

The alary muscles may approach the heart in three ways: they may run along the surface of the heart wall parallel to the long axis; they may pass over or under the heart and continue to the other side; or they may make contact with a cardiac cell directly (McCann, 1970).

The alary muscles are innervated in some insects, such as the cockroach, *Blatta orientalis* L. (Alexandrowicz, 1926), bee (Morison, 1928), silkworm (Ke, 1932) and mosquito (Jones, 1954). A very small cell or nucleus (perhaps a nerve cell) is found near the middle of each alary muscle of *Chironomus* (Miall and Hammond, 1900).

Although striated muscle is believed to be the only muscle of the Insecta (Edwards, 1960) and Zawarzin had already illus-
trated the striation of the alary muscle of *Aeschna* in 1911, the striation of the alary muscle was still doubted by some workers, such as Ellis (1969). Recently, the striated nature of the muscle has been clearly confirmed with the help of the electron microscope (Sanger and McCann, 1968a). Actually, under the high power of the light microscope the striation of this muscle is shown clearly enough in most cases.

Nutting (1951) studied Orthopteroid hearts and indicated that the presence or absence of one or both mesothoracic and metathoracic alary muscles is largely governed by the development of the flight musculature. For instance, non-flying Tettigonoioidea have 12 pairs while the strongly flying ones only have 10 pairs. In other words, the meso- and meta-thoracic pairs are absent or markedly smaller in winged forms where the thoracic musculature is better developed. But in many other Orthopteroid groups, this relationship is not found.

If the alary muscle is responsible for holding the dorsal vessel in position in the dorsal sinus, and performing some cardiac activity, it should be in some way in contact with the heart. The myomuscular junction between the alary muscular cell and the cardiac muscular cell has been demonstrated in the moth *Hyalophora cecropia* (L.) by Sanger and McCann (1968a).

Jones (1954) studied the effect of starvation on the
mosquito heart and observed that the heart of the dying larva swayed from one side of the mid-dorsal line to the other, suggesting that the alary muscles were unable to maintain proper tension on the heart.

Various workers have proposed that the alary muscles actually initiate the heart beat, as in Chironomus (de Wilde, 1948), or that they have no active function, as in Dytiscus marginalis L. or P. americana (Beard, 1953). However, there is no general agreement as to how alary muscles function or what effective contribution they make to the cardiac cycle." (McCann, 1970).

4. The Pericardial Cells

The pericardial cells of the insect, as their name implies, surround the surface of the pulsatile dorsal vessel (Bowers, 1964) and often extend onto the underlying pericardial septum and alary muscles (Smith, 1968), but they are also scattered throughout the body and even in the appendages (Snodgrass, 1935). This kind of cell exists in all examined insects, without exception.

The pericardial cells were first studied by Leydig (1866). During the period between 1870 and 1920, the pericardial cells became a great controversial subject among the light microscopists.
Many different concepts of their nature and function were proposed: 1) Site of hematosis (Graber, 1871), 2) Nerve cells (Lowne, 1891), 3) Hematopoietic organs (Cuénot, 1891), 4) Supporting cells of the heart (Massonat, 1909), 5) Gland cells homologous to peritracheal cells (Verson, 1910), 6) Large celled glands (Sikora, 1916), 7) Hepatic cells (all cited from Hollande, 1921), etc.

In 1886, Kowalevsky found that the pericardial cells of dipterous larvae absorbed injected ammonia carmine from the blood and suggested that they served as a 'storage kidney'. This led Bruntz (1903) to propose that the cells be named 'nephrocytes'. But the excretory function was not accepted by Hollande (1921). The pericardial cells were also called diaphragm cells by Snodgrass (1935). The most common names still being used are 'pericardial cell' and 'nephrocyte'.

Heymons (1885) indicated that the pericardial cells are derived from mesodermal tissue and from the same parts of the mesodermal layers that give rise to the heart and the dorsal diaphragm (cited from Snodgrass, 1935).

Their size, number, exact position and number of nuclei vary from one order of insects to another. For instance, they
may be 150 μ in length in the moth \textit{H. cecropia} (Sanger and McCann, 1968b), 50 μ in the grasshopper \textit{Melanoplus differentialis differentialis} (Thomas) (Kessel, 1962), but in \textit{Geocorisa}e there are very few which are over 30 μ (Hinks, 1966).

They are generally diminished in number and in many cases are absent around the aorta (McCann, 1970), but clusters of pericardial cells were seen in the aorta of the aphid \textit{Drepanosiphum platanoides} Schrank (Johnson, 1963). Mostly, they are located on the outer surface of the heart, but in all examined species of \textit{Heteroptera} the pericardial cells occur within the lumen of the dorsal vessel (Hinks, 1966). Those within the heart are referred to as 'endocardial cells' (Jones, 1964).

The pericardial cells may be mononucleate as in \textit{Icerya purchasi} Mask. (Pesson, 1951), binucleate or with three or four nuclei as in \textit{S. oryzae} (Murry and Tieg, 1935), or with up to seven nuclei as in the moth \textit{H. cecropia} (Sanger and McCann, 1968b). However, they are large and binucleate in general and are readily observed in all instances (Hollande, 1921).

The pericardial cells, according to their location, are either divided into two groups, the periesophageal and disseminated cells as in \textit{Pediculus humanus humanus} L. (Nuttall and Keilin, 1921), or into three groups: periesophageal, pericardial and
parietal cells as in *Cimex lectularius* L. (Puri, 1924).

The cytoplasm of the pericardial cell is vacuolated and contains many mitochondria, Golgi bodies, lysosome-like organelles (Novikoff, 1961) and other granules, which vary in size from time to time. Sometimes they have yellow, pink, red, brown, black and other pigments (Hollande, 1921; Kessel, 1962). The inclusions may be droplets, or needle-shaped, slender or irregular in form (Hollande, 1921). These inclusions are very high in protein and lipoprotein content and low in free lipid (Hollande, 1921; Kessel, 1962). The cytoplasm is either neutrophilic or acidophilic. The acid reaction can be easily demonstrated by injection with blue litmus, when the cells turn the absorbed litmus red (Kowalevsky, 1889).

The secretory cycle of the pericardial cells has been separated into four stages; the cells are said to be capable of renewing the cycle (Hollande, 1921). Schwinck (1951) indicated that the pericardial cells may be secretory during the early stages of the maturation of the ovaries.

In *Calliphora*, the pericardial cells undergo phagocytosis during metamorphosis (Kowalevsky, 1889).

Their property of taking up injected electronegative colloids from the blood of *B. mori* larva (i.e. 'micro-phagocyt-
tosis') led Lesperon (1937) to propose that the pericardial cells perform a function comparable with the vertebrate reticuloendothelial system.

Wigglesworth (1965) states that "if the pericardial cells play a part in excretion it is likely to be rather in the intermediary metabolism of waste substances". Blood pigment in *Rhodnius* hemolymph is taken up by the pericardial cells and first converted into a brown modified haematin and then into a green pigment and then into biliverdin and stored in the pericardial cell throughout its life (Wigglesworth, 1943). There are the same changes which happen to haemoglobin in the Kupffer cells of the reticuloendothelial system in the liver (Wigglesworth, 1965).

Hollandé (1921) suggested that the pericardial cell may regulate the hemolymph pressure. Davey (1961a) found that the pericardial cell of *P. americana* can be stimulated by the corpus cardiacum to produce a pharmacologically active factor (indolalkylamine) which then elevates the heart rate.

In some insects, the number of the pericardial cells increases before ecdysis during nymphal or larval life (Hollandé, 1921; Lüscher and Engelmann, 1960; Kessel, 1962), but in other insects they are constant.
The ultrastructure of the pericardial cell has been revealed by the use of the electron microscope during the past decade (Kessel, 1961, 1962; Bowers, 1964; Mills and King, 1965; and Sanger and McCann, 1968b). Some of the proposed physiological functions may be confirmed with this help. For example, the taking up of protein has definitely been proved to occur via the 'coated vessels' and 'tubular elements' (Bowers, 1964; Sanger and McCann, 1968b).

5. The Phagocytic Organs

Kowalevsky and his students had already studied the phagocytic organs (ventral diverticula) of A. domesticus in 1894. They noticed two pairs of such organs in both the first and second abdominal segments. They supposed that these organs were a center for the manufacture of leucocytes and therefore gave them the name 'lymphatic glands' but they did not give any figures for these organs.

Cuénot (1896) found three pairs of phagocytic organs in Gryllus campestris L. which were located from the first to the third abdominal segments. In Gryllotalpa vulgaris Latr. they were located in the metathorax and in the first, second and third abdominal segments, he also found five pairs of phagocytic organs in Acridium aegyptium and Forficula auricularia L. which were located in the first five abdominal segments.
The above results were misunderstood by McIndoo (1939) when he considered all the phagocytic organs as segmental vessels. He said "Kowalevsky (1894) apparently saw pairs of segmental vessels in both the first and the second abdominal segments of a cricket...". Again he said "Cuénot (1896) seems to have found segmental vessels in many Orthoptera, but he called them phagocytic organs."

Dawydoff (1904) studied the phagocytic organs of the Locustidae (the modern term should be 'Tettigoniidae') and noted that fat body cells were always present in the phagocytic organs. This led him to consider that the phagocytic organs were developments of part of the fat body and their altered structure was related to their new function, that is, to phagocytose foreign particles. The fat body cell of insects normally has no phagocytic function. He (1904b) also studied Gymnogryllus, and found that besides the three large pairs in the first to third segments, there were five other much smaller phagocytic organs which were situated symmetrically on each side of the heart from the fourth to eighth segments, which he designated 'complementary phagocytic glands'. The first pair of these small organs communicated with the heart cavity, the others were completely isolated from the heart.

Philiptschenko (1907) found that some Apterygota such as Ctenlepisma sp. (Thysanura) also contain phagocytic organs.
Nutting (1951) examined a number of Orthoptera and concluded that phagocytic organs are peculiar to a small number of saltatorial families of the Orthoptera. Such species possess four pairs, e.g. the mole cricket, *Gryllotalpa hexadactyla* Perty, the wingless bush cricket *Hoplosphyrum boreale* Scudder, *Cycloptilum comprehens fortior* Hebard, *Pterophylla camellifolia* (Fab.) and *Liparoscelis* sp. The larger brown bush cricket, *Paroecanthus aztecs* Sauss., has three pairs and the small ground cricket, *Allonemobius fasciatus* (DeGeer) and *Microcentrum rhombifolium* (Sauss.), two pairs. All of these phagocytic organs are located at the anterior part of the abdomen and are triangular in form, except in *H. boreale* and *C. comprehens fortior* where they differ only in gross form, being flattened, blind tubules. They are supported by a delicate diaphragm, and are terminally attached to the tergum by connective tissue fibers.

From the above literature, it is clear that all these organs appear to be histologically very similar from group to group, differing only in number and position. In general, the organs seem to be pouches whose cavities communicate may with the cavity of the heart, and the principal cells of these organs are phagocytic cells.
It is indeed surprising that among insects, such important phagocytic organs are only known in a very small number of species, and have been studied so little.

6. Innervation

The gross innervation of the insect heart is known for only a few insects, and there is considerable variation from group to group (Bullock and Horridge, 1965). Recently, while many workers have studied the lateral cardiac nerves of the insect heart, the emphasis has been particularly on the ultrastructure of the individual cardiac nerve cell body and its physiological function (Edwards and Challice, 1960; Johnson, 1966; Miller and Thomson, 1968.)

Zawarzin (1911) first observed the innervation of the insect heart. He treated dragonfly (Aeschna) larvae with methylene blue and then described its segmental (which he called 'seiten-nerven') and lateral cardiac nerves (which he called heart nerves) with their ramifications.

Alexandrowicz (1926) used the Rongalite white preparation for a study of the innervation of the cockroach heart (B. orientalis, but he called it Periplaneta). He found there were ten pairs of segmental nerves and two lateral heart nerves running along each side of the heart tube. More than forty ganglionic
or nerve cells were distributed along the lateral cardiac nerves. The rami cardiac dorsales reach the heart on its dorsal side.

Some lateral cardiac nerves are without nerve cell bodies, e.g. Aeschna (Zawarzin, 1911) and B. mori (Kuwana, 1932). On the other hand, some lateral cardiac nerves contain cell bodies, e.g. the honeybee A. mellifera (Rehm, 1939), and the cockroach (Alexandrowicz, 1926; McIndoo, 1939, 1945). The nerve cell bodies in the cockroach are usually near the junction of the segmental and lateral cardiac nerve (Miller, 1968a), rather evenly, singly or in pairs (Alexandrowicz, 1926). Their shape is more or less elongated.

Opoczyńska-Sembratowa (1936) has described in the walking-stick (C. morosus) the lateral cardiac nerve cell body. The surface was surrounded by the aborization of other cardiac nerve cells and of the segmental cardiac axons.

The lateral cardiac nerve cell bodies are bipolar, both processes running along the heart and giving branches to the heart muscle. Tripolar nerve cells are found in C. morosus (Opoczyńska-Sembratowa, 1936) which also send a process to the alary muscle.
The innervation of the alary muscle has been demonstrated in the cockroach (Alexandrowicz, 1926; McIndoo, 1945), in the silkworm (Ke, 1932), and mosquito (Christophers, 1960; Jones 1954). The segmental heart nerve fibers always enter the alary muscle near the heart.

McIndoo (1945) examined the innervation of several insect hearts and concluded that the cockroach (P. americana) heart may be considered to be well innervated, the silkworm (B. mori) heart is only fairly well innervated, and the southern armyworm (Prodenia eridania (Cram.)) heart, where no lateral cardiac nerve could be found, is poorly innervated.

This incomplete account of the innervation of insect hearts shows the great variability of the insect group in this regard. As far as is known, no information has been published on the innervation of the hearts of Gryllidae.

B. THE PHARMACOLOGY OF THE INSECT HEART

The pharmacology of the vertebrate heart has been thoroughly studied for a long time. On the contrary, very little attention has been paid to the invertebrates (except molluscs), and only relatively little work has been done with the insect heart. Therefore, the pharmacology of the insect heart is still in an unsatisfactory state.
The drugs most often used with insects are those which are implicated as transmitter substances in the vertebrate, i.e., acetylcholine (Ach), adrenalin (epinephine) or noradrenalin (epinephine), and 5-hydroxytryptamine (serotonin). These three substances have all been demonstrated to occur in insect tissues. Ach is said to accelerate neurogenic hearts, inhibit innervated myogenic hearts and to have no effect on the non-innervated myogenic hearts. Adrenalin and 5-hydroxytryptamine usually excite the hearts but have no effect in some cases.

1. Cholinergic Drugs

By definition neurons containing and releasing Ach are called cholinergic neurons (Dale, 1933). Therefore, it can be considered that drugs such as Ach and its analogues, e.g. nicotine, muscarine, atropine, curare (for structural formulae see Appendix I) which may stimulate or inhibit these cholinergic neurons, are cholinergic drugs.

a. The action of acetylcholine

This substance occurs in various parts of animals (even in plants) but is chiefly found in the nervous system and in particularly highly concentration in the ganglionic area (Welsh, 1939).
It has been already identified in many insects, such as the blowflies, Calliphora erythrocephala Meig, and Lucilia sericata Meig. (Lewis, 1953), the honeybee (Augustinsson and Grahan, 1954), the housefly Musca domestica L. (Chefurka and Smallman, 1956), and P. americana (Colhoun, 1958).

Ach is a high energy compound and in vertebrates, its synthetic pathway is as follows:

\[
\text{ATP} + \text{acetate} \rightarrow \text{adenyl acetate} + \text{pyrophosphate}
\]

\[
\text{adenyl acetate} + \text{coenzyme A} \rightarrow \text{acetyl coenzyme A} + \text{AMP}
\]

\[
\text{citrate} \rightarrow \text{Acetyl coenzyme A}
\]

\[
\text{pyruvate} \rightarrow \text{Acetyl coenzyme A}
\]

\[
\text{Acetylcoenzyme A} + \text{choline} \xrightarrow{\text{acetylase}} \text{acetylcholine} + \text{coenzyme A}
\]

Ach is destroyed (hydrolyzed) by esterases, particularly by cholinesterases (Florey, 1966).

Ach has two distinct effects in vertebrates. One, in which the action of Ach is imitated by nicotine is called the 'nicotinic action of Ach' and the other, in which muscarine can replace Ach is called the 'muscarinic action of Ach' (Florey, 1966).

Concerning the pharmacology of the insect heart, Ach can be considered the only drug which has received much attention.

In most insects, Ach has a stimulating action. Hamilton (1939) observed that Ach stimulated the intact heart of
M. differentialis with a threshold concentration of $10^{-14}$ M. Concentrations higher than $10^{-14}$ M caused a very marked chronotropic increase and an inotropic decrease, followed by irregular beating. A concentration of $10^{-8}$ M caused an acceleration which diminished very quickly. With a concentration of $10^{-2}$ M the stimulation was maintained for 15 to 20 minutes. Eventually the initial stimulation gradually decreased and caused slow, rhythmic movement of the alary muscles. The response of the alary muscles was eliminated by cutting them.

The isolated segments of the heart also respond in a similar way to the intact M. differentialis heart. This indicates that Ach does not act on a single localized cardiac center (Beard, 1953).

Davenport (1949) found that some hearts of the Stenopel-matus longispina Brunner were accelerated only at a concentration of $10^{-6}$; higher concentrations induced a transitory systolic tetany.

Krijgsman and Krijgsman-Berger (1951) studied the heart of P. americana and noted that Ach over a wide range of concentrations showed a strongly stimulating action and stated that the action of Ach on the cockroach is strikingly different from its action on the vertebrate heart, in which it is well known to cause an inhibition of the myogenic pacemaker.
Their results also defined the cholinergic property of the pacemaker and suggested that Ach is a normal neurohumor of this neurogenic mechanism.

Naidu (1955) also studied the cockroach and suggested that the stimulating action of Ach in low concentration is on the myocardium, not on the cardiac neurones.

Metcalf, Winton and Fukuto (1964) found that Ach was about 40 times more effective than other analogous cholinergic substances and suggested again that the cardiac ganglionic cells control the rate of heartbeat and that the synapses of these cells have receptor sites specifically complementary in structures to the Ach molecule. Obviously, these cholinergic synapses of the heart are not insulated against the action of the externally applied Ach by the lipid sheath as are the synapses of the insect central nervous system (Twarog and Roeder, 1956, 1957, Winton, Metcalf and Fukuto, 1958).

Miller (1968b) showed that Ach at $10^{-9}$ M increases the rate of firing of isolated cardiac ganglion cells. At $10^{-8}$ M there is an initial increase followed by depression. At $10^{-7}$ M the firing decreases to zero. He hypothesized that Ach acts on the cardiac ganglion cell, not at a synapses membrane, but rather at an unspecified cholinergic sensitive site, because the experiments have not yet revealed the presence of a choli-
nergic synapse in the cockroach cardiac nervous system.

The hearts of Chaoborus (Florey, 1951), and Tenebrio (Butz, 1962) were also accelerated by Ach.

In some non-innervated hearts, such as Galleria (Millman, 1938), Anopheles (Jones, 1956) Ach has no effect.

Yamasaki and Ishii (1950) found that Ach caused the Locusta heart to stop in systole at concentration of $10^{-5}$ M (cited from Jones 1964).

i. potentiated by eserine

In general, eserine by itself is much less active in the invertebrates than in vertebrates (Jones, 1956).

Eserine has no effect on some insects, such as Stenopel-matus (Davenport, 1949) and Anopheles (Jones, 1956).

As mentioned before Ach is destroyed by cholinesterase (ChE); and eserine acts as a powerful anti-cholinesterase, thereby prolonging the action of Ach. Usually, the principal effects of eserine are made when eserine treatment is followed by Ach (Jones, 1956).

Many insects, and especially active insects, contain large amounts of ChE, e.g. various flies, the honeybee, ants, and
cockroaches (Metcalf, March and Maxon, 1955). Theoretically, these insects should be markedly effected by eserine alone, as has been already proved for the cockroach. Naidu (1955) found that $5 \times 10^{-6}$ to $10^{-5}$ eserine induces a vigorous stimulation of the heartbeat of the cockroach. This accelerating action of eserine is blocked by atropine but not by nicotine. As well as potentiating the action of Ach, eserine also potentiates the action of nicotine (Naidu, 1955).

On the other hand, Yamasaki and Ishii (1950) noted that eserine did not potentiate the action of Ach on the Locusta heart (cited from Jones, 1964).

ii. antagonized by curare

In vertebrates, curare acts as a well-known blocking agent on the motor end plates although not acting in a specific way on the heart (Florey, 1966). According to Goodman and Gilman (1955), the mode of action of curare is the competition with Ach for acceptance by the cholinergic receptors of the motor end plates, with no depolarization of the cell membrane and consequently no contraction of the muscle fiber. Some workers (Roeder, Kennedy and Samson, 1947, Roeder, 1948) indicated that curare has no effect on insect neuromuscular junctions or
neuromuscular transmission. But McCann (1966) proved that curare also blocked the neuromuscular junctions of insects.

Many studies have showed that this drug itself is not effective on many insect hearts. For example, Davenport (1949) found that curare at $10^{-4}$ gave no immediate effect on the _Stenopelmatus_ heart; the rate depressed only after long perfusion.

Although this drug causes only a very slight action when applied to the insect heart alone, it greatly reduces the action of subsequently applied Ach. Krijgsman and Krijgsman-Berger (1951) showed that pretreatment with $1.5 \times 10^{-4}$ curare for 25 mins. then abolished the effect of $5 \times 10^{-7}$ Ach, which alone can give an immediate stimulation.

Typical non-innervated hearts such as that of _Anopheles_ are not affected by curare (Jones 1956).

iii. antagonized by atropine

Atropine, which abolishes the muscarinic action of Ach on the vertebrate cholinergic system, is generally considered to have a direct action in blocking the Ach receptor site (Florey, 1966).

In many insects, the effect of atropine also indicates a counteraction of Ach.
Krijgsman and Krijgsman-Berger (1951) found that atropine alone at $2.5 \times 10^{-5}$ sometimes can induce a slight temporary increase in rate on the cockroach heart followed by an incomplete contraction and decrease of frequency. $5 \times 10^{-5}$ caused an immediate lack of coordination and after some time a very slow irregular beating or diastolic standstill.

Naidu (1955) also found that atropine abolishes the action of Ach. Atropine alone at $2 \times 10^{-3}$ to $5 \times 10^{-4}$ depresses the heartbeat of the cockroach, at $10^{-4}$ it temporarily increases the heartbeat rates and at $5 \times 10^{-5}$ there is an immediate and prolonged increase in frequency.

In *Stenopelmatus*, at concentrations under $10^{-5}$, the isolated heart continues to beat for hours with a decreased but constant rate, the antagonistic action to applied Ach only occurring after long perfusion with atropine (Davenport, 1949).

Hamilton (1939) observed that in the grasshopper, concentrations less than $10^{-4}$ M did not give any effect but at higher concentrations produced a slightly chronotropic decrease; $10^{-2}$ M atropine was toxic and stopped the heart immediately. He also showed that atropine prevented the action of applied Ach on the heart, but only temporarily abolished the responses of the alary muscles.
That atropine blocks subsequently applied Ach also has been proved by Miller (1968b) in the cockroach.

Atropine has no effect on the myogenic heart, such as that of Anopheles (Jones, 1956).

Florey (1951) stated that $10^{-3}$ and $2 \times 10^{-3}$ atropine accelerated the Chaoborus heart.

The action of atropine is reversible.

b. The action of nicotine

In vertebrates, it is known that this alkaloid has no specific action on the myogenic pacemaker of the heart, but it stimulates powerfully the cholinergic systems in low concentrations and blocks in high concentrations.

This diphasic action (stimulation with low and blocking with higher concentration) also occurs in some insect hearts. Yeager and Grahan (1937) noted that nicotine causes an initial stimulation without depression on the cockroach heart at low concentrations, initial stimulation followed by partial depression at relatively intermediate concentrations, and stimulation followed by complete depression and paralysis at relatively high concentrations.
Davenport (1949) found in *S. longispina* that the action of nicotine was similar to its action on the cockroach heart. Concentrations as high as $10^{-4}$ M brought about a sudden rate increase and rise in tone leading to transtomy systolic tetany, followed by a depression in rate. Lower concentrations accelerated the heart without depression.

The heart of the southern armyworm, *P. eridania*, is less sensitive to nicotine than that of the cockroach. There is no great effect even up to 0.1%. However, it is similarly stimulated and depressed, although final stoppage occurs in diastole rather than in systole (Yeager and Gahan, 1937). This marked difference in response of the cockroach and southern armyworm may be due to difference either in their possession of intrinsic ganglionic cells or in the resistance to nicotine of various neural or muscular components (Yeager and Gahan, 1937). Yeager (1938) suggested that nicotine decreases the ability of the heart to relax, and so systole always predominates.

With the heart of *M. differentialis*, Hamilton (1939) found that all concentrations from $10^{-4}$% to 1% produced a marked inotropic stimulation, but the rate response to nicotine was variable. Low concentrations often produced an initial transitory stimulation in rate, followed by a decrease below the normal rate, and high concentrations caused a slight chronotropic decrease.
The effect of nicotine on the cockroach was also demonstrated by Krijgsman and Krijgsman-Berger (1951) and Miller (1968b). Miller suggested that the sites of action are both the ganglionic cell and neurosecretory neurons.

Naidu (1955) indicated that nicotine causes the cardiac neurons of the cockroach to release Ach. He observed that nicotine $5 \times 10^{-5}$ produced an acceleration of the heartbeat which declined with prolonged exposure until eventually no effect was produced on reapplication. Recovery occurred with sufficient washing. This reversible reaction has also been shown by others (Yeager and Gahan, 1937; Davenport, 1949; Hamilton, 1939).

Hexamethonium iodide antagonises the stimulating effects of low concentration of nicotine (Naidu, 1955; Miller, 1968b). Since atropine also reduces the action of nicotine, it is suggested that the ganglia excited may be chiefly cholinergic.

Nicotine is not an antagonist of Ach in the vertebrates; but in the cockroach nicotine may antagonize the stimulating action of high but not of low concentrations of Ach (Krijgsman, 1952).

In the intact animal, Kirschner (1932) found that the fumes of burning tobacco (presumably nicotine) stopped the heart of *Aphis tulipae* Fonscolombe in two to three minutes.
Coon (1944) injected 0.75% and 1% nicotine into the America cockroach and found the heart still beat long after the appendages were paralyzed, but the normal circulation was greatly changed. The amplitude of the heartbeat was reduced.

Florey (1951) found that nicotine increased the rate of the intact heart of Chaoborus larvae from 14-24 beats to 19-33 beats per minute.

Jones (1956) found that neutralized nicotine $10^{-2}$ to $10^{-3}$ slightly stimulated the heart rates of fasting Anopheles larvae and their cardiac rhythm then resembled that in normal feeding larvae.

c. The action of muscarine

Muscarine is said to imitate the action of Ach and is a parasympathetic activator of vertebrates. It acts upon the heart and the visceral smooth muscle of the alimentary tract. It is antagonized by parasympathetic inhibitors such as atropine (Florey, 1966).

Dogiel (1877) immersed the whole larval mosquito, Corethra, in a muscarine solution and found it was without effect; but this might have been due to the drug never having penetrated to the heart.
In *S. longispina* Brunner, muscarine from $10^{-6}$ to $10^{-3}$ also did not produce any effect on the heartbeat (Davenport, 1949).

Miller and Metcalf (1968) found that the denervated cockroach heart is unresponsive to arecoline (which is said to be similar in action to muscarine) at concentrations up to $10^{-3}$ M and therefore suggested that arecoline acts on the cardiac nervous system.

2. Adrenergic Drugs

Applying Dale's definition of cholinergic neurons, the neurons containing and releasing adrenalin are called adrenergic neurons.

The main pathway for the biosynthesis of these catecholamines is as follows:

\[
\text{Tyrosine} \xrightarrow{\text{Dopa}} \xrightarrow{\text{dopa decarboxylase}} \xrightarrow{\text{Dopamine}} \xrightarrow{\text{dopamine hydroxylase}} \text{Noradrenalin} \xrightarrow{\text{N-methylation}} \text{Adrenalin} (\text{see Appendix 2}).
\]

Some nervous tissue can only perform the synthesis to the formation of dopamine, some can carry on until noradrenalin, but the vertebrate adrenal medulla and amphibian sympathetic neurons can complete the process and produce adrenalin (Florey, 1966).
There is no evidence that the above mechanism occurs in insects, even though some species, such as *Tenebrio*, *Vanessa*, *Musca*, *Apis* (Östluud, 1954), and *Pieris brassicae* L. (von Euler, 1961), have been found to contain adrenalin and noradrenalin. Dopamine also has been identified in insects (Welsh, 1957), but so far there is no certain demonstration that these chemicals are natural neurohumors in insects.

It is known that mammalian sympathetic neurons release noradrenalin as a transmitter, and that the amphibian sympathetic neurons release adrenalin. The action of noradrenalin releasing neurons can be imitated only by noradrenalin, not by adrenalin; similarly, the adrenalin releasing neurons can only be imitated by adrenalin (Florey, 1966).

a. The action of adrenalin

Only a few experiments with adrenalin have been done with the insect hearts. Krijgsman and Krijgsman-Berger (1951) indicated a stimulating action of adrenalin with a threshold at \(10^{-7}\), the hearts stopping in systole at \(10^{-4}\). In vertebrates, adrenalin stimulates the heart by acting on the sympathetic nerve. Therefore, he suggested that the cockroach heart might also have an adrenergic property. This stimulating action can be paralyzed by ergotamine, which is a well known inhibitor of adrenergic systems. This was confirmed by the results of Naidu (1955).
The stimulating effect of adrenalin was also observed by Florey (1951) on Chaoborus larvae, Wixforth (1924) on Culex pipiens L. larvae.

There is no effect on the Anopheles heart (Jones, 1956), no effect on the Telea moth (McCann, 1962, cited from Jones, 1964), and no effect on the denervated cockroach heart (Miller and Metcalf, 1968).

However, Davenport (1949) noted that $10^{-6}$ adrenalin is inhibitory to the heart of Stenopelmatous and causes immediate diastolic arrest at $10^{-5}$, this stoppage recurring periodically even after washing with saline.

The stimulating action also can be partially antagonized by rotenone but not by nicotine or atropine (Naidu, 1955).

b. The action of dopamine

Dopamine will elevate the rate of beating of the isolated heart of P. americana with a threshold at $10^{-8}$ M. This stimulation is not inhibited by semicarbazide (which inhibites dopa) but is antagonized by 2-bromo derivative of LSD (BOL) (Davey, 1963). This is similar to the action of serotonin, which may have the same action on the heart muscle.

Dopamine increased the rate of beating of the denervated
cockroach hearts, which suggested that it acts on the myocardium (Miller and Metcalf, 1968).

McFarlane (1967) indicated that dopamine accelerated the young adult cricket heart and inhibited the old heart.

3. 5-Hydroxytryptamine

The action of 5-hydroxytryptamine (5-HT) had been known for years before it was identified. This compound used to be called enteramine and serotonine. The latter name is still commonly used (Florey, 1966).

It occurs in the nervous system of some vertebrates and invertebrates (Welsh and Moorhead, 1960). There is some suggestive evidence that 5-HT is the transmitter substance of certain animals (Florey, 1966).

The pharmacological studies of 5-HT are almost limited to vertebrates and molluscs (Florey, 1966). 5-HT has circulatory effects on mammals varying from species to species (Prosser and Brown, 1962).

Molluscan hearts are said to be more highly sensitive to 5-HT than any others. Sugi and Matsunami (1966) found that 5-HT stimulates the visceral pacemaker of the Ciona intestinalis, but reduced the hypobranchial pacemaker.
There is evidence that some insects such as the cockroach contains 5-HT (Colhoun, 1963). But pharmacological studies related to 5-HT of insect heart are very few. Davey (1961b) found that 5-HT accelerates the heartbeat of the cockroach P. americana with a threshold at $10^{-8}$ M. The action of 5-HT is antagonized by LSD (lysergic acid diethylamide) in vertebrates, but in the cockroach LSD itself brings about a stimulation in heartbeat leading to systolic arrest at high concentrations (Davey, 1964). The effect of 5-HT on the cockroach is antagonized by BOL (Davey, 1961b).

Miller and Metcalf (1968) studied the denervated cockroach heart and found that the denervated heartbeat was increased after perfusing with 5-HT.

McFarlane (1967) found that the 5-HT acts like Ach tending to inhibit the old cricket hearts and to accelerate the young adult cricket hearts.
III. MATERIALS AND METHODS

The house cricket, *A. domesticus* was cultured using the method of Ghouri and McFarlane (1958). Adults were maintained at 28 ±2°C. and 50% R.H. in groups of 15–20 in one gallon glass jars. Oviposition dishes were placed in the jars at weekly intervals to permit the females to oviposit regularly.

A. HISTOLOGICAL STUDY

The heart and associated tissues were studied by vital staining and by sections of the adult animals.

1. Vital Staining

   a. Rongalite white

For studying the innervation of the heart and the distribution of the alary muscle Rongalite white was used. This kind of leuco methylene blue has more advantages than the ordinary methylene blue because the latter tends to stain indiscriminately. The formula is as follows: Methylene blue 0.5% - 100 ml, concentrated Hydrochloric acid - five drops and Rongalite - three grams. (Rongalite is composed of various hydrosulfite and formaldehyde combinations and can be obtained from Edward Gurr Ltd., London, England.). The acid is added to the Methylen blue solution and then the Rongalite (procedure from ESBE
Laboratory Supplies, Toronto, Canada). The mixture is warmed until it changes color from deep blue to pale yellow or colorless. This takes about five minutes. The mixture is then cooled and filtered. It is necessary to allow the mixture to stabilize for a few days before use. It is stored in the refrigerator as a stock solution.

The usual procedure followed began with the injection of one part of the above stock solution to three parts of modified Bélar's Ringer (Buck, 1953). (The composition of the Ringer was: 9 g NaCl, 0.2 g CaCl₂, 0.2 g KCl, 0.2 g NaHCO₃, and 1 g glucose in one liter glass distilled water.) The injection was made through the pleural membrane into the abdomen with a gauge-27 hypodermic needle. Into each cricket about 0.1 cc was injected, the amount depending roughly on the size of the insect.

The colorless Rongalite white became blue again (oxidized) in the cricket body. In most instances, about five hours was sufficient for a satisfactory distribution of the stain, but usually the specimens were left overnight. Injection of this solution, when carefully done, rarely caused death within a day, while many insects lived for several days without apparent ill effect.
The crickets were then dissected by cutting along the pleural membrane on one side of the abdomen and extending into the anterior end of the thorax. Unwanted viscera were removed carefully (Fig. 1). The carcass was placed in 8% ammonium molybdate for at least 12 hours to fix the methylene blue. After fixation, it was placed in running tap water for ten hours or longer to wash out the excess ammonium molybdate.

The specimens were then transferred directly to 100% alcohol for 12 hours, then xylene for 12 hours, and mounted whole in Canada balsam or from 100% alcohol into Euparol and examined under the dissecting microscope.

Some specimens were pinned on transparent Glycerine-Jelly slides for study at higher magnifications.

It was found desirable to starve the insects for a few days before use, as the fatty tissue was much reduced and this was an advantage in studying the segmental nerves.

b. Indian ink

The injection of 3% Indian Ink in Ringer solution gave a very good demonstration of the phagocytic organs. After three hours, the animals were dissected as before and transferred to 100% alcohol without washing and then to xylene and Canada balsam or from fresh dissections to glycerine.
2. Section Staining

For the detailed study of the associated organs of the heart, serial cross and longitudinal sections were made.

The insect was cut along both pleural membranes; therefore, only the dorsal part was observed. The specimens were fixed in alcoholic or aqueous Bouin's fixative, washed, dehydrated, and embedded in paraffin wax.

The sections were cut at 3 μ to 10 μ.

The stains used were: 1) Delafield's Hematoxylin and Eosin, 2) Ehrlich's Hematoxylin and Eosin, 3) Iron Hematoxylin and Orange G, 4) Silver Nitrate, 5) Mallory's triple stain, 6) Thionin, 7) Van Giesen, 8) Acid fuchsin.

Generally speaking, Mallory's triple stain gave the best results for general purposes. The procedure of Pantin (1964) was used, except that the time was cut to 5 sec. in 1% acid fuchsin, 10 sec. in 1% phosphomolybdic acid, and 10 sec. in Mallory's stain.

B. PHARMACOLOGICAL STUDY

Both young and old adult house crickets were studied. The 'young' heart referred to in this thesis is actually the heart
of the two-day-old adult, and the 'old' heart is from the
eight-week-old adult. It was found through preliminary experi­
ments that the eight-week-old heart from insects reared under
prevailing laboratory conditions matched pharmacologically
with McFarlane's (1967) six-week-old heart.

The method used was the same as previously described by
McFarlane (1967). The semi-isolated heart was prepared as for
the above histological study. This preparation consisted of
only the dorsum of the abdomen and the thorax together with
the dorsal diaphragm containing the heart, phagocytic organs,
alar muscles, pericardial cells and some other tissues. This
was therefore isolated from any central nervous effects or
hormonal control. Then the preparation was pinned cuticle
side down to a microscope slide covered with paraffin wax
(Fig. 2), and this slide was transferred immediately into an
aerated Ringer solution bath (Fig. 3). A bank of four baths
was prepared. The Ringer solution was changed after 30 min.
The heart was observed through a travelling microscope and
the rate was recorded by using a stop watch.

In most cases the 'normal' rate was established after one
hour from operational shock. The 'normal' rate was the latter
count of two consecutive counts, for one minute each, which
had a range not greater than two beats per minute.
After the 'normal' rate was established, the test drug (or an equal volume of Ringer solution alone in the control group) was added to the bath and the observations immediately repeated. A change in rate of 5% was considered significant.

The test drugs were Acetylcholine chloride (ACh), Physostigmine (Eserine), Nicotine, Atropine sulfate (Atropine), Noradrenalin bitartrate (Noradrenalin), Arecoline HBr (Arecoline), 3,4-dihydroxyphenylethylamine HCl (Dopamine), d-tubocurarine chloride pentahydrate (Curare) and 5-hydroxytryptamine creatinine sulfate monohydrate (Serotonin). The first three were obtained from British Drug Houses (Canada) Limited, and the last five from Nutritional Biochemicals Corp. Curare was obtained from two sources: Sigma Chemical Co., and Nutritional Biochemicals Corp.

The concentrations used for most test drugs were from $10^{-8}$ to $10^{-4}$ M and these drugs were diluted in Bélár's Ringer solution. All tests were conducted at room temperature and each heart was used for only one test.
FIGURE 1 and FIGURE 2
FIGURE 3.

Equipment for the pharmacological study.

A.) Heart in Bêlår's Ringer bath

B.) Travelling microscope

C.) Air pump
IV. RESULTS AND DISCUSSION

A. HISTOLOGY

1. The Dorsal Vessel

As in all other insects, the dorsal vessel of the house cricket is the principal organ of circulation. It is a delicate, transparent tube, usually straight but sometimes describing a wavy course. In comparison with other insect hearts, it can be considered of a simple typical type. It lies immediately under the hypodermis of the mid-dorsal line, extending from the ninth abdominal segment and tapering down anteriorly to the thorax. It ends blindly at the posterior end (Fig. 4). The total length is about 2 cm.

In the dissected specimen, the dorsal vessel in the prothorax and mesothorax is very difficult to follow because it is concealed by strongly developed thoracic muscles and abundant fat tissue. It is impossible to move all these unwanted tissues without disturbing the delicate dorsal vessel. However, this can be shown with sections. The dorsal vessel in the prothorax is far from the body wall and is surrounded by masses of fat cells and muscles (Fig. 5). The dorsal vessel in the mesothorax moves dorsally to the body wall (Fig. 6), but its ventral side is still surrounded by thick thoracic muscles.
In the metathoracic part, it is surrounded by a thin layer of muscle and fat tissue.

The dorsal vessel in the thorax is a simple tube without chambers (Fig. 7), and is smaller in diameter than in the abdomen, except for two pairs of enlargements present in the mesothorax and metathorax. As seen in sections, the narrow dorsal vessel of the thorax expands very widely in these two parts.

Dorsally, the dorsal vessel of the abdomen may make contact with the body wall directly without any special connections or filaments. Or there may be some space or fat tissue between the body wall and heart wall, the pericardial cells being scattered in this area. Ventrally the dorsal vessel makes contact with the dorsal diaphragm membrane directly (Fig. 8).

Some insects are said to be without a striated muscular dorsal vessel. The dorsal vessel of the cricket is definitely composed of striated muscle (Fig. 9). This is arranged in a single circular layer. Several large nuclei may be seen in this muscular layer. There is no histological difference between the dorsal vessel wall of the thorax and abdomen (Fig. 10).
The blood cells are usually found to lie free in the lumen of the dorsal vessel or attached to the inner side of the muscular layer (Fig. 11).

The dorsal vessel is accompanied by a pair of dorsal longitudinal tracheae.

In the Rongalite white preparation two to four rows of small blue areas appear on the surface of the dorsal vessel (Fig. 12), which are similar to those on the heart of mosquitoes under hematoxylin stain and which were called 'pigment cells' by Jones (1954). No corresponding structures appear in sections of the heart. The nature of these structures is quite unknown.
FIGURE 4 and FIGURE 5
FIGURE 4

Horizontal longitudinal section, showing the posterior end of the heart. Am. alary muscle; Ep. epiproct; Ht. heart; Pc. pericardial cell. 300X.

FIGURE 5

Cross section, showing that the dorsal vessel in the prothorax is surrounded by abundant fat cells. 1200X.
FIGURE 6 and FIGURE 7
FIGURE 6.
Cross section, showing the dorsal vessel in the mesothorax.
F. fat cell; M. muscle. 300X.

FIGURE 7.
Longitudinal (Sagittal) section of the dorsal vessel in the thorax. 120X
FIGURE 8 and FIGURE 9
FIGURE 8.
Cross section of the dorsal vessel in the abdomen.
Bw. body wall; Dd, dorsal diaphragm membrane; Ht. heart.
300X.

FIGURE 9
Cross section, showing the striation of the heart wall.
1,200X.
FIGURE 10 and FIGURE 11
FIGURE 10
Horizontal longitudinal section, showing the single circular muscular layer of the dorsal vessel in the thorax. 600X.

FIGURE 11.
Cross section, showing some blood cells (arrow) attached to the heart wall. 1,200X.
FIGURE 12 and FIGURE 13
FIGURE 12.
Rongalite white preparation, showing the blue square (arrow) on the surface of the heart wall. 1,200X.

FIGURE 13
Horizontal longitudinal section, showing the ostial valves. 1,200X.
2. The Ostial Valves

In all the fresh or prestained dissection specimens examined, the ostial valves were very difficult to detect, but in other insects, such as the cockroach, the ostial valves are very easily seen even with a fresh non-stained dissection specimen. Only in a few cases can the ostial valves of the house cricket be seen very clearly with the Rongalite white preparation; two slit-like openings with two pairs of lip-like valves were seen. The ostia are located laterally, the ostial valves extending inwards, then forwards. When the heart contracts, these valves closed together during systole and are forced apart during distole. The blood then runs into the heart but not out through the ostia.

However, there is no difficulty in detecting the ostial valves in sections. Longitudinal, horizontal and cross sections clearly show that the valves are simply extensions of the heart wall (Fig. 13, Fig. 14). Therefore, as part of the heart wall, these ostial valves also consist of striated muscles. Sometimes the dorsal vessel becomes wider at the portion with the ostial valves (Fig. 15). Two consecutive ostial valves divide the simple dorsal vessel into chambers. No ostial valves can be found in the thorax.
FIGURE 14 and FIGURE 15
FIGURE 14.

Cross section, showing the ostial valves. 1,800X

FIGURE 15.

Horizontal longitudinal section, showing that the heart becomes wider in the region of the ostia. 1,200X.
3. The Dorsal Diaphragm

a. The diaphragm membrane

The dorsal diaphragm membrane of the house cricket is not much different from that of most other Orthoptera. It is a very thin, well-developed, fenestrated web with a serrate edge (Fig. 16). This membrane is quite broad, occupying half of the dorsal width, spreading among the alary muscles and all the area between the alary muscle. The membrane ends posteriorly in the ninth abdominal segment, just below the cardial end of the dorsal vessel, and stretches forwards into the mesothoracic part.

The margins of the diaphragm membrane are narrowed into points and, combined with alary muscles, attach to the front border of the terga. Thus, this membrane separates a definite pericardial sinus from the hemocoel.

From sections, this diaphragm membrane appears as a thin membrane, making contact with the ventral side of the dorsal vessel directly (Fig. 8).
FIGURE 16.

Whole mount, showing the fenestrated (arrow) diaphragm membrane. 300X.
b. The alary muscles

The dorsal vessel of the house cricket is held in position mainly by the alary muscles and abundant connective tissue fibers. In total, there are 11 pairs of alary muscles, nine pairs in each of the first nine abdominal segments, two pairs in the mesothorax and metathorax. The metathoracic pair is the longest and widest one, but it seems weaker than the others. The last pair is the smallest one; the muscles join each other at the caudal end, just below the heart tube, and unite with the diaphragm membrane.

These alary muscles are arranged very symmetrically, one on each side of the heart in every segment (Fig. 17), on the other hand, the alary muscles of many other insects appear very irregular. The alary muscle begins at a point on the anterior tergal border close to the intersegmental fold. From this attached point, the muscle expands into a triangular fibrous sheet, and connects with bundles of connective tissue fibers, which then branch and anastomose until they meet the ventral surface of the heart wall (Fig. 18). Actually, one pair of alary muscles crosses two segments.

Mallory's triple stain gave a striking distinction between the muscular portion and the connective tissue fibers.
The muscular portion stains red and the connective tissue portion stains blue. The red portion at the attachment is deeper than at the base which is close to the heart surface. This demonstrated that the muscular part is concentrated at the apex (Fig. 19).

The alary muscle resembles any other body muscle of insects, that is, in being striated. This striation is hard to see when the alary muscle is attached to the body wall. After cutting the alary muscles free from the attached point, the muscles shortened into wide bands. Then, in this relaxed state, the cross striation became very distinct (Fig. 20). The large elongate nuclei are separate and easily seen.

The alary muscles and the connective tissue fibers are invested by numerous pericardial cells.
FIGURE 17 and FIGURE 18
FIGURE 17.
Whole mount, showing the symmetrically arranged alary muscles. 300X.

FIGURE 18.
Whole mount, showing the network of connective tissue fibers at the ventral surface of the heart wall, Ht, heart. 1,200X.
FIGURE 19 and FIGURE 20
FIGURE 19.
Whole mount of alary muscles, showing that the muscular part is concentrated at the apex. 300X.

FIGURE 20.
The striation of the alary muscles. 1,200X.
4. The Pericardial Cells

The pericardial cells are always intermingled with other tissues under ordinary circumstances and are not easily distinguished from other cells. But they can be easily distinguished from any other tissues with certain dyes. As with other insects, the pericardial cells of the house cricket also have a characteristic appearance.

In the natural condition, these cells are colorless. With Mallory's triple stain, the cytoplasm stains purplish-blue and the nucleus stains red. The whole cell is ovoid or kidney-shaped. They are very large cells, being approximately 40 μ in diameter. Most of them are monocleate or binucleate (Fig. 21), but some of them have three nuclei. The periphery of the cell stains intensely because it contains many more inclusions than the central reticular network region. A clear area is always found around the central spherical nucleus or nuclei. A single nucleolus and other large granules also can be seen in the nucleus (Fig. 22).

These cells are discrete or irregularly loosely packed together in the pericardial sinus, while the pericardial cells of certain other insects, e.g. Drosophila melanogaster are serially arranged in 20-25 rows on each side of the heart.
The size of this cell in the house cricket is more or less the same from the anterior to the posterior end of the dorsal vessel, but in many other insects the pericardial cells in the abdomen are larger than in the thorax or head, e.g. aphids *Myzus persicae* Sulz (Bowers, 1964) and fruit flies (Miller, 1965).

The number of pericardial cells in the thorax is much less than in the abdomen.

Numerous pericardial cells are enmeshed in and attach to the alary muscle fibers and fine connective tissue fibers. This can be seen clearly with the Rongalite white whole mount.

Most of the pericardial cells are scattered in the area which is covered by the dorsal diaphragm, but some of them can extend to other parts beyond the dorsal diaphragm.

The pericardial cells occur chiefly on the lateral sides of the heart wall, and are also scattered along the ventral side, but only few of them appear dorsally. Moreover, they only appear outside the heart wall, and have never been found in the lumen of the heart, as in *Nepa* (Hamilton, 1931), in which the pericardial cells are located both inside or outside of the heart.
The arrangement of the pericardial cells in the house cricket heart is quite different from individual to individual. But they do not appear to migrate appreciably, and it is believed that they occupy fairly constant positions in the body cavity.

The rapid removal of dyes shows the phagocytic characteristic of the pericardial cells, a property common to these cells in other insects.
FIGURE 21 and FIGURE 22
FIGURE 21
Cross section, showing the mononucleate and binucleate pericardial cells. 3,000X.

FIGURE 22.
Pericardial cells, showing the nucleolus and large granules in spherical nucleus, G, granule; N, nucleus; N1, nucleolus.
5. The Phagocytic Organs

The simple tubular heart of house cricket is complicated by the presence of phagocytic organs (ventral diverticula). These phagocytic organs are very well developed in all the post-embryonic stages (Fig. 23).

Normally, there are three pairs, which are located from the first abdominal segment to the third abdominal segments. They are more or less opposite to one another on each side of the heart. The first pair is elliptical in shape, and much smaller than the next two pairs (Fig. 24). The second and third pairs are very large, arising from a position near the heart and extending laterally to the edge of the dorsal diaphragm. Near the heart they are narrow but become enlarged toward their distal ends to form a triangular fan shape. Both anterior and posterior edges lie on the borders of the same segment. Therefore, they just fill the spaces between two adjacent pairs of alary muscles.

In many individuals, there are only two pairs, the first aborted pair being completely absent (Fig. 25). Occasionally, there are three and a half or four pairs. The abnormal extra organ (or organs) usually occurs in the fourth or fifth abdominal segment (Fig. 26), but they might occur anywhere. This
is not in agreement with the observations of Kowalevsky (1894), who found only two pairs of these organs in the house cricket, and these were located in the first and second abdominal segments.

The organs may be demonstrated particularly clearly after the injection of Indian ink. The phagocytic organs take up the ink from the hemolymph and clean the hemocoele much like a scavenger. The longer the time interval allowed, the cleaner the body cavity would be (Fig. 27).

Definitely, the phagocytic organs are not the same as segmental vessels. An additional experiment shows even the external aspects are quite different. After injection of Rongalite white into the cockroach (P. americana), the segmental vessels remain colorless while several fine blue nerve fibers run the full length of the vessel. This exactly corresponds to the results of McIndoo (1945). In the cricket, the phagocytic organs appear deep blue in color without any colorless area. They are also different in sections, the segmental vessel having a muscular wall, while the phagocytic organ is limited by a membranous pocket. No nerves can be found in the phagocytic organs of the cricket.

Contrary to the finding of Nutting (1951), the surfaces of the phagocytic organs of the cricket are well supplied with
fine tracheoles. Therefore, they are metabolically quite active.

In cross section, the phagocytic organs appear as long tapering sacs (Fig. 28). There is an opening from the heart leading to the sac (Fig. 29). Phagocytic cells compose the tissue. They are so compact that no cell boundary can be clearly seen. The ventral side of the organs is always united with the dorsal diaphragm. It is here suggested that the blood percolates through these phagocytic cells, then diffuses out through the membranous walls. This agrees with Nutting's (1951) suggestion that the phagocytic organs serve as blood filters.

After trying several stains, such as Ehrlich's Haematoxylin and Eosin, Mallory's triple stain or Van Giesen, there is the impression that the phagocytic cell somewhat resembles the pericardial cell because it always took up the same stains. The phagocytic cells are smaller and more irregular than the pericardial cells, but with one or more nuclei bigger than the nuclei of the pericardial cell. The cytoplasm is also denser than the cytoplasm of the pericardial cell.
That the phagocytic organs are haemopoietic was suggested by Kowalevsky (1894). Unfortunately, lacking physiological evidences, one can only consider these organs as being phagocytic.
FIGURE 23 and FIGURE 24
FIGURE 23.
Indian ink injection, showing the phagocytic organs in various post-embryonic stages.
A.) 20-day old nymph
B.) 30-day old nymph
C.) 40-day old nymph
D.) Adult

FIGURE 24
Indian ink injection, showing three pairs of phagocytic organs located in the first three abdominal segments.
20X.
FIGURE 25 and FIGURE 26
FIGURE 25.

Indian ink injection, showing two pairs of phagocytic organs located in the 2nd and 3rd abdominal segments. 10X.

FIGURE 26.

Indian ink injection, showing some abnormal extra phagocytic organs 1.5X.
FIGURE 27 and FIGURE 28
FIGURE 27.

Phagocytic organs after different intervals from injection with Indian ink. Left: one hour after injection; right: three hours after injection. 1.5X.

FIGURE 28.

Cross section, showing the sac-like phagocytic organ. 1,500X.
FIGURE 29
FIGURE 29.

Cross section, arrow showing an opening from the heart leading to the phagocytic organ. 300X.
6. Innervation

It has been known for a long time that methylene blue or its modifications works capriciously, but when it works it gives very beautiful results. Therefore, this vital stain is still the most commonly used method for gross innervation. It has been used recently, for example, by Plotnikova (1967) for the innervation of the gut of Locusta migratoria L., Seabrook (1968) for the innervation of the terminal abdominal segments of the Schistocerca gregaria (Forskal) and Finlayson and Osborn (1968) for the abdominal segmental nerves of the C. morosus.

In the house cricket, after Rongalite white treatment, both nerve and muscle took up the stain, but the nerve was much more obvious than the muscle. Two lateral cardiac nerve trunks were easily to be seen running along either side the length of the heart, and fusing into one at its posterior end, i.e. at the 9th abdominal segment.

The nerve cell bodies of the lateral cardiac nerve trunks could be seen sometimes very clearly with this preparation (Fig. 30), but it was never possible to see all of such nerve cells in one preparation.
There are three ganglia of the ventral nerve cord located in the thorax and five ganglia in the abdomen. There are 12 pairs of segmental nerves, three in the thorax and nine in the abdomen. All the segmental nerves spring from the ventral nerve cord and finally join with the lateral cardiac nerves (Fig. 31). The position of these ventral ganglia is shown in Figure 31. The ganglion which is located in the metathorax gives nerves to the metathorax and to the 1st and 2nd segments of the abdomen. The ganglion which is located in the 1st abdominal segment gives off nerves to the 3rd abdominal segment. The ganglia which are located in the 3rd, 4th and 5th abdominal segments give off nerves to 4th, 5th and 6th abdominal segments. The terminal large ganglion, which increase about 40-fold in volume in the adult as compared with the first instar (Gymer and Edwards, 1967), is located in the 7th segment where it borders on the 8th segment.

This arrangement of ventral abdominal ganglia agrees with the description for the same insect by Panov (1966). The transposition of the ganglia of the nerve cord during embryonic and postembryonic development of *A. domesticus* has been studied by Panov and is shown in Figure 32.

If the ontogenetic development of the ventral nervous system in the house cricket is as above, then it is clear that the
metathoracic ganglion is the fusion of metathoracic and 1st and 2nd ganglia. The following ganglia, i.e. 3rd, 4th, 5th, and 6th move from their original places but still give nerves back to the original segments. The final abdominal ganglion appears to result from the fusion of the 7th, 8th, and 9th ganglia because no 10th segmental serve can be found in A. domesticus. The relation between the segmental nerves and their corresponding nerve cords is shown in Figure 31.

The rami of the segmental nerves innervating the dorsal muscles and alary muscles is shown in Figure 33.

The nerve cell bodies are very easily demonstrated by Ehrlich's Hematoxylin & Eosin or by Silver Staining because of their intense staining and very large size. They are always a little distance from the heart wall (Fig. 34), but their processes reach the heart wall directly. The smallest fibers were not observed in the sections.
FIGURE 30.
Whole mount after Rongalite white injection, showing the lateral nerve and the cardiac nerve cell (arrow). 600X.
FIGURE 31.

Diagram of dorsal vessel and ventral nervous system, showing the relation between the segmental nerves and their corresponding nerve cords. Tl - T11, pro-, meso-, and metathorax; A1 - A9, first to ninth abdominal segments. Ht, heart; Ln, lateral nerve; Nc, nerve cell; Sn, segmental nerve; Vg, ventral ganglion; Vc, ventral cord.
FIGURE 32.
FIGURE 32.

Transposition of the nerve cord during embryonic and post-embryonic development (redrawn after Panov, 1966).
FIGURE 33 and FIGURE 34
FIGURE 33.
Rongalite white injection, showing the segmental nerve branch to the dorsal muscles. 300X.

FIGURE 34.
Cross section, showing a large nerve cell (arrow). 1,200X.
B. PHARMACOLOGY

1. The Normal Beat

In the semi-isolated preparation, the house cricket hearts stop or beat weakly and irregularly at the beginning, due to post-operative shock. In most cases, it begins to beat more strongly and more regularly after one hour of recovery. This 'normal' beat can be maintained for four to five hours (or even longer) in Bélár's Ringer solution. The pumping mechanism was observed as rhythmic systolic and diastolic movements of the heart wall.

As mentioned in methods, the heart, prepared in this way, is isolated from the central nervous system and therefore is influenced only by the autonomic centers in the lateral cardiac nerves. This preparation also presumably is without any hormonal effect.

A number of factors can affect the heartbeat, such as activity, starvation, drugs, size, PH, temperature, etc. So, different individuals may have different heartbeats; or the same individual may produce different heartbeats under different conditions; but a given individual always has a fairly
constant beat under the same conditions. The following pharmacological studies were conducted under as similar conditions as possible, therefore making large numbers of animals unnecessary.

Generally speaking, the average rate of beating of young hearts was approximately 76 beats per minute, as determined from 84 insects; the average rate of old hearts was 87 beats per minute, as determined from 85 insects. That is, the old hearts usually beat faster and also stronger than the young hearts in Ringer solution and at room temperature. A simple average does not, however, consider the distinct impression that age differences were practically nil during the summer months, but more pronounced during the winter.

All the present results demonstrated that Bêlâr's Ringer solution is very suitable for the house cricket. Experiments showed that the cricket hearts also beat very well without any adverse effect in 'two times calcium' Bêlâr's Ringer, but not in 'four times calcium'. The latter high calcium caused the heart beats to be irregular and weak or even stop. But this weakness can be reversed by increasing the potassium five times, simply due to the antagonism between these monovalent and divalent cations.
There was a difference in the temperature response of young and old hearts. At $23 \pm 0.5^\circ\text{C.}$, there was no significant difference between the heartbeats of the young or old crickets. On the other hand, there was a highly significant difference (significant at the 1% level, Table 1) between the young and the old hearts at a temperature of $26 \pm 0.5^\circ\text{C.}$, the old heart beating faster. These results, as much as any other, show that the old heart is basically different from the young heart.
### TABLE 1

**EFFECTS OF TEMPERATURE ON YOUNG AND OLD HEARTS**

<table>
<thead>
<tr>
<th>Temperature</th>
<th>23 ± 0.5°C.</th>
<th>26 ± 0.5°C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Young</td>
<td>Old</td>
</tr>
<tr>
<td>Replication</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>77</td>
<td>58</td>
</tr>
<tr>
<td>2</td>
<td>69</td>
<td>86</td>
</tr>
<tr>
<td>3</td>
<td>73</td>
<td>62</td>
</tr>
<tr>
<td>4</td>
<td>67</td>
<td>75</td>
</tr>
<tr>
<td>5</td>
<td>80</td>
<td>67</td>
</tr>
<tr>
<td>6</td>
<td>62</td>
<td>77</td>
</tr>
<tr>
<td>7</td>
<td>94</td>
<td>83</td>
</tr>
<tr>
<td>8</td>
<td>73</td>
<td>70</td>
</tr>
<tr>
<td>9</td>
<td>77</td>
<td>56</td>
</tr>
<tr>
<td>10</td>
<td>76</td>
<td>67</td>
</tr>
<tr>
<td>11</td>
<td>62</td>
<td>78</td>
</tr>
<tr>
<td>12</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>92</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>82</td>
<td></td>
</tr>
</tbody>
</table>

Total: 810 1233 1215 1368
n: 11 16 15 14
Mean: 74 77 81 98
S.D.: 9.1 10.0 11.4 14.3

t: 0.301 (P = 0.8 < 0.7) ** N.S. 3.479 (P = 0.01 < 0.001)**

**N.S.** No significant difference

** Highly significant difference
2. The Action of Acetylcholine

As shown in Table 2, Figure 35, Figure 36, the young cricket hearts started to be accelerated at $10^{-6}$ Ach (16.8 ± 4.2%) and the old hearts tended to be inhibited at the same concentration (8.3 ± 1.2%). At $10^{-5}$, all the young hearts were accelerated (34.0 ± 7.4%); only three of the eight old hearts increased in rate, but five old hearts were inhibited (20.1 ± 4.5%). None of the young hearts were inhibited at $10^{-5}$ Ach. In other words, the young heart is very sensitive to acceleration, and the old heart is sensitive to inhibition. This experiment is essentially a repetition of McFarlane's (1967) work.

It was mentioned that in the Bêlôr's Ringer solution, the young hearts beat slower and less regularly than the old ones. After treatment with Ach, the young hearts beat more regularly and increased both in frequency and amplitude, that is, the young hearts tended to be similar to the old hearts.

Histologically, no difference can be found between the old and the young hearts, under the light microscope. There might be some difference under the electron microscope, particularly in the cardiac neuron membranes. It is possible that, in the young heart, Ach acts on the postsynaptic membrane of the cardiac ganglionic cell and alters the membrane properties
so that it increases the permeability of small cations (such as sodium) so as to depolarize the membrane sufficiently for a propagated impulse to arise. The action on the old heart is difficult to understand, but it may be that Ach produces hyperpolarization and inhibition of the rhythmic beat, in the same way as it affects the myogenic vertebrate heart, or it may act directly on the myocardium.

When the concentration of Ach was increased beyond $10^{-4}$, some of the young hearts were arrested in systole. This is probably due to 'Ach block'.
**TABLE 2**

**EFFECTS OF ACH ON SEMI-ISOLATED HEARTS OF A. DOMESTICUS**

<table>
<thead>
<tr>
<th>Age</th>
<th>Total No.</th>
<th>'Normal' Average</th>
<th>Conc.</th>
<th>Accelerated No.</th>
<th>Mean ± S.E.</th>
<th>Inhibited No.</th>
<th>Mean ± S.E.</th>
<th>Unaffected No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young</td>
<td>8</td>
<td>74</td>
<td>$10^{-6}$</td>
<td>5</td>
<td>16.8 ± 4.2%</td>
<td>0</td>
<td>--</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$10^{-5}$</td>
<td>8</td>
<td>34.0 ± 7.4%</td>
<td>0</td>
<td>--</td>
<td>0</td>
</tr>
<tr>
<td>Old</td>
<td>8</td>
<td>97</td>
<td>$10^{-6}$</td>
<td>1</td>
<td>6.4</td>
<td>4</td>
<td>8.3 ± 1.2%</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$10^{-5}$</td>
<td>3</td>
<td>12.0 ± 0.8%</td>
<td>5</td>
<td>20.1 ± 4.5%</td>
<td>0</td>
</tr>
</tbody>
</table>
FIGURE 35.
The percentage of hearts of both young and old which were accelerated by two concentrations of acetylcholine.
PERCENT ACCELERATED

ACH CONC.
FIGURE 36.
The percentage of hearts of both young and old which were inhibited by two concentrations of acetylcholine.
In Table 3, Figure 37, it is indicated that eserine itself at $10^{-5}$ M increased the heartbeat of some young hearts. It greatly potentiated the action of subsequently applied Ach without any latent period. It induced all young hearts to be accelerated at $10^{-8}$ M Ach. The percentage accelerated was raised proportionately to the increase of the concentration of Ach. This sharp rise in the frequency is also accompanied by an obvious increase in amplitude. All the young hearts stopped in systole at $10^{-5}$ M Ach. Some stoppage occurred after an irregular and fibrillar beat.

The old hearts were not so sensitive to eserine alone as the young hearts. It may be noted from Table 2 that the old hearts were not responsive to $10^{-5}$ M eserine alone. The action of subsequently administered Ach was also modified by prior treatment with eserine. Few of the hearts were accelerated, and the percentage increase was not as high as with the young hearts. One of the five was inhibited at $10^{-7}$ and $10^{-6}$ M Ach, and all of them were inhibited at $10^{-5}$ M, and stopped at $10^{-4}$ M.

Ach is known to be destroyed by esterases, particularly by cholinesterases. These cholinesterases (ChE) are divided into two groups: Acetylcholinesterase (AchE) and non-specific
(or pseudo-) cholinesterases (Florey, 1966). The AchE is one of the most active and specific enzymes for Ach. It can hydrolyze one Ach molecule in just 40 microseconds (Lawler, 1961). In other words, it can destroy 1,500,000 molecules Ach per minute. This rapid reaction is inhibited by several drugs called 'anticholinesterases'; eserine is a well-known anticholinesterase and more specific than others. Eserine inactivates AchE, preventing the hydrolysis of Ach, therefore the Ach accumulates immediately in synaptic zones and extends postsynaptic ganglionic membrane depolarization and therefore greatly intensifies its action.

The literature indicated that AchE has already been demonstrated to be present in many insects. It has not yet been demonstrated to occur in the cricket, but from the present results, there is little doubt that it is present.

The potentiation of Ach by eserine in young hearts is also shown in Table 4, Figure 38, Figure 39 once again. The threshold of Ach was lowered from $10^{-7}$ M to $10^{-8}$ M after eserinization by $10^{-6}$ M eserine. All young hearts completely stopped in systolic tetany when the Ach concentration reached $10^{-5}$ M. It should be noted that both $10^{-5}$ M and $10^{-6}$ M eserine caused a systolic tetany at $10^{-5}$ M Ach.
TABLE 3

EFFECTS OF ACH ON ESERINIZED SEMI-ISOLATED HEARTS OF A. DOMESTICUS

<table>
<thead>
<tr>
<th>Age</th>
<th>Total No.</th>
<th>'Normal' Average</th>
<th>Conc.</th>
<th>Accelerated (%)</th>
<th>Inhibited (%)</th>
<th>Unaffected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No.</td>
<td>No.</td>
<td>No.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mean ± S.E.</td>
<td>Mean ± S.E.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10^{-5} M Eserine</td>
<td>3</td>
<td>10.5 ± 2.4</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10^{-8} M Ach</td>
<td>5</td>
<td>14.7 ± 3.4</td>
<td>0</td>
</tr>
<tr>
<td>Young</td>
<td>5</td>
<td>73</td>
<td>10^{-7} M</td>
<td>5</td>
<td>21.6 ± 5.1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10^{-6} M</td>
<td>5</td>
<td>35.6 ± 6.6</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10^{-5} M</td>
<td></td>
<td></td>
<td>All Stop</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10^{-4} M</td>
<td></td>
<td></td>
<td>All Stop</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10^{-5} M Eserine</td>
<td>0</td>
<td>--</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10^{-8} M</td>
<td>0</td>
<td>--</td>
<td>1</td>
</tr>
<tr>
<td>Old</td>
<td>5</td>
<td>94</td>
<td>10^{-7} M</td>
<td>2</td>
<td>10.0 ± 1.6</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10^{-6} M</td>
<td>3</td>
<td>24.5 ± 6.2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10^{-5} M</td>
<td>0</td>
<td>--</td>
<td>2(49.0 ± 1.0) &amp; 3 stop</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10^{-4} M</td>
<td></td>
<td></td>
<td>All Stop</td>
</tr>
</tbody>
</table>
FIGURE 37.

Effect of acetylcholine on the rate of beating of eserinized young and old hearts.
TABLE 4
EFFECTS OF ACH ON CURARIZED AND ESERINIZED HEARTS OF A. DOMESTICUS

<table>
<thead>
<tr>
<th>Group</th>
<th>Total No.</th>
<th>&quot;Normal&quot; Average</th>
<th>Conc. of Ach</th>
<th>Accelerated (%)</th>
<th>Inhibited (%)</th>
<th>Unaffected No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>10^-8 M</td>
<td>No. Mean ± S.E.</td>
<td>No. Mean ± S.E.</td>
<td></td>
</tr>
<tr>
<td>Ringer</td>
<td>20</td>
<td>66</td>
<td>10^-8 M</td>
<td>1   7.0</td>
<td>0</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10^-7 M</td>
<td>5   10.3 ± 5.9</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10^-6 M</td>
<td>15  14.6 ± 2.3</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10^-5 M</td>
<td>18  24.1 ± 3.2</td>
<td>1 stop</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10^-4 M</td>
<td>13  50.0 ± 5.7</td>
<td>7 stop</td>
<td>0</td>
</tr>
<tr>
<td>Curare</td>
<td>20</td>
<td>67</td>
<td>10^-8 M</td>
<td>9   12.1 ± 3.0</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10^-7 M</td>
<td>18  15.1 ± 3.0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10^-6 M</td>
<td>19  21.1 ± 3.2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10^-5 M</td>
<td>2   31.6 ± 4.6</td>
<td>18 stop</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10^-4 M</td>
<td></td>
<td></td>
<td>All stop</td>
</tr>
<tr>
<td>Eserine</td>
<td>20</td>
<td>64</td>
<td>10^-8 M</td>
<td>14  12.9 ± 1.8</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10^-7 M</td>
<td>19  18.8 ± 2.6</td>
<td>1 stop</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10^-6 M</td>
<td>14  35.5 ± 3.3</td>
<td>6 stop</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10^-5 M</td>
<td>0   --</td>
<td>18 stop</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2 (18.9 ± 3.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10^-4 M</td>
<td></td>
<td></td>
<td>All stop</td>
</tr>
</tbody>
</table>
FIGURE 38
FIGURE 38.

Effect of acetylcholine on the rate of beating of eserinized and curarized young house cricket hearts.
FIGURE 39
FIGURE 39

Mean increase in rate of beating (with standard error) of acetylcholine-treated young hearts.
PERCENT INCREASE IN RATE

- eserine
- curare
- ringer

ACH CONC. (M)
b. The action of curare

i. potentiated the action of Ach in vitro

In the literature review, it was mentioned that curare is a well-known neuromuscular blocking agent in the vertebrate. It has also been clearly shown by several workers that curare antagonizes the action of subsequently applied Ach in certain insect hearts. The explanation for this interference is that curare alkaloids compete with Ach for its receptor. The process is probably analogous to the competitive inhibition of an enzyme, Ach being the normal substrate and curare the competitive inhibitor.

The first experiment with curare showed that with $10^{-4}$ M curare alone, there was no significant effect on either the old or young hearts (Table 5, Figure 40). Ach was then added immediately after curare counts were taken. It was found that there was no blockage to the applied Ach function at all, but instead an obvious potentiation. The threshold of Ach was lessened from $10^{-7}$ to $10^{-8}$ M, three of the ten young hearts being accelerated at $10^{-8}$ M Ach. The percentage of hearts accelerated increased in a manner proportional to the increments of the Ach concentration. All the young hearts were
accelerated at $10^{-6}$ M with a percentage of $30.5 \pm 1.7\%$.

Higher than $10^{-6}$ M, some of the young hearts started to stop in systole due to Ach block. None of the young hearts were beating at $10^{-4}$ M Ach.

The above stimulatory action of curare on the young cricket heart was again proved by a blind experiment. Three different treatments were done at the same time. The first group was treated with Bêlâr's Ringer solution followed by $10^{-8}$ M to $10^{-4}$ M Ach, the second and third groups were similarly treated after previous treatment with $10^{-6}$ M eserine and $10^{-4}$ M curare (Table 4, Figure 38). The results showed that with curarized hearts Ach was about $10 - 100$ times as effective as with the Bêlâr's Ringer control. It reacted in exactly the same pattern as the $10^{-6}$ M eserine, although the latter was more powerful than curare. Figure 39 shows the mean accelerating percentage at the different concentrations of Ach.

Longer treatments with curare for 0.5 hr. and 1 hr. also indicated a potentiating action of Ach.

Concentrations of curare up to $10^{-2}$ M still gave a positive potentiation of subsequently administered Ach.
An additional test showed that curare blocked the effect of Ach in *M. femur-rubrum* (Table 6, Figure 41) as in most other insects. Therefore, the curare acts differently in different insects even in the same order.

However, the potentiating action of curare was not apparent on the old house cricket heart.

One possible explanation for this result may be that curare in the young house cricket heart acted as an anticholinesterase in a direct manner. This action of curare on cholinesterase has been studied by various workers (see e.g. Župančić, 1965).
### TABLE 5

**EFFECTS OF \( \text{ACH} \) ON CURARIZED SEMI-ISOLATED HEARTS OF \( \text{A. DOMESTICUS} \)**

<table>
<thead>
<tr>
<th>Age</th>
<th>Total No.</th>
<th>'Normal' Average</th>
<th>Conc.</th>
<th>No.</th>
<th>Mean ± S.E. (%)</th>
<th>No.</th>
<th>Mean ± S.E. (%)</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young</td>
<td>10</td>
<td>67.5</td>
<td>( 10^{-4}) M Curare</td>
<td>0</td>
<td>--</td>
<td>0</td>
<td>--</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>( 10^{-8}) M Ach</td>
<td>3</td>
<td>6.5 ± 0.6</td>
<td>1</td>
<td>5.6</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>( 10^{-7}) M Ach</td>
<td>7</td>
<td>19.0 ± 4.2</td>
<td>0</td>
<td>--</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>( 10^{-6}) M Ach</td>
<td>10</td>
<td>30.5 ± 1.7</td>
<td>0</td>
<td>--</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>( 10^{-5}) M Ach</td>
<td>8</td>
<td>43.1 ± 8.3</td>
<td>1 Stop</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>( 10^{-4}) M Ach</td>
<td>-----</td>
<td>-----</td>
<td>-------</td>
<td>-------</td>
<td>-----</td>
</tr>
<tr>
<td>Old</td>
<td>9</td>
<td>75</td>
<td>( 10^{-4}) M Curare</td>
<td>0</td>
<td>--</td>
<td>0</td>
<td>--</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>( 10^{-8}) M Ach</td>
<td>1</td>
<td>6.1</td>
<td>1</td>
<td>6.2</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>( 10^{-7}) M Ach</td>
<td>1</td>
<td>13.0</td>
<td>0</td>
<td>--</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>( 10^{-6}) M Ach</td>
<td>4</td>
<td>12.7 ± 4.0</td>
<td>1</td>
<td>10.8</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>( 10^{-5}) M Ach</td>
<td>1</td>
<td>47.0</td>
<td>5</td>
<td>72.0 ± 16.9</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>( 10^{-4}) M Ach</td>
<td>-----</td>
<td>-----</td>
<td>-------</td>
<td>-------</td>
<td>-----</td>
</tr>
</tbody>
</table>
FIGURE 40
FIGURE 40.

Effect of acetylcholine on the rate of beating of both curarized young and old hearts.
### TABLE 6

**EFFECTS OF CURARE AND ACH ON HEARTS OF M. FEMUR-RUBRUM**

<table>
<thead>
<tr>
<th>Group</th>
<th>Total No.</th>
<th>'Normal' Average</th>
<th>Conc.</th>
<th>Accelerated</th>
<th>Inhibited</th>
<th>Unaffected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No.</td>
<td>Mean ± S.E.(%)</td>
<td>No.</td>
</tr>
<tr>
<td>Ringer</td>
<td>7</td>
<td>88</td>
<td>10⁻⁸ M 1</td>
<td>5.0</td>
<td>0</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10⁻⁷ M 2</td>
<td>8.5 ± 2.5</td>
<td>0</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10⁻⁶ M 4</td>
<td>6.3 ± 0.5</td>
<td>0</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10⁻⁵ M 7</td>
<td>11.1 ± 1.6</td>
<td>0</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10⁻⁴ M 6</td>
<td>31.2 ± 3.2</td>
<td>0</td>
<td>--</td>
</tr>
<tr>
<td>Curare</td>
<td>7</td>
<td>89</td>
<td>10⁻⁸ M 1</td>
<td>5.2</td>
<td>2</td>
<td>10.0 ± 2.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10⁻⁷ M 1</td>
<td>5.2</td>
<td>3</td>
<td>10.8 ± 2.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10⁻⁶ M 2</td>
<td>9.7 ± 4.6</td>
<td>2</td>
<td>10.2 ± 3.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10⁻⁵ M 3</td>
<td>21.0 ± 9.8</td>
<td>0</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10⁻⁴ M 4</td>
<td>12.5 ± 5.4</td>
<td>1</td>
<td>28.0</td>
</tr>
</tbody>
</table>
FIGURE 41

Effect of acetylcholine on the rate of beating of curarized Melanoplus femur-rubrum hearts.
ii. curare alone causes a blockage in vivo

In vivo, injection with curare into the house cricket indeed produced a vertebrate-like flaccid paralysis. Several minutes, about five to ten minutes, after injection with 0.05 cc. $10^{-4}$ M curare, spasmodic twitching started in the posterior end of the body (in the female, it was particularly obvious at the ovipositor), then the hind legs, and there was some fluttering of the wings and antennae. Sometime after the cricket lost its balance and therefore walked very slowly and unsteadily. Eventually, this sluggish movement also stopped; the cricket was then completely paralyzed. Placed on its back, it was unable to turn itself over. However, the heart continued to beat apparently normally. No significant mortality occurred, and normal activity was restored with 12 hrs. If the volume injected was increased to 0.1 cc., then the time needed for recovery was longer. The site of injection seemed not to be related to the first responsive location, the response always occurring firstly at the posterior end. These in vivo results more or less agreed with those of Larsen et al (1966), with a little difference, i.e., the site of injection was said to be very important to the first symptom.

McCann (1966) has shown that when the blowfly is paralyzed
by the injection of curare, and the neuromuscular junction then examined by electrophysiological techniques, that there is a true neuromuscular block of the curare type. Why this occurs only after the injection of curare is not understood.

c. Antagonized by atropine

A concentration of atropine of $10^{-4}$ M did not have an appreciable effect on the cricket heart. It did however antagonize the action of subsequently applied Ach, as is the case with most other insects.

Table 7, Figure 42 clearly show that the stimulating effect of Ach on the young cricket heart was reduced by pretreatment with $10^{-4}$ M atropine. Most of the young hearts were unaffected by Ach at any concentration. Only a small number of them still showed an acceleration with a very low percentage increase. Some of the young hearts even changed their original accelerating effect of Ach into an inhibition. (None of the young hearts was inhibited by Ach alone.) This interaction suggested that this Ach-like compound abolished the action of Ach at the synapses of the ganglionic cells of the cardiac nerve, i.e. by acting directly upon the receptor site of Ach.

This antagonistic action of atropine gave further evidence for the neurogenic and particularly for the cholinergic proper-
ties of the pacemaker of the young cricket heart.

Atropine at the same concentrations also gave more or less the same results on the old cricket hearts as on the young hearts. The old hearts seemed more sensitive to atropine alone.
### TABLE 7

**EFFECTS OF ACH ON ATROPINIZED SEMI-ISOLATED HEARTS OF A. DOMESTICUS**

<table>
<thead>
<tr>
<th>Age</th>
<th>Total No.</th>
<th>'Normal' Average</th>
<th>Conc.</th>
<th>Accelerated (%)</th>
<th>Inhibited (%)</th>
<th>Unaffected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mean ± S.E.</td>
<td>Mean ± S.E.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>No.</td>
<td></td>
<td>No.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mean ± S.E.</td>
<td>No.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td>12</td>
<td>77</td>
<td>$10^{-4}$ M Atropine</td>
<td>1</td>
<td>8.3</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$10^{-7}$ M Ach</td>
<td>1</td>
<td>8.3</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$10^{-6}$ M Ach</td>
<td>3</td>
<td>7.9 ± 1.7</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$10^{-5}$ M Ach</td>
<td>1</td>
<td>19.0</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$10^{-4}$ M Ach</td>
<td>4</td>
<td>11.6 ± 5.8</td>
<td>2</td>
</tr>
<tr>
<td>Old</td>
<td>12</td>
<td>81</td>
<td>$10^{-4}$ M Atropine</td>
<td>3</td>
<td>8.4 ± 6.8</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$10^{-7}$ M Ach</td>
<td>3</td>
<td>11.6 ± 1.8</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$10^{-6}$ M Ach</td>
<td>1</td>
<td>9.2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$10^{-5}$ M Ach</td>
<td>1</td>
<td>9.2</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$10^{-4}$ M Ach</td>
<td>2</td>
<td>8.0 ± 0.9</td>
<td>5</td>
</tr>
</tbody>
</table>
FIGURE 42.

Effect of acetylcholine on the rate of beating of atropinized young and old hearts.
3. The Action of Nicotine

Nicotine did not produce an appreciable effect on either young or old hearts until $10^{-4}$ M (Table 8, Figure 43, Figure 44).

This absence of effect in low concentrations and stimulation at higher concentrations was unlike the effect of nicotine in vertebrates or in some other insects, e.g. the cockroach, where nicotine is said to act specifically on the synapses of the cholinergic cardiac ganglionic cells.

An additional check was carried out on the cockroach. Whereas the cricket heart was unaffected by nicotine at concentration of $10^{-8}$ to $10^{-5}$ M, the cockroach heart was stimulated at $10^{-6}$ M and nicotine caused an extremely vigorous beating of cockroach hearts at $10^{-5}$ M and $10^{-4}$ M. The original beating was elevated by more than 100%. This result agreed with other workers on the cockroach but the effect was much greater. This was probably due to the different original Ringer. In this above check, cockroach hearts also were immersed in Bêlår's Ringer solution.

This low sensitivity of the cricket heart to nicotine suggested that the cricket heart might be muscarinic.
<table>
<thead>
<tr>
<th>Age</th>
<th>Total No.</th>
<th>'Normal' Average</th>
<th>Conc.</th>
<th>Accelerated (%)</th>
<th>Inhibited (%)</th>
<th>Unaffected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No.</td>
<td>Mean ± S.E.</td>
<td>No.</td>
<td>Mean ± S.E.</td>
</tr>
<tr>
<td>Young</td>
<td>12</td>
<td>81</td>
<td>$10^{-8}$ M</td>
<td>0 --</td>
<td>0 --</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$10^{-7}$ M</td>
<td>0 --</td>
<td>0 --</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$10^{-6}$ M</td>
<td>1 7.9</td>
<td>1 7.2</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$10^{-5}$ M</td>
<td>0 --</td>
<td>1 7.2</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$10^{-4}$ M</td>
<td>3 11.0 ± 0.0</td>
<td>0 --</td>
<td>9</td>
</tr>
<tr>
<td>Old</td>
<td>11</td>
<td>76</td>
<td>$10^{-8}$ M</td>
<td>0 --</td>
<td>1 6.0</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$10^{-7}$ M</td>
<td>0 --</td>
<td>1 8.4</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$10^{-6}$ M</td>
<td>0 --</td>
<td>2 7.4 ± 2.3</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$10^{-5}$ M</td>
<td>0 --</td>
<td>2 8.8 ± 3.3</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$10^{-4}$ M</td>
<td>5 13.6 ± 4.0</td>
<td>1 9.6</td>
<td>5</td>
</tr>
</tbody>
</table>
FIGURE 43.
FIGURE 43.

The percentage of hearts of both young and old which were accelerated by different concentrations of nicotine.
FIGURE 44

The percentage of hearts of both young and old which were unaffected by different concentrations of nicotine.
PERCENT UNAFFECTED

NICOTINE CONC. (M)
4. The Action of Arecoline

Table 9, Figure 45, indicated that arecoline caused a sudden increase in the beating of the young house cricket heart at concentrations of $10^{-6}$ M and $10^{-5}$ M. Only one of the twelve hearts was unaffected. All the young hearts then suddenly stopped at $10^{-4}$ M. None of the young hearts could be affected below $10^{-6}$ M.

Old hearts reacted similarly, but were less sensitive to arecoline.

Arecoline (here used to replace muscarine) can imitate the action of Ach. That this is the case with the cricket heart is suggested by the fact that similar concentrations of the two substances gave similar effects.

If the arecoline can completely take the place of Ach, then an inhibitory effect should be found after application of arecoline to the old hearts. In fact, a contradictory result was obtained. The old hearts were also stimulated by arecoline in the same way as the young hearts.
TABLE 9

EFFECTS OF ARECOCINE ON SEMI-ISOLATED HEARTS OF A. DOMESTICUS

<table>
<thead>
<tr>
<th>Age</th>
<th>Total No.</th>
<th>'Normal' Average</th>
<th>Conc.</th>
<th>Accelerated (%)</th>
<th>Inhibited (%)</th>
<th>Unaffected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No.    Mean ± S.E.</td>
<td>No.    Mean ± S.E.</td>
<td>No.</td>
</tr>
<tr>
<td>Young</td>
<td>12</td>
<td>79</td>
<td>10^-8 M</td>
<td>0    --</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10^-7 M</td>
<td>0    --</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10^-6 M</td>
<td>11   14.8 ± 2.0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10^-5 M</td>
<td>11   23.5 ± 3.4</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10^-4 M</td>
<td>--</td>
<td>--</td>
<td>All Stop</td>
</tr>
<tr>
<td>Old</td>
<td>13</td>
<td>93</td>
<td>10^-8 M</td>
<td>0    --</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10^-7 M</td>
<td>0    --</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10^-6 M</td>
<td>4    10.5 ± 1.5</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10^-5 M</td>
<td>11   17.3 ± 3.0</td>
<td>1  9.0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10^-4 M</td>
<td>--</td>
<td>--</td>
<td>All Stop</td>
</tr>
</tbody>
</table>
FIGURE 45.
The percentage of hearts of both young and old which were accelerated by different concentrations of arecoline.
5. The Action of Noradrenalin

Noradrenalin stimulates the cricket heart with a threshold of $10^{-7}$ M for the young hearts, and $10^{-6}$ M for the old ones (Table 10, Figure 46). In concentrations greater than $10^{-4}$ M, the hearts tended to be inhibited.

These effects are probably due to the non-specific action of noradrenalin on the myocardium.
TABLE 10

EFFECTS OF NORADRENALINE ON SEMI-ISOLATED HEARTS OF *A. DOMESTICUS*

<table>
<thead>
<tr>
<th>Age</th>
<th>Total No.</th>
<th>'Normal' Average</th>
<th>Conc.</th>
<th>Accelerated (%)</th>
<th>Unaffected No.</th>
<th>Mean ± S.E.</th>
<th>Inhibited (%)</th>
<th>Unaffected No.</th>
<th>Mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young</td>
<td>10</td>
<td>78</td>
<td>10^{-8} M</td>
<td>0</td>
<td>--</td>
<td>0</td>
<td>--</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10^{-7} M</td>
<td>1</td>
<td>22.0</td>
<td>0</td>
<td>--</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10^{-6} M</td>
<td>3</td>
<td>15.0 ± 8.3</td>
<td>0</td>
<td>--</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10^{-5} M</td>
<td>8</td>
<td>18.6 ± 3.4</td>
<td>0</td>
<td>--</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10^{-4} M</td>
<td>6</td>
<td>17.0 ± 2.9</td>
<td>2</td>
<td>6.3 ± 1.1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Old</td>
<td>10</td>
<td>89</td>
<td>10^{-8} M</td>
<td>0</td>
<td>--</td>
<td>0</td>
<td>--</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10^{-7} M</td>
<td>0</td>
<td>--</td>
<td>0</td>
<td>--</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10^{-6} M</td>
<td>2</td>
<td>6.1 ± 0.3</td>
<td>0</td>
<td>--</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10^{-5} M</td>
<td>8</td>
<td>9.3 ± 1.2</td>
<td>0</td>
<td>--</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10^{-4} M</td>
<td>2</td>
<td>11.3 ± 2.7</td>
<td>1</td>
<td>21.0</td>
<td>7</td>
<td></td>
</tr>
</tbody>
</table>
FIGURE 46.
The percentage of hearts of both young and old which were accelerated by different concentrations of noradrenalin.
6. The Action of Dopamine

Young cricket hearts started to be accelerated by dopamine at $10^{-7}$ (Table 11, Figure 47). The frequency increased with the concentration. One out of the seven hearts was inhibited at $10^{-5}$.

Dopamine did not cause any of the old hearts to be accelerated except for two at $10^{-6}$ with a very low percentage increase ($7.0 \pm 1.7\%$). It did cause an inhibitory effect on the old heart, which started at $10^{-7}$. The number of inhibited hearts increasing with the concentration (Table 11, Figure 48). The rate of the beat decreased with the concentrations also.

This phenomenon is similar to that of Ach, that is, the young hearts tended to beat more strongly and regularly than the old ones, after treatment with dopamine. It might be suggested that dopamine acts on the cardiac nervous system like Ach.

Dopamine is a metabolic intermediary of noradrenalin and is expected to have a response similar to noradrenalin. But the results showed that they did not act completely the same.
### TABLE 11

**EFFECTS OF DOPAMINE ON SEMI-ISOLATED HEARTS OF *A. DOMESTICUS***

<table>
<thead>
<tr>
<th>Age</th>
<th>Total No.</th>
<th>'Normal' Average</th>
<th>Conc.</th>
<th>Accelerated (%)</th>
<th>Inhibited (%)</th>
<th>Unaffected No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No.</td>
<td>Mean ± S.E.</td>
<td>No.</td>
</tr>
<tr>
<td>Young 8</td>
<td>74</td>
<td>10^{-7}</td>
<td>5</td>
<td>9.1 ± 3.0</td>
<td>0</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10^{-6}</td>
<td>7</td>
<td>11.7 ± 1.7</td>
<td>0</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10^{-5}</td>
<td>6</td>
<td>33.0 ± 4.6</td>
<td>1</td>
<td>14.0</td>
</tr>
<tr>
<td>Old 8</td>
<td>86</td>
<td>10^{-7}</td>
<td>0</td>
<td>--</td>
<td>2</td>
<td>8.7 ± 0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10^{-6}</td>
<td>2</td>
<td>7.0 ± 1.6</td>
<td>4</td>
<td>15.0 ± 2.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10^{-5}</td>
<td>0</td>
<td>--</td>
<td>7</td>
<td>21.0 ± 4.1</td>
</tr>
</tbody>
</table>
FIGURE 47
FIGURE 47.

The percentage of hearts of both young and old which were accelerated by different concentrations of dopamine.
DOPAMINE CONC.

PERCENT ACCELERATED

young

old
FIGURE 48.

The percentage of hearts of both young and old which were inhibited by different concentrations of dopamine.
7. The Action of 5-Hydroxytryptamine

The cricket heart is very sensitive to 5-HT. Table 12, Figure 49 show that four of the seven young hearts began to be stimulated at $10^{-8}$, and all but one at $10^{-7}$. At $10^{-6}$ five of them were inhibited.

Half of the old hearts were accelerated with the lowest concentration, but this caused only a very low percentage increase ($6.6 \pm 0.7\%$). After the concentration was increased ten times, most of the old hearts were inhibited ($31.3 \pm 4.8\%$) (Figure 50). They changed their original strong and regular beats into flaccid vibration. All the old hearts stopped in systole at $10^{-6}$.

These results agree with the work of McFarlane (1967), that is, the young hearts were accelerated by 5-HT, but the old hearts tended to be inhibited at the same concentration. 5-HT therefore resembles Ach in its action on the cricket heart.

The possible mechanism of action of this drug may be directly on the heart muscle or may be on the cardiac nerves. This function is not presently known.
## TABLE 12

**EFFECTS OF SEROTONINE ON SEMI-ISOLATED HEARTS OF *A. DOMESTICUS***

<table>
<thead>
<tr>
<th>Age</th>
<th>Total No.</th>
<th>'Normal' Average</th>
<th>Conc.</th>
<th>Accelerated (%)</th>
<th>Inhibited (%)</th>
<th>Unaffected No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No.</td>
<td>Mean ± S.E.</td>
<td>No.</td>
<td>Mean ± S.E.</td>
</tr>
<tr>
<td>Young</td>
<td>7</td>
<td>88</td>
<td>10^-8</td>
<td>4 13.6 ± 1.8</td>
<td>1</td>
<td>6.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10^-7</td>
<td>6 34.5 ± 2.2</td>
<td>1</td>
<td>16.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10^-6</td>
<td>2 43.0 ± 1.0</td>
<td>5</td>
<td>44.0 ± 9.3</td>
</tr>
<tr>
<td>Old</td>
<td>9</td>
<td>91</td>
<td>10^-8</td>
<td>4 6.6 ± 0.7</td>
<td>1</td>
<td>6.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10^-7</td>
<td>1 7.4</td>
<td>6</td>
<td>31.3 ± 4.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10^-6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

163
FIGURE 49.

The percentage of hearts of both young and old which were accelerated by different concentrations of serotonin.
FIGURE 50
FIGURE 50.

The percentage of hearts of both young and old which were inhibited by different concentrations of serotonin.
V. CONCLUSIONS AND SUMMARY

The heart of the house cricket is a very simple one. In the thoracic part, it is a slender tube and in the abdominal part, it is divided by pairs of ostial valves into chambers and ends in the ninth abdominal segment. This dorsal vessel is suspended in the pericardial cavity by eleven pairs of symmetrically arranged alary muscles and connective tissue fibers. The heart wall is one single muscular layer, and it, as well as the alary muscles, are clearly striated. Mononucleate and multinucleate pericardial cells are attached to the surface of the heart wall and invest the alary muscles and the connective tissue fibers. The heart is well innervated by two lateral cardial nerves and 12 pairs of segmental nerves. The cardiac ganglionic cells spread along the lateral cardial nerves. A delicate membrane which with the alary muscles is called the dorsal diaphragm separates the dorsal sinus from the perivisceral sinus. The dorsal longitudinal tracheae lie in the pericardial sinus above the diaphragm. Two to three pairs of phagocytic organs are located in the first three abdominal segments.

Pharmacologically, if only according to Prosser's (1942) suggestion that hearts accelerated by Ach are neurogenic,
hearts inhibited are myogenic innervated and hearts unaffected are noninnervated myogenic, it can be concluded that all the young cricket hearts are neurogenic and most of the old hearts are myogenic innervated. But actually this above suggestion is not enough to distinguish neurogenic and myogenic hearts. Miller and Metcalf (1968) denervated the cockroach heart and after testing with several drugs concluded that the classic neurogenic cockroach heart is actually myogenic with extensive nervous control instead of being purely neurogenic. The young house cricket heart might be similar, that is basically myogenic, but also with a neurogenic pacemaker.

Furthermore, the action of Ach on young cricket hearts is muscarine-like, i.e. arecoline (muscarine) accelerates, Ach is antagonized by atropine, and nicotine does not mimic the action of Ach. Other drugs such as noradrenalin, dopamine and 5-HT all give an excitatory effect on the young hearts, to cause the young hearts to beat more strongly and regularly, i.e. similar to the old ones. In addition, both eserine and curare potentiate the action of Ach.

Generally speaking, the data from young hearts are quite consistent. On the other hand, in the case of old hearts,
many contradictory and unexplained 'exceptions' create confusion, although there is no doubt from the temperature response studies of a fundamental difference between old and young hearts. For some drugs, there does exist some difference between the old and the young hearts. But for some other drugs, many of the old hearts still respond in the same way as the young hearts, although they are always less sensitive. What is clear is that 1) cholinergic synapses are probably present in the cardiac nervous system, because of the response to eserine, and 2) aging involves this cholinergic system, at least in part, because of the varying responses to Ach and curare. The precise changes in the cholinergic system during aging are beyond the scope of the techniques used in the present study. Certainly an electrophysiological study of the heart system would help to explain the observed effects of the drugs.
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APPENDIX 1

The structural formulae of the cholinergic drugs.

1. Acetylcholine

\[
\text{N} - \text{CH}_2\text{-CH}_2 - 0 - \text{CO} - \text{CH}_3
\]

\[(\text{CH}_3)_3\]

2. Nicotine

\[
\text{CH}_3
\]

3. Muscarine

\[
\text{H}_3\text{C} - \text{CH}_2\text{N}^+\text{(CH}_3)_3
\]

\[\text{HO}\]

4. Atropine

\[
\text{CH}_3 - \text{N} - \text{CH}_2 - 0 - \text{CO} - \text{CH}_2\text{-OH}
\]

5. d-Tubocurarine

\[
\text{N}^+\text{(CH}_3)_2
\]

\[\text{OCH}_3\]

6. Eserine

\[
\text{CH}_3\text{-NH-CO-O-}
\]

\[\text{N}^+\text{H}\]

\[\text{CH}_3\text{ CH}_3\]
APPENDIX 2

Main pathway of biosynthesis of catecholamine

Tyrosine

Dopa

Dopamine

Noradrenalin

Adrenalin