

Chapter 5

INSECT MODELS FOR BIOMEDICAL RESEARCH

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“For many problems there is an animal on which it can most conveniently be studied” — the August Krogh Principle.¹

I. INTRODUCTION

The vast numbers of species of insects and other arthropods offer tremendous possibilities for medical research. Meglitsch included the insects in his statement; “It is no accident that nearly all truly basic zoological discoveries have been based on studies of invertebrates.”²

The major advantages of using insect models include the ease and low cost of rearing large numbers of specimens. The more commonly used laboratory insects can be reared or purchased at a fraction of the cost of mice, rats, and other laboratory animals. Reduced per diem costs and space requirements also result in significant savings. The rapid reproduction and maturation and the large number of offspring from a single male-female pairing of some species are distinct advantages over vertebrate models. The potential number of descendants from a single pair of insects, such as the housefly *Musca domestica*, is 10^{18} in a matter of months, permitting research designs using multicellular animals which usually are viewed to be restricted to single-cell organisms.² The flexibility afforded by use of short-lived species also can be exploited using many insect models. Experiments often can be run in months or a few years using large genetically homogeneous populations. The ability to use large numbers of test specimens can be exploited to arrive at highly significant statistical results and the detection of low-frequency occurrences. Opportunities for studies in embryology are especially promising because of the detailed knowledge of egg and larval development in some insect species. Since it is often easier to isolate physiological or pharmacological systems in insect models, these usually can be studied more simply in insects. Invertebrate tissue cultures, although initially difficult to establish, usually can be handled more easily than vertebrate systems.³ The use of animals lower on the evolutionary scale also reduces objections by antivivisectionist and animal rights groups, a major concern of scientists today.

II. INSECTS AS A GROUP

A. MORPHOLOGY AND PHYSIOLOGY

Insects differ in their morphology and physiology from mammals in a number of ways.⁴ In particular, insects possess an external exoskeleton rather than bone — the only vertebrate tissue they lack.² This chitinous structure provides a great deal of protection against a number of environmental stresses, such as desiccating conditions, chemicals, and pressure. Within this exoskeleton is the body cavity (hemocoel) which contains systems for digestion, circulation, respiration, excretion, innervation, and reproduction (see Figure 1). Unlike mammals, there is an open blood system with a dorsal heart and blood (hemolymph) which contains no hemoglobin. The hemolymph is responsible for a variety of transportation and immunological functions. Insect respiration is provided by a branching series of tubes called the tracheal system and by passive diffusion of oxygen to individual cells. Analogous to the vertebrate liver is a tissue known as insect fat body. This group of specialized cells is enclosed in a membranous sheath and is important in insect metabolism. Insects have a well-developed neuromuscular system. The insect organs and muscles are innervated through a series of ganglia that form a ventral nerve cord (see Figure 2). The nervous system is similar to that of mammals in having a blood-brain barrier and cholinergic synapses; however, the neuromuscular junctions are glutaminergic, unlike the vertebrate cholinergic junctions. Reproductive mechanisms in insects are quite species specific, but in general the two sexes mate via a complex chemical, visual, and tactile communication system.⁴

B. HUSBANDRY AND ECONOMICS OF REARING

The development and use of animal models for biomedical research depend upon the

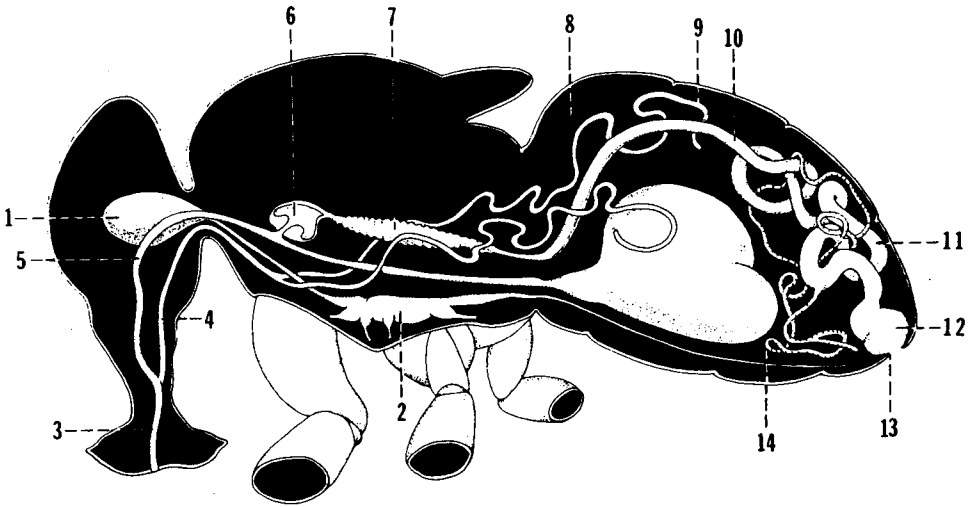


FIGURE 1. Longitudinal cross-section of an adult housefly (diagrammatic) showing gross internal organization: (1) esophageal ganglion, (2) compound thoracic ganglion, (3) pharynx, (4) salivary duct, (5) esophagus, (6) proventriculus, (7) stomach, (8) hemocoel, (9) salivary gland, (10) proximal intestine, (11) distal intestine, (12) rectum, (13) anus, and (14) Malpighian tubule. (Modified after Patton⁶⁶ and West.⁶⁷)

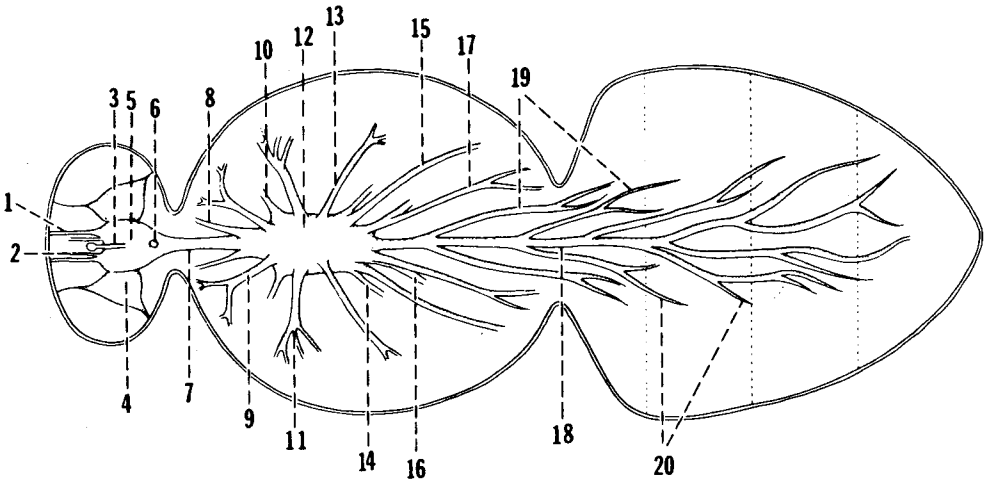


FIGURE 2. Gross nervous system of an adult housefly (diagrammatic): (1) antennary nerve, (2) pharyngeal nerve, (3) ocellar nerve, (4) optic peduncle, (5) cephalic ganglion, (6) space for esophagus, (7) cephalo-thoracic nerve cord, (8) cervical nerve, (9) prothoracic dorsal nerve, (10) prothoracic crural nerve, (11) mesothoracic dorsal nerve, (12) compound thoracic ganglion, (13) accessory mesothoracic dorsal nerve, (14) mesothoracic crural nerve, (15) metathoracic dorsal nerve, (16) metathoracic crural nerve, (17) accessory metathoracic dorsal nerve, (18) abdominal nerve cord, (19) abdominal nerves of thoracic origin, and (20) abdominal nerves of local origin. (Modified after Hewitt⁶⁸ and West.⁶⁷)

production of the needed specimens which must meet quality control requirements within specific cost restrictions. The great advantage of insects for use in biomedical studies is the ease with which these biological organisms can be reared.

Successful rearing is dependent upon a detailed knowledge of the biology, behavior, habitat, and nutritional requirements of the insect species selected. This knowledge has been expanded greatly in the past few decades, with numerous descriptions appearing in the

literature on rearing methods and diets for selected insects and other arthropods.⁵ Some of the most widely used insects are often those most easily reared, such as the flies, Lepidoptera larvae, and other insects of economic importance. The housefly *M. domestica* is reared easily on CSMA (Chemical Specialties Manufacturers Association), a diet that provides year-round rearing on an efficient medium. The commercial availability of artificial and defined diets for some lepidopteran larval species significantly reduces the trouble and expense of feeding. Quality control of diet ingredients is essential to ensure proper insect nutrition at the lowest possible cost. A list of the more important references on insect diets is given by Singh.⁵

The containers and enclosures used for rearing often dictate the success of the operation. Desirable characteristics of rearing containers include economics, barrier to microbial contaminations and pathogens, allowance for gas exchange, moisture regulation, visibility and accessibility, convenience of handling and harvesting, and ease of cleaning and disinfection or disposal.⁶

The rearing procedures usually described are designed for the production of hundreds to thousands of specimens per week. Mechanized mass rearing systems also have been developed where the number of insects reared is measured in millions per week. As part of a sterile-male screwworm eradication program, approximately 500 million flies were produced per week.⁷ However, production on this scale requires uniquely designed facilities to meet the needs of controlled environments, mechanized handling methods and control of pathogens, contaminants, and respiratory hazards.⁸

III. DISCOVERIES AND APPLICATIONS IN BIOMEDICAL SCIENCES

A. GENETICS

The study of genetics in multicellular organisms has progressed rapidly during the past 80 years. The fruit fly *Drosophila melanogaster* has become the best-known model for laboratory and field studies of genetics. This insect was used first as the basis for amplifying Mendelian genetics and giving it its present form. In 1910, Morgan⁹ at Columbia University reported the crisscross nature of sex linkage in *Drosophila* and, more importantly, set the standards of excellence for experimental work in genetics.¹⁰ Dobzhansky¹¹ was the first scientist to integrate the results of laboratory and field studies with the predictions arising from mathematical theory such as the Hardy-Weinberg law. Since Morgan's initial report in 1910,⁹ it is estimated that over 25,000 articles dealing with *Drosophila* have been published and that the literature would double every 12 years.¹²

The advantages of using the fruit fly *Drosophila* as a model for the study of genetics are many.¹² The adult fly is small, readily handled, and breeds prolifically in the laboratory and in the field. Conditions for rearing the flies are simple, cheap, and readily controlled. The life cycle is short, about 9 d, and thousands can be produced in a small space. There are only four haploid chromosomes, and the polytene chromosomes of the salivary glands of larvae are gigantic and show a characteristic banding pattern. These patterns facilitate the detection of chromosomal rearrangements, the mapping of gene deficiencies, and the subsequent cytological localization of genes. Since the homologous chromosomes do not undergo crossing over in the germ cells of the male,¹³ the genetic procedures employed are simplified greatly. This insect can serve as host to a variety of viruses,¹⁴ thereby allowing the study of the genetics of host-parasite interactions. *D. melanogaster* flourishes upon many media; however, a synthetic, minimal medium has been developed upon which flies can be reared aseptically.¹⁵ Schneider¹⁶ has developed a medium for *in vitro* cell cultures of fly-derived cells. Massive collections of hereditary variations in flies have been developed, and stocks of many of the mutants can be obtained from various workers in the field. Finally,

an encyclopedic body of information on *Drosophila* genetic studies is readily available through indexed bibliographies, such as those by Herskowitz.¹⁷⁻²¹ Without question, these attributes make this insect model one of the more important findings in the field of genetics, as well as in modern science.

B. MUTAGENICITY TESTING

The fruit fly *D. melanogaster* has also proved to be extremely useful in testing materials for mutagenicity, and the literature on this subject is abundant.²² Studies of mutation induction in *Drosophila* began with Muller's experiments with X-rays in 1927.²³ In the years after World War II, the mutagenic effects of radiation were studied extensively, and Auerbach and co-workers were the first to detect chemical mutagenesis by mustard gas and formaldehyde using *Drosophila*.^{23,24}

The wealth of specific test strains, special markers, inversions, and other rearrangements make it possible to test for most of the genotoxic end points relevant to human hazards using *Drosophila*. These range from recessive lethals or visible point mutations and small deletions to translocations, duplications, meiotic or mitotic recombinations, and dominant lethals or chromosome loss as an indication of open, unrepaired breaks, chromosome damage, and aneuploidy.^{22,25,26} Testing for the different types of mutations often can be conducted simultaneously if desired. The life cycle of *D. melanogaster* is short enough to permit rapid analysis of many progeny but long enough to distinguish between chronic, acute, and fractionated doses.²² Since the fruit fly is a multicellular eukaryote, it possesses a cellular and chromosomal organization more akin to mammals than the bacteria sometimes used for the initial screening of mutagens. The overlap between the mutagenic and carcinogenic potential of many classes of chemicals tends to make the distinction between the two an artificial one.^{25,26}

Indirect mutagens and carcinogens require activation by the microsomal enzyme systems present in the mammalian liver. Mutagens of this kind register as negative in microbial test systems unless host-mediated assays or plating on microsomal extracts from mammalian tissues are employed. Mammalian-like detoxification pathways have been demonstrated in *Drosophila* and are capable of facilitating similar enzymic reactions to those from mammalian liver. Thus, the use of *Drosophila* is convenient for detecting indirect mutagens and short-lived metabolites. Over 50 compounds, falling into 9 different groups, that all require metabolic activation for the manifestation of their mutagenic and carcinogenic properties have been tested in *Drosophila* and yielded positive responses.^{26,27}

Many of the advantages of using the fruit fly listed in the preceding section apply also to mutagenicity testing. Toxicity testing using a housefly model is described in Chapter 6.

C. PATHOGEN PRODUCTION

Many human pathogens, such as bacteria, protozoa, rickettsia, viruses, and helminths, multiply in various insects. These insect hosts may be involved in the natural transmission of certain pathogens to man. Insect-borne diseases, such as malaria, trypanosomiasis, and dengue, account for the loss of millions of people each year, particularly in tropical areas. Scientists, however, have learned to take advantage of this pathogen-insect relationship in disease diagnosis.

A unique application exploiting parasite development in insect vectors is xenodiagnosis. The causative organism of some arthropod-transmitted diseases often occurs only sparsely in human blood, making nonacute forms of the disease difficult to diagnose by recovery of the parasite. Xenodiagnosis involves the feeding of noninfected insects on the patient. After incubation and multiplication in the insect's body, the parasite, if present, may be recovered and examined. Xenodiagnosis is used most commonly in the detection of trypanosomes causing Chagas' disease (American trypanosomiasis) in the gut and feces of conenose bugs

fed 1 to 2 weeks earlier on patients.²⁸ More recently, phlebotomine sand flies and simuliid black flies have been used for the diagnosis of New World leishmaniasis²⁹ and onchocerciasis,³⁰ respectively.

Insects are used also in the laboratory confirmation of certain human viral illnesses, such as those caused by the dengue viruses. Dengue is one of the most important arthropod-borne viruses that occurs in man because of the high numbers of individuals infected and because it may cause mortality in children. The four viruses that cause this disease, however, are among the most difficult to detect and propagate in the laboratory. They are not very pathogenic when inoculated into the brain of a newborn mouse and may require many serial passages to produce signs of illness in mice. The application of cell culture techniques for detection led to more sensitive assays, but not all four virus types would produce consistently cytopathic effects which could be detected in the cultured cells. Upon discovery that the dengue virus grew to high titers in certain mosquitoes, workers began inoculating virus into mosquitoes to develop a more sensitive detection system.³¹

The use of mosquitoes to assay dengue viruses offers a considerable advantage in sensitivity whether the viruses are present in mosquitoes, in sera from naturally infected humans, or have been adapted to cell cultures or mice.³² The discovery that male mosquitoes, such as *Aedes aegypti*, are as sensitive to infection as females, offers a significant advantage in safety, since males cannot transmit the infection should they escape. It was shown also that *Toxorhynchites* mosquitoes, a genus that does not feed on blood and is extremely large, could be infected with the virus. This mosquito currently is the genus of choice for the laboratory confirmation of the four dengue viruses.³¹

D. NEUROENDOCRINE CONTROL MECHANISMS

Insect metamorphosis has been a fascinating phenomenon from ancient times. However, it was not until 1922 when an insight into this phenomenon was gained by Kopec.³³ He showed that a chemical factor had to be released from the brain of the gypsy moth larva, *Lymantria dispar*, to cause pupation. This was the first evidence in the animal kingdom that the nervous system was involved in the endocrine control of growth and development. We now know that the vertebrate hypothalamic-hypophyseal complex provides the same coordination of the nervous and endocrine activities as the pars intercerebralis-corpora cardiaca complex of insects. The first evidence on the mode of action of steroid hormones at cellular and molecular level came from the studies of Clever and Karlson in the 1960s on the polytene chromosomes of a fly, *Chironomus* sp.³⁴ The role of cyclic nucleotides in insect hormone action provides a commonality in the mode of action of insect hormones with those of mammals such as serotonin.³⁵ The discovery that RNA and protein syntheses were important to the action of insect hormones has yielded basic information of great significance to the mode of action of hormones in general.

E. ANTIMALARIAL DRUGS

Insects have proved very effective in the screening of potential drugs, in particular, with antimalarial compounds. Following World War II and our experience with malaria in vast numbers of military troops serving in tropical areas, malaria research centered on the development of more effective drugs. At this time, there was the need for newer testing methods to seek out compounds with antimalarial activity. The need derived from the fact that testing methods using the vertebrate hosts of avian and simian malarias, ordinarily used for preliminary evaluation of compounds for antimalarial activity, failed to show a consistent relation between the activity in animal models and that in human beings. For example, paludrine had a prophylactic effect against the avian malaria *Plasmodium gallinaceum* but not against the human malaria *P. vivax*. Consequently, preliminary evaluation of this compound required the use of experimentally infected human volunteers. Furthermore, other

compounds were not being considered because of their lack of activity against avian or simian malaria and may have been overlooked because they had not been tested against the human malarials.³⁶

This need for drug-testing methods which showed drug effects in human malarials was met, in part, by studies in which various antimalarial compounds were administered to the mosquito hosts (*Anopheles quadrimaculatus*, *Aedes aegypti*) of *Plasmodium falciparum* and other malarials, and in which drug action was evaluated by its morphological and physiological effects on the various stages of the malaria cycle within the mosquito.³⁷⁻³⁹ As a result of these studies, a specific relation in drug action between the mosquito and the human-liver cycle of malaria was shown. Those compounds that had a prophylactic action in the mosquito, also had a prophylactic effect in the human being. As a consequence of this relation, it became possible to evaluate compounds for prophylactic activity against human malarials by using mosquitoes infected with human malarial strains as the test animal. This reduced the need for tests of drug activity in other animal models.³⁶

In addition to the reduced need for animal testing, this insect model made possible a greatly expanded and accelerated malaria drug testing program at a comparatively low cost. With the discovery that drugs tested against avian malaria in the mosquito reliably predicted possible curative activity against *P. vivax*, this insect model was considered even more useful.³⁶ However, in the 1960s the discovery of several new nonhuman primate models led to decreased utilization of the insect model, although it was and still is a valid and much less expensive model.

F. BIOLUMINESCENCE

Insects have been used also to study the fate of various biochemical components like adenosine triphosphate during bioluminescence. Self-luminescence, not involving bacteria, occurs in insects from the orders Collembola, Homoptera, Diptera, and Coleoptera.⁴ Bioluminescence has been characterized best in the common North American firefly, *Photinus pyralis*. Firefly luciferase catalyzes the adenosine triphosphate (ATP)-dependent oxidative decarboxylation of luciferin (LH₂), resulting in the production of light (*hν*) as shown in the reaction where P denotes the product oxyluciferin:



The reaction catalyzed by this enzyme has a quantum yield of 0.88 with respect to LH₂, making it the most efficient bioluminescent reaction known.⁴⁰ Firefly luciferase is useful in a variety of applications. Because of its specificity for ATP, firefly luciferase can be used to measure the amount of ATP present in biological samples without interference from other nucleotide triphosphates.⁴¹ Using luciferase isolated from fireflies, in conjunction with suitably sensitive liquid scintillation counters or biometers, less than 1 fmol (10⁻¹⁵ mol) of ATP can be detected.⁴² The level of endogenous ATP in a cell may be used as an index of its energy status and is therefore useful in metabolic and physiological studies. Estimates of cell numbers in microbial and tissue cultures may be obtained after determining the ATP per cell under defined conditions and measuring total ATP in a sample of culture.⁴³ This has served as a basis for rapidly quantitating bacteria in urine, milk, wine, and polluted waters, with sufficient sensitivity to detect the ATP contents of as few as 10 colony-forming units (CFU) per milliliter.⁴⁴ Replacing radiolabels (e.g., ¹²⁵I) with luciferin- or firefly luciferase-conjugated ligands in a bioluminescent immuno- or affinity-assay, can result in increased assay sensitivity, elimination of hazardous radiolabeled compounds, increased speed of the assay, and decreased cost per assay.⁴⁵ Commercially available firefly luciferase reagents for use in these assays have been evaluated by Leach and Webster.⁴⁶

IV. AREAS OF POTENTIAL RESEARCH

A. GENERAL CONSIDERATIONS

Insects, by their enormous species diversity and antiquity, present a wide choice of biological parameters. There appears to be no common ancestry between mammals and insects. Interestingly, the basic biological functions are essentially similar in these diverse animal groups. Among the various insect species, cockroaches may be considered as relatively primitive, while bees and flies may be considered more advanced in terms of evolution. Vertebrate evolution, of course, is much more recent than that of insects; however, certain basic mechanisms are conserved throughout the animal kingdom. Therefore, the chances are high of finding a body function or a control mechanism of biomedical interest in insects. Based on the current status of our knowledge on comparative physiology, biochemistry, and molecular biology, the following areas of research appear promising for biomedical purposes.

B. SPECIFIC AREAS

1. Insectan Antibiotics

Because of their long history of survival on this planet, insects may be looked at as the founders of successful defensive mechanisms. They possess a complex, multicomponent, active defensive system that is regulated and coordinated by several distinct cell populations.^{47,48} They exhibit cellular and humoral defensive mechanisms as well as the acquisition of a protected ("immune") state to bacterial infections.

The insect immunocytes (hemocytes) are efficient in eliminating bacteria, fungi, nematodes, and other foreign particles by either phagocytosis, nodule formation, or encapsulation. The recognition of foreignness is thought to be mediated by certain hemolymph proteins (agglutinins; lectins) that function as opsonins.⁴⁹ Lectins, which may play a role in the receptor-mediated endocytosis, also have been found on the cell surface of insect hemocytes.⁴⁹⁻⁵²

Insect immunocytes, namely, plasmatocytes and granulocytes, are functionally comparable to vertebrate (mammalian) B- and T-lymphocytes.⁴⁸ The plasmatocytes perform the analogous killer function and helper-cell-independent cytotoxic function of the T-lymphocytes. The plasmatocytes also perform the functions of vertebrate macrophages. The granulocytes perform the analogous functions of the B-lymphocytes as well as the suppressor functions of the T-lymphocytes. A detailed hypothesis on the evolution of these immunocytes from a primitive arthropod granulocyte was proposed recently.⁴⁸

Insect hemolymph is rich in a polyphenoloxidase that catalyzes, among others, the oxidation of tyrosine to 3,4-dihydroxyphenylalanine. It has been proposed that the activation of this enzyme may have a role in the recognition of foreign particles.⁵³

A broad spectrum of antibiotic proteins and peptides are known to be synthesized by insects in response to bacterial infections. For example, the cecropins (3.5 to 4 kDa) and attacins (20 to 23 kDa) in the hemolymph of silkworm, *Hyalophora cecropia*, are bactericidal.⁵⁴⁻⁶⁰ The site of synthesis of these proteins or related bactericidal proteins appears to be the fat body. Insect lysozymes exhibit properties (thermostability, pH optima, and ionic strength optima) similar to those of chicken egg white lysozyme.^{57,61-63}

In spite of some significant progress made in the past decade in our understanding of invertebrate immunity, our present knowledge of cellular recognition and mediation of immune response is lagging severely behind that of mammalian immunity. Future research, therefore, should concentrate on cell-surface and humoral molecules, their characterization, synthesis, regulation, and possible specificity against human pathogens and toxins. Because of the absence of mammalian-type diversification of cell functions, the insect immunocytes may provide an array of molecules for both basic and applied research in immunology. As a reward, one might be able to identify antibiotic molecules that are very different from

TABLE 1
Homologous/Analogous Aspects Between the Neuroendocrine Systems of Insects and Vertebrates*

Insects	Vertebrates
Neuroendocrine System	
Axoplasmic neurosecretion flow	Axoplasmic neurosecretion flow
Paired groups of neurosecretory cells in the protocerebrum	Hypothalamic neurosecretory center
Corpus cardiacum	Posterior lobe of pituitary gland
Corpus allatum	Adenohypophysis
	Anterior lobe of hypophysis
Chemistry of Neuropeptides	
Peptidergic neurosecretions; allatostatin/allatinhibin	Oxytocin, vasopressin, somatostatin
Corpus cardiacum secretions	Substance P, glucagon, insulin, secretin
Proctolin	β -Endorphin
Control of Reproductive Activity	
Synthesis of vitellogenins is extraovarial	Synthesis of vitellogenins is extraovarial
Vitellogenins synthesized in fat body	Vitellogenins synthesized in liver
Reproduction cyclic	Reproduction cyclic
Vivipary/ovovivipary	Pregnancy
Reproductive quiescence terminated by denervation of corpus allatum	Reproductive quiescence terminated by denervation of mammary gland or hypophysectomy
Egg diapause hormone secreted by subesophageal ganglion or other parts	Embryonic diapause (delayed implantation of fertilized egg) controlled by hypothalamic-adenohypophysial system

* Modified after Reference 65.

those of mammals and perhaps were never acquired by the mammals through the evolutionary process, either deliberately or accidentally. For example, the inability of the human immunodeficiency virus (HIV) to replicate in insect cells⁶⁴ might lead to a novel insectan molecular weapon against this deadly virus now threatening millions of people.

2. Neuroendocrine System

“The episodic events, including the molting cycle and metamorphic transformations that lead to the emergence of adult insects, are programmed with greater precision than the developmental steps leading to maturity in most vertebrates. The cyclicity in the reproductive activity of the females of certain insect species resembles that of mammals.”⁶⁵ Some of the homologies and analogies are shown in Table 1. The identity and precise biological activity of many neurosecretory materials are currently under investigation in many laboratories. One can conclude at this point that the insects possess a very complex array of neurosecretions that may not be very different from those of mammals. It is hoped that future research efforts will be directed toward a clear understanding of these historical molecules and a better understanding of our own molecular systems.

V. SUMMARY

Insects as models for biomedical research offer attractive alternatives to the use of higher

animals, particularly in light of dwindling research dollars and increasing protests by animal rights groups. A major advantage of using insect models is the ease with which large numbers of specimens can be reared; reduced per diem costs and space requirements result in significant savings. The flexibility afforded by use of short-generation species also can be exploited using insect models, and experiments can be run with large genetically homogeneous populations. Although insects differ from mammals in their morphology and physiology in a number of ways, it is often easier to isolate physiological or pharmacological systems in insect models.

Scientists have taken advantage of insect models in the past and have made significant discoveries in the biomedical sciences using them. The fruit fly *Drosophila melanogaster* has become the best-known model for laboratory and field studies of genetics. An encyclopedic body of information on *Drosophila* genetic studies is readily available through indexed bibliographies, proving that this insect model is one of the more important findings in the field of genetics. The fruit fly model has been also valuable in testing materials for their mutagenic and carcinogenic properties. Scientists have learned to use insect-pathogen transmission models to screen antipathogen chemical compounds and to diagnose certain human diseases. In addition, insect models have been used to study such diverse fields as the mode of action of steroid hormones and bioluminescence. For example, the role of cyclic nucleotides in insect hormone action provides a basis for studies on the animal hormone serotonin, and determinations of total ATP using insect luciferase have facilitated the estimation of low bacterial numbers in urine, milk, wine, and water. Current emphasis on utilizing insects as models for biomedical research has been in the fields of immunology and neuroendocrinology.

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