COMPREHENSIVE INSECT PHYSIOLOGY BIOCHEMISTRY AND PHARMACOLOGY

Executive Editors

G A KERKUT
L I GILBERT

Volume 10

BIOCHEMISTRY

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Foreword

Aristotle was enchanted by the phenomenon of insect metamorphosis and the early microscopists such as Robert Hook, Marcello Malpighi, Anton van Leeuwenhoek, René de Réaumur and Pieter Lyonet were fascinated by the structure and function of the different parts of insects and made some of the first important contributions to our knowledge of insect physiology. More detailed functional studies were made by Borelli in his book "De Motu Animalium", published in 1680, and his interpretation of insect walking patterns remained in our textbooks until 1955.

In general, the 18th and 19th century research workers were more concerned with the morphology and classification of insects, though physiologists such as Claude Bernard and naturalists such as John Lubbock and Henri Fabre were always interested in the functional analysis of insects.

One of the milestones in the study of insect physiology was the publication by Wigglesworth of his small book on insect physiology in 1934. This was stimulated by an appreciation of the way in which studies on the basic physiology of insects were necessary before one could understand and ultimately control the activity of insect pests of man and crops.

Wigglesworth initially studied medicine and then carried out research at the London School of Hygiene and Tropical Medicine. His innate gift for planning simple but fundamental experiments on *Rhodnius* led rapidly to an increase in our knowledge about moulting, the control of larval and adult stages, and provided the foundation for insect endocrinology. Furthermore he inspired a group of co-workers who later played a key role in the application of modern techniques to solve the problems of insect physiology and biochemistry.

Wigglesworth's "Insect Physiology" was followed by a more detailed and full-sized textbook, "Principles of Insect Physiology", which was published in 1947 and is now in its 7th edition (1972).

The three-volume edition of "Physiology of Insecta", a multi-authored work edited by Morris Rockstein, was published in 1964 and a new edition in six volumes followed in 1973.

The study of insect biochemistry developed more slowly, partly because there was no special distinction between physiology and biochemistry; the investigator just used the methods available for his studies. David Keilin started his studies working on insects: "From 1919 onwards I had been actively engaged in the study of the anatomy of the respiratory system, respiratory adaptation and respiration of dipterous larvae and pupae. Among the vast amount of material I was investigating, special attention was given to the larvae of *Gasterophilus intestinalis*". For these studies Keilin developed a method for the spectroscopic analysis of respiratory pigments of insect pupae under the microscope, which ultimately led to the discovery of the cytochromes.

The pteridines were discovered in insect pigments, and the one gene–one enzyme hypothesis of Beadle and Tatum, which was the cornerstone of molecular biology, was a result of biochemical and genetic analysis of *Drosophila*.

The rapid expansion of biochemistry after 1945 led to many more workers studying insect biochemistry and the first textbook on the subject by Darcy Gilmore was published in 1961. This was followed by the multi-authored "Biochemistry of Insects", edited by Morris Rockstein, in 1978.

The first evidence that a steroid hormone acts at the level of the gene came from the studies of Clever and Karlson in the 1960s on the puffing by the polytene chromosomes of *Chironomus*.

Though insect physiologists and biochemists initially published their papers in journals such as *Biological Bulletin*, *Journal of Biological Chemistry*, *Biochemical Journal*, *Journal of Physiology*, *Journal of Experimental Zoology*, *Journal of Experimental Biology*, *Roux' Archiv für Entwicklungsmechanik*, and *Zeitschrift für vergleichende Physiologie*, the great expansion of insect physiology and biochemistry from 1945 onwards led to the establishment of journals and other periodicals specialising in insects, such as the *Journal of Insect Physiology*, *Insect Biochemistry*, *Annual Review of Entomology*, and *Advances in Insect Physiology*.

It is also fitting to mention the work of other pioneers in the study of insect physiology and biochemistry, such as Autrum, Bounhiol, Bodenstein, Butenandt, Chadwick, Dethier, Fraenkel, Fukuda, Joly, Lees, Karlson, Kopec, Piepho, Richards, Roeder, Berta Scharrer, Snodgrass and Williams; these and many others laid the foundations of the subject and all following research workers have stood on the shoulders of these giants.

In July 1980 a meeting was held at Pergamon Press in Oxford to discuss the possibility of publishing a series of volumes on insect physiology, biochemistry and pharmacology. The idea was to produce 12 volumes that would provide an up-to-date summary and orientation on the physiology, biochemistry, pharmacology, behaviour and control of insects that would be of value to research workers, teachers and students. The volumes should provide the reader with the classical background to the literature and include all the important basic material. In addition, special attention would be given to the literature from 1950 to the present day. Emphasis would be given to illustrations, graphs, EM pictures and tabular summaries of data.

We were asked to act as Executive Editors and by December 1980 we had produced a 27-page booklet giving details of the aims and objectives of the project, details of the proposed volumes and chapters, suggested plans within the chapters, abbreviations, preparation of diagrams and tables, and journal citations to ensure uniformity of presentation as far as possible. This booklet was sent to authors of the chapters and their comments invited. By the middle of 1981 most of the chapters had been assigned to authors and the project was under way. The details of the volumes and the chapters they contain are given on the following pages so that the reader can see the contents of each of the other volumes.

In addition, there is a final volume, Volume 13, which is the Index Volume. Although each volume will contain its own subject index, species index and author index, Volume 13 will contain the combined subject, species and author indexes for all 12 text volumes so that any material in these volumes can be rapidly located.

All references in the volumes are given with full titles of papers, journal, volume, and first and last pages. The references to the authors in the text are given with their initials so that it is clear that the text refers to D. Smith and not, say, to A. Smith. There are more than 50,000 references to the literature, more than 10,000 species of insect referred to, and all should be readily found in the 12 different volumes.

There are 240 authors of the 200 chapters in the volumes and they have produced a series of very readable, up-to-date, and critical summaries of the literature. In addition, they have considered the problems associated with their subject, indicated the present state of the subject and suggested its developmental pathway over the next decade.

We are very grateful to our colleagues for the efficient way that they have met the challenge and the deadlines in spite of their many other commitments.

This series of volumes will be very useful to libraries, but an important case can be made that the books should be considered as research instruments. A set of volumes should also be available in the laboratory for constant reference. They will provide the research worker with an account of the literature and will always be instantly available for consultation. For this reason they should be considered as research equipment equally important as microscopes, oscilloscopes or spectrophotometers.

The volumes should save research workers many weeks of time each year in that not only will they provide an awareness of the literature and the background, and so save valuable research time, but the full index to authors, subject and species, and the full literature references, should also make it much easier to write reports and papers on their own new research work.

It is hoped that these volumes will do much to strengthen the case for insects as a source of research material, not only because insects are important medical and agricultural pests (over 200 million people at present have malaria: insects eat or destroy about 20% of planted food crops), but also because in many cases insects are the ideal unique research material for studying and solving fundamental biological problems.

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Preface to Volume 10

In reviewing a recent Ciba symposium on the neuropharmacology of insects, P. N. R. Usherwood asked: "What are the factors that combine to make insect neuropharmacology an interesting and important topic in science?" Substituting biochemistry for neuropharmacology, the same question can be asked, and the respective answers are, in principle, not very different. However, because of its more extensive scope, insect biochemistry encompasses a broader range of interests with a correspondingly wider range of answers entailing three approaches to the study of insect biochemistry.

The first approach, which may be termed the zoologist's viewpoint, is the study of insects for their own sake. Considering that insects occur in such vast numbers throughout the globe, the variety of different species, and their economic and medical importance, this approach scarcely needs any justification. If such investigations, prompted by an intrinsic interest in insect biology, lead to results of fundamental and/or general applicability, then so much the better. Thus Keilin's curiosity regarding the fate of larval haemoglobin in the bot-fly *Gastrophilus* resulted in his discovery of the cytochromes; Fraenkel's dietary experiments on growth of the larval mealworm *Tenebrio* culminated in identification of a new co-factor, carnitine or vitamin B_T, while Roth and Porter's electron microscopy of yolk uptake by oöcytes of the mosquito *Aedes aegypti* was the first description of cellular coated vesicles which have since become the object of widespread interest.

By contrast, to the biochemist insects are of interest only insofar as they can be used as "model systems" demonstrating some unusual biochemical reaction or product, or evincing some general reaction or product to an exaggerated degree. For example, Sir Gowland Hopkins' interest in the nature of the white pigment on the wings of a butterfly leading to the discovery of a new class of compounds, the pteridines, and indirectly to folic acid; the structure and biosynthesis of silk, obviously restricted to Arthropods; and while the identification of the toxins of insect venoms is still largely at the descriptive stage, the seminal studies of Kreil and co-workers on the biosynthesis of the polypeptide melittin of bee venom are undoubtedly the forerunner of a whole field of endeavor.

The third category are the molecular biologists and geneticists, for whom *Drosophila* has become a veritable cornucopia of experimental genes and mutants exploited to a degree far surpassing comparable findings for any other insect species.

The majority of chapters in this volume fall within the first category and four in particular deal with various kinds of metabolism. The first of these, Intermediary Metabolism (D. Candy), may be defined broadly as the chemical events intervening between the uptake of food, water and oxygen and the final metabolic end-products (excretion). The Krebs cycle, operative in insects, plays a central role in these reactions; but little attention has been paid to the mechanisms whereby many insects accumulate relatively high concentrations of Krebs cycle organic acids. Historically, it is interesting to note that apparently the first demonstration of carbon dioxide fixation by living organisms (lepidopteran pupae) was by Maria von Linden in 1905.

An important component of insect exoskeleton is the polysaccharide chitin — a polymer of *N*-acetylglucosamine units — and the principal "blood sugar" of most insects is the disaccharide trehalose. These facts, together with the flight muscle glycerol-3-phosphate cycle and the use of polyols in cold-hardiness, are the predominant features of insect Carbohydrate Metabolism (S. Friedman).

The term lipid encompasses a heterogeneous group of many substances characterized by being relatively insoluble in water but soluble in so-called fat solvents. Because of their diversity, obvious importance and increasing research, three chapters are devoted to lipid biochemistry. Steroids apart, the composition, synthesis and breakdown of lipids are reviewed under Lipid Metabolism (R. Downer); the transport of lipid in the form of diacylglycerol and carrier proteins (lipophorins), from the primary tissue of synthesis (fat body) to the sites of utilization, is discussed by H. Chino (Lipid Transport).

The preceding three chapters are of special relevance in relation to insect flight. Depending upon the genus, carbohydrates and/or lipids, and in certain instances the amino acid proline, are the primary fuels for flight energy. Further, it is now known that mobilization of the requisite fuel is under hormonal control. Flight physiology and integration of the various biochemical processes involved are reviewed by A. M. Th. Beenakkers *et al.* (Biochemical Processes Directed to Flight Muscle Metabolism). The actual flight muscle machinery is discussed by K. Maruyama (Biochemistry of Muscle Contraction). The extremely rapid wing beat frequency of the smaller insects is due not to any unique features of flight muscle *per se*, but depends largely on the mechanical oscillatory properties of the rigid thorax.

Steroids (J. Svoboda and M. Thompson) are of special importance because, *inter alia*, insects lack enzyme systems for sterol biosynthesis (*i.e.* they require an exogenous source of steroid), but can perform a wide range of steroid interconversions, *viz.* esterification, desaturation, saturation, dealkylation, *etc.* Moreover, the importance of ecdysteroids as hormones further accentuates the significance of steroid metabolism in the Insecta.

Insects have long been noted for having exceptionally high concentrations of free amino acids and peptides, while during metamorphosis larval proteins are degraded with a concomitant synthesis of adult proteins. These and related topics are covered by P. Chen in Amino Acids and Protein Metabolism, a chapter which sets the stage for the Molecular Biology [of] Protein Synthesis (M. S. Kaulenas). The mechanisms of protein synthesis in insects are basically similar to those of other eukaryotes, but in certain cases insect material such as particular cells specialized for the rapid production of one or more highly specific gene products, *e.g.* the silkmoth galea cocoonase, or the egg chorion proteins, have proven exceptionally suitable for probing various aspects of this complicated process. The *Bombyx mori* silkgland is another example of a cell highly specialized to produce a single gene product, *viz.* fibroin, and probably more is known about the biochemistry and molecular biology of silk synthesis than of any other insect protein. An entire chapter, Silk Synthesis (J. Prudhomme *et al.*), is devoted to this topic.

As pointed out by S. J. Berry in Insect Nucleic Acids, the importance of *Drosophila* in contemporary molecular genetics has not generated concomitant progress in the biochemistry of these nucleic acids. Nevertheless, an impressive body of data exists, and even though much of it pertains to the genus *Drosophila*, the fruit fly is merely a paradigm for what may be anticipated in other insects.

Several insects easy to rear in the laboratory also have relatively short life cycles, and these are eminently suitable for the study of the aging process. A variety of parameters such as chemical composition, enzyme activities, flight performance, environmental effects, *etc.*, as a function of age, are reviewed by R. S. Sohal (Aging in Insects). For much the same reasons, over 60 years ago T. H. Morgan selected *Drosophila* as an ideal animal for laboratory genetic studies which subsequently included population genetics. The earlier morphological variations have now been expanded to include enzyme polymorphisms as a consequence of newer biochemical techniques, especially gel electrophoresis, and the "Central Dogma" of molecular biology. A. J. Zera *et al.* (Allozymes and Biochemical Adaptation) discuss how the study of several allozymes whose individual polypeptide components are coded by separate genes, have furthered our understanding of population genetics.

Several related proteins widely distributed among insects have been termed Insect Storage Protein (L. Levenbook), signifying their probable function, or, more recently, arylphorins because they are so rich in aryl residues. These proteins accumulate in the last larval instar, and are utilized during metamorphosis. The biology and biochemistry of these proteins are the subject of this chapter. Another, quite different group of proteins, first discovered in *Drosophila*, is the heat-inducible Heat Shock Proteins (N. Petersen and H. Mitchell). The function of these proteins is still unknown; they are not peculiar to insects, but insects are exemplary organisms for research on this special type of gene expression.

Until relatively recently, insect neurochemistry has lagged behind neurophysiology as a topic of investigation, but this is no longer the case. Aside from the well established cholinergic system, glutamate, γ-aminobutyric acid, various biogenic amines and small peptides have been identified as neurotransmitters. Y. Pichon and R. Manaranche (Biochemistry of the Nervous System) review this aspect of insect biochemistry. Another wide range of compounds such as ommochromes, melanins, pteridines, carotenoids and flavonoids are important in a different context, namely, as insect pigments. Their structure, metabolism and functions are the subject of H. Kayser's review on Pigment.

To protect themselves against predators or to immobilize their prey, insects have evolved a veritable armamentarium of toxic compounds, many of which are neurotoxins. The biology of venomous arthropods, the chemistry of the toxins, and their pharmacological actions are summarized by E. Zlotkin (Toxins Derived from Arthropod Venoms Specifically Affecting Insects).

The last chapter, Metabolism and Characterization of Insect Cell Cultures by W. F. Hink *et al.*, deals with the characterization and metabolism of established cell lines from several insect orders. Assuming a pure line is derived from a known cell type, then such cells become amenable to such experiments as influence of the medium including hormonal effects, developmental properties, metabolic reactions, *etc.*, under precisely known conditions that cannot be duplicated *in situ*.

In the absence of any "crystal ball", it is presumptuous to predict the future trends of insect biochemistry. One might hazard a guess, however, that important advances will be made in the following areas: (1) Genes coding for a variety of characterized gene products cloned from insects other than *Drosophila* to facilitate an understanding of the modes of action of juvenile hormone(s) and ecdysteroids at the molecular level. (2) The developmental biochemistry of insect metamorphosis, with special reference as to how larval tissues are broken down and the resulting "building blocks" are utilized by the developing imaginal discs. (3) The structure and biosynthesis of the insect exoskeleton. (4) The biochemical basis of resistance to insecticides. But even without such prognostications, if this book can arouse the interests of biochemists or molecular biologists who had previously ignored insects as experimental animals, then at least one of its purposes will have been achieved.

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1 INTRODUCTION

This chapter has three main functions. The first is to introduce the topic of intermediary metabolism and to indicate those aspects of insect metabolism that are of particular interest. Many of these aspects will be covered in more detail in later chapters of this volume. The second function is to review in greater depth the tricarboxylic acid cycle and its regulation in insects. The third function is to show how the