THE BIOCHEMISTRY OF INSECTS

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PREFACE

In an address in 1935, on the occasion of his appointment as Honorary President of the London Natural History Society, Sir Frederick Gowland Hopkins recalled that his first scientific paper, in 1878, was concerned with the biology of the bombardier beetle, and that his interest in biological chemistry was first aroused by a desire to know the composition of the violet-coloured vapour ejected by this beetle. Apparently he never returned to this original object of curiosity, and it was almost eighty years before the bombardier's chemical armament was analyzed and found to consist of quinones, probably by-products of biochemical mechanisms of protein tanning of great importance in the biology of insects. Later, Hopkins initiated some research on the water-soluble pigments of the wings of pierid butterflies, which he identified as purine derivatives. Had he followed the chemistry and biology of these compounds more closely, he might have discovered the pteridines, a group of widespread occurrence in nature, the biological significance of which is only now being revealed. These anecdotes are mentioned with the object of showing the intellectual stimulus to biochemical research that the insects can provide, and of demonstrating that the open-minded scientific pursuit of the lines of inquiry so opened up can lead to discoveries of far-reaching biological importance.

The view of the biochemist as a naturalist, which Sir Frederick Hopkins championed, is not so popular today, and, in fact, many biochemists would probably regard the subject of insect biochemistry as something close to a contradiction in terms. The concept of the unity of biochemistry is well known, and the view is widely held that most biochemical mechanisms were established very early in evolutionary history and are the same throughout all the natural taxonomic groups of animals and plants. As a consequence, many textbooks of biochemistry, in arranging material according to *biochemical* principles, mix together information drawn from widely differing biological sources. This method is both inevitable and unimpeachable in the present state of development of the science, but it does violate the biologist's concept of the complexity of natural forms and physiological adaptations, and tends to shift the emphasis in biochemistry to the chemistry of reactions rather than the chemistry of organisms. A book on the biochemistry of insects may be thought of as reversing in some small degree this over-simplification in biochemical literature and the over-emphasis of the chemical as opposed to the biological approach, and this must serve as my justification, on purely scientific grounds, for writing it. From a more practical standpoint, no such justification is needed. The major part of the world's fauna is classified in the Insecta, and a large number of scientists throughout the world are engaged on studies of all aspects of the biology of insects. Of recent years an ever-increasing stream of information on the life processes of insects at the biochemical level has appeared, and the need for a summary of this literature has become apparent not only to insect biochemists and those engaged in the related field of insecticide toxicology, but also to entomologists in general.

This being the first text in the field, its aim has been, as far as is practicable, a complete coverage of existing knowledge, and the book is not directed at any restricted audience. On the other hand, I have attempted more than a mere compendium of information, and have aimed rather at a critical synthesis, writing in what I considered to be a necessary background of biological and biochemical principles, and indicating profitable lines for future work. I hope, therefore, that the book may have value both as a teaching text and a working manual for investigators in insect biochemistry.

I would like to thank the Executive of the Commonwealth Scientific and Industrial Research Organization for permission to write this book. I am particularly indebted to Mr. John Calaby for his help in reading proofs, and to the following friends and colleagues who read and criticized parts of the manuscript: Dr. Mervyn Griffiths, Dr. Cyril Appleby, Professor W. P. Rogers, Dr. Lucile Smith, and Dr. D. F. Waterhouse. Some of the figures were photographed by Mr. Don Wilson, of the Division of Entomology. My thanks go also to numerous authors, whose names are acknowledged in the text, for permission to use their figures. Most of the illustrations were prepared by my wife, Betsey, whose encouragement during the writing was a constant source of needed strength.

DARCY GILMOUR

July, 1960

INTRODUCTION

THE material presented in this book is organized into three sections. The first of these, called Energy Metabolism, deals with the insect as a machine, burning the fuel supplied by its diet, and deriving from this combustion the energy for biological syntheses, movement, and so on. It considers the nature of the food necessary for the development of insects, its preparation in the gut for absorption into the cells, the long chain of degradative processes by which the compounds derived from the food are oxidized in the cells, the conservation of the energy derived from this oxidation and its use in the performance of biological work of various kinds, and the nature of the end-products of the degradative sequence. The second section, called Intermediate Metabolism, includes a number of biochemical transformations not directly concerned with the energy-yielding or energy-using processes. The material in this section is divided along chemical lines. The last section, which is very short, is concerned mostly with hormonal regulations of metabolism, which in insects have been studied mostly in connection with morphogenesis.

These divisions are, of course, artificial. The same thermodynamic principles govern the reactions considered in Sections 2 and 3 as in Section 1, although they are not considered from the viewpoint of energy transformations. Modern biochemistry no longer recognizes the distinction between catabolism and anabolism, since the very mechanism of the conservation of energy within the organism is the coupling of exergonic with endergonic reactions. Nevertheless, it is convenient to group together all the mechanisms concerned *primarily* with the winning and expenditure of energy and to treat catabolic and anabolic aspects separately. Unifying principles are less easily found in the material included in Section 2, perhaps because the biochemical mechanisms have been explored less thoroughly. This section includes a great deal of early work on the chemistry of insect products, which, although providing a fundamental basis for future work, does not contribute much to biochemistry in its modern dynamic sense. On the other hand, the section does include some aspects of insect metabolism in which biological transformations have been thoroughly studied, such as, for instance, the synthesis of the phenoxazine pigments, and the quinone tanning of proteins, which have yielded important information on biochemical mechanisms peculiar to insects. Work on insects may in the future have much of general interest to offer in the sphere of biological regulation and control, which is considered in Section 3, but so far there is little of a concrete nature to record.

A short word is appropriate at this point on the names used in the text for the many species of insects which have been studied biochemically. In writing about insects, any biologist who is not an insect taxonomist must suffer some embarrassment in the task of trying to give to each its correct name. As the over-enthusiastic efforts of the earlier entomologists are corrected by the taxonomists, names change with sometimes alarming rapidity, and the unwary biochemist may be caught referring to several different names as different insects, when, in fact, only one species is involved. The problems posed by this confusion have been avoided to some extent by the use of either well-established common names, such as "the housefly," for *Musca domestica*, or the generic name only to denote a well known and much studied member of a genus, such as *Blattella* for *Blattella germanica*. Where a specific name is mentioned, the author's name or abbreviation has been omitted as inappropriate for a biochemical text. These practices will give pain to some entomologists, but the alternative elaboration of names could well interrupt the flow of the biochemical theme. It is hoped that the bibliographical references are complete enough that any reader who wishes to satisfy himself about a specific name can go to the original paper and from there on into the taxonomic literature.

CONTENTS

SECTION 1

ENERGY METABOLISM

CHAPTER I

NUTRITION

THE study of nutrition provides information of several kinds to the biochemist. In the first place, by establishing the major constituents of the natural diet of an animal it defines the starting material for the long chain of catabolic processes from which is derived the energy for life and growth. More importantly, experimental data on absolute nutritive requirements demonstrate the animal's ability to synthesize metabolites, and further, insofar as the accessory growth factors are known to be linked with definite biochemical mechanisms, the necessity for or independence of such factors can give a clue to the kinds of metabolic pathways operating, and may even indicate the presence or absence of specific enzymes. Finally, the kind of lesion produced in an animal starved of an essential nutrient may yield information on the site of action and metabolic function of the nutrient.

Many insects survive and grow on what appears at first sight to be an extremely restricted diet. One thinks, for instance, of the termites living in dry wood, the wax moth which feeds on the wax and debris of bee combs, and the clothes moth which lives on wool. In all cases that have been investigated critically, however, it is found that this apparent limitation of diet is not complete, in that the insects, like other animals, require a number of vitamins and sources of organic nitrogen in the form of essential amino acids. Where the diet is very restricted these constituents are found to be supplied by micro-organisms either in the food or in the bodies of the insects. In the case of many termites, for instance, the gut contains a rich fauna of Protozoa which besides digesting cellulose probably also provides the essential metabolites which are absent from the insect's diet. Experiment has shown that the clothes moth cannot survive on a diet of uncontaminated wool, or the wax moth on pure wax. Other insects, such as the cockroach, *Blattella*, and various grain beetles, which have a high degree of independence of the vitamins which are necessary in the diet of nearly all animals, have been shown or are believed to owe their independence to the synthetic activities of yeasts or bacteria which the insects harbor within their own cells.

Although examples such as this indicate that any conclusions with regard to the synthetic ability of insects based on a cursory or uncritical examination of the diet are apt to be misleading, it is still nevertheless true that a number of insects do derive almost all their energy from a food supply of a very restricted nature. This in itself is an indication that the metabolic pathways for the interconversion of fats, carbohydrates, and proteins are well developed in many insects. In others, however, these capabilities are less well expressed. It is to be expected that metabolic patterns will differ in important details between insect species, just as they do between different species of mammals.

The diversity of the diet of insects and their successful invasion of many ecological niches not shared by other animals has created a great deal of interest in insect nutrition, so that the literature on the subject is large. Much of this is of a general nature, and being of little interest to the biochemist, will not be considered here. The gathering of information on the absolute dietary requirements of insects ideally requires that experiments be performed under sterile conditions with the animals living on a chemically defined diet. Such conditions have been met in only a small number of the published studies on insect nutrition, and it happens that most of the insects reared successfully in this way have been members of the Diptera. A large body of other important information on nutrition has been derived from studies on flour- and grain-eating insects whose diet is so dry as to be for all practical purposes sterile. This review will be based largely on these two bodies of information; for a more extensive treatment of the literature the reader is referred to the reviews of Trager[1] and Lipke and Fraenkel.[2]

Carbohydrate

Carbohydrate, which forms a large part of the diet of many insects, is usually not essential, but a few insects cannot develop without it. Such exceptions are found among insects adapted to a diet with a very high carbohydrate content. Thus, of the pests of stored food products, *Tribolium*, *Lasioderma*, and *Ptinus* do not need carbohydrate, while *Tenebrio*, *Ephestia kühniella*, and *Oryzaephilus* fail to grow in its absence.[3-6] This physiological difference has its reflection in the food preferences of the insects, those needing carbohydrates being restricted to the diets of high carbohydrate content (cereal, dried fruit, etc.), whereas the others attack a wider variety of foods. Adults of the flies *Calliphora erythrocephala* and *Lucilia cuprina* do not survive without carbohydrate in the food.[7, 8] Another fly, *Pseudosarcophaga affinis*, which lives as a parasite in the spruce bud worm, also needs carbohydrate when grown on an artificial diet.[9]

Several workers have studied the nutritive value of a series of carbohydrates for those insects in which lack of carbohydrate causes a marked reduction in the life span. Most of this material is considered in the chapter on digestion, but it is appropriate to include in this section information on the nutritive value of carbohydrates which may be presumed to be absorbed in the gut unchanged, such as the hexoses, pentoses, and sugar alcohols. Of the hexoses, glucose and fructose have high nutritive values for most insects, mannose and galactose are used by some,[7, 8, 10, 11] but are of little or no value to several species,[12-14, 141] and sorbose is not used by any insect so far studied. Similarly, the sugar alcohols mannitol and sorbitol are used by several, but not all, insects. The pentoses are in general poorly used, but *Stegobium* grows well on several,[15] and a number of flies can make some use of xylose.[7, 8, 10] These results are difficult to interpret, since the lack of food value may have been due to the insect's failure to absorb the sugars, but it does seem that some insects may lack the enzymes needed to introduce sugars other than glucose and fructose into the glycolytic process.

In *Tenebrio*, several of the sugars which are not used are actually harmful, inhibiting the utilization of greater quantities of starch.[14] It is not known whether the site of action of this inhibitory effect is in the gut wall, or whether it represents an interference with the further metabolism of absorbed carbohydrate.

FAT

The larval stages of most insects store up the greater part of their reserve energy supplies as fat, and it is clear that the ability to synthesize fat from other constituents of the diet is widely developed. This ability has been studied in the case of *Aedes*, which stores fat if fed on either a protein or a carbohydrate diet.[16] Fat has been shown to be an essential article of diet in the case of moths of the genus *Ephestia*. In the absence of fat, larvae of three species of *Ephestia* grow slowly and the adults fail to emerge from the pupal cases.[17] The deficiency in the diet can be overcome by the addition of linoleic acid ($C_{18}H_{32}O_2$). *Ephestia* thus resembles the rat in its dependence on a dietary supply of unsaturated fatty acid, but differs from the rat in that it can use isolinoleic acid in place of linoleic. Of the naturally occurring, long-chain, unsaturated fatty acids, linolenic acid ($C_{18}H_{30}O_2$) is effective in *Ephestia* but not oleic acid ($C_{18}H_{34}O_2$), while arachidonic acid ($C_{20}H_{32}O_2$), a fatty acid of animal origin which is probably the most effective for curing fat deficiency in the rat, has no effect on adult emergence in the moth.[18]

It has been suggested that the fatty acid required in the diet of *Ephestia* has an essential role in the production or proper functioning of the molting fluid, which, in insects, is secreted between the old and new cuticles at the time of molting. The evidence for this rests on the fact that in a complete deficiency of fat the adult moth, although fully formed, remains imprisoned in the pupal case, whereas larvae reared on diets containing suboptimal quantities of fat produce adults with varying degrees of malformation of the wings. The malformation consists of the absence of some or all of the wing scales, which, on the emergence of the moth, remain stuck to the inner surface of the pupal skin.

The fat requirements of a number of insects have been tested,[5, 17, 19, 20] but until recently it was only in *Ephestia* that a positive effect had been obtained. Even the closely related moth *Plodia interpunctella*, which lives on the same food as some species of *Ephestia*, can grow in the complete absence of fat. The pink boll worm[21] and larvae of the fly *Pseudosarcophaga*,[9, 22] however, have been shown to need fat in the diet, and since these requirements can be met by a mixture of fatty acids, they probably reflect a block in metabolism similar to that occurring in *Ephestia*.

The nutrition of the wax moth, *Galleria mellonella*, the larvae of which burrow into the comb of beehives, is of interest, since it is one of the very few animals that regularly consume quantities of wax. It has been shown that wax is not essential in the diet of *Galleria*,[23] and in fact, the moth has been reared on a diet from which fat, but not cholesterol, had been extracted by treatment with light petroleum.[24] The larvae of *Galleria* digest and use the beeswax which is normally part of their diet, approximately 40% of the wax ingested being retained in the insect's body.[25, 26]

Amino Acids

The study of the amino acid requirements of insects has been handicapped by the difficulty of formulating a completely "synthetic" or chemically defined food on which any insect will grow. Much of the earlier work on insect nutrition was done with diets which included either casein or yeast, or both, and while quite satisfactory growth of some insects was obtained on such diets, the replacement of the natural products with pure vitamins and amino acids usually had a harmful effect which could not be related to the lack of any known factor, but was usually ascribed to a lack of "balance" in the constituents of the artificial diet. For instance, it has been shown that it is possible to grow *Tribolium confusum* as well on a diet containing a mixture of the 19 common amino acids of proteins (with carbohydrate, salts and pure vitamins) as on one containing casein,[27] but *Dermestes* and *Tenebrio*

TABLE 1

AMINO ACID REQUIREMENT OF INSECTS

Amino acid	*Blattella*[29, 30] ♂ ♀	*Chilo*[31]	*Aedes*[32]	*Drosophila*[33]	*Calliphora*[34]	*Phormia*[35]	*Pseudosarcophaga*[9]	*Apis* (adult)[36]	*Tribolium*[27, 37, 38]	*Attagenus*[39]
Arginine	+	+	+	+	+	+	+	+	+	+
Histidine	+	+	+		+	+	+	+	+	+
Isoleucine	+ +	+	+	+	+	+	+	+	+	+
Leucine	+ +	+	+		+	+	+	+	+	+
Lysine	+ +	+	+		+	+	+	+	+	+
Methionine	−	+	+		+		+	+	+	+
Phenylalanine	− −	+	+		+	+	+	+	+	+
Threonine	− −	+	+		+	+	+	+	+	+
Tryptophan	+	+	+		+	+	+	+	+	+
Valine	+	+			+	+	+	+	+	
Alanine	+ +			−	−		−	−	−	
Aspartic acid	− −			−	−		−		−	
Cystine	−		+		−		−	−	−	
Glycine	−		+	+	±		±	−	−	
Glutamic acid	− −				−		−	−	−	
Hydroxyproline	− −			−	−		−	−	−	
Proline	+ −			−		+	−	−	−	
Serine	+ −			−	−		−	−	−	
Tyrosine	− −			−	−		−	−	−	

KEY: + indicates essential; − indicates not needed; ± indicates some growth-promoting activity; numbers after insect names are literature references.

completely fail to grow when amino acids are substituted for casein. On the other hand, a number of insects have been grown successfully under sterile conditions on a diet that is as nearly as possible completely defined chemically, and in one case, at least, such an artificial diet produced more rapid growth than did the natural diet of the insect.[28]

Data on the amino acid requirements of insects are collected in Table 1. While it seems to be generally true that the amino acids required in the diet by insects are the same 10 that are essential for the rat and other animals, namely, arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine, nevertheless certain other specific requirements have been well established. Thus, glycine is essential for several members of the Diptera, cystine for *Aedes*, and alanine for *Blattella*. Proline is essential for *Phormia* and male *Blattella*, and serine also for male *Blattella*, although neither of these amino acids seems to be needed by the female cockroach. Other reports have indicated the possible existence of unusual synthetic mechanisms in insects; for instance, the ability of *Aedes* to use tyrosine in place of phenylalanine,[32] of *Phormia* to use cystine in place of methionine,[40] and of *Blattella* to synthesize its requirements of methionine, phenylalanine, and threonine.[29, 30, 41] In several instances the addition of all the other amino acids listed in Table 1 has been shown to produce an improvement in the growth of insects, but since none of them had any effect when added to or removed from the mixture singly they have been concluded to be not essential. Synthesis of all of these nonessential amino acids, with the exception of hydroxyproline, has been demonstrated in the rice stem borer, *Chilo simplex*.[31]

These bald statements of amino acid requirements, however, tend to obscure the variation in the level of response obtained by different workers in the field of insect nutrition. The designation of an amino acid as essential for a particular animal usually means that the amino acid is required for the animal's full development or adult survival, and that it is not synthesized by the tissues either from other amino acids or from simpler precursors. This criterion seems to be satisfied in the case of many of the insects studied. Thus, in *Tribolium*,[27] the effect of the omission of any one of the 10 amino acids considered to be essential for the rat is that very few of the larvae on the deficient diet survive for 20 days, and none pupate (normal length of larval life 29 days). Similar deficiency effects have been observed in *Aedes*,[32] *Drosophila*,[33] and *Pseudosarcophaga*.[9] In *Attagenus*, although survival is fair on deficient diets, the weights of the survivors are usually down to about one-tenth of those of normal larvae.[39] In the case of *Blattella*,[29, 30] however, the deficiency effects, when observed at all, are of a much milder nature. In this insect, the absence from the diet of any one of the amino acids listed as essential in Table 1 results only in a slowing of the growth rate, insects on all diets surviving unto the twelfth week, so that it is only by a statistical treatment of nymphal weights that a dietary requirement is established. Moreover, there is direct evidence that many of the amino acids, so established as required in the diet, can be produced in the insect's tissues. Only by the omission of alanine and leucine have reasonably well marked deficiency effects been established, and it is clear that, of these amino acids, alanine can be produced in the insect's tissues. It must be conceded, therefore, that *Blattella* is capable of synthesizing many, or all, of the amino acids it requires, and that omission of any one amino acid from its diet merely strains metabolic resources, rather than imposes an insurmountable metabolic block.

Even in the case of *Tribolium*, the well defined effects of amino acid deficiencies are largely obscured by the addition to the diet of 1% of yeast, an amount which adds only insignificantly to the total amount of certain of the essential amino acids. This suggests that in *Tribolium*, also, some ability to synthesize amino acids may exist and that the observed failure of such synthetic mechanisms in the absence of yeast may be due to a deficiency of another, as yet unresolved, sort.

It is probable that in *Blattella* many of the synthetic processes of which it is apparently capable are performed by its flora of microscopic intracellular symbiotes. *Blattella* is well provided with intracellular inclusions of what are generally conceded to be symbiotic micro-organisms, located in special cells called mycetocytes or bacteriocytes.[42] It is reasonable to suppose that the synthetic mechanisms which are a normal feature of the metabolism of bacteria and plants rather than of animals reside in these symbiotes. Similar inclusions have been proved to be a source of vitamins in other insects, and it is probable, though by no means certain, that they could provide essential amino acids also. Although from the point of view of the biochemist, it is important to know exactly where the synthetic mechanisms are located, the insect biologist may be excused the belief that the distinction between insect and symbiote tissue is largely immaterial. The intracellular symbiotes, transmitted from generation to generation through the egg, are as much a part of the organism *Blattella* as are the mitochondria, and in fact, in the past there has been some confusion about where to make a distinction between these two sorts of intracellular inclusion. The possible role of its symbiotes in nutrition renders no less remarkable the extraordinary selfsufficiency of the cockroach, which is capable of surviving without a source of organic sulfur or of the more complex amino acids.

It is of interest that the amino acid nutrition of *Pseudosarcophaga*, a parasite which spends its larval life in the tissues of an insect, an environment very rich in free amino acids, is so similar to that of the free-living forms. In addition to the usual 10 amino acids, in the absence of any one of which development ceases completely, *Pseudosarcophaga* requires glycine for optimal growth, but this requirement is in line with that of other Diptera investigated. Some evidence of an increasing dependence on dietary amino acids is seen in the fact that the absence of either alanine, serine, or tyrosine results in a small but statistically significant reduction in growth rate.

Since much of the information so far available on the amino acid metabolism of insects is derived from nutritional studies, it is appropriate at this point to deal with the amino acids individually in greater detail.

Glycine

$H_2N.CH_2.COOH$

The simplest of all the amino acids, glycine is readily synthesized by most animals, but seems to be an essential nutrient for many of the Diptera. It is required in the diet of *Aedes*[32] and *Drosophila*,[33] and its omission from the diet of the parasite *Pseudosarcophaga*[9] or the blowfly *Calliphora*[34] retards growth, although some individuals reach maturity without it. The only dipterous insect found to have no requirement for glycine is *Phormia*.[35, 40] Glycine and serine are interconvertible in higher animals, but serine cannot replace glycine in the diet of *Aedes*. Neither can creatine, although the tripeptide glutathione (glutamylcysteinylglycine) is partially effective as a replacement for glycine. There is some suggestion that the requirement for glycine is connected with its role as a detoxicant for other constituents of the unnatural synthetic diet,[33] but the evidence suggests that in Diptera there is a failure to synthesize this amino acid, at least in the quantities required for normal growth.

Alanine

$$\begin{array}{l} CH_3.CH.COOH \\ \quad\;\;\;\, | \\ \quad\;\; NH_2 \end{array}$$

Alanine is readily formed from pyruvic acid by transamination, and can be synthesized by most animals. In *Blattella*, however, a diet deficient in alanine produces a pronounced retardation in growth. Alanine shares with leucine the distinction of being the most essential amino acid for this insect. Failure to synthesize the amino acid is not complete, however, since it can be detected in tissue extract of roaches grown on a diet free from alanine.[30] Absence of alanine from the diet