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CHAPTER 2

HEMOLYMPH: COMPOSITION

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I. INTRODUCTION

Insects are known to possess only one extracellular fluid, bathing the cells and circulating throughout the body; the term "hemolymph" is thus more accurate than "blood" to designate this body fluid.

Insects have given up the physiological association between respiratory and circulatory systems, the tracheal system insuring the arrival of oxygen in the immediate vicinity of the cells. The hemolymph is thus not concerned with oxygen transport nor with the transport of CO_2. Exceptions to this rule are found in the case of some chironomids, the hemolymph of which carries a hemoglobin.

However, this is not the only peculiarity of insect hemolymph and the data accumulated mainly during the last decade have revealed that it is entirely different, especially from the biochemical point of view, from the body fluids of all other animal phyla. The most striking peculiarities are, as will be emphasized in this chapter, the tendency to the replacement of the inorganic osmolar effectors, usually Na^+ and Cl^-, by organic molecules, especially free amino acids and organic acids, the very special pattern of cationic composition characterizing several orders, the seat of gluconeogenesis and the unique form of hemolymph carbohydrate, namely trehalose, the presence of organic phosphates, and of a wide variety of enzymes, and so on.

However, these biochemical characteristics are generally more deeply marked in the more specialized insect orders than in the more primitive ones. The modern taxa of the class Insecta may thus be considered, according to the views of taxonomists, as representing a collection of the successive evolutionary levels, the most original and specialized biochemical features being fully exploited by, for instance, the larval forms of Lepidoptera.

In the present chapter, we shall pay particular attention to some aspects of the physiological role and the adaptive significance of the main biochemical constituents of insect hemolymph, considered especially from an ecobiochemical point of view. Clotting in the hemolymph is discussed in a separate chapter (see Grégoire, Chapter 3, this volume). Other physical or chemical properties of insect hemolymph, such as specific gravity, surface tension, gas content and gas transport, hydrogen ion concentration and oxidoreduction will be omitted from the present review, little recent information having been made available on these subjects since the reviews of Buck (1953) and Wyatt (1961).

II. OSMOTIC PRESSURE

The osmotic pressure of the hemolymph is generally somewhat higher than that of mammalian blood. The values obtained by different authors, and carefully compiled by Sutcliffe (1963) show that the osmotic pressure, expressed in terms of freezing point lowering, generally ranges from −0.5° to −0.9°C. Minimal values have been obtained in the case of *Ephemera danica* larvae (−0.504°C), of three Trichoptera larvae (−0.38° to −0.455°C) and of *Tipula montium* larvae (−0.443°C). Higher values have been observed in the larvae of *Popillia japonica* (−1.03°C) and *Ephestia kühniella* (−1.130°C). The high values observed during pupal life in some Lepidoptera are not surprising, owing to the increasing amount of hydrolytic products resulting from histolysis.

Insects are able to regulate the osmotic pressure of their body fluids. The role of the different solutes as osmolar effectors is considered in the following section. However, a direct effect of the relative ambient humidity on the osmotic pressure of the hemolymph has been demonstrated in *Tenebrio molitor* (from −0.8°C to −1.3°C: Marcuzzi, 1955, 1956). Some insects show a considerable increase of the hemolymph osmotic pressure during overwintering, owing to the accumulation of glycerol (for instance, *Monema flavescens;* Asahina *et al.*, 1954).

In contrast to the blood of vertebrates, the sum of the inorganic cations and anions does not account for the total osmotic pressure. Free amino acids, organic acids and other organic molecules play an important role as osmolar effectors, especially in the most specialized endopterygote orders (see below).

III. OSMOLAR EFFECTORS

It is well known that, in most other animal phyla, the osmotic pressure of the body fluid is insured by inorganic constituents, among which sodium is generally the main cation, and chloride the main anion. The situation is more complicated and sometimes entirely different in the case of insects.

As Sutcliffe (1962, 1963) has pointed out, and as it appears from examination of Figs. 1 and 2, the participation of inorganic cations and anions in the osmotic pressure of the hemolymph tends to decrease with

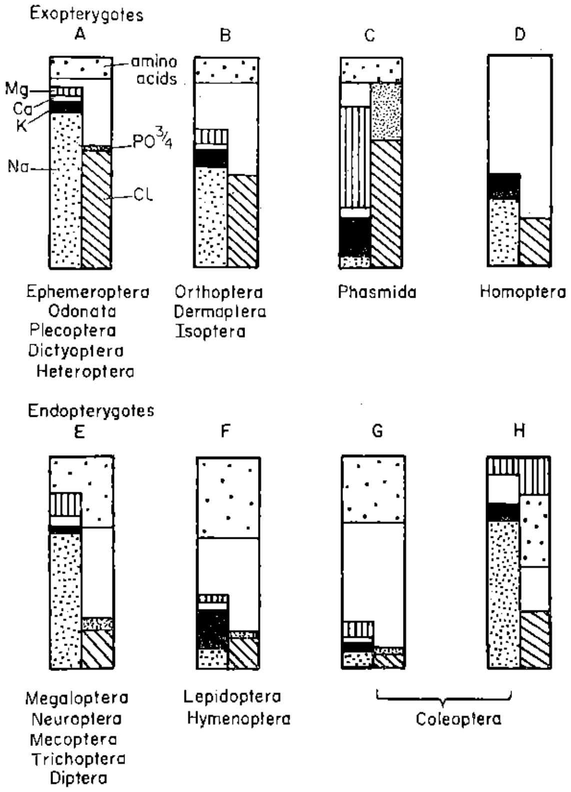

FIG. 1. Osmotic effects of components illustrated as percentages of the total osmolar concentration of hemolymph in pterygote insects. Each block in the figure is visualized as two vertical sections, each section representing 50% of the total osmolar concentration. The percentage contributions of cations are illustrated in the left-hand section, with sodium at the base (stippled), followed by potassium (black area), calcium (white area), and magnesium (vertical stripes). Anions are illustrated in the right-hand section, with chloride at the base (oblique stripes) followed by inorganic phosphate (fine stippling). Where possible, free amino acids are illustrated in equal proportions in both sections (coarse stippling). The large blank area in each block represents the proportion of the total osmolar concentration that must be accounted for by other components of the hemolymph. (Sutcliffe, 1963.)

the evolutionary level of the insect. Among the most primitive Insecta (Apterygota), *Petrobius maritimus* shows a hemolymph composition very similar to that of other arthropods, with the nearly exclusive participa-

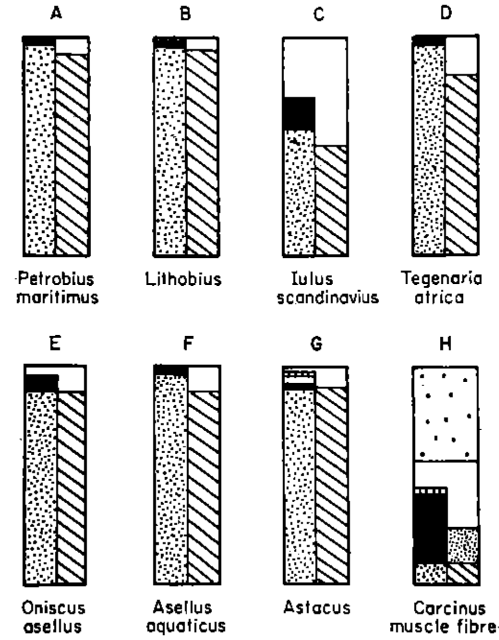

FIG. 2. Osmotic effects of components illustrated as percentages of the total osmolar concentration of blood in: (A) an apterygote insect, (B) a chilopod, (C) a diplopod, (D) an arachnid, (E–G) crustaceans. (H) illustrates the osmotic effects of components in the muscle fiber of *Carcinus maenas*. Conventions as in Fig. 1. (Sutcliffe, 1963.)

tion of Na and Cl as osmotic effectors (Lockwood and Croghan, 1959). In most primitive pterygote Insecta, all of which are exopterygotes (Ephemeroptera, Odonata, Dictyoptera, Heteroptera, and, to a lesser extent, in Orthoptera, Isoptera, and Dermaptera), the sum of the four cations contributes to nearly half of the osmotic pressure, Na playing the principal role, while the concentrations of K, Ca, and Mg are very low. In these orders, chloride is the main anion, inorganic phosphates and organic molecules being in low concentration. In these insects, the situation is not very different from that found in other animals, and Sutcliffe (1963) has suggested that "hemolymph with this type of composition represents the basic type of hemolymph in pterygote insects."

In the Phasmidae, the situation is very similar, but Mg takes the place of Na as the principal osmotic effector, and inorganic phosphates are more abundant.

A third case is represented by the following endopterygote orders: Megaloptera, Neuroptera, Mecoptera, Trichoptera, and Diptera. The sum of the cations is also responsible for nearly half of the osmotic pressure, with Na as the principal effector, but chloride has a minor importance and is partially replaced by amino acids and other small organic molecules.

In Lepidoptera, Hymenoptera, and many Coleoptera, the importance of cations, as well as that of chloride, is considerably reduced, organic molecules playing the main role as osmolar effectors. These groups, in which the highest values of amino acid participation are found, are also recognized by Duchâteau *et al.* (1953) as being highly specialized by the existence of very low values of the Na index, and of very high values of the Mg and K indices.

Figures 1 and 2 clearly illustrate the biochemical evolution of insects, as far as hemolymph osmolar effectors are concerned. The great similarity between the body fluid composition of the apterygote *Petrobius* and the other Arthropoda is an excellent indication of the fact that primitive insects emerged from the common arthropodial trunk with an internal medium of the "basic" types, that is with sodium chloride as the almost sole osmolar effector. The same type of hemolymph composition has been kept by the modern Palaeoptera, as well as by the three orders originally derived from three distinct stocks of Neoptera exopterygotes (according to Jeannel, 1949): Plecoptera, Dictyoptera, and Heteroptera. But, in these primitive insects, we may find some indication of the evolutionary tendencies developed later in the more specialized insects: a slight reduction of the sodium chloride and the incorporation of small organic molecules in the bulk of the hemolymph constituents. This tendency develops considerably in the endopterygotes; the monophyletic origin of this group suggests that the increasing utilization of free amino acids (and other organic molecules) in replacement of chloride occurred very early in the evolution of endopterygotes, probably prior to the divergence of the "panorpoïd complex."

It appears that two different tendencies are to be seen during the evolution of the different orders from the "panorpoïd complex": one of these being the conservation of a high amount of inorganic cations, the other tendency (represented by Hymenoptera, Lepidoptera, and many Coleoptera) being the strong decrease of inorganic cations in the hemolymph. According to Sutcliffe (1963), this last specialization probably occurred independently on at least two occasions, these three orders being derived independently from the panorpoïd line.

In the matter of osmotic regulation, insects are not able to control the concentration of inorganic ions in their hemolymph when placed in a more diluted or concentrated medium. However, osmoregulation takes place to some extent through the modification of the aminoacidemia.

This is the case for dragonfly larvae and for *Dytiscus marginalis* adults (Schoffeniels, 1960). Osmotic and ionic regulation in insects are discussed thoroughly in another chapter (see Shaw and Stobbart, Chapter 4, Section II,C, this volume).

IV. INORGANIC CATIONS

A concept commonly current among biochemists is that the inorganic composition of the medium of the cells has to comply with definite relative proportions of Na, K, Ca, and Mg if they are to be capable of maintaining these cells in life. Baldwin, in his book "An Introduction to Comparative Biochemistry" (3rd ed., 1948) devotes 8 pages to this concept and writes ". . . instead of being surprised that the bloods of different animals resemble each other so closely, we must realize that it could not have been otherwise. The composition of the blood has remained the same because the conditions under which life is possible have remained the same." In his recent book, "The Nature of Biochemistry" (1962) Baldwin underlines this concept again: "Even the cells and organs of animals whose ancestors, like our own, became independent of the sea many millions of years ago, cannot tolerate for long any appreciable departure from the normal, sea-water-like composition of the blood as far as Na^{+}, K^{+} and Ca^{++} are concerned. This necessary internal constancy is something that *has* to be maintained." Insect hemolymph contradicts this statement in many cases, as shown by the data presented in Table I.

Table I is an exhaustive recapitulation of the numerous data obtained by different authors. The results are expressed in milliequivalents per liter, and in per cent of the sum of the cations ("indices"). For each order, the data concerning larval, pupal, and adult stages are presented separately. It may be seen that the indices vary as follows: Na: from 4.4 to 90; K: from 1 to 53.4; Ca: from 2 to 37.6 and Mg: from 0.3 to 75. The significance of the different types of cationic patterns may be discussed from several points of view.

A. Ontogenic Modifications of Cationic Pattern

It must be emphasized that a definite picture of the hemolymph cationic patterns is still difficult to present for each order, owing to the lack of representative data for the different developmental stages. With the exception of a few species, data have been accumulated for only one stage in each order. Table I shows, for instance, that the cationic hemolymph composition of Homoptera and Heteroptera is only known for

TABLE I

INORGANIC CATIONS IN THE INSECT HEMOLYMPH

Insect	mcq/liter Na	K	Ca	Mg	Sum of cations	Indices (% of the sum) Na	K	Ca	Mg	References
APTERYGOTES										
Petrobius maritimus	208	5.8								Lockwood and Croghan (1959)
EXOPTERYGOTES —PALEOPTERA										
Ephemeroptera										
Larvae: *Ephemera danica*	103	18								Sutcliffe (1962)
Odonata										
Larvae: *Aeschna grandis*	145	9	7.5	7.5	169	85.7	5.3	4.4	4.4	Sutcliffe (1962)
A. cyanea	142	8	—	—						Sutcliffe (1962)
A. sp.	143	4.3	16	—						Duchâteau *et al.* (1953)
Aeschna sp.	134.7	5.4	7.5	6.0	153.6	87.8	3.5	4.9	3.8	Duchâteau *et al.* (1953)
Aeschna sp.	179.3	4.5	20.4	12.3	216.5	82.8	2.1	9.4	5.7	Duchâteau *et al.* (1953)
Libellula depressa	178.3	3.8	18.4	12.0	212.5	83.9	1.8	8.7	5.6	Duchâteau *et al.* (1953)
Libellula sp.	152.0	—	16.0	—						Duchâteau *et al.* (1953)
Libellula sp.	—	—	7.5	4.8						Clark and Craig (1953)
Agrion (Calopteryx) sp.	158.0	9.0	—	—						Boné (1944)
Agrion virgo	140	8	—	—						Sutcliffe (1962)
Agrion virgo	145	9	—	—						Sutcliffe (1962)
Enallagma cyathigerum	139	14	—	—						Sutcliffe (1962)
Adults: *Aeschna cyanea*	120	21	—	—						Sutcliffe (1962)
Agrion virgo	145	27.5	—	—						Sutcliffe (1962)

Insect	Na	K	Ca	Mg	Sum of cations	Na	K	Ca	Mg	References
EXOPTERYGOTES —POLYNEOPTERA										
Dictyoptera										
Larvae: *Periplaneta americana*	100	15.4	3.3	—						Tobias (1948 a)
Adults: *Periplaneta americana*	157	7.6	4.2	5.4	174.2	90.1	4.3	2.4	3.1	Van Asperen and Esch (1956)
Periplaneta americana	—	—	8.5	22.9						Clark and Craig (1953)
Periplaneta americana	—	—	17.8	13.5						Van Asperen and Esch (1954)
P. australasiae	—	—	19.4	14.8						Van Asperen and Esch (1954)
Blabera fusca	—	—	20.2	15.7						Van Asperen and Esch (1954)
Isoptera										
Larvae: *Cryptotermes havilandi*	103	28								Sutcliffe (1963)
Zootermopsis angusticollis	—	—	8.6	17.6						Clark (1958)
Adults: *Zootermopsis angusticollis*	—	—	16.8	34.8						Clark and Craig (1953)

Aeschna sp.	134.7	5.4	7.5	6.0	153.6	87.8	3.5	4.9	3.8	Duchâteau *et al.* (1953)
Aeschna sp.	179.3	4.5	20.4	12.3	216.5	82.8	2.1	9.4	5.7	Duchâteau *et al.* (1953)
Libellula depressa	178.3	3.8	18.4	12.0	212.5	83.9	1.8	8.7	5.6	Duchâteau *et al.* (1953)
Libellula sp.	152.0	—	16.0	—						Duchâteau *et al.* (1953)
Libellula sp.	—	—	7.5	4.8						Clark and Craig (1953)
Agrion (Calopteryx) sp.	158.0	9.0	—	—						Boné (1944)
Agrion virgo	140	8	—	—						Sutcliffe (1962)
Agrion virgo	145	9	—	—						Sutcliffe (1962)
Enallagma cyathigerum	139	14	—	—						Sutcliffe (1962)
Adults: *Aeschna cyanea*	120	21	—	—						Sutcliffe (1962)
Agrion virgo	145	27.5	—	—						Sutcliffe (1962)
EXOPTERYGOTES										
—POLYNEOPTERA										
Dictyoptera										
Larvae: *Periplaneta americana*	100	15.4	3.3	—						Tobias (1948 a)
Adults: *Periplaneta americana*	157	7.6	4.2	5.4	174.2	90.1	4.3	2.4	3.1	Van Asperen and Esch (1956)
Periplaneta americana	—	—	8.5	22.9						Clark and Craig (1953)
Periplaneta americana	—	—	17.8	13.5						Van Asperen and Esch (1954)
P. australasiae	—	—	19.4	14.8						Van Asperen and Esch (1954)
Blabera fusca	—	—	20.2	15.7						Van Asperen and Esch (1954)
Isoptera										
Larvae: *Cryptotermes havilandi*	103	28								Sutcliffe (1963)
Zootermopsis angusticollis	—	—	8.6	17.6						Clark (1958)
Adults: *Zootermopsis angusticollis*	—	—	16.8	34.8						Clark and Craig (1953)
Plecoptera										
Larvae: *Perla bipunctata*	127	12								Sutcliffe (1962)
Dinocras cephalotes	117	10								Sutcliffe (1962)
Cheleutoptera										
Adults: *Carausius mororus*	21.0	25.0								Boné (1944)
Carausius mororus	14.0	16.0								Ramsay (1953 b)
Carausius mororus ("serum")	11	18	7	108	144	7.6	12.5	4.8	75	Ramsay (1955)
Carausius mororus	8.7	27.5	16.2	145.0	197.4	4.4	13.9	8.2	73.5	Duchâteau *et al.* (1953)
Carausius mororus	15	18	15	106	154	9.7	11.6	9.7	68.8	Wood (1957)
Orthoptera										
Larvae: *Chorthippus parallelus*	72	30								Sutcliffe (1963)
Locusta migratoria migratorioides	60.0	12.0	17.2	24.8	114.0	52.6	10.5	14.9	21.9	Duchâteau *et al.* (1953)
Schistocerca gregaria	81.3	5.3	17.8	34.6	139.0	58.6	3.8	12.8	24.9	Duchâteau *et al.* (1953)
Adults: *Anabrus simplex*	21.9	15.4	3.0	1.4	41.7	52.5	36.9	7.2	3.4	Pepper *et al.* (1941)
Chortophaga viridifasciata	108.9	3.4	2.8	21	136.1	80	2.5	2.0	15.4	Barsa (1954)
Gryllotalpa gryllotalpa	233.7	7.3	28.0	10.4	297.4	83.6	2.6	10.0	3.7	Duchâteau *et al.* (1953)
Gryllotalpa gryllotalpa	174.0	11.0								Boné (1944)
Locusta migratoria migratorioides	67.4	9.0	15.2	27.0	118.6	56.8	7.6	12.8	22.8	Duchâteau *et al.* (1953)

TABLE I (continued)

Insect	meq/liter Na	K	Ca	Mg	Sum of cations	Indices (% of the sum) Na	K	Ca	Mg	References
EXOPTERYGOTES										
—POLYNEOPTERA (cont.)										
Locusta migratoria migratorioides	75	20								Ramsay (1953)
Locusta migratoria migratorioides	74	15								Ramsay (1953)
Locusta migratoria migratorioides	102	22								Ramsay (1953)
Romalea microptera	56.5	17.9								Tobias (1948 b)
Stenobothrus stigmaticus	61.0	62.0								Boné (1944)
Stenopelmatus longispina	—	—	12.1	29.2						Clark and Craig (1953)
Tettigonia viridissima	83.0	51.0								Boné (1944)
Dermaptera										
Adults: *Forficula auricularia*			32.9							Clark and Craig (1953)
PARANEOPTERA										
Homoptera										
Adults: *Cinara cilicia*	—	—	21.4	30.4						Clark and Craig (1953)
Jassidae gn. sp.	59	21								Sutcliffe (1963)
Heteroptera										
Adults: *Gerris najas*	142.0	8.0								Boné (1944)
Notonecta kirbyii	—	—	31.0	18.5						Clark and Craig (1953)
Notonecta obliqua	155	21	—	—						Sutcliffe (1962)
Corixa punctata	112	31	—	—						Sutcliffe (1962)
Hesperocorixia larigata	—	—	7.8	3.5						Clark and Craig (1953)
Rhodnius prolixus	158.0	6.0	—	—						Ramsay (1953)
Rhodnius prolixus	158.0	4.0	—	—						Ramsay (1953)
Triatoma infestans	—	—	40.9	1.5						Clark and Craig (1953)
Triatoma megista	133.0	5.0	—	—						Boné (1944)
Triatoma neotomae	—	—	16.5	1.0						Clark and Craig (1953)
Triatoma phyllosoma	—	—	13.3	1.2						Clark and Craig (1953)
Triatoma protracta	—	—	29.5	1.3						Clark and Craig (1953)
Cimex lectularius	139.0	9.0	—	—						Boné (1944)
Oncopeltus fasciatus	—	—	13.9	52.1						Clark and Craig (1953)
Palomena prasina	22.0	42.0	—	—						Boné (1944)
Unknown stage: *Oncopeltus fasciatus*	39.5	20.5	11	—						Mullen (1957)
OLIGONEOPTERA										
Megaloptera										
Larvae: *Sialis lutaria*	109	5	15	38	167	65.2	3	9	22.7	Shaw (1955)
Planipennia (= Neuroptera)										
Larvae: *Myrmeleon formicarius*	143.5	8.7	12.1	31.3	195.6	73.3	4.4	6.1	16.2	Duchâteau *et al.* (1953)
Adults: *Osmylus fulvicephalus*	92	40	—	—	—	—	—	—	—	Sutcliffe (1963)
Mecoptera										
	94	38	—	—	—	—	—	—	—	Sutcliffe (1963)

Adults: *Cinara cilicia*	—	—	21.4	30.4						Clark and Craig (1953)
Jassidae gn. sp.	59	21								Sutcliffe (1963)
Heteroptera										
Adults: *Gerris najas*	142.0	8.0								Boné (1944)
Notonecta kirbyii	—	—	31.0	18.5						Clark and Craig (1953)
Notonecta obliqua	155	21	—	—						Sutcliffe (1962)
Corixa punctata	112	31	—	—						Sutcliffe (1962)
Hesperocorixia larigata	—	—	7.8	3.5						Clark and Craig (1953)
Rhodnius prolixus	158.0	6.0	—	—						Ramsay (1953)
Rhodnius prolixus	158.0	4.0	—	—						Ramsay (1953)
Triatoma infestans	—	—	40.9	1.5						Clark and Craig (1953)

Triatoma megista	133.0	5.0	—	—						Boné (1944)
Triatoma neotomae	—	—	16.5	1.0						Clark and Craig (1953)
Triatoma phyllosoma	—	—	13.3	1.2						Clark and Craig (1953)
Triatoma protracta	—	—	29.5	1.3						Clark and Craig (1953)
Cimex lectularius	139.0	9.0	—	—						Boné (1944)
Oncopeltus fasciatus	—	—	13.9	52.1						Clark and Craig (1953)
Palomena prasina	22.0	42.0	—	—						Boné (1944)
Unknown stage: *Oncopeltus fasciatus*	39.5	20.5	11	—						Mullen (1957)
OLIGONEOPTERA										
Megaloptera										
Larvae: *Sialis lutaria*	109	5	15	38	167	65.2	3	9	22.7	Shaw (1955)
Planipennia (= Neuroptera)										
Larvae: *Myrmeleon formicarius*	143.5	8.7	12.1	31.3	195.6	73.3	4.4	6.1	16.2	Duchâteau *et al.* (1953)
Adults: *Osmylus fulvicephalus*	92	40	—	—	—	—	—	—	—	Sutcliffe (1963)
Mecoptera										
Adults: *Panorpa communis*	94	38	—	—	—	—	—	—	—	Sutcliffe (1963)
Trichoptera										
Larvae: *Anabolia nervosa*	101	17								Sutcliffe (1962)
Chaetopteryx villosa	63.9	9								Boné (1944)
Limnephilus stigma	83	14								Sutcliffe (1962)
Philopotamus montanus	109	21								Sutcliffe (1962)
Phryganea sp.	69	7								Sutcliffe (1962)
Phryganea sp.	92.0	6.8	14.4	51.0	164.2	56.0	4.1	8.8	31.1	Duchâteau *et al.* (1953)
Diptera										
Larvae: *Tipula montium*	115	7								Sutcliffe (1952)
Tipula paludosa + *oleracea*	84.8	8.2	12.3	16.0	121.3	69.9	6.8	10.1	13.2	Duchâteau *et al.* (1953)
Dictenidia bimaculata	39.6	3.7	13.8	14.5	71.6	53.3	5.2	19.3	20.2	Duchâteau *et al.* (1953)
Chironomus sp.	104.3	2.1	10.5	14.6	131.5	79.3	1.6	8.0	11.1	Duchâteau *et al.* (1953)
Chironomus sp.	92.0	8.0								Boné (1944)
Tabanide sp.	151.0	5.0								Ramsay (1953)

TABLE I (continued)

Insect	meq/liter Na	K	Ca	Mg	Sum of cations	Indices (% of the sum) Na	K	Ca	Mg	References
EXOPTERYGOTES										
OLIGONEOPTERA (cont.)										
Pegomya sp.	26.0	58.0								Boné (1944)
Eristalomyia tenax	100.0	7.9	12.0	13.3	133.2	75.1	5.9	9.0	10.0	Duchâteau *et al.* (1953)
Gasterophilus intestinalis	206.0	13.0	7.0	38.0	264.0	78.0	4.9	2.6	10.6	Levenbook (1950)
Calliphora erythrocephala	148.0	37.0								Boné (1944)
Pupae: *Calliphora erythrocephala*	139.6	26.1	20.8	34.3	220.8	63.2	11.8	9.9	15.6	Duchâteau *et al.* (1953)
Adults: *Stomoxys calcitrans*	128.0	11.0								Duchâteau *et al.* (1953)
Eristalis tenax	193.2	20.9								Original
Lepidoptera										
Larvae: *Cossus cossus*	—	15.5	27.7	40.4						Duchâteau *et al.* (1953)
Cossus cossus	18.4	35.4	51.5	48.0	153.3	12.0	23.1	33.6	31.3	Duchâteau *et al.* (1953)
Yponomeuta evonymella	3.2	23.3	17.1	29.7	73.3	4.4	31.8	23.3	40.5	Duchâteau *et al.* (1953)
Nymphula nymphaeata	40	29								Sutcliffe (1962)
Ephestia kuehniella	17.0	60.0								Boné (1944)
Ephestia kuehniella	32.6	32.7	41.2	51.1	157.6	20.7	20.8	26.1	32.4	Duchâteau *et al.* (1953)
Galleria mellonella	26.5	36.3	24.4	33.3	120.7	22.0	30.1	20.2	27.7	Duchâteau *et al.* (1953)
Phalera bucephala	5.9	49.2	34.2	79.8	169.1	3.5	29.1	20.2	47.2	Duchâteau *et al.* (1953)
Euproctis chrysorrhoea	17.9	44.5	20.6	87.9	170.9	10.5	26.0	12.1	51.4	Duchâteau *et al.* (1953)
Phryganidia californica	—	—	8.5	52.1						Clark and Craig (1953)
Apamea sordens	—	38.7	17.1	56.8						Duchâteau *et al.* (1953)
Laphygma exigua	—	—	5.4	56.1						Clark and Craig (1953)
Prodenia praefica	—	—	6.5	64.3						Clark and Craig (1953)
Phlogophora meticulosa	12.3	34.9	35.5	68.4	151.1	8.1	23.1	23.5	45.3	Duchâteau *et al.* (1953)

Insect	Na	K	Ca	Mg	Sum of cations	Na	K	Ca	Mg	References
Prodenia eridania	22.3	39.7	18.4	14.3	94.7	23.5	41.9	19.4	15.1	Babers (1938)
Peridoma margaritosa	—	—	8.7	85.2						Clark and Craig (1953)
Estigmene acraea	—	—	5.1	10.2						Clark and Craig (1953)
Amathes xanthographa	24.1	29.2	40.4	104.2	197.9	12.2	14.8	20.4	52.6	Duchâteau *et al.* (1953)
Triphaena pronuba	16.1	35.6	56.0	70.9	178.6	9.0	19.9	31.4	39.7	Duchâteau *et al.* (1953)
Mamestra brassicae	4.3	53.6	17.9	99.2	175.0	2.5	30.6	10.2	56.7	Duchâteau *et al.* (1953)
Diataraxia oleracea	13.1	43.1	31.9	78.7	166.8	7.9	25.8	19.1	47.2	Duchâteau *et al.* (1953)
Melanchra persicariae	10.8	40.3	19.1	79.0	149.2	7.2	27.0	12.8	53.0	Duchâteau *et al.* (1953)
Hypocrita jacobaeae	7.3	34.6	25.0	86.7	153.6	4.8	22.5	16.3	56.4	Duchâteau *et al.* (1953)
Spilosoma lutae	3.3	56.2	31.4	38.5	129.4	2.6	43.4	24.3	29.7	Duchâteau *et al.* (1953)
Bombyx mori	14.0	35.0	—	—						Boné (1944)
Bombyx mori	12.2	35.9								Tobias (1948b)
Bombyx mori: 3rd instar	3.4	41.8	24.5	80.8	150.5	2.2	27.8	16.3	53.7	Duchâteau *et al.* (1953)
Bombyx mori: 4th molt	6.0	39.4	15.0	88.0	148.4	4.0	26.5	10.1	59.3	Bialaszewicz and Landau (1938)
Bombyx mori: 5th instar	14.6	46.1	24.5	101.0	186.2	7.8	24.7	13.1	54.2	Bialaszewicz and Landau (1938)
Bombyx mori: prenymphs	8.2	59.2	26.5	92.5	186.4	4.3	32.1	14.2	44.2	Bialaszewicz and Landau (1938)
Antheraea mylitta	1.3	49.7	21.9	37.7	110.6	1.2	44.9	19.8	34.1	Bialaszewicz and Landau (1938)
Actias selene	4.8	51.3	25.5	60.0	141.6	3.4	36.2	18.0	49.4	Bialaszewicz and Landau (1938)

Nymphula nymphaeata	40	29								Sutcliffe (1962)
Ephestia kuehniella	17.0	60.0								Boné (1944)
Ephestia kuehniella	32.6	32.7	41.2	51.1	157.6	20.7	20.8	26.1	32.4	Duchâteau *et al.* (1953)
Galleria mellonella	26.5	36.3	24.4	33.3	120.7	22.0	30.1	20.2	27.7	Duchâteau *et al.* (1953)
Phalera bucephala	5.9	49.2	34.2	79.8	169.1	3.5	29.1	20.2	47.2	Duchâteau *et al.* (1953)
Euproctis chrysorrhoea	17.9	44.5	20.6	87.9	170.9	10.5	26.0	12.1	51.4	Duchâteau *et al.* (1953)
Phryganidia californica	—	—	8.5	52.1						Clark and Craig (1953)
Apamea sordens	—	38.7	17.1	56.8						Duchâteau *et al.* (1953)
Laphygma exigua	—	—	5.4	56.1						Clark and Craig (1953)
Prodenia praefica	—	—	6.5	64.3						Clark and Craig (1953)
Phlogophora meticulosa	12.3	34.9	35.5	68.4	151.1	8.1	23.1	23.5	45.3	Duchâteau *et al.* (1953)

Prodenia eridania	22.3	39.7	18.4	14.3	94.7	23.5	41.9	19.4	15.1	Babers (1938)
Peridoma margaritosa	—	—	8.7	85.2						Clark and Craig (1953)
Estigmene acraea	—	—	5.1	10.2						Clark and Craig (1953)
Amathes xanthographa	24.1	29.2	40.4	104.2	197.9	12.2	14.8	20.4	52.6	Duchâteau *et al.* (1953)
Triphaena pronuba	16.1	35.6	56.0	70.9	178.6	9.0	19.9	31.4	39.7	Duchâteau *et al.* (1953)
Mamestra brassicae	4.3	53.6	17.9	99.2	175.0	2.5	30.6	10.2	56.7	Duchâteau *et al.* (1953)
Dialaraxia oleracea	13.1	43.1	31.9	78.7	166.8	7.9	25.8	19.1	47.2	Duchâteau *et al.* (1953)
Melanchra persicariae	10.8	40.3	19.1	79.0	149.2	7.2	27.0	12.8	53.0	Duchâteau *et al.* (1953)
Hypocrita jacobaeae	7.3	34.6	25.0	86.7	153.6	4.8	22.5	16.3	56.4	Duchâteau *et al.* (1953)
Spilosoma lutae	3.3	56.2	31.4	38.5	129.4	2.6	43.4	24.3	29.7	Duchâteau *et al.* (1953)
Bombyx mori	14.0	35.0	—	—						Boné (1944)
Bombyx mori	12.2	35.9								Tobias (1948b)
Bombyx mori: 3rd instar	3.4	41.8	24.5	80.8	150.5	2.2	27.8	16.3	53.7	Duchâteau *et al.* (1953)
Bombyx mori: 4th molt	6.0	39.4	15.0	88.0	148.4	4.0	26.5	10.1	59.3	Bialaszewicz and Landau (1938)
Bombyx mori: 5th instar	14.6	46.1	24.5	101.0	186.2	7.8	24.7	13.1	54.2	Bialaszewicz and Landau (1938)
Bombyx mori: prenymphs	8.2	59.2	26.5	92.5	186.4	4.3	32.1	14.2	44.2	Bialaszewicz and Landau (1938)
Antheraea mylitta	1.3	49.7	21.9	37.7	110.6	1.2	44.9	19.8	34.1	Bialaszewicz and Landau (1938)
Actias selene	4.8	51.3	25.5	60.0	141.6	3.4	36.2	18.0	49.4	Bialaszewicz and Landau (1938)
Sphinx ligustri	—	34.7	30.5	57.5						Duchâteau *et al.* (1953)
Celerio (Deilephila) euphorbiae	—	20.0	36.0	—						Heller and Moklowska (1930)
Pieris rapae	11.0	39.0	—	—						Boné (1944)
Pieris rapae	—	96.4	41.0	66.6						Duchâteau *et al.* (1953)
Pieris brassicae	—	19.7	16.6	92.5						Brecher (1929)
Pieris brassicae	9.0	30.0	—	—						Ramsay (1953)
Pieris brassicae	5.0	27.0	—	—						Ramsay (1953)
Aglais (Vanessa) urticae	22.0	43.0	—	—						Boné (1944)
Junonia coenia	—	—	5.2	29.1						Clark and Craig (1953)
Papilio machaeon	13.6	45.3	33.4	59.8	152.1	8.9	29.8	22.0	39.3	Duchâteau *et al.* (1953)
Pupae: *Dasychira pudibunda*	3.0	51.8	33.9	74.1	162.8	1.9	31.8	20.8	45.5	Duchâteau *et al.* (1953)
Cucullia absinthii	9.8	48.7	32.5	51.2	142.2	6.9	34.2	22.9	36.0	Duchâteau *et al.* (1953)
Bombyx mori	21.7	54.9	29.5	87.5	193.6	11.2	28.4	15.2	45.2	Duchâteau *et al.* (1953)

TABLE I (continued)

Insect	meq/liter Na	K	Ca	Mg	Sum of cations	Indices (% of the sum) Na	K	Ca	Mg	References
EXOPTERYGOTES										
OLIGONEOPTERA (cont.)										
Bombyx mori	11.3	41.5	24.0	69.4	146.2	7.7	27.7	16.4	47.4	Bialaszewicz and Landau (1938)
Graellsia isabellae	6.2	46.2	17.5	29.1	99.0	6.3	46.7	17.7	29.3	Duchâteau *et al.* (1953)
Tropaea luna	4.4	52.8	31.4	48.8	137.4	3.2	38.4	22.9	35.5	Duchâteau *et al.* (1953)
Eudia (Saturnia) pavonia	3.0	39.2	14.2	—						Drilhon (1934)
Saturnia pyri	3.9	41.0	14.5	—						Drilhon (1934)
Antheraea (Attacus) pernyi	11.4	43.6	16.5	—						Drilhon (1934)
Telea polyphemus	9.8	34.6	16.2	—						Drilhon (1934)
Telea polyphemus	2.5	59.3	11.6	73.8	147.2	1.7	40.2	7.8	50.0	Carrington and Tenney (1959)
Samia walkeri	2.6	42.2	18.8	65	128.6	2	32.8	14.6	50.5	Barsa (1954)
Samia cecropia	7.0	50.0	14.7	—						Drilhon (1934)
Philosamia cynthia	7.5	36.9	28.5	52.1	125.0	6.0	29.5	22.8	41.7	Duchâteau *et al.* (1953)
Philosamia (Samia) walkeri	2.6	42.1	18.8	65.0	128.5	2.0	32.8	14.6	50.6	Gese (1950)
Endromis versicolora	1.3	32.8	28.0	44.0	106.1	1.2	30.9	26.4	41.5	Duchâteau *et al.* (1953)
Smerinthus ocellatus	5.4	34.8	15.5	—						Drilhon (1934)
Celerio (Deilephila) euphorbiae	—	10–18	16–32	—						Heller and Moklowska (1930)
Hyloicus pinastri	tr.	35.0	15.0	46.0						Brecher (1929)
Sphinx ligustri	2.6	52.8	16.4	49.2	121.0	2.1	43.6	12.6	40.7	Duchâteau *et al.* (1953)
Sphinx ligustri	3.0	54.1	40.9	50.0	148.0	2.0	36.6	27.6	33.8	Duchâteau *et al.* (1953)
Sphinx ligustri	4.3	48.4	15.0	—						Drilhon (1934)
Mimas tiliae	3.2	39.2	129.7	15.6	127.7	2.5	30.7	23.3	43.5	Duchâteau *et al.* (1953)
Deilephila elpenor	4.7	27.4	41.0	89.3	142.4	2.9	16.9	25.2	55.0	Duchâteau *et al.* (1953)
Pieris brassicae	—	37.4	10.6	52.9						Brecher (1929)

Insect	Na	K	Ca	Mg	Sum of cations	Na	K	Ca	Mg	References
Adults: *Bombyx mori*	14.3	36.1	14.5	44.6	109.5	13.0	32.9	13.2	40.7	Bialaszewicz and Landau (1938)
Telea polyphemus	tr.	54.1	—	72						Carrington and Tenney (1959)
Coleoptera										
Larvae: *Dytiscus* sp.	115	20								Sutcliffe (1952)
Colymbetes fuscus	127	19								Sutcliffe (1952)
Popillia japonica	20.2	9.5	15.8	38.8	84.3	24.0	11.3	18.7	46.0	Ludwig (1951)
Cetonia aurata	51.3	18.6	22.8	80.0	172.7	29.7	10.8	13.2	46.3	Duchâteau *et al.* (1953)
Tenebrio molitor	86.0	45.0	—	—						Boné (1944)
Tenebrio molitor	77.0	32.0	—	—						Ramsay (1953)
Tenebrio molitor	54.0	53.0	—	—						Ramsay (1953)
Timarcha tenebricosa	—	48.1	46.4	165						Duchâteau *et al.* (1953)
Timarcha tenebricosa	1.6	46.9	72.2	158.0	278.7	0.6	16.8	25.9	56.7	Duchâteau *et al.* (1953)
Leptinotarsa decemlineata	3.5	65.1	47.5	188.3	304.4	1.1	21.4	15.6	61.9	Duchâteau *et al.* (1953)
Leptinotarsa decemlineata	9.6	50.3	155.0	198.0	412.9	2.3	12.2	37.6	48.0	Duchâteau *et al.* (1953)
Leptinotarsa decemlineata	2.0	54.9	43.4	146.9	247.2	0.8	22.2	17.6	59.4	Duchâteau *et al.* (1953)
Adults: *Cicindela maritima*	162.0	9.0	—	—						Boné (1944)
Scaphinotus sp.	—	—	10.9	18.7						Clark and Craig (1953)
Dytiscus marginalis	133.0	10.0	—	—						Boné (1944)

Philosamia (Samia) walkeri	2.6	42.1	18.8	65.0	128.5	2.0	32.8	14.6	50.6	Gese (1950)
Endromis versicolora	1.3	32.8	28.0	44.0	106.1	1.2	30.9	26.4	41.5	Duchâteau *et al.* (1953)
Smerinthus ocellatus	5.4	34.8	15.5	—						Drilhon (1934)
Celerio (Deilephila) euphorbiae	—	10–18	16–32	—						Heller and Moklowska (1930)
Hyloicus pinastri	tr.	35.0	15.0	46.0						Brecher (1929)
Sphinx ligustri	2.6	52.8	16.4	49.2	121.0	2.1	43.6	12.6	40.7	Duchâteau *et al.* (1953)
Sphinx ligustri	3.0	54.1	40.9	50.0	148.0	2.0	36.6	27.6	33.8	Duchâteau *et al.* (1953)
Sphinx ligustri	4.3	48.4	15.0	—						Drilhon (1934)
Mimas tiliae	3.2	39.2	129.7	15.6	127.7	2.5	30.7	23.3	43.5	Duchâteau *et al.* (1953)
Deilephila elpenor	4.7	27.4	41.0	89.3	142.4	2.9	16.9	25.2	55.0	Duchâteau *et al.* (1953)
Pieris brassicae	—	37.4	10.6	52.9						Brecher (1929)

Adults: *Bombyx mori*	14.3	36.1	14.5	44.6	109.5	13.0	32.9	13.2	40.7	Bialaszewicz and Landau (1938)
Telea polyphemus	tr.	54.1	—	72						Carrington and Tenney (1959)
Coleoptera										
Larvae: *Dytiscus* sp.	115	20								Sutcliffe (1952)
Colymbetes fuscus	127	19								Sutcliffe (1952)
Popillia japonica	20.2	9.5	15.8	38.8	84.3	24.0	11.3	18.7	46.0	Ludwig (1951)
Cetonia aurata	51.3	18.6	22.8	80.0	172.7	29.7	10.8	13.2	46.3	Duchâteau *et al.* (1953)
Tenebrio molitor	86.0	45.0	—	—						Boné (1944)
Tenebrio molitor	77.0	32.0	—	—						Ramsay (1953)
Tenebrio molitor	54.0	53.0	—	—						Ramsay (1953)
Timarcha tenebricosa	—	48.1	46.4	165						Duchâteau *et al.* (1953)
Timarcha tenebricosa	1.6	46.9	72.2	158.0	278.7	0.6	16.8	25.9	56.7	Duchâteau *et al.* (1953)
Leptinotarsa decemlineata	3.5	65.1	47.5	188.3	304.4	1.1	21.4	15.6	61.9	Duchâteau *et al.* (1953)
Leptinotarsa decemlineata	9.6	50.3	155.0	198.0	412.9	2.3	12.2	37.6	48.0	Duchâteau *et al.* (1953)
Leptinotarsa decemlineata	2.0	54.9	43.4	146.9	247.2	0.8	22.2	17.6	59.4	Duchâteau *et al.* (1953)
Adults: *Cicindela maritima*	162.0	9.0	—	—						Boné (1944)
Scaphinotus sp.	—	—	10.9	18.7						Clark and Craig (1953)
Dytiscus marginalis	133.0	10.0	—	—						Boné (1944)
Dytiscus marginalis	tr.	32.0	26.0							Drilhon and Busnel (1943)
Dytiscus marginalis	140.0	5.0								Ramsay (1953)
Dytiscus marginalis	126	14								Sutcliffe (1962)
Dytiscus marginalis	165.2	6.4	22.5	37.5	231.6	71.3	2.8	9.7	16.2	Duchâteau *et al.* (1953)
Cybister sp.	143.5	7.3	38.2	51.8	240.8	59.6	3.0	15.9	21.5	Duchâteau *et al.* (1953)
Hydrophilus piceus	123.7	4.3	24.5	46.8	199.3	62.1	2.1	12.3	23.5	Duchâteau *et al.* (1953)
Hydrophilus piceus	120.7	13.9	23.0	44.2	201.8	59.8	6.9	11.4	21.9	Florkin (1943)
Hydrophilus piceus	—	21.2	21.5							Drilhon and Busnel (1937)
Geotrupes stercorosus	119.1	16.0	17.8	49.8	202.7	58.7	7.9	8.8	24.6	Duchâteau *et al.* (1953)
Melolontha melolontha	113.0	5.8	15.3	41.3	175.4	64.4	3.3	8.7	23.6	Duchâteau *et al.* (1953)
Melolontha melolontha	6.0	49.0	—	—						Boné (1944)
Meloe strigulosus	—	—	26.6	156.7						Clark and Craig (1953)
Lytta molesta	—	—	—	185.8						Clark and Craig (1953)

TABLE I (concluded)

Insect	meq/liter Na	K	Ca	Mg	Sum of cations	Indices (% of the sum) Na	K	Ca	Mg	References
EXOPTERYGOTES										
OLIGONEOPTERA (cont.)										
Coelocnemis dilaticollis	—	—	27.1	10.0						Clark and Craig (1953)
Agelastica alni	17.0	47.0	—	—						Boné (1944)
Timarcha tenebricosa	12.2	54.9								Original
Leptinotarsa decemlineata	—	28.5	—	—						Drilhon and Busnel (1937)
Hymenoptera										
Larvae: *Pteronidea ribesii*	1.6	43.4	17.5	60.7	123.2	1.3	35.2	14.2	49.3	Duchâteau *et al.* (1953)
Tenthredinide sp.	6.0	55.0								Boné (1944)
Neodiprion sertifer	3	38								Sutcliffe (1963)
Vespula germanica	48.0	41.0	—	—						Boné (1944)
Vespula germanica	26.0	56.4	18.7	23.6	124.7	20.9	45.2	15.0	18.9	Duchâteau *et al.* (1953)
Apis mellifica	10.0	45.0	—	—						Boné (1944)
Apis mellifica	10.9	30.5	18.2	20.5	80.1	13.6	38.1	22.7	25.6	Duchâteau *et al.* (1953)
Apis mellifica	5.0	24.4	7.5	15.8	52.7	9.5	46.3	14.2	30.0	Bishop, Briggs and Ronzini (1925)
Pupae: *Formica rufa*	14.7	50.3	14.9	21.6	101.5	14.5	49.6	14.7	21.2	Duchâteau *et al.* (1953)
Vespula germanica	22.8	60.8	11.2	19.0	113.8	20.0	53.4	9.9	16.7	Duchâteau *et al.* (1953)
Adults: *Vespula pensylvanica*	—	—	7.1	1.0						Clark and Craig (1953)
Vespula germanica	93	18.2	1.8	2.6	115.6	80.4	15.7	1.5	2.2	Original
Vespula germanica	153.5	21.9	2.2	0.5	178.1	86.1	12.3	1.2	0.3	Original
Apis mellifica	47.1	27.1	17.8	1	93	50.6	29.1	19.1	1	Original

adults, whereas that of Trichoptera a
known only for larvae or pupae. This fac
by many authors, who discussed the sys
cance of the hemolymph cationic compo
different ontogenic positions.

The assumption, made by the authors
of the hemolymph does not vary signifi
based in a few cases, mainly exopteryg
of both larval and imaginal stages hav
position (see Table I: Odonata: *Aesc*
Dictyoptera: *Periplaneta americana;* C
This seems also to be true in the case
mori and *Dytiscus* sp.

However, a reexamination of the
(Florkin and Jeuniaux, 1963; see also
that the cationic composition of the hen
metamorphosis, in this order. In the
bees and wasps, the Na index is indc
K ranges from 38 to 53, and that of M
these proportions are reversed, the Na
(50 to 86), and those of K and Mg cons
0.3 to 2.2).

It is clear that one must be careful b
developmental stages of one insect or
tained with representatives of only o

B. Hemolymph Cationic Patte

From the data in Table I, it may b
acteristic patterns, bearing in mind th
tered, and that ontogenic variations ar

1. Apterygotes: the only important
2. Exopterygotes Paleoptera: in
is the most important cation (103 t
being of a very low concentration (
to be true for larvae as well as for adu
3. Exopterygotes Polyneoptera: w
is also the most important ion, but
become more concentrated than in
bothrus stigmaticus and *Tettigonia*
similar to that of Na. The situation
adults.

adults, whereas that of Trichoptera and Lepidoptera is practically known only for larvae or pupae. This fact seems to have been neglected by many authors, who discussed the systematic or phylogenetic significance of the hemolymph cationic composition by comparing animals of different ontogenic positions.

The assumption, made by the authors, that the cationic composition of the hemolymph does not vary significantly during metamorphosis, is based in a few cases, mainly exopterygotes, in which the hemolymphs of both larval and imaginal stages have approximately the same composition (see Table I: Odonata: *Aeschna cyanea* and *Agrion virgo;* Dictyoptera: *Periplaneta americana;* Orthoptera: *Locusta migratoria*). This seems also to be true in the case of two endopterygotes: *Bombyx mori* and *Dytiscus* sp.

However, a reexamination of the situation among Hymenoptera (Florkin and Jeuniaux, 1963; see also Table I) led to the conclusion that the cationic composition of the hemolymph is greatly altered during metamorphosis, in this order. In the larval and pupal hemolymph of bees and wasps, the Na index is indeed only 10 to 20, while that of K ranges from 38 to 53, and that of Mg from 16.7 to 30. In the adults, these proportions are reversed, the Na indices being consistently higher (50 to 86), and those of K and Mg considerably lower (K: 12 to 29; Mg: 0.3 to 2.2).

It is clear that one must be careful before generalizing on the different developmental stages of one insect order with the separate results obtained with representatives of only one or two stages.

B. Hemolymph Cationic Patterns of the Different Orders

From the data in Table I, it may be proposed to recognize some characteristic patterns, bearing in mind that the sampling is obviously scattered, and that ontogenic variations are often ignored.

1. Apterygotes: the only important cation is Na.

2. Exopterygotes Paleoptera: in Ephemeroptera and Odonata, Na is the most important cation (103 to 179 meq/liter), the other cations being of a very low concentration (less than 30 meq/liter). This seems to be true for larvae as well as for adults.

3. Exopterygotes Polyneoptera: with the exception of *Carausius*, Na is also the most important ion, but K, Ca, and especially Mg tend to become more concentrated than in Paleoptera. In some cases (*Stenobothrus stigmaticus* and *Tettigonia viridissima*), the K concentration is similar to that of Na. The situation seems to be the same in larvae and adults.

Cheleutoptera are characterized by a completely different pattern, in which Mg replaces Na almost entirely.

4. Exopterygotes—Paraneoptera: the hemolymph of larvae has not been studied. In adults, the situation is not very different from that found in other exopterygotes, with the exception of *Oncopeltus fasciatus* (Mg: 52.1 meq/liter) and of *Palomena prasina,* in which the K concentration is twice that of Na.

5. In the Oligoneoptera, Megaloptera, Neuroptera, Mecoptera, Trichoptera, and Diptera, Na is also the main cation (indices: from 53 to 79). There seems to be no fundamental difference between the ontogenic stages.

6. Coleoptera: the available data are particularly diverse. It may tentatively be proposed to consider the existence of three groups. In the first group, Adephaga, both larval and adult hemolymphs contain a high proportion of Na (110 to 165 meq/liter) and a low proportion of K, Ca, and Mg, a pattern similar to that found in Polyneoptera. In a second group, corresponding presumably to the Phytophaga, and in which only Chrysomelidae have been studied, the hemolymph of both stages contain a very low amount of Na, while K, Ca, and especially Mg are at high concentration. This pattern is similar to that found in Lepidoptera. Finally, a third group may be presumed, in which the adult hemolymph contains more Na and less K and Mg than the larval hemolymph (for instance: Scarabaeidae).

7. Lepidoptera: as far as larval and pupal hemolymphs are considered, Lepidoptera are characterized by a low proportion of Na (from traces to 30 meq/liter: indices 2 to 23), higher proportions of K, of Ca (generally from 10 to 60 meq/liter) and chiefly of Mg (30 to 100 meq/liter: indices from 30 to 50). The spectrum of Na concentration is situated below the lowest limit of the values recorded for animals outside the class of insects (with one exception: *Anodonta*). The spectrum of Mg concentration can be superimposed on the spectrum found in sea animals, but is situated above the highest values recorded for fresh water or terrestrial invertebrates and for vertebrates. This is also the case for potassium. The hemolymph of larval and pupal stages of Lepidoptera thus appears with a very specialized cationic pattern different from that of other animal phyla.

The cationic pattern of adult hemolymph is only known in the case of two species: *Bombyx mori* and *Telea polyphemus.* These data seem to indicate that adult hemolymph does not differ from that of the larvae or pupae.

8. Hymenoptera: in larvae and pupae of Symphyta and Aculeata, the most important cations are K and Mg. The situation is very different

in the adult Aculeata, in which
index (50 to 80), less K (index:
Ca and Mg.

C. Ion Binding

In order to account for the no
such insects with a hemolymph ri
postulated that an important pro
free ions, in the hemolymph, b
Bishop *et al.*, 1925; Buck, 1953;
is not the case for *Antherea poly*
evidence was detected for any
the Ca and Mg were bound to m
1959).

D. Dietetic Relationships

For Boné (1944) as for Tobias
types of cationic pattern is diete
would tend to have high Na, an
in their hemolymph. This relatio
some insects (grasshoppers, *Ti*
trupes, etc.) contradict this state

Insects, being mainly terres
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appear in Table II are the bee
cossus which eats wood, and
wax comb in the beehive. The
trates all the cations of honey
potassium, the magnesium and t
its sodium.

From this survey, it can be s
some insects have a high potass
sequence of eating foliar food a

in the adult Aculeata, in which the cationic pattern shows a high Na index (50 to 80), less K (index: 12 to 30) and only minute amounts of Ca and Mg.

C. Ion Binding

In order to account for the normally functioning excitable tissues in such insects with a hemolymph rich in K and poor in Na, several authors postulated that an important proportion of the cations do not exist as free ions, in the hemolymph, but in a combined form (Barsa, 1954; Bishop *et al.*, 1925; Buck, 1953; Clark and Craigh, 1953). However, this is not the case for *Antherea polyphemus,* in the hemolymph of which no evidence was detected for any binding of K, while 15–20 per cent of the Ca and Mg were bound to macromolecules (Carrington and Tenney, 1959).

D. Dietetic Relationships

For Boné (1944) as for Tobias (1948), the explanation of the different types of cationic pattern is dietetic. In their opinion, zoophagous insects would tend to have high Na, and phytophagous insects high K and Mg in their hemolymph. This relationship appears clearly in most cases, but some insects (grasshoppers, *Tipula* larvae, *Hydrophilus* adults, *Geotrupes,* etc.) contradict this statement, as Boné himself pointed out.

Insects, being mainly terrestrial and therefore unable to absorb cations from a fluid habitat, can only rely on food to insure the steady state of the concentration of cations in their hemolymph, which is the result of the equilibrium between ingestion and excretion. It is therefore indicated to compare the concentrations of these cations, per 1000 gm of fresh food, or per 1000 ml of hemolymph. Table II shows that when the insects considered are phytophagous, the specialized pattern of cations in hemolymph is always due to the dilution of potassium and calcium, and to the concentration of magnesium. With respect to sodium, we can see that either concentration, dilution or concentration takes place. The nonphytophagous insects with the specialized pattern which appear in Table II are the bee larva, eating honey, the larva *Cossus cossus* which eats wood, and *Galleria mellonella* which feeds on the wax comb in the beehive. The table shows that the bee larva concentrates all the cations of honey, while *Cossus* and *Galleria* dilute the potassium, the magnesium and the calcium of their food and concentrate its sodium.

From this survey, it can be seen that the concept according to which some insects have a high potassium and a low sodium content as a consequence of eating foliar food and others have a high sodium and a low

TABLE II

COMPARISON BETWEEN THE CATIONIC COMPOSITION OF THE FOOD AND THE HEMOLYMPH OF INSECTS

Food and Organism	meq/kg Fresh food or /liter hemolymph				Σ	Indices			
	Na	K	Ca	Mg	Cations	Na	K	Ca	Mg
Lettuces (*Lectuca sativa*)[a]	13.0	87.2							
Periplaneta americana, adults[a]	113.0	25.6							
Romalea microptera, adults[a]	56.5	17.9							
Ivy, leaves (*Hedera helix*)[f]	35.9	147.6	665.0	53.1	900.6	4.0	16.4	73.8	5.8
Privet, leaves (*Ligustrum vulgare*)[f]	46.4	152.1	824.5	39.9	1062.9	4.4	14.3	77.6	3.8
Carausius morosus, adults[f]	8.7	27.5	16.2	145.0	197.4	4.4	13.9	8.2	73.5
Horse blood, total[b]	84.8	31.4	1.7	3.3	121.4	69.9	25.9	1.6	2.7
Gasterophilus intestinalis, larvae[c]	206.0	13.0	7.0	38.0	264.0	78.0	4.9	2.7	14.4
Poplar, wood (*Populus* sp.)[f]	16.0	126.0	1471.0	113.9	1726.9	0.9	7.3	85.2	6.6
Cossus cossus, larvae[f]	18.4	35.4	51.5	48.0	153.3	12.0	23.1	33.6	31.3
Wax[f]	12.8	347.2	257.0	215.3	832.4	1.5	41.7	30.9	25.9
Galleria mellonella, larvae[f]	26.5	36.3	24.4	33.5	120.7	22.0	30.1	20.2	27.7
Mulberry tree, leaves[e]	11.3	59.0							
Bombyx mori, larvae[e]	12.2	35.9							
Carrot, leaves (*Daucus carota*)[f]	25.6	176.9	214.5	35.6	422.6	5.7	39.1	47.4	7.9
Papilio machaon, larvae[f]	13.6	45.3	33.4	59.8	152.1	8.9	29.8	22.0	39.3
Potato, leaves (*Solanum tuberosum*)[f]	tr	144.5	128.6	85.9	359.0	—	40.3	35.8	23.9
Leptinotarsa decemlineata, adults[f]	3.5	65.1	47.5	188.3	304.4	1.1	21.4	15.6	61.9
Leptinotarsa decemlineata, adults[f]	2.0	54.9	43.4	146.9	257.2	0.8	22.2	17.6	59.4
Currant-bush, leaves (*Ribes grossulariae*)[f]	tr	249.1	271.2	53.6	573.9	—	43.4	47.3	9.3
Pteronidea ribesii, larvae[f]	1.6	43.4	17.5	60.7	123.2	1.3	35.2	14.2	49.3
Honey[d]	4.7	13.1	2.7	1.8	22.3	21.1	58.7	12.1	8.1
Apis mellifica, larvae[f]	10.9	30.5	18.2	20.5	80.1	13.6	38.1	22.7	25.6

[a] Tobias (1948). [b] Aberhalden (1898). [c] Levenbook (1950). [d] McCance and Widdowson (1946).
[e] Tobias (1948b). [f] Duchâteau *et al.* (1953).

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E. Phylogenetic Relationships

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F. Adaptive Significance of the

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potassium content because they do not consume this kind of food, is not acceptable.

E. Phylogenetic Relationships

Duchâteau *et al.* (1953) proposed a hypothesis involving both phylogenetic and dietetic considerations in order to explain the diversity of cationic patterns. According to the classic views of insect taxonomy, the cationic pattern of Palaeoptera (high sodium type) is considered as a primitive pattern among insects, not dissimilar from that of other animal taxa and of apterygotes, if we consider the "indices" of each cation (see Table I).

The pattern found in other insect orders, especially in Lepidoptera, is strikingly different from the type defined above, and appears as a special evolutionary development, found also in other advanced groups, such as certain Coleoptera and in the larval stages of Hymenoptera. This specialized type appears as a systematic characteristic, linked to the genotype controlling the synthesis of the enzymes playing a role in the regulation. We can take into consideration the notion of the evolution of Lepidoptera, Coleoptera, and Hymenoptera parallel to the evolution of the angiosperms, and suggest that the speciation along this phylogenic line has been accompanied by a kind of regulation of the steady state of the cationic concentrations in the hemolymph, leading to a low sodium, a high potassium and a high magnesium pattern.

When the insects of these specialized groups adapt themselves secondarily to another form of food, as for example in the case of the wasp and bee larvae, of *Cossus* and of *Galleria,* this ecological change supposes the acquisition of new regulatory processes, maintaining the specialized pattern.

On the other hand, it is true that insects belonging to the orders which have not acquired the specialized type can very well adopt phytophagous habits without acquiring the pattern of cationic concentrations which is found in Lepidoptera and Hymenoptera. Clearly, this pattern is not a question of food, it is a question of taxonomy.

F. Adaptive Significance of the Specialized Cationic Pattern

The muscles of *Carausius morosus* and of Lepidoptera larvae function well and show action potentials in salines of a composition reproducing the cationic pattern of their hemolymph. This points to the fact that the mechanism of neuromuscular transmission must be of such a nature as to allow the muscle function to take place in media containing a high concentration of potassium, an extremely high concentration of magnesium, and almost no sodium. Hoyle (1954) suggests that mechanisms similar to those of Crustacea could be adapted to function in such media while the vertebrate mechanism could not be adapted. Hoyle also suggests that the type of cationic pattern of the "specialized" insects may be a way of reducing spontaneous activity and speed of movement. For instance, the level of potassium in phytophagous insects is reduced by fasting and it has been suggested by Hoyle that effects of this kind may be at work in building up the hypertensive excited state of migratory locusts (Ellis and Hoyle, 1954; Hoyle, 1954).

It appears that insects have on several occasions developed a regulation of the inorganic constituents of hemolymph in which the cationic pattern is not compatible with the function of the nerves and muscles of species belonging to other categories of insects or other animals.

This specialization appears, as already pointed out, as being linked with speciation parallel with the development of angiosperms. The ecological interest of the acquisition of the specialized hemolymph type may perhaps be linked with a behavioral aspect of relative inactivity, maintaining the larval stages in the midst of abundant food, as is the case for caterpillars.

From this point of view, it is particularly interesting to note the striking modification of the ratio Na/K during the metamorphosis of bees and wasps, leading from the resting larvae, with the specialized type of cationic pattern, to the well-known active adults, with a hemolymph containing large amounts of Na.

It seems, therefore, that the adaptations to an entirely vegetable diet, and to a sedentary life in the midst of food, has been developed independently in different orders, and generally as a particular feature of larval stages. The adult stages generally retain the basic and primitive cationic pattern. According to their phylogenetic position and to the specialized pattern of both larval and adult hemolymphs, the Coleoptera of the family Chrysomelidae, and probably also the Lepidoptera, are, among the insecta, the most fully adapted to phytophagous habits.

V. INORGANIC ANIONS AND ION BALANCE

The participation of the different inorganic anions in the equilibration of cations is illustrated in Table III. The concentration of Cl^-, $H_2PO_4^-$, and HCO_3^- are given in meq/liter, and also expressed by their "indices," that is in per cent of the sum of the four inorganic cations.

With respect to the concentration of the Cl^- anions, we may recognize two categories; in exopterygotes, the Cl^- concentration is always high (about 100 meq/liter or more) and neutralizes 50–82% of the total

TABLE III

INORGANIC ION CONCENTRATION OF THE HEMOLYMPH AND CATION-ION BALANCE IN SOME REPRESENTATIVE SPECIES

Species	Stage	Sum of cations meq/liter	Anions, meq/liter			Anions "indices"			References
			Cl^-	$H_2PO_4^-$	HCO_3^-	Cl^-	$H_2PO_4^-$	HCO_3^-	
Exopterygotes									
Odonata: *Aeschna grandis*	Larvae	169	110	4	15	65	2.3	8.8	Sutcliffe (1962)
Dictyoptera: *Periplaneta americana*	Adults	174.2	144	—	—	82.6	—	—	Van Asperen and Esch (1954)
Orthoptera: *Locusta migratoria*	Adults	118.6	97.6	—	—	82.3	—	—	Duchâteau *et al.* (1953) Hoyle (1954)
Cheleutoptera: *Carausius morosus*	Adults	197.4[a]	93[b]	40[c]	—	47.1	20.2	—	[a]Duchâteau *et al.* (1953) [b]May (1935) [c]Ramsay (1955a)
[illegible]	Adults	154	101	16	—	65.5	10.4	—	Wood (1957)

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Carausius morosus	Adults	154	101	16	—	65.5	10.4	—	Wood (1957)
Endopterygotes									
Megaloptera: *Sialis lutaria*	Larvae	167[a]	31[a]	5[b]	15[a]	18.5	3	6	[a]Shaw (1955) [b]Sutcliffe (1962)
Diptera: *Gasterophilus intestinalis*	Larvae	264	14.8	4	14.5	5.6	1.5	5.7	Levenbook (1950)
Lepidoptera: *Bombyx mori*	Larvae	150	21	3	—	14	2	—	Buck (1953)
Prodenia eridania	Larvae	94.7	34	5.8	—	35.9	6.1	—	Babers (1938)
Samia walkeri	Pupae	128.6	10.4	3.5	—	8	2.7	—	Gese (1950)
Telea polyphemus	Pupae	147.2	19.5	—	—	13.2	—	—	Carrington and Tenney (1959)
Coleoptera: *Dytiscus marginalis*	Adults	231.6[a]	44[a]	2.8[b]	—	19	1.2	—	[a]Sutcliffe (1962) [b]Buck (1953)
Popillia japonica	Larvae	84.3	19	4.9	—	22.5	5.8	—	Ludwig (1951)
Hymenoptera: *Apis mellifica*	Larvae	52.7	33	10.3	—	62.6	19.5	—	Bishop *et al.* (1925)

inorganic cation equivalents. In endopterygotes, on the contrary, the Cl^- concentration is generally less than 40 meq/liter, and its index varies from 5.6 to 36, with the exception of the bee larvae. In the latter case, however, the concentration is not higher than in other endopterygotes, the high index resulting from the very low concentration of inorganic cations.

The part played by the inorganic phosphates (calculated in Table III as $H_2PO_4^-$, a value probably somewhat inferior to the reality) and the bicarbonate ions is of only minor importance in cation binding, with the exception of *Carausius morosus* (Table III), in which the phosphates seem to contribute largely to the ion balance, and of the cricket *Anabrus simplex,* in which the phosphates concentration appears sufficient to balance almost entirely the sum of the cations (Pepper *et al.,* 1941).

In conclusion, the sum of the anions Cl^-, $H_2PO_4^-$ and HCO_3^- balances approximately the sum of the cations in the hemolymph of exopterygotes. The deficit of anion-cation balance in the hemolymph of most endopterygotes reveals the part played by inorganic molecules in the neutralization of the cations. This role seems to be mainly assumed by organic acids, the free amino acids making rather a net contribution to the cationic than to the anionic phase of the hemolymph, according to the pH and the nature of the amino acid concerned (Wyatt, 1961).

VI. ORGANIC ACIDS

During recent years, new information has been brought to the knowledge of organic acids in insect hemolymph, a question almost entirely ignored since the former work of Tsuji (1909).

The main organic acids found in the insect hemolymph belong to the substrates of the tricarboxylic acids cycle enzymes: citrate, α-ketoglutarate, succinate, fumarate, malate, etc. It appears from the data so far available, that these organic acids are generally more concentrated in the larval hemolymph of endopterygotes than in the adult hemolymph and in the exopterygotes.

Citrate has been detected by Levenbook and Hollis (1961) in 15 species. In the 13 species of endopterygotes studied (Coleoptera, Hymenoptera, Diptera, and Lepidoptera), citrate is more concentrated in larvae than in adult hemolymph (for instance: *Phormia regina:* 12.5 mM in larvae, with 0.44 and 0.33 mM in adults; *Sarcophaga bullata:* 10.3 mM in larvae, with 2.6 mM in adults; *Prodenia eridania:* 20.5 mM in larvae, with 4.7 in adults). Data obtained for exopterygotes are of

0.73 mM (*Periplaneta americana* larvae: Levenbook *et al.*, 1961), 1.6 mM (*Leptocoris trivittatus:* Levenbook *et al.*, 1961) and 2.3 mM (*Rhodnius prolixus:* Patterson, 1956).

Among the other acids of the tricarboxylic acids cycle, α-ketoglutarate, malate, fumarate, succinate, and oxaloacetate have been observed in the larval hemolymph of *Gasterophilus intestinalis* (Levenbook and Wang, 1948; Levenbook, 1950; Nossal, 1952), *Bombyx mori* (Fukuda and Hayashi, 1953, 1958) and *Hyalophora cecropia* (Sayigh and Wyatt, in Wyatt, 1961). The presence of pyruvate is not clearly established in the hemolymph of *Bombyx mori,* but large amounts have been found in *Antherea pernyi* (23–31 mM: Burova, 1953), as well as in *Hyalophora cecropia* (Wyatt, 1961). Other organic acids are probably also present: glyoxylic and aceto-acetic acids have been detected in *B. mori* larvae (Fukuda and Hayashi, 1958).

These organic acids play an important role in the cationic balance, at least in the endopterygote larvae. In *Gasterophilus intestinalis,* the sum of 6 organic acids so far identified amounts to 123 meq/liter, and accounts for 46.5% of the sum of the inorganic cations. In *Hyalophora cecropia,* the total of the different organic acids of the hemolymph amounts to 25–35 meq/liter. In *Bombyx mori* larvae, citrate alone, amounting to 32.1 mM (Levenbook and Hollis, 1961) assumes about 34% of the cation binding.

According to Levenbook and Hollis (1961), the large amount of organic acids in endopterygote larvae is not directly related to alimentary habits. The hemolymph citrate of *Prodenia eridania* is not affected by a change of diet, but is doubled after injection of fluoroacetate, as a result of the inhibition of aconitase. Citrate and other organic acids are undoubtedly "endogenous" in origin. The existence of all the enzymes of the tricarboxylic acids cycle in larval tissues leads to the conclusion that the accumulation of organic acids in the hemolymph appears as the consequence of a disproportional rate between acid production and acid oxidation and/or utilization (Levenbook, 1961).

VII. ORGANIC PHOSPHATES

According to Wyatt (1961), one of the most interesting peculiarities of insects is the high concentration of phosphates in their hemolymph. These phosphates are essentially organic in nature, and acid-soluble. An extensive study of organic phosphates has been carried out in the case of *Hyalophora cecropia* hemolymph (Wyatt, 1958; Wyatt and Kalf, 1957; Wyatt, Meyer and Kropf, 1958), by ion-exchange chromatography. In diapausing pupae, α-glycerophosphate, phosphoethanolamine and phosphocholine are the main components, with the respective concentrations of 8.5 and 9 mM. Their presence in the hemolymph is not the result of histolysis, but of a biosynthesis, as shown by incorporation of P^{32}. In *Bombyx mori*, α-glycerophosphate does not occur, but sorbitol-6-phosphate and glucose-6-phosphate have been detected in relatively large amounts (Kondo and Watanabe, 1957).

VIII. CARBOHYDRATES AND RELATED SUBSTANCES

It has been known for a long time that insect hemolymph generally contains little amounts of fermentable sugars, almost no saccharose, and little if any glycogen. The reducing power of the hemolymph is sometimes relatively high, but the greater part of this reducing power is due to substances nonsaccharidic in nature, such as ascorbic acid, α-ketonic acids, uric acid, tyrosine, and other phenols, and doubtless also many other unknown substances.

The explanation of such an unusually low concentration of fermentable sugars in an internal medium arose from the discovery, by Wyatt and Kalf (1956, 1957) of the existence in the hemolymph of a nonreducing dimer of α-glucose, trehalose, in high concentration. Hemolymph trehalose appears to be a form of carbohydrate transport peculiar to the class of Insecta.

A. Fermentable Sugars

The data concerning the amount of substances fermentable by yeast are presented in Table IV. The nature of these substances has been determined in only a few instances (Table IV). In the adult bee, the fermentable substances are fructose (30 to 40%) and glucose (60–80%) (Von Czarnovsky, 1954). Fructose is also present in rather large amounts in the hemolymph of *Gasterophilus intestinalis* (Levenbook, 1947, 1950) and glucose in that of *Phormia regina*, in which its concentration increases in the adult stage (Evans and Dethier, 1957). The high levels of fructose and glucose appear however to be exceptional in the hemolymph of insects.

B. Trehalose

The concentration of trehalose in a number of representative insects is shown in Table IV. Trehalose is generally present in large amounts in the hemolymph of all the insects studied so far, with the remarkable

TABLE

CONCENTRATION OF TOTAL FERMENTABLE
MG/100 ML), OF GLUCOSE, FRUCTOSE, AN
OF INSECTS (MG

Species	Stage	Fermentab sugars (as glucose)
Dictyoptera		
Periplaneta americana[12]	?	30
Leucophaea maderae[13]	?	65
Orthoptera		
Schistocerca gregaria	Larvae	—
Coleoptera		
Hydrophilus piceus	Adults	5–31[5]
Popillia japonica	Larvae	69[11]
Chalcophora mariana	Larvae	—
Ergates faber	Larvae	—
Hymenoptera		
Diprion hercyniae[14]	Larvae	—
Apis mellifica	Adults	1000–400
Lepidoptera		
Phalera bucephala	Larvae	40[6]
Prodenia eridania	Larvae	11[1]
Bombyx mori	Larvae	9–28[4]
	Pupae	18–50[4]
	Adults	16[4,5]
Deilephila euphorbiae	Larvae	Traces[7]
Deilephila elpenor	Pupae	—
Galleria mellonella	Larvae	—
Hyalophora cecropia[14]	Larvae	—
Hyalophora cecropia	Pupae	—
	Adults	—
Telea polyphemus[14]	Larvae	—
Diptera		
Gastrophilus intestinalis[9,10]	Larvae	95
Calliphora erythrocephala[9]	Larvae	—
Phormia regina[3]	Larvae	—
	Adults	—

[1] Babers, 1938; [2] Duchâteau and Florkin, 1959
[5] Florkin, 1937; [6] Hemmingsen, 1924; [7] Heller a
1956; [9] Levenbook, 1947; [10] Levenbook, 1950; [11]
[14] Wyatt and Kalf, 1957; [15] Wyatt, Loughheed

exception of the larvae of *Phormia*
The presence of trehalose in the hen
characteristic of the class of insects.

In vertebrates the cells generally
the circulatory form of the carbohy

osphate, phosphoethanola-
onents, with the respective
esence in the hemolymph
ynthesis, as shown by in-
ophosphate does not occur,
phate have been detected
tanabe, 1957).

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TABLE IV

CONCENTRATION OF TOTAL FERMENTABLE SUGARS (EXPRESSED IN GLUCOSE, MG/100 ML), OF GLUCOSE, FRUCTOSE, AND TREHALOSE IN THE HEMOLYMPH OF INSECTS (MG/100 ML)

Species	Stage	Fermentable sugars (as glucose)	True glucose	Fructose	Trehalose
Dictyoptera					
Periplaneta americana[12]	?	30	—	—	—
Leucophaea maderae[13]	?	65	—	—	580–780
Orthoptera					
Schistocerca gregaria	Larvae	—	—	Traces[9]	800–1500[8]
Coleoptera					
Hydrophilus piceus	Adults	5–31[5]	—	—	500–700[2]
Popillia japonica	Larvae	69[11]	—	—	—
Chalcophora mariana	Larvae	—	—	—	4700–5200[2]
Ergates faber	Larvae	—	—	—	3200[2]
Hymenoptera					
Diprion hercyniae[14]	Larvae	—	28	—	926
Apis mellifica	Adults	1000–4000[16]	600–3200[16]	200–1600[16]	600–1200[2]
Lepidoptera					
Phalera bucephala	Larvae	40[6]	—	—	—
Prodenia eridania	Larvae	11[1]	—	—	—
Bombyx mori	Larvae	9–28[4,5]	1–3[15]	1–2[15]	400–500[15]
	Pupae	18–50[4,5]	3–5[15]	1–2[15]	202[15]
	Adults	16[4,5]	—	—	—
Deilephila euphorbiae	Larvae	Traces[7]	—	—	—
Deilephila elpenor	Pupae	—	—	—	800–1900[2]
Galleria mellonella	Larvae	—	21[14]	—	1700[14]
Hyalophora cecropia[14]	Larvae	—	—	—	1200
Hyalophora cecropia	Pupae	—	0–8	—	400–600
	Adults	—	—	—	650–1150
Telea polyphemus[14]	Larvae	—	—	—	1306
Diptera					
Gastrophilus intestinalis[9,10]	Larvae	95	10	184–294	—
Calliphora erythrocephala[9]	Larvae	—	—	Traces	—
Phormia regina[3]	Larvae	—	70–125	—	Absent
	Adults	—	up to 600	—	598

[1] Babers, 1938; [2] Duchâteau and Florkin, 1959; [3] Evans and Dethier, 1957; [4] Florkin, 1963b; [5] Florkin, 1937; [6] Hemmingsen, 1924; [7] Heller and Moklowska, 1930; [8] Howden and Kilby, 1956; [9] Levenbook, 1947; [10] Levenbook, 1950; [11] Ludwig, 1951; [12] Todd, 1957; [13] Todd, 1958; [14] Wyatt and Kalf, 1957; [15] Wyatt, Loughheed and Wyatt, 1956; [16] Von Czarnowsky, 1954.

exception of the larvae of *Phormia regina* (Evans and Dethier, 1957). The presence of trehalose in the hemolymph appears as a biochemical characteristic of the class of insects.

In vertebrates the cells generally contain little glucose; glucose is the circulatory form of the carbohydrate cellular food; it is mainly of endogenous origin, the product of a gluconeogenesis principally performed by the liver, and which has its final point in the blood. Glucose enters the cells by crossing the membrane as hexose-6-phosphate. Insects, on the other hand have in their hemolymph trehalose as the circulating form of the saccharidic cellular food. The cells of most insect tissues use glucose, the liberation of which is carried out inside the cells by the action of the enzyme trehalase.

During the intermolts, trehalose is stable in the internal medium, the trehalase of the hemolymph being inhibited (Friedman, 1961). The cells of most tissues (with the exception of epidermis) contain an active trehalase (Kalf and Rieder, 1958; Howden and Kilby, 1956; Zebe and McShan, 1959; Duchâteau-Bosson *et al.,* 1963) and may thus utilize trehalose for their metabolism. This has been unequivocally demonstrated in the case of muscle activity (Evans and Dethier, 1957; Clegg and Evans, 1961; Bücher and Klinkenberg, 1958). At the breaking of diapause, the trehalose contents of the pupae of *Deilephila elpenor* are also greatly reduced (Duchâteau and Florkin, 1959).

On the other hand, the trehalose concentration of the hemolymph falls rapidly during molting in *Schistocerca gregaria* (Howden and Kilby, 1956). According to Candy and Kilby (1961, 1962), the hemolymph trehalose is used not only for metabolic purposes, but also for providing carbohydrate material during chitin synthesis by the epidermis at each molt. However, epidermal cells appear to lack trehalase (Zebe and McShan, 1959; Duchâteau *et al.,* 1963). They use glucose, liberated by the enzymic hydrolysis of trehalose, a hydrolysis performed not inside the cells, but outside in the hemolymph. The supply of glucose from the trehalose of the hemolymph has been investigated by Duchâteau *et al.* (1963); this mechanism is illustrated by Fig. 3, showing the variations of trehalose concentration and of trehalase activity in the hemolymph of *Bombyx mori* during the end of larval and the beginning of pupal life.

The amount of blood trehalose sharply decreases at each molt, and also during the fasting period corresponding to spinning. The fall of blood trehalose during the molts corresponds to the increase of glucose (Florkin, 1936) observed at the same period. It is related to the release, probably of hormonal nature, of the inhibition of the trehalase present in an inactive state in the hemolymph. In the fat-body, an inverse relationship exists between glycogen and trehalose, the former disappearing almost completely at each molt, while the amount of trehalose tends to remain at nearly constant level (Duchâteau *et al.,* 1963). On the other hand, the bulk of fat-body is consumed to a large extent, during the periods of chitin synthesis. These observations suggest that the

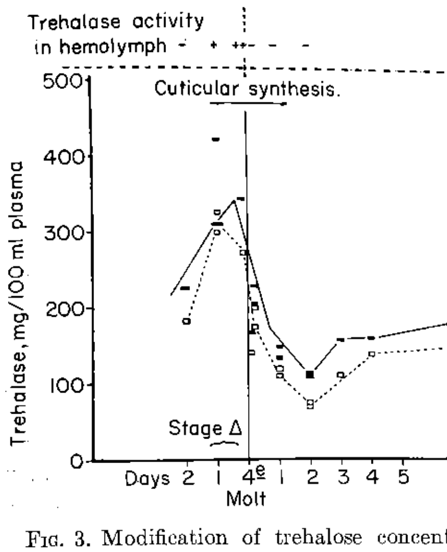

FIG. 3. Modification of trehalose concen
hemolymph of *Bombyx mori* (from Duchâ
anthrone reactive material, in mg trehalo
mg/100 ml.

trehalose level of the hemolymph i
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body and the hemolymph of *Peripla*

C. Glycogen

There are only small amounts of
according to Wyatt (1961), the sul
the classic methods are, as far as ins
ably of a different chemical nature
glycoproteins.

D. Amino Sugars

There are only a few studies bea
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have been detected, sometimes in la
Tenebrio molitor (Marcuzzi, 1955),
Wyatt, 1960) and of *Bombyx mori* la
1956). The concentration of acety
the silkworm *B. mori* varies at eac
to 40 mg/100 ml (Jeuniaux, unpu

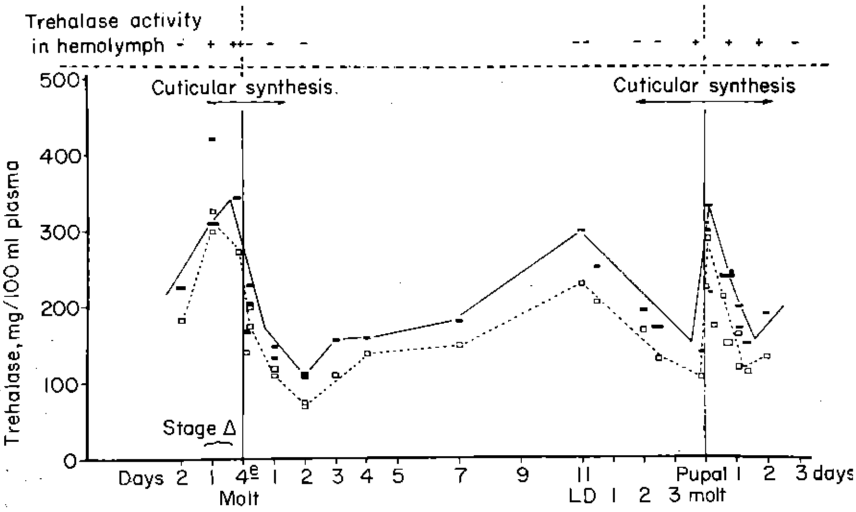

FIG. 3. Modification of trehalose concentration and of trehalase activity in the hemolymph of *Bombyx mori* (from Duchâteau *et al.* 1963). ■———■ : anthrone reactive material, in mg trehalose/ml; □-----□ : trehalose, mg/100 ml.

trehalose level of the hemolymph is supplied at the expense of the glycogen of the fat-body. This conclusion is supported also by the recent experiments of Steele (1963) who demonstrated the effects of a hyperglycemic hormone on the glycogen and trehalose contents of the fat-body and the hemolymph of *Periplaneta americana*.

C. Glycogen

There are only small amounts of glycogen in the insect hemolymph; according to Wyatt (1961), the substances estimated as glycogen by the classic methods are, as far as insect hemolymph is concerned, probably of a different chemical nature, such as other polysaccharides or glycoproteins.

D. Amino Sugars

There are only a few studies bearing on amino sugars in the insect hemolymph. Substances related to hexosamines or acetylhexosamines have been detected, sometimes in large amounts, in the hemolymph of *Tenebrio molitor* (Marcuzzi, 1955), of *Hyalophora cecropia* (Carey and Wyatt, 1960) and of *Bombyx mori* larvae (Wyatt, Longhead and Wyatt, 1956). The concentration of acetylhexosamines in the hemolymph of the silkworm *B. mori* varies at each molting period, increasing from 2 to 40 mg/100 ml (Jeuniaux, unpublished). These variations are undoubtedly related to the resorption by the epidermis of cuticular breakdown products (Jeuniaux, 1963).

E. Glycerol

The existence of high amounts of glycerol in the hemolymph as well as in the tissues of several insects may be considered as an adaptation to cold-hardiness. In *Hyalophora cecropia*, Wyatt and Meyer (1959) have shown that glycerol is not present in the larval hemolymph, but accumulates gradually during diapause to reach a level of about 300 mM; then it disappears rapidly when diapause is broken. The production of glycerol appears as resulting from a modified glycolytic pathway. Glycerol is not a permanent constituent of insect hemolymph, and some species related to *H. cecropia* possess only little if any glycerol in their hemolymph. An exhaustive review of cold-hardiness in insects has been presented by Salt (1961).

IX. NITROGENOUS CONSTITUENTS

The insect hemolymph does not markedly differ from that of vertebrates with respect to its protein-nitrogen, but its very high aminoacidemia seems to be one of its most exceptional peculiarities. Therewith, the hemolymph stores sometimes relatively high amounts of the end-products of the nitrogen metabolism: uric acid, allantoin, allantoic acid, urea, and ammonia. Uric acid is often very concentrated, sometimes near saturation, and crystals are commonly found in the hemolymph. According to the absence of allantoicase in insect tissues, urea does not derive from allantoic acid, but probably from arginine, under the action of arginase (Garcia *et al.*, 1956; Kilby and Neville, 1957). Ammonia is mainly found in aquatic species.

The similarity between the amino acid composition of both hydrolyzed and nonhydrolyzed plasma after deproteinization (with the exception of the dicarboxylic acids which are partly in the form of their amides in the hemolymph) indicates that the peptide content is generally low (Florkin, 1959). Peptides, however, seem to be more abundant in the hemolymph of *Drosophila* (Hadorn and Mitchell, 1951).

X. FREE AMINO ACIDS

During the last 10 years, considerable information has been obtained concerning the nature and the concentration of free amino acids in the hemolymph, thanks to the improvement of quantitative techniques

such as microbiological method and th
of Moore and Stein. In the case of tl
convenient to consider the results as r
trations of amino acids, owing to the
method often gives results slightly but s

It is not possible to summarize brief
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from Table V, which gives some of t
available,[1] that, in spite of a very wid
clusions may be drawn.

A. Total Concentration

A high aminoacidemia is a characteri
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values generally much lower than thos
the exception of *Gasterophilus* larva).

The increasing importance of free am
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and Coleoptera. In these insects, cont
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B. Relative Concentration of th

As it appears from the comparison
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Arginine is essentially derived from its
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centrations of the different amino aci

[1] Other data may be found for the followi
(1948); Orthoptera: Benassi *et al.* (1959); Ben
and Dubreuil (1953); Auclair (1959); Pra
Lepidoptera: Auclair and Dubreuil (1953); C
(1956); Irreverre and Levenbook (1960); Col
Pochedley (1956, 1958); Diptera: Chen and H
(1950).

such as microbiological method and the ion-exchange chromatography of Moore and Stein. In the case of the former technique, it is more convenient to consider the results as reflecting the "apparent" concentrations of amino acids, owing to the fact that the chromatographic method often gives results slightly but significantly lower.

It is not possible to summarize briefly the numerous data available, and systematization appears to be impossible. However, it can be seen from Table V, which gives some of the more complete analysis now available,[1] that, in spite of a very wide variability, the following conclusions may be drawn.

A. Total Concentration

A high aminoacidemia is a characteristic of the class of insects. However, this character is clearly more accentuated in endopterygotes than in exopterygotes. In the three exopterygotes studied so far (Table V), the sum of the 15 amino acids ranges only from 293 to 636 mg/100 ml, values generally much lower than those found in endopterygotes (with the exception of *Gasterophilus* larva).

The increasing importance of free amino acids as hemolymph constituents appears, as already pointed out, as an evolutionary tendency developed in the most evolved groups, such as Lepidoptera, Hymenoptera, and Coleoptera. In these insects, contrary to what obtains in vertebrates and other invertebrates, the composition of the internal medium is thus similar to that of the cells. This pattern is quite fitted for an internal medium which is rapidly tapped upon for the construction of new cells, at the time of molting and metamorphosis.

B. Relative Concentration of the Different Amino Acids

As it appears from the comparison between hydrolyzed and nonhydrolyzed dialyzed plasma, aspartic and glutamic acids exist mainly in the form of their amides: asparagine and glutamine (Florkin, 1959). Arginine is essentially derived from its phosphagen, arginine-phosphoric acid. Exopterygote and endopterygote insects differ by the relative proportions of the hemolymph amino acids. In exopterygotes, the concentrations of the different amino acids are of the same order (from

[1] Other data may be found for the following orders: Odonata: Raper and Shaw (1948); Orthoptera: Benassi *et al.* (1959); Benassi *et al.* (1961); Dictyoptera: Auclair and Dubreuil (1953); Auclair (1959); Pratt (1950); Hemiptera: Pratt (1950); Lepidoptera: Auclair and Dubreuil (1953); Chen and Hadorn (1954); Wyatt *et al.* (1956); Irreverre and Levenbook (1960); Coleoptera: Auclair and Dubreuil (1953); Pochedley (1956, 1958); Diptera: Chen and Hadorn (1954); Hackman (1956); Pratt (1950).

TABLE V

DISTRIBUTION AND CONCENTRATION OF FREE AMINO ACIDS IN THE HEMOLYMPH OF SOME REPRESENTATIVE INSECTS (MG/100 ML HYDROLYZED PLASMA)[a]

Amino acids	Exopterygotes			Hymenoptera	Coleoptera[b]		Diptera	Lepidoptera				
	Aeschna sp. larvae	*Carausius morosus* adults	*Locusta migratoria* nymphs	*Apis mellifica* larvae	*Hydrophilus piceus* adults	*Popillia*[h] *japonica* larvae	*Gasterophilus* larvae	*Euproctis chrysorrhoea* larvae[c]	*Smerinthus ocellatus* larvae[c]	*Saturniidae*[e] pupae	*Sphyngidae*[f] pupae[d]	*Papilio machaon* pupae
Alanine	46	10–60	34	58	60	146–187	—	33	27	7–300	16–250	103–213
Arginine	19–27	17–19	24	50–74	7–11	48–81	8	44–58	10	107–243	59–576	126–127
Aspartic acid (total)	4–13	6–14	13	32–33	17–18	42–47	14	9–22	27	4–36	5–55	14–10
Glutamic acid (total)	32–63	50–77	166	308–347	131–195	309–526	314	302–343	202	83–468	62–240	202–226
Glycine	22–54	23–31	97	72–84	17–26	288–325	5	48–94	52	20–82	4–57	48
Histidine	7–21	55–58	30	17–30	8–12	169–225	1	107–161	83	23–196	3–127	71–89
Isoleucine	16–18	7–13	21	20–24	8–25	36–54	8	15–32	12	14–83	20–65	40–56
Leucine	22–29	10–14	21	25–30	7	20–25	7	13–23	8	15–108	14–73	56–80
Lysine	6–14	20–28	47	74–104	20–24	29–94	8	50–105	77	113–471	64–433	325–401
Methionine	4–13	9–13	6	19–23	3	3–12	7	1–13	8	11–148	25–81	122–163
Phenylalanine	5–11	6–9	11	8–12	6–7	13–17	7	8–15	9	7–72	8–49	24–43
Proline	12–41	10–16	62	368–418	122–283	264–507	16	129–157	23	62–478	28–230	146–256
Threonine	12–23	29–40	20	27–49	12–17	11–29	23	30–54	34	1–136	20–82	47–57
Tyrosine	3–13	5–8	28	3	2–9	11–37	22	0–5	36	2–76	8–146	4–5
Valine	23–29	22–25	48	58–59	11–20	94–150	15	29–49	83	34–127	22–105	101–120
Total	399.0	293–424	636.0	1239.0	445–721	1723–2162	465[g]	870–1164	700	1124–1989	515–1819	1575–1769
Serine	24		49		22–35							

[a] The values have been rounded to the unity. From Duchâteau and Florkin (1959) and Shotwell *et al.* (1963).

[b] Other species studied: *Leptinotarsa decemlineata*.

[c] Other species studied: larvae of *Cossus cossus* (sum of the 15 amino acids: 938 mg/100 ml); *Amathes xanthographa* (1027 mg/100 ml), *Triphaena pronuba*. (1.352 mg/100 ml), *Imbrasia macrothyris* (497 mg/100 ml) and *Pseudobunaea seydeli* (709 mg/100 ml).

[d] Other pupae studied: *Lasiocampa quercus* (sum of 15 amino acids: 2317 to 2430 mg/100 ml), *Euproctis chrysorrhoea* (1066 mg/100 ml) and *Smerinthus ocellatus* (1645 mg/100 ml).

[e] 15 species belonging to the genus *Citheronia, Eacles, Saturnia, Antheraea, Actias, Hyalophora, Philosamia*.

[f] Species studied: *Deilephila elpenor, Sphinx ligustri, Celerio euphorbiae, Laothoe populi, L. austanti, and L. populi × austanti*.

[g] Without alanine.

[h] Shotwell *et al.* (1963).

Valine	23–29	22–25	48	58–59	11–20	94–150	15	20–49	83	34–127	22–105	101–120
Total	399.0	293–424	636.0	1239.0	445–721	1723–2162	465[g]	870–1164	700	1124–1989	515–1819	1575–1769
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[g] Without alanine.

[h] Shotwell *et al.* (1963).

10–60 ml/100 ml); "total" glutamic acid and glycine are somewhat more concentrated in *Locusta* hemolymph (see Table V).

In endopterygotes, on the contrary, the different amino acids may be present at very different concentrations:

(1) "Total" aspartic acid and phenylalanine, and also leucine and isoleucine, always occupy a minor place in the amino acid pool of the insect hemolymph.

(2) "Total" glutamic acid and proline (the latter with only a few exceptions) generally take the most important quantitative place in the amino acid pool.

(3) The other amino acids may be present at more or less high concentrations, according to the species considered.

C. Modifications of the Amino Acid Pattern

It appears, from a general survey, that a characteristic amino acid pattern cannot be ascribed to any kind of taxonomic group as a biochemical character, according to the very high variations observed between the different genus of a given family, or even between the different species of a given genus (see for instance, the extensive study of Saturniidae and Sphyngidae by Duchâteau and Florkin, 1958). Moreover, every species shows great modifications of its aminoacidemia during its development, especially during metamorphosis. The pattern of amino acid concentration is more constant in the case of diapausing pupae of Lepidoptera, as a result of a steady state easily maintained at lowered metabolism, mainly controlled by internal factors in a nonfeeding, nonmetamorphosing individual (Duchâteau and Florkin, 1958). The aminoacidemia of an insect species may therefore be defined as being a succession of steady states expressed by a succession of patterns specific to the different instars of this species and to particular ecological or physiological events. An example of the metabolic alteration of the aminoacidemia is given by the silkworm *Bombyx mori,* which has been the most intensively studied from this point of view.

D. Effects of Molting, Diet, Histolysis, Silk Secretion, and Pupation on the Aminoacidemia of the Silkworm

The origin and the fate of the different amino acids has been studied by following the effects of the removal of silk glands, coupled or not with starvation experiments, by the study of the incorporation into the silk of radioactive amino acids, and so on. The results may be summarized as follows (Fig. 4).

1. The silk gland utilizes only a few kinds of free amino acids from the hemolymph in order to synthetize the fibroin: these are glycine,

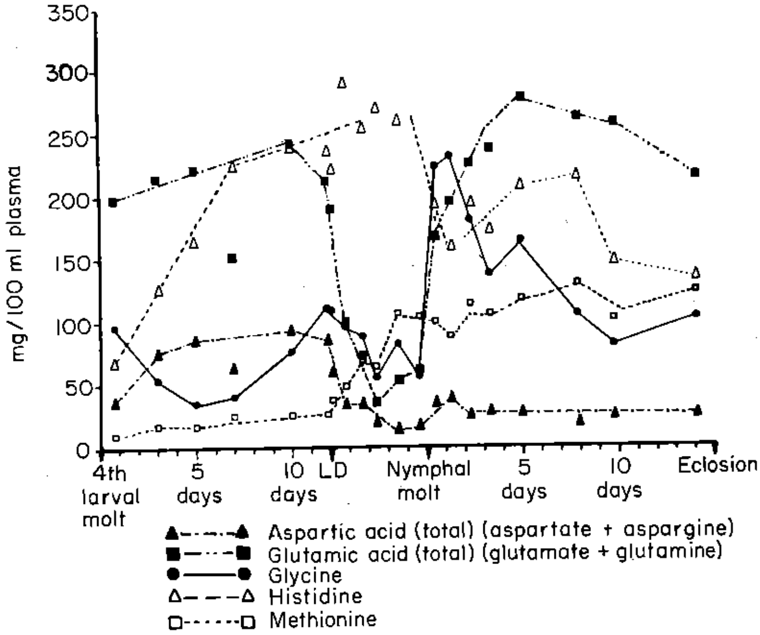


FIG. 4. Variation of the concentrations of three "sericigenous" amino acids, of histidine and of methionine in the hemolymph of the silkworm *Bombyx mori* during the fifth larval stage, the spinning and the metamorphoses. (Jeuniaun *et al.*, 1961.)

aspartic, and glutamic acids (mainly in the form of their amides), serine, threonine, and proline, but no significant amounts of alanine nor of phenylalanine. The removal of silk glands produces indeed a considerable accumulation of these "sericigenous" amino acids in the hemolymph, at the end of the fifth larval stage (Duchâteau *et al.*, 1959; Bricteux-Grégoire *et al.*, 1959a; Bricteux-Grégoire *et al.*, 1959b; Duchâteau-Bosson *et al.*, 1960; Duchâteau-Bosson *et al.*, 1961a). After injection of radioactive glycine or threonine, the isotopic carbon is incorporated into the fibroin not only as glycine, but also to a lesser extent as alanine and serine (Bricteux-Grégoire *et al.*, 1959). As shown also by radioactive experiments, glutamic and aspartic acids are mainly used by the silk gland for the biosynthesis of the alanine of fibroin (Bricteux-Grégoire *et al.*, 1960).

2. The "sericigenous" amino acids of the hemolymph are mainly of dietary origin. During the first 5 or 6 days of the fifth larval stage, that is, during the half of the feeding period of the last larval intermolt, some of these amino acids, especially glycine, are stored in tissues, and

their concentration in the hemolymph
During the second part of the feedin
maintain a steady state, the utilization
of the hemolymph by the silk glands
supplies (Fig. 4).

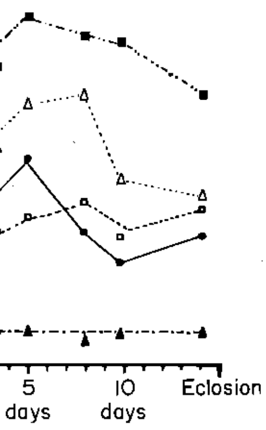
3. The period of spinning, which c
taneous starvation, is characterized b
amino acid pattern. The concentration
acids, threonine, serine, and proline f
state is established, reflecting the bal
tion by the silk gland and the supply
has been clearly demonstrated, by rad
of dietary origin, "stored" in the tis
5th instar, is laid down at the time
the fibroin at the end of the silk threa

4. When the secretory activity of the
amino acids liberated by histolysis a
hemolymph by the silk gland; their c
generally attains the initial values obse

5. Histidine concentrations vary in
accumulates in the hemolymph durin
tration remains at its higher level (
spinning period, when the other am
After spinning, the concentration o
the increase of the sericigenous amin
The variations of histidine, and to a
been interpreted as being a compe
some way the osmotic pressure of
mains relatively constant (Jeuniaux

6. The other amino acids (alanine,
and phenylalanine), are not utilized
silk gland, as shown by experiments
gland. Their concentrations decreas
period as a result of the spontaneou
centration during the pupal molt is
steady state is generally maintained
(Duchâteau-Bosson *et al.*, 1961b).

7. The concentration of tyrosine
during the whole life course. Accum
days preceding each molt (up to 8
a sharp and sudden decrease followi



"sericigenous" amino acids, of
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their concentration in the hemolymph remain more or less constant. During the second part of the feeding period, there is a tendency to maintain a steady state, the utilization of the "sericigenous" amino acids of the hemolymph by the silk glands being balanced by the alimentary supplies (Fig. 4).

3. The period of spinning, which corresponds to a period of spontaneous starvation, is characterized by a marked modification of the amino acid pattern. The concentration of glycine, glutamic and aspartic acids, threonine, serine, and proline falls to low values. A new steady state is established, reflecting the balance between amino acid utilization by the silk gland and the supply from the lysis of the tissues. It has been clearly demonstrated, by radioactive experiments, that glycine of dietary origin, "stored" in the tissues during the beginning of the 5th instar, is laid down at the time of spinning and incorporated into the fibroin at the end of the silk thread (Fukuda and Florkin, 1959).

4. When the secretory activity of the silk glands stops, the sericigenous amino acids liberated by histolysis are no longer withdrawn from the hemolymph by the silk gland; their concentration increases rapidly and generally attains the initial values observed before silk secretion (Fig. 4).

5. Histidine concentrations vary in an opposite direction: histidine accumulates in the hemolymph during the fifth instar, and its concentration remains at its higher level (up to 300 mg/100 ml) during the spinning period, when the other amino acids are depleted (Fig. 4). After spinning, the concentration of histidine decreases, parallel to the increase of the sericigenous amino acids (Duchâteau *et al.*, 1960). The variations of histidine, and to a lesser extent, of methionine, have been interpreted as being a compensatory mechanism regulating in some way the osmotic pressure of the hemolymph, which indeed remains relatively constant (Jeuniaux *et al.*, 1961).

6. The other amino acids (alanine, lysine, leucine, isoleucine, valine, and phenylalanine), are not utilized to any appreciable degree by the silk gland, as shown by experiments involving the removal of the silk gland. Their concentrations decrease somewhat during the spinning period as a result of the spontaneous starvation. Their increasing concentration during the pupal molt is a consequence of histolysis. A new steady state is generally maintained during the rest of the pupal stage (Duchâteau-Bosson *et al.*, 1961b).

7. The concentration of tyrosine in the hemolymph varies widely during the whole life course. Accumulation takes place within the few days preceding each molt (up to 80 mg/100 ml), and is followed by a sharp and sudden decrease following each molt. These variations are

related to the utilization of tyrosine in the protein-tanning and melanization of the new cuticle (Duchâteau-Bosson *et al.*, 1962). Similar observations have been noted in the case of the puparium formation of *Sarcophaga* (Fraenkel and Rudall, 1947).

E. D and L Forms of Amino Acids

The free amino acids of the hemolymph are usually of the L configuration. A few exceptions are known: for instance, in *Oncopeltus fasciatus*, the hemolymph contains large amounts of the D isomer of alanine, a substance that does not exist in the food of this insect (Auclair and Patton, 1950). Free D-serine has been detected in the hemolymph of larvae, pupae, and adults of different Lepidoptera (*Bombyx mori, Hyalophora cecropia* and *Antheraea pernyi*); the D-isomer is more abundant in the pupae, in which it may account for up to 70% of the total free serine of the hemolymph (Srinivasan, Corrigan and Meister, 1962). D-alanine has not been found in these Lepidoptera, while D-serine does not occur in *Oncopeltus* hemolymph.

XI. PROTEINS

The protein concentration in insect hemolymph is similar to that of the blood of man and other vertebrates, and generally higher than that of the internal fluids of other invertebrates. The average protein constant is of 5 gm/100 ml in Hymenoptera, 3–4 gm/100 ml in Coleoptera, 2 gm/100 ml in Lepidoptera and 1 gm/100 ml in Orthoptera (Florkin, 1936a).

In recent years, considerable attention has been paid to the characterization of hemolymph proteins, using electrophoresis on paper or in agar and starch gels, ultracentrifugation, immunoelectrophoresis, etc. The already numerous data have been summarized by Wyatt (1961) and by Gilbert and Schneiderman (1961). The characterization of the different fractions as albumins, α- and β-globulins, and so on, on the basis of their electrophoretic mobility has been criticized by Dénucé (1958). These studies are in full development, and there is now little to say about the physicochemical properties of the hemolymph proteins.

The electrophoretic pattern of hemolymph proteins is used by some authors for taxonomic purposes, these patterns being, in a given family, more similar for the species of the same genus than for species belonging to different genus (see, for instance, Benoit and Van Sande, 1959; Brezner and Enns, 1958; Van Sande and Karcher, 1960; Stephen, 1956; Martin and Cotner, 1934). Hemolymph proteins show also some differences, according to the sex of the individuals (Stephen and Steinhauer, 1957).

The protein pool of the hemolymph is said to function as a reserve, source of the protein synthesis of the adult stage during pupal life (Heller, 1932). In the Lepidoptera, indeed, the level of hemolymph proteins generally increases during the larval life, and then decreases at the end of the pupal instar. The protein pool is also said to be the source of the free amino acids of the hemolymph, especially during starvation (Beadle and Shaw, 1950), the amino acid concentration remaining approximately constant.

It is not clear whether or not antibodies proteinic in nature are formed in the insect hemolymph. Earlier reports of Steinhaus (1949) and other workers have not been confirmed recently. A phenomenon of immunization does however occur in some Lepidoptera (Briggs, 1958; Stephens, 1959), but the nature of this immunity seems rather different from that known in vertebrates.

The only well-defined proteins of the insect hemolymph are those exhibiting enzymic properties. The number of different enzymes or isoenzymes in the hemolymph is surprisingly high. Although we do not possess any quantitative estimate of the relative concentration of these proteins enzymic in nature, it appears that they represent an important portion of the hemolymph proteins (Laufer, 1960). Some of the enzymic activities are nearly as high as those of the tissues, so that the presence of these enzymes in the hemolymph cannot be considered as necessarily resulting from a leakage from the tissues, as it occurs in mammals. The exact role of these enzymes in the hemolymph requires, however, further demonstration. Laufer (1961), in the case of proteolytic enzymes, and Jeuniaux (1961), in that of chitinolytic systems, both suggested that these enzymes may function in the histolysis which occurs at the time of molting and metamorphoses.

A. Hydrolases

The roughly qualitative studies of Arvy and Gabe (1946a,b) and the more accurate studies of Laufer (1960a,b) indicate the existence of different hydrolytic activities in the hemolymph of Odonata, Cheleutoptera, Dermaptera, Orthoptera, Coleoptera, and Lepidoptera. It is clear that amylases, esterases (lipases) and one or more proteolytic enzymes do generally occur in the insect hemolymph. Glucosidases able to hydrolyze sucrose and maltose are found in the hemolymph of *Bombyx mori* (Yamafuji, 1934a) in addition to amylase (Yamafuji, 1934a, 1935), the activity of which is a biochemical characteristic of the different races. In *B. mori*, the amylases of the hemolymph and of the gut are two very different isoenzymes, according to their different properties (optimum pH, activation and inhibition, etc.; Ito *et al.*, 1962).

The enzymes of the chitinolytic system also occur in the insect hemolymph. Chitobiase is present in high concentrations during the whole life course of *B. mori*, while chitinases can be detected at the beginning of the pupal life (Jeuniaux, 1961). Chitinases have also been identified in the hemolymph of *Periplaneta americana* adults, in which they reach concentrations higher than that in saliva, digestive juices and gut tissues (Waterhouse and McKellar, 1961). Their role in the hemolymph remains obscure.

The presence and the role of trehalase in the hemolymph have been discussed above (see Trehalose).

B. Phosphatases

Organic phosphates are broken down rather rapidly in the hemolymph plasma of *Gasterophilus* (Levenbook, 1950) and of *H. cecropia* (Wyatt, 1958). In *B. mori* larvae, the hemolymph contains a hexose-1-phosphatase (Faulkner, 1955) and an alkaline phosphatase (Itabashi, Koide and Shimura, 1913). A number of phosphatases have been detected in the hemolymph of *H. cecropia* and *Philosamia cynthia* (Laufer, 1960).

C. Transaminases

Aspartic-α-ketoglutaric transaminase occurs in the hemolymph of *Celerio euphorbiae* and of *Bombyx mori*, but its activity is many times lower than that of fat body or muscles (Belzecka *et al.*, 1959; Bheemenvar and Sreenivasaya, 1952).

D. Enzymes of Carbohydrate Metabolism

According to Faulkner (1955), the hemolymph is a likely site of the metabolism of carbohydrates, the activity of hexose-1-phosphatase, "malic" enzyme (TPN linked dehydrogenase) and polyoldehydrogenase being intermediate between those of fat-body and gut tissue. Malic dehydrogenase and isocitric dehydrogenase, both TPN dependent, have been found in high concentration in the larval and adult hemolymphs of *Tenebrio molitor;* glutamic, α-glycerophosphate, glucose, and lactic dehydrogenases seem to be lacking in the larval but present in the adult hemolymph of this species (Prota, 1961). The presence of multiple forms of malic dehydrogenase, lactic dehydrogenase and α-glycerophosphate dehydrogenase has been observed in *Hyalophora cecropia* and *Samia cynthia* hemolymph; the variations of the relative importance of the different isoenzymes have been followed during the pupal life and the development of the adult (Laufer, 1961).

E. Oxidases

Phenoloxidases (or tyrosinases) are
lymph of insects, and are responsible
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discussed by Ito (1953). In Diptera as
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Xanthine-oxidase has been found in
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F. Other Enzymes

Catalase is present in *B. mori* hemol
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XII. PIGM

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Among the numerous pigments wh
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E. Oxidases

Phenoloxidases (or tyrosinases) are uniformly present in the hemolymph of insects, and are responsible for the rapid darkening of the hemolymph when exposed to air. The presence of inhibitors has been discussed by Ito (1953). In Diptera as well as in *Bombyx mori*, hemolymph tyrosinase seem to be present in the form of a proenzyme, "protyrosinase," activated by a proteic activator (Onishi, 1959). In *Calliphora erythrocephala,* the metamorphosing hormone ecdysone controls the biosynthesis of the proteic activator of the prophenoloxydase (Karlson and Schweigger, 1961), whereas this hormone has no effect on the secretion of the proenzyme or on the activity of the enzyme itself (Karlson and Schmid, 1955).

Xanthine-oxidase has been found in the larval and adult hemolymphs of *Tenebrio molitor* (Prota, 1961)

F. Other Enzymes

Catalase is present in *B. mori* hemolymph, and more active in males than females (Matsumura, 1935).

XII. PIGMENTS

The function and properties of hemoglobins in *Chironomid* larvae which attracted considerable attention in the last decade, has been thoroughly discussed by Buck (1953).

Among the numerous pigments which give to the hemolymph its specific color, only a few have been identified, viz., α-carotene, riboflavine and flavine nucleotides in *Hyalophora cecropia* (Chefurka and Williams, 1952), flavones, flavines, fluorescyanine, and folic acid in *B. mori* (Drilhon, 1951; Drilhon and Busnel, 1951). The presence of chlorophyll as the pigment of green hemolymphs is doubtful. In the hemolymphs of larvae of *Pieris rapae, Cacoecia australana* and *Amphipyra sanguinipuncta* (Lepidoptera), the green color is due to the presence of a yellow chromoprotein, the prosthetic groups of which are β-carotene and lutein, and of a blue chromoprotein, the prosthetic group of which seems to be mesobiliverdin (Hackman, 1952). A similar composition has been observed in the case of the green hemolymph of the solitary phases of *Locusta migratoria* and *Schistocerca gregaria* (Goodwin and Srisukh, 1951). But the green color of the hemolymph of the bug *Nezara viridula* is due to a β-carotene-protein complex and a blue pigment resembling anthocyanine (Hackman, 1952).

XIII. CONCLUSION

Considered from the ecological point of view, insects are the only invertebrates able to live in dry environments and able to fly. The hemolymph is their only extracellular fluid. They have given up the physiological association between the respiratory and the circulatory systems, the tracheal system ensuring the arrival of oxygen to all cells. Insects are therefore not bound to the maintenance of a definite blood volume and they can rely on blood water to insure their survival in dry media. They can, in spite of the variations of blood volume, regulate the osmotic pressure in the hemolymph by changing the amino acid concentration. The aminoacidemia is high and the nonprotein nitrogenous components of hemolymph are mainly made up of the components of the amino acid pool. The proteins of insect hemolymph probably lack the oncosmotic and nutritive components in Mammalian plasma: they are mainly made up of enzymes. The hemolymph of insects appears therefore with the characteristics of a fluid tissue, with its own metabolism, revealing a composition more similar to that of the intracellular fluid than to that of the blood of vertebrates. Inorganic cations and anions are, especially in the most specialized endopterygote orders, replaced by amino acids and organic acids.

By its nature as a container of a number of reserve or transport materials, the most peculiar of which being trehalose, in constant exchange relations with the fat body, hemolymph fits the life of organisms in which feeding is interrupted during certain life phases or during diapause, in relation to factors of the environment or to ecological adaptations corresponding to different periods of development.

References

Abderhalden, E. (1898). *Z. Physiol. Chem.* **25**, 65.

Arvy, L., and Gabe, M. (1946a). *Compt. Rend. Soc. Biol.* **140**, 73

Arvy, L., and Gabe, M. (1946b). *Compt. Rend. Soc. Biol.* **140**, 757.

Asahina, E., Aoki, K., and Shinozaki, J. (1954). *Bull. Entomol. Res.* **45**, 329–339.

Auclair, J. L. (1959). *J. Insect Physiol.* **3**, 127–131.

Auclair, J. L., and Dubreuil, R. (1953). *Can. J. Zool.* **31**, 30.

Auclair, J. L., and Patton, R. L. (1950). *Rev. Can. Biol.* **9**, 3.

Babers, F. H. (1938). *J. Agr. Res.* **57**, 697.

Barsa, M. C. (1954). *J. Gen. Physiol.* **38**, 79.

Beadle, L. C., and Shaw, J. (1950). *J. Exptl. Biol.* **27**, 96–109

Belzecka, K., Raczynski-Bojanowska, and Heller, J. (1959). *Acta Biochim. Polon.* **6**, 195–203.

Benassi, C. A., Colombo, G., and Allegri, G. (1961). *Biochem. J.* **80**, 332.

Benassi, C. A., Colombo, G., and Peretti, G. (1959). *Experientia*, **15**, 457–458.

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ochem. J. **80,** 332.
perientia, **15,** 457–458.

Benoit, P. L., and Van Sande, M. (1959). *Ann. Soc. Belg. Méd. Trop.* **39,** 135–143.
Beutler, R. (1936). *Z. Vergleich. Physiol.* **24,** 71.
Bheemeswar, B., and Sreenivasaya, M. (1952). *Current Sci. India,* **21,** 253.
Bialaszewicz, K., and Landau, C. (1938). *Acta Biol. Exptl.* **12,** 307.
Bishop, G. H., Briggs, A. P., and Ronzoni, E. (1925). *J. Biol. Chem.* **66,** 77.
Boné, G. J. (1944). *Ann. Soc. Roy. Zool. Belg.* **75,** 123.
Boné, G. J. (1944). *Ann. Soc. Belg. Méd. Trop.* **24,** 229.
Brecher, L. (1929). *Biochem. Z.* **211,** 40.
Brezner, J., and Enns, W. R. (1958). *J. Kansas Entomol. Soc.* **31,** 241–246.
Bricteux-Grégoire, S., Dewandre, A., Florkin, M., and Verly, W. G. (1959). *Arch. Intern. Physiol. Biochim.* **67,** 693–698.
Bricteux-Grégoire, S., Dewandre, A., and Florkin, M. (1960). *Biochem. Z.* **333,** 370–376.
Bricteux-Grégoire, S., Duchâteau, Gh., Florkin, M., and Jeuniaux, Ch. (1959a). *Arch. Intern. Physiol. Biochim.* **67,** 586–596.
Bricteux-Grégoire, S., Florkin, M., and Jeuniaux, Ch. (1959b). *Arch. Intern. Physiol. Biochim.* **67,** 182–184.
Briggs, J. D. (1958). *J. Exptl. Zool.* **138,** 155–188.
Bücher, Th. and Klingenberg, M. (1958). *Angew. Chem.* **70,** 552.
Buck, J. B. (1953). *In* "Insect Physiology" (K. D. Roeder, ed.) pp. 147–190. Wiley, New York
Burova, A. A. (1953). *Uchenye Zapiski Moskov. Gosundarst. Pedagog. Inst.* **77,** 33–66. Cited by Wyatt, *Ann. Rev. Entomol.* (1961).
Candy, D. J., and Kilby, B. A. (1961). *Biochem. J.* **78,** 531.
Candy, D. J., and Kilby, B. A. (1962). *J. Exptl. Biol.* **39,** 129–140.
Carey, F. G., and Wyatt, G. R. (1960). *Biochim. Biophys. Acta* **41,** 178–179.
Carrington, C. B., and Tenney, S. M. (1959). *J. Insect Physiol.* **3,** 402–413.
Chen, P. S., and Hadorn, E. (1954). *Rev. Suisse Zool.* **61,** 437–451.
Clark, E. W. (1958). *Ann. Entomol. Soc. Amer.* **51,** 142–154.
Clark, E. W., and Craig, R. (1953). *Physiol. Zool.* **26,** 101.
Clegg, J. S., and Evans, D. R. (1961). *J. Exptl. Biol.* **38,** 771–792.
Czarnowski, C. von. (1954). *Naturwissenschaften* **41,** 577.
Dénucé, J. M. (1958). *Z. Naturforsch.* **13b,** 215–218.
Drilhon, A. (1934). *Compt. Rend. Soc. Biol.* **115,** 1194.
Drilhon, A. (1951). *Compt. Rend.* **232,** 1876.
Drilhon, A., and Busnel, R. G. (1937). *Compt. Rend. Soc. Biol.* **124,** 806–807.
Drilhon, A., and Busnel, R. G. (1943). *Bull. Soc. Zool. France* **71,** 185.
Drilhon, A., and Busnel, R. G. (1951). *Compt. Rend. Soc. Biol.* **232,** 182.
Duchâteau-Bosson, Gh. Bricteux-Grégoire, S., Florkin, M., and Jeuniaux, Ch. (1960). *Arch. Intern. Physiol. Biochim.* **68,** 275–280.
Duchâteau, Gh., and Florkin, M. (1958). *Arch. Intern. Physiol. Biochim.* **66,** 573–591.
Duchâteau, Gh., and Florkin, M. (1959). *Arch. Intern. Physiol. Biochim.* **67,** 306–314.
Duchâteau-Bosson, Gh., Florkin, M., and Jeuniaux, Ch. (1960). *Arch. Intern. Physiol. Biochim.* **68,** 327–338.
Duchâteau, Gh., Florkin, M., and Jeuniaux, Ch. (1959). *Arch. Intern. Physiol. Biochim.* **67,** 173–181.
Duchâteau, Gh., Florkin, M., and Leclercq, J. (1953). *Arch Intern. Physiol. Biochim.* **61,** 518–549.
Duchâteau-Bosson, Gh., Jeuniaux, Ch., and Florkin, M. (1961a). *Arch. Intern. Physiol. Biochim.* **69,** 369–373.

Duchâteau-Bosson, Gh., Jeuniaux, Ch., and Florkin, M. (1961b). *Arch. Intern. Physiol. Biochim.* **69**, 485–492.
Duchâteau-Bosson, Gh., Jeuniaux, Ch., and Florkin, M. (1962). *Arch. Intern. Physiol. Biochim.* **70**, 287–291.
Duchâteau-Bosson, Gh., Jeuniaux, Ch., and Florkin, M. (1963). *Arch. Intern. Physiol. Biochim.* **71**, 566–576
Ellis, P. E., and Hoyle, G. (1954). *J. Exptl. Biol.* **31**, 271–279.
Evans, D. R., and Dethier, V. G. (1957). *J. Insect Physiol.* **1**, 3–17.
Faulkner, P. (1955). *Biochem. J.* **60**, 590.
Faulkner, P. (1956). *Biochem. J.* **64**, 430–436.
Faulkner, P. (1956). *Biochem. J.* **64**, 436–441.
Faulkner, P. (1956). *Nature* **178**, 921.
Faulkner, P. (1958). *Biochem. J.* **68**, 374–380.
Florkin, M. (1936a). *Compt. Rend. Soc. Biol.* **123**, 1024.
Florkin, M. (1936b). *Compt. Rend. Soc. Biol.* **123**, 1249.
Florkin, M. (1937). *Bull. Soc. Chim. Biol.* **19**, 990.
Florkin, M. (1943). *Bull. Soc. Roy. Sci. Liège,* p. 301.
Florkin, M. (1958). *Proc. Intern. Congr. Biochem. 4th Vienna, Symp. XII,* pp. 63–73.
Florkin, M., and Jeuniaux, Ch. (1963). *Life Sci.* **12**, 982–985.
Fraenkel, G., and Rudall, K. M. (1947). *Proc. Roy. Soc. London Ser. B* **134**, 111–143.
Friedman, S. (1961). *Arch. Biochem. Biophys.* **93**, 550–4.
Fukuda, T., and Florkin, M. (1959). *Arch. Intern. Physiol. Biochim.* **67**, 185–189.
Fukuka, T., and Hayashi, T. (1953). *Sanshi Kenkyu* **4**, 32–33.
Fukuda, T., and Hayashi, T. (1958). *J. Biochem. Japan* **45**, 469–474.
Garcia, I., Tixier, M., and Roche, J. (1956). *Compt. Rend. Soc. Biol.* **150**, 468.
Gese, P. K. (1950). *Physiol. Zool.* 23, 109.
Gilbert, L. I., and Schneiderman, H. A. (1961). *Amer. Zoologist* **1**, 11–51.
Goodwin, T. W., and Srisukh, S. (1951). *Biochem. J.* 48, 199.
Hackman, R. H. (1952). *Arch Biochem. Biophys.* **41**, 166.
Hackman, R. H. (1956). *Australian. J. Biol. Sci.* **9**, 400–405.
Hadorn, E., and Mitchell, H. K. (1951). *Proc. Natl. Acad. Sci. U.S.* **37**, 650–665.
Heller, J. (1932). *Biochem. Z.* **255**, 205.
Heller, J., and Moklowska, A. (1930). *Biochem. Z.* **219**, 473.
Hemmingsen, A. M. (1924). *Skand. Arch. Physiol.* **45**, 204
Howden, G. F., and Kilby, B. A. (1956). *Chem. Ind.* Dec., 1453–1454.
Howden, G. F., and Kilby, B. A. (1960). *J. Insect Physiol.* **4**, 258–269.
Hoyle, G. (1954). *J. Exptl. Biol.* **31**, 260.
Irreverre, F., and Levenbook, L. (1960). *Biochim. Biophys. Acta* **38**, 358–360.
Itabashi, H., Koide, F., and Shimura, K. (1953). *J. Agr. Chem. Soc. Japan* **27**; 53.
Ito, T. (1954). *Japan. J. Zool.* **11**, 253–260.
Ito, T., Mukaiyama, F., and Tanaka, M. (1962). *J. Sericult. Sci. Japan* **31**, 228–234.
Jeannel, R. (1949). *In* "Traité de Zoologie" (P. P. Grassé, ed.) vol. 9, pp. 1–17. Masson, Paris.
Jeuniaux, Ch. (1961). *Arch. Intern. Physiol. Biochim.* **69**, 750–751.
Jeuniaux, Ch. (1963). "Chitine et Chitinolyse," 181 pp. Masson, Paris.
Jeuniaux, Ch., Duchâteau-Bosson, Gh., and Florkin, M. (1961). *Arch. Intern. Physiol. Biochim.* **69**, 617–627.
Kalf, G. F., and Rieder, S. V. (1958). *J. Biol. Chem.* **230**, 691–698.
Karlson, P., and Schmid, H. (1955). *Z. Physiol. Chem.* **300**, 35.
Karlson, P., and Schweiger, A. (1961). *Z. Physiol. Chem.* **323**, 199–210.

Kilby, B. A., and Neville, E. (1957). *J. Exptl.*
Kondo, Y., and Watanabe, T. (1957). *Nippon*
Kuwana, Z. (1937). *Japan J. Zool.* **7**, 273–303.
Laufer, H. (1960a). *Proc. Intern. Congr. Ento*
Laufer, H. (1960b). *Ann. N.Y. Acad. Sci.* **89**, 4
Laufer, H. (1961). *Ann. N.Y. Acad. Sci.* **94**, 82
Levenbook, L. (1947). *Nature* **160**, 465.
Levenbook, L. (1950). *Biochem. J.* **47**, 336–34
Levenbook, L. (1961). *Arch. Biochem. Biophy*
Levenbook, L., and Hollis, W. W. Jr. (1961).
Levenbook, L., and Wang, Y. L. (1948). *Natu*
Lockwood, A. P. M., and Croghan, P. C. (195
Ludwig, D. (1951). *Physiol. Zool.* **24**, 329.
Marcuzzi, G. (1955). *R. C. Acad. N. Lincei* (
Marcuzzi, G. (1956). *R. C. Acad. N. Lincei* (
Martin, S., and Cotner, F. B. (1934). *Ann. E*
Matsumura, S. (1935). *Bull. Sericult. Silk Ind*
May, R. M. (1935). *Bull. Soc. Chim. Biol.* **17**
Mullen, J. A. (1957). *Nature* **180**, 813–814.
Nossal, P. M. (1952). *Biochem. J.* **50**, 349–3
Ohnishi, E. (1959). *J. Insect Physiol.* **3**, 219.
Patterson, D. S. P. (1956). *Arch Intern. Phys*
Pepper, J. H., Donaldson, F. T., and Hasting
Po-Chedley, D. S. (1956). *Trans. N.Y. Acad.*
Po-Chedley, D. S. (1958). *J. N.Y. Entomol.*
Pratt, J. J. (1950). *Ann. Entomol. Soc. Amer*
Prota, C. D. (1961). *J. N.Y. Entomol. Soc.* 6
Ramsay, J. A. (1953). *J. Exptl. Biol.* **30**, 358–
Ramsay, J. A. (1955a). *J. Exptl. Biol.* **32**, 18
Ramsay, J. A. (1955b). *J. Exptl. Biol.* **32**, 20
Ramsay, J. A. (1956). *J. Exptl. Biol.* **33**, 697–
Raper, R., and Shaw, J. (1948). *Nature* **162**,
Salt, R. W. (1961). *Ann. Rev. Entomol.* 6, 5
Schoffeniels, E. (1960). *Arch. Intern. Physio*
Shaw, J. (1955). *J. Exptl. Biol.* **32**, 353.
Shotwell, O., Bennett, G. A., Hall, H. H., *J. Insect Physiol.* **9**, 35–42.
Srinivasan, N. G., Corrigan, J. J., and Meist
Steele, J. E. (1963). *Gen. Comp. Endocrinol*
Steinhaus, E. A. (1949). "Principles of I New York.
Stephen, W. P. (1958). *Proc. Intern. Con*
Stephen, W. P., and Steinhauer, A. L. (1957
Stephens, J. M. (1959). *Can. J. Microbiol.* 5
Sutcliffe, D. W. (1962). *J. Exptl. Biol.* **39**, 3
Sutcliffe, D. W. (1963). *Comp. Biochem. Ph*
Tobias, J. M. (1948a). *J. Cellular Comp. Ph*
Tobias, J. M. (1948b). *J. Cellular Comp. Ph*
Todd, M. E. (1957). *J. N.Y. Entomol. Soc.*
Todd, M. E. (1958). *J. N.Y. Entomol. Soc.*

Kilby, B. A., and Neville, E. (1957). *J. Exptl. Biol.* **34**, 276–289.
Kondo, Y., and Watanabe, T. (1957). *Nippon Sanshigaku Zasshi,* **26**, 298–305.
Kuwana, Z. (1937). *Japan J. Zool.* **7**, 273–303.
Laufer, H. (1960a). *Proc. Intern. Congr. Entomol. 11th Vienna III,* 194–200.
Laufer, H. (1960b). *Ann. N.Y. Acad. Sci.* **89**, 490–515.
Laufer, H. (1961). *Ann. N.Y. Acad. Sci.* **94**, 825–835.
Levenbook, L. (1947). *Nature* **160**, 465.
Levenbook, L. (1950). *Biochem. J.* **47**, 336–346.
Levenbook, L. (1961). *Arch. Biochem. Biophys.* **92**, 114–121.
Levenbook, L., and Hollis, W. W. Jr. (1961). *J. Insect Physiol.* **6**, 52–61.
Levenbook, L., and Wang, Y. L. (1948). *Nature* **162**, 731.
Lockwood, A. P. M., and Croghan, P. C. (1959). *Nature* **184**, 370–371.
Ludwig, D. (1951). *Physiol. Zool.* **24**, 329.
Marcuzzi, G. (1955). *R. C. Acad. N. Lincei (Cl. Sci. Fis. Mat. Nat.).* **18**, 654
Marcuzzi, G. (1956). *R. C. Acad. N. Lincei (Cl. Sci Fis. Mat. Nat.)* **20**, 492–500.
Martin, S., and Cotner, F. B. (1934). *Ann. Entomol. Soc. Amer.* **37**, 372–383.
Matsumura, S. (1935). *Bull. Sericult. Silk Ind.* **7**, 5–8.
May, R. M. (1935). *Bull. Soc. Chim. Biol.* **17**, 1045.
Mullen, J. A. (1957). *Nature* **180**, 813–814.
Nossal, P. M. (1952). *Biochem. J.* **50**, 349–355.
Ohnishi, E. (1959). *J. Insect Physiol.* **3**, 219.
Patterson, D. S. P. (1956). *Arch Intern. Physiol. Biochim.* **64**, 681–683.
Pepper, J. H., Donaldson, F. T., and Hastings, E. (1941). *Physiol. Zool.* **14**, 470.
Po-Chedley, D. S. (1956). *Trans. N.Y. Acad. Sci.* **19**, 19–22.
Po-Chedley, D. S. (1958). *J. N.Y. Entomol. Soc.* **66**, 171–177.
Pratt, J. J. (1950). *Ann. Entomol. Soc. Amer.* **43**; 573.
Prota, C. D. (1961). *J. N.Y. Entomol. Soc.* **69**, 59–67.
Ramsay, J. A. (1953). *J. Exptl. Biol.* **30**, 358–369.
Ramsay, J. A. (1955a). *J. Exptl. Biol.* **32**, 183.
Ramsay, J. A. (1955b). *J. Exptl. Biol.* **32**, 200.
Ramsay, J. A. (1956). *J. Exptl. Biol.* **33**, 697–708.
Raper, R., and Shaw, J. (1948). *Nature* **162**, 999.
Salt, R. W. (1961). *Ann. Rev. Entomol.* **6**, 55–74.
Schoffeniels, E. (1960). *Arch. Intern. Physiol. Biochim.* **68**, 507–508.
Shaw, J. (1955). *J. Exptl. Biol.* **32**, 353.
Shotwell, O., Bennett, G. A., Hall, H. H., Van Etten, C. H., and Jackson, R. W. *J. Insect Physiol.* **9**, 35–42.
Srinivasan, N. G., Corrigan, J. J., and Meister, A. *J. Biol. Chem.* **237**, 3814.
Steele, J. E. (1963). *Gen. Comp. Endocrinol.* **3**, 46–52.
Steinhaus, E. A. (1949). "Principles of Insect Pathology," 757 pp. McGraw-Hill, New York.
Stephen, W. P. (1958). *Proc. Intern. Congress Entomol 10th Montreal.* **1**, 395–400.
Stephen, W. P., and Steinhauer, A. L. (1957). *Physiol. Zool.* **30**, 114–120.
Stephens, J. M. (1959). *Can. J. Microbiol.* **5**, 203–228.
Sutcliffe, D. W. (1962). *J. Exptl. Biol.* **39**, 325–344.
Sutcliffe, D. W. (1963). *Comp. Biochem. Physiol.* **9**, 121–135.
Tobias, J. M. (1948a). *J. Cellular Comp. Physiol.* **31**, 125–142.
Tobias, J. M. (1948b). *J. Cellular Comp. Physiol.* **31**, 143–148.
Todd, M. E. (1957). *J. N.Y. Entomol. Soc.* **65**, 85–88.
Todd, M. E. (1958). *J. N.Y. Entomol. Soc.* **66**, 135–143.

Tsuji, C. (1909). *Sanji Hokoku* **35**, 1–24.
Van Asperen, K., and Van Esch, I. (1954). *Nature* **174**, 927.
Van Asperen, K., and Van Esch, I. (1956). *Arch. Neerl. Zool.* **11**, 342–360.
Van Sande, M., and Karcher, D. (1960). *Science* **131**, 1103–1104.
Waterhouse, D. F., and McKellar, J. W. (1961). *J. Insect Physiol.* **6**, 185–195.
Wood, D. W. (1957). *J. Physiol. London* **138**, 119–139.
Wyatt, G. R. (1958). *Proc. Intern. Congr. Biochem. 4th Vienna,* Symp. XII, 161–178.
Wyatt, G. R. (1961). *Ann. Rev. Entomol.* **6**, 75–102.
Wyatt, G. R., and Kalf, G. F. (1956). *Federation Proc.* **15**, 388.
Wyatt, G. R., and Kalf, G. F. (1958). *Proc. Intern. Congr. Entomol. 10th Montreal.* **2**, 333.
Wyatt, G. R., and Kalf, G. F. (1957). *J. Gen. Physiol.* **40**, 833–847.
Wyatt, G. R., Loughheed, T. C., and Wyatt, S. S. (1956). *J. Gen. Physiol.* **39**, 853–868.
Wyatt, G. R., Meyer, W. L., and Kropf, R. (1958). *Federation Proc.* **17**, 1345.
Wyatt, G. R., and Meyer, W. L. (1959). *J. Gen. Physiol.* **42**, 1005–1011.
Yamafuji, K. (1933). *Bull. Agr. Chem. Soc. Japan* **9**, 19–23.
Yamafuji, K. (1934a). *Bull. Agr. Chem. Soc. Japan* **10**, 19–23.
Yamafuji, K. (1934b). *Bull. Agr. Chem. Soc. Japan* **10**, 119–127.
Zebe, E. C., and McShan, W. H. (1959). *J. Cell. Comp. Physiol.* **53**, 21–29.

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