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CHAPTER 2

Hemolymph—Arthropoda

Charles Jeuniaux

I. Introduction

The open circulatory system of Arthropoda is presumably derived from the highly organized closed system of their annelidan or pre-annelidan ancestors and can thus be considered as being a case of regressive evolution. In Arthropoda, the blood fills up the main body cavity, a cavity which does not correspond to a primitive coelomic space, but to sinuses or cavities burrowed within the body tissues. Tissues are thus in direct contact with the blood. This system of cavities should be considered as a hemocoel, and the blood, combining to some extent the characters of a circulatory fluid and those of an interstitial lymph, should be called hemolymph.

From a physiological point of view, the hemolymph of Arthropoda differs from the internal fluids of Annelida (true blood and coelomic fluid) by the minor role played by hydrostatic pressure in the control of body shape and movements, due to the presence of a rigid integument. The hemolymph acts, however, as a hydrostatic skeleton in most insect larvae, in some crustaceans with thin cuticles, such as *Artemia*, and above all in every species during the few hours following ecdysis, a very crucial step in the development, during which the integument remains soft and unhardened.

The other functions generally fulfilled by the blood in other animal phyla are also carried out by the hemolymph—transport of food material for cell life and growth, of excretory products, of hormones, of coagulating material. In primitive marine Arthropoda and in modern Crustacea and Arachnida, the circulatory system is also associated with the respiratory system and is the carrier of the respiratory pigments. The hemolymph of marine Arthropoda does not greatly differ from the bloods of other invertebrate or vertebrate phyla.

But the striking originality of the evolution of Arthropoda, i.e., the successful adaptation of insects to terrestrial life, is accompanied by a number of drastic modifications of the primitive characteristics of the hemolymph. The most conspicuous morphological adaptation is the abandonment of the branchial respiratory system and the adoption of a tracheal system, metamerically distributed (at least primitively), which insures the direct arrival of gaseous oxygen in the immediate vicinity of the cells. As a result, the hemolymph has lost the physiological role of oxygen carrier, in Myriapoda as well as in Insecta.

Another important evolutionary tendency of hemolymph chemistry is the replacement of the seawater-like composition by a mixture of organic and inorganic osmolar effectors, among which small organic molecules increase considerably in concentration in the most evolved orders of insects. Moreover, the relative proportions of the inorganic

cations are deeply modified parallel to this tendency, as will be emphasized later.

Most recent publications have thrown light on the fact that the chemical composition of the hemolymph may depend more or less directly on the nature of the environment and varies during development. In any case, one can obviously speak of chemical metamorphosis as well as depicting the well known morphological aspects of this phenomenon. Despite these ontogenetic and phenotypic variations, taxonomic inferences can often be based on hemolymph composition. Since the hemolymph is the carrier of every kind of chemical from one point of the body to another, a review of hemolymph chemistry should be concerned with a number of substances in transit, the specific role of which is better considered elsewhere in this treatise. Some of the most important and peculiar aspects of hemolymph physiology and chemistry are treated in Chapters 9 and 10 of Volume V and Chapters 3, 4, 8, and 9 of Volume VI.

II. Blood Volume, Osmotic Pressure, and Osmolar Effectors

We shall mainly limit this chapter to the evolution of the general pattern of the osmolar effectors in the hemolymph.

A. VARIATIONS OF HEMOLYMPH VOLUME

With respect to the dry weight of the whole body, the volume of the hemolymph varies widely according to age and developmental events. These variations have been carefully followed in crabs by Drach (1939) and in the desert locust Schistocerca gregaria by Lee (1961). In every case, there is an increase in blood volume at the time of ecdysis, promoting an increased hydrostatic pressure that contributes to the expansion of the cuticle prior to hardening. On the other hand, when locusts are fed a very dry food, there is a loss of blood volume, but no loss of cellular water (Lee, 1961); this observation supports the view of Mellanby (1939) that the hemolymph is a reserve of water for the tissues.

B. VALUE OF THE OSMOTIC PRESSURE

In aquatic Arthropoda, this value depends upon the concentration of the external medium, and upon the specific osmoregulatory abilities of the animal (see Chapter 9 of Volume V). In Arachnida, the osmotic pressure of the hemolymph of the scorpion Androctonus australis has been estimated at −0.99°C (freezing point depression) or ±530 mosmole/liter (Bricteux-Grégoire et al., 1963). Sutcliffe (1963) has compiled the values obtained in insects by different authors. These

values generally range from —0.5° to —0.9°C (freezing point depression). Aquatic larvae often exhibit minimal values (*Ephemera danica*, —0.504°C; caddis fly larvae, —0.38 to —0.48°C); but higher values have been observed in the pupae of Lepidoptera (up to —1.130°C in *Ephestia kuehniella*), probably because of the increasing amount of soluble hydrolytic products resulting from histolysis.

C. EVOLUTIONARY TRENDS AT THE LEVEL OF HEMOLYMPH OSMOLAR EFFECTORS

In the marine ancestors of Arthropoda, as in all the other marine Invertebrata with internal fluid, the osmotic pressure of the blood was nearly equal to that of seawater and essentially composed of inorganic constituents, mainly sodium and chlorine. This situation is retained by recent marine Crustacea. In the crustaceans adapted to fresh water or to terrestrial life, the osmotic pressure of the hemolymph is lowered, but the relative importance of the inorganic ions is not impaired. This seems to be true in the case of Arachnida and Myriapoda (Fig. 1) (Sutcliffe, 1963), with the possible exception of Diplopoda (*Iulus*).

In the most primitive insect so far studied, the apterygote *Petrobius maritimus* (Fig. 1), the ions of sodium and chlorine still make up almost the entire total osmotic pressure of the hemolymph (Lockwood and Croghan, 1959). But the evolution of insects is characterized by a tendency toward the replacement of inorganic ions by small organic molecules as osmolar effectors (Sutcliffe, 1962, 1963) (Fig. 2). In the less evolved pterygotes (Paleoptera and most Polyneoptera) (Fig. 2A and B), the sum of the inorganic cations amounts to nearly half the osmotic pressure, while chlorine remains the most important anion; a small but not negligible part of the osmotic pressure is made up by organic molecules. This is, according to Sutcliffe (1963), the basic type (type I) of hemolymph in pterygote insects.

A very similar type is that of Phasmida (Fig. 2C), but magnesium takes the place of sodium in the cationic pattern, and the amount of inorganic phosphates is exceptionally high.

A third type is that of the less evolved orders of Oligoneoptera and of Diptera (Fig. 2E); it is characterized by (1) the sharp reduction of chloride concentration, (2) a marked increase in the concentration of the small organic molecules, (3) a status quo as far as inorganic cations are concerned. Owing to the monophyletic origin of endopterygotes, it has been postulated that this increasing utilization of organic substances as osmolar effectors appeared very early in the evolution of the Oligoneoptera, probably prior to the divergence of the panorpoid complex.
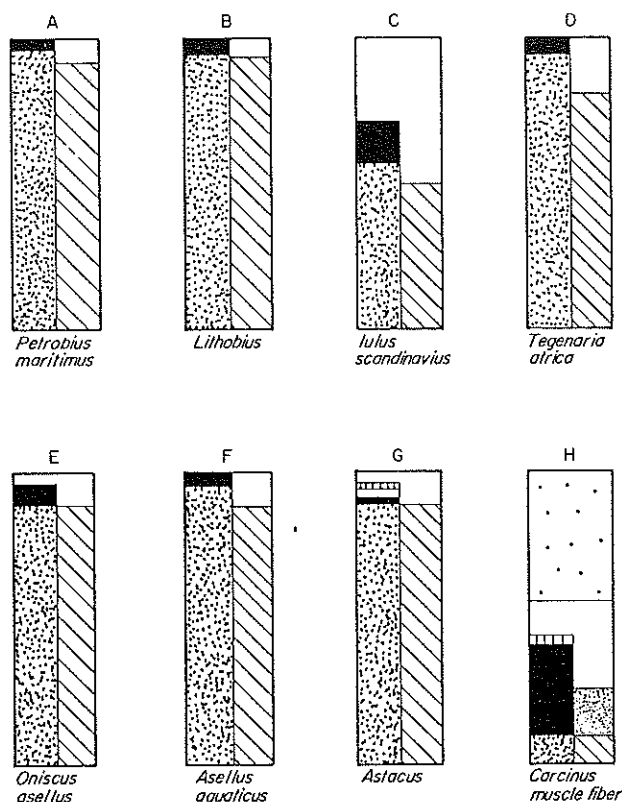
FIG. 1. Osmotic effect of the principal organic and inorganic components, illustrated as percentage of the total osmolar concentration, in the hemolymph of some Arthropoda. (A) an apterygote insect; (B) a chilopod; (C) a diplopod; (D) an arachnid; (E) an isopod (Crustacea); (F) an amphipod (Crustacea); and (G–H) decapods (Crustacea). Conventions as in Fig. 2. (Sutcliffe, 1963.)

The most specialized type of hemolymph (type IV of Sutcliffe, 1963) (Fig. 2F,G,H) is exhibited by Lepidoptera, Hymenoptera, and many Coleoptera. The importance of the inorganic cations and anions as osmolar effectors is considerably reduced, the main role being played by small organic molecules, such as free amino acids. According to Sutcliffe (1963), this specialization is probably polyphyletic in origin, the orders of Coleoptera, Hymenoptera, and Lepidoptera being derived independently from distinct stocks of primitive Oligoneoptera.

D. VARIATIONS OF THE OSMOTIC PRESSURE

Disregarding regulation in the course of osmotic adaptation (see Chapter 9 of Volume VI), we shall briefly evoke some aspects of osmotic

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Exopterygotes

Ephemeroptera
Odonata
Plecoptera
Dictyoptera
Heteroptera

Orthoptera
Dermaptera
Isoptera

Phasmida

Homoptera

Endopterygotes

Megaloptera
Neuroptera
Mecoptera
Trichoptera
Diptera

Lepidoptera
Hymenoptera

Coleoptera
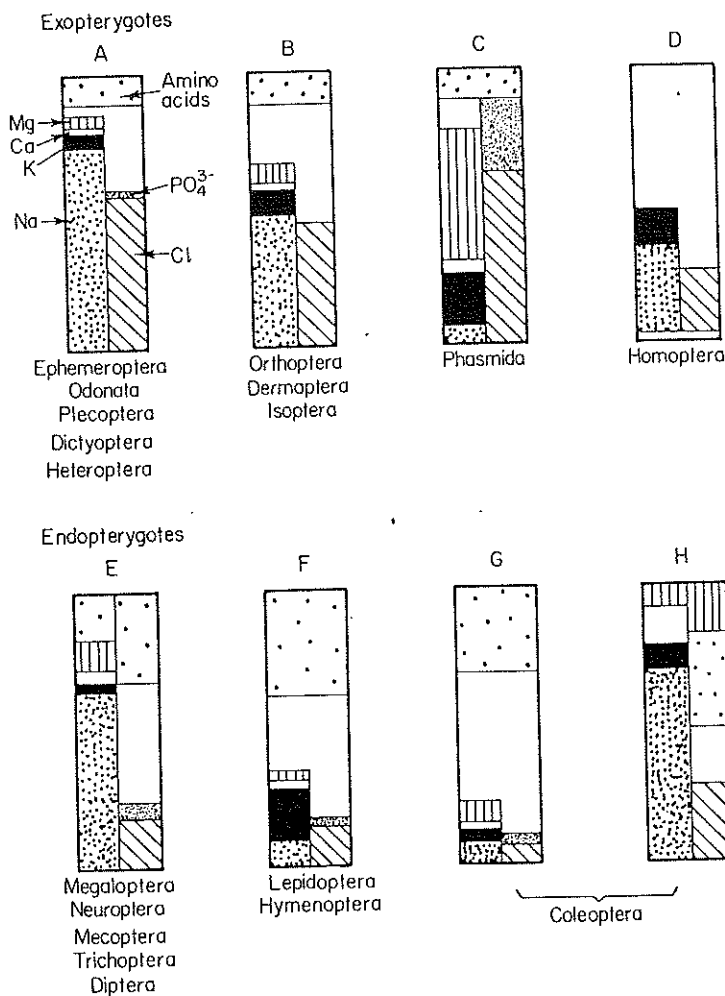
Fig. 2. Osmotic effect of the principal components in the hemolymph of pterygote insects. Each block is visualized as two vertical sections, each representing 50% of the total osmolar concentration; the cations are illustrated in the left-hand section, the anions in the right-hand section. The large blank area represent the osmolar concentration of unidentified components (Sutcliffe, 1963).

pressure variation due to factors other than salinity of the external medium.

1. Influence of Molting

Generally speaking, the molting process itself does not seem to affect deeply the osmotic pressure of the hemolymph. In many terrestrial ar-

thropods, the total body volume increases by the "swallowing" or breathing of air at the time of ecdysis. In other species (dragonfly nymphs, Shafer, 1923; locusts, Lee 1961), there is a rise in the blood volume, but the effects on the osmotic pressure have not been investigated.

The absorption of water by Crustacea at the time of ecdysis has been explained by some workers as being the result of the existence of a hyperosmotic hemolymph shortly before ecdysis (Baumberger and Olmsted, 1928; Baumberger and Dill, 1928). This hypothesis has been rejected by most recent authors. In *Carcinus maenas* for instance, the hemolymph remains isosmotic during the intermolt period (C_4–D_1 of Drach's terminology) and becomes slightly but significantly hyperosmotic during the premolt period (stages D_3–D_4 of Drach). The osmotic pressure reaches a maximum of 107.5% with respect to seawater (Robertson, 1960). The weight of the absorbed water following ecdysis has been estimated at 66% of the premolt total fresh weight. [Other values are 42% of the premolt total fresh weight in *Uca pugilator* (Guyselman, 1953); 94% in *Cancer pagurus;* and 125% in *Maia squinado* (Drach, 1939).] It has been calculated (Robertson, 1960) that this water uptake is surely not due to an osmotic effect alone. A premolt *Carcinus* weighing 50 gm. can absorb only 1.5 gm. water osmotically, whereas about 36 gm. water are actually absorbed.

The absorbed fluid is not pure water but a solution having approximately the same ionic composition as seawater (Robertson, 1939; Drach, 1939). The sites of the absorption mechanisms are not located only at the gills (Travis, 1954) but are distributed on the whole surface of the epidermis (Dandrifosse, 1966). As a result of this considerable absorption of seawater-like fluid following ecdysis, the hemolymph osmotic pressure is reduced from 107.5% (with respect to seawater) to 102.9% in *Carcinus* (Robertson, 1960). Similar observations have been made in the case of *Maia squinado* and *Cancer pagurus* (Jeuniaux, 1961b).

2. Influence of Dehydration and Rehydration

This problem has been investigated by Djajakusumah and Miles (1966) on the locust *Chortoicetes terminifera*. In starved larvae dehydrated for 24 hours, there is a loss of over 25% of the hemolymph volume, but there is little change in osmotic pressure, due to a concomitant lowering of the amount of solutes. When the dehydrated insects are allowed to imbibe water, the volume of the hemolymph increases rapidly and reaches values higher than before dehydration. The osmotic pressure again remains constant, due to a rise in the concentration of osmotic effectors, among which amino acids play an important role. This is in

good agreement with the observations of Marcuzzi (1955, 1956) on *Tenebrio molitor*.

3. Influence of Silk Secretion

The relative stability of the osmotic pressure of the hemolymph of *Bombyx mori* larvae during spinning is partly due to the compensatory effect of histidine and methionine, which increase in concentration in the hemolymph, whereas the sericigenous amino acid concentration decreases sharply (Jeuniaux *et al.*, 1961; see also Section XI,C).

III. Inorganic Cations

A. ONYCHOPHORA

In *Peripatopsis moseleyi*, the index of sodium, in percentage of the sum of the concentrations of the cations, is of about 90% (88.2–96.3 meq/liter). The concentration of the other cations is very low (potassium, 3.46–5.14 meq/liter; calcium, 2.4–7.2 meq/liter; magnesium, less than 1.2 meq/liter) (Robson *et al.*, 1966).

B. CRUSTACEA

1. General Cationic Pattern

The hemolymph of marine Crustacea is approximately isotonic with respect to seawater, while freshwater species are always hypertonic. In both cases, the nature and the relative proportions of the inorganic cations are similar to those found in seawater, as shown in Table I. Minor differences can be pointed out:

1. The magnesium concentration is generally lower in the blood of Crustacea than in seawater, with the remarkable exception of the anomuran *Lithodes maia*.

2. The calcium concentration is slightly higher in the hemolymph of marine species than in seawater.

3. In freshwater species, the absolute amounts of calcium in the blood are of the same order as in seawater (see also Table II); consequently, the calcium index amounts to 11–13% of the total cation concentration, a value two to four times higher than in marine forms.

4. Apart from the existence of lower magnesium values in Brachyura than in Macrura and especially than in the anomuran *Lithodes maia* (Florkin, 1960), no taxonomic characteristics are to be found at the level of the cationic patterns.

However, the cationic pattern of the hemolymph of the Isopoda is characterized by the relatively high proportions of calcium (50–70

TABLE I

INORGANIC COMPOSITION OF THE HEMOLYMPH OF SOME MARINE CRUSTACEA COMPARED WITH LOCAL SEAWATER, AND OF SOME FRESHWATER SPECIES[a]

Origin and species	Sum of cations (meq/liter)	Indices (% of the sum of the cations)				Author
		Na	K	Ca	Mg	
From Heligoland (seawater)	592.2	79.0	1.8	3.4	15.6	Bethe and Berger, 1931
Cancer pagurus (Brachyura)	638.4	84.7	2.8	3.8	8.5	Bethe and Berger, 1931
Carcinus maenas (Brachyura)	662.4	87.1	1.9	3.9	8.0	Bethe and Berger, 1931
Hyas araneus (Brachyura)	620.2	82.0	2.0	3.8	12.0	Bethe and Berger, 1931
From Maine (seawater)	553.6	82.7	1.5	3.5	12.2	Cole, 1940
Cancer borealis (Brachyura)	536.8	85.6	1.9	4.3	8.2	Cole, 1940
Homarus americanus (Macrura)	516.6	88.1	1.8	6.6	3.5	Cole, 1940
From New York (seawater)	505.8	75.7	1.8	5.1	17.4	Cole, 1940
Callinectes sapidus (Brachyura)	531.8	85.5	2.5	7.4	3.6	Florkin, 1960
Homarus americanus (Macrura)	529.1	87.1	1.7	7.2	3.4	Florkin, 1960
From Millport, Scotland (seawater)	569.3	77.1	1.6	3.4	17.9	Robertson, 1939
Lithodes maia (Anomura)	619.6	76.9	2.0	4.0	17.1	Robertson, 1949
Cancer pagurus (Brachyura)	596.7	84.2	2.0	4.6	9.2	Robertson, 1939
Homarus gammarus (Macrura)	567.0	89.8	2.5	5.2	2.5	Robertson, 1939
Nephrops norvegicus (Macrura)	570.8	90.6	1.3	4.9	3.2	Hemmingsen, 1924
Palinurus elephas (Macrura)	615.4	88.4	1.7	4.4	5.5	Robertson, 1949
Freshwater forms						
Eriocheir sinensis (Brachyura) (acclimated to fresh water)	359.1	86.0	1.6	10.5	1.9	Smith, cited by Cole, 1940
Astacus astacus (Macrura)	188.4	82.7	1.5	13.0	2.7	Bogucki, 1934
Astacus astacus (Macrura)	215.3	85.1	1.5	11.4	2.0	Smith, cited by Cole, 1940
Procambarus clarkii (Macrura)	194.8	82.4	3.0	12.6	2.1	Schlatter, 1941

[a] After Florkin (1960).

meq/liter in halophilous species, 30–39 meq/liter in terrestrial spec
and of magnesium (up to 110 meq/liter in *Tylos latreillei*); the va
tions of the magnesium concentration are perhaps related to the de
of activity of the species so far considered (Lagarrigue, 1969).

2. *Influence of Molting*

Variations of the cationic composition, other than those occurring
ing osmoregulatory processes (see Chapter 9, of Volume V), are ma
due to molting. An increase in the calcium concentration in the he
lymph just before molting occurs in all the marine or freshwater spe
so far studied; this increase amounts to 13–39% of the intermolt va
in *Maia squinado* (Drilhon, 1935), 80% in *Panulirus argus* (Travis, 19
30% in *Carcinus maenas* (Robertson, 1960), and about 50% in *Astc*
spp. [0.3–0.4 mg. calcium per milliliter in winter to 0.6 mg. calc
per milliliter in spring (Damboviceanu, 1932; Havel and Kleinh
1951)].* The level of calcium falls during the few days following
ecdysis. These variations are obviously related to the metaboliza
of calcium reserve material for the mineralization of the cuticle.
importance of gastroliths in the calcium balance has been studied
Chaisemartin (1964, 1965) in *Astacus pallipes,* and the storage of
cium before molting, by Graf (1964, 1966, 1968) in the Amphip

The cations sodium and magnesium contribute to a lesser extent
Carcinus maenas, to the increase of the osmotic pressure of the he
lymph before ecdysis (Robertson, 1960). In *Homarus vulgaris,* Gl
(1968) noticed a rise of the magnesium concentration before ecd
but sodium remained constant, while the potassium level decrease
this time. Following ecdysis, water absorption, and cuticle mineral
tion, a net decrease in the magnesium and calcium concentration
observed in *Panulirus* (Travis, 1955), *Carcinus* (Robertson, 1960)
Homarus (Glynn, 1968).

C. CHELICERATA

The cationic inorganic composition of the hemolymph of scorpi
does not differ greatly from that of Crustacea having approximately
same osmotic pressure, such as crayfishes, except for the lower calc
concentration and the higher magnesium concentration (Table II).
stated by Bricteux-Grégoire *et al.* (1963), the osmotic pressure of
blood is due almost entirely to the ions of sodium and chlorine, v
small organic molecules contributing only to a very limited extent

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* It is said that in the crab *Sesarma haematocheir,* which is exceptional an
Brachyura in its aptitude to form gastroliths, the level of calcium in the b
rises to forty to fifty times that of intermolt stages (Numanoi, 1939).

TABLE II

INORGANIC COMPOSITION OF THE HEMOLYMPH OF SOME SCORPIONS (ARACHNIDA) COMPARED WITH THAT OF SOME FRESHWATER CRUSTACEA

Species	Osmotic pressure (freezing point depression) (°C)	Cation concentration (mM/liter)				Anions concentration (mM/liter)		Reference
		Na	K	Ca	Mg	Cl	SO₄	
Heterometrus fulvipes								
♂♂	−0.85	221.7 ± 52.5	1.40 ± 0.83	6.68 ± 1.86	10.08 ± 3.35	251.4 ± 44.3	1.21 ± 0.66	Padmanabhanaidu, 1966.
♀♀	−0.85	197.8 ± 34.7	1.85 ± 0.84	5.52 ± 2.24	10.83 ± 3.16	277.1 ± 63.1	1.04 ± 0.71	
Androctonus australis ♂♂ + ♀♀	−0.99	263	8.0	2.4	—	267	—	Bricteux-Grégoire et al., 1963.
Androctonus australis ♂	—	—	—	7.6 ± 1.17	—	—	—	Goyffon and Ridet, 1968
♀	—	—	—	7.3 ± 1.12	—	—	—	
Astacus astacus	−0.81	173–220	5.2	10.4	2.6	195	—	Scholles, 1933
Procambarus clarkii	−0.64	160 (serum)	5.8	12.2	2.0	117	—	Lieneman, 1938

the osmotic pressure. The cationic pattern is only slightly influenced by the sex (Padmanabhanaidu, 1966; Goyffon and Ridet, 1968).

D. INSECTA

Most of the numerous data published concerning the cationic composition of insect hemolymph are incomplete, with one or more of the four principal cations not listed. On the basis of the exhaustive tabulations presented by Florkin and Jeuniaux (1964) and by Jeuniaux and Naoumoff (1971), Tables IV–VI summarize the most interesting data and can lead to the conclusions discussed below.

1. Ontogenetic Variation of the Cationic Pattern

Analytical data for each developmental stage of a given taxon are scarce; this situation must be kept in mind when discussing the systematic or phylogenetic significance of hemolymph composition. In many groups, important differences between larval and adult stages of a single species have been observed; this is the case for bees and wasps (Florkin and Jeuniaux, 1963), for some Coleoptera, and for many Lepidoptera (Naoumoff and Jeuniaux, 1970a), as shown in Table III.

2. Phylogenetic Signification of Cationic Patterns

As stated by Duchâteau *et al.* (1953), the cationic patterns in insects are not a question of food, but a matter of taxonomy. More precisely, the cationic pattern must be related to the evolutionary level attained by the taxon. The most primitive pattern, very similar to that found in the other classes of Arthropoda and other animals, is characterized by a high sodium index and very low potassium, calcium, and magnesium indices. This pattern is found in Apterygota and in the Paleoptera (Odonata and Ephemeroptera) at every developmental stage (Table IV). In the more recent and evolved orders of endopterygotes, such as Lepidoptera, the cationic pattern is highly specialized, with a very low sodium index and very high potassium and/or magnesium indices (Table V).

The order Coleoptera offers another good demonstration of the significance of the hemolymph cationic patterns. In beetles belonging to the primitive suborder of Adephaga (Carabidae, Dytiscidae), the cationic pattern is always of the primitive type, with high sodium index, while the most evolved Phytophaga (Chrysomelidae, Curculionidae, Cerambycidae) exhibit the specialized type of hemolymph with a very high magnesium index (see Table VI).

The tendency of replacing sodium by magnesium is obviously polyphyletic, being observed in Cheleuthoptera (Phasmida) within the sec-

TABLE III

SELECTED EXAMPLES OF THE MODIFICATIONS IN THE CATIONIC PATTERN OF THE HEMOLYMPH, FROM LARVAL TO IMAGINAL STAGES OF SOME INSECT SPECIES[a]

Taxon	Species	Stage	Indices (% of the sum of cations in meq/liter)				References
			Na	K	Ca	Mg	
Exopterygotes; Orthoptera	Locusta migratoria migratorioides	"nymphs"	52.6	10.5	14.9	21.9	Duchâteau et al., 1953
		adults	56.8	7.6	12.8	22.8	
Endopterygotes; Hymenoptera	Apis mellifica	larvae	13.6	38.1	22.7	25.6	Duchâteau et al., 1953
			9.5	46.3	14.2	30.0	Bishop et al., 1925
		adults	50.6	29.1	19.1	1.0	Florkin and Jeuniaux, 1963
	Vespula germanica	larvae	20.9	45.2	15.0	18.9	Duchâteau et al., 1953.
		adults[b]	80.4-86.1	12.3-15.7	1.2-1.5	0.3-2.2	Florkin and Jeuniaux, 1963
Lepidoptera	Barathra brassicae	larvae[c]	4.9-6.4	16.6-19.0	12.1-14.1	62.6-64.6	Naoumoff and Jeuniaux, 1970
		adults	14.7	41.4	10.3	33.7	
	Bombyx mori	larvae[d]	0.2-2.6	15.8-41.3	4.9-18.8	51.2-75.6	Naoumoff and Jeuniaux, 1970
		adults	8.3	35.6	12.9	43.2	
Coleoptera Scarabaeidae	Oryctes boas	larvae[e]	2.8-14.4	4.2-7.0	8.3-13.8	64.7-60.3	Naoumoff and Jeuniaux, 1971
		adults	44.7	8.8	9.1	37.4	
Chrysomelidae	Timarcha tenebricosa	larvae	0.6-1.0	14.4-16.8	9.8-25.9	56.7-74.8	Jeuniaux and Naoumoff, 1971
		adults	0.4	17.3	8.6	73.7	
Curculionidae	Rhyncophorus palmarum	larvae[c]	3.2-5.8	21.7-23.0	6.7-11.3	55-68.4	Jeuniaux and Naoumoff, 1971
		adults	21.3	21.0	9.8	47.8	
Cerambycidae	Ergates faber	larvae[c]	12.6-20.1	19.5-20.5	7-9.4	53.4-54.5	Jeuniaux and Naoumoff, 1971
		adults	22.3	12.3	6.6	58.8	

a Indices are calculated from values given in Tables IV-VII.
b Values obtained with two different series of wasps.
c Extreme values obtained with three different series of larvae.
d Extreme values, obtained with eleven different series, from the beginning of the second larval instar to the end of the fifth larval instal.
e Extreme values, obtained with four different individual larvae.

tion of Polyneoptera, in Polyphaga within the order of Coleoptera, and in Lepidoptera but not in Diptera in the panorpoid complex. This trend can be considered a case of orthogenetic evolution.

3. Significance of the Cationic Pattern Modifications during Metamorphosis

Returning to the problem of the ontogenetic transformations of the hemolymph cationic composition, we can stress the fact that in every species of endopterygotes so far studied, the hemolymph cationic pattern of the larva is of a more specialized type than that of the adult. Such a chemical metamorphosis is in good agreement with the evolutionary significance of the cationic pattern, based on taxonomic considerations. The larva of endopterygotes clearly represents an adaptive round-about way, instead of an ontogenetic recapitulation of an ancestral phylogenetic level. From this point of view, three cases can be cited: (1) insects with the primitive type of cationic pattern in adults and larvae (all the Paleoptera; most Polyneoptera with the exception of Phasmida; all the Coleoptera Adephaga); (2) insects in which the adult stage has kept the primitive composition while the larva shows a more specialized cationic pattern (the Hymenoptera *Apis* and *Vespula*, the rhinoceros beetle *Oryctes boas*) (see Table III); (3) insects with a specialized hemolymph cationic pattern throughout the whole development, the larval hemolymph being nevertheless often of a still more specialized type with respect to sodium/magnesium proportions. The last condition is found in the most recent, typically phytophagous groups, such as Lepidoptera and Coleoptera Phytophagoidea. The pupal hemolymph composition regularly occupies an intermediate position between the larval and adult types.

4. Dietetic Relationships

An earlier explanation of the different types of cationic patterns in insects was dietetic. Indeed, in many cases, carnivorous insects have a high sodium index, while phytophagous insects have higher potassium and/or magnesium indices (Boné, 1944a,b; Tobias, 1948a,b). As shown by Duchâteau *et al.* (1953), however, each species actually concentrates or dilutes the cations supplied by the food eaten, the result being the regulation of a steady state, which characterizes this species.[*] Thus,

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[*] The sodium/potassium ratio in the hemolymph of *Periplaneta americana*, however, can be slightly modified by a prolonged diet of lettuces (Pichon and Boistel, 1963), but the activity of the insects is lowered in such conditions.

the concept of a direct relationship between the cationic pattern and the nature of the food must be abandoned. However, the evolution of the most recent groups of endopterygotes has been obviously simultaneous with that of angiosperms; it can be suggested that the adaptation of these insects to such a new and abundant source of food has been favored by the acquisition of a new type of cationic composition of the hemolymph, easier to control with such a type of food supply.

5. Ion Binding

It has been postulated that a more or less important fraction of the cations of the hemolymph, especially potassium and magnesium, are in a combined form (Bishop *et al.*, 1925; Clark and Craigh, 1953; Barsa, 1954). Such a situation would account for the normal functioning of excitable tissues in insects with a specialized type of hemolymph. Experimental results have shown that the binding of cations is generally not important. In *Antherea polyphemus* pupae, only 15–20% of the calcium and magnesium is bound to macromolecules (Carrington and Tenney, 1959). In the hemolymph of *Galleria mellonella*, 6.6–8.8% of the total sodium is bound to proteins, while the proportions of bound potassium, calcium, and magnesium are, respectively, 1.2–2%, 9–13%, and 15–25% (Plantevin, 1967). This is consistent with the observation of Wharton and Lola (1969) on the increase of sodium concentration in the blood of cockroaches injected with egg white lysozyme.

6. Description of the Cationic Pattern of the Different Insect Orders (see Tables IV–VI)

Apterygota—The hemolymph is surely of the primitive type, sodium being the only important cation; however, a complete analysis of the cationic pattern is lacking.

Paleoptera—In Odonata (and presumably in Ephemeroptera, too), the cationic pattern is of the primitive type (sodium index = 80–90%) in larvae as well as in adults.

Polyneoptera—With the exception of the phasmids, the orders so far studied (Dictyoptera, Isoptera, Orthoptera) have a hemolymph of the primitive type (sodium index = 52–90%). However, in Orthoptera, there is a slight tendency to increase the concentration of potassium, calcium, and magnesium. The larval hemolymph of locusts does not differ from that of the adult. The characteristics of the blood of Plecoptera and Dermaptera need to be elucidated.

TABLE IV

INORGANIC CATIONS IN THE HEMOLYMPH OF APTERYGOTE AND EXOPTERYGOTE INSECTS[a]

Species	Cation concentration (meq/liter)				Sum of cat-ions	Indices (% of the sum)				References
	Na	K	Ca	Mg		Na	K	Ca	Mg	
Apterygotes										
Petrobius maritimus	208	5.8	—	—	—	—	—	—	—	Lockwood and Croghan 1959
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
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.	170.3	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
Agrion (Calopteryx) sp.	158.0	9.0	—	—	—	—	—	—	—	Boné, 1944a
Agrion virgo	140–145	8–9	—	—	—	—	—	—	—	Sutcliffe, 1962
Enallagma cyathigerum	139	14	—	—	—	—	—	—	—	Sutcliffe, 1962
adults:										
Aeschna cyanea	120	21	—	—	—	—	—	—	—	Sutcliffe, 1962
Agrion virgo	145	27.5	—	—	—	—	—	—	—	Sutcliffe, 1962
Polyneoptera										
Dictyoptera										
larvae:										
Periplaneta americana	100	15.4	3.3	5.4	174.2	90.1	4.3	2.4	3.1	Tobias, 1948a
adults:										
Periplaneta americana	157	7.6	4.2	14.8	—	—	—	—	—	Van Asperen and Van Esch, 1956
P. australasiae	—	—	19.4	15.7	—	—	—	—	—	Van Asperen and Van Esch, 1954
Blabera fusca	—	—	20.2	—	—	—	—	—	—	Van Asperen and Van Esch, 1954
Leucophaea maderae	100	9.7	8.2	3.6	121.5	82.3	8.0	6.6	2.9	Todd, 1958
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, 1958
Plecoptera										
adults:										
Perla bipunctata	127	12	—	—	—	—	—	—	—	Sutcliffe, 1962
Dinocras cephalotes	117	10	—	—	—	—	—	—	—	Sutcliffe, 1962
Cheleutoptera										
adults:										
Carausius morosus	11	18	7	108	144	7.6	12.5	4.8	75	Ramsay, 1955b
Carausius morosus	8.7	27.5	16.2	145.0	197.4	4.4	13.9	8.2	73.5	Duchâteau et al., 1953
Carausius morosus	15	18	15	106	154	9.7	11.6	9.7	68.8	Wood, 1957

TABLE IV (Continued)

	Species	Cation concentration (meq/liter)				Sum of cations	Indices (% of the sum)				References
		Na	K	Ca	Mg		Na	K	Ca	Mg	
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
adults:	Schistocerca gregaria	81.3	5.3	17.8	34.6	139.0	58.6	3.8	12.8	24.9	Duchâteau et al., 1953
	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
	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
	Ramalea microptera	56.5	17.9	—	—	—	—	—	—	—	Tobias, 1948b
	Stenobothrus stigmaticus	61.0	62.0	—	—	—	—	—	—	—	Boné, 1944a
	Stenopelmatus longispina	—	—	12.1	29.2	—	—	—	—	—	Clark and Craig, 1953
	Tettigonia viridissima	83.0	51.0	—	—	—	—	—	—	—	Boné, 1944a
Dermaptera adults:	Forficula auricularia	96	13	32.9	—	—	—	—	—	—	Sutcliffe, 1952
Paraneoptera **Homoptera** adults:	Cinara cilicia	—	—	21.4	30.4	—	—	—	—	—	Clark and Craig, 1953
	Jassidae gn. sp.	59	21	—	—	—	—	—	—	—	Sutcliffe, 1963
Heteroptera adults:	Gerris najas	149.0	8.0	—	—	—	—	—	—	—	Boné, 1944a
	Notonecta kirbyii	—	—	31.0	18.5	—	—	—	—	—	Clark and Craig, 1953
	Notonecta obliqua	155	21	—	—	—	—	—	—	—	Sutcliffe, 1962
	Coriza punctata	112	21	—	—	—	—	—	—	—	Sutcliffe, 1962
	Hesperocorixa lorigata	—	—	7.8	3.5	—	—	—	—	—	Clark and Craig, 1953
	Rhodnius prolixus	158.0	4.0–6.0	—	—	—	—	—	—	—	Ramsay, 1953
	Triatoma infestans	—	—	40.9	1.5	—	—	—	—	—	Clark and Craig, 1953
	Triatoma megista	133.0	5.0	—	—	—	—	—	—	—	Boné, 1944a
	Triatoma spp.	—	—	13.3–29.5	1.0–1.3	—	—	—	—	—	Clark and Craig, 1953
	Cimex lectularius	139.0	9.0	—	—	—	—	—	—	—	Boné, 1944a
	Oncopeltus fasciatus	—	—	13.9	52.1	—	—	—	—	—	Clark and Craig, 1953
	Palomena prasina	22.0	42.0	—	—	—	—	—	—	—	Boné, 1944a
unknown stage:	Oncopeltus fasciatus	39.5	20.5	11	—	—	—	—	—	—	Mullen, 1957

a Incomplete data dealing with only some of the four cations have been omitted when complete data are available.

Charles Jeuniaux

TABLE V

INORGANIC CATIONS IN THE HEMOLYMPH OF ENDOPTERYGOTE INSECTS (OLIGONEOPTERA) EXCEPT COLEOPTERA

Species	Cation concentration (meq/liter)				Sum of cat-ions	Indices (% of the sum)				References
	Na	K	Ca	Mg		Na	K	Ca	Mg	
Megaloptera larvae:										
Sialis lutaria	109	5	15	38	167	65.2	3	9	22.7	Shaw, 1955
Planipennia (= Neuroptera) larvae:										
Myrmeleon formicarius	148.5	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, 1944a
Limnophilus marmoratus	63	3.8	11.6	80.6	159.1	39.7	2.4	7.3	50.7	Beaujot et al., 1970
Limnophilus politus	69.5	9.9	11.5	167.0	256.8	26.9	3.6	4.5	65.0	Beaujot et al., 1970
Philopotamus montanus	109	21	—	—	—	—	—	—	—	Sutcliffe, 1962
Philopotamus ludificatus	98.9	12.6	20.5	190.0	322.0	30.7	3.9	6.3	59.0	Beaujot et al., 1970
Phryganea sp.	92.0	6.8	14.4	51.0	164.2	56.0	4.1	8.8	31.1	Duchâteau et al., 1953
Hydropsyche pellucidula	99.3	9.8	16.0	10.5	135.4	73.3	7.1	11.8	7.7	Beaujot et al., 1970
Oligotricha ruferus	72.7	11.1	15.0	41.3	140.1	51.8	7.8	10.7	29.5	Beaujot et al., 1970
Diptera larvae:										
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
Tabanidae gn. sp.	151.0	5.0	—	—	—	—	—	—	—	Ramsay, 1953
Pepomya sp.	26.0	58.0	—	—	—	—	—	—	—	Boné, 1944a
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, 1944a
pupae: *Calliphora erythrocephala*	139.6	26.1	20.8	34.3	220.8	63.2	11.8	9.3	15.6	Duchâteau et al., 1953
adults: *Stomoxys calcitrans*	128.0	11.0	—	—	—	—	—	—	—	Duchâteau et al., 1953
Eristalis tenax	193.2	20.9	—	—	—	—	—	—	—	Florkin and Jeuniaux, 1963

TABLE V (Continued)

Species	Cation concentration (meq/liter)				Sum of cations	Indices (% of the sum)				References
	Na	K	Ca	Mg		Na	K	Ca	Mg	
Lepidoptera (see also Table III)										
larvae:										
Cossus cossus	18.4	35.4	51.5	48.0	153.3	12.0	23.1	33.6	31.3	Duchâteau et al., 1953
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
Euproctis chrysorrhoea	17.9	44.5	20.6	87.9	170.9	10.5	26.0	12.1	51.4	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
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 lutea	3.3	36.2	31.4	58.5	129.4	2.6	43.4	24.3	29.7	Duchâteau et al., 1953
Antheraea mylitta	1.3	49.7	21.9	37.7	110.6	1.2	44.9	19.8	34.1	Bialaszewiz and Landau, 1938
Actias selene	4.8	51.3	25.5	60.0	141.6	3.4	36.2	18.0	40.4	Bialaszewiz and Landau, 1938
Papilio machaon	13.6	45.3	33.4	59.8	152.1	8.9	29.8	22.0	39.3	Duchâteau et al., 1953
Dasychira pudibunda	3.0	51.8	33.9	74.1	162.8	1.9	31.8	20.8	45.5	Duchâteau et al., 1953
pupae:										
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
Sphinx ligustri	2.6	52.8	16.4	49.2	121.0	2.1	43.6	12.6	40.7	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
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
Euproctis chrysorrhoea	18.6	31.6	9.6	47.0	106.9	17.4	29.6	9.0	43.9	Naoumoff and Jeuniaux, 1970

a Data concerning only some of the four principal cations have generally been omitted. Representative data concerning Lepidoptera have been selected from the exhaustive tabulation given by Florkin and Jeuniaux (1964).

Cheleutoptera—The only examined species, *Carausius morosus*, is characterized by the exceptionally high proportion of magnesium (index = 68–75%).

Homoptera and Heteroptera—No complete analysis of the cationic pattern in a single species is available. The scattered data suggest a primitive type of cationic composition in most species.

Oligoneoptera—Among Oligoneoptera, the Megaloptera, Planipennia, Mecoptera, and Diptera possess primitive cationic patterns (sodium indices = 53–80%), probably in larvae as well as in adults.

Trichoptera—Only the larval hemolymph composition is known, and it appears to be highly variable (especially with respect to magnesium). It seems that the evolutionary tendency of "replacing" sodium by magnesium in the most evolved endopterygotes is more or less marked in Trichoptera.

Lepidoptera—The evolutionary tendency initiated in Trichoptera is completely realized in most species. The cationic pattern of every stage is of the evolved type, with a sodium index lower than 20%. The proportions of potassium, calcium, and magnesium are variable, but magnesium or potassium and sometimes both are the most important cations.

Coleoptera—The cationic pattern of the hemolymph seems to be highly characteristic for a given taxon, but more detailed and numerous data are needed before a generalization can be made, except in the case of the following taxa.

In Adephaga, the cationic pattern is of the primitive type, with sodium index between 60–70% and magnesium index lower than 20%. Larval hemolymph does not appear to differ from that of adults.

The hemolymph of Polyphaga is always of a more evolved type, with sodium index lower than 60% and magnesium index higher than 20%. From this point of view, the Hydrophilidae and the Cantharidae appear to be the less specialized families. The Tenebrionidae occupy an intermediary position, sodium and magnesium indices having approximately the same values (30–45%) in larvae as in adults.

In the Phytophagoidea (Curculionidae, Chrysomelidae, Cerambycidae), both adult and larva exhibit a typical pattern of the most evolved type (very low sodium and high magnesium), but this characteristic is still more accentuated in larvae.

Hymenoptera—According to the data given by Bishop *et al.* (1925), Clark and Craig (1953), Duchâteau *et al.* (1953), and Florkin and Jeuniaux (1963, 1964), the cationic pattern of adult Aculeata is of the primitive type, while the larvae of the same suborder

TABLE VI

INORGANIC CATIONS IN THE HEMOLYMPH OF COLEOPTERA[a]

		meq/Liter					Indices: (% of the sum)				References
	Species	Na	K	Ca	Mg	Sum of cations	Na	K	Ca	Mg	
Adephaga											
Carabidae											
adults:	*Peronia madida*	133.6	12.6	2.1	21.1	171.6	77.9	7.4	1.2	17.3	Jeuniaux and Naoumoff, 1971
	Nebria brevicollis	144.2	11.8	12.0	18.1	186.1	77.5	6.3	6.4	9.7	Jeuniaux and Naoumoff, 1971
Dytiscidae											
larvae:	*Dytiscus sp.*	115	20	—	—	—	—	—	—	—	Sutcliffe, 1962
	Colymbetes fuscus	127	19	—	—	—	—	—	—	—	Sutcliffe, 1962
adults:	*Dytiscus marginalis*	165.2	6.4	22.5	37.5	231.6	71.3	2.8	9.7	16.2	Sutcliffe, 1962
	Dytiscus marginalis ♂♂	89–105	6–8	11–13	19–25	141–145	63–72	4–5	8–9	13–25	Jeuniaux and Naoumoff, 1971
	Dytiscus marginalis ♀♀	99–117	5–8	13–14	19–20	152–156	70–75	3.5–5	9	13–15	Jeuniaux and Naoumoff, 1971
	Cybister sp.	143.5	7.3	98.2	51.8	240.8	59.6	3	15.9	21.5	Duchâteau et al., 1953
Polyphaga											
Silphidae											
adults:	*Silpha tristis*	132.2	13.5	10.7	89.5	245.8	53.7	5.5	4.3	36.4	Jeuniaux and Naoumoff, 1971
Coccinellidae											
adults:	*Coccinella 7-punctata*	6.4	26.3	8.3	57.4	98.4	6.5	27.7	9	58.5	Jeuniaux and Naoumoff, 1971
Hydrophilidae											
adults:	*Sphaeridium scarabaeoides*	90	12.6	44	52.5	199.1	45.2	6.7	22.1	26.4	Jeuniaux and Naoumoff, 1971
	Hydrophilus piceus	123.7	4.3	24.5	36.8	199.3	65.1	2.1	12.3	23.5	Duchâteau et al., 1953
Malachiidae											
adults:	*Malachius viridis*	45.1	39.1	14.0	82.5	168.2	26.8	22	8.7	49.1	Jeuniaux and Naoumoff, 1971
Cantharidae											
adults:	*Cantharis cryptica*	79.4	32.5	14.6	50.4	176.9	44.9	18.3	8.3	28.5	Jeuniaux and Naoumoff, 1971
	Cantharis fusca	78.1	6.8	10.5	44.2	139.6	56	4.8	7.5	31.7	Jeuniaux and Naoumoff, 1971
Elateridae											
adults:	*Selatosomus latus*	94.9	19.8	25.2	131.4	271.3	35	7.3	9.3	48.4	Jeuniaux and Naoumoff, 1971
Tenebrionidae											
larvae:	*Tenebrio molitor*	71–75	38.7	11–13	76–83	200–208	36	19	0	38–40	Jeuniaux and Naoumoff, 1971
pupae:	*Tenebrio molitor*	64.3	37.4	13.1	88.1	202.9	31.7	18.5	6.5	43.4	Jeuniaux and Naoumoff, 1971
adults:	*Tenebrio molitor*	87.2	30.1	9.9	58.3	185.4	40.7	16.1	5.3	31.4	Jeuniaux and Naoumoff, 1971
	Blaps mucronata	84–85	21–32	11–16	60–85	188–208	44–45	11–17	6–8	32–41	Jeuniaux and Naoumoff, 1971

TABLE VI *(Continued)*

Species	meq/Liter				Sum of cations	Indices: (% of the sum)				References
	Na	K	Ca	Mg		Na	K	Ca	Mg	
Lucanidae										
larvae: *Platycerus caraboides*	13.8	12.6	69	169	264.4	5.2	4.8	26.4	63.9	Jeuniaux and Naoumoff, 1971
adults: *Platycerus caraboides*	59	4.5	14.6	123.6	201.7	29.3	2.2	7.3	51.3	Jeuniaux and Naoumoff, 1971
Geotrupidae										
adults: *Geotrupes stercorosus*	119.1	16	17.8	49.8	202.7	58.7	7.9	8.8	24.6	Duchâteau et al., 1953
Scarabaeidae										
larvae: *Popillia japonica*	20.2	9.5	15.8	38.8	84.3	24	11.3	18.7	48	Ludwig, 1951
Cetonia aurata	51.3	18.6	22.8	80	172.7	29.7	10.8	13.2	46.3	Duchâteau et al., 1953
Oryctes boas	5.5–24	8–11.7	10–23	108–212	160–270	2.8–14.4	4.2–7	8.3–13.8	65–80	Jeuniaux and Naoumoff, 1971
adults: *Oryctes boas*	69	13.6	14	57.5	154.1	44.7	8.8	9.1	37.3	Jeuniaux and Naoumoff, 1971
Melolontha melolontha	113	5.8	15.3	41.3	175.4	64.4	3.3	8.7	23.6	Duchâteau et al., 1953
Phytophagoidea (see Table III)										
Chrysomelidae										
larvae: *Leptinotarsa 10-lineata*	2–3.5	55–65	43–47	147–188	247–304	0.8–1.1	21–22	15–17	59–62	Duchâteau et al., 1953
Timarcha tenebricosa	1.6	46.9	72.2	158	278.7	0.6	16.8	25.9	56.7	Duchâteau et al., 1953
adults: *Timarcha tenebricosa*	1.4	54.8	27.3	233.6	317.1	0.4	17.5	8.6	73.7	Jeuniaux and Naoumoff, 1971
Gastroidea viridula	18.4	40.8	6	118.9	186	9.9	21.9	4.3	63.9	Jeuniaux and Naoumoff, 1971
Curculionidae										
adults: *Phyllobius urticae*	33.1	15	11.2	138	187.2	17.7	8	6	68.4	Jeuniaux and Naoumoff, 1971
Rhyncophorus palmarum (see Table III)										
Cerambycidae										
larvae: *Ergates faber* (see Table III)										
Leptura sp.	13.4	33.8	28	110.2	185.4	7.2	18.3	15.1	59.4	Jeuniaux and Naoumoff, 1971

a Selected representative examples; for a more exhaustive tabulation, see Florkin and Jeuniaux (1964) and Jeuniaux and Naoumoff (1971).

present a highly specialized pattern, with a particularly high potassium index. Other suborders need more detailed study.

IV. Inorganic Anions

A. CRUSTACEA

The chloride ion concentration is always high; this anion neutralizes 80–95% of the total inorganic cations. According to Florkin (1960), in Decapoda, the chloride index of the plasma of Macrura is higher (90–98.7% of the total cations) than in Brachyura (77–90.3%). In marine Crustacea, sulfate also plays a role in the cationic balance; its concentration varies from 2.6 meq/liter (in *Palaemon*, Parry, 1954) and 4.5 meq/liter (*Ligia*, Parry, 1953) to 40 meq/liter (*Uca*, Green *et al.*, 1959), but generally lies between 15 and 25 meq/liter. For a detailed table of the sulfate concentration in the plasma of marine Crustacea, see Robertson (1949, 1953).

Inorganic phosphates in the blood amount to about 0.45 meq phosphorus per liter in *Panulirus argus* (Travis, 1955). In this species, as in *Astacus* and *Maia* (Damboviceanu, 1932; Drilhon, 1935), a rise in phosphate concentration can occur at the time of molting but is always followed by a significant fall following the molt. This is probably due to inanition coupled with cuticular hardening and phosphorus store depletion in the hepatopancreas (Travis, 1955). In *Homarus vulgaris*, Glynn (1963) reports a significant rise in the organic phosphate concentration before molting (up to 64 mg. phosphorus per liter of blood).

B. ARACHNIDA

In scorpions, the chloride concentration is high and neutralizes nearly 100% of the total inorganic cations. The mean values are 267 meq/liter in both *Androctonus australis* (Bricteux-Grégoire *et al.*, 1963) and *Heterometrus fulvipes* (Padmanabhanaidu, 1966). In the latter species, the chloride content is higher in females (277 meq/liter) than in males (251 meq/liter), as is the case for sodium content. The sulfate concentration in *Heterometrus* blood is very low (1.10 mM).

C. INSECTA

The concentration of inorganic anions in some representative insects is shown in Table VII. The chloride concentration is high in all the exopterygotes (90–150 meq/liter) and neutralizes about 50–83% of the inorganic cations. On the contrary, endopterygotes are characterized by low chloride concentrations (generally less than 40 meq/liter). The anions of dihydrogen phosphate and bicarbonate play only a minor role

TABLE VII

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

Species	Stage	Sum of cations (meq/liter)	Anion concentration (meq/liter)			Anion indices (% of the sum of the cations)			References
			Cl^-	$H_2PO_4^-$	HCO_3^-	Cl^-	$H_2PO_4^-$	HCO_3^-	
Exopterygotes									
Odonata: *Aeschna grandis*	Larva	169	110	4	15	65	2.3	8.8	Sutcliffe, 1962
Dictyoptera: *Periplaneta americana*	Adult	174.2	144	—	—	82.6	—	—	Van Asperen and Van Esch, 1954
Orthoptera: *Locusta migratoria*	Adult	118.6	97.6	—	—	82.3	—	—	Duchâteau et al., 1953
Cheleutoptera: *Carausius morosus*	Adult	197.4(1)	93(2)	40(3)	—	47.1	20.2	—	(1) Duchâteau et al., 1953 (2) May, 1935 (3) Ramsay, 1955a
Carausius morosus	Adult	154	101	16	—	65.5	10.4	—	Wood, 1957
Endopterygotes									
Megaloptera: *Sialis lutaria*	Larva	167(1)	31(1)	5(2)	15(1)	18.5	3	6	(1) Shaw, 1955 (2) Sutcliffe, 1962
Diptera: *Gasterophilus intestinalis*	Larva	264	14.8	4	14.5	5.6	1.5	5.7	Levenbook, 1950a
Lepidoptera: *Bombyx mori*	Larva	150	21	3	—	14	2	—	Buck, 1953
Prodenia eridania	Larva	94.7	34	5.8	—	35.9	6.1	—	Babers, 1938
Samia walkeri	Pupa	128.6	10.4	3.5	—	8	2.7	—	Gese, 1950
Teleo polyphemus	Pupa	147.2	19.5	—	—	13.2	—	—	Carrington and Tenney, 1959
Coleoptera: *Dytiscus marginalis*	Adult	231.6(1)	44(1)	2.8(2)	—	19	1.2	—	(1) Sutcliffe, 1962 (2) Buck, 1953
Popillia japonica	Larva	84.3	19	4.9	—	22.5	5.8	—	Bishop et al., 1925
Hymenoptera: *Apis mellifica*	Larva	52.7	33	10.3	—	62.6	19.5	—	Bishop et al., 1925

in the cation balance, with the exception of the stick insect *Carausius morosus* (see Table VII) and of the cricket *Anabrus simplex* (Pepper *et al.*, 1941) in which inorganic phosphates contribute largely to the ion balance. Either storage at low temperatures or anoxia, increase the inorganic phosphate concentration in the blood of the larvae of *Anagasta kuehniella* (Sømme, 1966).

The bicarbonate anion is the principal form of carbon dioxide transport in most insects; the amount of bicarbonate in the hemolymph appears to be directly proportional to the amount of gaseous carbon dioxide (Levenbook, 1950b,c; Levenbook and Clark, 1950). According to Wyatt (1961), the deficit in the ion balance of the hemolymph of most endopterygotes is mainly made up by organic acids.

V. Carbohydrates and Related Substances

A. CRUSTACEA

1. Nature of the Hemolymph Carbohydrates

Glucose is generally considered to be the exclusive reducing sugar in the blood of Crustacea. A recapitulative table of the "glucose" concentration in Crustacea is given by Florkin (1960). The wide range of values obtained (1.0–182 mg. "glucose" per 100 ml.) suggests that starvation or feeding directly influences the blood sugar level. For a detailed study of carbohydrate metabolism, see Chapter 5 of Volume V.

Recent investigations using chromatographic and enzyme-specific methods for true glucose have shown that glucose generally represents only a fraction of the hemolymph reducing sugars—50–60% of the total reducing sugars in *Homarus americanus* (Telford, 1965). The other reducing sugars in the blood of the crayfish *Orconectes virilis* (50 mg./100 ml.) are glucose 6-phosphate, maltose, maltotriose, maltotetraose, and a galactan derivative (McWhinnie and Saller, 1960). In *Homarus americanus*, Telford (1965, 1968) notes that eight different types of soluble sugars can occur in the hemolymph, but none except glucose appears in all the samples so far studied. Table VIII shows the relative frequency of occurrence of these sugars in eighty specimens belonging to four different stages of the molting cycle. It is interesting to note that mannose, when present, occurs at a relatively high concentration (about 15 mg./100 ml.).

2. Quantitative Variations during Life Cycle

Renaud (1949) first suggested that molting could profoundly modify the concentration of the blood sugars. Blood "glucose" falls during the

premolting stages in *Armadillium vulgare,* an isopod (Nambu *et al.,* 1960). In Decapoda, however, the results obtained by several authors (see, for instance, Drilhon, 1935; Renaud, 1949; McWhinnie and Scheer, 1958; McWhinnie and Saller, 1960; Telford, 1968) in different species are too diverse to allow any generalization. The discrepancies are probably due to the failure of correct identification of molting stages, to the influence of ecological factors, and to the diversity of analytical methods used so far. In addition to these sources of errors, the existence

TABLE VIII

FREQUENCY OF OCCURRENCE OF EIGHT SUGARS
IN THE HEMOLYMPH OF *Homarus americanus*[a]

		Stage of the molting cycle[c]			
Sugar	Rg[b]	C_4 (intermolt)	D_0–D_4 (premolt)	B_2 (postmolt)	C_1–C_3 (late postmolt)
Maltotriose	0.06	4	2	1	—
Trehalose	0.17	20	20	16	19
Unknown	0.23	20	20	11	18
Maltose	0.29	5	4	—	3
Galactose	0.79	3	7	—	2
Glucose	1.00	20	20	20	20
Mannose	1.24	2	—	—	—
Fructose	1.37	3	6	—	1

[a] Eighty specimens belonging to four different molting stages (twenty per stage). From Telford (1968).

[b] Rg = mobility relative to glucose.

[c] Following the nomenclature of Drach (1939).

of a circadian or tidal rhythm in the blood sugar concentration must be borne in mind (Dean and Vernberg, 1965). Seasonal variations do not seem to occur at this level in Crustacea (McWhinnie and Saller, 1960).

B. ARACHNIDA

In scorpions, there is a great discrepancy between the results obtained by different workers; trehalose, estimated by anthrone after alkaline digestion, is claimed to be the main sugar (28.75 mg./100 ml.) in the hemolymph of *Androctonus australis* (Bricteux-Grégoire *et al.,* 1963), while this sugar has not been identified at all by thin layer chromatography in the same species by Schantz *et al.* (1969) who found only

glucose (420 mg./liter). Moreover, it is said (Kanungo and Nanda, 1961) that a nonidentified pentose is the only reducing sugar in the hemolymph of another scorpion *Palamnaeus bengalensis*. More detailed information is obviously needed.

C. INSECTA

1. Nature of the Carbohydrates Present in the Hemolymph

With very few exceptions (see below), the reducing power of the hemolymph of insects is not due to fermentable sugars such as glucose, as is the case in most other animals, but to nonsaccharidic substances, such as ascorbic acid, α-ketonic acids, uric acid, different amino acids (principally tyrosine), and other unknown substances (Howden and Kilby, 1961). The hemolymph contains almost no saccharose. It is a well known fact since the works of Wyatt and Kalf (1956, 1957) that the hemolymph sugar is trehalose, a nonreducing dimer of α-glucose occurring in all the insect species so far studied. The numerous data concerning the concentration of this sugar in the hemolymph have been tabulated by Florkin and Jeuniaux (1964); the trehalose concentrations range from 202 mg./100 ml. in the pupae of *Bombyx mori* to 4700–5200 mg./100 ml. in the larvae of the buprestid *Chalcophora mariana*. The only exceptions are those of *Phormia regina*, in which trehalose is absent in larvae but present in pupae and adults (D. R. Evans and Dethier, 1957; Wimer, 1969), and of another dipteran *Agria affinis* (Fall), in which very small amounts of trehalose, about 1–2% of total blood carbohydrate, have been found (Barlow and House, 1960).

The utilization of trehalose as the unique circulating form of saccharidic cellular food is a very general characteristic of the class of insects, but probably is not exclusively specific to this class. Trehalose is synthetized by many different invertebrates; it is occasionally present in the blood of some Crustacea, and it has been reported to have been found in the hemolymph of a scorpion (see above).

Glucose and fructose generally exist only as traces in the hemolymph of insects. High amounts of blood glucose have, however, been found in *Apis mellifica* adults (600–3200 mg./100 ml.) (von Czarnowsky, 1954), in *Phormia regina* (larvae, 70–125 mg./100 ml.; adults, up to 600 mg./100 ml.) (D. R. Evans and Dethier, 1957), and in *Agria affinis* (larvae, 80% of total carbohydrate in the form of glucose) (Barlow and House, 1960). High levels of fructose occur in *Apis mellifica* adults (200–1600 mg./100 ml.) (von Czarnowsky, 1954) and in *Gasterophilus intestinalis* larvae (184–294 mg./100 ml. (Levenbook, 1947, 1950a).

Mannose and pentoses (probably arabinose and ribose) have been detected in the blood of the larvae of a dipterous parasite *Agria affinis*, reared on synthetic diets, but these sugars amounted to only a few percent of the total carbohydrate content (Barlow and House, 1960).

The insect hemolymph contains little if any glycogen. According to Wyatt (1961) and to Howden and Kilby (1961), the substances in the hemolymph of insects estimated as glycogen by classical methods are probably other polysaccharides or glycoproteins.

The sex may influence the total blood sugar concentration; in *Periplaneta americana* the concentration is 1188 mg./100 ml. in males and only 551 mg./100 ml. in females (Hilliard and Butz, 1969).

2. Quantitative Modifications and Hormonal Control

The trehalose of the hemolymph is absorbed and used by the cells of most tissues, due to an intracellular trehalase. The cells of the epidermis seem to lack trehalase (Zebe and McShan, 1959; Duchâteau-Bosson *et al.*, 1963). The cyclic activity of these cells (at every molting period) is parallel to the variation of the activity of a trehalase present in the blood, which remains inhibited during the whole intermolt period (Friedman, 1961). The inhibition of the hemolymph trehalase is suppressed at the beginning of every molting period, which induces a fall in the trehalose concentration of the blood (Howden and Kilby, 1956; Duchâteau-Bosson *et al.*, 1963). Glucose resulting from trehalose hydrolysis is rapidly removed from the blood by the cells and is used not only for metabolic purposes, but also for providing carbohydrate material during chitin synthesis by the epidermis (Candy and Kilby, 1961, 1962). An example of the quantitative variations of trehalose concentration together with the activity of trehalase in the hemolymph of an insect, the silkworm *Bombyx mori*, is given in Fig. 3.

It has been demonstrated that an inverse relationship exists between glycogen and trehalose concentrations in the fat body, the former decreasing sharply at each molt or during starvation, while the amount of trehalose in the fat body and in the blood tends to remain at a constant level (Duchâteau-Bosson *et al.*, 1963; Saito, 1963). The gluconeogenetic pathways in the fat body are described in Chapter 6 of Volume V. Suffice it to say here that the supply of trehalose by the fat body to the hemolymph, the rate of trehalose synthesis, and the activity of the blood trehalase are controlled by a hormone, called hyperglycemic hormone, which is produced by the corpora allata (Steele, 1961, 1963), and are moreover subjected to end product inhibition (Murphy and Wyatt, 1965; Friedman, 1967).

3. Influence of Diet

By contrast to the concept of a blood sugar homeostasis, some recent workers observed a more or less profound influence of the nature of the diet on the concentration and even on the chemical nature of the sugars in the hemolymph. In the mature larvae of *Agria affinis* reared on chemically defined diets, an increase in the glucose content of the diet augmented the concentration of the blood carbohydrates, in which glucose represents 80%, without qualitative modification (Barlow and
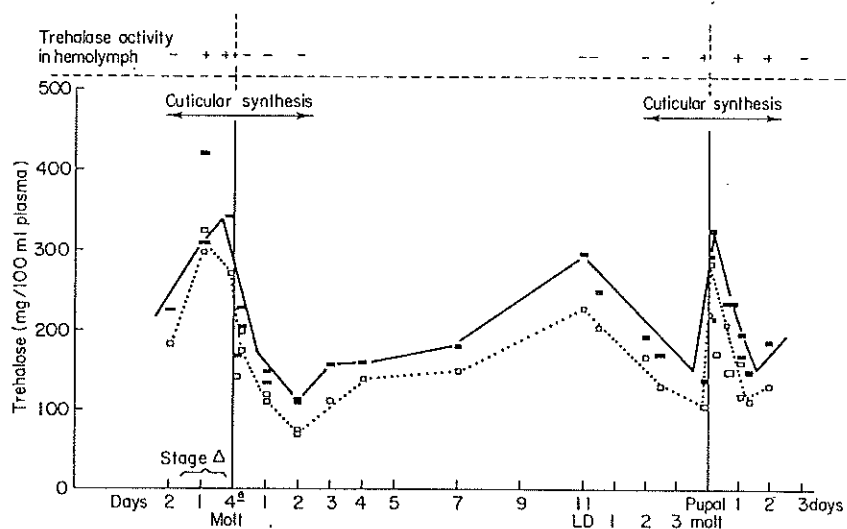
FIG. 3. Modification of trehalose concentration and of trehalase activity in the hemolymph of *Bombyx mori*. Solid line = anthrone reactive material; broken line = trehalose, (Duchâteau-Bosson *et al.*, 1963).

House, 1960). This was also the case for locusts reared on a fructose-rich food (peas); fructose accounted for more than half of the 100% increase in blood carbohydrate concentration (Hansen, 1963). It is most surprising to note that when these locusts (*Locusta migratoria* and *Schistocerca gregaria*) had free access to growing wheat, the total carbohydrate concentration was higher than under natural conditions, but trehalose could not be obtained from the hemolymph, while crystalline maltose and cellobiose were isolated and identified (Hansen, 1964).

4. Circadian Variation and Influence of Environmental Factors

The blood sugar level in a given species can also show circadian fluctuations, as seen in *Gryllus domesticus* (Nowosielsky and Patton,

1964) or in *Periplaneta americana* (Hilliard and Butz, 1969). Moreover, low temperatures (0°C for 7 days) can increase the trehalose concentration in the hemolymph of the larvae of *Nematopogon personnellus* from 94 mM to 208 mM; in the same species, anoxia for 6 hours produces the same result (94 mM–201 mM trehalose, Sømme, 1967). But opposite effects have been obtained by placing the larvae of *Anagasta kuehniella* (Sømme, 1966) under the same conditions.

VI. Organic Acids

A. Crustacea

Lactic acid is present in the blood of *Homarus americanus;* in quiet water the concentration of lactic acid in the hemolymph is between 0.61 and 0.71 mg./100 ml., but the values are five times as high after rearing during 4 days in running water (Steward *et al.*, 1966).

B. Insecta

The hemolymph of insects contains extraordinarily large amounts of organic acids (Tsuji, 1909). They are mainly substrates of the tricarboxylic-acid cycle enzymes—citrate, α-ketoglutarate, succinate, fumarate, malate, and oxaloacetate. All these organic acids probably play an important role in the ionic balance. In *Gasterophilus* larvae (Levenbook and Wang, 1948; Levenbook, 1950a; Nossal, 1952), their sum contributes to about 50% of the anion titer (Wyatt, 1961).

Citrate seems to be a permanent constituent of the hemolymph (Levenbook and Hollis, 1961), but it is more abundant in the larvae of endopterygotes than in the adults. For instance, in two species of Diptera, *Sarcophaga bullata* and *Phormia regina*, the citrate concentration of the hemolymph was, respectively, 10.3 and 12.5 mM in the larvae, as opposed to 2.6 and 0.33–0.44 mM in the adults. Citrate is less abundant in the hemolymph of exopterygote insects (0.73 M in *Periplaneta americana;* 1.66 M in *Leptocoris trivittatus;* 2.3 M in *Rhodnius prolixus*) (Levenbook and Hollis, 1961; Patterson, 1956).

The other organic acids cited above have been found occasionally, but are generally less abundant. However, large amounts of pyruvate have been detected in the blood of the pupae of *Antheraea pernyi* (up to 23–31 mM) (Burova, 1953) and of *Hyalophora cecropia* (Wyatt, 1961), glyoxylic and acetoacetic acids in the larval hemolymph of *Bombyx mori* (Fukuda and Hayashi, 1958), and volatile fatty acids, mainly acetic acid (34–46 mg./100 ml.) in the larval hemolymph of *Popillia japonica* (Stubblefield *et al.*, 1966).

The organic acids of the hemolymph are endogenous in origin. The level of hemolymph citrate in *Prodenia eridania* is not modified by a change of diet (Levenbook and Hollis, 1961). The infection of the larvae of *Popillia japonica* by *Bacillus popilliae* increases the concentration of malic, glycolic, tartaric, pyruvic, and glyoxylic acids (Stubblefield *et al.*, 1966).

VII. Alcohols

The presence of glycerol in the hemolymph of overwintering insects during their diapausing stage can be considered as a type of biochemical adaptation to cold hardiness exceptional in the animal kingdom. Glycerol accumulates at phenomenal levels in the hemolymph of *Hyalophora cecropia* (up to 300 mM) and is rapidly metabolized when diapause is broken (Wyatt and Meyer, 1959). This is true in the case of many other insects, whatever the diapausing developmental stage, which can be the embryo in the case of *Bombyx mori* (Chino, 1957, 1958). Exposure of nondiapausing insects to low temperatures can increase the concentration of blood glycerol, as in the case of normally diapausing species (Wilhelm, 1960; Sømme, 1966, 1967).

Among polyhydric alcohols, sorbitol has been found in overwintering eggs and embryos of *Bombyx mori* (Chino, 1958) and in the hemolymph of the larvae of the Lepidoptera *Eurosta solidaginis* and *Laspeyresia strobilella* (Sømme, 1966, 1967).

VIII. Lipids

A. CRUSTACEA

As far as we know, the data of Damboviceanu (1932) are the only ones available, and they concern three species of Decapoda exclusively. The concentration of total lipids in the hemolymph is between 110 and 590 mg./100 ml. Cholesterol (12–49 mg./100 ml.), cholesterol esters (4–30 mg./100 ml.), and phospholipids (traces to 54 mg./100 ml.) have been identified.

B. INSECTA

The total lipid content of the insect hemolymph is generally between 1.5 and 5.5%, but can vary considerably during development (Nowosielski and Patton, 1965; Nelson *et al.*, 1967) and during flight, at least in the case of locusts (Beenakkers, 1965; Mayer and Candy, 1969). The increase of the lipid level of the hemolymph during locust flight

is mediated by a peptide hormone, adipokinetic hormone, secreted by the corpora allata (Mayer and Candy, 1969). Starvation, on the contrary, does not seem to affect the total concentration or the composition of the blood lipids (Wlodawer and Wisniewska, 1965), except in the case of phosphorylethanolamine after prolonged starvation in *Galleria mellonella* larvae (Lenartowicz and Niemierko, 1964).

The qualitative identification of lipids in the insect hemolymph has been made recently by some workers. In *Galleria mellonella*, phospholipids represent 22%, sterols (including cholesterol) 15%, unesterified fatty acids 8%, and neutral glycerides about 50% (Wlodawer and Wisniewska, 1965). Phosphatidylcholine represents 60% of the phospholipid fraction and phosphatidylethanolamine only 20%.

In the cricket *Acheta domesticus*, cholesterol is the principal sterol of the hemolymph (0.13–0.23%). The total sterol concentration falls in the adult female. Phosphatidylethanolamine is the principal phospholipid, with a mean concentration of 0.44%. Linoleic, oleic, and palmitic acids constitute 88.6% of the total fatty acids (Wang and Patton, 1969).

The fatty acid composition of the tri-, di-, and monoglycerides in the hemolymph of *Periplaneta americana* is modified during development, as is the concentration of these lipids. The observed changes suggest that the young adult utilizes mainly the triglycerides composed of saturated fatty acids (Nelson *et al.*, 1967).

IX. Organic Phosphates

One of the most interesting biochemical peculiarities of insects is the high concentration of phosphates in their hemolymph, mainly in the form of acid-soluble organic phosphates. In *Hyalophora cecropia* pupae, for instance, the concentration of total acid-soluble phosphorus amounts to 26–44 mM (Wyatt *et al.*, 1963). The organic phosphates generally found in the insect hemolymph are α-glycerophosphate, phosphorylethanolamine, glycerophosphoethanolamine, phosphorylcholine, sorbitol 6-phosphate, and glucose 6-phosphate (Wyatt, 1959; Wyatt and Kalf, 1957, 1958; Wyatt *et al.*, 1963; Kondo and Watanabe, 1957). Small amounts of uridine diphosphate-*N*-acetylglucosamine and of some other uridine diphosphate derivatives have been detected in the blood of *Hyalophora cecropia* (Wyatt *et al.*, 1963).

The concentration and composition of the organic phosphate fraction of the hemolymph are greatly modified during the course of development and metamorphosis, by diapause (Heller, 1931; Wyatt *et al.*, 1963), by storage at low temperatures or by anoxia in *Anagasta kuehniella* (Sømme, 1966).

X. Hydrocarbons

The presence of hydrocarbons in insects has been shown recently (Chefurka and Williams, 1951; Beament, 1955; Dennell and Malek, 1956). The identification of hydrocarbons in the cockroach hemolymph has been performed by Baker *et al.* (1960, 1963) on *Periplaneta americana*, and by Acree *et al.* (1965) on *Blattella germanica*. In both species, *cis,cis*-6,9-heptacosadiene was the principal hydrocarbon (about 60% of the total), followed by 3-methylpentacosane and pentacosane, together with small amounts of heptacosane, nonacosane, and C_{41}–C_{43} hydrocarbons. Fluctuations according to age and sex—twice as much hydrocarbons in females as in males (Baker *et al.,* 1963; Acree *et al.,* 1965)—as well as circadian fluctuations (Turner and Acree, 1967) have been observed in cockroaches.

XI. Free Amino Acids

The existence of high concentrations of free amino acids in the hemolymph is one of the biochemical characteristics by which insects differ the most markedly from all other animals. The increasing importance of free amino acids as osmolar effectors of the hemolymph in Arthropoda has been considered from an evolutionary point of view at the beginning of this chapter (see Section II,C). The fundamental role of these substances in osmoregulatory processes is studied in Chapters 9 and 10 of Volume V.

A. CRUSTACEA

The aminoacidemia in Crustacea is as low as that of vertebrates; the sum of the concentrations of the principal free amino acids amounts to 80 mg./100 ml. in *Astacus astacus* (Duchâteau-Bosson and Florkin, 1961) and 64.1 mg./100 ml. in *Homarus gammarus* (Camien *et al.,* 1951), both data being obtained by the microbiological method; it amounts to 26.1 mg./100 ml. in *Cancer irroratus*, and 21.7 mg./100 ml. in *Homarus americanus*, according to Stevens *et al.* (1961), using column chromatography.

The pattern of the free amino acid pool in the blood seems to be highly different from species to species. For instance, 50% of the total amino-acid concentration is accounted for by glutamic acid and alanine in *Astacus astacus*, by glycine and alanine in *Homarus gammarus*, by proline, alanine, and serine in *Homarus americanus*, and by proline, glycine, arginine, and serine in *Cancer irroratus*. Taurine is sometimes

an important constituent of the hemolymph (4.2–6.1 mg./100 ml.) in *Cancer irroratus* (Stevens *et al.*, 1961).

B. CHELICERATA

Table IX compares the free amino acid concentration and composition of the blood in the marine species *Limulus polyphemus* (Stevens *et al.*, 1961; Bricteux-Grégoire *et al.*, 1966) to that of the hemolymph of the scorpion *Androctonus australis* (Bricteux-Grégoire *et al.*, 1963). The aminoacidemia of the scorpion is similar to that of Crustacea, but that of *Limulus* is much lower. In another scorpion species, *Heterometrus fulvipes*, the amino acid concentration seems to be of the same magni-

TABLE IX
FREE AMINO ACIDS IN THE HEMOLYMPH OF TWO CHELICERATA

	Concentration (mg./100 ml.)		
		Limulus polyphemus (Horseshoe crab)	
Amino acids	*Androctonus australis* (Scorpion)[a]	Stevens *et al.* (1961)	Bricteux-Grégoire *et al.* (1966)
Alanine	2.48	0.14–0.18	1.4
Arginine	4.92	0.49–0.56	0.8
Asparagine + aspartic acid	3.32	0.16–0.21	1.1
Glutamine + glutamic acid	15.80	0.26–0.33	0.3
Glycine	5.37	0.22–0.29	0.0
Histidine	1.60	—	0.3
Isoleucine	1.26	0.10–0.12	0.4
Leucine	0.97	0.17–0.21	0.2
Lysine	2.96	0.46–0.74	0.2
Methionine	—	0–0.04[b]	0.2
Phenylalanine	1.61	0.04–0.09	0.2
Proline	9.40	0.83–1.50	0.9
Serine	4.28	0.61–0.66	—
Threonine	3.44	0.15–0.17	0.2
Tyrosine	1.64	0.06–0.08	0.3
Valine	3.49	0.17–0.35	0.0
Sum of amino acids	62.54	4.39–5.01	6.5
Taurine	40.3	0.12–0.17	—

[a] Bricteux-Grégoire *et al.* (1963).
[b] In the form of methionine sulfoxide.

tude as that of *Limulus* (5.22 mg./100 ml.) (Padmanabhanaidu, 1966). It is obvious, however, that both species are characterized by a low aminoacidemia when compared with insects (Table X). The high level of aminoacidemia thus appears clearly as a biochemical taxonomic characteristic of the class of insects and is not to be directly related to ecological adaptation to terrestrial life. In scorpions, the most abundant amino acid in the hemolymph is glutamic acid (free and in the form of glutamine), but taurine obviously plays an important role, owing to its high concentration (see Table IX).

C. INSECTA

1. General Considerations

The total concentration of free amino acids in the hemolymph is generally lower than 700 mg./100 ml. in exopterygotes and higher than 700 mg./100 ml. in endopterygotes. The amino acids are usually of the L-configuration, but large amounts of D-alanine have been detected in the hemolymph of *Oncopeltus fasciatus* (Auclair and Patton, 1950) and D-serine in the blood of different Lepidoptera (Srinivasan *et al.*, 1962), where it may account for 70% of the total free serine.

From the numerous data so far available, it is almost impossible to give a synthetic picture of the amino acid pool in the different taxa. The free amino acid composition of the hemolymph is indeed highly variable within a given family or genus (Duchâteau and Florkin, 1958), and even in a given species, the successive stages, or instars, of development can exhibit very distinct patterns. As stated by Florkin and Jeuniaux (1964), "the aminoacidemia of an insect species may 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."

A selection of some representative data, given in Table X, shows the great diversity of the amino acid patterns among insects. A general tendency in exopterygotes is the relatively uniform concentration of every amino acid in a given species, while widely scattered values are observed in endopterygotes.

Besides the sixteen amino acids listed in Table X, some other free amino acids or related substances have occasionally been identified, such as methionine sulfoxide in *Periplaneta americana* (Stevens, 1961); ornithine, cystathionine, and 3-hydroxykynurenine in *Bombyx mori* (Kondo, 1959; Makino *et al.*, 1954); S-methylcysteine in *Prodenia eridania* (Irreverre and Levenbook, 1960); L-lanthionine and L-cysta-

Charles Jeuniaux

TABLE X

| | | | | | Concentration | |
| Amino acids | Exopterygotes | | | | Hymenoptera: | Coleoptera |
	Aeschna sp. larva	*Carausius morosus* adult	*Periplaneta americana* adult[c]	*Locusta migratoria* nymph	*Apis mellifica* larva	*Hydrophilus piceus* adult
Alanine	46	10–60	7	34	58	60
Arginine	19–27	17–19	19	24	50–74	7–11
Aspartic acid (+ asparagine)	4–13	6–14	2	13	32–33	17–18
Glutamic acid (+ glutamine)	32–63	50–77	24	166	308–347	131–195
Glycine	22–54	23–31	53	97	72–84	17–26
Histidine	7–21	55–58	23	30	17–30	8–12
Isoleucine	16–18	7–13	6	21	20–24	8–25
Leucine	22–29	10–14	7	21	25–30	7
Lysine	6–14	20–28	11	47	74–104	20–24
Methionine	4–13	9–13	4	6	19–23	3
Phenylalanine	5–11	6–9	6	11	8–12	6–7
Proline	12–41	10–16	42	62	368–418	122–283
Threonine	12–23	29–40	8	20	27–49	12–17
Tyrosine	3–13	5–8	25	28	3	2–9
Valine	23–29	22–25	11	48	53–59	11–20
Total	399	293–424	248	636	1239	445–721
Serine	24		14	49		22–35

[a] From Duchateau and Florkin (1958), Shotwell *et al.* (1963), and Stevens (1961).

[b] Values have been rounded to the unity.

[c] Stevens (1961).

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

[e] Other species studied: larva of *Cossus cossus* (sum of the fifteen amino acids = 938mg./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.).

thionine in *Bombyx mori* (Rajagopal Rao *et al.*, 1967); α-aminoisobutyric acid, homoarginine, and hydroxyproline in *Attacus ricini* (Pant and Agrawal, 1964); hydroxyproline in the larvae of *Apis mellifera* (Pratt, 1950; Lue and Dixon, 1967); and taurine in *Musca domestica, Oncopeltus fasciatus* (Pratt, 1950), and *Periplaneta americana* (Stevens, 1961).

DISTRIBUTION AND CONCENTRATION OF THE PRINCIPAL FREE AMINO
ACIDS IN THE HEMOLYMPH OF SOME REPRESENTATIVE INSECTS[a]

(mg./100 ml. hydrolyzed plasma)[b]

Coleoptera		Lepidoptera				
Popillia japonica larva[d]	Diptera: Gasterophilus larva	Euproctis chrysorrhoea larva[e]	Smerinthus ocellatus larva[e]	Saturniidae pupa[f,g]	Sphyngidae pupa[f,h]	Papilio machaon pupa
140–187	—	33	27	7–300	16–250	103–213
48–81	8	44–58	19	107–243	59–576	126–127
42–47	14	9–22	27	4–36	5–55	14–19
309–526	314	302–343	202	83–468	62–240	202–226
288–325	5	48–94	52	20–82	4–57	48
169–225	1	107–161	83	23–196	3–127	71–89
36–54	8	15–32	12	14–83	20–65	40–56
20–25	7	13–23	8	15–108	14–73	56–80
29–94	8	50–105	77	113–471	64–433	325–401
3–12	7	1–13	8	11–148	25–81	122–163
13–17	7	8–15	9	7–72	8–49	24–43
264–507	16	129–157	23	62–478	28–230	146–256
11–29	23	30–54	34	1–136	20–82	47–57
11–37	22	0–5	30	2–76	8–146	4–5
94–150	15	29–49	83	34–127	22–105	101–120
1723–2162	465[i]	870–1164	700	1124–1989	515–1819	1575–1769

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

[g] Fifteen species belonging to the genera *Citheronia, Eacles, Saturnia, Antheraea, Actias, Hyalophora,* and *Philosamia.*

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

[i] Without alanine.

2. Influence of Diet and Starvation

The nature of the diet largely contributes to the alteration of the free amino acid pattern within a given species, both quantitatively and qualitatively. This has been demonstrated by transferring phytophagous

insects from their usual host plant to another plant (Irrevere and Leven-book, 1960) or by rearing larvae of *Sarcophaga* on biscuit or on meat (Singh, 1965). The experimental addition of certain amino acids to the diet of an insect modifies its aminoacidemia; for instance, when DL-methionine is fed to the cockroach *Blatella germanica*, α-aminobutyric acid, histidine, and lysine increase in the hemolymph (Auclair, 1959). Similar results have been obtained when certain amino acids are added to the pea plants eaten by aphids (Maltais and Auclair, 1962).

The effect of starvation is variable; in *Aeschna* and *Bombyx mori* larvae, the concentration of most of the free amino acid decreases, while that of some increases significatively (Duchâteau and Florkin, 1958; Kondo and Watanabe, 1957). In other cases (*Anomala orientalis*, Po-Chedley, 1958; *Popillia japonica*, Ludwig and Wugmeister, 1953), the level of the total aminoacidemia rises after prolonged starvation, sometimes up to twice the normal values.

3. Quantitative Variations during Development, Metamorphosis, and Spinning in Lepidoptera

An exhaustive study has been performed by the laboratory of Florkin since 1958 on the free amino acids of the hemolymph in the silkworm *Bombyx mori*. Some amino acids, called sericigenous amino acids, are absorbed and directly utilized by the silk glands to synthesize fibroin. These sericigenous amino acids are glycine, asparagine, glutamine, serine, threonine, and proline, but not alanine nor phenylalanine (Duchâteau *et al.*, 1959; Bricteux-Grégoire *et al.*, 1959a,b; Duchâteau-Bosson *et al.*, 1960, 1961). Glutamine and asparagine are converted by the silk glands mainly to alanine (Bricteux-Grégoire *et al.*, 1960). The quantitative variations of these amino acids during the last larval instar and the cocoon spinning are shown in Fig. 4.

The sericigenous amino acids obtained from food are stored by incorporation into the body tissues during the beginning of the fifth instar, and then used by the glands during the spontaneous starvation which accompanies the cocoon spinning (Fukuda and Florkin, 1959). After spinning and pupation, the concentration of these amino acids increases rapidly in the hemolymph of the pupa, probably due to histolysis (Fig. 4).

The concentration of nonsericigenous amino acids in the hemolymph of *Bombyx mori* is also subjected to variations. Tyrosine accumulates during the few days preceding each molt and decreases sharply after molting (Duchâteau-Bosson *et al.*, 1962) or after the formation of the puparium in *Sarcophaga* (Fraenkell and Rudall, 1947); tyrosine is used for protein tanning and melanization of the cuticle. Histidine and methio-

nine concentrations increase when the sericigenous amino acids decrease (Fig. 4); these modifications probably regulate to some extent the osmotic pressure of the hemolymph (Jeuniaux et al., 1961). The concentrations of other free amino acids such as alanine, isoleucine, leucine, lysine, and valine are mainly affected by histolysis during pupation.

The fluctuations of the amino acid pattern of the hemolymph have been followed throughout the development of other Lepidoptera
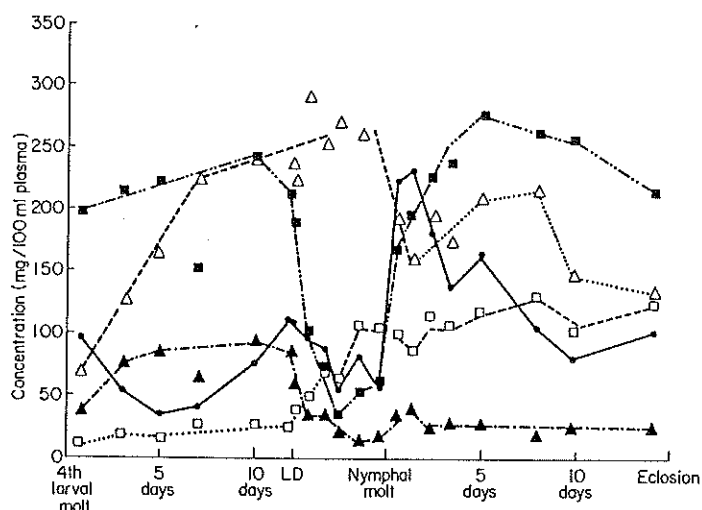

FIG. 4. Variation of the concentration of some free amino acids in the hemolymph of the silkworm Bombyx mori during the fifth larval instar, the spinning, the pupal stage, and the metamorphosis. Solid triangle = aspartic acid (total) (aspartate + aspargine); solid square = glutamic acid (total) (glutamate + glutamine); solid circle = glycine; open triangle = histidine; open square = methionine. (After Jeuniaux et al., 1961.)

(Prodenia eridania, Levenbook, 1962; Philosamia ricini, Pant and Agrawal, 1965; Antheraea pernyi, Mansingh, 1967) and of the ant Formica polyctena (Brunnert, 1967).

4. Other Factors Influencing Aminoacidemia

The influence of diapause is generally difficult to separate from the modifications due to development. By comparing diapausing and non-diapausing populations of Antheraea pernyi, Mansingh (1967) observed that diapause itself induces a significant increase in the level of most of the free amino acids in the hemolymph, mainly histidine, alanine, and proline. The termination of diapause and the postdiapause development are characterized by a marked depletion in the concentrations

of these amino acids. The rise in alanine and proline concentratio₁
are attributed to the existence of anaerobic physiological conditions an
the depletion of the cytochrome system during diapause.

Anoxia for 6 hours and low temperatures ($-6°C$ for 3 weeks) bot
induce an accumulation of alanine (from 12 mM to 64–74 mM) in th
hemolymph of *Nemapogon personnellus* (Sømme, 1967).

Caste differentiation, or development of female polymorphism,
marked by profound variations of the hemolymph aspartic acid concer
tration in the honeybee (Lue and Dixon, 1967) and by significati۱
and reproducible modifications of the concentrations of α-alanine, g!
cine, glutamine and glutamic acid, proline, valine, arginine, and tauri₁
in the ant *Formica polyctena* (Brunnert, 1967).

5. Peptides

The peptide content of insect hemolymph is often reported to b
very low, owing to the great similarity generally observed between th
concentration of the free amino acids of hydrolyzed and nonhydrolyze
plasma after deproteinization. However, peptides are said to be relative₁
abundant in the hemolymph of *Drosophila* (Hadorn and Mitchell, 1951
About nineteen different peptides have been identified in the hemolymp
of *Phormia regina* by Levenbook (1966); they are mainly dipeptide
and they show remarkable change during larval growth; their maximu.
concentration occurs at the early third instar and decreases rapidly ju:
before pupation. Four peptides have been identified at very low concer
trations in the blood of *Periplaneta americana* (von Knorre, 1967).

XII. Proteins

Among the numerous proteins that are to be found in the hemolymp
of Arthropoda, two particular components have been easily identifiec
when present, owing to their evident properties—the respiratory pi:
ments and the coagulable proteins. The nature, composition, and propr
ties of these proteins are treated in Chapter 3 and 4 of Volume VI.

The remaining protein fraction of the Arthropod hemolymph has bee
resolved into a series of components with different electrophoretic proɪ
erties. Comparative studies of electrophoretic patterns of the hemolymp
proteins are numerous, principally in insects, and have been tentative₁
used in a number of taxonomic studies. But the actual role of the:
proteins has obviously been more difficult to understand. As far as ۱
know, one of these protein components has been recently identified i
the insect blood as playing a well defined role in vitellogenesis.

On the other hand, an important part of the protein fraction in tl

hemolymph is made up by enzymes, mainly hydrolases or enzymes of carbohydrate metabolism. Nevertheless, it is obvious that a complete understanding of hemolymph proteins will need further investigations. Problems dealing with immunology and serology will not be considered in this chapter; blood pigments are considered in Chapter 8 of volume VI.

A. CRUSTACEA

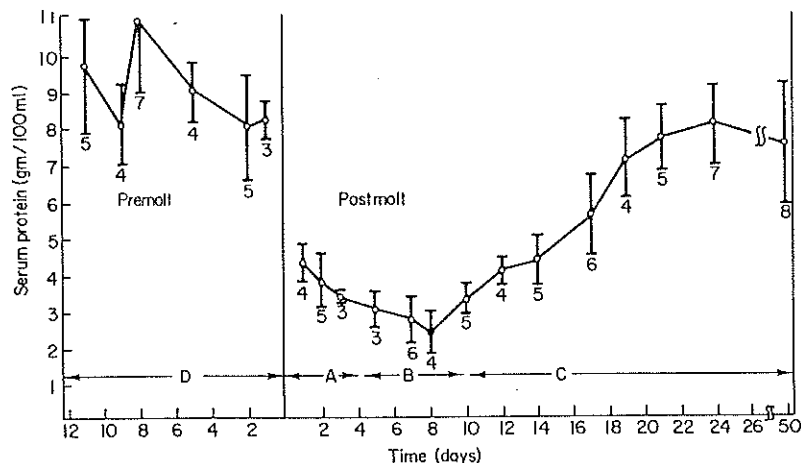The total concentration of proteins in the plasma generally varies from 1 to 6 gm./100 ml., but lower or higher values have been recorded

FIG. 5. Variation of the protein concentration in the hemolymph (serum) of *Homarus americanus*, during the molt cycle. Ecdysis is indicated by the vertical line at zero time. The number of individuals in each sample is indicated by the figures beside the points. (Glynn, 1968.)

occasionally (see Florkin, 1960). Intraspecific variations have been discussed by Leone (1953); they are especially explained by the physiological conditions preceeding or following the molts. There is generally an increase in protein concentration before the molt, followed by a sharp fall immediately after the ecdysis, due to the absorption of water and consecutive dilution of the blood (see Section II,D) (Drach, 1939; Damboviceanu, 1932; Travis, 1955; Robertson, 1960; Glynn, 1968). Figure 5 illustrates the variations of hemolymph proteins in *Homarus americanus* (Glynn, 1968).

The major part of the plasma proteins is formed by hemocyanin, which usually represents 80–95% of the total concentration, not only in Decapoda, but also in Isopoda and Amphipoda (Wieser, 1965).

Another part of the protein fraction of the plasma is made up by free enzymes, that is to say, enzymes that are not contained in blood cells and then liberated during coagulation, such as tyrosinase (Bhagvat and Richter, 1938; Pinhey, 1930). An amylase has been detected in the hemolymph of *Astacus* sp. (Damboviceanu, 1932) and in the serum of *Callinectes sapidus* (Horn and Kerr, 1969), a phosphatase in the blood of an unidentified crab (Roche and Latreille, 1934), and a chitobiase in the hemolymph of *Homarus vulgaris* (Jeuniaux, 1963). Carbonic anhydrase seems to be lacking in the blood of most Crustacea (Florkin, 1935a; Ferguson *et al.*, 1937) but is abundant in the hemolymph of *Ligia exotica* and of *Sesarma hematocheir* (Numanoï, 1941).

The blood of Decapoda presents pseudoperoxydasic and pseudophenoloxydasic activities, due to hemocyanin (Bhagvat and Richter, 1938; Zuckerkandl, 1953; Ghiretti, 1956). These activities have not been detected in *Euphausia superba* (the krill) in which hemocyanin is lacking (Decleir, 1968).

By starch gel electrophoresis, a "female-limited protein" component has been identified in the blood serum of *Callinectes sapidus;* this high density lipoprotein gives a yellow color to the blood, and is probably associated with egg production (Horn and Kerr, 1969). A detailed study of the relationships between blood proteins and carotenoid pigments has been recently undertaken by Ceccaldi (1967, 1968).

B. Insecta

The hemolymph of insects generally lacks both respiratory pigments and coagulable proteins. The total protein concentration of the plasma, after removal of hemocytes by centrifugation, is generally between 1.0 and 7.0 mg./100 ml. (Florkin, 1935b, 1936).

1. Quantitative Variations during Growth and Development

The proteinemia is subjected to variations that can differ markedly according to the taxa so far considered. The following picture can be drawn at the present time. In the cockroach (Dictyoptera) the total protein level is roughly constant during the end of larval life, but a fall is observed from adult molt throughout adult life. In the Diptera (*Culex, Phormia, Drosophila*) and in the Lepidoptera (*Bombyx, Samia, Galleria*), the total protein concentration is low at the beginning of the larval development and rises toward the end of larval life, together with an increase of the blood volume; the metamorphosis is characterized by a marked decrease of protein concentration through the adult stage, sometimes interrupted by periods of slight increase (Drilhon, 1954;

Denucé, 1957; Chen, 1956, 1958; Chen and Levenbook, 1966a; Laufer, 1960b).

Diapause may affect the proteinemia in the Lepidoptera *Ostrinia nubilalis* (Chippendale and Beck, 1966) but has no apparent effect on the level of hemolymph protein concentration in *Hyalophora cecropia* (Telfer and Williams, 1953). The particular case of proteinemia modifications in relation to caste differentiation and development has been studied by Liu and Dixon (1965) in the honeybee.

2. Identification of Protein Fractions

For about 20 years, the proteins of the insect hemolymph have been fractionated by paper or starch electrophoresis. The number of protein fractions is highly variable, not only according to the species so far considered (from two to twenty different fractions), but also within a single species, according to sex or ontogenetic stage. On the basis of their electrophoretic mobilities, these fractions have formerly been characterized as albumins, α- and β-globulins, etc., by comparison with the proteins of vertebrate blood. This procedure, criticized by Denucé (1957), has been given up by recent workers.

According to Siakotos (1960), most of the protein fractions are lipoproteins or glycolipoproteins containing protein, phospholipid, and carbohydrates, but more detailed information on the chemical composition of the hemolymph proteins is still lacking.

Marked changes in the electrophoretic patterns occur during ontogenetic development; they often include disappearance or appearance of some fractions during metamorphosis, indicating the possible morphogenetic role of some proteins. The ontogenetic patterns of hemolymph proteins have been characterized in *Hyalophora cecropia* and *Samia cynthia* by Laufer (1960) and in *Phormia regina* by Chen and Levenbook (1966a). The comparison of the protein electrophoretic patterns of the hemolymph of related species has been used frequently for taxonomic purposes.

3. Protein Pool of the Hemolymph as a Reserve

As Heller (1932) already pointed out, the proteins of the hemolymph can be utilized directly as a source of material for new protein synthesis, especially by the tissues of the developing adult in the pupa or nymph. This has been recently confirmed by experiments using radioactive labeled proteins in *Phormia regina* (Chen and Levenbook, 1966b) or in the fifth instar larvae of *Locusta migratoria* (Tobe and Loughton,

1969). This explains the decrease of protein concentration observed during the end of the pupal or nymphal life in many insects.

On the other hand, the hemolymph proteins can be sequestered by a number of tissues, mainly by the fat body, especially before pupation; they are stored within these tissues in the form of protein granules (Locke and Collins, 1966, 1967). Storage of two different protein components of the hemolymph by the fat body of *Pieris brassicae* before pupation has also been demonstrated by Chippendale and Kilby (1969).

The proteins of the hemolymph are not directly utilized for cuticle formation in *Rhodnius prolixus* (Coles, 1965), but the new adult cuticle formed at the end of the fifth larval instar by locusts is extensively labeled when these nymphs are injected with tritiated protein or leucine at the beginning of the instar (Tobe and Loughton, 1969).

4. Hemolymph Proteins and Vitellogenesis

The total hemolymph protein concentration decreases in the females of many insects, especially in cockroaches, during the vitellogenesis and the maturation of the oocytes, while it remains nearly constant in the males. The level of total protein then increases during the gestation period. These variations are not due to modifications of the hemolymph volume (Scheurer and Leuthold, 1969). Figure 6 shows the variations of protein concentration, hemolymph volume, and total hemolymph proteins during the sexual cycle of the females of the cockroach *Leucophaea maderae*.

By the use of autoradiographic methods, it has been confirmed that blood proteins are incorporated into the yolk of the oocytes. (Brier, 1962; Telfer and Melius, 1963; Ramamurty, 1964). The uptake of hemolymph proteins is a selective process; only some of the numerous different protein components of the hemolymph are actually absorbed by the oocytes (Telfer, 1960; Hill, 1962; Engelmann and Penney, 1966; Scheurer, 1969b). Moreover, a particular protein component is found exclusively in the hemolymph of the females, or at least at a level one thousand times higher in the females than in males (*Hyalophora cecropia*, Telfer, 1954; *Leucophaea maderae* fraction G, Scheurer, 1969a). Similarly, a lipoprotein fraction, more highly concentrated in *Periplaneta americana* females than in males, has been characterized by its neutral lipid and sterol content (Siakotos ,1960). This female protein is incorporated in the oocytes at the highest rate; its synthesis seems to be controlled by a hormone produced by the corpora allata (Sláma, 1964; Engelmann and Penney, 1966; Adiyodi, 1967; Scheurer, 1969a).

According to whether the mature female feeds or not, this female protein decreases in the hemolymph during egg maturation in the moth

Hyalophora cecropia (Telfer, 1954), or increases during the same period in the cockroach (Scheurer, 1969b). Experimental results suggest that the depletion of the female protein in moths is "one of the primary causes of the reduced vigor with which the later oocytes grow" (Telfer and Rutberg, 1960).

5. Influence of Diet

The level of the proteinemia decreases when newly emerged houseflies (*Musca domestica*) are carbohydrate fed but increases when they are milk fed. This increase is more highly pronounced in females than in males and mainly concerns one typical electrophoretic fraction, which could be the sex protein involved in the development of the ovaries or of the eggs (Bodnaryk and Morrison, 1966).

6. Proteins Associated with Molting Cycle

In *Locusta migratoria migratorioides*, among the nineteen protein fractions of the hemolymph electrophoretic pattern, one particular band regularly appears immediately prior to each molt and disappears soon thereafter, being absent during all the intermolt period and during the whole adult instar (McCormick and Scott, 1966a,b). In *Periplaneta americana*, in which a protein fraction is also said to be associated with the molting cycle (Steinhauer and Stephen, 1959), the modifications in the electrophoretic pattern suggest a definite relation between hemolymph proteins and cuticle (Fox and Mills, 1969). It has been postulated that this hemolymph protein involved in the molting process could be tyrosinase (Fox and Mills, 1969).

7. Proteins Associated with Spinning

In the hemolymph of the larvae of *Formica rufa* (Schmidt, 1965), and of *Bombyx mori* (Groulade *et al.*, 1961), a specific band of proteins is present just before and during spinning, suggesting that a protein fraction could be directly associated with silk production. It must be recalled, however, that the silk proteins are entirely synthesized by the silk glands from free amino acids absorbed from the hemolymph (see Section XI,C,3).

8. Enzymes

A high number of different enzymatic activities have been identified in the insect hemolymph; it appears that enzymes represent an important part of the blood proteins (Laufer, 1960). It has been suggested (Wyatt, 1961) that most of the enzymes present in the hemolymph result from

a. Protein concentration
   (118 determinations)
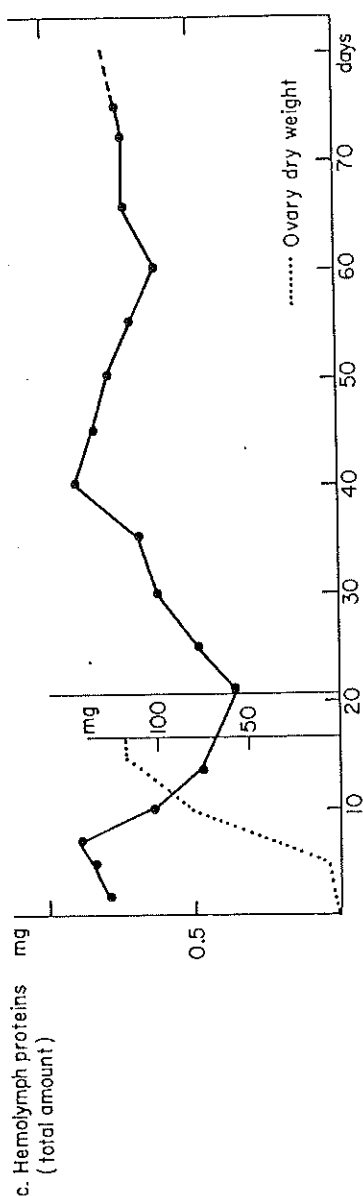
b. Hemolymph volume
   (191 determinations)
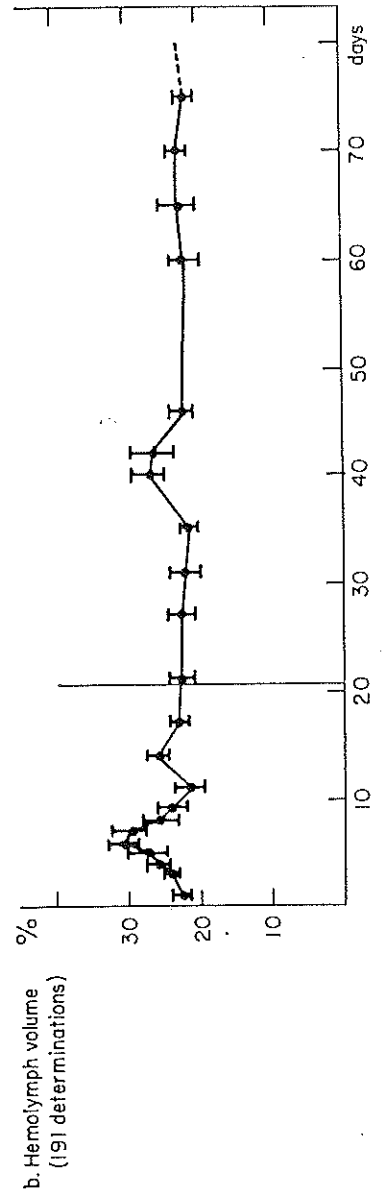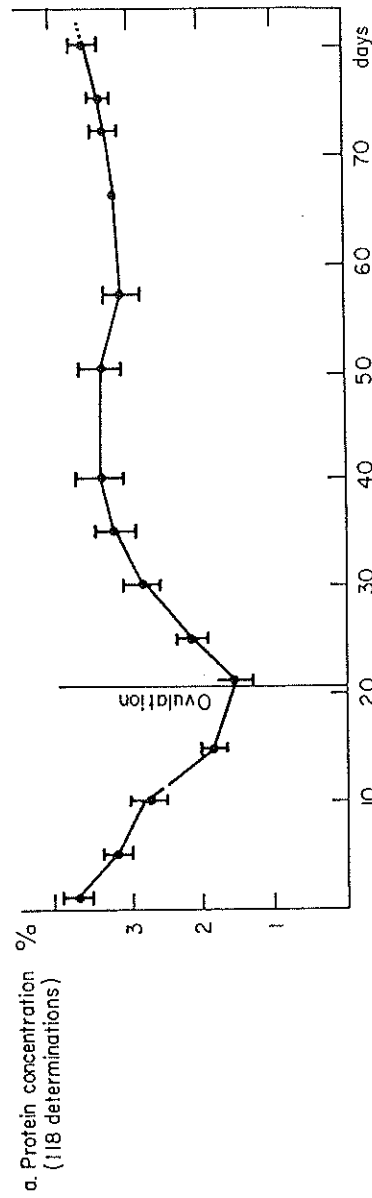
FIG. 6. Variation of (a) the total hemolymph protein concentration, (b) the total hemolymph volume, and (c) the total protein amount of hemolymph per individual during the sexual cycle of *Leucophaea maderae* females. (From Scheurer and Leuthold, 1969.)

a leakage from tissues, as in vertebrates. However, it must be mentioned that some of the enzymatic activities are higher in the hemolymph than in the tissues. Moreover, it has been shown, in *Bombyx mori*, that the amylases of the hemolymph and those of the gut are quite different isoenzymes (Ito *et al.*, 1962). Finally, a definite role in histolysis during metamorphosis can be attributed to hemolymph enzymes such as proteolytic enzymes (Laufer, 1961b) or chitinases (Jeuniaux, 1961a).

The enzymes detected so far in the insect hemolymph are mainly oxidoreductases or hydrolases. We shall refer below to the Enzyme Nomenclature (1965).

Oxidoreductases with NAD as acceptor are commonly found in the insect hemolymph (Laufer, 1961b; Prota, 1961; Chippendale and Beck, 1966): a lactate dehydrogenase (E.C. 1.1.1.27) in *Tenebrio molitor, Hyalophora cecropia*, and *Samia cynthia*; a malate dehydrogenase ("malic enzyme", E.C. 1.1.1.38 or 39) in the same species and in *Ostrinia nubilalis*; an isocitrate dehydrogenase (E.C. 1.1.1.42 or 43); a glutamate dehydrogenase (E.C. 1.4.1.2 or 3) in *T. molitor*; and a glucose dehydrogenase (E.C. 1.1.1.47) in *H. cecropia*. Other types of oxidoreductases have also been found in *T. molitor*: a glycerolphosphate dehydrogenase (E.C. 1.1.99.5) and a xanthine oxidase (E.C. 1.2.3.2) (Prota, 1961). A catalase (E.C. 1.11.1.6) is present in the blood of *B. mori* (Matsumura, 1935). These enzymes are present in the form of multiple isoenzymes, the quantitative variations of which have been followed throughout the life cycle of two moth species by Laufer (1960, 1961b).

o-Diphenoloxidases (E.C. 1.10.3.1) are universally found in the hemolymph of insects; they are responsible for the darkening of the blood when it is exposed to air. In Lepidoptera and Diptera these *o*-diphenoloxidases are transported by the hemolymph in the form of inactive precursors (Ohnishi, 1959; Hackman and Goldberg, 1967; J. J. T. Evans, 1967). The synthesis of the activator of this proenzyme is controlled by the hormone ecdysone (Karlson and Schweiger, 1961).

A unique transferase, an aspartate amino transferase (E.C. 2.6.1.1.) has been detected in the hemolymph of *Celerio euphorbiae* and of *B. mori* but with low activities when compared to muscles (Bheemeswar and Sreenivasaya, 1952; Belzecka *et al.*, 1959).

Hydrolases are abundant: amylases, esterases, and proteolytic enzymes of different types do generally occur in the hemolymph of insects (Arvy and Gabe, 1946a,b; Laufer, 1960b, 1961a; Clements, 1967; Tripathi and Dixon, 1963). *α*-Glucosidase (E.C. 3.2.1.20) also occurs in *B. mori* (Yamafuji, 1934), as well as chitobiase (E.C. 3.2.1.29) during the whole life, and chitinase (E.C. 3.2.1.14) exclusively during the pupal life (Jeuniaux, 1961a, 1963). Chitinases have also been observed in the

hemolymph of *Periplaneta americana* (Waterhouse and McKellar, 1961); they exhibit lysozyme-like activities (Powning and Irzykiewicz, 1967).

Trehalase (E.C. 3.2.1.28) is probably a permanent constituent of the protein pool of the hemolymph (see Section V,C).

A number of phosphatases have been identified in the hemolymph of different species of Lepidoptera (Itabashi *et al.*, 1953; Laufer, 1960).

Finally, deoxyribonucleases resembling DNase II of vertebrates (E.C. 3.1.4.6) have been isolated by zone electrophoresis from the blood of diapausing pupae of *Hyalophora cecropia* (Riechers *et al.*, 1969); they seem to be complexed with protein pigments.

XIII. Other Nitrogenous Constituents

The hemolymph of Arthropoda is, of course, the carrier of all the end products of cellular metabolism. Thus, the nonprotein and non-amino acid nitrogenous fraction of the hemolymph directly depends upon the nature of the nitrogen metabolism (see Chapter 7 of Volume V).

The blood of Crustacea Decapoda contains variable amounts of urea (0.1–10 mg. nitrogen per 100 ml.) and small amounts of uric acid (0.1–1.8 mg. nitrogen per 100 ml.), ammonia (0.3–2.5 mg. nitrogen per 100 ml.), and purines (0.4–2.1 mg. nitrogen per 100 ml.) (for a detailed tabulation of these data, see Florkin, 1960).

In the hemolymph of insects, uric acid is universally present, often very concentrated, sometimes saturated; indeed, crystals are often found in the hemolymph. The other nonprotein nitrogenous constituents of the blood are allantoin, allantoic acid, urea, and ammonia. Urea probably derives from arginine due to the action of an arginase present in the tissues, while allantoicase is lacking (Garcia *et al.*, 1956; Kilby and Neville, 1957).

Different kinds of physiological or ecological conditions can modify the concentration of these nitrogenous constituents in the hemolymph. Starvation reduces the concentration of uric acid in some species (Anderson and Patton, 1955; Ludwig and Cullen, 1956) but not in *Rhodnius prolixus* (Barrett and Friend, 1966). Ablation of the silk glands raises the level of uric acid in the hemolymph of *Bombyx mori* (Buonocore and Magnani, 1958). Molting increases the concentration of uric acid in *Rhodnius prolixus* (Barrett and Friend, 1966). According to Wang and Patton (1969), the amount of urea in the hemolymph changes with metamorphosis in the cricket *Acheta domesticus* (29.81 mg. per 100 ml. in the larvae, and 72.33 mg. per 100 ml. in the adult); this is also the case for uric acid (12.69 mg. per 100 ml. in larvae, 23.24 mg. per

100 ml. in adults), while ammonia remains at a low and constant level (7.08 and 7.32 mg. per 100 ml.).

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