Reprinted from:
THE PHYSIOLOGY OF INSECTA, VOL. V
2nd Edition

ACADEMIC PRESS, INC.
New York San Francisco London

Chapter 6

HEMOLYMPH: COMPOSITION

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I. Introduction

Insects are known to possess only one extracellular fluid circulating throughout the body. It flows within a system of cavities which do not correspond to primitive coelomic spaces, but to sinuses burrowed in the body tissues. The cells are thus in direct contact with the blood. The term "hemolymph" is therefore more accurate to designate this body fluid.

Insects have given up the physiological association between respiratory and circulatory systems; the tracheal system insures the arrival of oxygen in the immediate vicinity of the cells. The hemolymph is thus not concerned either with oxygen transport or with the transport of CO_2. Exceptions to this rule are found in the case of some chironomids, the hemolymph of which contains a hemoglobin.

However, this is not the only peculiarity of insect hemolymph; data accumulated mainly during the last decade have revealed that it is entirely different, especially biochemically, from the body fluids of all other animal phyla. The most striking peculiarities of insect hemolymph are the following, as will be emphasized in this chapter: (1) the tendency to replace the inorganic osmolar effectors, usually Na^+ and Cl^-, by organic molecules; (2) the very special pattern of cationic composition characterizing several orders; (3) the unique form of hemolymph carbohydrate, namely, trehalose; and (4) the presence of organic phosphates and of a wide variety of enzymes.

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

In the present chapter, we shall pay particular attention to some aspects of the physiological role and the adaptive significance of the main biochemical constituents of insect hemolymph, considered especially from

an ecobiochemical point of view. Clotting in the hemolymph is discussed in a separate chapter (see Grégoire, Chapter 7, this volume). Other physical or chemical properties of insect hemolymph, such as specific gravity, surface tension, gas content and transport, hydrogen ion concentration, and oxidation–reduction, will be omitted from the present chapter, since little recent information has been made available on these subjects subsequent to the reviews of Buck (1953) and Wyatt (1961).

II. Hemolymph Volume

The volume of the hemolymph varies widely according to age and developmental stages. As a rule the hemolymph volume increases, with respect to the dry weight of the body, during the end of every larval instar, until after ecdysis. The resulting increased hydrostatic pressure contributes to the rupture of the old cuticle and the expansion of the new integument prior to hardening. A short time after ecdysis, the hemolymph volume decreases sharply (Loughton and Tobe, 1969).

The hemolymph also plays a role as a reserve of water for tissues (Mellanby, 1939); thus, when locusts are fed a very dry food, there is a loss of blood volume, but no loss of cellular water (Lee, 1961).

III. Osmotic Pressure

The osmotic pressure, expressed in terms of freezing-point lowering, generally ranges from $-0.5°$ to $-0.9°C$ (Sutcliffe, 1963). Minimal values have been obtained in the case of *Ephemera danica* larvae ($-0.504°C$), of three Trichoptera larvae ($-0.38°$ to $-0.455°C$) and of *Tipula montium* larvae ($-0.443°C$). Higher values have been observed in the larvae of *Popillia japonica* ($-1.03°C$) and *Ephestia kühniella* ($-1.130°C$).

Generally, insects are able to regulate the osmotic pressure of the hemolymph. For instance, during dehydration, the hemolymph volume decreases, but the osmotic pressure remains constant, due to a concomitant lowering of the concentration of solutes. The reverse phenomenon is observed during rehydration (Djajakusumah and Miles, 1966).

Different cases can be observed during metamorphosis. In *Bombyx* mori (Jeuniaux *et al.*, 1961) and *Antheraea pernyi* (Fyhn and Saether, 1970), the osmotic pressure almost remains at a constant level throughout the development, thanks to a regulation of the concentration of the free amino acids of the hemolymph. On the contrary, in *Galleria mello-*

nella, the osmotic pressure decreases sharply prior to pupal molt and during pupal life, and increases at the time of adult ecdysis (Czaja-Topinska and Klekowski, 1970). Some insects show a considerable increase of the hemolymph osmotic pressure during overwintering, owing to the accumulation of glycerol *(Monema flavescens,* Ashahina *et al.,* 1954).

IV. Osmolar Effectors

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

As Sutcliffe (1962, 1963) has pointed out, and as it appears from examination of Figs. 1 and 2, the participation of inorganic cations and anions in the osmotic pressure of the hemolymph tends to decrease with the evolutionary (phylogenetic) level of the insect. Among the most primitive Insecta (Apterygota), *Petrobius maritimus* shows a hemolymph composition very similar to that of other arthropods, with the nearly exclusive participation of Na^+ and Cl^- as osmotic effectors (Lockwood and Croghan, 1959). In most primitive pterygote Insecta, all of which are exopterygotes (Ephemeroptera, Odonata, Dictyoptera, Heteroptera, and, to a lesser extent, in Orthoptera, Isoptera, and Dermoptera), the sum of the four cations contributes to nearly half of the osmotic pressure, Na^+ playing the principle role while the concentrations of K^+, Ca^{2+}, and Mg^{2+} are very low. In these orders, chloride is the main anion, inorganic phosphates and organic molecules being in low concentration. In these insects, the situation is not very different from that found in other animals, and Sutcliffe (1963) has suggested that "hemolymph with this type of composition represents the basic type of hemolymph in pterygote insects."

In the Phasmidae, the situation is very similar, but Mg^{2+} takes the place of Na^+ as the principal osmotic effector, and inorganic phosphates are more abundant.

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

In Lepidoptera, Hymenoptera, and many Coleoptera, the importance

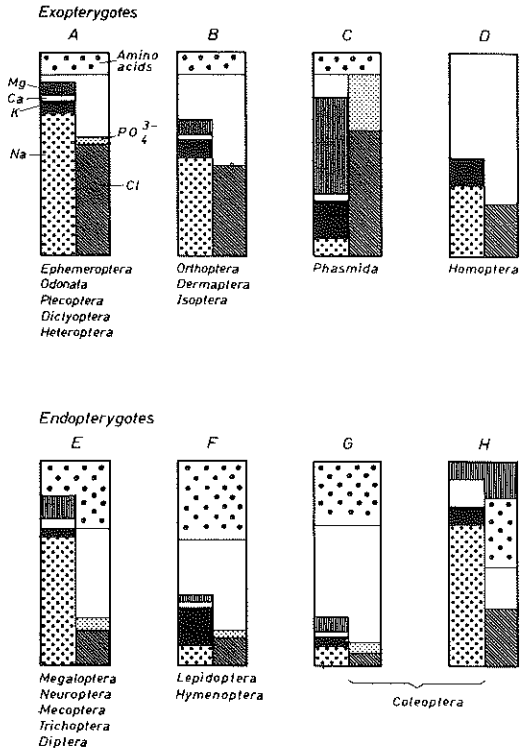

Fig. 1. Osmotic effects of components illustrated as percentages of the total osmolar concentration of hemolymph in pterygote insects. Each block in the figure is visualized as two vertical sections, each section representing 50% of the total osmolar concentration. The percentage contributions of cations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

of cations, as well as of chloride, is considerably reduced, organic molecules playing the main role as osmolar effectors. These groups, in which the highest values of amino acid participation are found, are also recognized by Duchâteau *et al.* (1953) as being highly specialized by the existence of very low values of the Na^+ index, and of very high values of the Mg^{2+} and K^+ indices.

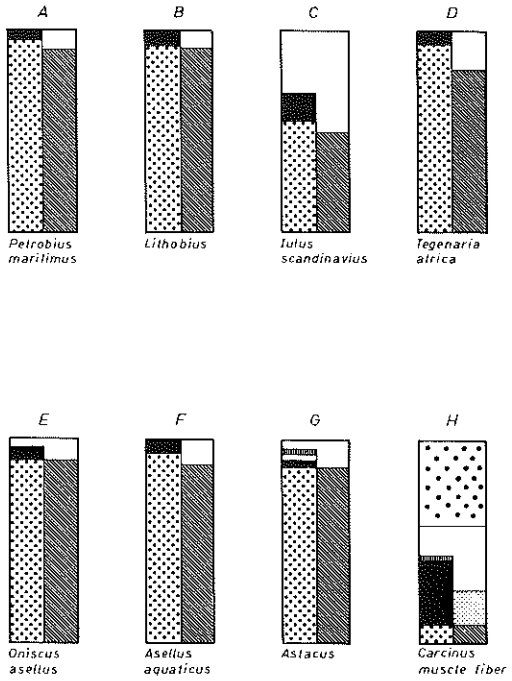

Fig. 2. Osmotic effects of components illustrated as percentages of the total osmolar concentration of blood in (A) an apterygote insect, (B) a chilopod, (C) a diplopod, (D) an arachnid, (E)–(G) crustaceans. (H) illustrates the osmotic effects of components in the muscle fiber of *Carcinus maenas*. Conventions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

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

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

In the matter of osmotic regulation, some insects are not able to control the concentration of inorganic ions in their hemolymph when placed in a more diluted or concentrated medium. However, osmoregulation takes place to some extent through the modification of the aminoacidemia. This is the case for dragonfly larvae and for *Dytiscus marginalis* adults (Schoffeniels, 1960).

V. Inorganic Cations

A concept commonly current among biochemists is that the inorganic composition of the medium of the cells has to comply with definite relative proportions of Na, K, Ca, and Mg if they are to be capable of maintaining these cells in life (Baldwin, 1948, 1962). Insect hemolymph contradicts this statement in many cases, as shown by the data presented in Table I.

Table I is an exhaustive recapitulation of the numerous data reported by different authors. The results are expressed in milliequivalents per liter, and in percent of the sum total of all cations ("indices"). For each order, the data concerning larval, pupal, and adult stages are presented separately. The significance of the different types of cationic patterns may be discussed from several points of view.

A. Ontogenic Modifications of Cationic Pattern

It must be emphasized that a definite picture of the hemolymph cationic patterns is still difficult to present for each order, owing to the lack of representative data for the various developmental stages. With the exception of a few species, data have been accumulated for only one stage in

TABLE I

INORGANIC CATIONS IN THE INSECT HEMOLYMPH

Insect	mEq/liter Na	K	Ca	Mg	Sum of cations	Indices (% of the sum) Na	K	Ca	Mg	References
APTERYGOTES										
Petrobius maritimus	208	5.8								Lockwood and Croghan (1959)
EXOPTERYGOTES										
Paleoptera										
Ephemeroptera										
Larvae : *Ephemera danica*	103	18								Sutcliffe (1962)
Odonata										
Larvae : *Aeschna grandis*	145	9	7.5	7.5	169	87.7	5.3	4.4	4.4	Sutcliffe (1962)
A. cynaea	142	8	—	—						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.	179.3	4.5	20.4	12.3	216.5	82.8	2.1	9.4	5.7	Duchâteau *et al.* (1953)
Libellula depressa	178.3	3.8	18.4	12.0	212.5	83.9	1.8	8.7	5.6	Duchâteau *et al.* (1953)
Agrion (Calopteryx) sp.	158.0	9.0	—	—						Boné (1944)
Agrion virgo	140	8	—	—						Sutcliffe (1962)
Enallagma cyathigerum	139	14	—	—						Sutcliffe (1962)
Adults : *Aeschna cyanea*	120	21	—	—						Sutcliffe (1962)
Agrion virgo	145	17.5	—	—						Sutcliffe (1962)
Polyneoptera										
Dictyoptera										
Larvae : *Periplaneta americana*	100	15.4	3.3	—						Tobias (1948a)
Adults : *Periplaneta americana*	157	7.6	4.2	5.4	174.2	90.1	4.3	2.4	3.1	Van Asperen and Esch (1956)
Periplaneta americana	—	—	8.5	22.9						Clark and Craig (1953)
P. australasiae	—	—	19.4	14.8						Van Asperen and Esch (1954)
Blabera fusca	—	—	20.2	15.7						Van Asperen and 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 (1953)
Plecoptera										
Larvae : *Perla bipunctata*	127	12	—	—						Sutcliffe (1962)
Dinocras cephalotes	117	10	—	—						Stucliffe (1962)
Cheleutoptera										
Adults : *Carausius morosus* ("serum")	11	18	7	108	144	7.6	12.5	4.8	75	Ramsay (1955a,b)
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)
Orthoptera										
Larvae : *Chorthippus parallelus*	72	30	—	—						Sutcliffe (1963)
Locusta migratoria migratorioides	60.0	12.0	17.2	24.8	114.0	52.6	10.5	14.9	21.9	Duchâteau *et al.* (1953)
Schistocerca gregaria	81.3	5.3	17.8	34.6	139.0	58.6	3.8	12.8	24.9	Duchâteau *et al.* (1953)

(continued)

TABLE I *(continued)*

Insect	mEq/liter Na	K	Ca	Mg	Sum of cations	Indices (% of the sum) Na	K	Ca	Mg	References
Adults : *Anabrus simplex*	21.9	15.4	3.0	1.4	41.7	52.5	36.9	7.2	3.4	Pepper *et al.* (1941)
Chortophaga viridifasciata	108.9	3.4	2.8	21	136.1	80	2.5	2.0	15.4	Barsa (1954)
Cryllotalpa 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)
Locusta migratoria migratorioides	75–102	15–22								Ramsay (1953)
Romalea microptera	56.5	17.9	—	—						Tobias (1948b)
Stenobothrus stigmaticus	61.0	62.0	—	—						Boné (1944)
Stenopelmatus longispina	—	—	12.1	29.2						Clark and Craig (1953)
Tettigonia viridissima	83.0	51.0	—	—						Boné (1944)
Dermaptera										
Adults : *Forficula auricularia*	96.0	13.0	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*	142.0	8.0	—	—						Boné (1944)
Notonecta kirbyii	—	—	31.0	18.5						Clark and Craig (1953)
Notonecta obliqua	155	21	—	—						Sutcliffe (1962)
Corixa punctata	112	31	—	—						Sutcliffe (1962)
Hesperocorixia larigata	—	—	7.8	3.5						Clark and Craig (1953)
Rhodnius prolixus	158.0	4.0–6.0	—	—						Ramsay (1953)
Triatoma infestans	—	—	40.9	1.5						Clark and Craig (1953)
Triatoma megista	133.0	5.0	—	—						Boné (1944)
Triatoma neotomae	—	—	16.5	1.0						Clark and Craig (1953)
Triatoma phyllosoma	—	—	13.3	1.2						Clark and Craig (1953)
Triatoma protracta	—	—	29.5	1.3						Clark and Craig (1953)
Cimex lectularius	139.0	9.0	—	—						Boné (1944)
Oncopeltus fasciatus	—	—	13.9	52.1						Clark and Craig (1953)
Palomena prasina	22.0	42.0	—	—						Boné (1944)
ENDOPTERYGOTES										
Oligoneoptera										
Megaloptera										
Larvae : *Sialis lutaria*	109	5	15	38	167	65.2	3	9	22.7	Shaw (1955)
Planipennia (=Neuroptera)										
Larvae : *Myrmeleon formicarius*	143.5	8.7	12.1	31.3	195.6	73.3	4.4	6.1	16.2	Duchâteau *et al.* (1953)
Adults : *Osmylus fulvicephalus*	92	40	—	—	—	—	—	—	—	Sutcliffe (1963)
Mecoptera										
Adults : *Panorpa communis*	94	38	—	—	—	—	—	—	—	Sutcliffe (1963)

(continued)

TABLE I *(continued)*

Insect	mEq/liter Na	K	Ca	Mg	Sum of cations	Indices (% of the sum) Na	K	Ca	Mg	References
Trichoptera										
Larvae : *Anabolia nervosa*	101	17	—	—	—	—	—	—	—	Sutcliffe (1962)
Chaetopteryx villosa	63.9	9	—	—	—	—	—	—	—	Boné (1944)
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	9.3	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	9.6	16.0	10.5	135.4	73.3	7.1	11.8	7.7	Beaujot *et al.* (1970)
Oligotricha ruficrus	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 montium*	115	7	—	—						Sutcliffe (1952)
Tipula paludosa + *oleracea*	84.8	8.2	12.3	16.0	121.3	69.9	6.8	10.1	13.2	Duchâteau *et al.* (1953)
Dictenidia bimaculata	39.6	3.7	13.8	14.5	71.6	53.3	5.2	19.3	20.2	Duchâteau *et al.* (1953)
Chironomus sp.	104.3	2.1	10.5	14.6	131.5	79.3	1.6	8.0	11.1	Duchâteau *et al.* (1953)
Tabanidae gn. sp.	151.0	5.0	—	—						Ramsay (1944)
Pegomya sp.	26.0	58.0	—	—						Boné (1944)
Eristalomyia tenax	100.0	7.9	12.0	13.3	133.2	75.1	5.9	9.0	10.0	Duchâteau *et al.* (1953)
Gasterophilus intestinalis	206.0	13.0	7.0	38.0	264.0	78.0	4.9	2.6	10.6	Levenbook (1950)
Calliphora erythrocephala	148.0	37.0	—	—						Boné (1944)
Pupae : *Calliphora erythrocephala*	139.6	26.1	20.8	34.3	220.8	63.2	11.8	9.9	15.6	Duchâteau *et al.* (1953)
Adults : *Stomoxys calcitrans*	128.0	11.0	—	—						Duchâteau *et al.* (1953)
Eristalis tenax	193.2	20.9	—	—						Florkin and Jeuniaux (1964)
Lepidoptera										
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)
Yponomeuta evonymella	3.2	23.3	17.1	29.7	73.3	4.4	31.8	23.3	40.5	Duchâteau *et al.* (1953)
Nymphula nymphaeata	40	29	—	—						Sutcliffe (1962)
Ephestia kuehniella	32.6	32.7	41.2	51.1	157.6	20.7	20.8	26.1	32.4	Duchâteau *et al.* (1953)
Galleria mellonella	26.5	36.3	24.4	33.3	120.7	22.0	30.1	20.2	27.7	Duchâteau *et al.* (1953)
Phalera bucephala	5.9	49.2	34.2	79.8	169.1	3.5	29.1	20.2	47.2	Duchâteau *et al.* (1953)
Euproctis chrysorrhoea	17.9	44.5	20.6	87.9	170.9	10.5	26.0	12.1	51.4	Duchâteau *et al.* (1953)
Phryganidia californica	—	—	8.5	52.1						Clark and Craig (1953)
Apamea sordens	—	38.7	17.1	56.8						Duchâteau *et al.* (1953)
Laphygma exigua	—	—	5.4	56.1						Clark and Craig (1953)

(continued)

TABLE I *(continued)*

Insect	mEq/liter Na	K	Ca	Mg	Sum of cations	Indices (% of the sum) Na	K	Ca	Mg	References
Prodenia praefica	—	—	6.5	64.3						Clark and Craig (1953)
Phlogophora meticulosa	12.3	34.9	35.5	68.4	151.1	8.1	23.1	23.5	45.3	Duchâteau *et al.* (1953)
Prodenia eridania	22.3	39.7	18.4	14.3	94.7	23.5	41.9	19.4	15.1	Babers (1938)
Peridoma margaritosa	—	—	8.7	85.2						Clark and Craig (1953)
Estigmene acraea	—	—	5.1	10.2						Clark and Craig (1953)
Amathes xanthographa	24.1	29.2	40.4	104.2	197.9	12.2	14.8	20.4	52.6	Duchâteau *et al.* (1953)
Triphaena pronuba	16.1	35.6	56.0	70.9	178.6	9.0	19.9	31.4	39.7	Duchâteau *et al.* (1953)
Barathra brassicae	4.3	53.6	17.9	99.2	175.0	2.5	30.6	10.2	56.7	Duchâteau *et al.* (1953)
Diataraxia oleracea	13.1	43.1	31.9	78.7	166.8	7.9	25.8	19.1	47.2	Duchâteau *et al.* (1953)
Melanchra persicariae	10.8	40.3	19.1	79.0	149.2	7.2	27.0	12.8	53.2	Duchâteau *et al.* (1953)
Hypocrita jacobaeae	7.3	34.6	25.0	86.7	153.6	4.8	22.5	16.3	56.4	Duchâteau *et al.* (1953)
Spilosoma lutae	3.3	56.2	31.4	38.5	129.4	2.6	43.4	24.3	29.7	Duchâteau *et al.* (1953)
Bombyx mori : 3rd instar	3.4	41.8	24.5	80.8	150.5	2.2	27.8	16.3	53.7	Duchâteau *et al.* (1953)
Bombyx mori : 4th molt	6.0	39.4	15.0	88.0	148.4	4.0	26.5	10.1	59.3	Bialaszewicz and Landau (1938)
Bombyx mori : 5th instar	[illegible]									Duchâteau *et al.* (1953)
Bombyx mori : prenymphs	8.2	59.2	26.5	92.5	186.4	4.3	32.1	14.2	44.2	Duchâteau *et al.* (1953)
Antheraea mylitta	1.3	49.7	21.9	37.7	110.6	1.2	44.9	19.8	34.1	Duchâteau *et al.* (1953)
Actias selene	4.8	51.3	25.5	60.0	141.6	3.4	36.2	18.0	49.4	Duchâteau *et al.* (1953)
Sphinx ligustri	—	34.7	30.5	57.5						Duchâteau *et al.* (1953)
Pieris rapae	11.0	39.0	—	—						Boné (1944)
Pieris rapae	—	96.4	41.0	66.6						Duchâteau *et al.* (1953)
Pieris brassicae	—	19.7	16.6	92.5						Brecher (1929)
Pieris brassicae	9.0	30.0	—	—						Ramsay (1953)
Aglais (Vanessa) urticae	22.0	43.0	—	—						Boné (1944)
Junonia coenia	—	—	5.2	29.1						Clark and Craig (1953)
Papilio machaon	13.6	45.3	33.4	59.8	152.1	8.9	29.8	22.0	39.3	Duchâteau *et al.* (1953)
Pupae : *Dasychira pudibunda*	3.0	51.8	33.9	74.1	162.8	1.9	31.8	20.8	45.5	Duchâteau *et al.* (1953)
Cucullia absinthii	9.8	48.7	32.5	51.2	142.2	6.9	34.2	22.9	36.0	Duchâteau *et al.* (1953)
Bombyx mori	21.7	54.9	29.5	87.5	193.6	11.2	28.4	15.2	45.2	Duchâteau *et al.* (1953)
Bombyx mori	11.3	41.5	24.0	69.4	146.2	7.7	27.7	16.4	47.4	Bialaszewicz and Landau (1938)
Graellsia isabellae	6.2	46.2	17.5	29.1	99.0	6.3	46.7	17.7	29.3	Duchâteau *et al.* (1953)
Tropaea luna	4.4	52.8	31.4	48.8	137.4	3.2	38.4	22.9	35.5	Duchâteau *et al.* (1953)
Telea polyphemus	2.5	59.3	11.6	73.8	147.2	1.7	40.2	7.8	50.0	Carrington and Tenney (1959)

(continued)

TABLE I *(continued)*

Insect	mEq/liter Na	K	Ca	Mg	Sum of cations	Indices (% of the sum) Na	K	Ca	Mg	References
Samia walkeri	2.6	42.2	18.8	65	128.6	2	32.8	14.6	50.5	Barsa (1954)
Philosamia cynthia	7.5	36.9	28.5	52.1	125.0	6.0	29.5	22.8	41.7	Duchâteau *et al.* (1953)
Philosamia (Samia) walkeri	2.6	42.1	18.8	65.0	128.5	2.0	32.8	14.6	50.6	Gese (1950)
Endromis versicolora	1.3	32.8	28.0	44.0	106.1	1.2	30.9	26.4	41.5	Duchâteau *et al.* (1953)
Smerinthus ocellatus	5.4	34.8	15.5	—						Drilhon (1934)
Hyloicus pinastri	tr.	35.0	15.0	46.0						Brecher (1929)
Sphinx ligustri	2.6	52.8	16.4	49.2	121.0	2.1	43.6	12.6	40.7	Duchâteau *et al.* (1953)
Sphinx ligustri	3.0	54.1	40.9	50.0	148.0	2.0	36.6	27.6	33.8	Duchâteau *et al.* (1953)
Mimas tiliae	3.2	39.2	127.7	15.0	127.7	2.5	30.7	23.3	43.5	Duchâteau *et al.* (1953)
Deilephila elpenor	4.7	27.4	41.0	89.3	142.4	2.9	16.9	25.2	55.0	Duchâteau *et al.* (1953)
Adults : *Barathra brassicae*	15.6	43.9	10.8	38.7	106	14.7	41.4	10.3	33.7	Naoumoff and Jeuniaux (1970)
Bombyx mori	14.3	36.1	14.5	44.6	109.5	13.0	32.9	13.2	40.7	Bialaszewicz and Landau (1938)
Bombyx mori	7.7	33.1	12.0	40.3	99.2	8.3	35.6	12.9	43.2	Naoumoff and Jeuniaux (1970)
Telea polyphemus	tr.	54.1	—	72						Carrington and Tenney (1959)
Coleoptera										
Carabidae										
Adults : *Feronia madida*	133.6	12.6	2.1	21.1	171.6	77.9	7.4	1.2	17.3	Jeuniaux and Naoumoff (1973)
Nebria brevicollis	144.2	11.8	12.0	18.1	186.1	77.5	6.3	6.4	9.7	Jeuniaux and Naoumoff (1973)
Dystiscidae										
Larvae : *Dystiscus* 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	19–35	141–145	63–72	4–5	8–9	13–25	Jeuniaux and Naoumoff (1973)
Dytiscus marginalis ♀♀	92–117	5–8	13–14	19–20	132–156	70–75	3.5–5	9	13–15	Jeuniaux and Naoumoff (1973)
Cybister sp.	143.5	7.3	38.2	51.8	240.8	59.6	3	15.9	21.5	Duchâteau *et al.* (1953)
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 (1973)
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 (1973)
Hydrophilidae										
Adults : *Sphaeridium scarabaeoides*	90	12.6	44	52.5	199.1	45.2	6.7	22.1	26.4	Jeuniaux and Naoumoff (1973)
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	14.6	82.5	168.2	26.8	22	8.7	49.1	Jeuniaux and Naoumoff (1973)

(continued)

TABLE I *(continued)*

Insect	mEq/liter Na	K	Ca	Mg	Sum of cations	Indices (% of the sum) Na	K	Ca	Mg	References
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 (1973)
Cantharis fusca	78.1	6.8	10.5	44.2	139.6	56	4.8	7.5	31.7	Jeuniaux and Naoumoff (1973)
Elateridae										
Adults : *Selatosomus latus*	94.9	19.8	25.2	131.4	271.3	35	7.3	9.3	48.4	Jeuniaux and Naoumoff (1973)
Tenebrionidae										
Larvae : *Tenebrio molitor*	71–75	38.7	11–13	76–83	200–208	36	19	6	38–40	Jeuniaux and Naoumoff (1973)
Pupae : *Tenebrio molitor*	64.3	37.4	13.1	88.1	202.9	31.7	18.5	6.5	43.4	Jeuniaux and Naoumoff (1973)
Adults : *Tenebrio molitor*	87.2	30.1	9.9	58.3	185.4	40.7	16.1	5.3	31.4	Jeuniaux and Naoumoff (1973)
Blaps mucronata	84–85	21–32	11–16	60–85	188–208	44–45	11–17	6–8	32–41	Jeuniaux and Naoumoff (1973)
Lucanidae										
Larvae : *Platycerus caraboides*	13.8	12.6	69	169	264.4	5.2	4.8	26.4	63.9	Jeuniaux and Naoumoff (1973)
Adults : *Platycerus caraboides*	59	4.5	14.6	123.6	201.7	29.3	2.2	7.3	51.3	Jeuniaux and Naoumoff (1973)
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	5.3–24	8–11.7	19–23	108–212	166–270	2.8–14.4	4.2–7	8.3–13.8	65–80	Jeuniaux and Naoumoff (1973)
Adults : *Oryctes boas*	69	13.6	14	57.5	154.1	44.7	8.8	9.1	37.3	Jeuniaux and Naoumoff (1973)
Melolontha melolontha	113	5.8	15.3	41.3	175.4	64.4	3.3	8.7	23.6	Duchâteau *et al.* (1953)
Phytophagoidea										
Chrysomelidae										
Larvae : *Leptinotarsa 10-lineata*	2–3.5	55–65	43–47	147–148	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	17.3	8.6	73.7	Jeuniaux and Naoumoff (1973)
Gastroidea viridula	18.4	40.8	6	118.9	186	9.9	21.9	4.3	63.9	Jeuniaux and Naoumoff (1973)
Curculionidae										
Larvae : *Rhyncophorus palmarum*	3.9	22–44	7–18	70–91	102–160	3–6	22–28	7–11	55–68	Jeuniaux and Naoumoff (1973)
Adults : *Rhyncophorus palmarum*	27.3	26.9	12.5	61.2	127.9	21.3	21.0	9.8	47.8	Jeuniaux and Naoumoff (1973)
Phyllobius urticae	33.1	15	11.2	138	187.2	17.7	8	6	68.4	Jeuniaux and Naoumoff (1973)
Cerambycidae										
Larvae : *Leptura* sp.	13.4	33.8	28	110.2	185.4	7.2	18.3	15.1	59.4	Jeuniaux and Naoumoff (1973)
Ergates faber	16–38	27–37	12–13	75–101	131–190	12–20	19–20	7–9	53–54	Jeuniaux and Naoumoff (1973)
Adults : *Ergates faber*	43.5	23.9	12.9	114.9	195.2	22.3	12.3	6.6	58.8	Jeuniaux and Naoumoff (1973)

(continued)

TABLE I *(continued)*

Insect	mEq/liter Na	K	Ca	Mg	Sum of cations	Indices (% of the sum) Na	K	Ca	Mg	References
Hymenoptera										
Larvae : *Pteronidea ribesii*	1.6	43.4	17.5	60.7	123.2	1.3	35.2	14.2	49.3	Duchâteau *et al.* (1953)
Neodiprion sertifer	3	38	—	—						Sutcliffe (1963)
Vespula germanica	26.0	56.4	18.7	23.6	124.7	20.9	45.2	15.0	18.9	Duchâteau *et al.* (1953)
Apis mellifera	10.9	30.5	18.2	20.5	80.1	13.6	38.1	22.7	25.6	Duchâteau *et al.* (1953)
Apis mellifera	5.0	24.4	7.5	15.8	52.7	9.5	46.3	14.2	30.0	Bishop *et al.* (1925)
Papae : *Formica rufa*	14.7	50.3	14.9	21.6	101.5	14.5	49.6	14.7	21.2	Duchâteau *et al.* (1953)
Vespula germanica	22.8	60.8	11.2	19.0	113.8	20.0	53.4	9.9	16.7	Duchâteau *et al.* (1953)
Adults : *Vespula pensylvanica*	—	—	7.1	1.0						Clark and Craig (1953)
Vespula germanica	93	18.2	1.8	2.6	115.6	80.4	15.7	1.5	2.2	Florkin and Jeuniaux (1963)
Vespula germanica	153.5	21.9	2.2	0.5	178.1	86.1	12.3	1.2	0.3	Florkin and Jeuniaux (1963)
Apis mellifera	47.1	27.1	17.8	1	93	50.6	29.1	19.1	1	Florkin and Jeuniaux (1963)

each order. Table I shows, for instance, that the cationic hemolymph composition of Homoptera and Heteroptera is known only for adults, whereas that of Trichoptera and Lepidoptera is known only for larvae or pupae. This fact seems to have been neglected by many authors, who have discussed the systematic or phylogenetic significance of the hemolymph cationic composition by comparing animals of different ontogenetic positions.

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

However, a reexamination of the situation among Hymenoptera (Florkin and Jeuniaux, 1963), Lepidoptera (Naoumoff and Jeuniaux, 1970), and Coleoptera (Jeuniaux and Naoumoff, 1973) led to the conclusion that, in many species of Endopterygotes, the cationic pattern of the larva is of a more specialized type than that of the adult. In the larval hemolymph, indeed, the Na index is significantly lower and the Mg index is higher than in the adult hemolymph (see, for instance, the cases of *Apis mellifera*, *Vespula germanica*, *Barathra brassicae*, or *Oryctes boas*, in Table I). In such cases, the larval stage can be considered, from a biochemical point of view as well as from morphological considerations, as being an adaptative round-about way, instead of an ontogenetic recapitulation of an ancestral phylogenetic position.

B. Hemolymph Cationic Patterns of the Different Orders

From the data in Table I, one can stress some characteristic patterns, bearing in mind that the sampling is obviously scattered, and that ontogenetic variations are often ignored.

1. Apterygotes: the only important cation is Na.

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

3. Exopterygote Polyneoptera: with the exception of *Carausius*, Na is also the most important ion, but K, Ca, and especially Mg tend to become more concentrated than in Paleoptera. In some cases (*Steno-*

bothrus stigmaticus and *Tettigonia viridissima),* the K^+ concentration is similar to that of Na^+. The situation seems to be the same in larvae and adults.

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

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

5. Among the Endopterygotes, the orders Megaloptera, Neuroptera, Mecoptera, and Diptera are characterized by a hemolymph of the primitive type, in which Na is the main cation (Na indices: from 53 to 79). There seems to be no fundamental difference between the ontogenic stages.

The situation is quite different in the other orders belonging to the Endopterygotes.

6. In Trichoptera, recently studied by Beaujot *et al.* (1970), the concentrations of Na in the larval hemolymph are comprised of between 60 and 90 mEq/liter, but the concentrations of Mg are highly variable, lying between the extreme values of 10.5 and 205 mEq/liter. As far as cationic patterns of larval hemolymph are concerned, Trichoptera appear to occupy an intermediate position between Mecoptera and Lepidoptera, a view which is in good agreement with taxonomic relationships generally admitted within the "panorpoid complex."

7. In Lepidoptera, the evolutionary tendency initiated in Trichoptera is achieved in most species: the sodium index in the larval hemolymph is generally lower than 20%, while the concentrations of magnesium and/or potassium are often very high.

The hemolymph cationic pattern is slightly but significantly modified at the time of metamorphosis. According to its higher Na index and lower Mg index, the cationic pattern of the adult hemolymph corresponds to a less specialized type than that of larvae (Naoumoff and Jeuniaux, 1970).

8. Coleoptera: We propose to consider the existence of three types of cationic patterns in Coleoptera.

In the Adephaga (Carabidae, Dytiscidae), both larval and adult hemolymphs contain a high proportion of Na (Na index: from 60 to 77%) and a low proportion of K, Ca, and Mg, a primitive type of pattern similar to that found in Polyneoptera.

In the Polyphaga (ten families studied so far, excluding the Phytophagoidea), the hemolymph is of a more highly evolved type, characterized by an Na index generally lower than 60% and an Mg index higher than 20%. Differences between larval and adult hemolymphs are slight in the Tenebrionidae, but are clear cut in the Lucanidae and Scarabaeidae.

In the Phytophagoidae (Curculionidae, Chrysomelidae, Cerambycidae), both larval and adult hemolymphs are of a specialized type, with low Na index and high Mg index, but these characteristics are more accentuated in larvae than in adults.

9. In Hymenoptera, particularly in larvae and pupae of Symphyta and Aculeata, the most important cations are K and Mg. The situation is very different in the adult Aculeata, in which the cationic pattern shows a high Na index (50 to 80), less K (index: 12 to 30), and only minute amounts of Ca and Mg.

C. Ion Binding

In order to account for the normally functioning excitable tissues in such insects with a hemolymph rich in K and poor in Na, several authors postulated that an important proportion of the cations do not exist as free ions in the hemolymph, but in a combined form (Barsa, 1954; Bishop *et al.* 1925; Buck, 1953; Clark and Craigh, 1953). Experimental results have shown, however, that cation binding is generally not important. No evidence was detected for any binding of K in the hemolymph of *Antheraea polyphemus,* while only 15–20% of the Ca and Mg were bound to macromolecules (Carrington and Tenney, 1959). In the hemolymph of *Galleria mellonella* larvae, the following proportions of cations bound to proteins have been found: Na—6.6 to 8.8%; K—1.2 to 2.0%; Ca—9 to 13%; Mg—15 to 25% (Plantevin, 1967). In *Carausius morosus,* the Mg bound to proteins accounts for 12% of the total concentration of this ion; the other cations occur almost entirely in a free state. (Ch. Jeuniaux and T. Lenoir, unpublished).

D. Dietetic Relationships

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

Insects, being mainly terrestrial and therefore unable to absorb cations

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

From this survey, it can be seen that the concept, according to which some insects have a high potassium and a low sodium content as a consequence of eating foliar food, while others have a high sodium and a low potassium content because they do not consume this kind of food, is not acceptable.

E. Phylogenetic Relationships

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

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

TABLE II

Comparison between the Cationic Composition of Food and the Hemolymph of Insects

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

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

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

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

F. Adaptive Significance of the Specialized Cationic Pattern

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

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

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

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

It seems, therefore, that the adaptations to an entirely vegetable diet, and to a sedentary life in the midst of food, have been developed independently in different orders, and generally as a particular feature of larval stages. The adult stages generally retain a more primitive cationic pattern. According to their phylogenetic position and to the specialized pattern of both larval and adult hemolymphs, the Coleoptera of the superfamily of Phytophagoidea (Chrysomelidae, Curculionidae, and Cerambycidae) and the Lepidoptera are the most fully adapted to phytophagous habits.

VI. Inorganic Anions and Ion Balance

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

With respect to the concentration of the Cl^- anions, we may recognize two categories: in exopterygotes, the Cl^- concentration is always high (about 100 mEq/liter or more) and neutralizes 50–82% of the total inorganic cation equivalents. In endopterygotes, on the contrary, the Cl^- concentration is generally less than 40 mEq/liter, and its index varies from 5.6 to 36, with the exception of the bee larvae. In the latter case, however, the concentration is not higher than in other endopterygotes, the high index resulting from the very low concentration of inorganic cations.

The part played by the inorganic phosphates (calculated in Table III as $H_2PO_4^-$, a value probably somewhat inferior to the reality) and the bicarbonate ions is of only minor importance in cation binding, with the exception of *Carausius morosus* (Table III) and *Anabrus simplex* (Pepper *et al.,* 1941), in which the phosphates contribute largely to ion balance. Anoxia or storage at low temperatures increases the concentration of inorganic phosphates in the hemolymph of *Anagasta kuehniella* larvae (Sømme, 1966).

The HCO_3^- anion is the principal form of CO_2 transport, and its concentration in the hemolymph is directly proportional to the amount of gaseous CO_2 (Levenbook, 1950b,c, Levenbook and Clark, 1950).

In conclusion, the sum of the anions Cl^-, $H_2PO_4^-$, and HCO_3^- balances approximately the sum of the cations in the hemolymph of exopterygotes. The deficit of anion–cation balance in the hemolymph of

TABLE III

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

Species	Stage	Sum of cations (mEq/liter)	Anions (mEq/liter)			Anions "indices"			References
			Cl^-	$H_2PO_4^-$	HCO_3^-	Cl^-	$H_2PO_4^-$	HCO_3^-	
Exopterygotes									
Odonata: *Aeschna grandis*	Larvae	169	110	4	15	65	2.3	8.8	Sutcliffe (1962)
Dictyoptera: *Periplaneta americana*	Adults	174.2	144	—	—	82.6	—	—	Van Asperen and Esch (1954)
Orthoptera: *Locusta migratoria*	Adults	118.6	97.6	—	—	82.3	—	—	Duchâteau *et al.* (1953) Hoyle (1954)
Cheleutoptera: *Carausius morosus*	Adults	197.4[a]	93[b]	40[c]	—	47.1	20.2	—	Duchâteau *et al.* (1953) May (1935) Ramsay (1955a)
Carausius morosus	Adults	154	101	16	—	65.5	10.4	—	Wood (1957)
Endopterygotes									
Megaloptera: *Sialis lutaria*	Larvae	167[a]	31[a]	5[b]	15[a]	18.5	3	6	Shaw (1955) Sutcliffe (1962)
Diptera: *Gasterophilus intestinalis*	Larvae	264	14.8	4	14.5	5.6	1.5	5.7	Levenbook (1950)
Lepidoptera: *Bombyx mori*	Larvae	150	21	3	—	14	2	—	Buck (1953)
Prodenia eridania	Larvae	94.7	34	5.8	—	35.9	6.1	—	Babers (1938)
Samia walkeri	Pupae	128.6	10.4	3.5	—	8	2.7	—	Gese (1950)
Telea polyphemus	Pupae	147.2	19.5	—	—	13.2	—	—	Carrington and Tenney (1959)
Coleoptera: *Dytiscus marginalis*	Adults	231.6[a]	44[c]	2.8[b]	—	19	1.2	—	Sutcliffe (1962) Buck (1953)
Popillia japonica	Larvae	84.3	19	4.9	—	22.5	5.8	—	Ludwig (1951)
Hymenoptera: *Apis mellifera*	Larvae	52.7	33	10.3	—	62.6	19.5	—	Bishop *et al.* (1925)

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

most endopterygotes reveals the part played by inorganic molecules in the neutralization of the cations. This role seems to be mainly assumed by organic acids, the free amino acids making rather a net contribution to the cationic than to the anionic phase of the hemolymph, according to the pH and the nature of the amino acid concerned (Wyatt, 1961).

VII. Organic Acids

Extraordinarily large amounts of organic acids are found in the insect hemolymph, as has already been stated by Tsuji (1909). The chief organic acids found in the insect hemolymph belong to a group of compounds serving as the substrates of the tricarboxylic acids cycle enzymes i.e., citrate, α-ketoglutarate, succinate, fumarate, malate, and oxaloacetate. These organic acids are generally more abundant in the larval hemolymph of endopterygotes than in the adult hemolymph and than in the hemolymph of exopterygotes.

Citrate seems to be a permanent constituent of the insect hemolymph (Levenbook and Hollis, 1961). In the thirteen species of endopterygotes studied (Coleoptera, Hymenoptera, Diptera, and Lepidoptera), citrate is more concentrated in larvae than in adult hemolymph (for instance, *Phormia regina:* 12.5 m*M* in larvae, with 0.44 and 0.33 m*M* in adults; *Sarcophaga bullata:* 10.3 m*M* in larvae, with 2.6 m*M* in adults; *Prodenia eridania:* 20.5 m*M* in larvae, with 4.7 in adults). Data obtained for exopterygotes are of 0.73 m*M* (*Periplaneta americana* larvae: Levenbook *et al.*, 1961), 1.6 m*M* (*Leptocoris tribittatus:* Levenbook and Hollis, 1961), and 2.3 m*M* (*Rhodnius prolixus:* Patterson, 1956).

Among the other acids of the tricarboxylic acids cycle, α-ketoglutarate, malate, fumarate, succinate, and oxaloacetate have been observed in the larval hemolymph of *Gasterophilus intestinalis* (Levenbook and Wang, 1948; Levenbook, 1950a; Nossal, 1952), *Bombyx mori* (Fukuda and Hayashi, 1953, 1958), *Prodenia eridania* (Levenbook, 1961), and *Hyalophora cecropia* (Wyatt, 1961). The presence of pyruvate is not clearly established in the hemolymph of *Bombyx mori*, but large amounts have been found in *Antherea pernyi* (23–31 m*M*: Burova, 1953), as well as in *Hyalophora cecropia* (Wyatt, 1961). Other organic acids are occasionally present: glyoxylic and acetoacetic acids in *Bombyx mori* larvae (Fukuda and Hayashi, 1958), volatile fatty acids, mainly acetic acid, in *Popillia japonica* larvae (Stubblefield *et al.*, 1966).

These organic acids play an important role in cationic balance, at least in the endopterygote larvae. In *Gasterophilus intestinalis*, the sum of six organic acids so far identified amounts to 123 mEq/liter, and accounts

for 46.5% of the sum of the inorganic cations. In *Hyalophora cecropia,* the total of the different organic acids of the hemolymph amounts to 25–35 mEq/liter. In *Bombyx mori* larvae, citrate alone, amounting to 32.1 m*M* (Levenbook and Hollis, 1961), assumes about 34% of the cation binding.

According to Levenbook and Hollis (1961), the large amount of organic acids in endopterygote larvae is not directly related to alimentary habits. They are undoubtly endogenous in origin. Some modifications can be observed following infection by bacteria, i.e., the concentration of malic, tartaric, pyruvic, and glyoxylic acids increased in the larvae of *Popillia japonica* infected by *Bacillus popilliae* (Stubblefield *et al.,* 1966).

VIII. Organic Phosphates

According to Wyatt (1961), one of the most interesting peculiarities of insects is the high concentration of phosphates in their hemolymph (for instance, 26 to 44 m*M* total acid-soluble phosphorus in *Hyalophora cecropia* pupae, Wyatt *et al.,* 1963). These phosphates are essentially organic in nature, and acid-soluble. An extensive study of organic phosphates by ion-exchange chromatography shows that α-glycerophosphate, phosphorylethanolamine, glycerophosphoethanolamine, phosphorylcholine, sorbitol 6-phosphate and glucose 6-phosphate are the main components, at least in Lepidoptera (Kondo and Watanabe, 1957; Wyatt, 1958; Wyatt and Kalf, 1956, 1958; Wyatt *et al.,* 1963). Their presence in the hemolymph is not the result of a histolysis, but of a biosynthesis, as shown by the use of ^{32}P. Their concentration is greatly modified by metamorphosis, diapause, anoxia, or storage at low temperatures (Wyatt *et al.,* 1963; Sømme, 1966).

IX. Carbohydrates and Related Substances

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

The explanation of such an unusually low concentration of fermentable sugars in an internal medium arose from the discovery, by Wyatt and Kalf (1956, 1957), of the existence in the hemolymph of a nonreducing dimer of α-glucose, namely trehalose, in high concentration. Hemolymph trehalose appears to be a form of carbohydrate transport peculiar to the class Insecta, although this sugar can be found in the hemolymph of some Crustacea *(Homarus vulgaris)* (Telford, 1968).

A. Fermentable Sugars

The data concerning the amount of substances fermentable by yeast are presented in Table IV. The nature of these substances has been determined in only a few instances. In the adult bee, the fermentable substances are fructose and glucose (Von Czarnovsky, 1954; Alumot *et al.,* 1969). Fructose is also present in rather large amounts in the hemolymph of *Gasterophilus intestinalis* (Levenbook, 1947, 1950) and glucose in that of *Phormia regina,* (Evans and Dethier, 1957; Wimer, 1969). In the latter species, maltose also is present in the larval hemolymph (8 to 19 mg/100 ml), and increases sharply at the time of puparium formation (up to 65 mg/100 ml) (Wimer 1969). High amounts of glucose (80% of the total carbohydrates), fructose (5–6% of the total carbohydrates), and small amounts of trehalose, mannose, arabinose, and ribose, have been detected in the larval hemolymph of another Diptera: *Agria affinis* (Barlow and House, 1960). We tentatively propose to consider that the presence of high amounts of reducing mono- or disaccharides in the hemolymph is restricted to a number of species of Hymenoptera and Diptera well-adapted to sustained flight.

The composition of the diet sometimes modifies more or less deeply the concentration or the composition of the carbohydrate fraction of the hemolymph. This has been shown in *Agria affinis* reared on chemically defined diets with variable amounts of glucose (Barlow and House, 1960) and in locusts reared on peas or on growing wheat; in the latter cases, fructose, maltose, or cellobiose are detected in the hemolymph, sometimes at a high level, while trehalose disappears (Hansen, 1964).

B. Trehalose

The concentration of trehalose in a number of representative insects is shown in Table IV. Trehalose is generally present in large amounts in the hemolymph of all the insects studied so far, with the remarkable exceptions of the larvae of *Phormia regina* and *Agria affinis.*

In vertebrates the cells generally contain little glucose since glucose is the circulatory form of the carbohydrate cellular food; it is mainly of en-

TABLE IV

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

Species	Stage	Fermentable sugars (as glucose)	True glucose	Fructose	Trehalose
Dictyoptera					
Periplaneta americana[e]	?	30	—	—	—
Leucophaea maderae[m]	?	65	—	—	580–780
Orthoptera					
Schistocerca gregaria	Larvae	—	—	traces[f]	800–1500[h]
Coleoptera					
Hydrophilus piceus	Adults	5–31[e]	—	—	500–700[b]
Popillia japonica	Larvae	69[k]	—	—	—
Chalcophora mariana	Larvae	—	—	—	4700–5200[b]
Ergates faber	Larvae	—	—	—	3200[b]
Hymenoptera					
Diprion hercyniae[n]	Larvae	—	28	—	926
Apis mellifera	Adults (workers)	1000–4000[p]	600–3200[p]	200–1600[p]	600–1200[b]
Apis mellifera[r]	Adults (queens)		500–860	220–800	560–1200
Lepidoptera					
Phalera bucephala	Larvae	40[g]	—	—	—
Prodenia eridania	Larvae	11[a]	—	—	—
Bombyx mori	Larvae	9–28[d,e]	1–3[e]	1–2[e]	400–500[e]
	Pupae	18–50[d,e]	3–5[e]	1–2[e]	202[e]
	Adults	16[d,e]	—	—	—
Deilephila euphorbiae	Larvae	Traces[g]	—	—	—
Deilephila elpenor	Pupae	—	—	—	800–1900[b]
Galleria mellonella	Larvae	—	21[n]	—	1700[n]
Hyalophora cecropia[n]	Larvae	—	—	—	1200
Hyalophora cecropia	Pupae	—	0–8	—	400–600
	Adults	—	—	—	650–1150
Telea polyphemus[n]	Larvae	—	—		1306
Diptera					
Gasterophilus intestinalis[i,j]	Larvae	95	10	184–294	—
Calliphora erythrocephala[i]	Larvae	—	—	traces	—
Phormia regina[c]	Larvae	—	70–125	—	absent
	Adults	—	up to 600	—	598
Phormia regina[q]	Larvae	—	55–155	—	traces

[a] Babers (1938).
[b] Duchâteau and Florkin (1959).
[c] Evans and Dethier (1957).
[d] Florkin (1936b).
[e] Florkin (1937).
[f] Hemmingsen (1924).
[g] Heller and Moklowska (1930).
[h] Howden and Kilby (1956).
[i] Levenbook (1947).
[j] Levenbook (1950a).
[k] Ludwig (1951).
[l] Todd (1957).
[m] Todd (1958).
[n] Wyatt and Kalf (1957).
[o] Wyatt *et al.* (1956).
[p] VonCzarnowsky (1954).
[q] Wimer (1969).
[r] Alumot *et al.* (1969).

dogenous origin, the product of a gluconeogenesis principally performed by the liver. Glucose enters the cells by crossing the membrane as hexose 6-phosphate. In most insects, the trehalose of the hemolymph is absorbed and used by the cells of most tissues, due to an intracellular trehalase (Howden and Kilby, 1956; Evans and Dethier, 1957; Kalf and Rieder 1958; Bücher and Klinkenberg, 1958; Zebe and McShan, 1959; Clegg and Evans, 1961; Duchâteau-Bosson *et al.*, 1963).

Epidermal cells appear to lack trehalase (Zebe and McShan, 1959; Duchâteau-Bosson *et al.*, 1963). However, they use trehalose not only for metabolic purposes, but also for chitin synthesis (Candy and Kilby, 1961, 1962). As a matter of fact, the hemolymph trehalose is hydrolyzed to glucose at each molt by a trehalase present in the hemolymph. This trehalase is inhibited during the intermolts (Friedman, 1961), but the release of its inhibition at the beginning of every molting period induces a fall of the concentration of the trehalose in the hemolymph (Howden and Kilby, 1956; Duchâteau-Bosson *et al.*, 1963). An example of the quantitative variations of hemolymph trehalose during the larval development is shown in Fig. 3.

In the fat-body, an inverse relationship exists between glycogen and trehalose, the former disappearing almost completely at each molt, while the amount of trehalose tends to remain at nearly constant level

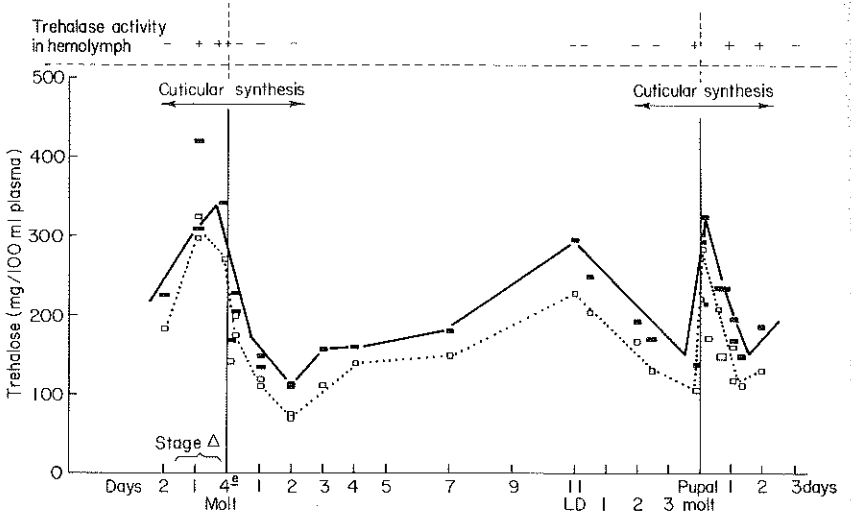

Fig. 3. Modification of trehalose concentration and of trehalase activity in the hemolymph of *Bombyx mori*. (From Duchâteau *et al.* 1963). ■—■: anthrone reactive material, in mg trehalose/100 ml; □ . . . □: trehalose, mg/100 ml.

(Duchâteau-Bosson *et al.*, 1963; Saito, 1963). On the other hand, the bulk of fat-body is consumed to a large extent during the periods of chitin synthesis. These observations suggest that the trehalose level of the hemolymph is supplied at the expense of the glycogen of the fat-body. The rate of trehalose synthesis by the fat-body, the supply of trehalose to the hemolymph, and the inhibition of the hemolymph trehalase are controlled by hormones, especially the hyperglycemic hormone, produced by the corpora allata (Steele, 1963), and by end-product inhibition (Murphy and Wyatt, 1965; Friedman, 1967).

The concentration of trehalose can moreover be subjected to circadian modifications (Nowosielsky and Patton, 1964, Hilliard and Butz, 1969) as well as to fluctuations due to anoxia or exposure to low temperature (Sømme, 1966, 1967).

C. Glycogen

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

D. Amino Sugars

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

E. Alcohols

The existence of high amounts of glycerol in the hemolymph as well as in the tissues of several insects is undoubtedly an adaptation to cold-hardiness. Glycerol doubtless plays the principal part in lowering supercooling points of the hemolymph. Accumulation of glycerol in the hemolymph has been observed in many overwintering insects, whatever the diapausing development stage, such as embryos in the case of *Bombyx*

mori (Chino, 1957, 1958), larvae of *Laspeyresia strobilella* (Sømme, 1967), old larvae and prenymphs in the nearctic Hymenoptera *Megachile relativa* (Krunic and Salt, 1971), pupae of *Hyalophora cecropia,* up to 300 m*M* (Wyatt and Meyer, 1959), or adults of beetles *(Pterostichus brevicornis,* Baust and Miller, 1970). Glycerol concentration decreases rapidly when diapause is broken.

Sorbitol, a polyhydric alcohol, has been found in overwintering embryos or larvae of some Lepidoptera (Chino, 1958; Sømme, 1966, 1967).

X. Hydrocarbons

A wide variety of hydrocarbons has been identified in the hemolymph of the cockroaches *Periplaneta americana* (Baker *et al.,* 1960, 1963) and *Blattella germanica* (Acree *et al.,* 1965). The principal hydrocarbon (about 60% of the total) is *cis,cis*-6,9-heptacosadiene, followed by pentacosane, 3-methylpentacosane, heptacosane, nonacosane, and C_{41}–C_{43} hydrocarbons. The tobacco hornworm, *Manduca sexta,* is able to synthesize the branched hydrocarbons (Nelson *et al.,* 1971). Circadian fluctuations have been observed (Turner and Acree, 1967). The amount of hemolymph hydrocarbons is significantly higher in the females than in the males of cockroaches (Baker *et al.,* 1963; Acree *et al.,* 1965).

XI. Lipids

Lipids are transported by the hemolymph from the midgut to the fat-body, where they are stored in more or less modified chemical species, and from the fat-body to the organs.

The total lipid content of the hemolymph generally lies between 1.5 and 5.5%. Wide variations of the lipids' concentration are observed during muscular activity (flight), development, or metamorphosis (Nowosielsky and Patton, 1965; Beenakkers, 1965; Nelson *et al.,* 1967; Mayer and Candy, 1969).

In *Galleria mellonella,* the phospholipids represent 22% of the total lipid content, sterols represent 15%, unesterified fatty acids 8%, and neutral glycerides about 50% (Wlodawer and Wisniewska, 1965). Cholesterol is the principal sterol in the hemolymph of *Acheta domesticus,* while phosphatidylethanolamine is the principal phospholipid (Wang and Patton, 1969).

The metabolic relations between the lipids transported by the hemolymph and those stored by the fat-body have been intensively studied by Wlodawer and Lagwinska (1967) in the waxmoth, and by Martin (1969) in *Pyrrhocoris apterus* and *Hyalophora cecropia;* it appears that assimilation, transfer, and storage of lipids by insects are realized through a wide variety of different ways.

XII. Nitrogenous Constituents

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

The similarity between the amino acid composition of both hydrolyzed and nonhydrolyzed plasma after deproteinization (with the exception of the dicarboxylic acids which are partly in the form of their amides in the hemolymph) indicates that the peptide content is generally low (Florkin, 1958). Peptides, however, seem to be more abundant in the hemolymph of *Drosophila* (Hadorn and Mitchell, 1951). A number of different peptides have been identified in the hemolymph of *Phormia regina* (Levenbook, 1966) and of *Periplaneta americana* (Von Knorre, 1967).

XIII. Free Amino Acids

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

* Other data may be found for the following orders: Odonata, Raper and Shaw (1948); Orthoptera, Benassi *et al.* (1959) and Benassi *et al.* (1961); Dictyoptera, Auclair and Dubreuil (1953), Auclair (1959), Pratt (1950), and Von Knorre (1967); Hemiptera, Pratt (1950); Lepidoptera, Auclair and Dubreuil (1953), Chen and Hadorn (1954), Wyatt *et al.* (1956), Irreverre and Levenbook (1960), Pant and Agrawal 1964, 1965), and Mansingh (1967); Coleoptera, Auclair and Dubreuil (1953) and Po-Chedley (1956, 1958); Diptera, Pratt (1950), Chen and Hadorn (1954), Hackman (1956, and Levenbook (1966).

TABLE V

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

	Exopterygotes				Hymenoptera	Coleoptera[b]		Diptera	Lepidoptera			
Amino acids	*Aeschna* sp. larvae	*Carausius morosus* adults	*Periplaneta americana* adults[d]	*Locusta migratoria* nymphs	*Apis mellifera* larvae	*Hydrophilus piceus* adults	*Popillia*[h] *japonica* larvae	*Gasterophilus* larvae	*Euproctis chrysorrhoea* larvae[c]	*Saturniidea*[e] pupae	*Sphyngidae*[f] pupae[d]	*Papilio machaon* pupae
Alanine	46	10–60	7	34	58	60	146–187	—	33	7–300	16–250	103–213
Arginine	19–27	17–19	19	24	50–74	7–11	48–81	8	44–58	107–243	59–576	126–127
Aspartic acid (total)	4–13	6–14	2	13	32–33	17–18	42–47	14	9–22	4–36	5–55	14–19
Glutamic acid (total)	32–63	50–77	24	166	308–347	131–195	309–526	314	302–343	83–468	62–240	202–226
Glycine	22–54	23–31	53	97	72–84	17–26	288–325	5	48–94	20–82	4–57	48
Histidine	7–21	55–58	23	30	17–30	8–12	169–225	1	107–161	23–196	3–127	71–89
Isoleucine	16–18	7–13	6	21	20–24	8–25	36–54	8	15–32	14–83	20–65	40–56
Leucine	22–29	10–14	7	21	25–30	7	20–25	7	13–23	15–108	14–73	56–80
Lysine	6–14	20–28	11	47	74–104	20–24	29–94	8	50–105	113–471	64–433	325–401
Methionine	4–13	9–13	4	6	19–23	3	3–12	7	1–13	11–148	25–81	122–163
Phenylalanine	5–11	6–9	6	11	8–12	6–7	13–17	7	8–15	7–72	8–49	24–43
Proline	12–41	10–16	42	62	368–418	122–283	264–507	16	129–157	62–478	28–230	146–256
Threonine	12–23	29–40	8	20	27–49	12–17	11–29	23	30–54	1–136	20–82	47–57
Tyrosine	3–13	5–8	25	28	3	2–9	11–37	22	0–5	2–76	8–146	4–5
Valine	23–29	22–25	11	48	58–59	11–20	94–150	15	29–49	34–127	22–105	101–120
Total	399.0	293–424	248	636.0	1239.0	445–721	1723–2162	465[g]	870–1164	1124–1989	515–1819	1575–1769
Serine	24		14	49		22–35						

[a] The values have been rounded to unity. (From Duchâteau and Florkin, 1959; Stevens, 1961; Shotwell *et al.*, 1963.)

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

[c] Other species studied: larvae of *Cossus cossus* (sum of the 15 amino acids : 938 mg/100 ml) ; *Amathes xanthographa* (1027 mg/100 ml), *Triphaena pronuba* (1352 mg/100 ml), *Imbrasia macrothyris* (497 mg/100 ml), *Pseudobunaea seydeli* (709 mg/100 ml), *Smerinthus ocellatus* (700 mg/100 ml).

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

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

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

[g] Without alanine.

[h] Shotwell *et al.*, 1963.

[i] Stevens, 1961.

A. Concentration in the Hemolymph

A high aminoacidemia is a characteristic of the class Insecta. However, this character is clearly more pronounced in endopterygotes than in exopterygotes. In the four exopterygotes, listed in Table V, the sumf of the fifteen amino acids ranges only from 293 to 636 mg/100 ml, values generally much lower than those found in endopterygotes (with the exception of *Gasterophilus* larva). The increasing importance of free amino acids as hemolymph constituents appears, as already pointed out, to be an evolutionary tendency developed in the most evolved groups, such as Lepidoptera, Hymenoptera, and Coleoptera.

The principal and almost permanent constituents of the amino acid pool of the insect hemolymph are the sixteen amino acids listed in Table V. As it appears from the comparison between hydrolyzed and nonhydrolyzed dialyzed plasma, aspartic and glutamic acids exist mainly in the form of their amides: asparagine and glutamine (Florkin, 1958). Arginine is essentially derived from its phosphagen, arginine-phosphoric acid. Other amino acids or related substances have been occasionally identified in the hemolymph of some insects: methionine sulfoxide and taurine in *Periplaneta americana* (Stevens, 1961), ornithine, L-cystathionine, 3-hydroxykynurenine, and L-lanthionine in *Bombyx mori* (Kondo, 1959; Makino *et al.*, 1954; Rajagopal Rao *et al.*, 1957), *S*-methylcysteine in *Prodenia eridania* (Irreverre and Levenbook, 1960), α-aminoisobutyric acid, homoarginine, and hydroxyproline in *Attacus ricini* (Pant and Agrawal, 1964), hydroxyproline in *Apis mellifera* larvae (Pratt, 1950; Lue and Dixon, 1967).

Exopterygote and endopterygote insects differ by the relative proportions of their respective hemolymph amino acids. In exopterygotes, the concentrations of the different amino acids are generally of the same order (from 10–60 ml/100 ml). In endopterygotes, on the contrary, the different amino acids may be present at very different concentrations:

1. "Total" aspartic acid (mainly in the form of asparagine), phenylalanine, leucine, and isoleucine, always occupy a minor place in the amino acid pool of the insect hemolymph.
2. "Total" glutamic acid (mainly in the form of glutamine), and proline (the latter with only a few exceptions) generally take the most important quantitative place in the amino acid pool.
3. The other amino acids may be present at more or less high concentrations, according to the species, or to the developmental stage considered.

B. Modifications of the Amino Acid Pattern

A characteristic amino acid pattern cannot be ascribed to any kind of taxonomic group as a biochemical character, according to the very high variations observed between the different genera of a given family, or even between the different species of a given genus (see, for instance, the extensive study of Saturniidae and Sphyngidae by Duchâteau and Florkin, 1958). Moreover, every species shows great modifications of its aminoacidemia during its development, expecially during metamorphosis, even in the case of diapausing pupae of some Lepidoptera (Mansingh, 1967). The aminoacidemia of an insect species may therefore be defined as being a succession of steady states expressed by a succession of patterns specific to the different instars of this species and to particular ecological or phyiological events. An example of the metabolic alteration of the aminoacidemia is given by the silkworm *Bombyx mori*, which has been the most intensively studied from this point of view.

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

The origin and the fate of the different amino acids have been studied by following the effects of the removal of silk glands, coupled or not with starvation experiments, by the study of the incorporation into the silk of radioactive amino acids, and by other investigations. The results may be summarized from Fig. 4 as follows:

1. The silk gland utilizes only a few kinds of free amino acids from the hemolymph in order to synthesize the fibroin. These are glycine, aspartic and glutamic acids (mainly in the form of their amides), serine, threonine, and proline, but no significant amounts of alanine or phenylalanine. The removal of silk glands produces a considerable accumulation of these "sericigenous" amino acids in the hemolymph, at the end of the fifth larval stage (Duchâteau *et al.*, 1959; Bricteux-Grégoire *et al.*, 1959a,b; Duchâteau-Bosson *et al.*, 1960, 1961). After injection of radioactive glycine or threonine, the isotopic carbon is incorporated into the fibroin not only as glycine, but also, to a lesser extent, as alanine and serine. Glutamic and aspartic acids are mainly used by the silk gland for the biosynthesis of the alanine of fibroin (Bricteux-Grégoire *et al.*, 1960).
2. The "sericigenous" amino acids of the hemolymph are mainly of dietary origin. During the first 5 or 6 days of the fifth larval stage (that is, during the half of the feeding period of the last larval intermolt), some of these amino acids, especially glycine, are stored in tissues, and their con-

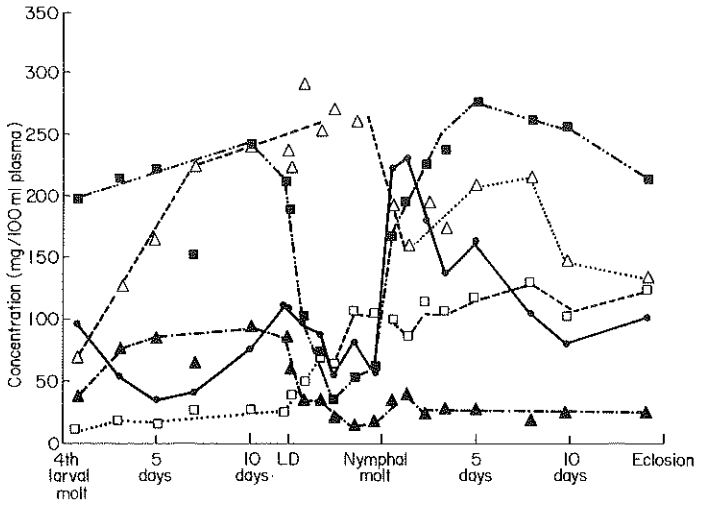

Fig. 4. Variation in the concentrations of three "sericigenous" amino acids, and of histidine and methionine in the hemolymph of the silkworm *Bombyx mori* during the fifth larval stage, spinning, and metamorphoses. (Jeuniaux *et al.*, 1961.)

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

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

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

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

6. The concentration of tyrosine in the hemolymph varies widely during the insect's whole life history. Accumulation takes place within the few days preceding each molt (up to 80 mg/100 ml), and is followed by a sharp and sudden decrease following each molt. These variations are related to the utilization of tyrosine in the protein-tanning and melanization of the new cuticle (Duchâteau-Bosson *et al.*, 1962). Similar observations have been noted during pupation of *Sarcophaga* (Fraenkel and Rudall, 1947) and of *Ephestia kühniella* (Blaich, 1969).

The changes of the free aminoacid pattern throughout the development of other Lepidoptera have been followed by Levenbook (1962), Pant and Agrawal (1965), and Mansigh (1967).

D. Other factors Influencing Aminoacidemia

The aminoacidemia is also more or less modified, both quantitatively and qualitatively, by changing the diet (Irreverre and Levenbook, 1960; Singh, 1965; Maltais and Auclair, 1962), by starvation (Kondo and Watanabe, 1957; Po-Chedley, 1958; Ludwig and Wugmeister, 1953), by anoxia and low temperatures (Sømme, 1967), and by caste differentiation in social insects such as the honey bee (Lue and Dixon, 1967) and the ant *Formica polyctena* (Brunnert, 1967).

E. D and L Forms of Amino Acids

The free amino acids of the hemolymph are usually of the L configuration. A few exceptions are known: D-alanine in *Oncopeltus fasciatus* (Auclair and Patton, 1950), and D-serine in three species of Lepidoptera, up to 70% of the total free serine in the hemolymph of the pupa (Srinivasan *et al.*, 1962).

XIV. Proteins

The protein concentration in insect hemolymph is similar to that of the blood of man and other vertebrates, and generally higher than that of the

internal fluids of other invertebrates. The average protein content is of 5 gm/100 ml in Hymenoptera and 3–4 gm/100 ml in Coleoptera, 2 gm/100 ml in Lepidoptera, and 1 gm/100 ml in Orthoptera (Florkin, 1936a).

In recent years, considerable attention has been paid to the characterization of hemolymph proteins, using electrophoresis on paper or in agar and starch gels, ultracentrifugation, immunoelectrophoresis, and other methods.

The number of different protein fractions is highly variable, according to species, caste (Lue and Dixon, 1965), sex (Stephen and Steinhauer, 1957; Kulkarni and Mehrotra, 1970), diet (Bodnaryk and Morrison, 1966; Dahlman, 1969), starvation (Feir and Krzywda, 1969), or ontogenetic stage. The characterization of the different fractions as albumins, α- and β-globulins, and so on, on the basis of their electrophoretic mobility has been criticized by Dénucé (1958). These studies are in full development, and there is now little to say about the physicochemical properties of the hemolymph proteins. In a study of *Schistocerca gregaria*, Kulkarni and Mehrotra (1970) report the approximate molecular weights, diffusion constants, values of Stoke's radius, and electrophoretic properties of 26 protein fractions of female and 21 fractions of male hemolymph.

The electrophoretic pattern of hemolymph proteins has been used by some authors for taxonomic purposes, these patterns being, in a given family, more similar for the species of the same genus than for species belonging to different genus.

The role played by some of the numerous proteins of the insect hemolymph has been brought into light in a few cases. The best defined hemolymph proteins are, however, those exhibiting enzymic properties.

A. Immunological Responses

Some hemolymph proteins seem to be involved in the phenomenon of protection against infection (Rehm, 1948; Gingrich, 1964; Marek, 1970). The role played by these proteins is discussed elsewhere in this treatise (see Chapter 9 of this volume).

B. Vitellogenesis

A few definite fractions of the protein pool are absorbed by the oocytes and incorporated into the yolk (Telfer, 1960; Brier, 1962; Hill, 1962; Telfer and Melius, 1963; Ramamurthy, 1964; Scheurer, 1969b). A protein component, exclusively found in the hemolymph of females, is

incorporated at the highest rate (Engelmann and Penney, 1966; Adiyodi, 1967). This "female protein" seems to be lipoproteic in nature (in the case of *Periplaneta americana:* Siakotos, 1960); it sometimes constitutes up to 50% of the total hemolymph proteins (in the bark beetle *Dendroctonus:* Sahota, 1970). The biosynthesis of the vitellogenic protein is probably controlled by a hormone produced by the corpora allata (Slama, 1964; Engelmann and Penney, 1966; Scheurer, 1969a).

The vitellogenic female protein of the hemolymph of *Leptinotarsa decemlineata,* which forms 70% of the yolk proteins, has been purified and its amino acid composition has been determined by de Loof and de Wilde (1970).

C. Enzymes

The number of different enzymes or isoenzymes in the hemolymph is surprisingly high. It appears that they represent an important portion of the hemolymph proteins (Laufer, 1960a,b). Some of the enzymic activities are nearly as high as those of the tissues, so that the presence of these enzymes in the hemolymph cannot be considered as necessarily resulting from a leakage from the tissues, as it occurs in mammals. However, the exact role of these enzymes in the hemolymph requires further demonstration.

A number of hydrolases have been identified: amylases, esterases, and proteolytic enzymes seem to be permanent constituents of the protein pool of the hemolymph. α-Glucosidase (Yamafuji, 1934), chitobiase, and chitinase (Jeuniaux, 1967, 1963; Waterhouse and McKellar, 1961) have also been detected. Proteolytic and chitinolytic systems may function in the histolytic processes which occur at the time of molting and metamorphosis (Laufer, 1961; Jeuniaux, 1961). The role of trehalase has been discussed above (see Section IX,B).

The hemolymph of some Lepidoptera contains different types of phosphatases (Itabashi *et al.,* 1953; Faulkner, 1955; Laufer, 1960a) and of transaminases (Belzecka *et al.,* 1959).

The hemolymph is a likely site of the metabolism of carbohydrates (Faulkner, 1955); the activity of the following oxidoreductases indeed has been demonstrated in the hemolymph of Lepidoptera (Laufer, 1961; Chippendale and Beck, 1966), of *Tenebrio molitor* (Prota, 1961), and of the honey bee (Tripathi and Dixon, 1969): lactate dehydrogenase, malate dehydrogenase, isocitrate dehydrogenase, glutamic dehydrogenase, glucose and glucose-6-phosphate dehydrogenases, glyceraldehyde-3-phosphate and glycerophosphate dehydrogenases, xanthine oxidase, and cat-

alase. These enzymes are present in the form of multiple isoenzymes. Their quantitative and qualitative variations according to developmental stage and caste differentiation have been followed in the honey bee by Tripathi and Dixon (1969).

Deoxyribonucleases are present in the hemolymph of diapausing pupae of *Hyalophora cecropia* (Riechers *et al.*, 1969).

Orthodiphenoloxidases (or tyrosinases) are responsible for the darkening of the hemolymph when exposed to air. In Diptera and Lepidoptera, they are present in the form of inactive proenzymes (Ito, 1954; Ohnishi, 1959; Karlson and Schweigger, 1961). Activation is controlled by protein activators liberated by the larval cuticle (Sekeris and Mergenhagen, 1964; Hackman and Goldberg, 1966) and by the cuticular lining of salivary glands (Geiger and Mitchell, 1966), while the cells of these glands produce a potent inhibitor (Thomson and Sin, 1970).

D. Variations during Development

In most insects so far studied, the total protein concentration increases during the larval life, then decreases at the end of the pupal instar and during the adult life (Drilhon, 1954; Denucé, 1957; Chen, 1956; Chen and Levenbook, 1966a; Laufer, 1960b). These quantitative variations suggest that the protein pool of the hemolymph may function as a reserve for the biosynthesis of the adult proteins or for providing the free amino acids of the hemolymph (Heller, 1932; Beadle and Shaw, 1950). This hypothesis has been confirmed by experiments with radioactive-labeled proteins (Chen and Levenbook, 1966b; Tobe and Loughton; 1969a,b). Specific hemolymph proteins can be sequestered by different tissues, principally by the fat-body, in which they are stored in the form of granules (Locke and Collins, 1966, 1967; Chippendale and Kilby, 1969).

Metamorphosis is often accompanied by qualitative variations of the electrophoretic pattern of the hemolymph protein pool (Laufer, 1960a,b; Chen and Levenbook, 1966a). Among the fourteen different protein fractions of the hemolymph of *Anthonomus grandis*, there is one fraction unique to the larval stage, and one unique to the pupal stage (Norman *et al.*, 1967). By means of immunoelectrophoretic methods, Lensky (1971a,b) was able to identify, in the honey bee, three main protein patterns, one present throughout the whole development, and two specific respectively to the larval and to the adult stages.

Modifications of the protein pattern during molting indicate that some relations exist between hemolymph proteins and cuticle (Fox and Mills, 1969). One of the nineteen protein bands of the hemolymph of *Locusta migratoria* regularly appears prior to each molt and disappears immediately after ecdysis; this fraction is absent during intermolts and adult life (McCormick and Scott, 1966a,b). The same is true in the case of *Periplaneta americana* (Steinhauer and Stephen, 1959).

XV. Pigments

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

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

XVI. Conclusion

Considered from the ecological point of view, insects are the only invertebrates able to live in arid environments and to fly. The hemolymph is their only extracellular fluid. They have given up the physiological association between the respiratory and the circulatory systems, the tracheal system insuring the arrival of oxygen to all cells. Insects are therefore not bound to the maintenance of a definite blood volume and they can rely on blood water to ensure their survival in dry media. They can, in spite of the variations of blood volume, regulate the osmotic pressure in the hemolymph by changing the amino acid concentration. Inorganic cations and anions are, particularly in the most specialized endopterygote orders, replaced by amino acids and organic acids. The aminoacidemia is

high and the nonprotein nitrogenous components of hemolymph are mainly made up of the components of the amino acid pool. The hemolymph of insects thus appears to have the characteristics of a fluid tissue, with its own metabolism, revealing a composition more similar to that of the intracellular fluid than to that of blood of vertebrates.

By its nature as a container of a number of reserve or transport materials, the most peculiar of which is trehalose, in constant exchange relations with the fat-body, hemolymph is suited to the life of organisms in which feeding is interrupted during certain life stages or during diapause.

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