INSECT HEMOLYMPH: A REVIEW

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The purpose of this paper is to give a generalized account of what is known concerning the hemolymph of insects with a bibliography of the more important works on the subject. It makes no attempt to include circulation nor any papers on arthropods other than insects. There are three published reviews available; one by Maluf (1939), which includes all arthropods; another by Wigglesworth in his textbook, the Principles of Insect Physiology (1939), and a third by Shulz, covering the tracheates in Hans Winterstein's Handbook (1925).

The circulating fluid of insects is termed hemolymph, because it is not enclosed within vessels except where it is moved through the dorsal vessel. For this reason it corresponds both to blood and lymph. However, since it is never so complex nor highly differentiated as the blood of higher animals, it is more like lymph in actual composition. The formed elements or cells are termed hemocytes. According to the latest classification there are 10 classes subdivided into 32 types (Yeager, 1945).

The first worker on insect hemolymph seems to have been H. Landois in 1864 who studied the crystallization of the fluid from 14 different insects. He described the different types of crystals, making many sketches. In addition, he made several other observations, namely that the blood consists of serum and corpuscles; it is usually water white, but often colored greenish, brownish, reddish, or yellowish; that the color very seldom harmonizes with the color of the adult insect; further that it contains protein, globulin, and iron. He also found that the larvæ are richer in hemolymph than adults and that poor fliers and long-lived mature adults have more than good fliers and short-lived adults. He stated that the proportion of body weight to blood weight, in the case of the larvæ, is four to one. In the light of subsequent investigation, Landois appears to have done a creditable piece of work.

Perhaps more time has been spent on the cytology of the hemo-

lymph than on any other one phase. In 1937 Maria Rooseboom published a thesis listing some 173 species in which the hemocytes had been described or included in a more generalized description. Some of the workers included Barrat and Arnold (1911), Blaustein (1935), Bruntz (1908), Cuénot (1891), Haber (1926), Hollande (1909, 1911), Hufnagel (1918), Kollman (1908), Landois (1864), Metalnikov (1908), Muttkowski (1923), and Paillot (1919).

Since Rooseboom's summary two of the papers to appear were by R. Ermin on *Periplaneta americana* and H. W. Jackson on *Tenebrio molitor,* both in 1939. Perhaps the newest approach in recent times has been the actual counting of blood cells by Tauber and Yeager (1934, '35, '36). Some estimation had occurred in previous literature, but not on so large a scale. They counted the total hemocytes from 502 individuals of 33 different species of Orthoptera, Odonata, Hemiptera, and Homoptera and 237 individuals of 29 species representing Neuroptera, Coleoptera, Lepidoptera, and Hymenoptera. From 220 counts of the field cricket, *Gryllus assimilas,* the average was 70,118 cells per cubic millimeter. The average for *Blatta orientalis* was 32,698 cells per cubic millimeter. In these studies they often obtained a wide range of counts but were able to duplicate averages. It was found that, as far as the orders with incomplete metamorphosis were concerned, the average count for the females was higher than that for the males. High hemocyte counts tended to be associated with some physiological or pathological condition such as ecdysis, oviposition or parasitism. In the Orthoptera the counts seemed to be higher for the adults than for the nymphs. However, the larval counts seemed higher than the adults for the orders with complete metamorphosis.

Many of the workers mentioned above established classifications of the hemolymph cells, of which probably the best known were those of Hollande (1911) and Paillot (1919). However, there were almost as many classifications as there were workers. Recently J. Franklin Yeager in 1945 published "The Blood Picture of the Southern Armyworm (*Prodenia eridania*)," which seems an excellent classification, at least for this particular order. As mentioned before he described 10 classes of hemocytes which were

then subdivided into 32 types. The changes of these different kinds of cells, which were studied during all stages of the life cycle, may serve to divide the cycle into early larval, late larval, metamorphic, and adult phases.

Some small attempts have been made to see if there is any affect made by poisons on the hemocytes. Tareev and Nenjukov in 1931 stated that "arsenite of sodium, when penetrating into the blood through the integument of the intestine, causes a change in the cellular elements, destroying their nuclei and dissolving the protoplasm." Shull *et al.*, in 1932, listed 34 inorganic and organic gaseous compounds of widely differing physical and chemical properties to which they subjected *Periplaneta americana,* in what they considered a probably lethal concentration, without producing a noticeable effect on coagulation or on the appearance of the cells. Pilat in 1935 added that intestinal poisoning by sodium arsenite and silico fluoride produced no change in the normal blood picture. As criteria he included the relative size and form of the nucleus, color reactions, and the ability for phagocytosis, because, as he pointed out, it is difficult to determine whether or not the hemocytes have been changed when a good classification of them is lacking. To my knowledge the latest paper on the changes effected by poisons was by Yeager and Munson in 1942, on the southern armyworm [*Prodenia eridania* (Cram.)]. They studied the affect on the blood cells and the hemocyte glycogen by such poisons as nicotine bentonite, rotenone, pyrethrum, phenothiazine, barium fluosilicate, sodium fluoride, calcium arsenate, paris green, etc. There were no significant changes noted due to nicotine bentonite, nicotine peat, rotenone, pyrethrum, and phenothiazine, although there were marked hemocyte changes involving cellular swelling, decrease in glycogen, formation of pseudopodia, appearance of numerous vacuoles and raggedness following the administration of arsenicals, fluorides and mercuric chloride.

To date little is known concerning the physiological functions of the hemocytes. However, it is known that immunity to diseases is due to the faculty of phagocytosis possessed by them. Workers on this phase include Chorine (1931), Hollande (1930), Metalnikov (1926), and Paillot (1933). The hemocytes also par-

ticipate in coagulation which will be discussed later. Several workers have noted that they store glycogen (Babers, 1938, and Yeager and Munson, 1941) and fat (Munson and Yeager, 1944).

As for physical properties of the hemolymph as a whole, the color has already been previously mentioned. It is usually straw colored, but often yellow, green, orange, or reddish. Muttkowski in 1923 listed a number of insects together with the life stage, color of blood, and type of food. He concluded that there was no correlation between the type of food and the hemolymph color, but that the color of the hemolymph of the immature was generally brighter than that of the adults. Often a difference in the color of the blood exists between the two sexes. Muttkowski (1923) mentions the fact that the female of *Dytiscus* has bright orange hemolymph while that of the male is clear yellow. Steche in 1912 listed 16 Lepidoptera in which the color of the hemolymph of the sexes differed. Geyer in 1913 attempted spectroscopic examination of the green color in the hemolymph of females and the yellow color of the males of certain caterpillars. He concluded that in the case of the females the green was a chlorophyll derivative; while, the males possessed only xanthophyll. He said that the difference in the color of the hemolymph of the two sexes occurred only in phytophagous species. This of course does not correlate with Muttkowski's work.

The volume of blood per body weight varies somewhat. Bishop (1923) gives 25–30 per cent of the body weight for the honey bee larva; Babers (1938), 0.17 to 0.2 ml. per army worm (*Prodenia eridania*) depending on age and type of food; Richardson, Burdette and Eagleson (1931), 31.2 cc. per 100 g. of body weight for *Bombyx mori* by the absorption method and 28.6 per 100 g. of body weight by the dry weight method and 41.0 cc. per 100 g. of body weight for *Galleria mellonella* by the dry weight method. Both these insects were in the larval state. Yeager and Tauber (1932) give 0.069 and 0.047 per g. of body weight, depending on the method, for *Periplaneta fulginosa.* Several other workers had made more or less approximations by bleeding. The difficulty lies in the complete removal of all the hemolymph.

Although the volumes in relation to the total body weight are fairly large as compared with vertebrate blood, the amount that

can be obtained for analyses seems amazingly small. Bishop, Briggs, and Ronzoni (1925) state that 30 drone honeybee larvæ or 50 to 60 worker larvæ yield only 1 cc. of hemolymph; Busnel and Drilhon (1937) that 100 individuals of *Leptinotarsa decimlineata* give only 0.15 cm.^{3}, Pepper, Donaldson, and Hastings (1941), however, obtained 0.05 to 0.10 ml. per 1 mormon cricket (*Anabrus simplex* Hald.).

Various reports have been made on the specific gravity. Among these are: Bishop (1923) for the honeybee larva, 1.045 g.; Barrat and Arnold (1911) for *Dytiscus marginalis,* 1.025 g. to 1.027 g. and for *Hydrophilus piceus,* 1.012 g.; Babers (1938) for the armyworm (*Prodenia eridania*), 1.032 g.; and Yeager and Fay (1935) for *Periplaneta americana,* 1.0163. These last investigators described a micromethod for determining the specific gravity of the hemolymph. They found no significant difference between nymphs and adults nor males and females.

Several workers have attempted to determine the hydrogen ion concentration of the hemolymph. Landois as early as 1864 reported that the blood of 14 species was alkaline. Muttkowski in 1923 found the blood to be alkaline to neutral to moist litmus paper; while, Haber in 1926 was still using litmus to test the hemolymph of 42 different species. Bodine in 1926 used a microhydrogen electrode so that individual studies could be conducted on a single individual from the day of hatching of a grasshopper until its death. He tables a whole series of pH values ranging from 6.23 to 7.11 and concludes that although considerable variation exists between the same and different species, one individual is at least constant for one day. There was no marked difference detected between different sexes, ages, or species. Kocian and Spacek (1934), on the other hand, found that the pH of the hemolymph of Coleoptera does vary with age and species from 6.2 to 7.2 and that carnivorous species have a higher pH than phytophagous ones. Demjanowski (1932), too, who ran up to 2,000 determinations on *Bombyx mori,* felt that the pH between the males and females differed from 0.02 to 0.05 points, the female through three developmental periods always being higher than the male. Other figures include 6.8 for the larva of the honeybee (Bishop, 1923); 6.8 to 7.2 by Hastings and Pepper (1943) for grasshop-

pers; 6.6 for the caterpillar and pupa of *Pieris brassicæ* by Brecher (1929), and 6.53 by Babers (1938) for the armyworm (*Prodenia eridania*). It would seem then that, in spite of the earlier workers, the pH inclines toward the acid side of neutrality.

Concerning the subject of bleeding, all workers agree that the hemolymph darkens on exposure to air, either to dark brown or black, and add that this is probably due to tryosinase contained in it. However, the literature concerning coagulation has been somewhat sparse, most workers saying incidentally that the blood does or does not coagulate. Muttkowski (1923), Yeager *et al.* (1932) and Yeager and Knight (1933) have offered the main contributions. The last pair named, following a study on 47 species set up three categories of insects: 1, those in which no coagulation takes place, namely Hymenoptera, Coleoptera and Lepidoptera; 2, those in which a clot is produced by leucocyte agglutination, namely Orthoptera, Homoptera, Coleoptera, Hymenoptera, Diptera, and Lepidoptera; and 3, those in which mainly a blood protein coagulation occurs although accompanied by some cell agglutination, namely Heteroptera, Orthoptera, Coleoptera, and Lepidoptera. Before this second study, Yeager had maintained that protein coagulation did not occur. During the coagulation process Yeager states that the blood cells may "lose their original . . . shapes, round up . . . form thread pseudopodia, agglutinate into clumps and disintegrate." According to Muttkowski (1923) the addition of potassium oxalate, while producing a calcium oxalate percipitate does not prevent the blood from clotting. Therefore, calcium is not necessary. Neither does the blood produce clotting when added to solutions of fibrinogin or thrombin from normal horse serum (Babers, 1938). The whole mechanism of clotting then is very different from what we know concerning the clotting of human blood. Clotting of the hemolymph can be prevented by immersion of the insect in water at 60° C. from 1 to 10 minutes or by an acetic acid vapor treatment (Shull and Rice, 1933) or a fatty acid vapor treatment (Shull, 1936).

Chemically, relatively little accurate work has been done, probably because of the small volume available for study from each insect. Many investigators found it necessary to collect hemo-

lymph from many individuals for a sample. Most workers devote little or no space to a discussion of the methods used, so that it is difficult to draw comparisons between species or orders. Earlier workers often ran analyses on the ash or on the ash of the total insect rather than on the hemolymph alone.

Considering first the inorganic constituents, it has been pointed out many times that sodium and chloride are low in percentage; while potassium, phosphorus, calcium and magnesium are present in relatively high concentrations. Recently Boné (1946) has contested this view as regards the Na/K ratio. According to Boné "the supposed biochemical hiatus between the insects and the rest of the animal kingdom clearly does not exist; the problem, open to experimental analysis, emerges of the variation of ionic regulation among members of a single zoological group." Vegetarian insects have more potassium ions than sodium ions; while carnivorous insects have more sodium than potassium ions.

As an example of analysis, Brecher in 1929 for the pupa of *Pieris brassicæ* lists for the following minerals expressed in milligrams per 100 cc.: Na, trace; K, 137.8; Ca, 33.0; Mg, 56; Cl, 59.5; and P, 66. Other investigators include Heller and Moklowska (1930), Babers (1938), Busnel and Drilhon (1937), and Bishop, Briggs and Ronzoni (1925). As contrasted with Brecher, Babers (1938) found 51.2 mg. per 100 cc. of Na in the larva of *Prodenia eridania;* while, Drilhon found 14.7 mg. per 100 for the various pupæ. No other investigators reported as high a figure for P, the next highest being 31 mg. per 100 cc. by Bishop *et al.* in 1925 for the honeybee larvæ, and the lowest 12 for the larva of *Deilephila euphorbiæ* by Heller and Moklowska in 1930. Clearly a variation must exist in different species of insects, so that analyses of a greater number of species seems indicated before drawing any sweeping conclusions. As for differences in the same species, Brecher (1929) found a slight difference between the male and female hemolymph content of potassium, calcium, magnesium, and chloride, but not phosphorus.

Muttkowski in 1923 reported Cu and Fe in insect hemolymph, but only qualitatively.

Of the organic constituents the amino acids are present in extremely high percentages as compared with human blood,

although total proteins in themselves are lower. For instance, Florkin in 1937 figured about 3.5 per cent total proteins for *Dixippus*. However, Bishop *et al.* (1925) figured 6.6 per cent for the larva of the honeybee. Duval, Portier, and Courtois (1928) reported the content of amino acids as follows: for the adult of *Dytiscus marginalis,* 1.34 g. per liter, and of *Hydrophilus piceus,* 1.46; for the pupa of *Attacus cynthia,* 3.27, of *Sphinx ligustri,* 3.22, of *Saturnia pyri,* 2.85, of *S. carpini,* 3.58; and of the larva of *Cossus cossus,* 2.34, all g. per liter. Amino acids are said to furnish the most effective fraction osmotically.

Several investigators have reported on the relative proportions of the nitrogenous products of protein degradation. For instance, Babers (1938) for the larva of *Prodenia eridania* gives 6.2 mg. per 100 cc. of urea, 14.8 mg. per 100 cc. of uric acid and 8.0 mg. per 100 cc. of creatin; Florkin (1937) for *Hydrophilus piceus,* 7.4 mg. per 100 cc. of urea and 8 to 15 mg. per 100 cc. of uric acid; and Bishop *et al.* (1925) for the honeybee larva, 5.3 mg. of uric acid, 1.1 mg. of creatin and 41.6 mg. of other nonidentified nonprotein nitrogen per 100 cc.

A great deal of time has been expended on whether or not insects contain a respiratory pigment such as the hemocyanin of molluscs, certain Crustacea, and some spiders and scorpions. Muttkowski, 1921, suggested that they might because of the large amount of copper he detected qualitatively. Among authors to refute this statement are Redfield (1934) in studies on the Florida grasshopper, Bishop (1925), who pointed out that the oxygen capacity of the honeybee larvæ is 0.2 to 0.8 per cent per volume and within the errors of experimental error, and Babers (1938). The latter points out first of all that some insects accumulate copper. Although the definite physiological function is unknown, it does not necessarily follow that copper is combined with a respiratory protein. Indeed, there is no method to distinguish qualitatively between dissolved oxygen and oxygen bound by a respiratory protein. Further, although copper in *Prodenia* is about 5 per cent, it occurs only to the extent of 1.99 per cent in the protein precipitate.

Hæmocyanin and hæmoglobin where they occur in invertebrate blood are said to be suspended in the plasma and not incorporated

in the corpuscles where they occur in vertebrate blood. This seems true of the larva of *Chironomous.* The larva of *Gastrophilus equi* has been proven to contain hemoglobin in the fat body and "Tracheal body." However, Hungerford (1922) and Bare (1928) say that oxyhæmoglobin in the genus *Buenoa* occurs in "definite bright red clusters of cells, enmeshed and closely associated with the tracheal system of the abdomen." These cells do not circulate, however, but "may fix and store oxygen."

Leitch (1916) and Harnisch (1927) have pointed out, however, that the hæmoglobin seems to dissociate oxygen only at low oxygen pressure, so that the respiratory proteins would seem of value not so much as storers, but as an aid in an environment with low oxygen content.

The fat content of blood may occur in lipomicrons, minute fat particles according to Haber (1926). Bishop, Briggs, and Ronzoni (1925) report 453 mg. per 100 cc. of total fat for the honeybee larva and that at the time of pupation the fat content rises a great deal. Compared with human values, this is high. However, the cholesterol value, being 35 mg. per 100 cc. in the feeding honeybee larva, is lower than humans.

Carbohydrates in insect hemolymph has been studied somewhat more than the fat content. A peculiarity here is that the hemolymph has a high percentage of reducing substances that are not glucose. Earlier workers presumed all reducing substances to be sugar, and hence gave results comparable to human blood sugar levels. According to Kuwana (1937) on the silkworm, Babers (1938) on *Prodenia eridania* and Florkin (1937) on *Hydrophilus,* about one half of the total reducing substances are something other than glucose. Figures for total reducing substances, sometimes calculated as glucose, include: 30.6 to 49.4 for various species of grasshoppers according to Blumenthal (1927); 127 for *Deilephila euphorbiæ* according to Heller and Moklowska (1930); 65.9 for *Prodenia eridania* according to Babers (1938) with 11.1 and 3.29 of glucose and glycogen, respectively; 24.43 for the larva of *Bombyx mori* with 0.4 to 4 of glucose according to Kuwana (1937) and 203 mg. for the feeding larva according to Bishop, Briggs and Ronzoni (1925). All these figures are calculated as mg. per 100 cc. Ronzoni and Bishop (1928) have shown that

blood sugar is utilized as a source of energy during spinning and pupation and the percentage naturally falls from 685, feeding, to 154, prepupa, to 20–80, pupa, mg. per 100 cc. Kuwana (1937) supports them in this statement as the blood sugar of the silkworm larva descends to zero when pupation begins and stays at zero through the pupal stage. At the same time, however, a great deal of glycogen is liberated into the hemolymph, the glycogen content rising from less than 10 mg. per 100 cc. to 2,800 (Ronzoni and Bishop, 1928). This glycogen must, of course, be hydrolyzed to glucose before being utilized by the tissues for energy. Studies in 1941 by Yeager and Munson show that glycogen is stored in the hemocytes for such a purpose, since blood cell glycogen increases during the different instars to the prepupa and then falls very rapidly.

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