DESCRIPTION OF HEMOCYTES AND THE COAGULATION PROCESS IN THE BOLL WEEVIL, ANTHONOMUS GRANDIS BOHEMAN (CURCULIONIDAE)[1]

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This report presents the results of a study of the hemocytes of the boll weevil, *Anthonomus grandis* Boheman. A knowledge of the hemocytes of healthy boll weevils was considered necessary before detailed study of the effect of various pathological conditions upon hemocytes could be conducted. Wittig (1962), in reviewing the pathology of insect blood cells, observed that diseases may affect hemocytes directly or indirectly. Knowledge of these effects may be useful not only in studying a microbial disease which attacks the hemocytes, but also as an aid to bioassaying a population for pathological conditions. Knowledge of the hemocytes of healthy insects is necessary for such comparisons.

Terminology

Classification of hemocytes provides a means of reference for further studies concerning their function, changes due to normal physiological processes or to abnormal conditions, and for comparison with hemocytes in other species. Classification systems have classically depended upon morphological characters. As Wigglesworth (1959) stated, determination of function should be most important. Nevertheless, recognition of hemocyte morphology is necessary before detailed functional studies can be conducted. Classification on a purely functional basis would result in much confusion, since a distinct cell type often has more than one function. Moreover, hemocytes of dissimilar morphology from different species have similar functions. Accordingly, classification of boll weevil hemocytes has been established on the basis of morphological similarities, following to a large extent the system used by Jones (1962). When a distinct hemocyte type was observed to transform *in vitro*, the variations in form were described; but these were not considered to constitute a distinct type of hemocyte, especially since *in vitro* changes may not have been a true representation of *in vivo* activity. This factor was further emphasized by the conditions which created artifacts during the investigation.

The types of hemocytes present in the boll weevil correspond to previously recognized hemocyte groups of other insects. We feel that this system of nomenclature may serve as a basis for common usage, by insect pathologists at least,

[1] In cooperation with Mississippi Agricultural Experiment Station. Accepted for publication October 8, 1964.

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until further data regarding origin and function warrant a change. Each insect species apparently has its own characteristic hemocyte picture. Basic hemocyte types exist, but morphological adaptations to varying physiological requirements and conditions occur. The reader is referred to the excellent reviews by Wigglesworth (1959) and Jones (1962) for a more extensive coverage. The literature which served as a basis for classification is reviewed briefly in the following paragraphs to indicate the reasons for usage of the terms and to relate the terms to those which have been considered synonyms.

Prohemocytes: Prohemocytes are generally characterized by having a small amount of cytoplasm, the nucleus comprising the greater amount of the cell volume. These cells develop into other types which presumably are then capable of performing functions of mature hemocytes. The term "prohemocytes" was used by Jones (1950) and Arnold (1952). Jones (1959) stated that these are the same as Yeager's (1945) proleucocytes. The term "prohemocyte" is proposed as the standard.

Plasmatocytes: Hemocytes usually characterized by their pleomorphic capability and varied functions, as well as being the most abundant in the host, have been termed "plasmatocyte" by Yeager (1945). Yeager recognized a large number of cell types which differed mainly in their morphology. Arnold (1952) used the term in a report in which he included many of Yeager's types under that term. Jones (1959) retained Yeager's classification of plasmatocyte but also retained "podocytes" and "vermiform cells." Jones (personal communication) reported that podocytes or vermiform cells had not been observed to alter their form *in vitro*. The term "plasmatocyte" was adopted in this study to designate the hemocytes which were pleomorphic, exhibited cytoplasmic variations, were capable of altering their shape *in vitro* and were obtained in corresponding shapes in fresh preparations of blood, were capable of phagocytosis and took part in a coagulation process. Until further study can determine separate, distinct cell types for these functions, it is proposed that this term be used to designate such hemocytes.

Adipohemocytes: Hemocytes with lipoid or other refractive inclusions were termed "spheroidocytes" by Yeager (1945) and Jones (1959). Later, Jones (1962) preferred the term "adipohemocytes," since "spheroidocytes" could be confused with a distinctly different type—"spherule cells." The term "adipohemocyte" is proposed as the standard. The reader is referred to this review for further discussion of adipohemocytes.

Spherule cells: Yeager (1945) described "eruptive cells," which Jones (1959) regarded as "spherule cells." Jones (1962) further reviewed the usage of this term. These cells contain one to several large, distinct, non-refringent, amorphous inclusions which often distend the cell membrane. These inclusions are often expelled into the hemolymph *in vitro* but are not associated with coagulation. They are a distinct and easily recognized type of cell. The term "spherule cell" is preferred because it adequately describes the distinctive appearance of the cell and does not relate to an activity which may or may not be an artifact, but which certainly can be affected by conditions attendant to the technique employed to study the cells.

Oenocytoids: Yeager (1945) described oenocyte-like cells free in the hemolymph. Arnold (1952) used the term oenocytoids. These have a small nucleus, a

compact cytoplasm which is usually opaque, are lightly basophilic or eosinophilic and frequently contain a small number of granules or crystals. The cell membrane may be lightly creased, have elaborate canaliculi, or be complexly folded (Hollande, 1911; Jones, 1959; Yeager, 1945). Oenocytoids are distinct from the non-circulating specialized cells known as oenocytes which are enormous acidophilic cells of ectodermal origin, usually segmentally arranged in the abdomen. The term "oenocytoids" is preferred to "oenocyte-like" cells for these reasons.

Materials and Methods

Larvae were used for the initial descriptive work and an attempt was made to select those of uniform size and physiological condition to eliminate possible variations due to growth or developmental stages. Later, hemocytes were examined from all larval stages and from adults. Larvae were utilized soon after collection from local cottonfields, to reduce artifacts which might have resulted from abnormal laboratory conditions for the insects. Larvae were placed ventral side up in modelling clay and fastened securely. A sealed #30 hypodermic needle was used to make a puncture at the midventral line, which was free of adipose tissue formation.

The primary method of observation was of wet mounts with a Leitz Ortholux [4] microscope by phase contrast, bright-field or ultraviolet illumination. A Metrimpex (Budapest) 3-D Condenser [4] (National Instrument Laboratories, Inc., 12300 Parklawn Drive, Rockville, Maryland, U. S. A.) was also used. Fixed and stained preparations were utilized for histochemical and comparative purposes.

Two methods were used for obtaining hemolymph and placing it on a coverslip. The direct method consisted of touching a coverslip to the exuding hemolymph. The capillary method involved collection in a capillary tube made from a 1-mm. capillary tube finely drawn and fire-polished. The hemolymph was expelled onto a coverslip. Approximately 1 to 3 μl. of hemolymph were easily obtained from larvae.

The coverslip was then inverted over a slide and supported by a thin ring of white petroleum jelly so that a deep layer of hemolymph, not compressed by the weight of the coverslip, was formed. A similar preparation was obtained by use of a Leitz culture trough slide (PHOLD).[4] Drying of the hemolymph was negligible and often a preparation remained satisfactory for 12 to 17 hours.

Some preparations were diluted about 1:1. Dilution of hemolymph was accomplished prior to placing the coverslip over the slide. The diluting solutions were: water, pH 7.3; 0.85% NaCl solution; 1.0% potassium oxalate solution; distilled water, pH 6.1; 0.5% trehalose solution in distilled water, final pH 5.8; phosphate buffer, pH 6.4. Fluorescent dyes were also added. Acridine orange, auramine 0, and rhodamine B were prepared at 1×10^{-3} concentration in phosphate buffers of pH 6.4 and 7.2. The phosphate buffers were prepared by mixing the proper amounts of 0.07 *M* Na_2HPO_4 and KH_2PO_4 solutions. No autofluorescence occurred.

Stained preparations were made by rapidly air-drying the fresh, unfixed hemolymph. Some preparations were then subjected to 40% formaldehyde or absolute

[4] The use of trade or proprietary names does not necessarily imply the endorsement of these products by the U. S. Department of Agriculture.

methanol fixation for several minutes. The stains used were Sudan IV, saturated iodine (Lugol's solution), Delafield's or Heidenhain's iron hematoxylin, fast green FCF, Giemsa, and Wright's blood stain.

Hemocyte Description

Prohemocytes. These were disc-shaped, pale gray, and nearly homogeneous by phase contrast. The nucleus occupied nearly the entire cell and only a thin band of peripheral cytoplasm was present. Cells averaged 7.0 μ $\pm$1.3 μ, range (R) 6.3–9.9 μ, number (N) = 11 (Plate I, Fig. 1). When stained with acridine orange, the large nucleus and small band of cytoplasm were very prominent. These cells were relatively rare and only in young first instars were they fairly numerous.

Variations of this cell were observed which formed a complete range of developmental stages. The cytoplasm increased in quantity and progressively became more optically dense to phase contrast. A small number of granular inclusions were often present and large granules or vacuoles were occasionally seen in cells near the end of maturation. The final product was indistinguishable from disc-shaped plasmatocytes.

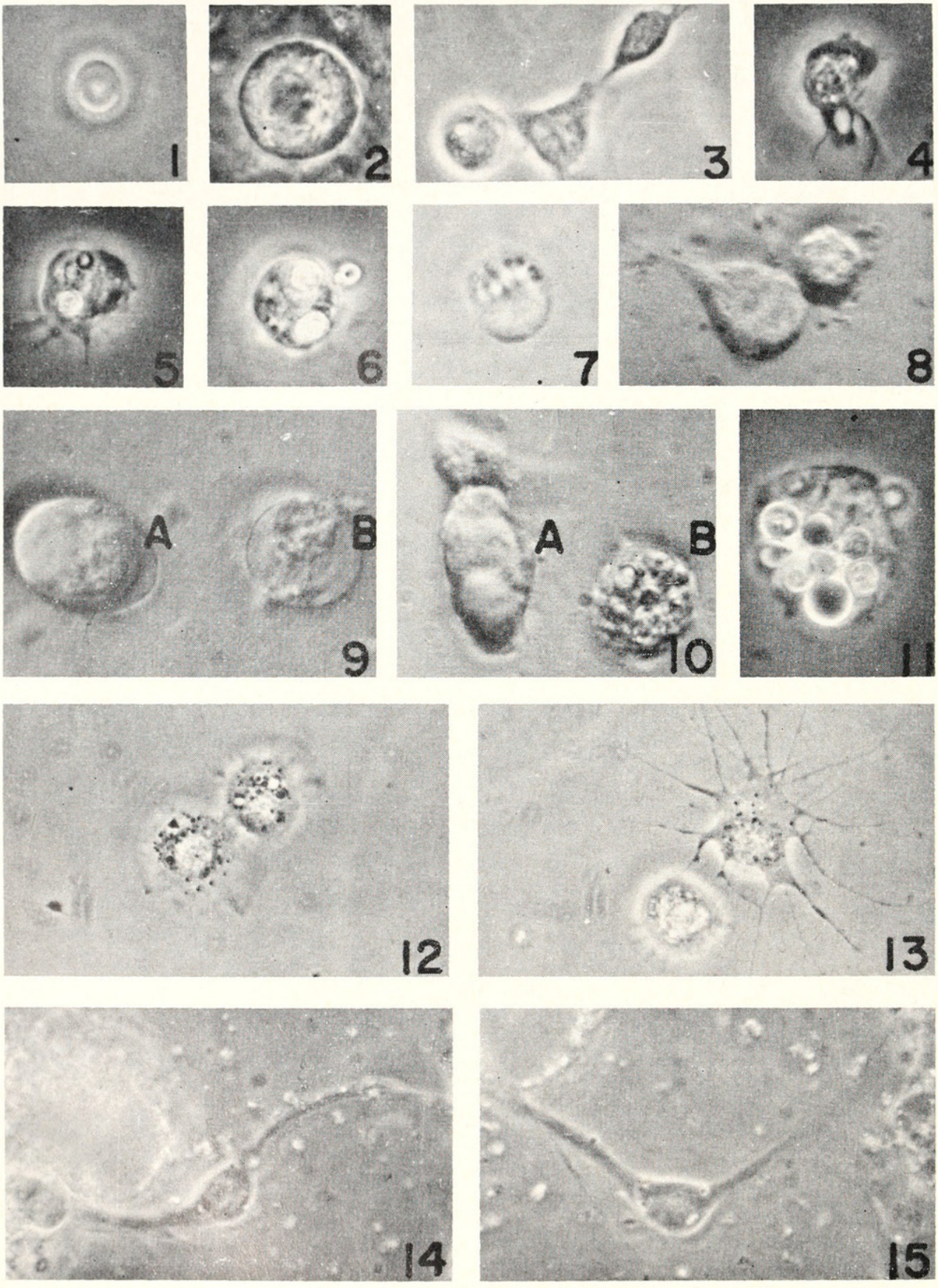
Plasmatocytes. Plasmatocytes were characterized mainly by their morphological variability. The cytoplasm was finely granular, dense, and uniform; or it contained various larger (about 1 μ) dark granules, exhibited areas of different density, and displayed small vacuoles or inclusions which were either gray or highly refractive by phase contrast. The nucleus was usually quite large and finely punctate or granulated. Plasmatocytes can become hyaline or highly refractive at their periphery. Disc-shaped plasmatocytes (Plate I, Fig. 2) measured 9.7 μ $\pm$1.9 μ (R = 6.4–15.3 μ, N = 75). The same data showed nuclei to measure 5.3 μ $\pm$1.05 μ (R = 3.4–8.5 μ).

A common variant form of plasmatocyte is shown in Plate I, Figures 5 and 6. These cells had a granular cytoplasm and sometimes contained small vacuoles, granular inclusions, or dark, gray, small inclusions. They were also seen with one or two highly refractile lipoid globules. These were termed adipoid plasmatocytes.

The frequency of cells with these inclusions was higher when free lipoid droplets were present in the hemolymph. Intentional disruption of adipose tissue, by rolling the larvae with pressure or by use of a needle to disrupt the tissue, produced a similar effect. The plasmatocytes were never observed ingesting lipoid droplets. The natural occurrence of free lipoid droplets was observed during the prepupal stage of larval development.

Another form was characterized by possession of one to several hyaloplasmic extensions (Plate I, Figs. 4 and 5). When movement of the fluid caused the cells to be carried along, they would often temporarily attach to the surface by these extensions. This form was observed to change into a disc-like shape or to attach at one point and elongate into a fusiform, or spindle-shaped, cell. This type was highly unstable *in vitro*.

The fusiform cell (Plate I, Fig. 3) was also seen upon immediate examination of a preparation, but was not as frequent as disc-shaped cells or cells with cytoplasmic extensions. The fusiform cell could also be formed directly from a disc-



All preparations were of fresh, unfixed, undiluted hemolymph.

Plate I

FIGURE 1. Prohemocyte. Phase contrast. 875 ×.

FIGURE 2. Disc-shaped plasmatocyte. Phase contrast. 1090 ×.

shaped plasmatocyte. A teardrop-shaped cell was also formed from disc-shaped plasmatocytes by elongation after attachment to the glass at the other end of the cell (Plate I, Fig. 8). The cytoplasm varied from dense and uniform but finely granulated to a differentiated cytoplasmic area of slight refractivity by phase contrast and containing small vacuoles, or dark granules. Extreme elongation resulted in a thinner cell in which these contents were more easily observed. The spindle-shaped cell often had tenuous, hyaline, filiform, cytoplasmic extensions from either end which exhibited a searching and probing motion. This activity was fairly slow and not whiplike. The incidence of these cells increased as the preparation remained undisturbed on the microscope stage.

Disc-shaped plasmatocytes were also capable of transformation into forms which played a major role in coagulation. They were observed to transform into cells which occasionally were flattened and expanded. The cytoplasm became hyaline at the periphery, accompanied by an extreme flattening of the cell. The cell membrane spread out quite thinly as the cytoplasm expanded onto the glass surface. Granules became very apparent. The edges of the expanded, hyaline cytoplasm ranged from scalloped to slightly pointed extrusions or to extremely long, filiform hyaline extensions, which were frequently very active (Plate I, Figs. 12 and 13). These morphological variations occurred only after the preparation was several minutes old.

Rapid protoplasmic streaming was observed, with granules being carried along in the internal currents. Many hemocytes engaged in extension, retraction, probing, and anastomosis with similar strands. This process may have originated from the form with several cytoplasmic extensions or the fusiform-shaped cell as well. Further description of this process is given under "Coagulation," in the section following this one.

Adipohemocytes. A very distinct hemocyte was often observed, replete with lipoid droplets to the extent that the cell membrane was distended and the cell had a bubbly appearance (Plate I, Figs. 10B and 11). These were classed as adipohemocytes as defined by Jones (1962). They measured 10.9 μ $\pm$1.1 μ, R = 5.6–17.4 μ,

FIGURE 3. Plasmatocytes of various shapes. Phase contrast. 700 ×.

FIGURE 4. Plasmatocytes with cytoplasmic extensions. Phase contrast. 875 ×.

FIGURE 5. Adipoid plasmatocyte with pseudopod-like extensions. Phase contrast. 875 ×.

FIGURE 6. Adipoid plasmatocyte with two lipoid inclusions. Phase contrast. 875 ×.

FIGURE 7. Small plasmatocyte with *Sarcina lutea* packets. Phase contrast. 1050 ×.

FIGURE 8. Disc-shaped and teardrop-shaped plasmatocytes. 3-D condenser-phase-objectives. 875 ×.

FIGURE 9. Spherule cells. A. Normal. B. Dead. Hyaline form with eccentric nucleus and "dancing" cytoplasmic particles. 3-D phase contrast. 875 ×.

FIGURE 10. A. Spherule cell in abnormal, elongated shape. B. Adipohemocyte. 3-D phase contrast. 875 ×.

FIGURE 11. Adipohemocyte. Phase contrast. 875 ×.

FIGURE 12. Flattening and expansion of a plasmatocyte on coverslip, showing granules in the cytoplasm. Phase contrast. 583 ×.

FIGURE 13. Plasmatocyte flattening onto coverslip and extending protoplasmic arms; cell membrane has become very thin and barely visible. Phase contrast. 583 ×.

FIGURE 14. Protoplasmic arm with well developed central body. Active streaming of protoplasm and movement of the body occurred. Phase contrast. 700 ×.

FIGURE 15. Same as Figure 14, taken about 10 seconds later. Bending of arms with central body as a fulcrum caused clump of cells to move in the preparation.

N = 22. Rhodamine B and Sudan IV were used to confirm the lipoid nature of these droplets. The frequency of these cells was always low, but increased during the prepupal stage and was correlated with the presence of free lipoids in the hemolymph. Complete transitional stages between an adipoid variant of a plasmatocyte with lipoid droplets and the hemocyte replete with lipoid droplets (adipohemocytes) were not observed.

Spherule cells. These were very large and highly refractile cells, round, oval, or distended by lobes of gray, homogeneous inclusions which appeared as dark clouds by phase contrast. Cells contained one to several of these inclusions, which distended the cell into irregular shapes (Plate I, Figs. 9A and 10A). Cells usually were unstable after about one hour *in vitro* and tended to rupture, ejecting amorphous material which rapidly dissipated. Some spherule cells never ruptured *in vitro*. After rupture, the nucleus, often previously obscured by the large inclusion, was very prominent, granular, and remained in the cell (Plate I, Fig. 9B). After intracellular disintegration the cell membrane was usually lined with granular debris. Measurements of 55 of these cells showed the longest axis to be 19.5 μ $\pm$9.8 μ (R = 11.9–34.7 μ) and the narrow axis measured 16.4 μ $\pm$4.8 μ (R = 11.9–29.6 μ). Twenty nuclei measured 6.0 $\pm$ 1.0 μ (R = 4.1–8.1 μ).

The nature of the spherules is unknown. They did not fluoresce with any of the fluorescent dyes. Of the other stains used, only fast green FCF and Wright's stain were accepted. The spherules stained red with the latter stain.

In vitro changes of spherule cells occurred in direct preparations. Cells which had one or two medium-sized homogeneous gray inclusions transformed into large "mature" spherule cells. The cells lost their granular appearance, and the diameter increased as the typical gray refractile lobes appeared. The transformation was rapid or required up to 30 minutes in some preparations. Cells which looked like plasmatocytes were observed to develop inclusions which resembled spherules *in vitro*. However, plasmatocytes were not observed to develop into typical spherule cells.

Oenocytoids. No cells resembling descriptions for oenocytoids (Arnold, 1952; Jones, 1962; Yeager, 1945) were observed in wet-mount preparations, and all hemocytes could be placed into the above categories. Fixed, stained smears of boll weevil hemolymph did produce distorted cells which could have been mistakn for oenocytoids because of their uniformly staining cytoplasm, very small (shrunken) nucleus, and apparent creases or striations in the cell membrane. However, prior observation of the slides in wet-mount preparations revealed that these were artifacts. A few such cells were also seen in wet mounts diluted with phosphate buffer, pH 6.4, or in partially dried wet mounts not ringed with white petroleum jelly. Many cells in these mounts were distorted and could not be recognized. Cells resembling oenocytoids were never observed in preparations free from observable artifacts.

Functional Processes

Phagocytosis. The plasmatocytes actively ingested foreign bodies. Larvae were injected into the hemocoel at the ventral side of abdomen with India ink, living *Escherichia coli* (Migula 1895) Castellani and Chalmers 1919, living *Sarcina lutea* (Schroeter 1886), or *S. lutea* stained with carmine. Hemolymph was drawn at

intervals up to one hour after injection. All forms of plasmatocytes were seen with ingested particles. Living *S. lutea* was used most frequently because the packets were easily discerned in hemocytes (Plate I, Fig. 7). Hemocytes with spherule bodies were also observed with phagocytosed bacteria. The typical mature spherule cells never exhibited phagocytosis.

Coagulation. A phenomenon occurred *in vitro* which resulted in formation of extensive networks of protoplasmic strands interconnecting with hemocytes. The process, seen *in vitro,* was first observed 15 minutes to an hour after a preparation was made and proceeded slowly for several hours. Usually about an hour after activity was first seen the networks became quite extensive. One 17-hour-old preparation was a dense mass of closely interwoven plasma fibers and fibroblast-like structures connecting hemocytes, many of which still retained their normal shape. The process was first detected by the occurrence of filaments which often had a filiform extension. (The term "filaments" was used by Jones, 1962. See also Yeager, 1945, "plastids.") They were homogeneous except for one or two small black granules in the bulb-shaped body. These appeared suddenly in great numbers in preparations which later became very active in production of networks. These bulb-shaped filaments originated from plasmatocytes.

Disc-shaped plasmatocytes underwent transformations, observed most frequently in capillary-obtained hemolymph diluted about 1:1 with pH 6.4 phosphate buffer, in which they flattened and expanded, or proceeded to produce filaments or cytoplasmic extensions.

The most frequent event was production of one to four protoplasmic strands. After a long strand had been formed with a bulb-shaped thickening, further extension distally occurred. The entire strand exhibited active protoplasmic streaming and movement of the granules. The distal portion engaged in a rapid extension-retraction movement, along with a probing motion in all dimensions. Whiplike movement of the strands was common.

Two more events occurred simultaneously and represented the major phase of network formation (Plate I, Figs. 14 and 15). The proximal portion of the strand became thickened as cellular contents flowed into it. The thickened proximal strand changed frequently from a plastic to a turgid state and back again. However, the distal portion remained hyaline and continued to move rapidly. The amorphous bulb-like region was retained and a second such region often was formed in the proximal area of the strand. The flow of cellular content resulted in formation of a fusiform body in the strand, with granular inclusions and an additional body which by phase contrast resembled a nucleus.

The second concurrent event was anastomosis of the distal portions to form the network. The extremely active tips often extended 10–50 μ in one direction to form a terminal bulb-like body, which increased greatly in size but remained hyaline, and also sent out a second pseudopodial extension from the thickened body. Then both strands could be rapidly withdrawn while a third originated from a new location on the thickened body (Plate II, Fig. 16). When the two strands touched each other they recurved along their entire length and joined into a single larger strand. The pseudopodia could become quite rigid and were occasionally observed to force their way between cells clumped together and to separate a cell from the clump. Plate I, Figures 14 and 15 show such activity. The photographs were

taken about 10 seconds apart. The result of the entire process was an extensive network in all dimensions, with newly formed thickened areas hardly distinguishable from fusiform plasmatocytes, and many areas of expanded protoplasm containing debris from parent cells. Extensive networks, formed one to two hours after removal of hemolymph and preparation of the slide, are shown in Plate II, Figures 17–19. More extensive formation frequently occurred after 6 to 17 hours.

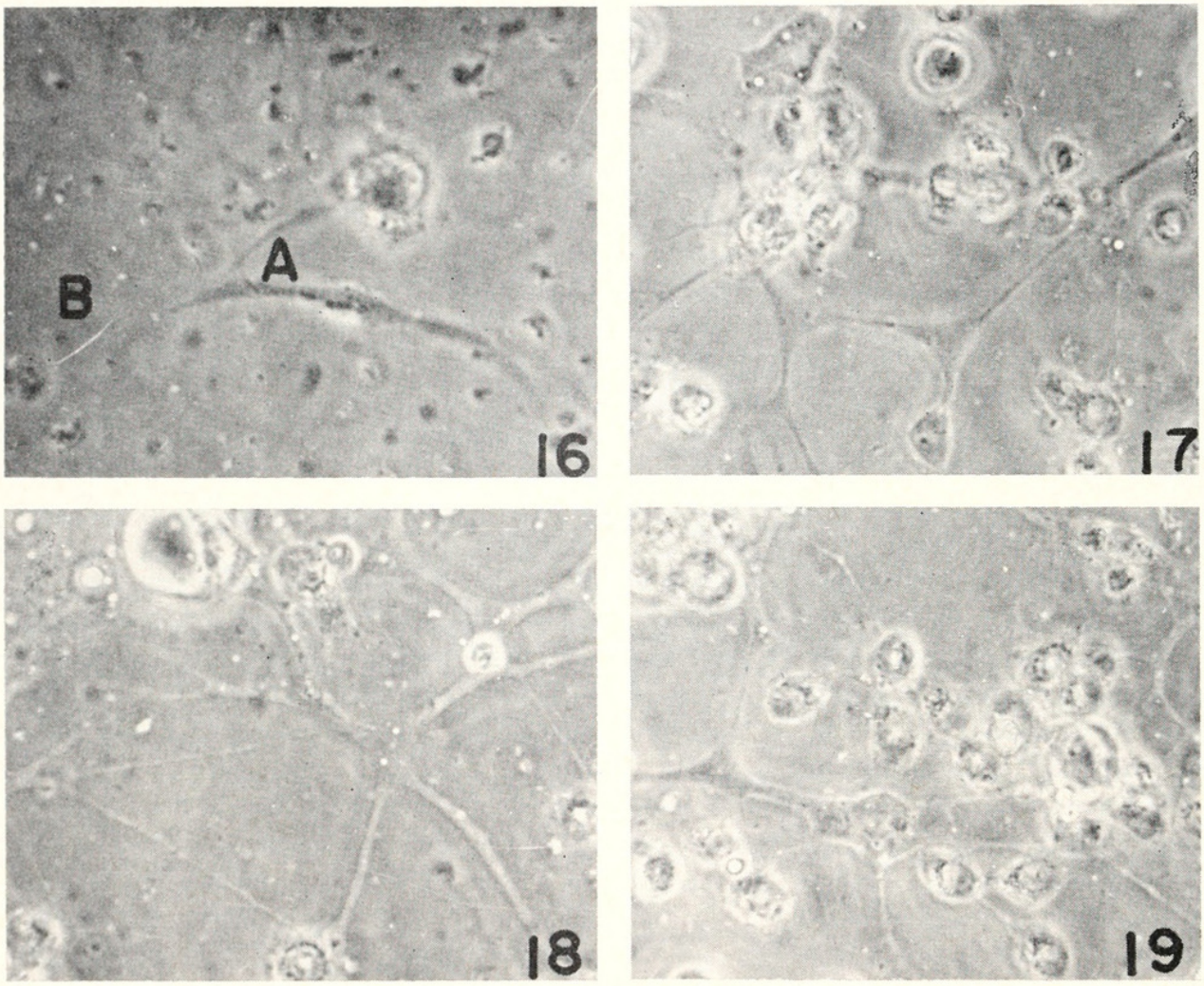


Plate II

FIGURE 16. Rapid recurving of two strands which have coalesced at A, and subsequent extension of a new strand at B. Phase contrast. 700 ×.

FIGURES 17, 18, 19. Extensive network formations 60–120 minutes after hemolymph withdrawal. Phase contrast. 350 ×.

Rupture of spherule cells occurred during this process and frequently was seen prior to the early stages of stranding and network formation. A definite relationship to initiation of the process by the erupted spherule cells was not established. However, protoplasmic stranding occurred in immediate proximity of the cells and even appeared to be attached to intact large spherule cells on several preparations. Their size and high refractivity prevented observance of the tenuous strands at the cell membrane, and no proof of actual connection was seen. From our observations, spherule cells do not initiate coagulation, nor do they play a role in coagulation

process, with the possible exception of serving as a physical body to which the strands may attach.

One important factor directly influencing the extent of network formation was the proximity of the plasmatocytes to each other. Strands were frequently seen which had extended apparently to the maximum extent possible. If no other strands were encountered, further activity ceased. Anastomosis with other strands appeared to rejuvenate or increase the vigor of the strands and promote continued formation.

Effect of Physical and Chemical Treatment on Hemocytes

Hemocyte morphology and *in vitro* transformations were altered by the physical action of hemolymph withdrawal and placement on the slide and by dilution with pH 6.4 phosphate buffer, 0.85% NaCl solution, 0.5% (w/v) trehalose in distilled water (final pH 5.8), 1% potassium oxalate (w/v) in distilled water (final pH 6.75), or tap water (pH 7.3). Although quantitative data regarding the extent of the effect on hemocytes were not obtained, comparisons were made between hemocytes observed in direct preparations and those in capillary preparations or hemolymph diluted about 1:1 with the above diluents. Study of hemocyte morphology and *in vitro* transformations by several techniques of sample preparation provided an indication of pleomorphic variability and recognition of artifacts imposed by technique.

The physical action of drawing hemolymph into a capillary tube and then dispensing it onto a coverslip involves tremendous rates of flow at the orifice. The direct method for allowing an exuding drop of hemolymph to flow onto a coverslip is much less rigorous. This latter, direct, method was considered to result in hemocytes most like those in natural *in vivo* conditions. However, interpretation of observations from this method must also be tempered when one is attempting to draw conclusions as to the *in vivo* hemocyte conditions. Plasmatocytes and spherule cells were affected by the physical forces involved in physical withdrawal of the hemolymph. Changes in appearance of adipohemocytes and prohemocytes due to technique were never detected.

The capillary method resulted in great changes in plasmatocyte morphology. Very few plasmatocytes observed immediately after sample preparation by this method exhibited cytoplasmic extensions or were spindle-shaped. Disc-shaped forms were dominant and the flattened and expanded forms were frequent. On the other hand, preparation by the direct method resulted in immediate observation of all pleomorphic forms of plasmatocytes.

Dilution of hemolymph caused variations in hemocyte morphology. Although quantitative tests were not conducted, comparison of hemocytes in preparations diluted with the above materials with those in direct, undiluted preparations gave clear indication of artifacts. In the trehalose or potassium oxalate solutions, disc-shaped plasmatocytes were inflated and internal structural details more distinct. When the pH 6.4 phosphate buffer was used, disc-shaped plasmatocytes underwent a very rapid transformation to spindle-shaped and pseudopodial forms. However, dilution with water drawn directly from the tap (pH 7.3) did not result in this increased activity. Surprisingly, water from the central distillation source of the

Laboratory was found to be pH 6.1. Attention to the pH of diluents may be quite important in a study of hemocyte morphology.

Discussion

The cells referred to as prohemocytes were not observed *in vitro* to change into mature cells. However, all intermediate stages from the typical prohemocyte described above to a spherical plasmatocyte were easily observed. As with all hemocytes, proof of origin may have to await application of tissue culture methods. Embryological study was not conducted. The reviews of Wigglesworth (1959) and Jones (1962) covered the origin of insect hemocytes. Imaginal discs, mitosis of hemocytes, and hemopoietic tissues have been reported as sources of post-embryonic replacement of hemocytes.

Plasmatocytes were the most abundant forms in all preparations. They were capable of extreme pleomorphism and varied considerably with regard to cytoplasmic inclusions. Close attention to nuclear size and placement, observation of *in vitro* changes, and intentional production of artifacts by various methods left no doubt as to the identity of all the observed forms. These hemocytes can carry on phagocytic activity, produce networks in a process which could be interpreted as coagulation, and possess different quantities and types of cytoplasmic inclusions. Because of transformation into many pleomorphic forms and variations of inclusion material, plasmatocytes are tentatively classed as a single category until further study proves other types to be valid end products of plasmatocyte differentiation.

The spherule cells are designated as a type of hemocyte because of their distinctive character. The slight phagocytic capability of spherule cells does not imply they are plasmatocytes because phagocytosis by pericardial cells and certain cells of adipose tissue has been reported (Cameron, 1934). Moreover, the function of phagocytosis has not been shown to be restricted to only one type of cell. Spherule cells were not demonstrated to play an active role in the coagulation process.

Adipohemocytes are considered a distinct type of cell because of their unique appearance and apparently unique function connected with lipid material. They were stable *in vitro* by all techniques employed. Although complete transitional stages from any other type of hemocyte, especially the adipoid plasmatocyte, were not observed, this does not establish the independent lineage of adipohemocytes. Further research may clarify the relationship between these hemocytes.

The process of coagulation was reviewed by Jones (1962) and Wigglesworth (1959). Grégoire (1951, 1955, 1959a, 1959b) studied the process in more than 400 species of insects. Four categories were described (Grégoire, 1951) and reviewed by Wigglesworth (1959). Type IV (no coagulation) was reported from two species of curculios (Grégoire, 1955). In a further study (1959b) he recorded Type IV from six additional species and observed the presence of hemocytes which exhibited the elongation, expansion, and retraction of flexuous protoplasmic arms, as well as formation of loose mesh-works of hemocyte protoplasm. In other studies (1959a) he reported network formation in many additional species of curculios. He also described "plastic hemocytes" which produced activity similar to that seen among plasmatocytes in *A. grandis*. Grégoire also reported prohemocytes, which probably correspond to those in the boll weevil, as well as other cells

similar to boll weevil plasmatocytes. Filaments with thickened regions, were also reported to take part in network formation. In the boll weevil, similar filamentous bodies originated from plasmatocytes and were the terminal portions of protoplasmic strands which frequently dissociated from the cell during the process of network formation.

The process of network formation did not occur in every preparation. It was most frequent when the direct method was utilized and the preparation appeared to be normal, or lacking in artifacts. The proximity of cells undergoing stranding was important to the extent of network formation finally attained. When strands failed to contact others, the process was not as extensive as when cells were close enough to allow extensive contact and fusion, which appeared to rejuvenate the strands involved. The importance of the number of cells to coagulation was also observed by Yeager and Knight (1933).

A very graphic example of the importance of recognition of artifacts and study by several techniques occurred when a hemolymph sample was diluted with pH 6.4 phosphate buffer. We observed thin plasma strands with adherent particles and granular plasma islands identical to those pictured by Grégoire (1951, Plate V, Figure 25). We saw this condition only once, and our attempts to repeat it were unsuccessful. Although Grégoire did not dilute the hemolymph, he allowed it to flow by capillary action between an unsupported coverslip and the slide. This incident serves merely to illustrate the necessity for studying hemocytes by several methods and attempting to recognize the possible alterations caused by technique and physical or chemical conditions.

The authors gratefully acknowledge the suggestions and comments of J. C. Jones in reading the manuscript.

Summary

1. Hemocytes of larval boll weevils, *Anthonomus grandis* Boheman, were classified on a morphological basis into four types. *Prohemocytes* had a large nucleus and a thin band of peripheral cytoplasm, which increased in quantity and became more optically dense to phase contrast as the prohemocytes matured. All gradations to spherical *plasmatocytes* were observed. *Plasmatocytes* were characterized by their great pleomorphic capability. The cytoplasm varied in that it was granular with fine or large granules, dense and uniform to phase contrast or heterogeneous with areas of different optical density as well as possessing vacuoles or various inclusions, in addition to being hyaline or refractive at the cellular periphery. Plasmatocytes assumed spherical, pseudopodial or fusiform, as well as irregular, shapes during a process of stranding or when flattening onto a glass surface. Plasmatocytes were phagocytic. Cells filled with lipoid globules were tentatively classed as *adipohemocytes*. A fourth type of cell, *spherule cells*, was characterized by the presence of one to several large globules which were amorphous and non-refractive to phase contrast. These cells were large and often distended by the inclusions.

2. A slow process of network formation was the only observed indication of coagulation. Plasmatocytes underwent extension stranding by extension, retraction,

probing, and anastomosis with protoplasmic arms from other cells to produce networks. The extent of these networks depended partially upon the proximity of stranding cells.

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