PARC NATIONAL ALBERT
(DEUXIÈME SÉRIE)
Fascicule 10

NATIONAAL ALBERT PARK
(TWEEDE REEKS)
Aflevering 10

HEMOLYMPH OF CURCULIONIDAE AND OF DIPTERA

OBSERVATIONS BY PHASE-CONTRAST AND ELECTRON MICROSCOPY

BY

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In most specimens belonging to 33 species of *Curculionidae* investigated previously (GRÉGOIRE, 1955, 1957; GRÉGOIRE and JOLIVET, 1957), coagulation of the plasma was not detected in the films of hemolymph spread out between slide and coverglass and examined in the optical conditions of the phase-contrast microscope.

On the other hand, GRÉGOIRE and JOLIVET (1957) reported, in the hemolymph of african weevils, alterations in a category of plastic hemocytes, resulting in constitution of loose networks, which extended over wide areas of the films. In 1911, HOLLANDE had already observed, in the hemolymph of the snout-beetle *Cionus fraxini* DE GEER, filamentous processes produced by considerable elongation of a category of hemocytes (phagocytes).

Similar reactions occurring in the hemolymph of insects in vitro have been reported as yet only in *Diptera* (GRÉGOIRE, 1955; JONES, 1956).

The present report is a comparative detailed description of these particular structures detected in a material of *Curculionidae*, most of them belonging to african species, and of *Diptera*.

MATERIAL AND METHODS.

The african weevils examined in the present study were captured by Dr. P. JOLIVET in Parc National Albert in 1954-1955. Two other african species, *Rhynchophorus phoenicis* FABRICIUS (13 specimens) and *Temno-*

schoita quadripustulata GYLL. (17 specimens) were kindly provided by the laboratories of I.N.E.A.C. (Yangambi). A few palearctic and neotropical species, exhibiting similar reactions, were included in the present material. The results have been compared to those collected in several species of palearctic Diptera and in one neotropical specimen.

In *Curculionidae*, especially in the insects of small size, collection of the hemolymph without contamination by tissues is difficult because of the hardness and rigidness of the integument. After severance of the antennas or of the legs, hemolymph does not generally flow out spontaneously from these appendages, but only when a rather strong pressure is exerted on the insect body. However, in the present study, several samples were correctly obtained by pushing the wing cases back to the thorax, and by applying the limpid — of golden yellow colour in most species — droplet of hemolymph issuing from the joints to the edge of a coverglass lying on a slide. Drops of hemolymph were also deposited directly on to the middle part of the slide and were immediately covered with the coverslip. Gentle pressure on the abdomen increased the size of the droplet, but was apt to induce contamination of the samples by foreign tissues. Uncontaminated transparent droplets of hemolymph were also obtained by squeezing slightly on the slide the severed end of the wings and by puncturing the abdomen with microneedles. The successive steps of the reactions were examined with a phase-contrast microscope (Wild M/10).

Samples of hemolymph were also collected on 200-mesh copper grids, previously coated with films of formvar (0.1 per cent in ethylene dichloride) and examined with a R.C.A., type E.M.U. electron microscope.

OBSERVATIONS.

CURCULIONIDAE.

Reactions of the hemocytes and of other particles in the films of hemolymph in vitro.

The following elements were recognized in the films of hemolymph immediately upon shedding and spreading out between slide and coverglass :

1. Small round hemocytes, with a large nucleus, surrounded by a narrow ring of cytoplasm. These cells may be identified with the macronucleocytes of small size, or prohemocytes of the literature (Textfig. 1, A 1).

2. Variously shaped hemocytes of larger size, with a nucleus relatively smaller than in the elements of category 1. Some of these hemocytes were oval, with short radiating spiky pseudopods. Spindle-shaped cells had straight expansions at the opposite sites of the cell body. The dark cytoplasm of these corpuscles contained from few to many granules (not drawn in Textfig. 1, A 2).

3. Large vesicular cells, with a small eccentric, coarsely outlined nucleus, a pale cytoplasm containing a few granules in active brownian motion. These hemocytes (Textfig. 1, A 3), unfrequent in weevil hemolymph, resembled the unstable hyaline hemocytes which play an important part in the inception of the coagulation of the plasma in the hemolymph of many other insects (GRÉGOIRE and FLORKIN, 1950; GRÉGOIRE, 1951, 1955, 1957). In lateral view in the films, these cells appeared as flat saucers with a central bulging elevation corresponding to the nucleus.

4. Particles of smaller size (0.5 to 0.9 microns) than the hemocytes of the three preceding categories, unrelated to them, and appearing as commas, rods with a central thickening, simple straight filaments, small dark vesicles with one or two short threadlike expansions, frequently disposed at obtuse angles with the central vesicle (Textfig. 1, B).

After a few seconds, the hemocytes and particles belonging to categories 2 and 4 underwent considerable modifications.

In some of the hemocytes, the hyaloplasm spread out into circular veil-like folded membranes (Plate 2, fig. 5) similar to the petaloid pseudopods described in invertebrate choanoleucocytes by LOEB (1903), by DEHORNE (1925) and by FAURÉ-FRÉMIET (1927, 1929).

In hemocytes of the same category (Plates I and II) the initially short cytoplasmic expansions grew slowly and steadily into tortuous filaments of considerable length (Plate I, figs. 1, 2, 2′), with vesicles, varicosities and

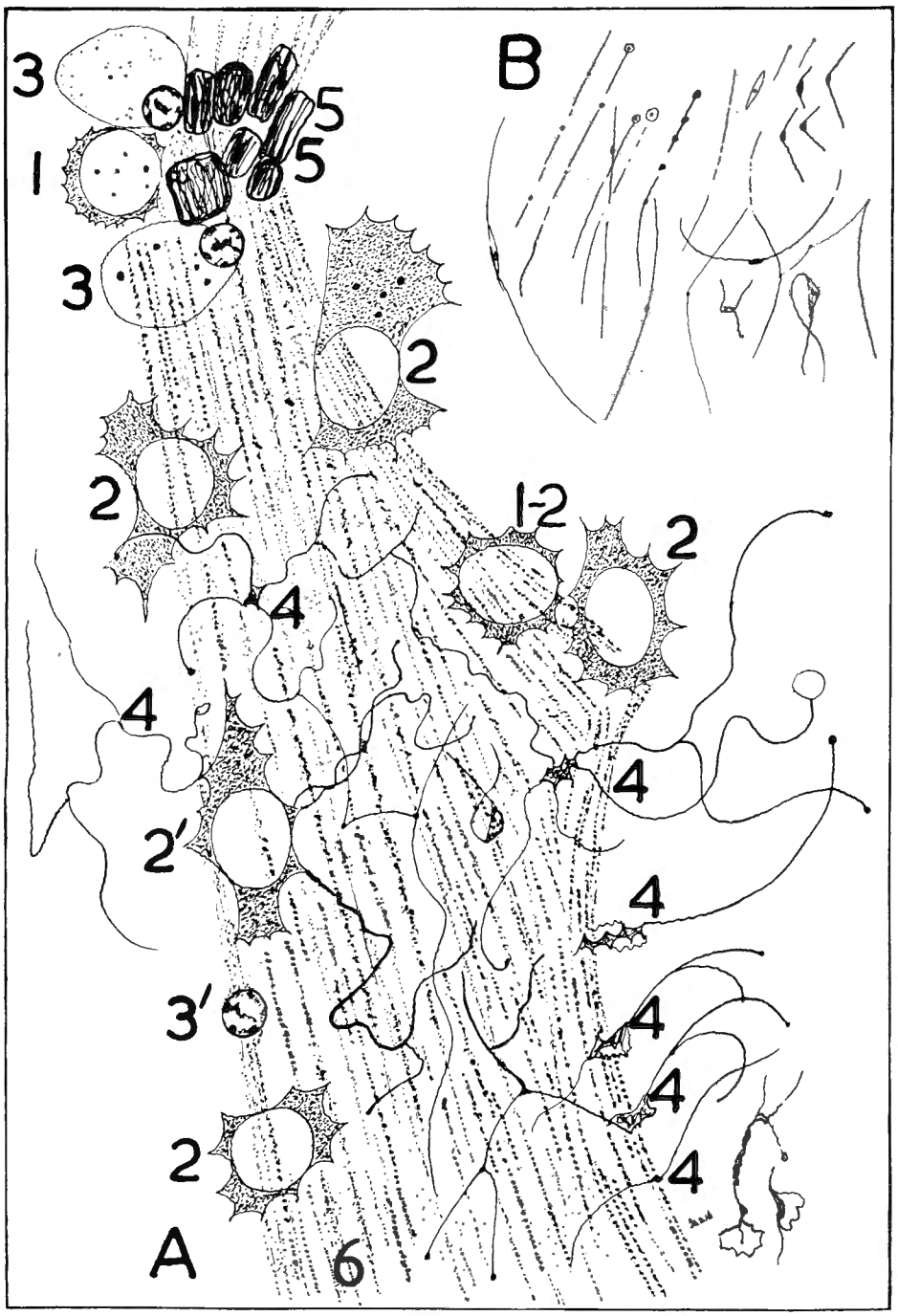

FIG. 1.

beadings scattered along their course, and with bulbous club-like thickenings at their extremities (Plate I, figs. 2, 2′, 3, 5). Some parts of these filaments became tubular or vesicular (Plate II, fig. 3). While growing, the filaments exhibited, sometimes during hours, continuous trepidations and jerks, elastic retractions followed by recurrence of extension. Without apparent hindrance on their way, the creeping threadlike expansions bent abruptly at various angles or underwent bifurcations into y-shaped structures. Like diverging twigs, secondary ramifications developed on the main branches and exhibited similar wrigglings (Plate I, fig. 5).

Within a few hours, considerable arborizations had grown from these hemocytes, which then closely resembled typical vertebrate nerve cells cultivated in vitro.

All these arborizations intercrossed and formed substantial networks or plexus-like structures (Plate I, fig. 4; Plate II, figs. 1, 3, 4).

Owing to their extreme plasticity, the structures described above were thoroughly altered or completely destroyed in the smears prepared for staining.

EXPLANATION OF TEXT FIGURE 1.

Rhynchophorus phoenicis FABRICIUS (*Curculionidae*).

Film of hemolymph spread out between slide and coverglass. The drawing, performed 150 minutes after shedding, has been combined from observations with the phase-contrast microscope on samples from several specimens of that species, and illustrates the two independent systems of reactions described in the text:

1. Constitution of *networks* by the cytoplasmic expansions, forming arborizations, of a category of plastic hemocytes (category 2 of the description), or plasmatocytes (A 2). Only one of these altered hemocytes (A 2′) has been represented on the drawing.

Subsidiary constitution of networks by slender expansions produced by undetermined particles (4, 4′), possibly pleomorphic growth forms of symbiotes. Immediately upon spreading out of the hemolymph, these particles appeared as represented on B (see description in text).

2. *Jellification of the plasma*, appearing as a fan-shaped, stretched and folded veil (A 6). Two hyaline hemocytes, closely resembling the unstable hyaline hemocytes which induce coagulation of the plasma in other groups of insects, are shown in A 3-3, anchored to debris of foreign material (A 5). In *Curculionidae*, these hemocytes are obviously less frequent than in many other groups of insects. In A 3′ (bottom right), one of these hemocytes is shown, reduced to its nucleus, after elongation of its cytoplasm into straight threadlike expansions, embedded in the plasma veils, and no more visible.

Emission of cytoplasmic expansions by hyaline hemocytes, followed by jellification of the plasma in the shape of veils, characterizes pattern II of the classification of the process of coagulation of the hemolymph in insects.

In *Rhynchophorus*, association of the two independent systems of reactions — cellular networks and jellification of the plasma — did not appear in all the specimens. In most *Curculionidae*, the networks developed by the plasmatocytes and by the small particles were the only reactions recorded.

The alterations in the small particles (category 4) consisted, like those observed in the hemocytes of category 2, of considerable increase in length of their expansions, with bendings and with emission of lateral branchings (Textfig. 1, A 4; Plate II, figs. 1 and 2). The arborizations developing from these alterations were more slender than those grown from the hemocytes of category 2 (Plate II, figs. 1 and 2).

Other undetermined particles, needle-like or blade-like in shape, incidentally found in the hemolymph of *Rhynchophorus phoenicis* (Plate IV, fig. 3), did not seem to take part in the formation of the slender networks.

The alterations described above were observed in the hemolymph of the following weevils :

Palearctic material :

Phytonomus fasciculatus L. (*Hypera zoilus* SCOP.); *Otiorrhynchus veterator* UYTTBERG; *Phyllobius urticae* LINNÉ.

African material :

Campyloscelus westermanni BOHEMAN; *Anomoederus interstitialis* FAUST; *Entypotrachelus micans* HUSTACHE; *Gasteroclisus auritus* BOHEMAN; *Gasteroclisus auricillatus* BOHEMAN; *Gasteroclisus prolongatus* MARSHALL; *Lixus bidentatus* KOLBE; *Brachyceropsis verrucosa* FABRICIUS; *Brachycerus kumbanensis* QUEDENFELDT; *Alcidodes orientalis* CHEVROLAT; *Alcidodes olivaceus* GERSTAECKER; *Alcidodes josephus* DUVIVIER; *Aedemonus eminentipunctatus* BOHEMAN, var. *griseus* HUSTACHE; *Rhadinomerus* sp.; *Borthus binotatus* MARSHALL; *Sipalinus guineensis* FABRICIUS; *Alcides* sp.; *Amphitmetus* sp.; *Rhynchophorus phoenicis* FABRICIUS; *Temnoschoita quadripustulata* GYLL.

Neotropical material :

Compsus sp.; *Heilipus* sp.; *Heilipus* sp.

The reactions in the hemocytes and in the particles were inconstant, absent or overlooked in the samples from *Sipalinus squalidus* KOLBE, and from the neotropical *Naupactus* sp. and *Metamasius* sp.

In 20 specimens of *Rhinopteryx foveipennis* THOMSON, the only species of *Brenthidae* available, the samples of hemolymph contained only scarce hemocytes, in which the alterations described in *Curculionidae* did not appear. Small particles were found in scarcer numbers.

Reactions in the plasma.

As reported in previous observations on *Curculionidae* (GRÉGOIRE, 1955, 1957; GRÉGOIRE and JOLIVET, 1957), the hemolymph remained fluid and visible alterations in the consistency of the plasma were absent in most samples

of hemolymph from that family, including those from *Temnoschoita quadripustulata* (17 specimens) and from 6 out of 13 specimens of *Rhynchophorus phoenicis* FABRICIUS, two species not represented in our former studies.

On the other hand, emission of cytoplasmic expansions by unstable hyaline hemocytes (category 3) (Textfig. 1, A 3 and 3'), development of fibrillar veil-like reactions in the plasma (Textfig. A 6; Plate II, fig. 2), — which are the main characters of pattern II in the classification of the process of hemolymph coagulation in insects (GRÉGOIRE, 1951, 1955, 1957) — were observed in 7 out of 13 specimens of *Rhynchophorus phoenicis* FABRICIUS. From these 7 specimens, 5 exhibited pattern II in a few samples only, while in other samples of the same insects no coagulation occurred. On the other hand, in 2 specimens, a substantial pattern II was recorded in all the samples. Upon shedding, the hemolymph of these 2 specimens became rapidly ropy and viscous (1).

In the hemolymph of *Rhinopteryx foveipennis* (*Brenthidae*), sparse changes in the consistency of the plasma in a few samples indicated that some degree of jellification might have taken place in these samples (see below and Plates III and IV).

At the periphery of several films, exposed to desiccation along the edges of the coverglass, precipitates developed in the shape of inelastic fibrillar meshworks, unrelated to the presence of hemocytes in the neighbourhood.

Preparations of jellified plasma, diluted with Meisenheimer's serum for insects, from *Rhynchophorus* and from *Rhinopteryx*, were examined with the electron microscope (Plates III and IV). In the electron micrographs, the clotted parts of hemolymph appear, as in preparations from several other insects (GRÉGOIRE, DUCHÂTEAU and FLORKIN, 1949; GRÉGOIRE and JOLIVET, 1957) in the shape of sponge-like masses or aggregates of microflocs against a background of small rounded particles, isolated or agglutinated into clumps. The clotted material, when incidentally drawn out, takes the shape of strings made of alined rounded particles.

Films of hemolymph from *Temnoschoita*, in which no modification in the plasma consistency could be detected by phase-contrast microscopy (pattern IV of the classification of the hemolymph coagulation in insects), were also examined with the electron microscope. Wide areas of the preparations consisted only of small granules, 15 to 20 millimicrons in diameter, difficult to distinguish from the granulations of the membrane of formvar. In other fields, sparse amorphous microflocs, invisible by phase-contrast microscopy, were scattered on the background.

(1) In the samples from *Brachycerus kumbanensis* QUEDENFELDT (GRÉGOIRE and JOLIVET, 1957, p. 31), sparse veils developed, but could not be ascertained unequivocally.

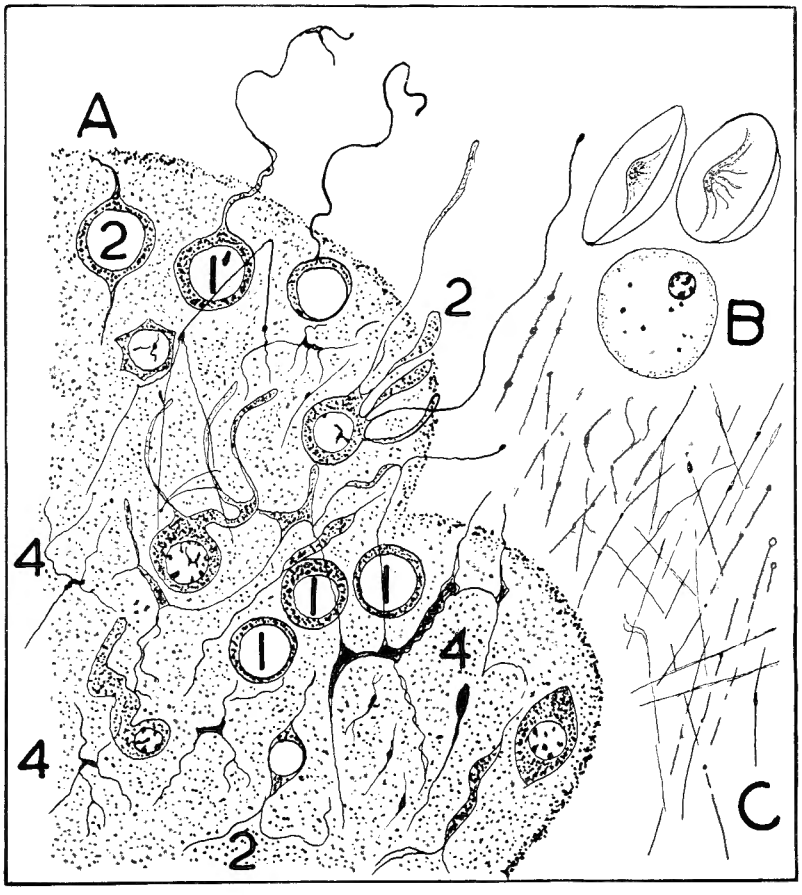

FIG. 2.

EXPLANATION OF TEXT FIGURE 2.

The drawing has been combined from photomicrographs with the phase-contrast microscope (see GRÉGOIRE, 1955, Pl. VIII, figs. 29 and 30) of several samples of hemolymph, spread out between slide and coverglass, from larval *Tipulidae* (gen. *Tipula* and *Dictenidia*).

Embedded in a substantial veil of jellified plasma (boundaries in the shape of curdy waves), the various structures observed in that material are shown : prohemocytes (A 1), transitional forms, with cytoplasmic expansions (A 1′), plastic hemocytes or plasmatocytes (A 2) with their expansions, forming the complex networks (not drawn) represented in the plates, small particles with their arborizations, possibly growth forms of pleomorphic symbiotes (A 4; C : immediately upon spreading out of the hemolymph). B : two hyaline hemocytes, appearing as floating saucers, before their alterations, and a clarified corpuscle, after discharge of cytoplasmic material (compare with Pl. VI, figs. 5, 6 and 7).

DIPTERA.

Reactions of the hemocytes and of other particles.

Plastic hemocytes (Plate V, figs. 1, 2, 4) resembling those described in *Curculionidae* (category 2), and particles smaller than hemocytes (Textfig. 2, A 4 and C; Plate V, fig. 5) were similarly involved in constitution of networks (Plate V, fig. 3; Plate VI, figs. 1, 3 and 4).

The reactions appeared in samples from specimens of the following species :

Palearctic material :

NEMATOCERA.

TIPULIDAE : *Tipula paludosa* MEIGEN (larvae); *Tipula oleracea* LINNÉ (larvae); *Dictenidia bimaculata* LINNÉ (larvae); undetermined xylophagous larvae; undetermined larvae, rootfeeders; undetermined aquatic larvae; undetermined adults.

BIBIONIDAE : *Bibio marci* LINNÉ (adult).

BRACHYCERA.

SYRPHIDAE : *Erystalomyia tenax* LINNÉ (adults).

SARCOPHAGIDAE : *Sarcophaga* sp. (adults).

TACHINIDAE : *Lucilia caesar* LINNÉ (adults).

MUSCIDAE : *Musca domestica* LINNÉ (adults).

CALLIPHORIDAE : *Calliphora erythrocephala* MEIGEN (adults).

Neotropical material :

LARVAEVORIDAE : *Ormiophasia buschkii* T.N.S. (adult).

Reactions in the plasma.

In agreement with former observations on insects from the same families (GRÉGOIRE, 1955, p. 121 and fig. 37), jellification of the plasma in the shape of substantial veils embedding all categories of structures, developed in the hemolymph of larval *Tipulidae* immediately upon shedding. Subsequently, the cellular networks described above developed within these veils (Textfig. 2). In several specimens, in which the reactions in the plasma were slower, alterations in a category of oenocytoid-like dark hyaline hemocytes were observed to precede these modifications in the plasma. The alterations in the hemocytes (Plate VI, figs. 5, 6, 7) consisted of swellings and shrinkages in succession, followed by sudden discharge of cytoplasmic

material, including granules, and clarification of the corpuscles, which finally appeared as rounded pale vesicles. Figure 7 of plate VI shows two altered hyaline hemocytes embedded in a transparent veil developed after their alterations.

DISCUSSION.

Two kinds of reactions have been observed in the films of freshly withdrawn hemolymph, spread out between slide and coverglass, from *Curculionidae* and from Diptera :

1. *Constitution of networks* over wide areas of the films by substantial arborizations developed by a category of plastic hemocytes, and subsidiarily, by corpuscles, of undetermined origin, and of smaller size than the hemocytes.

These modifications appeared consistently in the samples of hemolymph from both groups of insects.

The category of highly plastic hemocytes which build up these networks might be identified in the snout-beetles with the phagocytes observed by HOLLANDE (1911) in *Cionus fraxini*, characterized by production of filaments of considerable lengths, and in *Diptera* with the types 5 and 7 of ÅKESSON (1953) and with the plasmatocytes described by JONES (1956) in *Sarcophaga bullata* PARKER.

On the other hand, corpuscles closely resembling the altered hemocytes such as those illustrated in plate VI, figure 1, of the present paper, have been recorded by PAILLOT (1924, fig. 1; 1943), as « refringent bodies » in suspensions of the hemolymph of infected caterpillars of *Pieris brassicae*, and as large growth forms of pleomorphic bacterium-like symbiotes, floating freely in the hemolymph of the aphid *Drepanosiphum platanoides* (PAILLOT, 1930, 1933).

Some of the small particles are probably, in agreement with previous observations (HOLLANDE, 1911, in the weevil *Cionus*; JONES, 1956, in *Sarcophaga*; GRÉGOIRE and JOLIVET, 1957, in numerous specimens of *Curculionidae*) fragments detached from the expansions developed by the plastic hemocytes.

However, in the present and former materials of weevils and of *Diptera*, these small elements were recorded in the films immediately upon withdrawal of the hemolymph, before emission of threadlike processes had started in the plastic hemocytes, and obviously cannot proceed from those cells.

Consistent finding of these small elements in *Curculionidae* and in *Diptera*, without interference of any artifact of preparation of the hemolymph, suggests that these structures belong to the normal composition of the hemolymph.

As reported in extensive studies (see BUCHNER, 1930, 1933; PAILLOT, 1933; STEINHAUS, 1946, 1949), the hemolymph of probably all *Curculionidae* (including genera studied in the present material : *Brachycerus*, *Otiorhynchus* and *Rhynchophorus*) and of many *Diptera*, may harbour free symbiotes. Some of these symbiotes are discharged by disintegration of mycetocytes and of bacteriocytes. These symbiotes are highly pleomorphic and assume bacterium-like and filamentous shapes (Plate III, figs. 1, 2, 3). These bodies may undergo such variations in their structure, even in a same host individual, that they lose their original appearance.

2. *Jellification of the plasma* in the shape of veils, embedding all the hemocytes and their networks. These changes in the plasma were frequently preceded by alterations in a category of unstable hemocytes.

In *Curculionidae* as yet investigated, veil-like modifications in the plasma appeared inconstantly in the samples from 2 (*Rhynchophorus phoenicis* and possibly *Brachycerus kumbanensis*) out of 33 species. In the other species, changes in the plasma were not visible in the optical conditions of phase-contrast microscopy.

In *Temnoschoita*, in which the phase-contrast microscope did not reveal any modification of the plasma, discrete sponge-like masses, resembling the microflocs characterizing the plasma clots in other insects, such as *Rhynchophorus* and *Rhinopteryx* (see Plates III and IV), were observed whit the electron microscope. However, in *Temnoschoita*, these structures were in much scarcer amounts, and were not present over wide areas of the films.

The findings suggest that, in some insects characterized by pattern IV of the classification, changes, possibly corresponding to a small degree of coagulation, may take place in the films of hemolymph in vitro (see GRÉGOIRE, 1959). These changes do not alter perceptibly the fluidity of the hemolymph.

In *Diptera*, in agreement with our former results, immediate jellification of the hemolymph occurred in larval *Tipulidae*. Clotting of the blood of dipteran larvae has been reported by VIALLANES (1882), LOWNE (1890-1895), HEWITT (1914), JONES (1956).

In the snout-beetles and flies, in which that jellification of the plasma took place, alterations in hyaline hemocytes (category 3 of the present description) morphologically comparable to the coagulocytes of many other insects, preceded these changes.

In *Curculionidae* and in *Diptera* in which jellification of the plasma did not appear, the hemocytes of that category remained inert or their alterations were not followed by visible changes in the surrounding plasma.

The identity of these hemocytes has been examined in previous papers (GRÉGOIRE and FLORKIN, 1950; GRÉGOIRE, 1951, 1955 and 1957). In some

groups of insects (*Diptera*, *Lepidoptera*) they appear, before their alterations, as oenocytoid-like corpuscles. Oenocytoids have been reported in *Diptera* by THOMSON (1931), ROOSEBOOM (1938), DENNELL (1947), SÉGUY (1950) and RITZKI (1953).

SUMMARY AND CONCLUSIONS.

1. In *Curculionidae* and in *Diptera*, a category of hemocytes undergo in the films of freshly withdrawn hemolymph, spread out between slide and coverglass, alterations not shown by the other kinds of hemocytes. These elements, characterized by a high degree of plasticity, develop substantial cytoplasmic expansions, in the shape of arborizations resembling the dendritic expansions of nerve cells in vertebrates. These alterations result in constitution of networks over wide areas of the films. Subsidiarily, undetermined particles, of smaller size than the hemocytes, and possibly of symbiotic nature, undergo similar alterations and produce slender networks « in vitro ».

2. Inconstant coagulation of the plasma, with the characters of pattern II of the classification of the process of coagulation in insect hemolymph, occurred in two out of 33 species of *Curculionidae* investigated.

Immediate and substantial jellification of the plasma took place in larval *Tipulidae*, and was frequently observed to be preceded by alterations in a category of unstable hyaline hemocytes, morphologically similar to the fragile corpuscles or coagulocytes, which play an important part in the inception of coagulation in many other groups of insects.

3. The consistent development in such unrelated taxonomic groups, as are *Curculionidae* and *Diptera*, of closely similar reactions involving highly plastic hemocytes, contrasts with absence of such particular reactions in many other groups of insects as yet investigated.

In the specimens in which the plasma clots, these two independent systems of reactions — constitution of purely cellular networks on one hand, and jellification of the plasma on the other hand — are superimposed.

As pointed out by Jones, the systems of cellular networks might be of some importance in aiding healing of the wounds.

These formations might be especially efficient in the species characterized by absence or by sparse coagulation of the plasma. In these insects, the plastic hemocytes (plasmatocytes) might functionally compensate for the failure of the unstable hyaline hemocytes to induce coagulation in the plasma.

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We wish to express our sincere thanks to Professor V. VAN STRAELEN, President, to M. H. DE SAEGER, General Secretary and to Dr. E. LELOUP, Head of the Scientific Department, of the Institut des Parcs Nationaux du Congo Belge, for authorization of receiving by airmail fresh african insects, for defraying the expenses for the shipment of the material and for publication of the paper. I thank Dr. P. JOLIVET for collecting most *Curculionidae*.

We are indebted to M. F. JURION, General Director of the « Institut National pour l'Étude Agronomique du Congo Belge » (I.N.É.A.C.), for kindly arranging the most favorable conditions and for defraying the expenses of shipment of fresh african *Curculionidae*, to Dr. E. BUYCKX, Director of the Central Laboratories in Yangambi, for collecting and mailing the material of *Rhynchophorus* and *Temnoschoita* used in the present study, to Messrs. Drs. G. MARSHALL, British Museum, London, M. COLLARD, E. DERENNE, R. TOLLET, J. M. VRIJDAGH, for identification of the material, to Miss JEANINE COLLARD for carrying rapidly after capture several african specimens.

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Received February 14, 1959.

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PLATES

EXPLANATION OF PLATES I-VI.

The preparations (Plates I, II, V and VI) are films of hemolymph spread out between slide and coverglass, immediately upon shedding from severed appendages, and photographed in phase-contrast microscopy (WILD M/10).

Plates III and IV are electron micrographs of hemolymph diluted with Meisenheimer's serum for insects, deposited onto copper screens coated with films of formvar (0.1 per cent solutions in ethylene dichloride). After fixation and desiccation of the material, the preparations were shadowed with palladium.

PLATE I

EXPLANATION OF PLATE I.

FIG. 1. — *Rhadinomerus* sp. *(Curculionidae)* (1273).

Two hemocytes (category 2 of the present description) with threadlike, variously curved cytoplasmic expansions, before the stage of lateral branching. ×600.

FIGS. 2 and 2′. — *Alcidodes olivaceus* GERSTAECKER *(Curculionidae)* (1074).

Two hours after shedding of the hemolymph. Portions of threadlike cellular expansions of considerable length, drawn out by a plastic hemocyte (category 2). The expansions exhibited trepidations and jerks. Swellings were moving along the course of the filamentous process. Bottom left : beginning, in the shape of a bud, of a lateral arborization. ×600.

FIG. 3. — *Alcidodes olivaceus* GERSTAECKER (1074).

150 minutes after hemolymph withdrawal. Plastic hemocyte (category 2), in a more advanced stage of arborization than those represented in Figs. 1 and 2-2′. Note the numerous growing expansions with club-like thickenings at their endings. Blunt parts of the structures (center) were undergoing trepidations when the picture was recorded. ×600.

FIG. 4. — *Rhynchophorus phoenicis* FABRICIUS *(Curculionidae)* (1671).

3 hours after hemolymph withdrawal. Loose network developed by the cytoplasmic expansions and arborizations of several plastic hemocytes belonging to category 2. On the main expansions, lateral branchs and buds are starting growth. ×600.

FIG. 5. — *Rhynchophorus phoenicis* FABRICIUS (1668).

150 minutes after hemolymph withdrawal. A plastic hemocyte (category 2) with its systems of expansions. Granules and small dark vesicles were moving rapidly on the wriggling (unsharp : upper third of the picture) filamentous process. ×520.

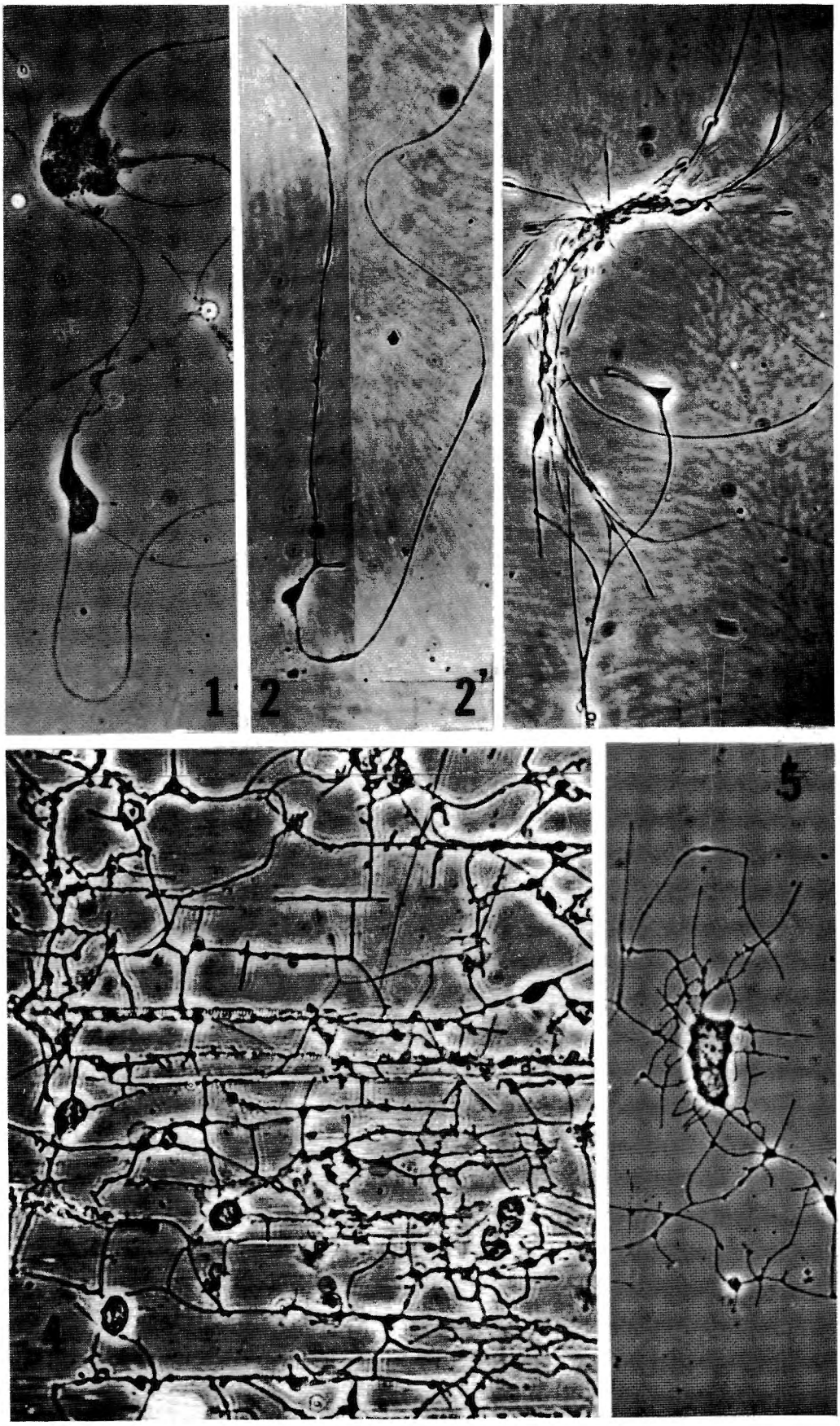
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PLATE II

EXPLANATION OF PLATE II.

FIG. 1. — *Gasteroclisus auricillatus* BOHEMAN (515) *(Curculionidae)*.

In that preparation, two kinds of networks were developed within 60 and 120 minutes by plastic hemocytes (category 2 : upper right half and bottom left of the picture) and by corpuscles smaller than hemocytes (category 4), scattered in the film, and appearing as rounded refringent granules, sometimes alined on filaments of various lengths (middle left part of the picture). The slender expansions of these corpuscles are faintly visible between the more robust trabeculae of the cellular expansions. (See electron micrographs of these structures on Figs. 1, 2 and 3 of Plate IV.) ×600.

FIG. 2. — *Rhynchophorus phoenicis* FABRICIUS (1666).

3 to 6 hours after hemolymph withdrawal. In that specimen, the shed hemolymph became immediately ropy and viscous, and jellification of the plasma took place rapidly. The picture shows a substantial, transparent and elastic, veil, in which hemocytes, with their arborizations, are embedded. Dark smaller corpuscles (category 4) are scattered in the veil. Compare with textfigure 1. ×600.

FIG. 3. — *Rhynchophorus phoenicis* FABRICIUS (1669).

7 hours after hemolymph withdrawal. In the samples of this specimen, the plasma remained fluid and jellification was not recorded. Modifications in the trabeculae of the cellular networks in the shape of vesicles and of tubules are shown. ×600.

FIG. 4. — *Lixus bidentatus* KOLBE *(Curculionidae)* (521).

2 hours after hemolymph withdrawal. Thick film of hemolymph. Cellular meshworks are shown at different levels of the film (unsharp trabeculae are out of focus). ×600.

FIG. 5. — *Lixus bidentatus* KOLBE (521).

2 hours after hemolymph withdrawal. Radiating filamentous processes of a plastic hemocyte, with simultaneous spreading out of the hyaloplasm in the shape of a circular and thinly folded membrane (petaloid pseudopod). ×520.

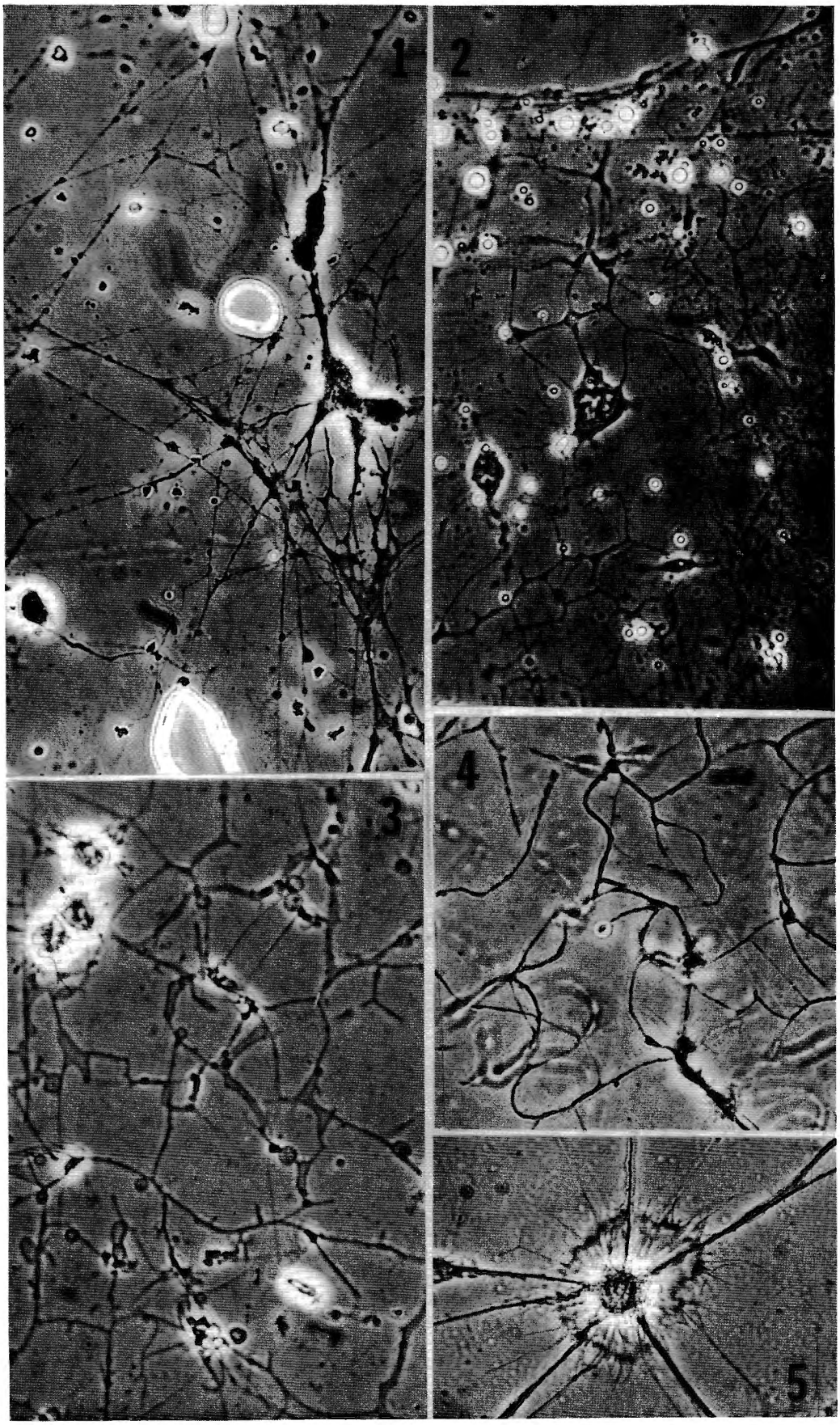
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PLATE III

EXPLANATION OF PLATE III.

FIGS. 1, 2 and 3. — *Rhynchophorus phoenicis* FABRICIUS (27/11/57, 5 DE and 6 B, 1656).

Film of hemolymph, shed from the joint of a wing case, diluted with MEISENHEIMER's serum for insects and allowed to clot on to a formvar film. The preparation, kept in a moist chamber for 17 minutes, was subsequently fixed for 31 hours with vapours of a 2 per cent solution of osmic acid, washed, dried, and shadowed with palladium.

Spore-like rounded or oval corpuscles, 500 millimicrons in diameter, are shown with their string-like tortuous expansions, about 60 millimicrons in diameter. Lateral buds appear on the main stem of the filaments. Some of these structures (bottom right) are flattened.

The filaments shown on these three micrographs form the slender networks, scarcely visible in the films observed with the phase-contrast microscope (see plate II, figs. 1 and 2). These undetermined structures are tentatively identified with growth forms of pleomorphic symbiotes (see discussion).

Cumulus-like aggregations of microflocs, or fragments of clotted diluted plasma, are scattered all over the preparations and appear agglutinated at random on the strings.

The pebble-like background consists of rounded bodies, 10-20 millimicrons in diameter, variously agglutinated and involved in the formation of the microflocs. ×16.500.

In *Temnoschoita*, larger spores (1-2 microns in diameter), with a mulberry-like appearance, flattened on the background (desiccation), and starting emission of buds and of filaments, were detected in a few preparations.

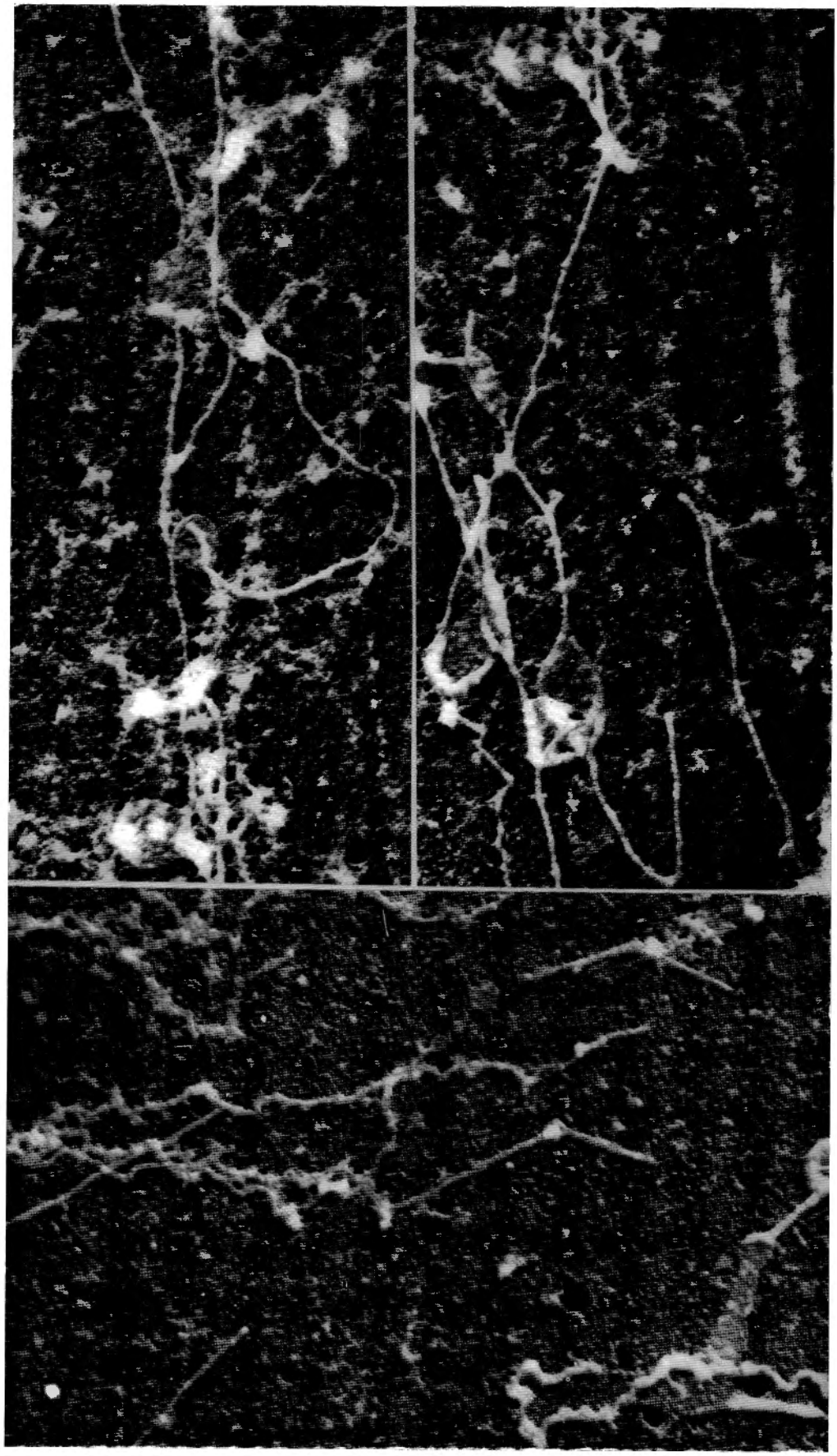

PLATE IV

EXPLANATION OF PLATE IV.

FIGS. 1 and 2. — *Rhinopteryx foveipennis* THOMSON *(Brenthidae)* (21/11/55, 4 A, 3 D, 1264).

Films of hemolymph, diluted with MEISENHEIMER's serum for insects, deposited on copper grids previously coated with formvar. Preparations kept in a moist chamber for 10 minutes, fixed in vapours of osmic acid for 10 hours, washed, dried, and shadowed with palladium.

As shown in *Rhynchophorus* (Plate III), microflocs of clotted hemolymph scattered or aggregated into cumulus-like masses, appear against a finely granular background. In figure 2, stretching of the clotting substances gave rise to fibril-like linear aggregations of rounded particles, 20 to 40 millimicrons in diameter. Similar particles are scattered at random on the background or are orientated into small chains of corpuscular units. ×16.500.

FIG. 3. — *Rhynchophorus phoenicis* FABRICIUS (14/2/58, 5 C, 1669).

Film of hemolymph, shed from the joint of a wing case, diluted with MEISENHEIMER's serum for insects and deposited on to a formvar film. The preparation was kept in a moist chamber for 2 hours, fixed for 23 hours with vapours of osmic acid, washed, dried, and shadowed with palladium.

Crystal- or needle-like structures, 600 to 2.200 millimicrons in length, are scattered with microflocs on the granular background.

Erected on their edges, these structures appear as spindle-shaped rods, and when lying on the background (upper left) as irregular blades. The very long shadows indicate that some of these corpuscles project obliquely upwards. These elements might be tentatively identified with bacterium-like symbiotes. Crystals of the salts used for dilution of the hemolymph have a different appearance. ×16.500.

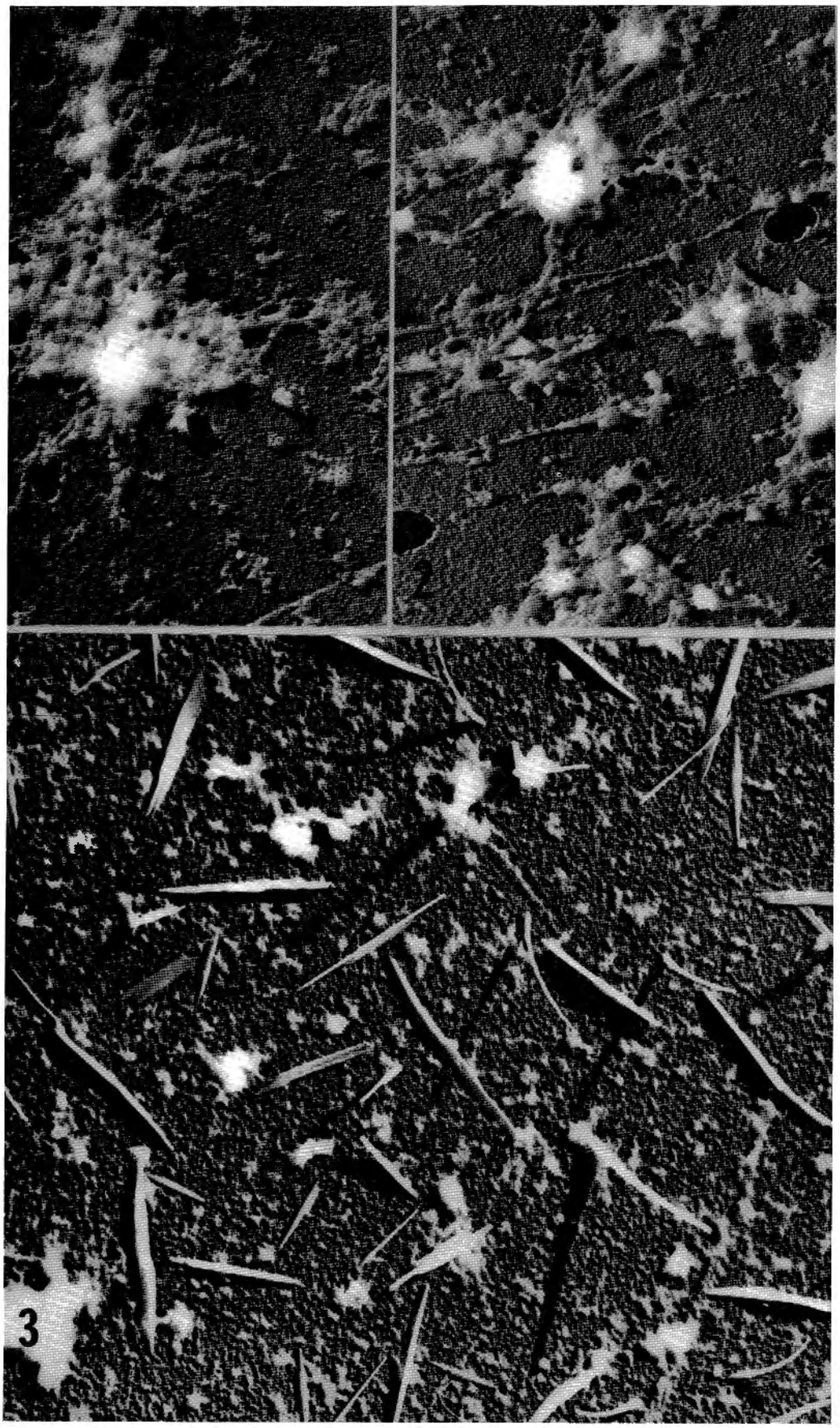
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PLATE V

EXPLANATION OF PLATE V.

FIG. 1. — *Calliphora erythrocephala* MEIGEN. *(Diptera)*, adult (1629).

Plastic hemocytes (plasmatocytes). morphologically comparable to category 2 of hemocytes in *Curculionidae* (compare with Plate I, fig. 3), with substantial dendritic expansions. One of these expansions is anchored by a sole to a physical interface (bubble). A wriggling branch appears on the bottom left part of the picture. On the right, a prohemocyte and a clarified hyaline hemocyte, similar in its appearance to the unstable hyaline hemocytes involved in the coagulation of the hemolymph in other insects. In previous (GRÉGOIRE, 1955, fig. 37) and in the present studies (see Plate VI, figs. 5, 6 and 7), these pale corpuscles were altered dark hyaline hemocytes. As in several adult *Diptera*, no modification developed in the plasma surrounding this altered hemocyte. ×600.

FIG. 2. — *Calliphora erythrocephala* MEIGEN. (1628).

Plastic hemocyte (plasmatocyte) starting emission of wriggling threadlike expansions, with club-like thickenings at their extremities. Compare with Plate I, figures 1, 2, 2′, 3 and 5, in *Curculionidae*. ×520.

FIG. 3. — Undetermined aquatic dipteran larva (1673).

Substantial network developed by the cytoplasmic expansions and arborizations of several plastic, granular hemocytes. Compare with similar network in *Curculionidae* (Plate I, fig. 4; Plate II, figs. 1, 2, 3, 4). ×600.

FIG. 4. — *Calliphora erythrocephala* MEIGEN. *(Diptera)* (1629).

Radiating straight cytoplasmic processes of a plastic hemocyte (plasmatocyte). A petaloid pseudopod is visible on the right. Compare with similar modifications in *Curculionidae* (Plate II, fig. 5). ×600.

FIG. 5. — Undetermined aquatic larva *(Diptera)* (1673).

Variously-shaped particles consistently observed in the films of hemolymph from *Curculionidae* and from *Diptera*, are shown floating in the hemolymph : extremely small dark rounded particles, spindle-shaped corpuscles, straight beaded filaments with branchings (see textfigs. 1 and 2). ×600.

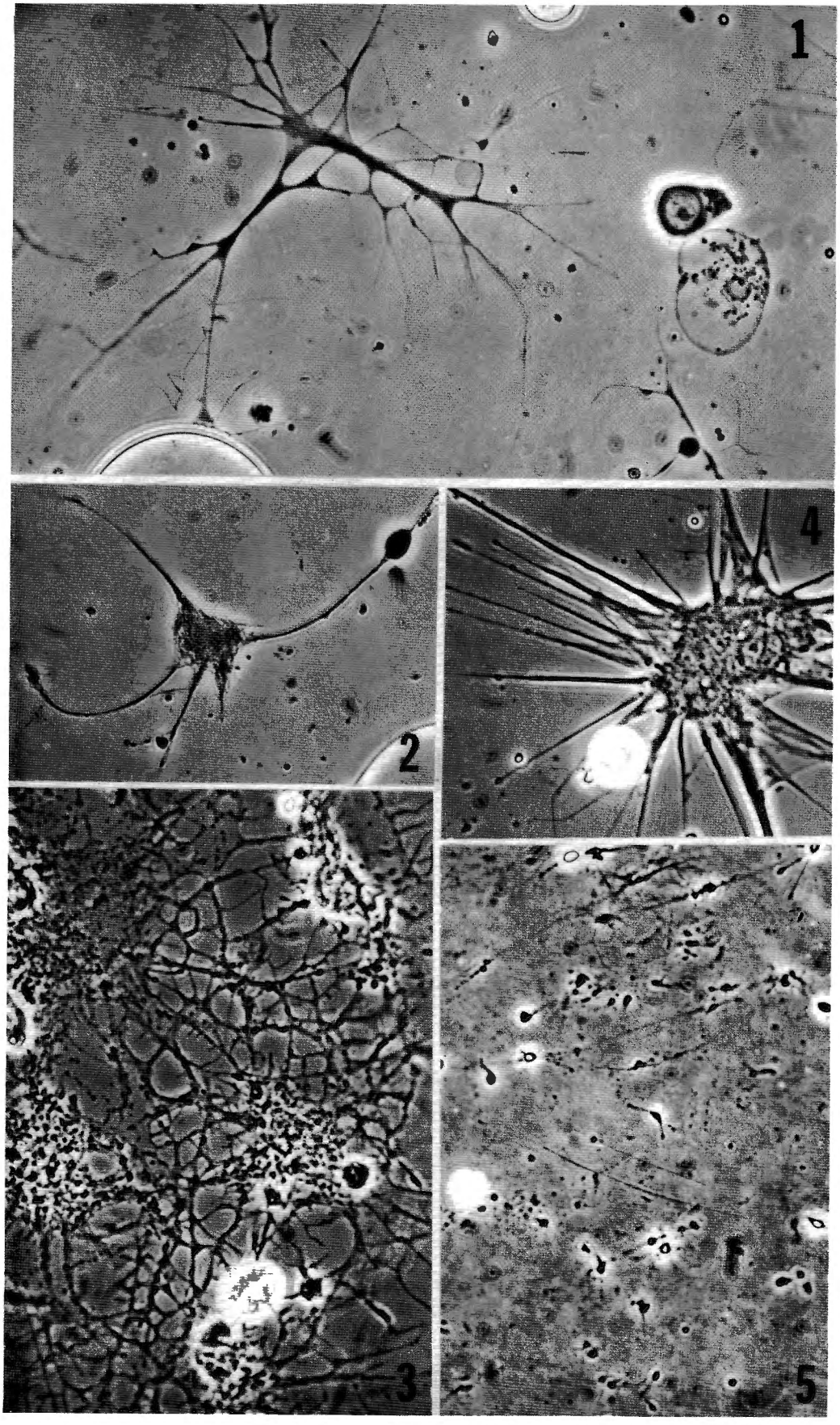
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PLATE VI

EXPLANATION OF PLATE VI.

FIG. 1. — *Tipulidae* sp. (*paludosa* MEIGEN. or *oleracea* LINNÉ) *(Diptera)* (561).

Larva. An intricate network produced by hemocytes drawn out into filaments, appears embedded in a substantial gel in which coarse granules are scattered. Compare with similar modifications in *Curculionidae* (Plate I, fig. 4 and Plate II, fig. 2). ×520.

FIG. 2. — *Tipula* sp., adult (1677).

Wriggling elastic filaments, sometimes of considerable length, floating freely in the still fluid hemolymph, 45 minutes after withdrawal. ×600.

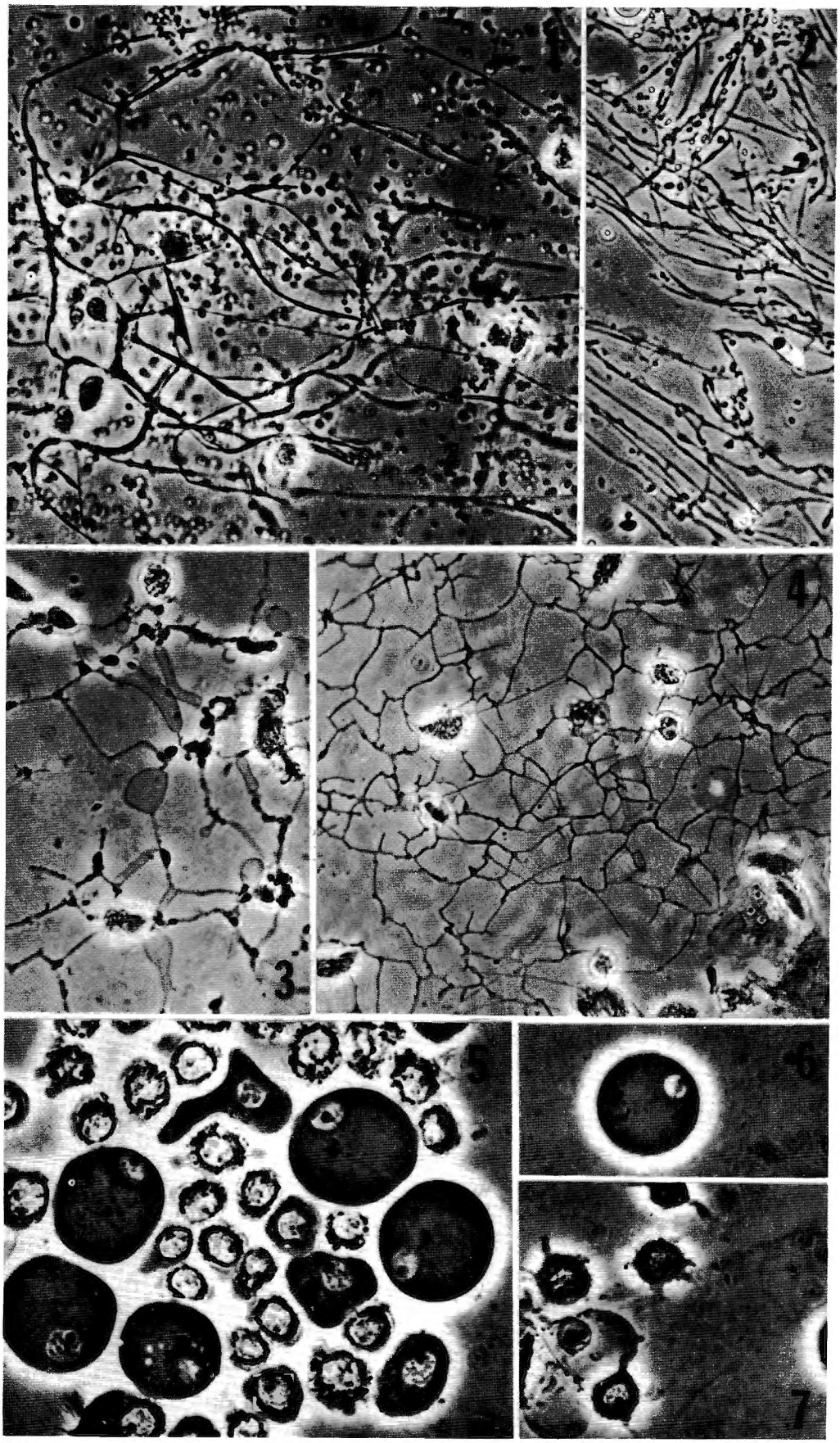
FIGS. 3 and 4. — *Tipulidae* sp., aquatic larva (1296).

Development of tubular and vesicular varicosities in the trabeculae of the cellular networks. Compare with identical pictures in *Curculionidae* (Plate II, fig. 3). Figure 4 : slender network of ramified expansions from hemocytes. Lateral buds are growing along the main branches. ×600.

FIGS. 5, 6, 7. — *Tipulidae* sp., undetermined larva (753-754).

Mixed with various granular hemocytes, five dark oenocytoid-like hyaline hemocytes surrounded by refractile halos. Alterations in these cells consisted of sharpening of the cell outlines, increase in size of the halo, while the corpuscle shrank (fig. 6), successive discharges of cytoplasmic substance into the plasma (such as that recorded in *Lepidoptera* : GRÉGOIRE, 1955, Plate XI, fig. 49), followed by disappearance of the halo, clarification of the hemocyte, which was transformed into a pale vesicle containing a few granules in active brownian motion (fig. 7; see also Plate V, fig. 1). ×800.

In figure 7, the veil-like jellification of the plasma is distinctly visible on the right of two clarified hyaline hemocytes, morphologically similar to the hemocytes shown in text figure 1, A 3, in *Curculionidae* and to the coagulocytes of other groups of insects. ×800.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