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STUDIES BY PHASE-CONTRAST MICROSCOPY ON DISTRIBUTION OF PATTERNS OF HEMOLYMPH COAGULATION IN INSECTS

(With One Plate)

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STUDIES BY PHASE-CONTRAST MICROSCOPY ON DISTRIBUTION OF PATTERNS OF HEMOLYMPH COAGULATION IN INSECTS[1]

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A category of hyaline hemocytes (coagulocytes) is playing an important part in the inception of the plasma coagulation in insect hemolymph (Grégoire and Florkin, 1950). Differences in the reactions of these corpuscles to contact with foreign surfaces and in those of the surrounding plasma were recorded previously for various insects (Grégoire, 1951). On the basis of these differences, a classification of the process of hemolymph coagulation in insects into four patterns of microscopic pictures has been suggested (Grégoire, 1951).

In former observations on the distribution of the patterns in 420 species of insects, predominance of one of these patterns has been recorded in several groups of various extension in the classification (Grégoire, 1955a). The material used in these studies consisted exclusively of representatives of insects from the Old World fauna (mostly European, and a few Mediterranean and African species).

The aim of the present investigations is to compare the previous results with data obtained from Neotropical species. In July and August 1954, during a stay at the Canal Zone Biological Area, the Smithsonian Institution's tropical preserve on Barro Colorado Island, the writer collected and examined samples of hemolymph from 630 insects, belonging to about 230 species.

METHODS

The samples of hemolymph were prepared by the procedure used in former studies (Grégoire, 1951, 1955). In most specimens the hemolymph issuing from severed or punctured appendages (antennae, legs, wings, joints of the wing cases) was dropped as rapidly as possible onto the edge of a cover glass lying on a slide and was allowed to spread out into films. Under optimal conditions, streaming of the

[1] This is No. 7 in the series of papers entitled "Blood Coagulation in Arthropods" published in various journals.

FIGS. 1-4.—The four tentative microscopical patterns of coagulation (schematic). The drawings have been combined from observations, by means of the phase-contrast microscope, of about 5,300 samples of hemolymph, in standard conditions of preparation of the films between slide and coverglass.

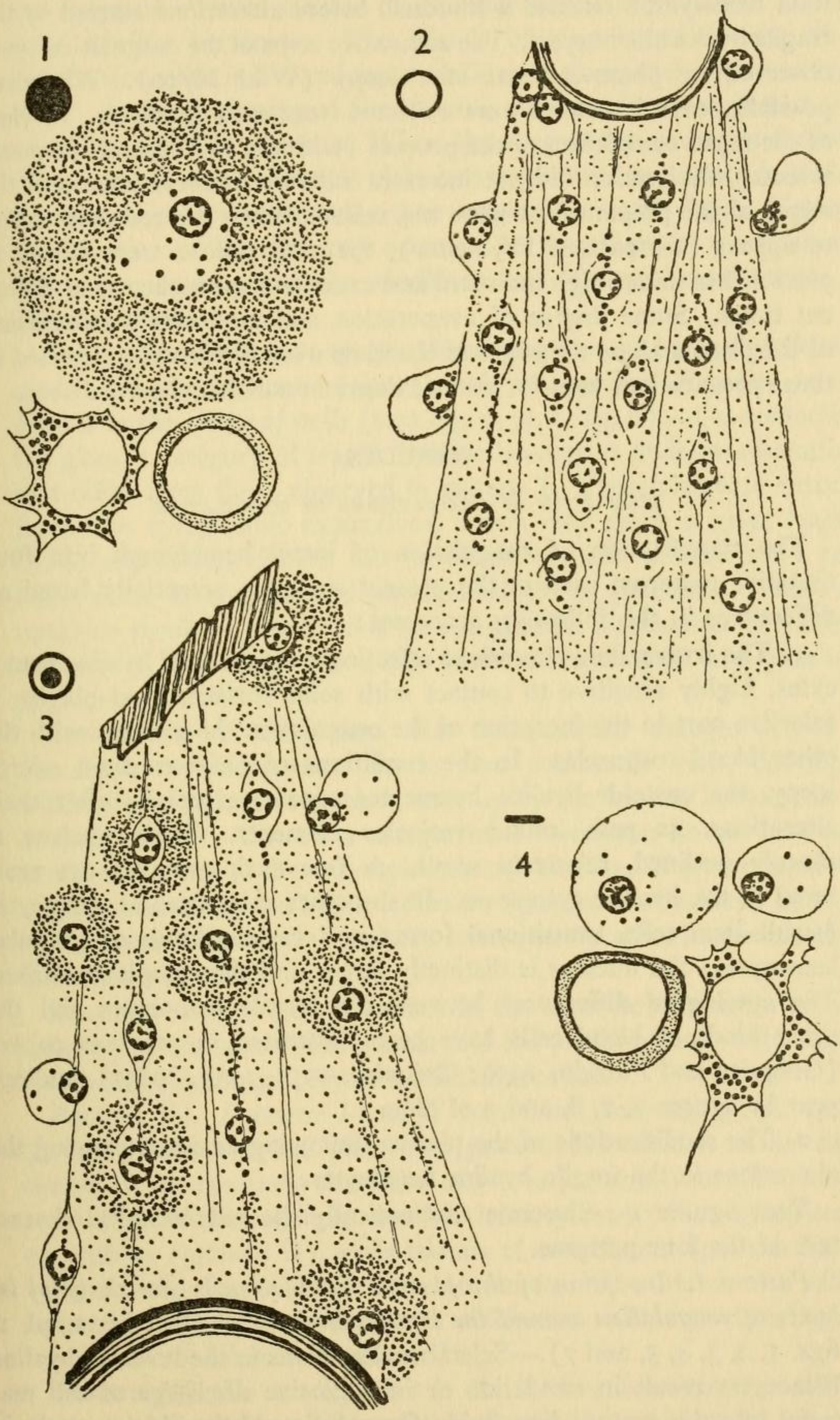
Fig. 1: *Pattern I.* Island of coagulation around a hyaline hemocyte (coagulocyte). A granular hemocyte and a macronucleocyte of small size are not involved in the process of coagulation. Extension of the coagulum around the island and reorganization of the granular clot into meshworks of granular fibrils have not been represented in the drawing. (Compare with photomicrographs in Grégoire and Florkin, 1950, pls. 1 to 10; Grégoire, 1951, figs. 1-3, 10-12, 14, 27, 28; 1953b, figs. 1, 2, 20, and 22; 1955a, figs. 1-6, 10, 15, 21, 23; 27; and in the present paper, pl. 1, figs. 1, 2, 3, 4, 5, and 7.)

Fig. 2: *Pattern II.* Extrusion of cytoplasmic expansions by hyaline hemocytes, which appear in the drawing elongated and reduced in size, except three corpuscles, bulging out at the periphery. The fan-shaped direction of the cytoplasmic filaments from an incidental bubble is induced in part by currents in the spreading film of hemolymph. However, this picture has also been observed to develop slowly, when the hemolymph was at a standstill. Reaction in the plasma in the shape of glassy elastic veils (no cell fibrin) inside of the cytoplasmic systems built up by the unstable hemocytes. The veils are frequently detected only by their stretched folds. By pressure exerted on the coverglass, the structures embedded in the veils move backward and forward, and preserve their relative positions and distances to each other. The other categories of hemocytes, agglutinated at random in strands along the highly adhesive cytoplasmic expansions of the fragile hemocytes, are not represented on the drawing. (Compare with photomicrographs in Grégoire, 1951, figs. 24-26; 1955a, figs. 17-19, 22, 26, 38, 39, 41; and in this paper, pl. 1, fig. 6.)

Fig. 3: *Pattern III.* Association of patterns I and II in the same film of hemolymph: extrusion of cytoplasmic expansions by the hyaline hemocytes, as in pattern II. Reaction of the plasma consisting of granular veils and of islands of coagulation. The islands appear within the veils as circular areas of greater density around the hyaline hemocytes. In this drawing, the reaction was initiated by alterations in hyaline hemocytes to contact with a foreign body (hatched). For the fan-shaped disposition of the structures, see above, pattern II. The other categories of hemocytes are not represented on the drawing. (Compare with photomicrographs in Grégoire, 1955a, figs. 11 and 20; and in this paper, pl. 1, fig. 9.)

Significance of pattern III might be questioned as representing merely a subsidiary variation of pattern II. However (see legend, plate 1, fig. 9, *Zophobas latticollis* Kraatz, Tenebrionidae), pattern III depicts actual differences appearing consistently in the microscopical picture of coagulation of the hemolymph, between groups of insects such as Cetoninae (typical pattern II) and Tenebrionidae (typical pattern III). On the other hand, as already pointed out (Grégoire, 1951), strong mechanical agencies are apt to give deceptive pictures of pattern III, by inducing extrusion of cytoplasmic expansions from hyaline hemocytes in insects in which these reactions do not develop spontaneously in the standard conditions of preparation of the films used for these studies (for instance, Orthopteroid complex).

FIG. 4: *Pattern IV.* No visible modification detected by means of the phase-contrast microscope in the plasma surrounding inert or altered hyaline hemocytes, similar in their appearance to the hyaline hemocytes playing a selective part in the coagulation of the plasma in the other patterns. (Compare with photomicrographs in Grégoire, 1951, figs. 29 and 30; 1955a, figs. 12, 13, 14, and 16.)



FIGS. 1-4.—(See legend on opposite page.)

fluid hemolymph reached a standstill before alterations started in the fragile hyaline hemocytes. The successive steps of the coagulation were observed by phase-contrast microscopy (Wild M/10). Whenever possible, several samples were collected from each specimen. In view of the rapid completion of the process (within a few seconds in many insects), desiccation did not interfere with the observations, and the edges of the preparations were not sealed. When the reactions failed to appear (see pattern IV, below), the preparations were stored in petri dishes under high moisture and examined subsequently at different times. Some degree of evaporation and moderate condensation of the plasma along the edges of the films were incidentally detected in those preparations kept for several hours in moistened petri dishes.

RESULTS

DESCRIPTION OF THE PATTERNS OF COAGULATION

The classification of coagulation of insect hemolymph into four tentative patterns, used in the present study, is essentially based on differences in the following processes:

1. The irreversible alterations affecting a category of hyaline hemocytes, highly sensitive to contact with solid surfaces, and playing a selective part in the inception of the coagulation, in contrast with the other blood corpuscles. In the conditions of phase-contrast microscopy, the unstable hyaline hemocytes appear, especially after their alterations, as pale, round vesicular elements. Their nucleus is sharply outlined, relatively small. A few dark granules are scattered in the hyaline cytoplasm. In the other categories of hemocytes (small stem cells, transitional forms, and various kinds of granular hemocytes) the nucleus is distinctly larger and the cytoplasm darker. The cytological differences between the fragile hemocytes and the other kinds of blood cells have been illustrated in previous papers (Grégoire and Florkin, 1950; Grégoire, 1951, 1953, 1955a), and appear in figures 1, 2, 8, and 9 of plate 1.

2. The modifications of the plasma, following or accompanying the alterations in the fragile hyaline hemocytes.

Text figures 1-4 illustrate schematically the microscopical characters of the four patterns.

Pattern I. Inception of the plasma coagulation in the shape of islands of coagulation around the hyaline hemocytes (text fig. 1; pl. 1, figs. 1, 2, 3, 4, 5, and 7).—Selective alterations in the unstable hyaline hemocytes result in exudation or in explosive discharge of cell material into the surrounding fluid. Coagulation of the plasma starts in

the shape of circular islands of granular consistency around the altered hyaline hemocytes. The islands of coagulation develop to a certain size, with individual and specific variations, then their increase stops. At the beginning of the process, the islands are scattered and separated by fluid channels. When the coagulation proceeds farther, the plasma in these channels clots into a granular substance in which the islands preserve generally their original size and shape. General solidification of the film may occur. The coagulum, of granular appearance, is progressively modified into delicate meshworks of granular fibrils.

Pattern II. Extrusion of cytoplasmic expansions by hyaline hemocytes, with development of cytoplasmic meshworks. Reaction in the plasma in the shape of veils (text fig. 2; pl. 1, fig. 6).—On contacting the glass, a category of fragile hyaline hemocytes undergo alterations that differ from those observed in pattern I. These elements extrude threadlike cytoplasmic expansions, which may reach a great length. These expansions exhibit intense thigmotropism toward solid particles, other hemocytes, and physical interfaces (bubbles). These alterations result in constitution of cytoplasmic meshworks of various complexity, on which the other kinds of hemocytes are passively agglutinated.

The reaction in the plasma after these cellular changes occurs in the shape of transparent, elastic, and contractile veils, developed within the cytoplasmic systems built up by the hyaline hemocytes, or in their vicinity. The other categories of hemocytes do not take part in the formation of these cytoplasmic meshworks. They are passively agglutinated along the highly adhesive cytoplasmic filaments sent out by the hyaline hemocytes and are subsequently embedded with them in the plasma veils.

In several insects the alterations in the unstable hemocytes are not followed by changes in the plasma and the modifications of the hemolymph in vitro consist only of a cellular reaction.

Pattern III. Patterns I and II combined (text fig. 3; pl. 1, fig. 9).—The microscopical picture of pattern III consists of an association of the reactions described above in patterns I and II. In the same film of hemolymph, hyaline hemocytes produce cytoplasmic expansions (pattern II) while islands of coagulation (pattern I) develop around the body of these corpuscles. The islands are either isolated or appear as denser areas within the veils characterizing the reaction in the plasma in pattern II.

Pattern IV. No modification in the hyaline hemocytes, or alterations not followed by visible reaction in the plasma (text fig. 4).—In

the hemolymph of various insects, hemocytes resembling in their cytological characters the fragile corpuscles involved in the other patterns do not visibly alter. They appear as large, pale vesicles in which a few dark particles are scattered. In several insects these elements are the remnants of darker refractile hyaline hemocytes, which undergo clarification after explosive discharge of a part of their cytoplasm. In the vicinity of these inert or altered hyaline hemocytes no change can be detected under the phase-contrast microscope in the consistency of the plasma, which remains permanently fluid.

DISTRIBUTION OF THE PATTERNS OF COAGULATION IN THE DIFFERENT GROUPS OF INSECTS INVESTIGATED

In the table below, the names of the species are followed by the numbers of specimens studied (adults, unless otherwise stated) and by the patterns of coagulation provisionally found predominant or representative on the basis of the microscopical study of several samples of hemolymph obtained from these specimens. Incidental findings of other patterns are also reported under "Comments."

In several taxonomic groups, the average pattern recorded on corresponding material from the Old World previously studied (Grégoire, 1955a; Grégoire and Jolivet, unpublished) follows the list of the Neotropical material in the table.

The patterns of coagulation described above have been represented in the table by the following symbols:

●: pattern I: inception of the plasma coagulation in the shape of islands of coagulation around hyaline hemocytes.

○: pattern II: development of cytoplasmic meshworks by hyaline hemocytes. Reaction in the plasma in the shape of veils.

⊖: pattern II: incomplete. Emission of cytoplasmic expansions, characterizing the reactions of the hyaline hemocytes in pattern II, but unaccompanied by formation of veils in plasma.

◉: pattern III: patterns I and II combined.

—: pattern IV: no visible coagulation.

( ): pattern incidentally or exceptionally recorded in limited fields of preparations exhibiting predominantly another pattern.

(?): microscopical characters of a pattern not clear-cut or equivocal.

Other abbreviations used: sp., species; spm., specimen.

Gradations in the intensity of the reactions, especially with regard to pattern I, are indicated by the following symbols: I poor (scarce fringes of clotted plasma around the altered hyaline hemocytes, with-

out extension of the coagulation; I (scattered islands of coagulation of various sizes, with moderate coagulation of the fluid in the channels); I*, I**, I*** (islands around all the hyaline hemocytes, substantial and general coagulation. In I***, the films appear to the naked eye with a bluish opalescent color).

MICROSCOPY (PARTICULAR REACTIONS)

Orthopteroid Complex.—Pattern I has been uniformly recorded in all the insects of the orthopteroid complex listed above. However, intensity in the reaction differed in the various groups: in this respect, Blattodea and Gryllidae exhibited the most substantial coagulation. These groups were followed, in order of decreasing intensity, by Mantodea, Phasmoptera, Tettigoniidae, and Acrididae.

In the present material, alterations in plasma appearing around hemocytes other than the fragile hyaline hemocytes were detected around a few macronucleocytes of small size (stem cells) in samples from *Paroecanthus podagrosus* and from *Phasma* sp. As already pointed out (Grégoire, 1951, 1955a) such reactions are exceptional.

Heteroptera.—As shown in the list, absence of visible change in the plasma was the predominant picture in all the specimens of the present material. The few modifications that might suggest subsidiary participation of a pattern other than pattern IV were equivocal.

In *Ghilianella* sp., *Triatoma dimidiata, Rhiginia* sp., *Apiomerus ochropterus* (Reduviidae), *Mecisthorhinus marmoratus* (Pentatomidae), dark oval hyaline hemocytes, surrounded by a refractile halo, underwent sudden clarification after explosive ejection of cytoplasmic substance into the surrounding fluid. These discharges did not bring about changes in the consistency of the plasma. Similar alterations have been described previously (Grégoire, 1955a) in various insects, especially in lepidopteran and dipteran larvae and in dipteran adults.

In several samples from different families, granular precipitates, unrelated to the presence of hemocytes in the vicinity, were found in films of hemolymph around air bubbles and along the edge of the coverglass. Though the preparations were maintained between the observations in petri dishes under high moisture, these modifications resulted probably from a slight degree of evaporation and condensation at the periphery of the films. Pressure exerted on these precipitates dispersed granular particles; this reaction is different from that taking place under similar mechanical agencies, in an actual coagulum, in which extension of the granular and fibrillar structures is followed by elastic contraction without dissociation.

TABLE 1.—*Patterns of Coagulation*

Orders, Families, Genera, and Species	Number of Specimens	Pattern of Coagulation Provisionally Representative or Predominant	Comments	Tentative Generalization
Orthopteroid Complex				
DICTYOPTERA				
BLATTODEA [1]				
Eublaberus posticus (Erichson)	3	●	** and ***	
Blaberus colosseus (Illiger)	2	●	** and ***	
" " (hatched larvae)	10	●	**	
Epilampra azteca Saussure	2	●		
Nyctibora noctivaga Rehn	1	●	**	●
" " (larva)	1	●	***	
Periplaneta brunnea Burmeister	1	●		
Phortioeca phoraspoides (Walker)	1	●	**	
Undet. sp. (larva)	1	●		
MANTODEA [2]				
Stagmomantis dimidiata (Burmeister) ♂	9	●	* in 3 spm.	
Angela guianensis Rehn ♂	2	●		
Pseudomiopteryx infuscata Saussure & Zehntner ♀	1	●	**	
Macromusonia (Catamusonia) conspersa (Saussure) ♂	1	●	Poor	●
Vates festae Giglio Tos ♂	1	●	**	
Phyllovates brevicornis (Stål) ♂	3	●	Poor to **	
Parastagmaptoptera serricornis Kirby ♂	1	●		
Choeradodis rhombicollis (Latreille) ♂	2	●	Poor	

KALOTERMITIDAE				
Cryptotermes sp. (winged nymphs) .	10	●		●
PHASMOPTERA [4]				
Stratocles forcipatus Bol. ♂ ♀.....	3	●		
Phasma sp. ♂.....	1	●		
Paradiapheromera strumosa Brunner von Wattenwyl ♂.....	3	●		
Pterinoxylus diffórmipes Serville ♂..	1	●		●
Bacteria ploiaria (Westwood) ♂....	1	●	**	
Bacteria sp. ♂.....	1	●		
Undet. sp.	1	●	Poor	
Undet. sp. (larva, 1.5 cm.).....	1	●		
ORTHOPTERA				
TETTIGONIIDAE [5]				
Hyperphrona sp. ♂.....	2	●		
Insara bolivari (Griffini) ♂ ♀.....	4	●		
Orophus sp. ♂.....	2	●		
Lamprophyllum micans Hebard ♂..	1	●		
Phaneroptera paronae (Griffini) ♀..	1	●		
Philophyllia guttulata Stål ♂.....	3	●		
Phylloptera dimidiata Brunner von Wattenwyl ♂ ♀.....	2	●		●
Acanthodes curvidens (Stål) ♂.....	1	●		
Balboa tibialis Brunner von Wattenwyl ♂	1	●	*	
Bliastes punctifrons Stål ♀.....	1	●		
Bliastes banksi Hebard ♂.....	1	●	Poor	

[1] Det. by Dr. C. Willemse. [2] Det. by Prof. L. Chopard. [3] Det. by Dr. Thos. E. Snyder. [4] Det. by Prof. L. Chopard.
[5] Det. by Dr. C. Willemse and Dr. M. Beier.

TABLE 1.—*Patterns of Coagulation*—continued

Orders, Families, Genera, and Species	Number of Specimens	Pattern of Coagulation Provisionally Representative or Predominant	Comments	Tentative Generalization
ORTHOPTERA (continued)				
TETTIGONIIDAE (continued)				
Cocconotus wheeleri Hebard ♂.....	1	●		
Ischnomela gracilis Stål (larva) ♀..	1	●		
Schedocentrus sp.	1	●		
Euceraia insignis Hebard ♂ ♀.....	3	●		
Gongrocnemis sp. (larva) ♀.....	1	●	**	
Drepanoxyphus minutus Brunner ♂.	1	●		
Idiathron majus Hebard ♂.....	1	●		
Microcentrum colosseum Brunner ♂.	1	●		
Microcentrum sp. ♂.....	1	●		
Peucestes championi Saussure & Pictet ♂	1	●		
Parabliastes punctifrons (Stål) ♀...	1	●		
Undet. sp. (Pseudophyllidae), larva, first stage ♂.....	1	●	Poor	●
Neoconocephalus maxillosus (Fabricius ♂ ♀.....	2	●		
Neoconocephalus triops (Linnaeus) ♂	1	●	*	
Neoconocephalus affinis (Beauvois) ♀	1	●		
Bucrates capitatus (De Geer) ♂.....	1	●		
Caulopsis microprora Hebard ♂....	1	●	Poor	
Copiphora brevirostris Stål ♀.....	2	●	*	
" " (larva) ♂...	1	●	Poor	
Eleven unidentified sp. (adults and larvae)	11	●		

GRYLLIDAE [6]				
Anurogryllus muticus (De Geer) ♀..	3	●		**
Aphonomorphus sp. ♀.....	1	●		
" " (not adult) ♀...	1	●		**
Ponca venosa Hebard ♀ ♂.....	2	●		**(♂)
Diatrypa sp. ♀.....	1	●		*
Paroecanthus podagrosus Saussure ♀	2	●		
Undet. sp.....	5	●		In 2 spms. **
ACRIDIDAE [7]				
Amblytropidia insignis Hebard ♂...	1	●		
" " (larva)....	1	●		Poor
Orphulella punctata (De Geer) 4♀, 1♂	5	●		
Orphulella punctata (larva).....	1	●		
Agriacris bilunata (Gerstaecker)) ♀.	1	●		**
Agriacris (*bilunata*) (Gerstaecker?) (larva) ♀	1	●		
Agriacris tricristata (Serville) ♂....	2	●		
" " (larva).....	1	●		
Agriacris sp. (larva).....	1	●		
Aidemona azteca (Saussure) ♀.....	1	●		Poor
" " (larvae) ♀ ♂.....	6	●		Poor
Copiocera harroweri Hebard or *specularis* Gerstaecker ♂.....	1	●		
Osmilia flavo-lineata (De Geer) ♀ ♂.	8	●		
Phaeoparia rotundata Stål.....	1	●		*
Schistocerca paranensis (Burmeister) ♂ ♀.....	2	●		
Schistocerca sp. (larva).....	1	●		Poor
Xyleus rosulentus (Stål) ♂ ♀.....	2	●		*(♀)

[6] Det. by Prof. L. Chopard. [7] Det. by Dr. C. Willemse.

TABLE 1.—*Patterns of Coagulation*—continued

Orders, Families, Genera, and Species	Number of Specimens	Pattern of Coagulation Provisionally Representative or Predominant	Comments	Tentative Generalization
ORTHOPTERA (continued)				
ACRIDIDAE (continued)				
Xyleus rosulentus (Stål) (larvae)	3	●		
Undet. sp.	2	●		
DERMAPTERA [8]				
Carcinophora americana (Palisot de Beauvois) ♀	2	●		●
Carcinophora sp. (probably *americana*) nymph	1	●	**	
Doru lineare (Eschscholtz) ♂	1	●	Poor	
Spongiphora croceipennis (Serville) ♂	1	●	*	
Former data and unpublished observations on material from the Old World: Blattodea (10 sp.), Mantodea (6 sp.). ISOPTERA (3 sp.), PHASMOPTERA (4 sp.), Tettigoniidae (9 sp.), Gryllotalpidae (1 sp.), Gryllidae (4 sp.), Tetrigidae (1 sp.), Acrididae (19 sp.), and DERMAPTERA (5 sp.)	525	●		*Orthopteroid Complex* Consistently pattern I. ● Coagulation, substantial in Blattodea, *Gryllotalpa,* and in Gryllidae, moderate to scarce in PHASMOPTERA, in Tettigoniidae and in Acrididae. (For diverging results in workers of a termite sp., see Grégoire, 1953, 1954.)
Hemipteroid Complex				
HEMIPTERA [9]				
REDUVIIDAE				
Ghilianella sp.	1	—		

Saica apicalis Osborn & Drake.....	3	—		
Triatoma dimidiata (Latreille).....	6	—		
Rhodnius pallescens Barber.....	2	—		
Sirthenea stria (Fabricius).....	2	—		Reduviidae
Rhiginia cruciata (Say).....	1	—		Pattern IV :— representative. In a few spm. (*Rhinocoris, Apiomerus, Ectrichodia, Ploegaster*) questionable pictures of pattern I recorded exceptionally in limited fields of preparations otherwise exhibiting pattern IV.
Rhiginia sp., possibly *cruciata* (Say) (nymph)	1	—		
Rhiginia sp.	1	—		
Apiomerus ochropterus Stål.....	1	—		
Apiomerus emarginatus Stål.....	1	—		
Apiomerus pilipes (Fabricius).....	2	—	(●?)	
Zelurus spinidorsalis (Gray).....	3	—		
Heza similis Stål.....	1	—		
Montina nigripes Stål.....	1	—		
Ploegaster gensana Kirkaldy.....	2	—	(●?)	
Brontostoma discus (Burmeister)...	1	—	(⊖)	
Mestor rufotuberculatus (Champion)	1	—		
Panstrongylus geniculatus (Latreille)	3	—		
Zirta sp.	1	—		
Former data and unpublished observations on specimens from the Old World (8 sp.)	12	—		
COREIDAE				
Acanthocephala sp.	1	—		
Undet. sp., prob. n.gen., n.sp. near *Staluptus*	1	—		Coreidae
Hyalmenus pulcher Stål.....	1	—		Pattern IV: —
Grammopoecilus flavicornis (Fabricius)	1	—		
Former data and unpublished observations (5 sp.).....	45	—		

[8] Det. by Prof. Dr. W. D. Hincks. [9] Det. by Dr. R. I. Sailer.

TABLE 1.—*Patterns of Coagulation*—continued

Orders, Families, Genera, and Species	Number of Specimens	Pattern of Coagulation Provisionally Representative or Predominant	Comments	Tentative Generalization
HEMIPTERA (continued)				
PENTATOMIDAE				
Mecistorhinus marmoratus (Erichson)	1	—		
Mecistorhinus piceus (Palisot de Beauvois)	1	—		
Berecynthus delirator (Fabricius)	1	—	(?)	
Proxys punctulatus (Palisot de Beauvois)	1	—		Pentatomidae
Loxa viridis (Palisot de Beauvois)	1	—		Pattern IV: —
Loxa flavicollis (Drury)	3	—		
Edessa rufomarginata (De Geer)	1	—	(?)	
Edessa celsa Distant	3	—		
Edessa sp.	1	—		
Neodine macraspis (Perty)	1	—		
Gen. near *Pharnus,* n.gen., n.sp.	1	—		
Acrosternum scutellatum (Distant)	4	—		
Former data and unpublished observations (22 sp.)	117	—		
CYDNINAE				
Prolobodes gigas Signoret	1	—		
Former data and unpublished observations on Nabidae (1 sp.), Gerridae (1 sp.), Pyrrhocoridae (1 sp.), Lygaeidae (2 sp.), Miridae (1 sp.), Naucoridae (1 sp.), Notonectidae (1 sp.), Corixidae (1 sp.)	83	—		Pattern IV: —

On Belostomatidae (4 sp.)	12	●	*** (— in 1 spm.)	Pattern I: ● very substantial coagulation
On Nepidae (4 sp.)	55	●	**	Pattern I: ● subtantial coagulation
HOMOPTERA [10]				
CICADIDAE [11]				
Fidicina mannifera (Fabricius)	2	●		
Fidicina sp.	1	●	Poor	Cicadidae
Fidicina sp.	1	●	**	Pattern I ●: substantial coagulation
Zammara calochroma Walker	2	●,●◉	***	
Zammara sp. near *calochroma* Walker	3	●		
Proarna championi Distant	1	●		
FULGORIDAE				
Calyptoproctus elegans (Olivier)	4	●	* to ***	Fulgoridae
Copidocephala guttata (White)	1	●	***	Pattern I ●: One of the most
Cathedra serrata (Fabricius)	1	●	**	substantial processes of co-
Laternaria servillei (Spinola)	2	●	***	agulation recorded among
Phrictus quinquepartitus Distant	3	●	***	all the insects investigated
Odontoptera carreñoi Signoret	1	●	***	
DICTYOPHARIDAE				
Hyalodicyton obtusifrons (Walker)	1	?	(●?)*	
CICADELLIDAE (Tettigellinae)				
Diestostemma sp.	2	●	*	Cicadellidae
Gypona notanda ? Fowler	1	○		Pattern I: ●
Oncometopia sp.	1	●		
Undet. spm. (Tettigellinae)	1	●	*(◉*?)	
NOGODINIDAE				
Biolleyana costalis (Fowler)	1	—		

[10] Det. by Dr. D. A. Young. [11] Det. by Miss Louise Russell.

TABLE 1.—*Patterns of Coagulation*—continued

Orders, Families, Genera, and Species	Number of Specimens	Pattern of Coagulation Provisionally Representative or Predominant	Comments	Tentative Generalization
HOMOPTERA (continued)				
FLATIDAE				
Carthaeomorpha rufipes Melichar...	1	—		
Neocerus sp., or rel. gen.....	1	—		
Former data on Cercopidae and Cicadellidae (4 sp.)	33	●◉		Substantial coagulation
COLEOPTERA				
ADEPHAGA				
CICINDELIDAE [12]				
Ctenostoma sp.	1	●	Possibly ◉	
Megacephala (*Tetracha*) *sobrina* Dejean	1	—		
Odontochila exilis Bates.....	1	●	Poor (◉?)	
Former data: *Cicindela campestris* Linnaeus	3	⊖	?	
CARABIDAE [13]				
Undet. sp. (Ozaeninae).....	1	—	(○)	
Undet. sp. (Ozaeninae).....	1	○	*(◉?)	
Undet. sp. (Ozaeninae).....	1	○	*(◉?)	
Undet. sp. (Ozaeninae).....	1	⊖		
Agra sp.	1	●◉	Poor	
Agra sp.	1	●	(◉)	
Agra sp.	2	●◉	**	
5 undet. sp.....	5	—	(⊖)	
Former data and unpublished observations (26 sp.)	45	Varied reactions		Predominance of a pattern recorded at the genus level: f.i. *Carabus* (4 sp.): Pattern III ◉ substantial

DYTISCIDAE				
No specimen captured				
Former data and unpublished observations (13 sp.)	67	Varied reactions	Predominance of a pattern recorded at the genus level: f.i. *Hydaticus* (6 sp.: Pattern IV) — *Dytiscus* (2 sp.: Pattern III) ◉ *Cybister* (2 sp.: Pattern I substantial) ●*	
POLYPHAGA				
HYDROPHILIDAE, HISTERIDAE, SILPHIDAE, CANTHARIDAE				
No specimen captured				
Former data and unpublished observations:				
Hydrophilidae (5 sp.)	50	—	Consistently recorded	Hydrophilidae Pattern IV: —
Histeridae (6 sp.)	13	○	(◉)	
Silphidae (8 sp.)	27	Varied reactions	Possibly predominance of a pattern at the genus level (*Necrophorus*: Pattern I)	
Cantharidae (7 sp.)	29	—	(○?)	
STAPHYLINIDAE [14]				
Eulissus chalybaeus Mannerheim	2	—		Staphylinidae Pattern IV: — consistently recorded
Former data and unpublished observations (10 sp.)	14	—		

[12] Det. by O. L. Cartwright. [13] Det. by G. Fagel. [14] Det. by Dr. R. E. Blackwelder.

TABLE 1.—*Patterns of Coagulation*—continued

Orders, Families, Genera, and Species	Number of Specimens	Pattern of Coagulation Provisionally Representative or Predominant	Comments	Tentative Generalization
POLYPHAGA (continued)				
PASSALIDAE [15]				Passalidae
Passalus punctiger Serville.....	3	Varied reactions	Mostly ⊖ or —; (●)	Probably pattern III: ◉ Large intraspecific variations. Coagulation scarce in many samples. Pattern I: ● unmixed or associated with pattern II ○ (=III ◉) detected in 6 out of 9 sp.
Verres cavicollis Bates.....	2	○		
Veturius platyrhinus Westwood....	2	○	— also recorded	
Veturius sp.	1	○		
Unpublished observations (5 African sp.)	25	●◉	Large intraspecific variations	
SCARABAEIDAE [15]				
Coprinae (Scarabaeinae)				
Canthon 7-maculatus Latreille.....	2	○	Poor or ⊖	Coprinae ●◉
Canthon sp. (?)	3	⊖ or —		
Former data and unpublished observations (18 sp.).....	39	●	(◉) in 13 out of 18 sp.	
Rutelinae				
Anomala granulipyga Bates.....	1	○		
Lagochile sparsa, subsp. *subandina* Ohaus	1	○	Probable	Rutelinae ○
Macraspis lucida Olivier.....	1	○		
Pelidnota notata Blanchard.....	7	○		
Phalangogonia sperata Sharp.....	1	○		
Trizogeniates foveicollis Ohaus.....	2	○		
Melolonthinae [16]				
Phyllophaga prolixa Bates [16] ♀.....	1	○		

Dynastinae				
Aspidolea singularis Bates.....	7	○	Poor or inc. in 2 spm.	
Cyclocephala carbonaria Arrow.....	1	○		
Cyclocephala signata Drury.....	1	○		Dynastinae
Dyscinetus frater Bates.....	20	○	Some*	○
Bothynus quadridens Taschenberg..	2	○	*	
Former data and unpublished observations: Aphodiinae (2 sp.); Geotrupinae (4 sp.); Rutelinae (3 sp.); Melolonthinae (5 sp.); Dynastinae (7 sp.); Trichiinae (4 sp.); Valginae (2 sp.), and Cetoninae (16 sp.)....	131	○		Pattern II ○ highly predominant, especially in Rutelinae, Dynastinae, Trichiinae, and Cetoninae. Pattern III ◉ incidental or questionable in scattered specimens
SANDALIDAE [17]				
Gen. and sp. unknown.....	2	—(?)	(●?)	
ELATERIDAE [18]				
Chalcolepidius near rugatus Candèze.	1	◉		
Chalcolepidius porcatus (Linnaeus).	1	○	**, possibly ◉	
Dicrepidius ramicornis (or near sp.) (Palisot de Beauvois).....	3	—		
Semiotus distinctus (Herbst) (or near sp.) (Chalcolepidinae).....	2	○	**, possibly ◉	
Former data and unpublished observations (11 sp.).....	21	Varied reactions		
LAMPYRIDAE				
Undet. sp.	1	—?		
Undet. sp.	1	—?		
Former data: *Lampyris noctiluca* Linnaeus	23	—		

[15] Det. by O. L. Cartwright. [16] Det. by Dr. M. W. Sanderson. [17] Det. by Geo. B. Vogt. [18] Det. by Ch. Jeuniaux.

TABLE 1.—*Patterns of Coagulation*—continued

Orders, Families, Genera, and Species	Number of Specimens	Pattern of Coagulation Provisionally Representative or Predominant	Comments	Tentative Generalization
HETEROMERA				HETEROMERA
TENEBRIONIDAE [19]				Pattern I ●, unmixed or associated with pattern II ○ (=pattern III ◉), recorded in all the sp. investigated
Nyctobates gigas (Linnaeus).....	1	●	Poor (◉)	
Zophobas latticollis Kraatz.....	2	◉		
Zophobas, probably *atratus* (Fabricius), *morio* of authors.....	2	◉		Generally substantial coagulation, especially in Meloidae
MELOIDAE [19]				
Epicauta grammica (Fischer von Waldheim)	7	●	***, (◉) in 1 spm.	
Former data and unpublished observations on Heteromera:				
Tenebrionidae (28 sp.).....	163	◉	● unmixed recorded in 2 sp.	Pattern III ◉
Lagriidae (3 sp.).....	6	●		
Monommidae (2 sp.).....	3	◉		
Oedemeridae (4 sp.).....	16	●		
Meloidae (6 sp.).....	23	●	Substantial coagulation. Pattern III ◉ recorded in several samples	
CERAMBYCIDAE [20]				
Prioninae				Prioninae
Stenodontes (Mallodon) molarius (Bates) ♀ ♂.....	5	●	***	Typical pattern I ●
Stenodontes (Mallodon) spinibarbis (Linnaeus) ♂ ♀.....	2	●	***	One of the most substantial processes of coagulation

Callipogon (Orthomegas) cinnamomeum (Linnaeus)	1	●	***	
Former data and unpublished observations (3 sp.).....	9	●	***	
Cerambycinae				
Chlorida festiva (Linnaeus).....	1	●◉	***	
Coleoxestia vittata (Thomson).....	1	●	*	Cerambycinae Pattern I ●(◉)
Malacopterus tenellus (Fabricius) ♂♀	4	●◉	*	
Nyssicus sp.	1	●	Poor	
Former data and unpublished observations (9 sp.).....	15	●	Pattern III ◉ recorded also in several samples. Moderate coagulation in various specimens	
Lamiinae				
Taeniotes scalaris (Fabricius).....	1	●		
Lochmaeodes sp.	1	●	*(◉)	
Acrocinus longimanus (Linnaeus)..	1	●	***	
Steirastoma sp., probably *meridionale* Aurivillius	1	●		Lamiinae Pattern I ●
Lagochirus araneiformis (Linnaeus).	1	●	**	
Lagochirus sp., near *binumeratus* Bates	2	●	***(◉)	
Acanthoderes circumflexus Jacquelin du Val	1	◉		
Former data and unpublished observations (4 sp.).....	9	●	Substantial coagulation. Pattern III ◉ recorded in a few samples	

[19] Det. by T. J. Spilman. [20] Det. by George B. Vogt.

TABLE 1.—*Patterns of Coagulation*—continued

Orders, Families, Genera, and Species	Number of Specimens	Pattern of Coagulation Provisionally Representative or Predominant	Comments	Tentative Generalization
BRUCHIDAE [21]				
Caryedes faldermanni (Mannerheim)	1	—		
CHRYSOMELIDAE [22] (br. sense)				
EUMOLPIDAE				
Typophorus sp.	1	—		
Eumolpus sp., probably *surinamensis* Fabricius	1	○		
ALTICIDAE				
Oedionychus sp.	1	—		
Former data and unpublished observations (41 sp.)	187			
Crioceridae (5 sp.)		— ?	Coagulation scarce, not detected in several spm.	
Eumolpidae (2 sp.)		—		
Chrysomelidae s.s. (18 sp.)		●◉		
CURCULIONIDAE [23]				
Exophtalmus jekelianus White	2	—		
Heilipus sp.	1	—		Curculionidae Pattern IV —
Rhinostomus barbirostris (Fabricius)	5	—		
Cosmopolites sordidus (Germar)	1	—		
Former data and unpublished observations (21 sp.)	89	—	(⊖?) recorded in 6 sp. ○ recorded in 1 sp.	
COLEOPTERA: General				Pattern II ○ (especially emission of cytoplasmic ex-

				pansions by the unstable hyaline hemocytes) unmixed or associated with pattern I ● (=pattern III ◉), recorded in 240 out of 354 sp. investigated
Panorpoid Complex				
NEUROPTERA [24]				
MYRMELEONTIDAE				
Undet. sp. (larva)	1	●	Poor	
MANTISPIDAE				
Mantispa phtisica Gerstaecker	3	—		
ASCALAPHIDAE				
Probably *Episperches* sp., near *vacuus* Gerstaecker	1	○	(◉?)	
Former data (NEUROPTERA):				
Chrysopa vulgaris Linnaeus	2	●	Poor or (?)	
LEPIDOPTERA (late larval stages)				LEPIDOPTERA
Megalopyge lanata Stål [25]	1	⊖	Or ○ poor	○
Undet. sp.	1	⊖		
Former data (20 families, 92 sp.)	331	○	Recorded in 83 out of 92 sp. Pattern III recorded in 10 out of 16 sp. of Saturniidae, in *Cossus*, and possibly in 1 sp. of Lasiocampidae and in 1 sp. of Sphingidae. Group differences recorded in the amount of coagulated material	

[21] Det. by George B. Vogt. [22] Det. by George B. Vogt. [23] Det. by Miss Rose Ella Warner. [24] Det. by Miss Sophy Parfin.
[25] Det. by James Zetek.

TABLE 1.—*Patterns of Coagulation*—continued

Orders, Families, Genera, and Species	Number of Specimens	Pattern of Coagulation Provisionally Representative or Predominant	Comments	Tentative Generalization
DIPTERA [26]				DIPTERA
Pantophtalmus conspicabilis Austen.	1	—		Adults: —
Former data:				
Tipulidae (larvae) (6 sp.).....	23	○	Instantaneous and substantial coagulation	
Fourteen sp. (adults and larvae) from 12 other families.....	112	—		
HYMENOPTERA [27]				
TENTHREDINIDAE				Tenthredinidae
No specimen collected.				
Former data (14 sp.).....	100	●◉	Instantaneous and substantial coagulation	●◉
BRACONIDAE				
Trigonophasmus, n.sp.	1	—	(?)	
FORMICIDAE				
Paraponera clavata (Fabricius) ♀...	2	●	* to ***	
" " " ♂...	5	●	* to ***	
" " " w....	9	●	***, (◉) in 1 spm.	
Eciton burchelli (Westwood) (br. sense) w.	20	○	*, (◉?)	
Eciton hamatum (Fabricius) w.....	8	○	(◉?)	
Eciton (*Eciton*) sp. w.....	8	○	(◉?)	
Atta cephalotes (Linnaeus) (?) w...	10	◉		
Atta sp. ♀.....	1	●		
Camponotus abdominalis (Fabricius) (br. sense) w.....	40	●◉		Formicidae Pattern I ● and/or III ◉

Camponotus abdominalis (Fabricius) (larvae)	5	—	(○)	
Camponotus sericeiventris (Guérin) (br. sense) ♀	1	●◉		
Camponotus sericeiventris (Guérin) w.	43	○	** in several spm. (◉)	
Camponotus sericeiventris (Guérin) (larvae)	20	—		
Former data (3 sp.)	43	●◉		
MUTILLIDAE				
Dasymutilla sp. ♂	1	◉	*	
Hoplomutilla xanthocerata (Smith) ♀	1	◉	Probable	Mutillidae Pattern III ◉
Pseudomethoca sp. ♀	1	◉		
VESPIDAE				
Apoica pallida var. *pallens* (Fabricius) ♂ ♀	4	◉	Probable. *** in 1 spm. Large individual variations	
Mischocyttarus melanarius (Cameron) ♂ ♀	5	—	● poor in 1 spm.	Vespidae Possibly patterns I ● and III ◉ Large intraspecific variations
Mischocyttarus melanarius (Cameron) (larvae)	5	—		
Mischocyttarus tolensis Richards, 1941	1	—	(⊖)	
Polistes canadensis var. *panamensis* Holmgren ♀	6	◉	Probable. Large variations; —recorded in several samples	
Polistes fuscatus Fabricius (Cape Cod) ♀	2	—		
Former data (4 sp.)	22	◉	● recorded in several spm.	

[26] Det. by Dr. W. W. Wirth. [27] Det. by K. V. Krombein, C. F. W. Muesebeck, and Dr. M. R. Smith.

TABLE 1.—*Patterns of Coagulation*—continued

Orders, Families, Genera, and Species	Number of Specimens	Pattern of Coagulation Provisionally Representative or Predominant	Comments	Tentative Generalization
HYMENOPTERA (continued)				
POMPILIDAE				
Anoplius a.amethystinus (Fabricius) ♂	1	◉		
Former data. *Anoplius viaticus* (Linnaeus)	1	●		
SPHECIDAE				
Chlorion mirandum (Kohl) ♀	2	●		Sphecidae
Chlorion singulare (Smith) 1♀	2	●	Poor	Pattern I ●
Stictia signata (Linnaeus) ♂	3	●		
Trypoxylon buscki Richards ♂	1	—	(?)	
COLLETIDAE				
Ptiloglossa fulvo-pilosa (Cameron) ♀	1	○	Poor or —	
APIDAE				Apidae
Centris vittata LePeletier ♀	1	—		Coagulation scarce, not detected in many specimens
Euplusia mexicana (Mocsary) ♀	1	⊖	Or —	
Former data (9 sp.)	36	○ poor, ⊖ and —	● recorded only in *Nomada flava* Panzer	
ODONATA [28]				
Probably *Pantala* sp. near *hymenaea* (Say) (Libellulidae, Libellulinae) adult ♂	1	—		
Former data (*Anisoptera*) (6 sp.)	55	Varied reactions recorded in adults; ●◉ in several larvae		

[28] Det. by Miss Sophy Parfin.

Homoptera.—Pattern I, with general solidification of the plasma, was consistently observed in Cicadidae, Cicadellidae and Fulgoridae. In the last family (*Laternaria, Phrictus*), coagulation of the hemolymph was especially substantial; the films of hemolymph were instantaneously transformed into opalescent bluish clots, embedding all the hemocytes (altered fragile hemocytes, numerous small macronucleocytes, and transitional forms to various types of granular hemocytes).

Coleoptera.—The various groups of Coleoptera listed in the table exhibited a great diversity in the reactions of their hemolymph in vitro. However, predominance of one of the patterns characterized several groups.

Dark hyaline hemocytes, undergoing clarification after discharge of substance (see Heteroptera above, and Grégoire, 1955a, discussion, p. 129), were observed in *Agra* sp. (Carabidae) and in *Veturius platyrrhinus* (Passalidae).

The reactions detected in Scarabaeidae (especially Melolonthinae, Rutelinae, Dynastinae) were essentially identical to those reported previously as representative of this family. Upon withdrawal, the hemolymph became immediately viscous and ropy. The hyaline hemocytes, relatively numerous (64 percent of the total hemogram in *Lagochile sparsa* Ohaus) and of small size, extruded spontaneously cytoplasmic expansions, soon embedded, like the other hemocytes, in the veil-like reaction developing in the plasma (pl. 1, fig. 6).

In *Zophobas latticollis* Kraatz, pattern III, characterizing several species of Tenebrionidae, developed with a special clarity: cytoplasmic expansions of the hyaline hemocytes and transparent glassy veils (pattern II) appeared immediately upon withdrawal of the hemolymph. The consistency of the veils became granular, while circular areas of greater density (islands of coagulation: pattern I) grew out around several hyaline hemocytes already involved in the constitution of cytoplasmic systems (pl. 1, fig. 9).

In the specimens of Sandalidae, the film of hemolymph consisted of a substantial syrupy granulum embedding tiny nuclei of altered unidentifiable hemocytes. The pattern of coagulation could not be safely established in these specimens.

In Lampyridae, dense suspensions of particles normally present in the hemolymph of these insects, as in other groups (Coccinellidae, various Chrysomelidae), interfered with the detection of the pattern of coagulation.

Among Cerambycidae, subfamily Prioninae (*Stenodontes, Calli-*

pogon) exhibited one of the most substantial coagulations recorded among all the insects examined in this and in the previous studies.

Hymenoptera.—Rapid collection of the hemolymph without contamination with foreign tissues was difficult in small specimens and in dry ones. In ants, large numbers of specimens were used, and the only samples not discarded were those in which a limpid drop of hemolymph could be collected and a rapid spreading out of the films performed.

In view of the scarcity of the species available and the large interspecific and intraspecific variations in the reactions observed in this order of insects, the predominant patterns could not be established safely for several species and groups. Some patterns actually recorded in a part of the samples correspond possibly to incomplete reactions.

Pattern I was observed in all the samples collected from all the females, males, and workers of *Paraponera clavata* (Formicidae) and *Chlorion* (Sphecidae), and pattern III in the three specimens of Mutillidae captured.

In larvae of *Camponotus sericeiventris,* numerous hemocytes were loaded with refractile inclusions, and no modification of the plasma appeared in that material.

The Hymenoptera listed in the table were characterized by the small size of their hemocytes and of the islands of coagulation around the hyaline hemocytes, even in the samples in which a substantial coagulation was recorded. In the latter preparations, a considerable extension of the coagulation took place from around the islands of coagulation, which appeared in the granular clots as small circular areas of greater density, centered by the fragile hyaline hemocytes and remaining distinct in the general coagulation of the plasma (pl. 1, figs. 3, 4, 5).

Lepidoptera (larvae).—In the two specimens of lepidopteran larvae, the reactions of the hemolymph in vitro were identical to those described and illustrated elsewhere in a large number of caterpillars (Grégoire, 1955, pp. 118-120 and pls. IX and X: pattern II, with large individual variations in the completion of the process, frequently incomplete, as in the two specimens listed in the table). In the films of hemolymph, refractile hyaline hemocytes underwent clarification after rupture of the cell boundaries and discharge of substance, as illustrated in figures 42-50 of the above-cited paper.

DISCUSSION

1. The four patterns used in the present study are an attempt to classify the disparities recorded in insects with regard to the micro-

scopical picture of films of clotting hemolymph, observed by phase-contrast microscopy in standard conditions of preparation.

Objection that these patterns might result from random artifacts has been examined elsewhere (Grégoire, 1955a).

In control observations, the clotting process was compared in films of hemolymph spread out under glass by the standard procedure and in clot plugs spontaneously formed at the wound site and gently squeezed under glass after completion of the process. In both instances, the microscopical alterations characterizing the same pattern were recorded. The standard conditions of preparation of the samples of hemolymph seem therefore to be a faithful reproduction of the alterations occurring during the undisturbed natural process.

2. Whatever each pattern might signify at the cytological [2] or biochemical level,[3] most results of the present and other studies [4] suggest

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[2] Among the factors implied in the process of coagulation, the patterns reflect actual inequalities between species and higher taxonomic groups in the degree of sensitiveness to contact with solid surfaces of the fragile hyaline hemocytes selectively involved in the inception of the coagulation, in the nature of the alterations undergone by these unstable cells, and in the rapidity with which these alterations develop. These differences affect the subsequent reaction of the plasma. As shown in the present and in previous studies, a similar degree in sensitiveness of the fragile hemocytes is frequently shared by insects belonging to a same group.

A tentative identification of the fragile hyaline hemocytes has been reported elsewhere (Grégoire, 1953a; 1955a, p. 129). A part of these corpuscles exhibits cytological features in common with the oenocytoids. In several groups (Odonata, Hemiptera-Heteroptera, various species of Coleoptera, lepidopteran and dipteran larvae, Trichoptera, and some Hymenoptera) these corpuscles appeared in the films of hemolymph in the shape of highly refractive or dark hyaline hemocytes, which undergo clarification after explosive discharge of substance. The same corpuscles differ, however, in other characters from the classical description of the oenocytoids (1955a, discussion, p. 131). On the other hand, the fragile hemocytes selectively involved in coagulation are referred to by Jones (1954) as cystocytes, in *Tenebrio molitor*.

[3] The scarcity of the data available at the present time does not enable one to establish whether actual biochemical differences characterize each of the four patterns, and especially the two aspects presented by the reactions in the plasma, the granular substance (in the islands of coagulation and in the areas of extension: pattern I), and transparent glassy veils (pattern II). As suggested by observations of films of varying thickness, it is unlikely, as reported elsewhere (Grégoire, 1955a, discussion, p. 128) that the twofold aspect of the plasma changes is related merely to differences in concentration or in thickness of the clotted films. The veils are not to be identified with the products of general disintegration of the hemocytes (cell fibrin).

An adequate test of the validity of the patterns would be to determine whether biochemical differences correspond to microscopical pictures as different as those consistently recorded, for instance, in insects belonging to the

that the patterns are not individual particularities, except in a few equivocal cases.[5] The patterns rather characterize species, more frequently taxonomic groups (genera, families, suborders, or orders).

Repeated samplings of hemolymph collected from several specimens of the same species, or from different species belonging to the same higher taxonomic category, made it possible to record consistently the same pattern in groups of various taxonomic importance such as the Orthopteroid complex (pattern I),[6] several families of Heteroptera (especially Reduviidae, Coreidae, Pentatomidae) (pattern IV), Belostomatidae and Nepidae (pattern I), three families of Homoptera (Cicadidae, Fulgoridae, Cicadellidae) (pattern I), among Coleoptera, Hydrophilidae (pattern IV), Staphylinidae (pattern IV), several subfamilies of Scarabaeidae (Rutelinae, Melolonthinae, Dynastinae, Geotrupinae, Trichiinae and Cetoninae) (pattern II), Heteromera (Tenebrionidae, Lagriidae, Monommidae, Oedemeridae and Meloidae; patterns I and III), Cerambycidae (pattern I), Curculionidae (pattern IV), several families of Lepidoptera (larvae; pattern II), Tenthredinidae (patterns I and III).

3. Other groups (Cicindelidae, Carabidae, Dytiscidae, Silphidae, Passalidae, Coprinae, Elateridae) exhibited large intraspecific and interspecific variations in the patterns of coagulation recorded. In view of the diversity of the reactions in these groups, the pattern representative or predominant could not be established with certainty. However, at the genus level predominance of a pattern appeared in genera such as *Carabus* (pattern III), *Agra* (pattern I), *Hydaticus* (pattern IV), *Dytiscus* (pattern III) *Cybister* (pattern I), *Necrophorus* (pattern I).

4. In the homogeneous groups listed above, the Neotropical material and the insects from the Old World supplied identical results

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orthopteroid complex (pattern I), in insects from several families of Scarabaeidae (Rutelinae, Melolonthinae, Dynastinae, Cetoninae) (pattern II), Heteromera (pattern III), and other groups of insects in which no visible modification could be detected in the plasma (e.g., Staphylinidae, Hydrophilidae, and many Heteroptera) (pattern IV) under the phase-contrast microscope.

[4] Grégoire (1951, 1955); Grégoire and Jolivet (unpublished). The total material investigated consists of approximately 5,300 samples of hemolymph, collected from 3,400 specimens belonging to about 850 species.

[5] In most of these cases, the scarcity of the material available suggests that individual variations, incomplete reactions, or accidental artifacts (mechanical agencies; see Grégoire 1955a, p. 124ff.) might confuse the actual pattern.

[6] In the highly homogeneous orthopteroid complex, differences in the intensity of the clotting reaction could be detected between several groups (see table).

with regard to the pattern of coagulation predominant or representative of the taxonomic category.

Such consistency suggests that the patterns of hemolymph coagulation are a character of taxonomic significance (in a broad sense). Whether that type of character is of more or less applicability in phylogenetical controversies, is a question left to competent phylogeneticists. It might, however, be stressed that the process of hemolymph coagulation is in no way related directly to any type of structural or ethological criteria commonly used for defining and grouping taxonomic categories. It is therefore of interest to check tentatively some taxonomic relationships on the basis of the presented data.

5. As pointed out elsewhere (Grégoire, 1955a, pp. 136-137), random coincidence does not seem to be entirely responsible for explaining some correlations between phylogenetic position of certain groups of insects and microscopical aspect of the coagulation of their hemolymph. In this respect, the Neotropical material examined here supports former tentative suggestions concerning most of these correlations.[7]

Pattern I has been heretofore uniformly recorded in Blattodea and in the other groups ranged within the orthopteroid complex. The mechanism involved in this pattern is identical to one of the types of coagulation described by Hardy (1892), Tait (1910, 1911), Tait and Gunn (1918), Numanoi (1938), and Grégoire (1955b) in crustacean blood, in which a special category of cells, the Hardy's explosive corpuscles, corresponding to the insect hyaline hemocytes or coagulocytes (Grégoire and Florkin, 1950), play a selective part in the inception of the coagulation of the plasma.

Pattern I has also been recorded among various unrelated groups of insects, especially in groups characterized by the retention of various primitive characters, such as the Homoptera. In this respect, the present study has brought information on groups not represented in the material previously investigated.

From these data, pattern I might be considered as a generalized primitive mechanism of coagulation of insect hemolymph.

The mechanism of coagulation illustrated in pattern II has been observed, unmixed or predominant, in relatively recent groups of

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[7] After the completion of this paper, the patterns of coagulation were recorded in 400 insects collected in September 1956 at Tingo María (Peru) and in October 1956 on Barro Colorado Island. The results are in agreement with those reported here, with regard to the predominance of one of the patterns in the following groups: Orthopteroid complex, Hemiptera, Homoptera, Scarabaeidae, Tenebrionidae, Cerambycidae, Curculionidae, Vespidae, and Diptera.

insects, such as Scarabaeidae (except Coprinae—see next paragraph) and lepidopteran larvae. As shown in the table, the present investigations have confirmed the predominance of the patterns previously reported for these groups.

6. In the samples of Neotropical species of Passalidae and of Coprinae, the islands of coagulation characterizing pattern I were absent or exceptionally recorded in the samples, while in the African specimens studied until now (Grégoire and Jolivet, unpublished), these islands of coagulation appeared frequently or consistently in many samples. In view of the scarcity of the material and the diversity in the reactions characterizing these two groups, random variations might be responsible for these divergences between the results.

SUMMARY

Coagulation of the hemolymph in vitro has been investigated by phase-contrast microscopy in 630 specimens from 230 Neotropical species of insects. The present material includes samples of hemolymph from insects belonging to 17 families not represented in previous related studies.

A tentative classification of the process of coagulation into four patterns, suggested previously, has been used, and the patterns characterizing provisionally each species have been determined and reported in tabular form.

The Neotropical material and the data collected formerly on species from the Old World (altogether approximately 850 species), supplied consistent results with regard to the predominance of some of the patterns in several taxonomic groups of various extension in the classification.

In a condensed form, the investigations on the distribution of the patterns of hemolymph coagulation in the different orders have shown: (1) In the Orthopteroid Complex, a great uniformity of reaction, in the shape of pattern I, possibly a generalized primitive mechanism of coagulation of the hemolymph; (2) in several families of Heteroptera, absence of a visible reaction in plasma (pattern IV), in striking contrast to two families of the same order, Belostomatidae and Nepidae, which exhibited a substantial coagulation (pattern I); (3) in Homoptera, a substantial reaction in the shape of pattern I, representative or predominant, in Cicadidae, Fulgoridae, and Cicadellidae; (4) in Coleoptera, as a taxonomic group, a large heterogeneity in the reactions. However, in this order, uniformity of reaction or predominance of a pattern was detected at the infraorder level, especially in Hydrophili-

dae (pattern IV), Staphylinidae (pattern IV), Scarabaeidae (pattern II, with the exception of Coprinae: patterns I and III), Heteromera (patterns I and/or III), Cerambycidae (pattern I), Curculionidae (pattern IV), and in a few genera reported in the table; (5) in Lepidoptera (larvae), predominance of pattern II, with the possible exception of Saturniidae (pattern III); (6) in Hymenoptera, occurrence of patterns I and III in several taxonomic groups, in Apidae, scarce coagulation or absence of plasma reaction.

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EXPLANATION OF PLATE 1

Films of hemolymph spread out between slide and coverglass, immediately upon shedding from severed appendages. Phase-contrast microscope (Wild M/10). Scale: 20 microns.

Figs. 1 and 2. *Stenodontes* (*Mallodon*) *molarius* Bates (Cerambycidae, Prioninae). (Pattern I***: very substantial coagulation.) All the hyaline hemocytes are surrounded by islands of coagulation. Considerable extension of the granular coagulum. In figure 1, three elements belonging to other categories of hemocytes are passively embedded in the clot.

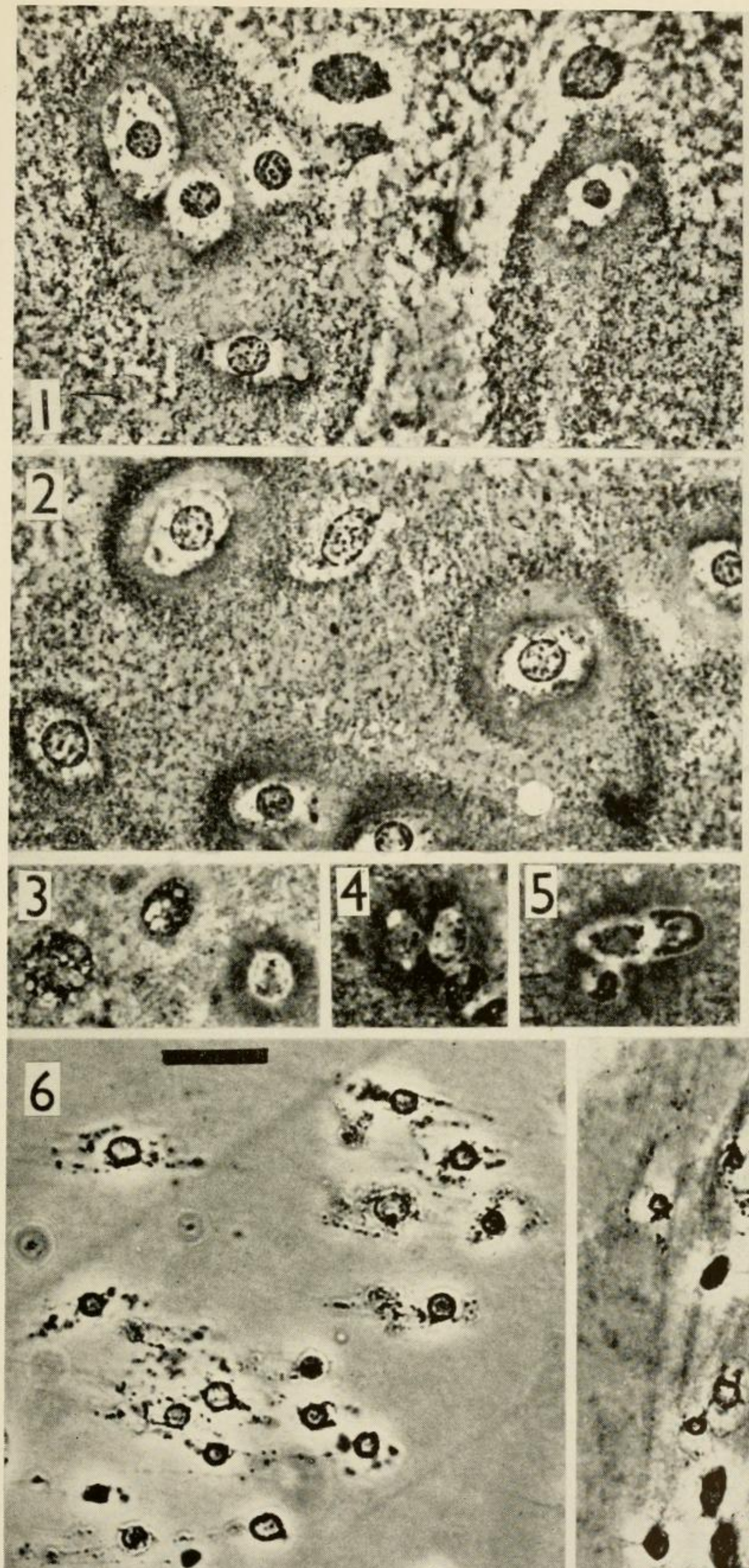
Figs. 3, 4, 5. *Paraponera clavata* (Fabricius) (Formicidae). (Pattern I ***). Islands of coagulation of small size. Extension of the coagulum. In figure 3, a small hyaline hemocyte (on the right) in the center of a small island of coagulation. On the left, two granular corpuscles embedded in the clot.

Fig. 6. Cetoninae sp. (Scarabaeidae). (Pattern II). Typical reaction of the hemolymph in vitro, as it appears in several subfamilies of this group (see table). Many hyaline hemocytes of small size with their cytoplasmic expansions are embedded in a substantial veil. Identical pictures were observed in several species listed in the table, especially in *Lagochile, Pelidnota, Phalangogonia, Trizogeniates, Aspidolea, Cyclocephala,* and *Dyscinetus.*

Fig. 7. *Phrictus quinquepartitus* Distant (Homoptera, Fulgoridae). (Pattern I***). Two hyaline hemocytes, each surrounded by an island of coagulation. Considerable extension of the coagulum between the islands, which preserve their size and shape.

Fig. 8. Pseudophyllidae (Tettigoniidae). Larva, first stage, male (Pattern I). The process of coagulation was slow and developed poorly in this specimen. When the picture was recorded, no reaction had yet developed in the plasma around the hyaline hemocyte shown in the center, between two other blood elements, including a granular hemocyte.

Fig. 9. Tenebrionidae sp. Picture representative of pattern III, predominant in this family (see description of the coagulation in *Zophobas latticollis* Kraatz, in the text, p. 27). Fan-shaped disposition of the threadlike cytoplasmic expansions of hyaline hemocytes, diverging from an air bubble on which these highly adhesive structures are anchored. Plasma reaction in the shape of a veil, with denser areas around hyaline hemocytes, corresponding to islands of coagulation. (Compare with Grégoire, 1955a, figs. 11 and 20. See legend of text figs. 2 and 3.)



(See explanation at end of text.)