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COAGULATION OF THE HEMOLYMPH
IN NEOTROPICAL INSECTS

By

CHARLES GRÉGOIRE

Department of Biochemistry, Institut Léon Fredericq
University of Liège, Belgium



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FURTHER OBSERVATIONS ON DISTRIBUTION OF PATTERNS OF COAGULATION OF THE HEMOLYMPH IN NEOTROPICAL INSECTS¹

BY CHARLES GRÉGOIRE

*Department of Biochemistry, Institut Léon Fredericq
University of Liège, Belgium*

The present paper is a contribution to a long-term inquiry on distribution of patterns of hemolymph coagulation in various arthropods, especially in insects.

The reactions of the main elements involved in the process of coagulation of the hemolymph—a category of unstable hyaline hemocytes (coagulocytes: Grégoire and Florin, 1950) and the plasma—differ in various insects. These differences, appreciated by phase-contrast microscopy, have been classified into four patterns of microscopic pictures (Grégoire, 1951).

The characters of these patterns may be described as follows:

Pattern I. Inception of the plasma coagulation in the shape of islands of coagulation around the hyaline hemocytes.—Selective alterations in the unstable hyaline hemocytes (shrinkages of the cell body and occasionally of the nucleus, sudden expansions, bulging of blisters and of blebs) 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; 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.

The mechanism involved in pattern I 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 crus-

¹ This is No. 9 in a series of papers entitled "Blood Coagulation in Arthropods" published in various journals.

tacean blood, in which a special category of cells, the Hardy's explosive corpuscles, corresponding to the insect hyaline hemocytes or coagulocytes, plays a selective part in the inception of the coagulation of the plasma.

Pattern II. Extrusion of cytoplasmic expansions by hyaline hemocytes, with development of cytoplasmic meshworks. Reaction in the plasma in the shape of veils.—On contacting the glass, a category of fragile hyaline hemocytes undergoes alterations that differ from those characterizing pattern I. These corpuscles extrude threadlike cytoplasmic expansions, sometimes of considerable length. These expansions are highly adhesive to solid particles (dust, chitinous debris), other hemocytes, and physical interfaces (bubbles). These alterations result in formation 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.

In various 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.—Association of the reactions taking place in patterns I and II characterizes the picture in pattern III. In the same film of hemolymph, hyaline hemocytes send out cytoplasmic expansions (pattern II) while islands of coagulation (pattern I) appear around the body of these corpuscles. When they develop within the veils, which characterize the reaction in the plasma in pattern II, the islands form circular, denser areas centered by the altered unstable corpuscles.

Pattern IV. No modification in the hyaline hemocytes, or alterations not followed by visible reaction in the plasma, in the optical conditions of phase-contrast microscopy.—In the pictures of this pattern, hemocytes resembling in their cytological characters the unstable corpuscles involved in the other patterns do not visibly alter. They appear as pale vesicles containing a few dark particles. In several insects, these corpuscles are the remnants of darker refractile, hyaline, frequently oenocytoid-like 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.

Specimens from more than 1,000 species of insects and of other arthropods have already been tested about the pattern of coagulation of their hemolymph or blood (Grégoire, 1951, 1953, 1955a, b, 1957, unpublished observations on palearctic insects (1957-1958); Grégoire and Jolivet, 1957). Predominance of one of the patterns has been observed in several taxonomic groups. In other groups, owing to the scarcity of the data available, or to large variations in the results, the pattern representative of a species or of a group at a supraspecific level could not be established.

The aim of the present study was to fill some gaps in the data. Four hundred Neotropical insects, belonging to 215 species, including 185 species not yet investigated, were collected and studied during visits to Tingo María, Peru (Estación Experimental Agrícola), August 1956, and to the Smithsonian Institution's tropical preserve on Barro Colorado Island (Canal Zone Biological Area), October 1956.

MATERIAL AND METHODS

The samples of hemolymph were mostly thin films prepared as soon as possible after capture. The hemolymph issuing from severed or punctured appendages (antennae, legs, wings, joints of the wing-cases) was placed immediately in contact with the edge of a cover glass lying on a slide and was allowed to spread out into films.

A phase-contrast optical equipment WILD M/10 was used for the observations (see Grégoire, 1955a, p. 105, and 1957, pp. 1 and 3).

RESULTS

DISTRIBUTION OF THE PATTERNS OF COAGULATION OF THE HEMOLYMPH IN INSECTS (TABLE I)

Detailed descriptions of the four patterns of coagulation of the insect hemolymph, used in the present study, have been given elsewhere (Grégoire, 1955a, p. 104; 1957, pp. 4-6 and text figs. 1-4).

In the table, 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 study of several samples of hemolymph obtained from these specimens. Incidental findings of other patterns are reported under "Comments."

In order to avoid duplication, the patterns recorded in the present study in 50 insects belonging to Neotropical species previously investigated (Grégoire, 1957) are reported in the notes, preceded by the date "(1957)."

The patterns of coagulation 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 the unstable hyaline hemocytes. Various degrees of extension of the process in the films.
- : 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 the plasma.
- ⊙: pattern III: patterns I and II combined.
- : pattern IV: no visible coagulation by phase-contrast microscopy.
- (): pattern incidentally or exceptionally recorded in limited fields of preparations exhibiting predominantly another pattern.
- (?): microscopical characters of a pattern not clear-cut or equivocal. Artifacts possibly involved.

Other abbreviations used: sp., species; spm., specimen; T., specimen captured and studied at Tingo María; B., specimen captured and studied on Barro Colorado Island.

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 a limited number of altered fragile hyaline hemocytes, without extension of the coagulation); I (scattered islands of coagulation of various sizes, with moderate coagulation of the fluid outside the islands); I*, I**, I*** (islands around all the hyaline hemocytes, substantial and general coagulation of the film).

TABLE I.—*Patterns of coagulation*

Material	Number of specimens	Patterns of coagulation or predominant in samples	Comments
<i>Orthopteroid Complex</i>			
DICTYOPTERA			
BLATTODEA ^{1, 2}			
<i>Periplaneta australasiae</i> (Fabricius) (adult and larva) (T.)	2	●	**
<i>Archimandrita tessellata</i> Rehn (B.)	1	●	
PHASMATIDAE ¹			
<i>Pseudophasma menius</i> Westwood ♂ (B.)	1	●	*
<i>Prisopus cerosus</i> Westwood (B.)	1	●	*
<i>Prisopus ariadne</i> Hebard (B.)	1	●	**
3 undet. sp. (2 adults, 1 larva) (T.)	3	●	(**)(***)
ORTHOPTERA			
TETTIGONIIDAE ¹⁻³			
<i>Scudderia paronae</i> (Griffini) (T.)	1	●	*
<i>Eupeucestes crassifolius</i> (Haan) ♀♂ (T.)	2	●	** (♂)
Undet. larva (Phaneropterinae) (T.)	1	●	
<i>Acanthodes aquilina</i> (Linnaeus) (B.)	1	●	*
<i>Microcentrum</i> sp.? (B.)	1	●	
<i>Neoxiphidion conocephalus saltator</i> (Saussure) ♀ (T.)	1	●	***
<i>Moncheca pretiosa</i> (Walker) (T.)	1	●	**
EUMASTACIDAE			
<i>Paramastax</i> sp. (T.)	3	●	
GRYLLIDAE ¹			
<i>Paragrillus temulentus</i> Saussure ♂ (B.)	1	●	**
GRYLLACRIDAE ¹			
<i>Abelona salvini</i> (Saussure and Pictet) ♂ (B.)	1	●	

¹ Det. by Dr. C. Willemse.² (Grégoire, 1957) *Epilampra azteca* Saussure (B.): I ***.³ (1957a) *Neoconocephalus affinis* (P. de B.) ♀ (B.): I (**); *Caulopsis microprora* Hebard (B.): I.

TABLE I.—*Patterns of coagulation*—continued

Material	Number of specimens	Patterns of coagulation representative or predominant in samples	Comments
ORTHOPTERA (continued)			
<i>PROSCOPIIDAE</i>			
<i>Apioscelis verrucosa</i> Brunner Von Wattenwyl ♀ (T.).....	1	●	poor
<i>ACRIDIDAE</i> ¹⁻⁴			
<i>Orphulella concinnula</i> Walker (T.)..	1	●	*
<i>Tetrataenia surinama</i> (Linnaeus) ♀♂♂ (T.)	3	●	poor
<i>Leptysmia insularis</i> (Bruner) (T.)..	1	●	poor
Undet. sp. (T.).....	1	●	**
<i>Dicaearchus</i> (gen. sp. nov.?) (T.)..	1	●	**
<i>Legua crenulata</i> Stoll (B.).....	1	●	**
DERMAPTERA ¹			
2 undet. sp. (T., B.).....	2	●	(**)
<i>Hemipteroid Complex</i>			
HEMIPTERA			
<i>REDUVIIDAE</i> ^{5, 6}			
<i>Saica meridionalis</i> Fracken and Bruner (B.)	1	—	
<i>Stenopoda cinerica</i> Laporte (B.)....	3	—	(○ poor or ⊖)
<i>Rasahus sulcicollis</i> (Serville) (B.)..	1	—	(● ?)
<i>Zelus</i> sp.? (nymph) (T.).....	1	—	
<i>Zelus</i> sp.? (nymph) (T.).....	1	—	
<i>Castolus subinermis</i> (Stål) (B.)....	1	—	
<i>Montina lobata</i> Stål (T.).....	2	—	
<i>Montina fumosa</i> (Stål) (T.).....	1	—	
<i>Brontostoma notatum</i> Stål (B.)....	1	—	
<i>Doldina bicarinata</i> Stål (T.).....	1	—	
<i>PYRRHOCORIDAE</i> ⁵			
<i>Largus balteatus</i> Stål (T.).....	1	—	
<i>Dysdercus incertus</i> Distant (T.)....	12	—	(⊖)

⁴ (Grégoire, 1957) *Copiocera specularis* Gerstaecker: 1; *Osmilia flavolineata* (de Geer) (T.): 1 poor; *Xyleus rosulentus* Stål, 3 larvae (T.): 1 (**); *Schistocerca paranensis* Burmeister (T.): 1 poor.

⁵ Det. by Dr. J. C. Lutz.

⁶ (Grégoire, 1957) *Saica apicalis* Osborn and Drake (B.): —; *Zelus spinidorsis* (Gray) (B.): — (II poor or incomplete); *Panstrongylus rufotuberculatus* (Champion) (B.): —; *Panstrongylus geniculatus* (Latreille), 3 spm. (B.): — (I?).

TABLE I.—*Patterns of coagulation*—continued

Material	Number of specimens	Patterns of coagulation or predominant in samples	Comments
HEMIPTERA (continued)			
PYRRHOCORIDAE (continued)			
<i>Dysdercus ruficeps</i> (Perty) (T.)...	I	—	(⊕)
<i>Dysdercus</i> sp.? (nymph) (T.).....	I	—	
COREIDAE ⁵			
<i>Phthia decorata</i> Stål (T.).....	I	—	
<i>Spartocera fusca</i> (Thunberg) (T.).	I	—	
<i>Plapigus foliaceatus</i> (Blanchard)			
(nymph) (T.)	2	—	
<i>Anasa haglundii</i> Stål (T.).....	I	—	
<i>Hypselonotus striatulus</i> (Fabricius)			
(T.)	I	—	
<i>Paryphes adelphus mutans</i> Horvath			
(T.)	I	—	
<i>Hyalymenus tarsatus</i> (Fabricius)			
(T.)	I	—	
<i>Leptocoris filiformis</i> (Fabricius)			
(B.)	2	—	
<i>Zoreva dentipes</i> (Fabricius) (T.)...	5	—	(⊕ ?)
<i>Zoreva spinifera</i> Stål (T.).....	2	—	
GELASTOCORIDAE ⁵			
<i>Nerthra peruviana</i> (Montandon)			
(T.)	I	—	(○)
PENTATOMIDAE ⁵⁻⁷			
<i>Symphylus deplanatus</i> (Herrich-Shäffer) (T.).....	I	—	
<i>Augocoris gomesii</i> Burmeister (T.).	I	—	
<i>Macropygium reticulare</i> (Fabricius)			
(T.)	2	—	
<i>Euschistus crenator</i> (Fabricius)			
(T.)	2	—	
<i>Euschistus</i> sp.? (nymph) (T.).....	I	—	(⊕ ?)
<i>Loxa picticornis</i> Horvath (B.).....	I	—	
<i>Peromatus</i> sp.? (B.).....	I	—	
<i>Edessa affinis</i> Dallas (T.).....	2	—	
<i>Edessa polymita</i> Distant (B.).....	I	—	
<i>Edessa</i> sp. #1 (?) (T.).....	I	—	

⁷ (Grégoire, 1957) *Mecistorhinus piceus* (Palisot de Beauvois) (T., B.), 2 spm.: —; *Edessa rufomarginata* De Geer, 4 spm. (B.): —; *Acrosternum scutellatum* Distant (T.): —; *Neodine macraspis* (Perty), (B.): —.

TABLE I.—*Patterns of coagulation—continued*

Material	Number of specimens	Patterns of coagulation representative or predominant in samples	Comments
HEMIPTERA (continued)			
PENTATOMIDAE (continued)			
<i>Edessa</i> sp.? (B.)	1	—	
<i>Edessa</i> sp.? (nymph) (T.)	1	—	
<i>Edessa</i> sp.? (nymph) (T.)	1	—	
MIRIDAE ⁵			
<i>Mimoncopeltus</i> , n. sp. (T.)	1	—	
HOMOPTERA			
CICADIDAE ⁸			
<i>Carineta</i> sp., near <i>boliviana</i> Distant ♂ (T.)	1	●	
FULGORIDAE ^{9, 10}			
<i>Copidocephala ornanda</i> (Distant) (B.)	1	●	***
<i>Odontoptera</i> sp. (B.)	1	●	***
<i>Diareusa annularis imitatrix</i> (Ossi-Nilson) (B.)	1	●	***
Gen. and sp. unknown (B.)	1	●	**
CIXIIDAE ⁹			
Gen. and sp. unknown (B.)	1	—	(?)
DICTYOPHARIDAE ⁹			
<i>Nersia florens</i> Stål (B.)	3	●	**
<i>Taosa herbida</i> (Walker) (B.)	1	●	*
Gen. and sp. unknown (B.)	1	●	***
MEMBRACIDAE ⁸			
<i>Stictolabus</i> sp. ♀ (T.)	1	●	**
CERCOPIDAE ⁸			
<i>Cephisus siccifolius</i> Walker ♀ (B.)	1	●	**
<i>Zulia</i> sp. #1 ♂♂ (T.)	2	●	**
<i>Zulia</i> sp. #2 ♀♂ (T.)	2	●	***
<i>Tomaspis</i> sp. #1 ♀ (T.)	1	●	(● ?)
<i>Tomaspis</i> sp. #2 ♂ (T.)	1	●	*** (●***)
<i>Tomaspis</i> sp. #3 ♀ (T.)	1	●	***
<i>Tomaspis</i> sp. #4 ♂ (T.)	1	●	***
<i>Tomaspis</i> sp. #5 ♂ (T.)	1	●	**
<i>Tomaspis</i> sp. #6 ♀ (T.)	1	●	***

⁸ Det. by Miss Louise M. Russell.⁹ Det. by Dr. D. A. Young; *Diareusa* by Dr. V. Lallemand.¹⁰ (Grégoire, 1957) *Calyptoproctus elegans* (Olivier), 2 spm. (B.): 1 ***; *Cathedra serrata* (Fabricius) (B.): 1 ***.

TABLE I.—*Patterns of coagulation—continued*

Material	Number of specimens	Patterns of coagulation representative or predominant in samples	Comments
HOMOPTERA (continued)			
CERCOPIDAE (continued)			
<i>Tomaspis</i> sp. #7 ♀♂ (T.).....	2	●*	*** (⊙)
<i>Tomaspis</i> sp. #8 ♂ (T.).....	1	⊙	***
<i>Tomaspis</i> sp. #9 ♂ (B.).....	1	—	(● ?)
CICADELLIDAE⁹			
Tettigellinae			
<i>Diestostemma nigropunctata</i> (Signoret) (T.)	1	●*	** (⊙)
<i>Diestostemma</i> sp. ♀ (T.).....	1	●*	**
<i>Baleja flavoguttata</i> (Latreille) (B.)	1	●*	**
Sp. unknown ♀ (T.).....	1	●*	
<i>Oncometopia</i> sp. # 1, sex anomaly (T.)	1	●*	poor (⊙)
<i>Oncometopia</i> sp. #2, normal ♀ (T.)	1	—	
Sp. unknown (B.).....	6	●*	* to **
Iassinae			
" <i>Gypona</i> " <i>decorata</i> Fowler (B.)....	2	●*	poor
<i>Gypona atitlana</i> Fowler (B.).....	1	—	(?) ; ● probable
<i>Gypona hebes</i> Fowler (B.).....	3	●*	poor to ** ; (⊙) ; — in 1 spm.
<i>Polana</i> sp. (B.)	1	—	(?) ; dry spm.
<i>Ponana</i> sp. (B.).....	3	—	(● ?) in 1 spm.
<i>Gyponana</i> sp. ♀ (B.).....	1	●*	probable.
<i>Negosiana</i> sp. #1 (B.).....	1	⊙	
<i>Negosiana</i> sp. #2 ♀ (B.).....	2	— (● ?)	● probable
FLATIDAE⁹⁻¹¹			
<i>Anormelis nigrolimbata</i> (Fowler) (B.)	8	—	(● poor)
<i>Flatormenis</i> sp. (?) (B.).....	2	—	(?)
<i>Paradascalia nietvi</i> (Distant) (B.)..	6	—	(● ?)
ISSIDAE⁹			
<i>Oronoqua</i> sp. (B.).....	1	—	

¹¹ (Grégoire, 1957) *Carthacomorpha rufipes* Melichar, 3 spm. (B.): I **; (—?) in 1 spm.

TABLE I.—*Patterns of coagulation*—continued

Material	Number of specimens	Patterns of coagulation representative or predominant in samples	Comments
COLEOPTERA			
ADEPHAGA			
CARABIDAE ¹²			
<i>Harpalinae</i> sp. #1 (B.)	I	—	
<i>Harpalinae</i> sp. #2 (B.)	I	—	
<i>Harpalinae</i> sp. #3 (B.)	I	⊖	
<i>Agra</i> sp. #1 (B.)	I	⊖	poor (—)
<i>Agra</i> sp. #2 (B.)	I	○	● probable
<i>Lebiini</i> sp. (T.)	I	—	
POLYPHAGA			
PASSALIDAE ^{13, 14}			
<i>Passalus (Neleus) interstitialis</i>			
Eschsch. (B.)	I	⊖—	
<i>Veturius</i> sp. (B.)	I	—	(⊖)
SCARABAEIDAE ¹³			
Coprinae (Scarabaeinae) ¹³			
<i>Canthon</i> sp. (T.)	I	○	
<i>Uroxys gorgon</i> Arrow (B.)	2	—	(⊖)
Rutelinae ¹³			
<i>Mesomerodon spinipenne</i> Ohaus			
(T.)	2	○	*
<i>Pelidnota chlorana</i> Erichson (T.)	2	○	
<i>Anomala virescens</i> Burmeister (T.)	2	○	(⊖)
<i>Anomala</i> sp. (T.)	3	○	
Dynastinae ^{13, 15}			
Gen. near <i>Bothynus</i> (T.)	I	○	
CEBRIONIDAE ¹⁶			
Gen. unknown (T.)	I	●	*
ELATERIDAE ¹⁷			
<i>Chalcolepidius</i> sp. (B.)	I	●	(●)
<i>Semiotus</i> sp. (B.)	I	●	
LYCIDAE ¹⁶			
<i>Lycus</i> sp. (T.)	I	?	(○ ?)
LAMPYRIDAE ¹⁶			
<i>Photinus</i> sp. (T.)	2	?	

¹² Det. by G. Fagel.¹³ Det. by O. L. Cartwright.¹⁴ (Grégoire, 1957) *Veturius platyrhinus* Westwood (B.): — (III?).¹⁵ (Grégoire, 1957) *Aspidolea singularis* Bates (B.): II.¹⁶ Det. by T. J. Spilman.¹⁷ Det. by Dr. Ch. Jeuniaux.

TABLE I.—*Patterns of coagulation—continued*

Material	Number of specimens	Patterns of coagulation representative or predominant in samples	Comments
POLYPHAGA (continued)			
<i>LYMEXYLIDAE</i> ¹⁸			
<i>Melittomma</i> sp. (B.).....	I	● or ⊙	
<i>ENDOMYCHIDAE</i> ¹⁹			
Probably <i>Amphix</i> sp. (T.).....	I	⊖	
<i>COCCINELLIDAE</i> ²⁰			
<i>Epilachna</i> sp. (T.).....	I	—	
<i>Monomeda marginata</i> (Linnaeus) (T.)	I	—	
<i>EROTYLIDAE</i> ¹⁹			
<i>Erotylus</i> , prob. <i>spectrum</i> Thomson (T.)	I	○	(⊙ ?)
Prob. <i>Homoeotelus</i> sp. (T.).....	I	⊖	*, ⊙ probable
Gen. unknown (T.).....	I	⊖	
Gen. unknown (T.).....	I	⊖	
<i>TENEBRIONIDAE</i> ²¹			
<i>Strongylium auratum</i> Laporte (T)..	I	⊙	probable
<i>MELOIDAE</i> ²²			
<i>CERAMBYCIDAE</i> ²³			
Prioninae			
<i>Stenodontes</i> sp. (T.).....	I	●	***
<i>Pyrodes</i> sp. (T.).....	I	●	*** (⊙)
Lamiinae			
<i>Desmiphora</i> sp. (B.).....	I	●	(⊙ ?)
<i>Estola</i> sp. (B.).....	I	●	poor
<i>Oreodera glauca</i> (Linnaeus) (B.)..	I	●	**
<i>Acanthoderes bivitta</i> White (B.)...	I	⊙	** (●),
<i>Lagocheirus</i> sp. #1 (B.).....	I	●	**
<i>Lagocheirus</i> sp. #2 (B.).....	I	●	***
<i>Colobothea</i> sp. (T.).....	I	●	**
<i>Charoides</i> sp. #1 (T.).....	I	⊙	***
<i>Charoides</i> sp. #2 (T.).....	I	●	**

¹⁸ Det. by Dr. J. G. Rozen.¹⁹ Det. by Dr. J. G. Rozen.²⁰ Det. by Dr. E. A. Chapin.²¹ Det. by T. J. Spilman. (Grégoire, 1957) *Zophobas* prob. *atratus* (Fabricius) (B.): III ** probable.²² Det. by T. J. Spilman. (Grégoire, 1957) *Epicauta grammica* (Fischer von Waldheim), 3 spm. (B.): I *** (III).²³ Det. by George B. Vogt. (Grégoire, 1957) *Taeniotes scalaris* (Fabricius) (B.): I (III).

TABLE I.—*Patterns of coagulation*—continued

Material	Number of specimens	Patterns of coagulation representative or predominant in samples	Comments
POLYPHAGA (continued)			
<i>CHRYSOMELIDAE</i> br. sense ²⁴			
<i>EUMOLPIDAE</i> : poss. near <i>Prionodera</i> sp. (T.).....			
	I	—	
Chrysomelinae			
<i>Doryphora</i> sp. (T.).....	I	—	(⊖)
<i>Stilodes</i> (?) sp. (T.).....	I	⊙	
<i>Cosmogramma</i> sp. (T.).....	I	(⊖ ?)	(—)
Galerucinae			
<i>Diabrotica</i> sp. (T.).....	I	—	
<i>Andrector</i> sp. (T.).....	I	—	
Alticinae			
<i>Oedionychus</i> sp. #1 (T.).....	2	—	
<i>Oedionychus</i> sp. #2 (T.).....	I	—	
Hispiinae			
<i>Oediopalpis guerini</i> Baly (B.).....	2	—	
Cassidinae			
<i>Cyclosoma tristis</i> Boheman (T.)....	I	⊖ —	
<i>Echoma</i> sp., prob. <i>aulica</i> Boheman (T.)	I	⊖	poor
<i>CURCULIONIDAE</i> ²⁵			
<i>Naupactus</i> sp. #1 (T.).....	4	—	
<i>Naupactus</i> sp. #2 (T.).....	I	—	
<i>Compsus</i> sp. (T.).....	3	—	
<i>Heilipus</i> sp. #1 (B.).....	I	—	
<i>Heilipus</i> sp. #2 (T. B.).....	3	—	
<i>Metamasius</i> sp. (T.).....	I	—	
<i>Panorpoïd Complex</i>			
NEUROPTERA-PLANNIPENNIA			
<i>MANTISPIDAE</i> ^{26, 27}			
<i>Climaciella semihyalina</i> (Serville)			
(B.)	I	—	(⊖ ?)

²⁴ Det. by George B. Vogt.²⁵ Det. by Miss Rose Ella Warner. (Grégoire, 1957) *Exophthalmus jekelianus* (White), 2 spm. (T., B.): —.²⁶ Det. by Miss Sophy Parfin.²⁷ (Grégoire, 1957) *Mantispa phthisica* Gerstaecker (B.): —.

TABLE I.—*Patterns of coagulation—continued*

Material	Number of specimens	Patterns of coagulation representative or predominant in samples	Comments
NEUROPTERA-SIALODEA ²⁶			
CORYDALIDAE			
<i>Corydalus</i> sp., near <i>armatus</i> Hagen			
♀ (B.)	1	●*	***
TRICHOPTERA			
HYDROPSYCHIDAE ²⁸			
Prob. <i>Leptonema</i> sp. ♂ (B.)	1	●	very poor (—)
LEPIDOPTERA			
<i>AMATIDAE</i> sp. (adult) ²⁹ (B.)	1	—	
<i>SATURNIIDAE</i> sp. (larva) (T.)	1	○	*
<i>ARCTIIDAE</i> sp. (larva) (T.)	1	○	poor or ⊖
DIPTERA			
LARVAEVORIDAE ³⁰			
<i>Ormiophasia bushkii</i> TNS.	1	—	
HYMENOPTERA			
ICHNEUMONIDAE ³¹			
<i>Netelia</i> sp. ♀ (B.)	1	—	possibly ●
FORMICIDAE ³²			
<i>Azteca</i> sp. #1 ♀ (B.)	3	—	
<i>Azteca</i> sp. #2 ♀ (B.)	4	—	
<i>Pachycondyla crassinoda</i> (Latreille)			
♀ (T.)	1	●	
<i>Dinoponera</i> sp. (worker) (T.)	1	●	*** (⊙)
<i>Labidus coecus</i> (Latreille) ♂ (B.) ..	1	?	possibly ⊙
VESPIDAE ^{33, 34}			
<i>Pachymenes</i> sp. (T.)	1	○	
<i>Polistes major weyrauchi</i> Bequaert			
(T.)	4	●	
POMPILIDAE ^{33, 35}			

²⁸ Det. by Dr. A. B. Gurney.²⁹ Det. by W. D. Field.³⁰ Det. by C. W. Sabrosky.³¹ Det. by Miss Luella M. Walkley.³² Det. by Dr. M. R. Smith. (Grégoire, 1957) *Paraponera clavata* (Fabricius) ♂ (B.): I**; *Camponotus sericeiventris* Guérin, br. sense, 4 workers (B.): possibly III.³³ Det. by K. V. Krombein.³⁴ (Grégoire, 1957) *Polistes canadensis panamensis* Holmgren, 4 spm. (B.): I.³⁵ (Grégoire, 1957) *Anoplius a-amethystinus* (Fabricius) (B.): III.

TABLE I.—*Patterns of coagulation*—concluded

Material	Number of specimens	Patterns of coagulation representative or predominant in samples	Comments
HYMENOPTERA (continued)			
<i>SPHECIDAE</i> ³³			
<i>Sceliphron fistulare</i> (Dahlbom) (B.)	I	●	**
<i>Stictia maculata</i> (Fabricius) (B.)	I	●	** (⊙)
ODONATA			
<i>AGRIONIDAE</i> ³⁶			
<i>Megaloprepus coeruleatus</i> (Drury) (B.)	I	—	
ARACHNIDA ³⁷			
Araneae			
<i>THERAPHOSIDAE</i>			
<i>Eury</i> (<i>Brachypelma</i>) sp. (B.)	I	—	
<i>THOMISIDAE</i>			
<i>Epicadus heterogaster</i> (Guérin) (B.)	I	—	
OPILIONES ³⁷			
<i>Cosmetidae</i> sp.	I	—	
PEDIPALPIDA ³⁷			
<i>Tarantula palmata barbadensis</i> Pocock (B.)	I	—	
<i>IXODIDAE</i> ³⁷			
<i>Amblyomma humerale</i> Koch ♂ (B.)	I	—	

³⁶ Det. by Dr. A. B. Gurney.³⁷ Det. by Dr. J. Cooreman.

MICROSCOPY

The microscopical features of the reactions which characterize the coagulation of the hemolymph in several supraspecific groups of insects (Orthopteroid Complex, Heteroptera, Homoptera, Scarabaeidae, Cerambycidae, Hymenoptera, Lepidoptera) have been described elsewhere (Grégoire, 1955a, pp. 109, 111, 115, 118, 123; 1957, pp. 7, 27, 28; Grégoire and Jolivet, 1957, pp. 28-33). They were also observed in the corresponding groups of the present material. A few particular reactions will be briefly mentioned below.

Phasmoptera.—As repeatedly pointed out (Grégoire, 1951, 1955a, 1957; Grégoire and Jolivet, 1957) the various categories of hemocytes are passively embedded in the coagulum initiated by the alterations

in the fragile hyaline hemocytes or coagulocytes. Modifications of the plasma induced around the former corpuscles are exceptional. Such modifications, recorded previously in two specimens of Neotropical stick insects (Grégoire, 1957, p. 7), were observed in *Prisopus cerosus* (table 1) around macronucleocytes of small size (stem cells), secondarily to the typical formation of islands of coagulation around the unstable hyaline hemocytes.

Heteroptera.—Granular precipitates, unrelated to the presence of hemocytes in the vicinity, recorded previously in the same group of insects, were observed in the present material in *Montina lobata*, *Saica apicalis* (Reduviidae), *Macropygium reticulare*, 3 species of *Edessa* (Pentatomidae), *Anasa haglundii*, *Zoreva dentipes* (Coreidae). A tentative interpretation of these occasional findings has been given elsewhere (Grégoire, 1957, p. 7).

Coleoptera.—The sequence in the alterations in the fragile hemocytes and in the plasma, characterizing pattern III (see Grégoire, 1957, p. 2 and text fig. 3), appeared with great clarity in the two specimens of Elateridae mentioned in table 1.

In the samples of hemolymph from *Compsus* sp., *Heilipus* sp., *Exophthalmus jekelianus* (Curculionidae), characterized, as shown in the table, by the absence of detectable alteration in the plasma, in the conditions of phase-contrast microscopy, a category of highly labile hemocytes, unrelated to the unstable hyaline hemocytes, underwent considerable modifications in their shape: immediately upon withdrawal and spreading out into films of the hemolymph, these hemocytes appeared spindle-shaped, with two straight expansions on both sides of the cell body. The expansions became progressively flexuous and exhibited continuous trepidations and jerks. They reached great lengths, bent suddenly at right angles, and sent out lateral ramifications in various directions. Simultaneous development of such changes in neighboring hemocytes resulted in constitution of loose meshworks in wide areas of the preparations. Similar labile hemocytes have been reported in African weevils (Grégoire and Jolivet, 1957, p. 32) and in Diptera by Grégoire (1955a) and Jones (1956). In the present material they appeared in *Ormiophasia bushkii* (Diptera).

Much smaller bipolar corpuscles, of unknown origin, unrelated to the labile elements described above, developed similar modifications. A detailed study of these corpuscles will be reported later.

Arachnida. Araneae.—In *Epicadus* and in *Eurypelma*, a category of hemocytes with coarse refractile granules scattered in their cytoplasm and highly sensitive to foreign surfaces underwent disintegration immediately upon shedding of the blood, in contrast to other

categories of more resistant blood cells, such as macronucleocytes of small size (stem cells) and other kinds of granular hemocytes. A similar "differential sensitiveness" has been formerly observed in extensive material of spiders (see Grégoire, 1955b).

DISCUSSION

DISTRIBUTION OF THE PATTERNS OF COAGULATION IN THE VARIOUS TAXONOMIC CATEGORIES OF INSECTS

Detailed accounts on the relationships between pattern of coagulation of the hemolymph and taxonomic category have been given in previous papers (Grégoire 1955a, pp. 132-137; 1957, pp. 28-32; Grégoire and Jolivet, 1957, pp. 34-37). In this respect, the information obtained in the present material supports our former conclusions. With one exception (*Carthaeomorpha rufipes*, see below), the pattern detected in the samples of hemolymph collected in the present study (table, notes) from 50 specimens belonging to 30 neotropical species already investigated (1957), were identical to those recorded previously.

1. *Orthopteroid Complex*.

That broad group constitutes a highly homogeneous category with regard to the pattern consistently recorded at the specific and at the supraspecific levels.

2. *Hemipteroid Complex*.

Hemiptera.—With the exception of Nepidae and Belostomatidae, studied previously (Grégoire, 1955a; Grégoire and Jolivet, 1957), all the specimens from 14 other families of Hemiptera investigated, including Reduviidae, Pyrrhocoridae (see 1955a), Coreidae, Gelastocoridae, Pentatomidae, Miridae of the present (38 species) and of former materials, exhibited consistently the pattern IV.

Homoptera.—The present material includes 41 species not investigated previously (Grégoire, 1955a, p. 110; 1957, pp. 15 and 16). Pattern I was predominant in Cicadidae, Fulgoridae, Dictyopharidae, Cercopidae, Cicadellidae, and was recorded in the only specimen of Membracidae captured, a family not yet investigated. In a few Cercopidae (see also 1955a, p. 110) and Cicadellidae, pattern I was associated with pattern II (= pattern III).

A substantial coagulation of the hemolymph, developing rapidly, sometimes instantaneously, characterized these families, with the

exception of Cicadellidae, and was especially conspicuous in Fulgoridae.

In Cicadellidae, the amount of clotted material varied greatly and appeared scarcer than in the other groups listed above.

Pattern IV was observed in the samples of Cixiidae, Flatidae, and Issidae. However, in Flatidae, pattern I was found in *Carthaeomorpha rufipes* (table, note 11), a species in which pattern IV had been recorded previously in the only specimen available (Grégoire, 1957, p. 16). Pattern I appeared also incidentally in *Anormelis nigrolimbata* and in *Paradascalia nietvi*. Pattern IV, observed to occur predominantly in the few samples examined till now, is then questionable as being representative of Flatidae, a family which requires further investigation.

3. *Coleoptera*.

The patterns predominant or representative in several groups formerly investigated were seen again in the present material: pattern II in Scarabaeidae (Rutelinae, Dynastinae), pattern III in Elateridae and in Tenebrionidae, pattern I in Meloidae (note 22), Cerambycidae (very substantial coagulation), pattern IV in Curculionidae.

Pattern I, alone or associated with pattern II (= pattern III) was recorded in specimens of Cebrionidae and of Lymexylidae, two families not represented in our former data.

In the other groups listed in the table, scarcity in the material, large variations at the individual, specific, and generic levels, already noticed previously, do not permit conclusions about the pattern predominant or representative of these groups.

In this and in former studies (Grégoire, 1957, p. 22; Grégoire and Jolivet, 1957, pp. 22 and 23), absence or scarcity in clotting substances was observed in several specimens of Eumolpidae and of Cassidinae.

In the present material, pattern III was recorded in one (*Stilodes*) out of 3 specimens of Chrysomelidae s.s., a family involving genera with obviously predominant patterns (see 1955, p. 114: *Chrysolina*, 7 species: patterns I and III; *Timarcha*, 5 species: patterns I and III).

4. *Panorpoïd Complex*.

The present results are in agreement with former data with regard to Mantispidae (pattern IV: see Grégoire, 1957, p. 23), Sialodea: *Corydalis* sp. (pattern I, instantaneous reaction: see 1955a, p. 115:

Sialis flavilatera L.); Trichoptera: *Leptonema* (pattern I: see 1955a, p. 116; Limnophilidae sp. and *Anabolia nervosa* Leach); larvae of Lepidoptera (pattern II, see 1955a, pp. 116-118; 1957, p. 23; Grégoire and Jolivet, 1957, p. 25), and adult Diptera (*Ormiophasis bushkii*: pattern IV, see Grégoire, 1955a, p. 121).

As already pointed out, pattern I frequently characterizes insects belonging to relatively archaic orders (Plecoptera, see 1955a, p. 107; Megaloptera, 1955a, p. 115).

5. *Hymenoptera*.

Patterns I and III are representative in several families of this order (Grégoire, 1955, pp. 122-123; 1957, pp. 24-26; Grégoire and Jolivet, 1957, p. 25). However, individual and specific variations may mask the representative pattern of the genus or of the family when only limited material is available.

In the present (note 32) and previous materials (1957, p. 24) of Formicidae, a substantial pattern I characterizes the genus *Paraponera*. Patterns I and III were also recorded, though not consistently, in several specimens of the genus *Camponotus* (1955a, p. 123; 1957, p. 24; Grégoire and Jolivet, 1957, p. 25).

On the other hand, no coagulation could be observed (pattern IV) in seven females of *Arteca* sp., from which the films of hemolymph were collected and prepared without interference of any artifact.

The present observations on Vespidae (note 34), Pompilidae (note 35) and Sphecidae are in agreement with those made previously (pattern I and/or III: 1955a, p. 123; 1957, pp. 25-26).

6. *Odonata*.

As in former studies (1955a, p. 107; 1957, p. 26), pattern IV was recorded in the only (adult) specimen of this order collected in the present material.

7. *Arachnida*.

Coagulation of the blood was not detected in the present and former specimens of Pedipalpa, Ixodidae (1955b, pp. 497-498). Pattern IV was also recorded, in this and in previous studies, in specimens of Opiliones and of *Brachypelma* (Theraphosidae, Araneae), while other specimens of the latter genus exhibited pattern II, sometimes substantial, sometimes incomplete (see 1955b, p. 495).

ON THE DISPARITIES IN THE REACTIONS OF COAGULATION OF THE
HEMOLYMPH RECORDED AT THE SUPRASPECIFIC, SPECIFIC,
AND INDIVIDUAL LEVELS

1. In contrast to the taxonomic categories characterized by a pattern of coagulation representative or predominant, other groups, especially Carabidae (Grégoire, 1955a, p. 111; 1957, p. 16; Grégoire and Jolivet, 1957, p. 12), exhibit such variations that, in spite of increased samplings, a representative pattern did not appear clearly in these groups at the family level, but provisionally at the generic or specific levels.

In that respect, incidental coincidences may be deceptive and suggest erroneously that a pattern is characteristic of a genus, when it may actually represent an incidental failure of the true pattern to appear with all its particularities in a set of specimens being provisionally, at the time of capture, in similar abnormal conditions. For instance, in three specimens belonging to three different species of the genus *Agra* (Carabidae), pattern II, incomplete in two of these specimens, was predominantly observed in the present study, while formerly, in three other species of the same genus, pattern I had been consistently found (Grégoire, 1957, p. 16). Pattern III, possibly dissociated in the individual samplings into its two components (patterns I and II), might be the representative pattern of the genus *Agra*. Other examples are furnished in Hymenoptera in the genera *Eciton* (1957, p. 24) and *Asteca* (table), in which the predominant patterns are possibly not the actual ones.

In families such as Lycidae, Lampyridae, Coccinellidae, Chrysomelidae (*Cosmogramma*), and Cassidinae (*Cyclostoma*), the observations were handicapped by the presence in the hemolymph of particles floating in considerable numbers, a finding already noticed (1955a, p. 106; 1957, p. 27; Grégoire and Jolivet, 1957, p. 30).

2. Divergences at the specific or individual level recorded in genera characterized by a pattern predominant or representative, appear, for instance, in specimens of Cicadellidae. However, the pattern characterizing the group was found incidentally in the samples (see under comments in the table).

At the individual level, pictures of another pattern were recorded incidentally in limited fields of preparations exhibiting a predominant pattern (Reduviidae: *Stenopoda*, *Rasahus*, *Dysdercus*; see also 1955a, p. 109; 1957, p. 13; Grégoire and Jolivet, 1957, pp. 10-11).

Tentative interpretation of these divergences have been presented elsewhere (1955a, pp. 111, 124, 126; 1957, discussion; Grégoire and

Jolivet, pp. 36 and 37). Artifacts of preparation are responsible for a part of the pictures recorded. Nutritional balance of the specimens at the time of capture, seasonal and pathological conditions, able to alter the sensitivity of the unstable hemocytes or the amounts of the coagulable substances in the hemolymph, are among the factors which might explain these discrepancies: change in the pattern of coagulation has been observed in infected insects belonging to species or to groups characterized in their normal conditions by another pattern (Acrididae, Dermaptera, Cerambycidae) (see Grégoire and Jolivet, 1957, p. 36). Similarly, in a specimen of *Gypona hebes* from the present material, exhibiting pattern IV (table, comments), the unstable hemocytes responsible for the inception of the coagulation contained unusual coarse granules, absent in the other normal specimens in which the pattern representative of the group was observed.

The present results support former conclusions (1957, p. 30) that the patterns of coagulation are not individual particularities, but rather characterize species, more frequently supraspecific categories.

DIVERGENCES BETWEEN NEOTROPICAL MATERIAL AND INSECTS FROM THE OLD WORLD

In 10 specimens belonging to 6 species of Neotropical Passalidae (1957, p. 18, and here, table I), pattern I was recorded exceptionally in one sample from a single species, while this pattern, unmixed or associated with pattern II (= pattern III), appeared in the 5 African species (25 specimens) available (Grégoire and Jolivet, 1957).

Pattern I, absent from the samples of Neotropical Coprinae (4 species, 8 specimens), was found, alone or associated with pattern II (= pattern III), in 12 (29 specimens) out of 17 African species examined (Grégoire and Jolivet, 1957), and was questionable in three other species (5 specimens).

These data might suggest the possibility of discrepancies, with regard to these two families, between Neotropical and Old World material. However, as already pointed out (Grégoire, 1957, p. 32), large individual variations characterize these families, especially Passalidae. Numerous samplings from insects of both origins, and belonging to genera and species more closely related than those available, are required before any conclusion might be drawn about the existence of such discrepancies.

SUMMARY

1. Coagulation of the hemolymph from 400 (mostly adult) specimens, belonging to 215 Neotropical species of insects, and including

185 species not yet investigated, has been observed on films *in vitro* by phase-contrast microscopy. In that material, the pattern of coagulation predominant in the samples or representative for the species or for the supraspecific taxonomic category has been recorded.

2. The material contained insects from 14 families poorly (Dictyopharidae, Cercopidae, Cicadellidae, Flatidae) or not (Gelastocoridae, Membracidae, Cixiidae, Issidae, Cembrionidae, Lymexylidae, Erotylidae, Hispididae, Corydalidae and Larvaevoridae) represented in previous studies.

3. Additional information obtained for the present paper was consistent with former data, with regard to the pattern predominant or representative, in the Orthopteroid Complex, in several families of Heteroptera (Reduviidae, Pyrrhocoridae, Coreidae, Pentatomidae, Miridae), of Homoptera (Cicadidae, Fulgoridae, Dictyopharidae, Cercopidae, Cicadellidae), of Coleoptera (Scarabaeidae, Elateridae, Tenebrionidae, Meloidae, Cerambycidae, Curculionidae), of Hymenoptera (Formicidae, Vespidae, Sphecidae).

4. In the families not represented in former investigations, pattern I was recorded in specimens of Cembrionidae and of Lymexylidae (Coleoptera).

5. Pattern I was also observed in specimens of Corydalidae (Sialodea) and of Hydropsychidae (Trichoptera), in agreement with previous results on palearctic representatives belonging to these groups.

6. Divergences in the reactions of coagulation observed in the present and in a former study between Neotropical and African Passalidae and Copridae (Coleoptera) require further investigations on more extensive material, owing to the large variations existing in these groups of insects.

7. The reactions of the blood *in vitro* observed in five specimens of Arachnida (Araneae, Ixodidae, Opiliones, Pedipalpa) are briefly mentioned in relation to previous results on more extensive material.

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