Types of Crustacean Blood Coagulation.

By

John Tait, M.D., D.Sc.

(From the Marine Laboratory, Plymouth, and the Physiological Laboratory of Edinburgh University.)

HAVING been engaged at intervals during the last few years in studying from a physiological point of view the coagulation of crustacean blood —Tait (08), (10, A and B), (11)—and finding more variation in this regard in different crustacea than has hitherto been recognized, it suggested itself to me to inquire if the observed differences are correlated with any special physiological peculiarities, and, further, if they depend in marked degree on phylogenetic relationship. It is not a simple matter to settle either of these questions, and I make no pretence to have done so. At the same time, I have thought it worth while to put my observations on record, in the hope that the matter may thus sooner arrive at a satisfactory settlement.

The literature of the subject and details regarding the technique of examination of the blood and other particulars I hope shortly to publish elsewhere. Suffice it here to state that at least three distinct modes of blood coagulation may be recognized in crustacea :—

A. Simple agglutination of the blood corpuscles without any subsequent jellying of the blood plasma. (This is probably the most primitive and essential device both in invertebrates and vertebrates for procuring arrest of hæmorrhage from a wound.)

B. Agglutination of the blood corpuscles with subsequent general jellying of the plasma.

C. Jellying of the plasma in two successive stages, the preliminary cell-agglutination being relatively insignificant. The first plasma coagulation consists of localized (primitively globular) clots, which occur around or in immediate relation to special blood corpuscles, originally discovered by Hardy (92), and by him named "explosive corpuscles." At a later stage a second jellying process occurs, which this time involves the whole of the remaining plasma.

Although it is not my intention to discuss in detail the basis on which crustacean coagulation is separated into these three types, it would be a mistake to convey the impression that a hard and fast line of separation can be drawn between them. The classification is arrived at by examining the blood always under the same conditions—that is to say, *entirely removed from the vessels and placed either in a dish or on a glass slide.* These are, in fact, the conditions under which observations on coagulation are generally made. The classification here given represents a more extended knowledge than that hitherto in vogue—originally due to Heim (92)—which recognizes two groups, viz. A and B above.

COAGULATION C.

It will be convenient to begin with coagulation C, which is associated with the presence of explosive cells, and which from its complexity must be regarded as a somewhat specialized form of coagulation.

This form seems to be specially frequent in Isopoda. I have found it in *Conilera cylindracea,* in *Idotea baltica* and *emarginata,* in *Ligia oceanica,* in *Oniscus,* and in one or more species of *Porcellio,* i.e. in individual members of three sub-orders out of six.* On the other hand, I have failed to find it in *Gnathia maxillaris,* in *Dynamene rubra,* in *Sphaeroma serratum,*† and in *Jaera marina,* though possibly owing to the fact that the amount of blood obtained from these relatively small specimens was too meagre to allow of a satisfactory examination.

Among Amphipoda the occurrence of coagulation C seems to be much less frequent. It is not present in *Gammarus marinus,* in *Gammarus pulex,* in *Orchestia littorea,* nor in certain specimens of *Caprella* examined by me. On the other hand, I have recorded its presence in *Gammarus locusta*—Tait (10, B). This was not, however, under the conditions above specified—that is to say, in blood wholly removed from the animal and examined separately on glass, nor have I as yet observed it under these conditions.

As regards the Mysidacea coagulation C is absent in at least one species of *Mysis* (the only member of this order examined by me).

Crustacean blood coagulation has been chiefly studied in the

* The zoological classification referred to in the present paper is that given by Calman in Part VII of Lankester's *Treatise on Zoology.*

† In the course of this work I found that *Sphaeroma serratum* (with black chromatophores) undergoes colour change in response to its background, like that undergone by *Idotea*—V. Bauer (05)—and by *Ligia*—Tait (10, C)—whereas *Oniscus* and *Conilera,* the latter possessed of orange and lemon-yellow chromatophores, do not.

Decapoda, and it is of especial interest to know if coagulation C occurs among them. Of this order I have examined *Pandalus montagui* and *P. brevirostris*, *Hippolyte varians* and *H. viridis*, *Palaemon serratus*, *Crangon vulgaris*, *Palinurus vulgaris*, *Homarus vulgaris*, *Astacus fluviatilis*, *Galathea squamifera* and *G. strigosa*, *Porcellana longicornis* and *P. platycheles*, *Eupagurus bernhardus* and *E. prideauxii*, *Ebalia tuberosa*, *Corystes cassivelaunus*, *Carcinus maenas*, *Portunus puber*, *P. marmoreus*, *P. arcuatus* and *P. depurator*, *Atelecyclus septemdentatus*, *Cancer pagurus*, *Xantho hydrophilus* and *X. incisus*, *Inachus dorynchus*, *Macropodia rostratus*, *Hyas coarctatus* and *Maia squinado*; and have found coagulation C only in two members of the list, viz. in Palinurus and in Astacus. It is thus an uncommon form of coagulation in the Decapoda, while it is doubtful if it occurs at all in the Brachyura.

The examples hitherto mentioned exhaust the crustacean forms in which I have looked for the presence of coagulation C. I shall now say a word or two regarding this form of coagulation in its physiological aspect.

The clotting associated with the presence of explosive cells is a sufficiently striking one. Thus the blood of Palinurus forms the stiffest jelly of any crustacean blood I have examined; Ligia blood also forms a specially firm coagulum; while the onset of coagulation is in all cases rapid. In the solidity of the jelly formed, coagulation C represents the most advanced form of coagulation to be met with in crustacea.

Assuming that the object of plasma coagulation is to provide an additional mechanism for stopping a wound (cell-agglutination being, as above indicated, the primary and most essential mechanism for this purpose) it would follow that, *ceteris paribus*, a hæmorrhage in an animal possessing explosive cells would cease sooner than a hæmorrhage in other crustacea. This being so, one would look in isopods, in Palinurus and in Astacus for some special cause necessitating the existence of an extraordinary mechanism for arrest of hæmorrhage.

One naturally thinks of the process of autotomy in this connection. The reflex surrender of damaged limbs is generally conceded to be a method of preventing undue loss of blood. Where the reflex is present in least degree one might expect on the whole to find the highest degree of coagulability of the blood, and *vice versa*.

Now isopods do not show autotomy. In Astacus the reflex is said to be present—Huxley (80)—though in my own experience and in that of others, e.g. Fredericq (83), it is not readily demonstrable even in specimens presumably fit and healthy. In isopods and in Astacus, therefore, the association is what one would *a priori* expect. In

Palinurus, however, whose blood forms such a stiff coagulum, the power of autotomy is present in very marked degree. Thus the form of coagulation associated with the presence of explosive cells does not imply absence or defective power of autotomy.

If, again, we direct our attention to the crustacea that possess incoagulable, or rather non-coagulating, blood-plasma (type A), we find the evidence equally contradictory. Thus in Maia and in Cancer, both of them forms with non-coagulating plasma, power of autotomy is marked. In the spider-crab, *Inachus dorynchus*, whose type of blood coagulation likewise falls under group A, the presence of autotomy is unusually difficult to demonstrate. It seems that no constant relationship exists between power of autotomy and any special form of blood coagulation.

The assumption with which we originally started, viz. that the property of coagulability in blood plasma exists or has been evolved for the sole purpose of arresting hæmorrhage, may however be unwarranted. All the microscopical observations made on the arrest of hæmorrhage from a vessel, whether in invertebrates or in vertebrates, including mammals, go to show that the opening is plugged chiefly by adhesion and agglutination of cells at the cut surface. The blood of a hæmophilic person forms a perfectly firm clot: in spite of the presence of the clot, however, blood continues to ooze for hours and even days from a wound. Again, we meet with coagulability in physiological fluids other than blood. Milk clots in the stomach: yet no one has suggested that this coagulation has a merely mechanical function. Considerations such as these warn us against drawing premature conclusions in regard to the purpose for which coagulability exists in the blood plasma. In circulating blood there are multitudinous chemical processes constantly going on, the nature of which is entirely hidden from us. Who can say that coagulability is not primarily concerned in some of these hidden processes?

As a matter of fact, when we compare the actual time taken for natural arrest of hæmorrhage from the terminal segment of one of the limbs of Maia and of Palinurus respectively, we find that, while the hæmorrhage is, to begin with, equally profuse in both cases, the Maia wound is closed as soon as the Palinurus wound. Maia blood is characterized by the absence of all plasma coagulation. Palinurus blood is highly coagulable. Until we have further knowledge as to the *raison d'être* of coagulability in blood plasma, attempts to correlate by *a priori* methods, different types of blood coagulation with special physiological conditions can be but shots in the dark.

I shall conclude this physiological discussion by referring shortly to two other conditions with which at one time I imagined coagulation C might possibly be correlated. Having discovered coagulation C in isopods long before I found it in any decapod, I looked for other physiological peculiarities characteristic of isopods as opposed to decapods. An obvious one is the peculiar method of moulting observable in isopods. A moulting isopod throws off first the covering of the abdomen along with that of the posterior three thoracic segments. At a later date the covering of the head and anterior four thoracic segments is got rid of.* In decapods the moulting process does not occur in two stages. Once again, a fact to which attention has not been called, so far as I know, isopods do not turn red on boiling as so many decapods do. The discovery, however, of coagulation C in decapods, which moult in a different fashion and turn red on boiling, disposed of any possibility of establishing a correlation with these two phenomena.

COAGULATIONS A AND B.

Before discussing coagulation C in its zoological bearing, it may be well to make some statements regarding the distribution of the other two forms of coagulation, viz. types A and B. To determine the existence of one or other of these two types a greater quantity of blood is necessary than when one looks merely for the presence of coagulation C. Partly for this reason my data are somewhat meagre, and refer only to fairly large crustacea of the order Decapoda. The results agree to some extent with those of Heim (92).

Coagulation A is present in *Cancer pagurus, Maia squinado, Inachus dorynchus, Macropodia rostratus*, and *Hyas coarctatus.*

Coagulation B is present in *Carcinus maenas, Palaemon serratus, Portunus puber*, and *Homarus vulgaris.*

The last four animals are arranged in order according to the extent and firmness of the plasma jelly that forms in their blood after withdrawal. In *Carcinus maenas* the bulk of the plasma remains indefinitely fluid; in Palaemon the jelly, which is soft, involves almost all the plasma; in Portunus there is a complete and fairly firm jelly; while the plasma of Homarus clots with exceptional firmness.

I should like to make two comments on these results. In the first place, there is a complete series of gradation of plasma coagulation to be observed in group B. The amount of plasma jelly formed in the blood of *Carcinus maenas* is so slight that we might almost

* I do not know that anyone has called attention to the fact that the separation between the two cast-off portions of the integument occurs just at the anterior limit of the heart, as determined by the researches of Delage (81).

include the animal under group A. This indicates a difficulty in drawing a sharp line of distinction between group A and group B. In the second place, the coagulation observable in Homarus blood (at the other end of the series) approaches, in regard to its mere firmness apart from the mechanism involved in its production, most nearly to the coagulation seen in the blood of Palinurus or of Ligia.

ZOOLOGICAL SIGNIFICANCE.

From what has been said it will be apparent that the various categories into which crustacea fall according to the form of blood coagulation observed in them do not coincide in any striking fashion with the subdivisions into which they are grouped by zoologists. Within the order Decapoda alone we meet with all three types of coagulation. When, within this order, we consider the animals characterized by one given type of coagulation, we find that they are not necessarily close allies from a morphological point of view. Thus Palaemon, Homarus, and Portunus, similar as regards blood coagulation, represent extremes of decapod structure; from a blood coagulation point of view, again, we should group Astacus with Palinurus rather than with Homarus, an arrangement that would appeal to no morphologist; and so on.

On the other hand, there are indications that one and the same type of blood coagulation may sometimes keep constant in the members of a given zoological group. The prevalence in so many isopods of coagulation C, a type rare in decapods, is one example. Another is the apparent universality of coagulation A among the Maiadae. Then, again, among the decapods coagulation C is confined to the macrurous Reptantia.

The question, so far as it concerns the zoologist, now comes to be: Do the facts above related afford any justification for utilizing the physiological method of inquiry in the task of deciding upon the inter-relationships of crustacea? It seems that while a valid case for the actual applicability of this method has not been established, a case has at least been established for the desirability of further research along this line. As a further justification for this standpoint, I would quote the following sentence from Calman (09):—

"The classification of the Decapoda is a very difficult problem, and none of the schemes hitherto proposed can be regarded as entirely satisfactory. The traditional classification of the group into the long-tailed Macrura and short-tailed Brachyura was established by Latreille in 1806; but the difficulty of defining these groups is shown by the varying limits which have been assigned to the intermediate group of Anomura established by Milne-Edwards in 1834. Boas, in 1880, was the first to make a radical departure from this system. He pointed out that the Brachyura and Anomura were only single branches of the Decapod stock, and by no means equal in systematic value to the Macrura, which included several other branches not more closely connected with each other. In other words, just as in the classification of the Malacostraca as a whole, so within the Order Decapoda, the retention of the primitive 'caridoid facies' does not necessarily imply close affinity between the groups exhibiting it."

Again, referring to the Amphipoda, which were ranked by Leach along with the Isopoda in his group Edriophthalmata, he says (p. 239): "It seems very likely that their affinity to the Isopoda is not so close as has been supposed."

These statements are quite in accordance with the grouping that one would adopt from considerations of blood coagulation.

PHYSIOLOGICAL EVOLUTION.

Lastly, and this concerns principally the physiologist, we have in Crustacea an exceptionally appropriate assemblage of types in which to study the evolution of blood coagulation. In the blood or body-fluid of all invertebrates apart from arthropods, the only form of "blood coagulation" that occurs is an agglutination of the corpuscles—Cuénot (91), see also Geddes (80); there is no jellying of the plasma. In arthropods alone among invertebrates we meet with a true jellying or solidification of "fibrinogen" normally present in solution in the plasma. Now, in the class Crustacea, and even within the order Decapoda, we find some animals whose blood does not jelly, others in which the jellying process is present but insignificant, and others again in which it is very conspicuous. In this group of animals, therefore, we have a readily available series of types showing every gradation of evolution from complete absence of jelly (e.g. the spider-crabs) to the occurrence of very firm jelly-coagulation (e.g. the lobster and the rock-lobster).

Further, in the same class of animals we find that the jellying process, when present in what is possibly its most developed state, is associated with the presence of corpuscles having special physiological attributes. Assuming that the functional peculiarities of these cells represent a high degree of selective adaptation, we have to inquire by what steps the specialization has been brought about. The physiological features in question cannot be supposed to have sprung suddenly into existence in a few special animals. The explosive property and the jelly-producing property must be present in various stages of development in the blood-cells of different Crustacea. Crustacean blood-cells offer excellent material for the study of functional evolution.

Generally speaking, such studies have been much neglected in the past. Keith Lucas in two notable communications (09, A, B) has recently sketched the lines on which such investigation should proceed, and indicated the general bearing of the results that may be expected thus to accrue. I hope shortly to adduce some facts related to the coagulation of crustacean blood, which further illustrate this question of functional evolution.

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