INTERNAL DEFENSES OF CRUSTACEA: A REVIEW[1]

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ABSTRACT

Studies of the internal defenses of Crustacea have a discontinuous history, which began in the late 1800's. Elaborate early studies of phagocytes and humoral factors in the hemolymph have been extended with renewed vigor during the past decade. As is true for other invertebrates studied, phagocytosis of foreign particles by fixed and mobile cells in the crustaceans is augmented by naturally occurring bactericidins, lysins, and agglutinins. A few instances of experimental enhancement of titers of such humoral factors by previous exposure to foreign protein have been reported. Specificity of natural and experimentally enhanced humoral factors is much lower than that of vertebrate immunoglobulins, but probably such factors act synergistically with cellular protective mechanisms, as they do in the vertebrates.

Phagocytosis by fixed and mobile cells in gills, pericardial sinus, and sinuses at the bases of appendages seems to be a principal defense perimeter in many crustaceans. Efficacy of phagocytes in destroying invading microorganisms varies, depending on the species of the microorganism, as well as host physiology and environmental factors. Phagocytic activity is enhanced by hemolymph factors, which, in addition to immobilizing and agglutinating the invading organisms, also sensitize them to phagocytosis.

Hemolymph factors, most of which seem to be of cellular origin, may also have bactericidal or lytic activity, leading to extracellular destruction of microbial invaders. A few recent studies indicate that effects of hemolymph factors may be enhanced experimentally by injection of killed or living microorganisms.

The number of known microbial diseases in crustaceans is greater than that known in most other invertebrate groups, with the possible exception of the Mollusca and Insecta. The number and depth of studies concerned with internal defense mechanisms of Crustacea are similarly greater than those of most other invertebrate groups—again with the possible exception of the mollusks and insects. One microbial pathogen of Crustacea that has received adequate attention is *Gaffkya homari*—a gram-positive coccus which causes a fatal septicemia in lobsters and is capable of infecting other decapods. An elaborate series of studies in several laboratories has elucidated many details of the host-parasite relationship and has also provided extensive information about the internal defenses of a number of the larger Crustacea. *Gaffkya* constitutes a test microorganism of choice for future studies of disease processes in crustaceans.

Thus the available information about cellular and humoral defenses of Crustacea against invasion by foreign protein constitutes a significant part of what we know about such processes in the invertebrates. Phagocytosis, augmented by humoral factors with low specificity, seems to be the fundamental means of internal protection in the crustaceans and in other groups of invertebrates as well.

Investigations of the internal defense mechanisms of invertebrates against disease have progressed with renewed vigor in the past decade. Literature has accumulated to the point where condensation and summarization of information about certain invertebrate groups, such as the Crustacea, seem justified.

The large number of published reports on internal defenses of Crustacea can provide a good indication of the extent of our knowledge of immunity in marine invertebrates. Beginning with the pioneering work on phagocytosis and the entire process of inflammation by Metchnikoff (1884, 1893, and 1905), and on humoral factors described in the very extensive (but sometimes poorly documented, in terms of procedures and data) work of Cantacuzène (1912-34), the Crustacea have often been animals of choice in studies of internal defense mechanisms. A significant

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body of literature has accumulated, with conspicuous bulges in the early years (1884-1930) and in the past decade (1960-70), but with a very narrow waist during the period 1930-60.

Studies of humoral defenses of the larger Crustacea during the past 5 years (1965-70) are curiously reminiscent of work reported by Cantacuzène and his associates during the period 1912 to 1934, with the very important difference that most of the modern work includes elements that were largely missing from earlier reports, such as details of procedures, supporting data, adequate controls, and attempts at quantitation of results.

Because so much of the literature produced before 1940 lacks adequate quantitation and fails to provide details of techniques used, it is often difficult to relate results to those of more recent studies. Bang (1967b) has deliberately addressed himself to a repetition of earlier studies but has used modern methods in an attempt to improve the relationship. Other recent reports, even though based on species other than those used in earlier studies, constitute reexaminations of the concepts and general findings of the early investigators.

It is difficult to determine why the early work on immune responses in invertebrates so effectively begun by Metchnikoff, Cantacuzène, Cuénot, Bruntz, and others during the late 19th century and the early 20th century seemed to lose impetus and then virtually cease until recently. It is apparent from the literature, though, that research on invertebrate defenses, initiated so auspiciously, receded for a number of decades to the backwaters and eddies of the mainstream of advances in immunology, which was concentrated on the homoiothermic vertebrates. This may be explained in part by a natural and necessary concentration of research interest on human and mammalian immune responses (most immunologists were—and still are—generally associated with medical schools and hospitals). Part of the explanation also may be that earlier work failed to disclose any defense mechanisms in invertebrates that seemed fundamentally or conceptually different from those that were being elucidated for the vertebrates. More importantly, the explanation may have been that much of the earlier work failed to indicate any immunologic responsiveness in the invertebrates tested. In spite of occasional successes, the "inability of invertebrates to respond to the introduction of antigen by formation of antibodies" became a sort of dogma among many of the early biologists, as was pointed out by Cantacuzène (1923b). Failures to find responses may have been due partly to choice of inoculum with negligible antigenicity in the experimental invertebrate. Two factors may have caused the recent resurgence of interest among biologists in comparative immunology (which is gradually beginning to include the lower vertebrates and the invertebrates): (1) an evident need to reexamine the conceptual and evolutionary basis of immune responses and (2) an interest in understanding the internal defenses of invertebrates, which allow them to survive in a microbe-rich environment even though they lack the specific antibody response characteristic of most vertebrates.

The subject matter of the present review is one that has been treated previously (Cantacuzène, 1923b; Huff, 1940; Baer, 1944; Bang 1967b; Levin, 1967; Tripp, 1969; Rabin, 1970b; Bang, 1970). Many of those papers, however, were broad considerations of the invertebrates as a whole, and discussions of internal defenses of Crustacea were often more or less incidental. It is interesting—as Good and Papermaster (1964) pointed out—that no review of invertebrate immunity has emphasized induced responses. A recent, and excellent, 2-volume text on the physiology of Crustacea (Waterman, 1960) does not include a detailed consideration of the very important subject of internal defenses, except for reference to hemocytes and phagocytosis (Maynard, 1960; Parry, 1960), and a consideration of hemolymph coagulation (Florkin, 1960).

The general plan for this review is to discuss some of the early literature, after a brief preliminary statement about concepts and terminology, and a summary of known diseases of Crustacea. More recent studies will then be considered by categories of cellular and humoral systems: phagocytic, bactericidal, lytic, agglutinating, precipitating, phage clearing, and anti-

toxic. A final section will attempt a detailed review of the demonstrated systems of internal defenses of lobsters and other crustaceans against the microbial pathogen *Gaffkya homari* —which is one of the best examples of a test system for invertebrates for which existing information is adequate.

CONCEPTS AND TERMINOLOGY

Before proceeding with an examination of internal defenses of crustaceans, a brief review of some of the terminology may be relevant. "Resistance" and "susceptibility" have often been used interchangeably and reciprocally, but as Schneider (1951) and Stauber (1961) pointed out, insusceptibility and resistance should probably be considered as separate biological phenomena. Insusceptibility refers to those existing external or internal features of an animal —morphological and physiological—which deny access to a potential pathogen, or prevent its successful establishment and survival. Resistance, on the other hand, has been defined by Read (1958) as "those changes in the physiological state of the host which represent a *response* to present or previous contact with the parasite or with a similar chemical entity."

Resistance (and its equivalent—"immunity"), defined as host response, can then be considered as "innate" (natural) or "acquired" (induced). Innate resistance includes responses to primary contact with a pathogen, and acquired resistance includes responses that develop after primary contact. The distinction between the two types of response is not, however, always as definitive as might be preferred. Acquired resistance is often characterized by enhanced responsiveness to subsequent contact with a pathogen.

Resistance, whether innate or acquired, may be cellular or humoral. The cellular defense mechanisms are based largely on activities of leucocytes (hemocytes of invertebrates) and include: *phagocytosis*—engulfment and often digestion of foreign particles, *leucocytosis* (*hemocytosis*), and *leucocytic* (*hemocytic*) *infiltration*—the mobilization of hemocytes in the blood stream and their migration to invaded or injured tissues; *coagulation*—the formation of cellular or extracellular clots to close gaps in the circulatory system and to immobilize microorganisms; and *encapsulation*—the surrounding of large masses of invading material by phagocytes and fibrocytes. Humoral defense mechanisms, which probably depend on cellular secretions or cell disruption, and act synergistically with cellular defenses, include agglutinating, lytic, precipitating, and bactericidal systems, and other activities (Figure 1).

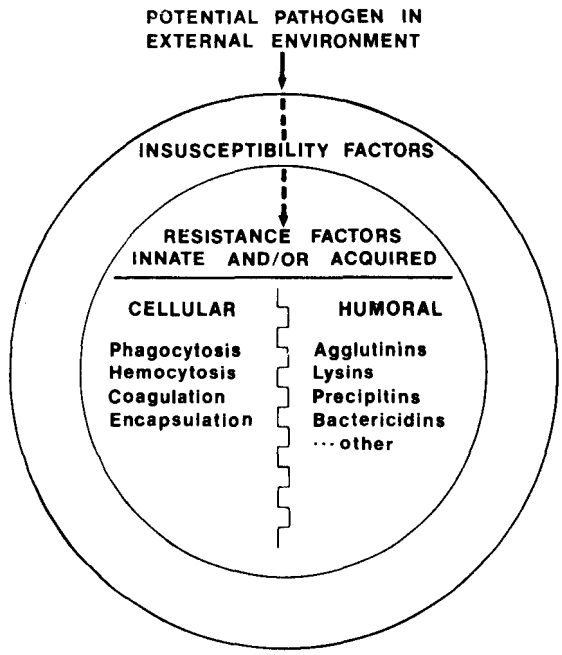

FIGURE 1.—Mechanisms of internal defense.

Antibodies (in the vertebrate sense of specific immunoglobulins) have not been demonstrated in invertebrates, although less specific antibody-like activity is common. Since antibodies have not been demonstrated, it is probably technically incorrect to use the term "antigen" with invertebrates. The semantics involved will be considered in the discussion section, but for convenience the term will be used in this paper. Furthermore, it must be made clear that when lysins, precipitins, agglutinins, etc. of invertebrates are discussed, no attempt is made to

homologize them with vertebrate factors. The terms merely indicate the type of activity produced.

DISEASES OF CRUSTACEA

An impressive array of diseases afflicting the Crustacea has been described (summarized in Sindermann, 1970, and Bang, 1970). A number of these diseases are of microbial etiology, and Koch's postulates have been satisfied for several of them. Some of the published reports concerning crustacean diseases include information about host defenses against infection, others do not. The literature also contains information about a variety of experimentally induced infections in Crustacea, many of them produced by microorganisms not known as pathogens in natural populations. Such experimental studies have been particularly useful in elucidating possible internal defense mechanisms—augmenting studies with known pathogens. The following summary is just that and is not intended as a detailed treatment of crustacean diseases. Some general background information on known diseases seems important, however, to any consideration of internal defense mechanisms.

The only virus disease of invertebrates reported in the scientific literature is one that occurs in crabs, *Portunus depurator*, on the French Mediterranean coast (Vago, 1966). Disease signs mentioned in his very brief paper included progressive darkening of the exoskeleton, paralysis, and death.

Bacterial infections of various Crustacea have been described, beginning with a disease of beach hoppers on the French coast caused by luminescent bacteria (Giard and Billett, 1889). Experimental infections were obtained by injecting cultured microorganisms, and some of the crustacean species tested exhibited varying degrees of resistance to experimental infection. Another luminescent disease was reported in sand fleas (*Talorchestia longicornis* and *Orchestia platinus*) from Woods Hole, Mass., by Inman (1927). Luminiscent bacilli were cultured, and experimental infections obtained. Luminiscent bacteria were also isolated from the digestive tracts of nonluminous sand fleas. A bacterial disease of *Gammarus marinus* was reported from England by Tait (1917), in which signs of disease included change in color of the infected amphipods from brown to opaque yellowish-white, reduction in numbers of blood cells, and absence of coagulation of hemolymph. Among the larger decapod Crustacea, a severe bacterial disease of lobsters caused by gram-positive cocci, *Gaffkya homari*, was recognized in 1947 (Snieszko and Taylor, 1947) and has been the subject of intensive studies since then (to be considered in detail later in this paper). Experimental infections and resultant mortalities of blue crabs, *Callinectes sapidus*, from Chesapeake Bay were reported by Krantz, Colwell, and Lovelace (1969) with *Vibrio parahemolyticus*. The microorganism has been isolated from mollusks, fishes, and sediments in various parts of the world and is known as a cause of human gastroenteritis in the Orient. Additionally, several examples of "shell disease"—erosion of the exoskeleton by chitin-destroying bacteria—are known in lobsters, crabs, and shrimps (Hess, 1937; Rosen, 1967, 1970; Anderson and Conroy, 1968).

Fungus diseases of Crustacea are surprisingly numerous in reports dating back to Metchnikoff (1884), who described fatal infections of *Daphnia* caused by *Monospora bicuspidata* and who first emphasized the crucial role of phagocytosis in determining the outcome of infection. A yeast infection in sand hoppers, *Talitrus*, from the coast of France, was reported by Herrmann and Canu (1891). Experimental infections from exposure to cultured microorganisms were fatal to *Talitrus* in 20 to 25 days. Phagocytosis was marked in such infections, and the hemolymph became milky in advanced cases. Crabs (*Carcinus maenas*), prawns (*Palaemonetes varians*), and crayfish (*Astacus fluviatulis*) were not susceptible to the experimental infections. Pixell-Goodrich (1928) described another yeast infection which was epizootic in *Gammarus* from a stream in England. The pathogen *Cryptococcus gammari* reproduced in the hemolymph and rendered it milky in color, coagulation was retarded, and heavily infected individuals died. Phagocytosis was active and sometimes successful in overcoming infections. Hypertrophy of fixed phagocytic cells was common.

One of the most severe, widespread, and long-continuing epizootics known in invertebrates has affected and still affects European crayfish. It is caused by the fungus *Aphanomyces astaci* (although other microorganisms have been variously associated with mortalities). Known as "Krebspest," the disease swept through crayfish populations of Europe beginning about the turn of the century (Schikora, 1906, 1926; Schäperclaus, 1935; Nybelin, 1935; Mannsfield, 1942; Unestam, 1965; Gordon, 1966). Apparently resistance differs among species—the American crayfishes, for example, seem less seriously affected by the pathogen in experimental studies.

Other fungus diseases of Crustacea include a systemic infection of pea crabs, *Pinnotheres*, from English sea mussels by *Leptolegnia marina* (Atkins, 1929, 1954a); a systemic disease of cultured prawns, *Palaemon serratus*, in England, caused by *Pythium* sp. (Anderson and Conroy, 1968); a gill infection of pandalid shrimp, *Dichelopandalus leptocerus*, from the western North Atlantic, caused by a chytrid-like microorganism (Uzmann and Haynes, 1969); and gill infections of lobsters, *Homarus vulgaris* and *Palinurus vulgaris*, in Italy, caused by *Ramularia branchialis* and *Didymaria palinuri*—both Fungi Imperfecti (Sordi, 1958).

Fungi also infect and destroy egg masses of Crustacea. Eggs of blue crabs, *Callinectes sapidus*, from Chesapeake Bay may be infected by *Lagenidium callinectes* (Couch, 1942; Newcombe and Rogers, 1947; Rogers-Talbert, 1948); and eggs of pea crabs are often infected by *Plectospira dubia* and *Pythium thalassium* (Atkins, 1954b, 1955).

Among the many protozoan diseases of Crustacea, those caused by microsporidans are probably the most destructive. *Nosema* sp. and *Plistophora cargoi* destroy body muscles of blue crabs (Sprague, 1965, 1966); *Nosema pulvis* and *Thelohania maenadis* infect muscles of green crabs, *Carcinus maenas* (Pérez, 1905a, 1905b, 1907). Other microsporidan infections of body muscles in Crustacea include those produced in *Gammarus* by *Theileria* sp. and *Nosema* sp. Necrotic muscle fibers containing microsporidan spores were destroyed by phagocytes (Pixell-Goodrich, 1928). Other pathological effects of microsporidans on gammarids have been recently reported by Bulnheim (1967) and Bulnheim and Vávra (1968). Crayfish muscles are attacked by Microsporida of the genera *Thelohania* and *Nosema* (Sprague, 1950b; Pixell-Goodrich, 1956; Sogandares-Bernal, 1962). Microsporida are also significant pathogens of shrimps. Sprague (1950a), Woodburn et al. (1957), Iversen and Manning (1959), Iversen and Van Meter (1964), and others have described infections of body muscles and gonads of shrimps from the Gulf of Mexico and European waters, caused by a number of representatives of the genera *Thelohania* and *Nosema*.

Other protozoan diseases of crustaceans include those caused by ciliates, an ameba, and gregarines. A ciliate, *Anophrys sarcophaga*, causes a fatal disease in shore crabs, *Carcinus maenas*, of Europe. The disease, and host responses to infection, will be considered in detail in a later section. Other parasitic ciliates occur in the hemolymph of Crustacea. *Paradinium* sp. and *Syndinium* sp. occur in calanoid copepods. *Syndinium* causes gonad destruction, while *Paradinium* colors the host a deep red (Gordon, 1966). *Hematodinium* sp. has also been reported by Gordon in *Carcinus*. A suctorian, *Ephelota gemmipara*, can seriously reduce production of lobster larvae (Dannevig, 1928, 1939). An ameboid parasite, *Paramoeba perniciosa*, causes a fatal disease (called "gray crab disease") in blue crabs from the Atlantic coast of the United States (Sprague and Beckett, 1966, 1968; Sprague, Beckett, and Sawyer, 1969; Sawyer, 1969). Hemolymph of infected crabs becomes cloudy and often incoagulable; in some individuals most of the cells in the hemolymph are amebae (Sawyer, Cox, and Higginbottom, 1970). A great number of gregarines occur in Crustacea of all kinds, but their pathogenicity seems slight, except for some destruction of the digestive epithelium resulting from heavy infections (Ball, 1938; Théodoridès, 1961, 1962; Tuzet and Ormières, 1961; Kruse, 1959a, 1959b).

Helminth diseases of crustaceans seem less abundant and less severe in their effects than those of microbial etiology. Trematode metacercariae encyst in muscles and hepatopancreas of

crabs, and larval cestodes, acanthocephalans, nematodes, and leeches occasionally have been reported from crabs, shrimps, and lobsters (Sindermann, 1970).

Crustaceans are frequently parasitized by other crustaceans—sometimes with serious effects on the host. Rhizocephalan barnacles are endoparasites of crabs, causing gonad degeneration and other morphological changes. Epicaridean isopods may produce similar changes in crabs and shrimps. Copepods sometimes parasitize crab eggs, as well as the gills of lobsters (Sindermann, 1970).

INTERNAL DEFENSE SYSTEMS

Studies of crustacean internal defenses published during the last decade have augmented earlier studies and have provided additional data to support generalizations and principles already enunciated, but none has yet provided the factual basis for new or different concepts. Because additional precision in terminology is now available, it is possible to consider the internal defense systems of Crustacea under the following headings: cellular (phagocytic), bactericidal, lytic, agglutinating, precipitating, phage clearance, antitoxic, and others. It should be obvious that these systems are not mutually exclusive and may often interact or even share components to protect the individual animal from invasion by potential pathogens. Largely for ease of description, the systems will be considered consecutively, even though many may act either in concert or simultaneously.

PHAGOCYTOSIS AND OTHER CELLULAR DEFENSES

The earliest study of phagocytosis in Crustacea concerned infections of *Daphnia* by the fungus *Monospora bicuspidata* (Metchnikoff, 1884). The fungus spores in the haemocoel were phagocytized and digested; the rapidity and vigor with which phagocytosis occurred determined the outcome of the infection. If some spores escaped phagocytosis, germinated, and formed conidia, the infection became generalized and the host died in a few days. If all the fungus spores were phagocytized and destroyed, the infection was arrested. Thus the speed and effectiveness of phagocytic action in some individuals, possibly mediated by humoral factors, determined survival. Absence of phagocytosis inevitably led to death.

Hemocytes of Crustacea were investigated by Cattaneo (1888b), Cuénot (1895, 1897, 1905), and Bruntz (1907). Cattaneo described the amebocytes of *Carcinus maenas;* Cuénot reported blood forming tissues—nodules of lymphoid cells in the blood sinuses—in decapods and described "phagocytic organs" in the hepatopancreas of decapods and amphipods; and Bruntz published an extensive paper on the hemocytes of many of the crustacean groups, distinguishing granular and hyaline hemocytes. Bruntz also described a "phagocytic organ" in gammarids; his 1907 paper reviewed an extensive series of his own studies (15 reports) published during the period 1903-1907 by the Societé Biologique de Paris. Other early studies of crustacean hemocytes include those of Hardy (1892), Tait (1918a, 1918b), and Tait and Gunn (1918).

Following the classical early studies of Metchnikoff, Cuénot, Bruntz, and others, which elucidated the critical role of phagocytes in the internal defenses against microorganisms, phagocytic cells have received greatest attention from vertebrate immunologists. General principles that have emerged from the more recent studies of phagocytosis in vertebrates undoubtedly apply as well to invertebrates. Among the papers that have contributed to understanding of phagocytosis are those of Wright and Douglas (1903), Wood, Smith, and Watson (1946), Wood (1953), Robineaux and Frederic (1955), Suter (1956), Rowley (1960), Rogers (1960), Evans and Karnovsky (1961), and Spector and Willoughby (1963). Reviews of phagocytosis have been published by Hirsch (1965) and Aarum (1967).

The mechanism of intracellular degradation of phagocytized microorganisms has been described in general terms for the vertebrates (Figure 2). Lysosomes—granules in the cytoplasm of phagocytes—contain antibacterial substances and hydrolytic enzymes (Cohn, Hirsch, and Wiener, 1963). The lysosome membrane

fuses with the vacuolar membrane within the phagocyte, releasing antimicrobial components into the vacuole (Robineaux and Frederic, 1955; Hirsch, 1965; Aarum, 1967). Evidence for comparable intracellular events in invertebrates is sparse, but Janoff and Hawrylko (1964) reported lysosomal enzymes in clams and starfish, and Eble (1966) found hydrolytic enzymes in oyster phagocytes.

Invading microorganisms are subjected to antimocrobial factors both inside and outside the phagocytes. Substances of presumed cellular origin, such as lysozyme, occur in the phagocytes and the plasma. A great array of such antimicrobial factors was identified in vertebrates (Skarnes and Watson, 1957; Elberg, 1960; Hirsch and Cohn, 1960; Landy, 1960; Coombs, Coombs, and Ingram, 1961; Mackaness, 1962; Miles, 1962), and some counterparts were recognized in invertebrates. McDade and Tripp (1967), for example, reported lysozymes in oyster hemolymph.

In the vertebrates, specific and nonspecific serum proteins increase the speed and effectiveness of phagocytosis—the opsonizing effect (Wright and Douglas, 1903; Suter, 1956; Rowley, 1960). Sensitization of bacteria with serum factors is not always a necessary prelude to phagocytosis, however, as was pointed out by Wood, Smith, and Watson (1946) and Wood (1953). In the absence of other host responses, early phagocyte activity may be important in preventing infection.

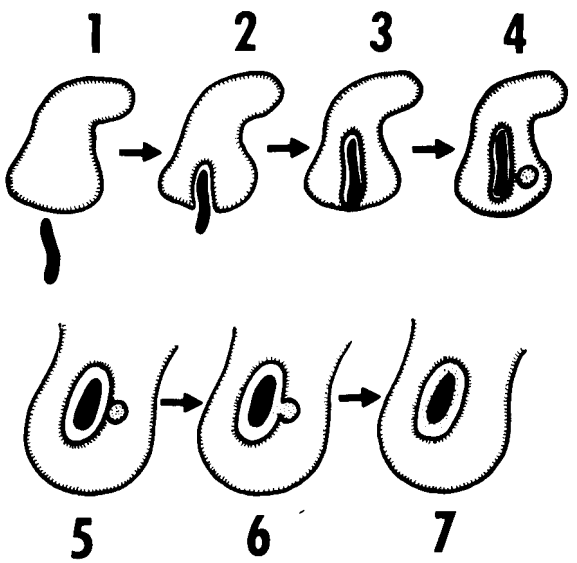

FIGURE 2.—Role of cell membrane and lysosome breakdown in phagocytosis. 1-4; phagocytosis; 5-7: lysosome activities. (Redrawn from Hirsch, 1965.)

Phagocytosis, then, constitutes the keystone to resistance. As Aarum (1967) mentioned, "...the organism's ability to oppose infection precisely follows the phagocytes' ability to function optimally. Resistance is lowered by a lowering of phagocytic activity." Phagocytosis can occur at the site of a lesion, in the filtering tissues and organs of the circulatory system, and (to a lesser extent) in the body fluid itself. Groups of fixed phagocytic cells are present in many crustaceans, most commonly in the sinuses and lacunae of the gills and at the bases of the legs.

Phagocytes agglutinate, aggregate, and cooperate in defense—forming nodules (the "nodules leucocytaires" of Cuénot, 1898), which are also known in annelids, mollusks, echinoderms, and other invertebrates. In a number of animals the nodules are brown, due to presence of large numbers of brown granules in the phagocytes, which may be excretion products or decomposition products. In *Gammarus*, the phagocytes composing the nodules secrete a clear yellowish chitinoid substance around the parasites. This secretion gradually becomes dark brown. The nodules appear as conspicuous black spots in infected individuals (Pixell-Goodrich, 1928) and are found frequently in gills and appendages. It should be clearly understood, however, that there are few detailed modern studies of phagocytosis in crustaceans or other invertebrates. Data from in vitro studies are particularly scarce, so there should be no implication that the kinetics, energetics, or other aspects of phagocytosis are fully understood.

Hemocytes of crustaceans and other invertebrates also act in other ways to protect the individual from overwhelming microbial invasion. Hemocytosis and hemocytic infiltration have been described in a number of invertebrate groups. Manifestations in invertebrates and vertebrates involve proliferation of hemocytes, changes in permeability of blood vessels, leakage

of blood fluids into tissues, adherence of hemocytes to blood vessel walls, and migration of hemocytes into tissues around areas of injury or parasitic invasion.

The involvement of hemocytes in coagulation or clot formation is a complex one, inasmuch as either cellular or extracellular clots may be formed. Intravascular cellular clots adhere to walls of blood vessels and spaces, producing stasis, and once they are formed, persist for some time. Extracellular clots, resulting from release of constituents of hemocytes, can inhibit microbial motion and thus render microorganisms more vulnerable to phagocytosis. Since Fredricq (1879) first pointed out that in Crustacea coagulation of hemolymph involves cell agglutination as well as plasma coagulation, others have demonstrated similar characteristics in a number of invertebrate groups. The release of a component from hemocytes and the role of this component in initiating coagulation of plasma were reported by a number of authors beginning with Halliburton (1885). Löwit (1889) observed the rapid disruption of hemocytes and the rapid clotting characteristic of most Crustacea and concluded that a causal relation existed. Hardy (1892), Tait and Gunn (1918), Tyler and Scheer (1945), and George and Nichols (1948) all provided data which supported the conclusion that a component from certain hemocytes acts with fibrinogen of plasma to form fibrin clots.

Bang (1967c, 1968) demonstrated that in the hermit crab, *Eupagurus longicarpus*, clots formed in at least two stages following injury—first a clumping and stickiness of hemocytes without change in shape or loss of granulation, then retraction of the clot and the development of a network of fibrous cell projections containing microtubules.

The abundant and elaborate literature on hemolymph coagulation in Crustacea was admirably summarized and evaluated by Florkin (1960). As he pointed out, coagulation has been considered by some authors to occur in two distinct phases—cellular coagulation and then plasma gelation—while other workers view coagulation as a continuous process in which plasma gelation begins around hemocytes. It was Florkin's conclusion that plasmatic coagulation was a one-step process in which fibrinogen of the plasma was acted upon by a coagulin released by the hemocytes. It seems equally possible, however, that more than one type of coagulable protein exists and that the categories of clots may be complex rather than simple. Florkin also reviewed the role of cellular clots in wound repair, emphasizing the importance of secretion of a chitin film over the wound area by underlying coagulated phagocytes.

Encapsulation is also a common form of cellular internal protection in invertebrates. Invading organisms, often relatively large, are surrounded by phagocytes and fibrocytes. The onset of encapsulation may be rapid, and the cellular aggregates may be resolved only very slowly.

In summary, the hemocytes function in a number of ways beyond phagocytosis, although the latter must be considered the dominant cellular defense mechanism:

1. Hemocytes are important in cellular infiltration of injured or diseased tissue.
2. They are important in clotting—either as participants in cellular clots, or by release of secretions or injury products which combine with plasma components to form extracellular clots.
3. The hemocytes are of primary importance to encapsulation.

A great variety of crustacean hemocytes have been described during the past several decades. Animals studied included crayfishes (George and Nichols, 1948; Toney, 1958; Wood and Visentin, 1967), blue crabs (George and Nichols, 1948; Toney, 1958), lobsters (Toney, 1958; Hearing and Vernick, 1967), and brine shrimp (Lochhead and Lochhead, 1941). Except for size differences, the principal distinction seemed to be presence or absence of granules in the cytoplasm. The hyaline hemocytes are usually smaller than the granular, and some of the hyaline cells probably develop into the granular types, since the intergrades have been noted (Cuénot, 1895). As was aptly pointed out by Rabin (personal communication), "The developmental relationships of one form of hemocyte to another which have been made amount to little more than edu-

cated guesses, since they have been based largely on static images which may not even represent the true cell pictures as they occur *in vivo*." Both types of cells (hyaline and granular) probably have physiological subtypes, as suggested by the work of Fisher-Piette (1931) in which explants of lobster hemopoetic tissues resulted in multiplication of two types of hyaline cells—adhesive ameboid and non-adhesive non-ameboid. Inclusions of granular cells, in addition to their defensive function mentioned earlier, may also provide nutrient material—as suggested by release of this material into the hemolymph during ovarian development (Lochhead and Lochhead, 1941). Hyaline hemocytes may also be transformed into connective tissue or endothelial cells of blood vessels (Danini, 1925, 1927; Debaisieux, 1952a, 1952b; Demal, 1953). Hemocyte physiology and biochemistry are areas where additional studies are needed, but the extreme fragility of certain cells once they are removed from the normal animal has undoubtedly been a major deterrent.

Phagocytic activity has been ascribed in varying degrees to most recognized categories of hemocytes (Haeckel, 1862; Hardy, 1892; Cuénot, 1895; Bruntz, 1905, 1907; Kollman, 1908; Tait and Gunn, 1918; George and Nichols, 1948; Toney, 1958; Rabin, 1970b).

In recapitulation, the phagocytes of vertebrate and invertebrate animals have been investigated widely since the late 19th century, and the blood cells of Crustacea have received at least proportionate study. Cellular defenses of Crustacea and other invertebrates are varied but center on the phagocyte and its activities. Important also are the humoral defenses, which will be considered in the following sections. Before proceeding to considerations of other than cellular defenses, however, it seems relevant to include an often overlooked perimeter of defense suggested by Miles (1962). Early suppression of microbial numbers may be due to microbicidal activity of the tissue cells themselves, either innate or induced, or to soluble antimicrobial substances in the intercellular fluid of the integument. As Miles pointed out, such pre-inflammatory cellular defenses in no way diminish the importance of phagocytes and humoral factors, but only provide an added perimeter of defense. Antimicrobial capacities of tissues as a whole, and of nonphagocytic cells in particular, may be a mainstay of nonspecific resistance—in both primary invasion and the determination of subsequent courses of infection. Tissue defenses of this nature in the invertebrates may be of great significance.

HUMORAL DEFENSE SYSTEMS

Early studies of humoral factors in Crustacea produced significant, but at times ambiguous, results. Noguchi (1903) found that sera of lobsters and horseshoe crabs possessed natural agglutinins against various vertebrate erythrocytes. After repeated injections, he was able to demonstrate an induced hemolysin in the horseshoe crab but not in the lobster. Fredericq (1910), using a variety of antigens, was unable to demonstrate precipitins in a number of decapods (*Homarus vulgaris, Palinurus vulgaris, Carcinus maenas, Portunus puber, Cancer pagurus*, and *Astacus fluviatilis*).

The early literature on humoral mechanisms of internal defense in invertebrates, and especially the crustaceans, was clearly dominated by Cantacuzène and his students. Cantacuzène, in a period covering almost 3 decades beginning in 1912, examined the broad picture of humoral defences of a large number of marine invertebrates. His work with Crustacea will be summarized in the next few pages as background for a consideration of subsequent studies.

Cantacuzène (1912a) reported (in a paper consisting essentially of a series of statements but little supporting data) the presence of natural agglutinins, lysins, and precipitins in serum of the hermit crab, *Eupagurus prideauxii*. Hemolysins for sheep and rabbit erythrocytes occurred in crab serum to a maximum titer of 250 and were destroyed by heat at 55° C. Agglutinins for rabbit erythrocytes persisted in dilutions beyond those at which hemolysis disappeared. Agglutinins for bacteria (*Escherichia coli* and *Vibrio cholerae*) were also present, as were weak precipitins against horse and rabbit sera. Cantacuzène also stated that comparable agglutinins, lysins, and precipitins were not present in *Pagu-*

rus striatus, which is closely related to *Eupagurus prideauxii*.

Cantacuzène (1923b), summarizing a decade of study of humoral defenses in invertebrates, found that the serum of the spider crab, *Maia squinado*, possessed natural agglutinins for mammalian red blood cells, with great individual variation from crab to crab. He observed, rather significantly, that agglutinins weakened or disappeared completely in crabs held in captivity for long periods. He noted, on injection of erythrocytes, that agglutinins disappeared completely during the first days after inoculation and did not return to their original titer for several weeks after the last dose. Cantacuzène also reported that rare individuals of *Maia*—invariably moulting females—possessed a lysin for mammalian red blood cells. *Maia* serum also possessed a strong lytic factor, but no agglutinins, against cholera vibrios.

Cantacuzène inoculated *Maia* with coelomic fluid of *Sipunculus nudus* (4 to 5 injections at intervals of 3 to 5 days) and found that the crabs produced first agglutinins and then lysins against the various injected sipunculid coelomic cells, including ova. The nature, intensity, and duration of response varied greatly among individuals. The lytic ability seemed more pronounced in females than in males, and more so in females approaching sexual maturity. Hemolysins against mammalian erythrocytes were also produced.

When he compared the lytic ability of *Maia* serum after injection with sipunculid fluid and mammalian erythrocytes, Cantacuzène found that response was more rapid and stronger with the former, and he concluded—on the basis of these and other studies—that mammalian red cells were only mediocre antigens for marine invertebrates. He attributed this weak antigenicity to coating of the injected mammalian erythrocytes in *Maia* and other invertebrates with a serum factor that interfered with subsequent reactions. Cantacuzène aptly referred to this as "mummification" of the red cells by invertebrate body fluids.

Concerning acquired immunity to bacterial infections in Crustacea, Cantacuzène (1923b) found that the crab *Maia squinado*, when inoculated with killed *Vibrio cholerae* or small doses of live vibrio, was able—12 days after the fifth injection—to survive the challenge with 20 times the dose of vibrio which had proved fatal to uninoculated control crabs.

Cantacuzène also reported on studies of the responses of *Maia squinado* to injections of gram-positive bacteria isolated from the crab's digestive tract. Inoculation was followed by reduction in numbers of amebocytes and, within 24 hr, by disappearance of most of the bacteria from the hemolymph. The bacteria were immobilized in the various phagocytic tissues, particularly in the branchial lacunae—at first they adhered to the cell surfaces, then amassed into small granules, and were finally engulfed by fixed and mobile phagocytes. The process of digestion of bacteria was slow, and still incomplete after 7 weeks. Clotting ability of the hemolymph decreased immediately after inoculation but returned to normal in 8 days. No agglutinins for the bacteria could be demonstrated in vitro, but the natural agglutinins for mammalian red blood cells (discussed earlier) disappeared. In vitro studies disclosed that the hemagglutinin coated the bacteria but did not cause their agglutination. The adherence in vivo of the bacteria to fixed phagocytic cells undoubtedly was enhanced by the sensitization. Some evidence for this was gained by adding macerated hypodermal or pericardial cells to a mixture of bacteria and crab serum in vitro. The cell fragments acted as centers for bacterial agglutination and immobilization, but the agglutinating ability was not conferred on the serum by the addition of cell fragments.

A different sequence of events was described for those crabs (*Maia*) in which the experimental infections progressed to death. Early immobilization of bacteria in lacunar cells was followed, in 8 to 15 days, by the appearance of encapsulated forms, invulnerable to destruction by phagocytes. The encapsulated bacteria multiplied, phagocyte numbers were reduced, and the clotting ability of the hemolymph diminished. By 10 to 20 days after inoculation, the hemolymph became incoagulable, the natural agglutinin for red cells disappeared, the connective tissue became gelatinous, and the animal died.

Cantacuzène (1923b) also examined the internal defenses of the hermit crab, *Eupagurus prideauxii*, which he had reported earlier to possess strong hemolysins and strong antibacterial agglutinins. Injected gram-negative bacilli were entrapped and immobilized on the cell surfaces of the branchial lacunae, and then phagocytized, as was the case with *Maia* described previously.

Cantacuzène (1923b) also reported that the sera of crabs, *Carcinus maenas*, infected by the rhizocephalan *Sacculina*, contained a factor absent from normal crabs. Using a standard complement fixation test, with extract of the rhizocephalan as antigen and with crab serum, Cantacuzène (1925b) was able to demonstrate an antibody-like response in parasitized crabs. Sheep cells were lysed in tubes with normal crab serum but not in those containing parasitized crab serum. Using a fine suspension of *Sacculina*, Cantacuzène found precipitating and agglutinating activity in the serum of parasitized crabs. The activity was not consistent, however, in that some sacculinized crabs lacked it. In a concurrent study, Lévy (1923) found that macerated sacculinids were toxic when injected into crabs, but that no antitoxic activity could be demonstrated in parasitized crabs. Both groups, normal and parasitized, died at about the same rate.

In an earlier study Cantacuzène (1913) reported that inoculation of the sacculinid parasite with gram-negative bacteria resulted in septicemia and death of the parasite within 1 week, and infection of the crab host by 10 days after inoculation. At about 5 days after inoculation, the crab hemolymph became incoagulable, and agglutinins appeared against the bacteria inoculated into the parasite. The antibacterial agglutinins were not present in sacculinized uninoculated control crabs.

Appreciable evidence for some degree of specificity of the natural agglutinins of Crustacea was accumulated by Cantacuzène. An agglutinin in *Maia* against mammalian red cells could be absorbed from crab serum by certain gram-positive bacteria but did not agglutinate them, nor did it agglutinate cholera vibrios. It did, however, strongly agglutinate typhoid bacilli. Agglutinins against vertebrate erythrocytes and certain bacteria were found in sera of *Homarus vulgaris*, *Eupagurus prideauxii*, and *E. bernhardus*, but the same antigens were not agglutinated by sera of *Cancer pagurus*, *Carcinus maenas*, *Portunus puber*, or *Galathea punctata*. The serum of *Eupagurus prideauxii* agglutinated mammalian red blood cells and amebocytes of *Maia* and *Buccinum*, but did not agglutinate the coelomic cells of sipunculids or ascidians.

And so Cantacuzène set the scene, by the early 1930's, for the continuation of broad and elaborate studies of humoral internal defenses of marine invertebrates—particularly the Crustacea—but the stage, with only a few notable exceptions, remained curiously empty and dark until the mid-1950's. Although research during the last decade emphasized species other than those studied by Cantacuzène (except for the work of F. Bang), it reinforced many of Cantacuzène's findings: that natural agglutinins and lysins, with some specificity, occur in Crustacea and other invertebrates, and that responses to foreign antigens can be induced in selected invertebrates—responses which are only partially specific. The increased precision and quantitation of tests, and the careful attention to controls, have improved the quality of the newer data, but have neither provided new concepts nor modified the general conclusions of Cantacuzène. The more recent literature on humoral defenses of Crustacea will be summarized by general categories in the following sections.

Bactericidal Systems

Natural bactericidins have been reported from a number of marine invertebrates (Bang, 1967b). Recently, increase in titers of bactericidal activity after inoculation with Formalin-killed bacteria was noted in West Indian spiny lobsters, *Panulirus argus*, and American lobsters, *Homarus americanus* (Evans et al., 1968; Acton, Weinheimer, and Evans, 1969). A bactericidal assay system described by Schwab and Reeves (1966) was used to quantitate the degree of response. In experiments with American lobsters held in seawater at 5° C, the peak of

bactericidal response against gram-negative bacilli was reached within 48 hr after inoculation, and high titers persisted throughout the observation period (11 days). In spiny lobsters, presumably maintained at significantly higher environmental temperatures at Bimini, the Bahamas (reported as 26°-28° C in a later paper), the primary bactericidal response to intracardial injection of living or killed suspensions of the same gram-negative bacilli (originally isolated from the digestive tract of spiny lobsters) reached a peak at about 36 to 48 hr after injection, then declined slowly for the following 2 weeks. Partial lack of specificity of the bactericidin was indicated by its appearance following injection of gram-positive bacilli and by its activity against *Salmonella typhosa* and *Escherichia coli*, as well as against the unidentified gram-negative bacillus used as the homologous test organism. The bactericidin was not active, however, against *Pseudomonas aeruginosa* or against three species of gram-positive bacteria.

A subsequent study (Weinheimer, Acton, Sawyer, and Evans, 1969) of the specificity of the spiny lobster bactericidin further indicated that the response was partially nonspecific. Low titers of the bactericidin against gram-negative bacilli could be demonstrated after injections of Formalin-killed type 2 pneumococci and bovine serum albumin. Formalin produced a pronounced adjuvant effect.

Secondary responses—those following reinjection of the same antigen after a lapse of time—of spiny lobsters to killed suspensions of gram-negative bacilli were also examined by Evans, Cushing, Sawyer, Weinheimer, Acton, and McNeely (1969). Titers of bactericidin were slightly but significantly higher after reinoculation than after primary inoculation, and the rate of secondary response (the number of hours to reach peak titer) seemed somewhat accelerated when compared with the primary response. Unfortunately, the number of animals tested was small. It seems important that similar observations be greatly extended—since, as the authors pointed out, the results were reminiscent of the specific anamnesis or immunological memory demonstrable in the immunoglobulin responses of vertebrates.

The spiny lobster bactericidin was apparently a large molecule, as suggested by resistance to dialysis and by Sephadex separations. Inactivation occurred at 65° C and activity was not restored by addition of unheated normal hemolymph. Activity was not reduced by treatment with EDTA or carageenin. These results indicate dissimilarity with vertebrate complement-based bactericidal systems, but the authors suggested that the bactericidin may represent a primordial immunoglobulin.

Noteworthy is that all the American lobsters and a number of the West Indian spiny lobsters used in the studies by Evans, Weinheimer, Painter, Acton, and Evans (1969) had demonstrable pre-existing titers of bactericidins against the gram-negative bacillus used in the experiments. Possibly the remainder of the spiny lobsters used in the studies could have had titers of bactericidins lower than were demonstrable by the methods used. Thus inoculation may not have "induced" the bactericidin but instead may have merely enhanced or increased the titers. A number of explanations were offered for the pre-existing titers of bactericidal activity, including trauma because of handling, and response to previous bacterial infection. Two relevant observations are that in many American lobsters, a rapid increase in bactericidal titer preceded death, and in studies of spiny lobsters, the bactericidal activity was found to be partially nonspecific, in that activity against other gram-negative bacteria was enhanced.

The conclusions reached by the research group that examined the spiny lobster bactericidin (Weinheimer, Acton, Sawyer, and Evans, 1969) are in accord with findings for other phyla: "These data suggest that, although invertebrates appear capable of antigenic recognition, the molecules synthesized may have broad specificity covering a wide range of antigenic determinants. Further studies will be necessary to ascertain whether these inducible substances represent primitive immunoglobulins. In any event, it would be surprising if they did not have a major role in defense of the animal against pathogenic microbes."

An important qualification was pointed out by Aarum (1967) regarding results obtained by ex-

perimental inoculation of large numbers of bacteria. Infectious agents are found in large numbers in the circulatory system only in septicemia, which occurs only after a growth phase of a virulent pathogen within the host, often within fixed or immobilized phagocytic cells. Rapid increase in pathogen numbers in the circulating body fluid is usually a precursor to death and indicates a failure of host defenses. Thus the artificial introduction of large numbers of microorganisms should not be expected to elicit normal responses in an animal. It should also be noted that insusceptibility barriers to infection, operative in the normal animal, may be bypassed by experimental inoculation, resulting in massive infection and death from microorganisms not otherwise known as pathogens. A further qualification mentioned by Aarum is that even the simplest experimental manipulation may change phagocytic abilities, so that the normal phagocytic response of an animal to pathogens may be far different from responses elicited experimentally.

Lytic Systems

A natural hemolysin for sheep erythrocytes in the hemolymph of West Indian spiny lobsters was reported by Weinheimer, Evans, Stroud, Acton, and Painter (1969). Low temperatures (0° and 4° C) inhibited lysis, which was best demonstrated at 25° and 37° C. Heating the lobster hemolymph to 52° C destroyed lytic activity. The hemolysin could be adsorbed on red cells or cell stroma at low temperatures. Red cells with the adsorbed lysin were lysed when the temperature was raised to 37° C, which indicated a possible enzymatic type of activity of this hemolytic system. The authors suggested a multiple step system, analogous to mammalian hemolytic systems, consisting of a single protein species which is first adsorbed on the surface of the sheep erythrocyte, and then—in one or more steps—lyses the cell.

A detailed study of the presence or absence of natural immunity to invasion of the hemolymph of crabs by a parasitic ciliate, *Anophrys sarcophaga*, was reported by Poisson (1930). The ciliate, which in shore crabs, *Carcinus maenas*, multiplies and causes death, was immobilized, agglutinated, and lysed in vitro by serum of several other crabs—particularly *Maia squinado*, *Eupagurus prideauxii*, and *Portunus puber*. Experimental inoculations of ciliates were cleared usually within several hours. It is interesting that the parasite, when experimentally introduced in another member of the Portunidae, *Portunus depurator*, multiplied and killed the experimental hosts just as it did in *Carcinus maenas*. Poisson attributed the reactions of crabs to the ciliate to expressions of a natural immunity, effected by agglutinating and lytic activity of the hemolymph.

Other lytic systems of Crustacea have received passing attention. Cantacuzène (1913) found that hemolymph of the hermit crab, *Eupagurus prideauxii*, possessed a heat-labile hemolysin, as well as precipitating and agglutinating activities. Cantacuzène (1921, 1923b) stated that injection of the spider crab, *Maia squinado*, with sheep erythrocytes produced hemolysins as well as agglutinins.

Agglutinating Systems

Natural hemagglutinins of the Australian freshwater crayfish, *Parachaeraps bicarinatus*, were examined by McKay, Jenkins, and Rowley (1969). Absorptions of hemolymph by erythrocytes of four mammalian and one avian species disclosed specificity, in that absorption by cells of one species still left agglutinins for cells of other species. The crayfish hemagglutinins were nondialysable and were inactivated at 57° C. In vitro studies with crayfish phagocytes and mouse erythrocytes disclosed that the crayfish hemagglutinins greatly enhanced the adhesion of the erythrocytes to the phagocytes—a specific opsonic effect. A similar effect was observed in vivo, apparently as a prelude to phagocytosis.

The recent studies support earlier reports of specific hemagglutinins in a number of invertebrate phyla (Tyler and Metz, 1945; Tyler, 1946; Sindermann and Mairs, 1959; Cushing, Calaprice, and Trump, 1963; Tripp, 1966) with specificities somewhat comparable to those of vertebrate natural isohemagglutinins and with some biological properties (such as enhancement of

adhesion and phagocytosis of erythrocytes by phagocytes) similar to those of vertebrate antibodies.

A study of natural agglutinins in the serum of California spiny lobsters, *Panulirus interruptus*, for blood and sperm cells of 54 species (representing 7 phyla) was published in 1945 by Tyler and Metz, and is still cited as one of the most comprehensive examinations of its kind for a marine animal. Agglutination was not found in 37 other species tested. Titers for positive reactions ranged from 8 to 256. The most interesting phase of the study was an extensive series of absorptions of spiny lobster serum by cells of many of the species tested. In each case, absorption of serum with cells of any single species removed agglutinins for all the species tested that belonged to the same group (Class) but left agglutinins for the cells of all other groups tested. Tyler and Metz concluded, on the basis of absorptions, that at least 10 class-specific agglutinins were present in the serum of the spiny lobster. A few cross-reactions occurred, and some reduction in titers resulted from absorptions with cells of other species, which suggested the presence of a number of reacting sites on the cells.

Bang (1967b) examined the responses of the spider crab, *Maia squinado*, to injections of *Anophrys*, a large ciliate pathogenic for shore crabs, *Carcinus maenas*, as part of a laudable attempt to repeat with modern methods some of the early French studies of invertebrate defenses. Sera of some spider crabs strongly agglutinated the ciliates, but that of others did not. Agglutination resulted from formation of a mucoid substance around the tail cilia. Crabs that lacked the agglutinin died from overwhelming infections of the hemolymph, whereas those with the agglutinin survived (Bang, 1962). When present, the agglutinin was apparently fairly constant, except that in some crabs it was lost spontaneously but temporarily at time of molting. Poisson (1930) had noted earlier that the hemolymph of *Maia* lysed the ciliates and that the hemolymph of the hermit crab, *Eupagurus prideauxii*, agglutinated them.

An agglutinin in the hemolymph of the hermit crab, *Paguristes ulreyi*, for human type O cells, and to a lesser extent for types A and B, was reported by Cushing (1967). A number of individuals lacker the hemagglutinin, and some of these possessed a serum factor which inhibited the action in vitro of the positive sera. The analogy to specific soluble substances found in sera of certain vertebrates was pointed out by Cushing, with the suggestion that further studies of this kind with other invertebrates might prove instructive. Cohen (1968) found agglutinins for human and other vertebrate erythrocytes in sera from coconut crabs, *Birgus latro*. Young crabs lacked the agglutinins.

The serum of the American lobster contains strong and specific natural agglutinins for an antigen present on the red blood cells of sea herring, *Clupea harengus* (Sindermann and Mairs, 1959). Detection of blood groups in this fish was aided by the use of the hemagglutinin—individual herring either had the antigen or lacked it. Titers reached as high as 256, and reactions paralleled those obtained with absorbed rabbit antisera and plant lectins (Sindermann, 1963). Erythrocytes of other clupeoid fishes tested were also strongly agglutinated by lobster sera (Sindermann, 1962).

Precipitating Systems

Production of precipitins by invertebrates has been reported only rarely. Osawa and Yabuubhi (1963), in a very brief paper, found that the "Homard americain, *Cambarus clarkii*" [probably *Homarus americanus*] did not produce agglutinins or lysins when injected with red blood cells but did produce weak precipitins (detectible by immunoelectrophoresis) after injection with serum from rabbits and goats. No information was given about dosage, injection schedules, time for response, or titers.

Stewart and Foley (1969) suggested that a "precipitin-like principle already present in the hemolymph" of the American lobster might be important in removal of foreign protein. Fluorescein-labelled bovine serum albumin (BSA) and lobster serum proteins were injected into lobsters held at 5° C, and the fluorescence level checked periodically for 6 days. With lobster serum proteins, an initial decline in fluorescence

within 2 hr after injection was followed by a plateau that remained constant for the duration of the experiment; with labelled BSA, however, the rate of clearance was concentration dependent, but the foreign protein declined to very low levels by 3 days after injection. Fluorescent material was apparently excreted in proportion to its disappearance from lobster hemolymph. Pinocytosis by phagocytes was not demonstrated, but tiny granular fluorescent accretions were observed in the hemolymph of lobsters injected with labelled BSA, beginning about 8 hr after injection. In vitro studies, in which a standard ring test with labelled or unlabelled BSA and lobster serum was used, disclosed a clearly discernible ring after 16 hr at room temperature, and a precipitate at the bottom of the tube after 36 hr. Precipitin titers of individual lobster sera ranged from 2 to 16 and were independent of the total protein concentration of the lobster serum. Dialysis of lobster serum did not change the titers, but precipitin activity was destroyed by heating above 50° C.

A number of interesting implications in the study were pointed out by Stewart and Foley. The clearance mechanism seemed able to distinguish between foreign and native proteins, and the capacity for clearance seemed high, as indicated by accelerated clearance of larger doses of BSA. Attempts to increase the levels of precipitin by previous injection of lobsters with BSA did not succeed, and in fact resulted in decreased precipitin levels in some individuals. The authors suggested that the hemolymph factor responsible for clearance of foreign protein may be maintained normally at low levels and may be supplemented by further secretions when required. They also suggested that the precipitin principle in the hemolymph may be the first of several steps or possibly the primary removal factor and that digestion and excretion may take place elsewhere—probably in the hepatopancreas.

The results of the studies of Stewart and Foley are in agreement with those of Teague and Friou (1964), who observed that injected foreign protein was rapidly removed from the hemolymph of the crayfish *Cambarus virilis*. Previous injection of the protein did not increase the clearance rate. Teague and Friou did not observe precipitin activity against injected bovine and human serum albumins but concluded that clearance resulted from nonspecific degradation of the foreign protein.

Other evidence of clearance of injected proteins but failure to induce heightened responsiveness in Crustacea was reported by Campbell and Garvey (1961). They mentioned that "It is also of interest that we have made many attempts to induce antibody formation in invertebrates, e.g., lobsters. We have been unsuccessful so far, but in every instance the antigens remained undigested and unchanged in the circulation and tissues for many months." Although not mentioned specifically, the lobsters were probably California spiny lobsters and the test antigens probably included BSA, since this was the principal antigen used in other studies reported in the same paper.

Phage Clearance

Taylor, Taylor, and Collard (1964) and Nelstrup, Taylor, and Collard (1968) presented some evidence (from two crabs) of an increase in the rate of secondary clearance of injected T_1 bacteriophage in the shore crab, *Carcinus maenus*. Clearance was not complete until after 2 weeks at 16° to 18° C, and no neutralizing antibody to T_1 phage was detected in the hemolymph. Primary inoculation with T_0 phage did not increase clearance rates for T_1 secondary injections. The small number of animals used in these experiments makes the conclusions highly tentative. The authors suggested the existence of a "phylogenetically more primitive type of immune response than the production of humoral antibody," but did not state clearly what the response was—except possibly that it was "an apparently purely cellular secondary response."

Studies of phage clearance by Cushing and McNeely (reported in Cushing, 1967) led to negative conclusions. Phage T_4 persisted for up to 168 days in the California spiny lobster and disappeared at a steady rate, uninfluenced by the size of the original inoculum. Two species of crabs tested also failed to clear bacteriophage.

Other negative findings for increased rate of phage clearance following inoculation in crayfish were reported by Teague and Friou (1964).

Antitoxic Activity

Little definitive information is available about antitoxic activity in invertebrates. As Huff (1940) pointed out, "Experimental demonstration of antitoxic action in invertebrates has failed for the most part because of lack of susceptibility of invertebrate cells for known toxins." Probably the best example of antitoxic phenomena in Crustacea was described by Cantacuzène (1925a) and Cantacuzène and Damboviceanu (1934a, 1934b). The hermit crab, *Eupagurus prideauxii*, exhibited resistance to nematocyst toxin of *Adamsia palliata*, a commensal coelenterate commonly found on the shell of the crab. When injected, the toxin had no effect on *E. prideauxii*, but it was lethal to many other Crustacea and to a number of other invertebrates tested, including the closely related hermit crab, *E. bernhardus*. Cantacuzène also found that serum of *E. prideauxii* could neutralize the coelenterate toxin when the two —serum and toxin—were mixed and injected into crab species susceptible to the toxin. The development of this antitoxic principle can be seen as a logical and necessary concomitant of the very close relationship of crab and anemone, but the question of whether this is an example of innate or acquired resistance has not been resolved.

Another example of coelenterate toxin lethal to crabs was reported by Lane, Coursen, and Hines (1961). Biologically active peptides in *Physalia* nematocyst toxins were tested, using fiddler crabs, *Uca pugilator*, as assay animals.

Except for the work with coelenterate toxins, evidence of antitoxins in invertebrates is weak. Stauber (1961) reported almost immediate removal of diphtheria toxoid from oyster blood, but Metchnikoff (1905) and Bengston (1924) found that tetanus and botulinus toxins remained in insect body fluids for several weeks without loss of toxicity. These studies must, of course, be viewed as most indecisive, since substances toxic to humans are not necessarily so to invertebrates. Reaction on the part of invertebrates could be identical to reaction against any other introduced foreign material.

Invertebrate responses to gram-negative bacterial endotoxins were the subject of a review by Levin (1967). The most striking activity of such endotoxins is the production, after experimental inoculation, of intravascular clots and the ensuing death of various crustaceans and other invertebrates. Antitoxic immunity has not been demonstrated, but, as Levin stated: "Endotoxin appears capable of activating complementary defense mechanisms in invertebrates, including aggregation of amoebocytes, coagulation, bacterial immobilization, and phagocytosis. All these may be operative through one type of cell—the amoebocyte."

Other Protective Systems

McKay and Jenkin (1969) examined resistance of the Australian freshwater crayfish, *Parachaeraps bicarinatus*, to a pathogenic *Pseudomonas* sp. and concluded that the animal was capable of an adaptive immune response. Their findings indicated lower mortality rates (after bacterial challenge) in animals inoculated with heat- and alcohol-killed vaccines as well as with endotoxin. Inoculation with vaccines prepared from other gram-negative bacteria also increased the level of resistance to the *Pseudomonas* infection, but vaccines from gram-positive bacteria did not—indicating some degree of specificity. A positive correlation was found between survival of challenged animals and the number of exposures to bacterial antigen; after four inoculations, the LD_{50} of immunized animals was nearly 100 times that of controls. Temperature also played a significant role in the onset, degree, and duration of protection induced by inoculation of animals with killed bacteria. At 26° C, onset of protection was rapid (1 day), reached a peak at 3 days, and almost disappeared by 12 days; at 19° C, onset was slower (2 days), reached a maximum at 4 days, and persisted for 12 days (the duration of the experiment); at 14° C, no protection was afforded. Inoculation of gram-negative endotoxin resulted in protection similar in appearance and duration to that

produced by killed vaccines. Although the terms "immunity" and "resistance" were used, the precise nature of the protection afforded by inoculation of vaccines and endotoxin was not described by the authors. In vitro experiments with hemolymphs of control and resistant crayfish disclosed no bactericidal or bacteriostatic effects, and McKay and Jenkin suggested that the most important effect of immunization may have been to increase the metabolic rate of the phagocytes [thereby stimulating phagocytosis].

Barker and Bang (1966), extending the earlier studies of Cantacuzène (1925b) with the shore crab, *Carcinus maenas*, and its rhizocephalan parasite *Sacculina carcini*, reported that inoculations of *Vibrio* sp. caused the hemolymph of the parasite to become incoagulable within 24 hr. Masses of gelled material containing bacteria were seen within body spaces. Septicemia and death, first of the parasite, and then often of the crab host, followed soon after.

Insusceptibility factors seem operative when certain parasites of invertebrates fail to develop. Michajłow (1938) and Baer (1944), for instance, found that larval cestodes, *Triaenophorus* and *Ligula*, penetrated the intestinal wall of a number of copepods, but developed only in certain species. In others, the larvae died and were phagocytized. Hedrick (1935) observed similar differences in survival of larval nematodes. Léger and Duboscq (1908) reported earlier that sporogony of the sporozoan *Agregata eberthi* (which occurs in the intestinal wall of crabs of the genus *Portunus*) took place readily in all species except *P. puber*, in which the parasite was quickly phagocytized after invading the intestinal wall.

INTERNAL DEFENSE MECHANISMS INVOLVED IN GAFFKAEMIA OF LOBSTERS

The American lobster, *Homarus americanus*, has an effective internal defénse system, consisting of active phagocytosis as well as agglutinating and bactericidal (or bacteriostatic) activity, against a number of injected bacteria. The protective system seems to fail completely only when challenged by *Gaffkya homari*—which is thus far the only bacterial pathogen known to develop systemic infections in lobsters and to kill them. Probably the most extensive series of reports concerned with responses of invertebrates to a particular pathogen is that dealing with the lobster (and other decapods) and the highly pathogenic gram-positive micrococcus *G. homari*. "Gaffkaemia"—the disease caused by *G. homari*—is enzootic in both the American lobster, *Homarus americanus*, and the European lobster, *H. vulgaris*, and has been reported to cause epizootics in captive populations of both species (Roskam, 1957; Goggins and Hurst, 1960; Gibson, 1961; Stewart and Rabin, 1970). Microorganisms with characteristics of *G. homari* have been isolated from shrimp (*Penaeus aztecus* from the Gulf of Mexico) and from crabs (*Carcinus maenas* and *Libinia emarginata* from New England and *Cancer irroratus* from eastern Canada), but the disease "gaffkaemia" is known only in lobsters. Early descriptions of the disease and its etiological agent (Hitchner and Snieszko, 1947; Snieszko and Taylor, 1947) have been followed during the past decade by studies in several laboratories, which used the lobster and the pathogen as a test system to elucidate responses to infection and other aspects of the host-parasite relationship. The possible course of infection in lobsters is summarized in Table 1. Snieszko and Taylor (1947) first satisfied Koch's postulates for the pathogen and demonstrated high mortality following inoculation of cultured *G. homari*. Stewart and MacDonald (1962) and Stewart et al. (1966) found that 40 to 60% of lobsters they examined from certain locations on the Canadian east coast were infected.

Studies by Harvey Rabin at Woods Hole and The Johns Hopkins University (Rabin, 1965; Rabin and Hughes, 1968) confirmed that lobsters inoculated with *Gaffkya* became septicemic within 2 days and died a few days later. Inoculation of gram-negative endotoxin 10 hr before exposure to the pathogen did not alter the course of infection. Prior inoculation of heat-killed *Gaffkya* cultures (24 hr before challenge) produced no protection.

In vitro studies with lobster serum as a culture medium disclosed that *Gaffkya* growth was

TABLE 1.—A proposed hypothesis to explain the course of *Gaffkya homari* infections in lobsters at 15° C.

Day	Development of gaffkaemia in lobsters
0	Bacteria gain access to tissues of lobsters as a result of injury which destroys the integrity of exoskeleton (or possibly the gut epithelium). Lobster hemocytes phagocytize *G. homari*.
2	Phagocytized bacteria may multiply within hemocytes. Hemocytes containing engulfed bacteria lodge in capillary and lacunar areas (heart, hepatopancreas, gills) of the lobster.
4	Hemocytes may be disrupted, releasing *G. homari* in hemolymph. This may result in rapid decrease in hemocyte numbers and logarithmic increase in bacterial numbers.
6	Hemolymph stimulates multiplication of released bacteria. Hemocyte numbers seem to be gradually reduced by continued phagocytosis and disruption of phagocytes.
8-10	Clotting mechanism (release of coagulin from hemocytes) is affected—possibly by reduction of hemocyte numbers—and clotting time is greatly prolonged.
12-14	Lobsters die from depletion of nutrient stores and utilization of this material by *Gaffkya*. Injured gaffkaemic lobsters may bleed to death. (The possibility of exotoxin has not been entirely eliminated, but there is no present evidence to suggest its existence.)

stimulated, while growth of a *Vibrio* (nonpathogenic to lobsters) was usually inhibited. Serum from lobsters which had been inoculated 24 hr earlier with killed *Gaffkya* still stimulated growth of the pathogen in vitro.

Rabin and Hughes (1968) tested resistance to *Gaffkya* in a variety of studies with lobsters and other marine arthropods. Findings with spider crabs (*Libinia emarginata*), rock crabs (*Cancer borealis*), and horseshoe crabs (*Limulus polyphemus*) were that most of the test animals cleared inoculated *G. homari*. In vitro studies with hemolymph disclosed either no apparent effect or only slight inhibition of growth of the pathogen by sera of spider and horseshoe crabs, and a slight stimulation of growth by sera of rock crabs.

The possible role of exotoxin was tested in lobsters by Rabin and Hughes with inoculation of filtrates of *G. homari* cultures. The filtrate had no effect when it was injected into the abdomen, but injection into the major joint of the chela induced autotomy or abnormal movements in over 50% of the lobsters treated.

Evidence of resistance to gaffkaemia was noted by Rabin and Hughes in a single lobster, which had been infected naturally before it was brought to the laboratory. Twelve days after capture the lobster was free of the pathogen. The animal was inoculated twice with increasingly larger dosages of *G. homari* and cleared the bacteria within 6 days—but died on the 11th day following the second challenge. The reactions indicated a partial resistance and an ability in some individuals to recover from gaffkaemia. It is interesting that serum from this presumably resistant lobster was similar to that of other lobsters tested in that it did not inhibit growth of *G. homari* in vitro.

Rabin and Hughes stated that the presence of *Gaffkya* infections did not damage the clotting mechanism—an observation quite different from that of Goggins and Hurst (1960), who found that reduction in amebocytes and a much prolonged clotting time were distinctive features of the disease. Stewart et al (1969) and Stewart and Rabin (1970) clarified these seemingly disparate observations by reporting that "coagulin" is released to initiate clotting by rupture of hemocytes and that the "concentration of plasma proteins, including fibrinogen, does not appear to decline significantly in gaffkaemic lobsters." An earlier report by Rabin and Hughes (1968) stated that when extract of lobster muscle was used as a coagulin source, recalcified clotting times were the same in diseased and normal animals. When these facts are combined, it can be concluded that the abnormally and persistently low hemocyte content of the hemolymph results in prolonged clotting time and does not indicate any deficiency in plasma constituents other than coagulin (Figure 3).

Studies carried on by James Stewart and his associates at the Halifax (Nova Scotia) Laboratory of the Fisheries Research Board of Canada have extended the work of Rabin and have provided the greatest number of contributions to the literature about the effects of *Gaffkya* disease on lobsters. In accord with earlier studies, infections usually were fatal, although rare individuals infected with *Gaffkya*-like organisms did survive (Stewart et al., 1966).

Cornick and Stewart (1968a) provided considerable relevant information about the host-parasite relationships of *Gaffkya* and lobsters. Experimental infections by inoculation, in which dosages as low as approximately 5 bacteria per

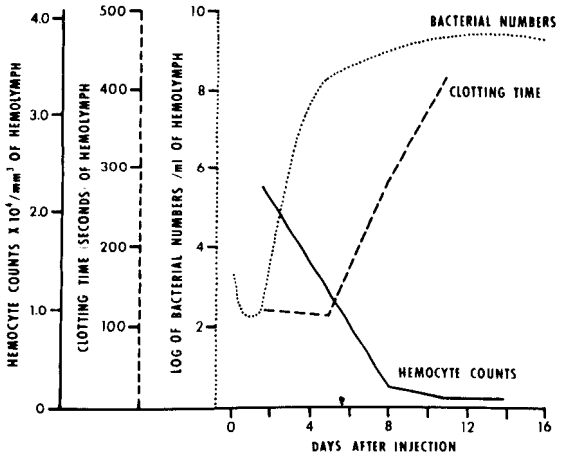

FIGURE 3.—Relations of hemocyte counts, clotting time, and bacterial numbers to time from experimental exposure of lobsters to *Gaffkya*. (Redrawn from figures in Stewart, Arie, Zwicker, and Dingle (1969) and Stewart and Rabin, 1970.)

lobster at 15° C were used, killed 90% of the test animals within 17 days. The absence of an effective host defense against *Gaffkya* was strongly indicated by the fact that the mean time to death was almost constant, regardless of dosage (Figure 4). Cornick and Stewart's studies disclosed additional facts that help to explain the pathogenicity of the bacterium to lobsters: *Gaffkya* resisted digestion in phagocytes and multiplied in the hemolymph; growth of *Gaffkya* was stimulated in vitro by serum of lobsters while growth of several other bacteria was inhibited; and *Gaffkya* was not agglutinated by lobster serum, though all other bacteria tested were agglutinated.

Presence in lobster hemolymph of effective defenses against bacteria other than *G. homari* was indicated by clearance within 30 days of inoculated suspensions of *Micrococcus conglomeratus*, *M. sedentarius*, *Achromobacter thalassius*, and *Gaffkya tetragena*. Since several of these bacteria are closely related to *G. homari* (which was not cleared), some specificity of the phagocytic or humoral protective mechanisms is strongly indicated.

Natural agglutinins in lobster serum were demonstrated against all bacteria tested (six genera), except for all strains of *G. homari*. Such agglutinins were of low titer, nondialysable, inactivated at 56° C, and seemed to be nonspecific (as suggested by the limited observation that a single absorption of serum by *Flavobacterium marinum* removed agglutinins for all other bacteria tested except *Brevibacterium* sp.).

Cornick and Stewart's observations on phagocytosis of *G. homari* are interesting and warrant further investigation. They found no phagocytized bacteria in hemolymph preparations 15 min after inoculation, but they did find fluorescent-dye-labelled bacteria in hemocytes in heart, liver, and gill tissues of experimental lobsters soon after inoculation. Circulating hemocyte numbers were reduced significantly within 15 min after bacterial inoculation but returned to normal levels after 5 hr. These data are in agreement with the statements of Maynard (1960), that phagocytes which have engulfed foreign material lodge in capillary and lacunar areas of the crustacean body, resulting in reduction in numbers of circulating hemocytes. Bang (1956) observed in tissues of *Limulus* injected with gram-negative bacteria a similar reduction in circulating hemocytes. In Cornick and Stewart's study long-term infections of lobsters were characterized by the presence of black nodules containing *G. homari* in tissue cells in the gills, swimmerettes, and ventral abdominal

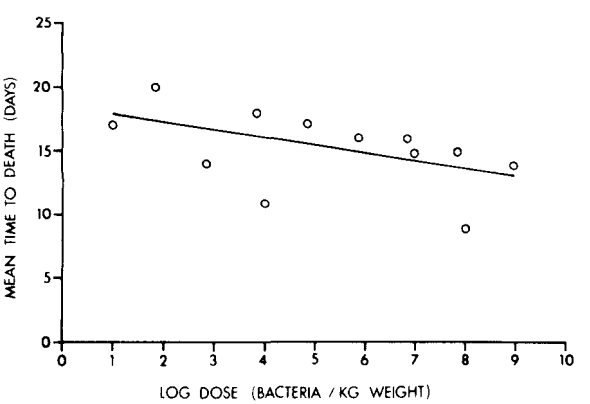

FIGURE 4.—Relation of dosage of *Gaffkya* to mean time to death (MTD) in lobsters (calculated line of best fit for mean time to death, using experimental groups of 10 lobsters each). (From Cornick and Stewart, 1968a.)

sinuses of the lobster. As Parry (1960) had pointed out earlier, this type of aggregation in gills is a common phenomenon in Crustacea. Poisson (1930) observed that, in the few crabs (*Carcinus maenas*) resistant to the parasitic ciliate *Anophrys sarcophaga*, masses of dead ciliates occurred in branchial lacunae, pericardial sinus, and hepatopancreatic sinuses. Degenerating ciliates eventually formed brownish cysts. Cantacuzène (1923b) observed a similar phenomenon in *Maia squinado* inoculated with bacteria. He pointed out that the lacunar tissue of the branchial lamellae of decapod crustaceans, with its many fixed phagocytes, acts as an extensive and effective bacterial filter.

Cornick and Stewart suggested that the development of polysaccharide capsules by *G. homari* in later stages of infection could be an important device that prevented destruction of the phagocytized bacteria and allowed multiplication of the pathogen. They pointed out, as evidence, that unencapsulated *G. homari* grown in culture were actively phagocytized. As Stewart and Rabin (1970) later reported, however, the unencapsulated cultured bacteria were also virulent. It may be that the capsule forms soon after the organisms are injected into the host. This observation of survival and growth of phogocytized encapsulated bacteria in lobsters is a direct counterpart of the inability of vertebrate phagocytes to destroy many encapsulated microorganisms, and parallels the earlier findings by Cantacuzène (1923b) of a fatal disease in crabs induced by encapsulated gram-positive bacteria.

Stewart, Dockrill, and Cornick (1969) examined certain insusceptibility factors affecting *Gaffkya* disease in lobsters. Destruction of the integrity of the integument seemed essential to transmission of the pathogen. Acidity of the gastric fluid was bactericidal and appeared to provide an effective barrier against oral infection. Previous attempts to infect lobsters by feeding infected material had been unsuccessful (Snieszko and Taylor, 1947; Wood, 1965a, 1965b; Rabin and Hughes, 1968).

Undoubtedly such insusceptibility factors are of definite importance to the epizootiology of gaffkaemia for several reasons: lobsters are cannibalistic; hemolymph of moribund gaffkaemic individuals contains about 10^9 organisms per ml (Stewart, Arie, Zwicker, and Dingle, 1969); and *Gaffkya* can be isolated consistently from lobster pounds, sea water, bottom mud, and slime of holding containers (Goggins and Hurst, 1960).

A thorough study of the effects of temperature on experimentally induced *Gaffkya* infections in American lobsters was reported by Stewart, Cornick, and Zwicker (1969). Mean time to death was inversely related to temperature (Figure 5). At 1° C no deaths attributable to experimental infections occurred; at 3° C mean time to death was 172 days; and at intermediate higher temperatures mean time to death decreased drastically to a minimum of 2 days at 20° C (which approaches the upper lethal tem-

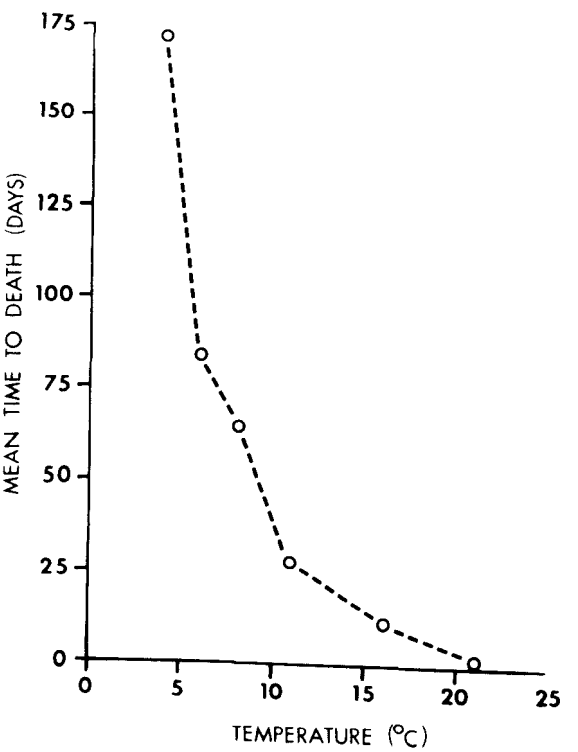

FIGURE 5.—Relation of temperature to mean time to death in lobsters experimentally exposed to *Gaffkya*. (From Stewart, Cornick, and Zwicker, 1969.)

perature for American lobsters). It is important to note (in view of the very low seasonal temperatures of waters in which lobsters live naturally) that at 1° C the pathogens persisted in the host in low numbers but with virulence unchanged, and they produced mortalities when the temperature increased. Experimentally infected lobsters were also sensitive to and died from rapid increases or decreases in environmental temperatures—although the temperature changes used in the experiments were probably greater than those that would normally be experienced in nature.

Findings in vivo were paralleled by in vitro results of growth of *Gaffkya* in lobster serum—with a more rapid increase to a peak of bacterial numbers with increasing temperature. The organism grew in culture at all the experimental temperatures (within a range of 1° to 20° C); at 1° C the bacterial growth curve was erratic—it decreased in numbers to the 30th day, then a log increase progressed to the 60th day, followed by a substantial decline. The important observation, of course, is that *G. homari* can survive within the range of environmental temperatures experienced by American lobsters and that the pathogen causes mortality more rapidly as temperature increases.

A concurrent physiological and biochemical study by Stewart, Arie, Zwicker, and Dingle (1969) and Stewart, Foley, and Ackman (1969), in which an attempt was made to define features of the infection that lead to death of lobsters, produced several interesting results. The pathogen lacked proteolytic, lipolytic, and fibrinolytic exoenzymes, suggesting that harmful effects are not caused by direct destruction of tissue. The authors observed that although in vitro growth of *G. homari* was limited by the carbohydrate level of the lobster serum medium used, presumably such a level would be maintained in vivo at the expense of other tissues. Drastic reductions in hepatopancreatic glycogen and hemolymph nonprotein nitrogen characterized later stages of the infection. No evidence of a toxin was detected, and the conclusion was that gaffkaemia is largely a wasting type of disease —that death from the disease was "a result of an unsuccessful competition on the part of the lobster for its own readily available storage material."

On the basis of experimental inoculations and subsequent mortalities, Bell and Hoskins (1966) suggested that *Gaffkya* might be pathogenic for the Dungeness crab, *Cancer magister*, and the shrimp *Pandalus platyceros* from the Pacific coast. That observation could be important in view of recent introductions of American lobsters (some possibly carrying *Gaffkya*) on the Canadian west coast. Other experimental studies (Cornick and Stewart, 1968b) indicated that the bacterium may also be pathogenic for east coast crabs (*Cancer irroratus*, *C. borealis*, and *Hyas coarctatus*). In vitro growth of the pathogen in crab sera was similar to that in lobster serum, suggesting susceptibility of the crabs. However, agglutinins for *G. homari*, which were demonstrated in the sera of one of the crab species (*C. irroratus*), might counteract the favorable bacterial growth in vivo and reduce the severity of infections in the crab. Cornick and Stewart extended their observations by inoculations of *C. irroratus* with suspensions of *G. homari*. After 49 days the surviving crabs (three) were found to be heavily infected (10^9 organisms/ml hemolymph). Passage through the crabs did not alter pathogenicity of *Gaffkya* to lobsters. A repetition of the crab inoculations with larger numbers of crabs provided some evidence of greater mortalities in experimental groups than in controls. Pathogenicity for rock crabs was less than for lobsters, as indicated by a mean time to death of 42 days in crabs, against only 18 days in lobsters. The authors mentioned the possible role of rock crabs as reservoirs of infection for lobsters, in view of reduced pathogenicity and prolonged mean time to death in crabs.

From the foregoing, it is apparent that experimental studies with *G. homari* have been numerous and varied and have provided significant insights about the internal defenses of Crustacea. Important areas for future study include determination of whether strains of the pathogen with different virulences exist, and determination of whether virulence may be increased by rapid passage through impounded lobster populations.

DISCUSSION

In the development of information and principles of invertebrate internal defenses, consistent and entirely natural attempts have been made to translate findings into the concepts and compartments constructed for the immune responses of vertebrates. The effort has led to some confusion of terminology and even to lack of agreement about definitions of immunity.

The concept of immunity in vertebrates has been admirably stated by Good and Papermaster (1964), who define immunity precisely and narrowly as "a biologic phenomenon embodying primary and secondary responses, with antibody synthesis and release, reactions of immediate and delayed allergy, and homograft immunity." They state: "To the time of writing [1964], adaptive immunologic responsiveness has not been demonstrated in the invertebrates." They then define adaptive immune responsiveness as "the ability to respond to antigenic material by production of specific combining substances, and to show an anamnestic response to these same antigens on subsequent exposure." Their continued exposition of immunity from the vertebrate point of view includes the following significant points:

1. "Adaptive immunity . . . is primarily a function achieving full expression late in phylogeny and ontogeny."
2. "The lymphoid cell family is the primary cellular basis for adaptive immune response in vertebrates . . . ."
3. "The possibility that another cell system may mimic adaptive immune responses in an invertebrate species cannot be excluded at this time."

A broader, more inclusive, concept of immunity has been suggested recently. If the broader definitions of terms proposed by several authors are accepted, the words "immunity" and "immune response," rather than careful circumlocutions, can be used with invertebrates. As an example, McKay and Jenkin (1969) stated that the Australian freshwater crayfish was capable of an "adaptive immune response." Such a capability is not possible within the confines of Good and Papermaster's definition of adaptive immunity as the production of specific immunoglobulins (a capacity which has been correlated with the occurrence of lymphoid tissue). Perhaps their definition is too restrictive and rigid, since a number of invertebrates do show responses that protect them from pathogens (hence they are adaptive).

Earlier definitions of antibodies and immunity allowed more latitude for inclusion of invertebrate responses. Cantacuzène (1923b), for example, considered as antibodies ". . . toute substance albuminoïde du plasma, douée ou non de spécificîté, qui, se fixant sur l'antigène, modifie les relations de contact de ce dernier, soit avec les cellules, soit avec les autres constituants chimiques des humeurs."

McKay, Jenkins, and Rowley (1969) stated ". . . to allow comparisons to be made between invertebrate phyla and the vertebrates . . . the definitions of the immune response should be as broad as possible and emphasis placed on the functional aspects . . . ." These authors suggest that such a definition of the immune response might be "the ability of the animal to respond to a foreign particle (whether it be truly foreign or unwanted self) by the production of specific proteins capable of reacting with the inducer, and the resultant of this reaction leading to phagocytosis."

Cushing (1967), in an excellent summarization of invertebrate immune mechanisms, stated "There is a growing consensus of observations supporting the view that while vertebrates and invertebrates may share some basic immune competences such as 'innate immunities' and phagocytic cells, it is indeed only within the vertebrates that the full capacity of adaptive immunity exists." This statement seems reasonable, and fits the confines of Good and Papermaster's narrow definition of adaptive immunity, but is too negative if a broader perspective of immunity—such as that proposed by McKay, Jenkins, and Rowley—is adopted.

Probably greater emphasis should be placed on the *adaptive* aspects of invertebrate internal defense processes. Substantial numbers of studies have indicated the existence of adaptive responses to experimental inoculations of for-

eign protein. Whether the response is in the form of specific immunoglobulin seems less significant than the degree of protection afforded to the individual by the adaptive response. In the broadest sense, the replacement of a protective constituent of the hemolymph after its utilization in preventing infection could be considered adaptive. For example, the precipitating factor for foreign protein found by Stewart and Foley (1969) in lobster serum (which decreases following experimental inoculation of BSA and which is unreactive against injected lobster serum) would be adaptive.

Considering immune responses of vertebrates and invertebrates, Good and Papermaster stated that the presence in vertebrates of lymphoid tissue and cells constitutes a basic distinction. On this basis, as Chadwick (1967) has pointed out, "It is highly unlikely that insects [or Crustacea or other invertebrates] do produce mammalian type antibody, or that the mechanism of any acquired response to antigenic stimulus could be likened to responses in higher animals in terms of the production of specific antibody globulins." Analogous tissues and cells exist in a number of invertebrate groups, however, as do analogous humoral responses without the extreme specificities of vertebrate globulins. Chadwick (1967) also stated that the ". . . immune response in an insect is not the consequence of an antigen-antibody-globulin reaction but more likely the result of the production of some, as yet undefined, principle in insect hemolymph which may contribute to its resistance." The same statement might be made about other invertebrates in which an induced response has been demonstrated.

Although somewhat beyond the confines of the present consideration of internal defenses of Crustacea, it might be well to call attention to recent tissue transplantation work of Cooper (1968, 1969a, 1969b, 1969c, 1969d) with annelids, which indicates a high degree of specificity of response and which suggests some similarities to vertebrate tissue graft responses. Cooper's (1969d) concluding statement is significant: "Further clarification of anamnestic responses to tissue transplants would confirm our views that at least two of the parameters of adaptive immunity [in the vertebrate sense], namely specificity and memory, did not evolve exclusively with the lower vertebrates."

It is obvious that modification, redefinition, or replacement of some conventional immunological terminology—particularly toward broader definitions—is needed if the invertebrates are included in comparative immunology. If we remove "antibodies" from invertebrate terminology we must also remove "antigen," since antibody response is part of the definition of antigen. An effective substitute for "antigen" (as suggested for insects by Hinton) (Chadwick, 1967) would be "immunogen." Chadwick also suggested replacement of "antibody" with such terms as "natural bactericidal substance," "specific inducible substance," and others. Furthermore, as stated earlier, it must be made clear that when "lysins," "precipitins," "agglutinins," and other humoral factors of invertebrates are discussed, identification with vertebrate factors is not intended—the terms are used merely to indicate the kind of activity produced (i.e., "lytic substance or activity," "precipitating substance or activity," etc.) regardless of the physiological-biochemical mechanism(s) involved.

When suitably qualified the "safe" general terms, therefore, include "resistance," "immunity," "immune response"; terms that can have general applicability and utility, if accepted in a general sense, include "agglutinin," "lysin," "precipitin"; specific vertebrate terminology, not applicable to invertebrates includes "antibody," "antigen," and "serological."

Beyond the establishment of working definitions of immunity in invertebrates, it seems appropriate to list a number of generalizations that seem warranted by the admittedly narrow base of evidence now available. Obviously, any generalization about a group as evolutionarily diverse as the invertebrates—or for that matter even of the Crustacea—must be in the form of a tenuous and easily retractable hypothesis (which may at times border on speculation), and the following statements are offered with these qualifications:

1. Resistance in the vertebrates seems primarily related to production of immunoglobulins

which combine with foreign protein to enhance the phagocytic process. Although specific immunoglobulins have not yet been demonstrated in invertebrates, an analogous protein system, of lower specificity but with functions similar to vertebrate immunoglobulins, is suggested. Natural (and in some cases, partially specific) agglutinins are common in invertebrate body fluids, and their titers in some species may be increased by exposure to specific antigens.

2. Resistance in the vertebrates, and in some invertebrates also, includes the production of bactericidins, lysins, and agglutinins. The appearance of, or the increase in, titers of such factors in certain invertebrates following exposure to foreign proteins may account in part for increased resistance to certain pathogens (McKay and Jenkin, 1969; Bang, 1967b).

3. Many of the bactericidal, bacteriostatic, lytic, and agglutinating properties seem to be conferred on the hemolymph of invertebrates by release of materials from hemocytes. The substances so released often seem not only less specific in their action than vertebrate antibodies, but also the stimulation of release may be much less specific. For example, the release of a lysin in sipunculids for the ciliate *Anophrys* can be stimulated by inoculation of certain bacteria (Bang, 1967a), and the release of a hemolysin in *Maia squinado* for sheep red blood cells can be induced by injection of sipunculid coelomic fluid (Cantacuzène, 1920a, 1923b).

4. Immune response in invertebrates, as best exemplified in insects and crustaceans, is often rapid in onset and disappearance—usually a matter of a few days.

5. As has been observed by a number of authors (Feng and Stauber, 1968; Stewart, Cornick, and Zwicker, 1969), environmental temperature is a critical factor in the host-parasite relationships of invertebrates. Temperature has been found experimentally to exert a significant effect on the appearance, degree, and duration of resistance to infection in certain invertebrates (McKay and Jenkin, 1969), just as it does in poikilothermic vertebrates (Bisset, 1946, 1947a, 1947b, 1948a, 1948b). Temperature affects the rate of growth and reproduction of microorganisms, the rate of production of toxic metabolites, and the utilization of nutrient derived from the host. Temperature can also affect the rate of phagocytosis and the rate of production of humoral defenses against infection. Thus the progress of infection and the outcome of disease represents a composite of enhancement or inhibition partly mediated by temperature.

6. Endotoxin has been found (in the vertebrates) to increase the metabolic rate of phagocytes and stimulate phagocytosis (Jenkins and Palmer, 1960; Whitby et al., 1961). A similar effect may be produced by endotoxin in the invertebrates. Thus, exposure to gram-negative bacteria which are so abundant in the sea (or to their endotoxins) may increase the level of nonspecific resistance to other gram-negative organisms or their endotoxins. This "nonspecific immunity," which is also known in the vertebrates (Rowley, 1956; Landy and Pillemer, 1956), may be of great significance in the invertebrates—in fact, it may be the basic mechanism of internal resistance to bacterial pathogens in the invertebrates.

7. Handling and experimental procedures rapidly induce bacteremias in a number of invertebrates. Rabin (1965), for example, found that almost half of all American lobsters used in his studies had bacteremias upon arrival in the laboratory. Cornick and Stewart (1966) found that about 20% of a large sample of lobsters had bacteria in their hemolymph. Isolates were principally *Micrococcus*, *Pseudomonas*, *Brevibacterium*, and *Achromobacter*—bacteria commonly found in the marine environment, and apparently nonpathogenic for lobsters. The acts of capture, transport, and impoundment of these and other marine animals may produce stresses and physiological changes (or mechanical damage) that permit entry of microorganisms common in the surrounding sea water.

8. There is some limited evidence that the process of phagocytosis, fully elaborated in the Protozoa, is enhanced in the vertebrates by nonspecific humoral factors which sensitize, agglutinate, immobilize, or otherwise increase the susceptibility of proteins to phagocytosis by fixed and mobile phagocytic cells. Although the opsonizing substances of invertebrates have not

been adequately characterized and the mechanisms involved have not been adequately elucidated, it may be speculated that in the vertebrates a greater degree of specificity of humoral factors has been added to the nonspecific mechanisms found in the invertebrates.

The proper role of specific vertebrate antibody as a possible augmentation of evolutionarily older nonspecific internal defenses was alluded to by Miles (1962). He stated ". . . it is fairly clear that antibody *per se* has little effect on the viability or metabolism of microbes with which it combines. It is effective in defense either because it neutralizes toxins, or because it makes the microbe susceptible to non-specific defense factors like complement or the phagocyte . . . . We may then properly consider antibody as accessory to the more fundamental nonspecific defense mechanisms . . . ." It should be emphasized, however, that much remains to be learned about the nonspecific humoral factors of vertebrates, as well as invertebrates.

9. Brown (possibly chitinoid) bodies or cysts in gills characterize later stages of a number of crustacean diseases. The sequence of events, after invasion by microorganisms, may include action of toxic or inhibitory factors in hemolymph, accretion of moribund or dead invaders in gill lacunae, phagocytosis of dead organisms, and formation of nodules or cysts containing dead organisms, and gradual phagocytic destruction of necrotic material.

10. An important point, as Bang (1967b) mentioned, is that the probability of discovering internal defense mechanisms is greater when disease phenomena are studied under natural conditions. In experimental work, microorganisms pathogenic to marine invertebrates should constitute test organisms of choice; microorganisms found in the environment (and which may be facultatively pathogenic) should be next in order of preference; and microorganisms or proteins which the marine animal is unlikely to encounter seem least instructive. There are valid experimental reasons, of course—such as the ease of recognition of bacteriophages—that often lead to selection of test microorganisms other than pathogens or potential pathogens. Whether these unusual choices are effective antigens is obviously a most important consideration.

Another extremely pertinent observation made by Bang (1967b) was: "The limited amount of information [concerning immunological responses of invertebrates] is, I believe, due mainly to the limited number of studies, and not to any lack of imagination on the part of evolutionary forces in developing protective mechanisms."

11. One final and very significant thought was proposed by Stauber (1961): "That so few examples of acquired resistance are known among invertebrates may even be quite logical. Because of their relatively short generation times, their usual small size and often enormous reproductive capacities, subsequent epizootics would be much more likely to be circumvented by the appearance of resistant stocks through natural selection . . . . Even with very high mortality rates a residual stock of animals under favorable conditions later might repopulate an area . . . . If this reasoning is adequate to explain the lack of evidence for the occurrence of acquired resistance in most of the invertebrates, perhaps those invertebrates with a long life span, like *Limulus* should be investigated more fully, as likely hosts capable of demonstrating acquired resistance."

It is interesting to note that it is precisely those invertebrates with a long life span which have received increased attention during the past several years, and that a few indications of acquired resistance have been reported.

Information about the internal defenses of crustaceans and other invertebrates may be summarized as follows:

The weight of evidence indicates a major defensive role in invertebrates for phagocytosis, augmented by relatively nonspecific innate or acquired humoral factors. Preformed substances released into the hemolymph from granular hemocytes seem to play a major role in humoral defenses of Crustacea, and probably other invertebrates as well. Thus the body fluids of many invertebrates contain natural bactericidins, agglutinins, lysins, and occasionally precipitins. Some limited evidence for augmenta-

tion of such defenses by exposure to foreign antigen exists. This evidence is primarily in form of increased titers following experimental inoculation. Specific acquired antibodies (immunoglobulins) have not been demonstrated in invertebrates, but induced antibody-like activity has been demonstrated in a few species. The fundamental difference seems to be in degree of specificity of response, which is significantly higher in the vertebrates. It is obvious that the synergistic action of phagocytes and humoral factors in the invertebrates, as well as in the vertebrates, constitutes the significant defense perimeter—but the degree of specificity of the humoral components is lower in the invertebrates. The master internal defense plan seems to be: foreign protein + humoral factor = recognition of foreignness and phagocytosis.

Cooper (1969c), concluding a very thought-provoking paper, stated: "It seems reasonable to conclude that invertebrates do possess immune systems, although the nature of the mechanisms is decidedly unknown. Reactions may be as numerous as the varied taxonomic groups, as is true of most rigorously studied vertebrates. Invertebrate cellular immunity may be closer to vertebrate reactions and may represent the more primitive responses. In the absence of classic vertebrate-type immunoglobulin in invertebrates, a real dichotomy would be evident in the evolution of immune responses. On the other hand, immunoglobulin precursors may be present."

Certainly the next decade will prove to be an exciting one in the study of invertebrate internal defense systems. The components of classical vertebrate immunity—present as analogues in invertebrates, and probably varying widely among phyla—provide an excellent background against which new findings may be evaluated.

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REFERENCES

AARONSON, S.
1956. A biochemical-taxonomic study of a marine micrococcus, *Gaffkya homari*, and a terrestrial counterpart. J. Gen. Microbiol. 15: 478-484.

AARUM, G. R.
1967. Fagocytose. Nor. Tannlaegeforenings Tid. 77: 243-254.

ACTON, R. T., P. F. WEINHEIMER, AND E. E. EVANS.
1969. A bactericidal system in the lobster *Homarus americanus*. J. Invertebr. Pathol. 13: 463-464.

ANDERSON, J. I. W., AND D. A. CONROY.
1968. The significance of disease in preliminary attempts to raise Crustacea in sea water. Proc. 3d. Symp. Mond. Comm. Off. Int. Epizoot. Etud. Mal. Poissons, Stockholm, Sep. No. 3, 8 p.

ARVY, L.
1952. Contribution à l'étude du sang et de la leucopoiése chez *Pachygrapsus marmoratus* Fabr. Ann. Sci. Nat. Zool. Biol. Anim. 14: 1-14.

ATKINS, D.
1929. On a fungus allied to the Saprolegniaceae found in the pea-crab *Pinnotheres*. J. Mar. Biol. Assoc. U.K. 16: 203-219.
1954a. Further notes on a marine member of the Saprolegniaceae, *Leptolegnia marina* n. sp., infecting certain invertebrates. J. Mar. Biol. Assoc. U.K. 33: 613-625.
1954b. A marine fungus *Plectospira dubia* n. sp. (Saprolegniaceae), infecting crustacean eggs and small Crustacea. J. Mar. Biol. Assoc. U.K. 33: 721-732.
1955. *Pythium thalassium* n. sp. infecting the egg-mass of the pea-crab *Pinnotheres pisum*. Trans. Br. Mycol. Soc. 38: 31-46.

AUSTREG, J.
1952. Contribution à l'étude morphologique et histochimique du sang de *Gammarus pulex* L. en fonction du sexe et de la mue. Bull. Soc. Zool. Fr. 77: 231.

BAER, J. G.
1944. Immunité et reactions immunitaires chez les

invertebrés. Schweiz. Z. Allg. Pathol. Bact. 7: 442-462.

BALBIANI, G.
1886. Études bactériologiques sur les Arthropodes. C. R. Séances Acad. Sci. (Paris) 103: 952-954.

BALL, G. H.
1938. The life history of *Carcinoectes hesperus* n. gen., n. sp., a gregarine parasite of the striped shore crab, *Pachygrapsus crassipes*, with observations on related forms. Arch. Protistenkd. 90: 299-319.

BANG, F. B.
1956. A bacterial disease of *Limulus polyphemus*. Johns Hopkins Med. J. 98: 325-351.
1962. Serological aspects of immunity in invertebrates. Nature (London) 196: 88-89.
1967a. Pathology Society Symposium: Defense Reactions in Invertebrates. Introduction. Fed. Proc. 26: 1664-1665.
1967b. Serological responses among invertebrates other than insects. Fed. Proc. 26: 1680-1684.
1967c. Blood clot formation in the antenna of the hermit crab *Pagurus longicarpus*. Biol. Bull. (Woods Hole) 133: 456-457.
1968. Cellular clots in *Pagurus longicarpus* and the possible role of microtubules. Biol. Bull. (Woods Hole) 135: 414.
1970. Disease mechanisms in crustacean and marine arthropods. *In* S. F. Snieszko (editor), A symposium on diseases of fishes and shellfishes, p. 383-404. Am. Fish. Soc., Spec. Publ. 5.

BARKER, W. H., JR., AND F. B. BANG.
1966. The effect of infection by gram-negative bacteria, and their endotoxins, on the blood-clotting mechanism of the crustacean *Sacculina carcini*, a parasite of the crab *Carcinus maenas*. J. Invertebr. Pathol. 8: 88-97.

BELL, G. R., AND G. E. HOSKINS.
1966. Experimental transmission of the lobster pathogen, *Gaffkya homari*, to Pacific crabs and prawns. Can. Soc. Microbiol. Abstr. 16th Annu. Meet., p. 21.

BENGSTON, I. A.
1924. Studies on organisms concerned as causative factors in botulism. Hyg. Lab. Bull. 132: 1-96.

BISSET, K. A.
1946. The effect of temperature on non-specific infections of fish. J. Pathol. Bacteriol. 58: 251-258.
1947a. Bacterial infection and immunity in lower vertebrates and invertebrates. J. Hyg. 45: 128-135.
1947b. Natural and acquired immunity in frogs and fish. J. Pathol. Bacteriol. 59: 679-682.
1948a. Natural antibodies in the blood serum of freshwater fish. J. Hyg. 46: 267-268.
1948b. The effect of temperature upon antibody production in cold-blooded vertebrates. J. Pathol. Bacteriol. 60: 87-92.

BRUNTZ, L.
1905. Étude physiologique sur les Phyllopodes Branchiopodes. Phagocytose et excrétion. Arch. Zool. Exp. Gén. 4: 37-48.
1907. Études sur les organes lymphöides, phagocytaires et excréteurs des Crustacés supérieurs. Arch. Zool. Exp. Gén. 7: 1-67.

BULNHEIM, H.-P.
1967. Mikrosporidieninfektion und Geschlechtsbetimmung bei *Gammarus duebeni*. Zool. Anz. 30: 432-442.

BULNHEIM, H.-P., AND J. VÁVRA.
1968. Infection by the microsporidian *Octosporea effeminans* sp. n., and its sex determining influence in the amphipod *Gammarus duebeni*. J. Parasitol. 54: 241-248.

CAMPBELL, D. H., AND J. S. GARVEY.
1961. The fate of foreign antigen and speculations as to its role in immune mechanisms. Lab. Invest. 10: 1126-1150.

CANTACUZÈNE, J.
1912a. Sur certains anticorps naturels observés chez *Eupagurus prideauxii*. C. R. Séances Soc. Biol. (Paris) 73: 663-664.
1912b. Observations relatives à certaines propriétés du sang de *Carcinus maenas* parasité par la sacculine. C. R. Séances Soc. Biol. (Paris) 74: 109-111.
1912c. Recherches sur la production expérimentale d'anticorps chez quelques Invertébrés marins. C. R. Séances Soc. Biol. (Paris) 74: 111-112.
1913. Sur la production d'anticorps artificiels chez *Eupagurus prideauxii*. C. R. Séances Soc. Biol. (Paris) 74: 293-295.
1919a. Étude d'une infection experimentale chez *Ascidia mentula*. C. R. Séances Soc. Biol. (Paris) 82: 1019.
1919b. Anticorps normaux et expérimentaux chez quelques Invertébrés marins. C. R. Séances Soc. Biol. (Paris) 82: 1087-1089.
1920a. Sur quelques réactions d'immunité chez les Invertébrés. Rev. Gén. Sci. 31e année: 353-357.
1920b. Formation d'hemolysins dans le serum de *Maia squinado* inoculées avec des hematies de Mammiferes. Existence dans ce serum d'une substance antagoniste qui empêche ou retarde hemolyse. C. R. Séances Soc. Biol. (Paris) 83: 1515-1514.
1921. Quelques remarques au sujet d'une infection experimentale chez *Maia squinado*. C. R. Séances Soc. Biol. (Paris) 84: 1007-1009.
1923a. Cytolysines et cytoagglutinins provoquées par l'inoculation de *Sipunculus nudus* chez *Maia squinado*. C. R. Séances Soc. Biol. (Paris) 89: 266-268.
1923b. Le probleme de l'immunite chez les Invertébrés. C. R. Séances Soc. Biol. (Paris) 75th Anniversary 88(Suppl.): 48-119.

CANTACUZÈNE—Con.
1925a. Immunité d'*Eupagurus prideauxii* vis-a-vis des poison de l'*Adamsia palliata*. C. R. Séances Soc. Biol. (Paris) 92: 1133-1136.
1925b. Réactions de crabe sacculine vis-a-vis d'une infection experimentale de la sacculine. C. R. Séances Soc. Biol. (Paris) 93: 1417-1419.
CANTACUZÈNE, J., AND A. DAMBOVICEANU.
1934a. Caractères biologiques de l'extrait des acconties d'*Adamsia palliata* après déprotéinisation. C. R. Séances Soc. Biol. (Paris) 117: 136-138.
1934b. Caractères physico-chemiques du poison des acconties d'*Adamsia palliata*. C. R. Séances Soc. Biol. (Paris) 117: 138-140.
CATTANEO, G.
1888a. Su di un infusorio ciliato, parassito del sangue del *Carcinus maenas*. Zool. Anz. 11: 456-459.
1888b. Sugli "amebociti" dei crustacei. Zool. Anz. 11: 452-455.
1889. Sulla struttura e sui fenomeni biologici delle cellule ameboidi del sangue nel *Carcinus maenas*. Atti Soc. Ital. Sci. Nat. Mus. Civ. Stor. Nat. Milano 31: 231-266.
CHADWICK, J. S.
1967. Serological responses of insects. Fed. Proc. 26: 1675-1679.
CHATTON, E.
1907. *Caullerya mesnili* n.g. n. sp. Haplosporidie parasite des Daphnies. C. R. Séances Soc. Biol. (Paris) 62: 529-531.
COHEN, E.
1968. Immunologic observations of the agglutinins of the hemolymph of *Limulus polyphemus* and *Birgus latro*. Trans. N.Y. Acad. Sci., Ser. II, 30: 427-433.
COHN, Z. A., J. G. HIRSCH, AND E. WIENER.
1963. The cytoplasmic granules of phagocytic cells and the degradation of bacteria. *In* A. V. S. de Reuck and M. P. Cameron (editors), Lysosomes, p. 126-144. Little, Brown Co., Boston.
COOMBS, R. A., A. M. COOMBS, AND D. C. INGRAM.
1961. The serology of conglutination and its relation to disease. Blackwell Sci. Publ., Oxford.
COOPER, E. L.
1968. Transplantation immunity in annelids. I. Rejection of xenografts exchanged between *Lumbricus terrestris* and *Eisenia foetida*. Transplantation 6: 322-337.
1969a. Chronic allograft rejection in *Lumbricus terrestris*. J. Exp. Zool. 171: 69-74.
1969b. Allograft rejection in *Eisenia foetida*. Transplantation 8: 220-223.
1969c. Neoplasia and transplantation immunity in annelids. Natl. Cancer Inst. Monogr. 31: 655-669.
1969d. Specific tissue graft rejection in earth worms. Science (Washington) 166: 1414-1415.
CORNICK, J. W., AND J. E. STEWART.
1966. Microorganisms isolated from the hemolymph of the lobster, *Homarus americanus*. J. Fish. Res. Board Can. 23: 1451-1454.
1968a. Interaction of the pathogen *Gaffkya homari* with natural defense mechanisms of *Homarus americanus*. J. Fish. Res. Board Can. 25: 695-709.
1968b. Pathogenicity of *Gaffkya homari* for the crab *Cancer irroratus*. J. Fish. Res. Board Can. 25: 795-799.
COUCH, J. N.
1942. A new fungus on crab eggs. J. Elisha Mitchell Sci. Soc. 58: 158-162.
CUÉNOT, L.
1891. Études sur le sang et les glandes lymphatiques dans le serie animale. 2e partie: Invertébrés. Arch. Zool. Exp. Gén. 9: 71-90.
1895. Études physiologiques sur les Crustacés Décapodes. Arch. Biol. Liège 8: 245-303.
1897. Les globules sanguins et les organes lymphoides des Invertébrés. (Revue critique et nouvelles recherches.) Arch. Anat. Microsc. Morphol. Exp. 1: 153-192.
1898. Études physiologiques sur les Oligochètes. Arch. Biol. 15: 114-124.
1905. L'organe phagocytaire des Crustacés Décapodes. Arch. Zool. Exp. Gén., Ser. 4, 3: 1-15.
CUSHING, J. E.
1967. Invertebrates, immunology and evolution. Fed. Proc. 26: 1666-1670.
CUSHING, J. E., N. L. CALAPRICE, AND G. TRUMP.
1963. Blood group reactive substances in some marine invertebrates. Biol. Bull. (Woods Hole) 125: 69-80.
CUSHING, J. E., AND D. H. CAMPBELL.
1957. Principles of Immunology. McGraw Hill Co., New York, 344 p.
DAMBOVICEANU, A.
1929. Recherches sur les constantes physico-chimiques du plasma des Invertébrés à l'état normal et en cours d'immunisation. I. Constantes du plasma de quelques Crustacés Décapodes à l'état normal. Arch. Roum. Pathol. Exp. Microbiol. 2: 5-38.
1932. Composition chimique et physico-chimique du liquide cavitaire chez les Crustacés Décapodes (Physiologie de la calcification). Arch. Roum. Pathol. Exp. Microbiol. 5: 239-309.
DANINI, E. S.
1925. Beiträge zur vergleichenden Histologie des Blutes und des Bindegewebes. III. Über die entzündliche Bindegewebsneubildung beim Flusskrebs (*Potamobius leptodactylus*). Jahrb. Morphol. Mikrosk. Anat. 3: 558-608.
1927. Beiträge zur vergleichenden Histologie des Blutes und des Bindegewebes. VI. Experimentell-histologische Untersuchungen über das Verhalten der Blutgefässwand beim Flusskrebs (*Potamobius leptodactylus*). Jahrb. Morphol. Mikrosk. Anat. 11: 565-597.

DANNEVIG, A.
1928. Beretning om Flødevigens utklekningsanstalt 1926-1927. Aarsberet. Nor. Fisk., p. 150-156.
1939. Beretning for Flødevigens utklekningsanstalt 1936-37. Aarsberet. Nor. Fisk., p. 70-75.

DEBAISIEUX, P.
1952a. Histologie et histogenèse chez *Chirocephalus diaphanus* Prév. (Phyllopode, Anostracé). Cell. Rec. Cytol. Histol. 54: 251-294.
1952b. Histologie et histogenèse chez *Argulus foliaceus* L. (Crustacé, Branchiure). Cell. Rec. Cytol. Histol. 55: 243-290.

DEMAL, J.
1953. Genèse et différenciation d'hémocytes chez *Palaemon varians* Leach. Cell. Rec. Cytol. Histol. 56: 85-102.

DUCCESCHI, V.
1903. Untersuchungen über die Blutgerinnung bei wirbellosen Tieren. Beitr. Chem. Physiol. Pathol. 3: 278-384.

DUCHÂTEAU, G., AND M. FLORKIN.
1954. La coagulation du sang des Arthropodes. IV. Sur le fibrinogène et sur la "coaguline" musculaire du Homard. Bull. Soc. Chim. Biol. 36: 295-305.

EBLE, A. F.
1966. Seasonal distribution of alkaline and acid phosphatase and nonspecific esterase in the normal and diseased American oyster as revealed by enzyme histochemistry. Am. Zool. 6: 339. (Abstr.)

ELBERG, S. S.
1960. Cellular immunity. Bacteriol. Rev. 24: 67-95.

EVANS, E. E., J. E. CUSHING, S. SAWYER, P. F. WEINHEIMER, R. T. ACTON, AND J. L. MCNEELY.
1969. Induced bactericidal response in the spiny lobster *Panulirus interruptus*. Proc. Exp. Biol. Med. 132: 111-114.

EVANS, E. A., B. PAINTER, M. L. EVANS, P. WEINHEIMER, AND R. T. ACTON.
1968. An induced bactericidin in the spiny lobster, *Panulirus argus*. Proc. Soc. Exp. Biol. Med. 128: 394-398.

EVANS, E. A., P. F. WEINHEIMER, B. PAINTER, R. T. ACTON, AND M. L. EVANS.
1969. Secondary and tertiary responses of the induced bactericidin from the West Indian spiny lobster, *Panulirus argus*. J. Bacteriol. 98: 943-946.

EVANS, W. H., AND M. L. KARNOVSKY.
1961. A possible mechanism for the stimulation of some metabolic functions during phagocytosis. J. Biol. Chem. 236: 30-32.

FAURÉ-FREMIET, E.
1927. Les amebocytes des Invertébrés à l'état quiescent et à l'état actif. Arch. Anat. Microsc. Morphol. Exp. 23: 99-173.

FENG, S. Y., AND L. A. STAUBER.
1968. Experimental hexamitiasis in the oyster *Crassostrea virginica*. J. Invertebr. Pathol. 10: 94-110.

FISCHER-PIETTE, E.
1931. Culture de tissus de Crustacés, la glande lymphatique du Homard. Arch. Zool. Exp. Gén. 74: 33-52.

FLORKIN, M.
1960. Blood chemistry. *In* T. H. Waterman (editor), The physiology of Crustacea, Vol. I, p. 141-159. Academic Press, New York.

FREDERICQ, H.
1879. Note sur le sang de l'Homard. Bull. Acad. Roy. Belg. Cl. Sci., Ser. 2, 47: 409-413.
1910. Les Invertébrés répondet-ils par la production d'anticorps aux injections de protéines étrangères agissant comme antigènes? Arch. Int. Physiol. Biochem. 10: 139-148.

GEDDES, P.
1880. On the coalescence of amoeboid cells into plasmodia, and on the so-called coagulation of invertebrate fluids. Proc. Roy. Soc. (London) 30: 252-255.

GEORGE, W. C.
1941. Comparative hemalotogy and the function of the leucocytes. Q. Rev. Biol. 16: 426-429.

GEORGE, W. C., AND J. NICHOLS.
1948. A study of the blood of some Crustacea. J. Morphol. 83: 425-443.

GIARD, A., AND A. BILLET.
1889. Observations sur la maladie phosphorescente des Talitres et autre Crustacés. C. R. Séances Soc. Biol. (Paris) 41: 593-597.

GIBSON, F. A.
1961. Gaffkaemia in stored lobsters. Int. Counc. Explor. Sea, Shellfish Comm. 58, 1 p. [Mimeo.]

GLAVIND, J.
1948. Studies on the coagulation of crustacean blood. Nyt Nordisk Forlag, Arnold Busck, Copenhagen, 137 p.

GOGGINS, P. L., AND J. W. HURST, JR.
1960. Progress report on lobster gaffkyaremia (red tail). Maine Dep. Sea Shore Fish., 9 p. [Mimeo.]

GOOD, R. A., AND B. W. PAPERMASTER.
1964. Ontogeny and phylogeny of adaptive immunity. Adv. Immunol. 4: 1-115.

GORDON, I.
1966. Parasites and diseases of Crustacea. Mem. Inst. Fondam. Afr. Noire 77: 27-86.

GREEN, J.
1959. Carotenoid pigment in *Spirobacillus cienkouski* a pathogen of Cladocera. Nature (London) 183: 56-57.

GREGOIRE, C.
1953. Blood coagulation in arthropods. Biol. Bull. (Woods Hole) 104: 373-393.
1962. Blood coagulation. *In* M. Florkin and H. S. Mason (editors), Comparative biochemistry, Vol. IV, Part B, p. 435-482. Academic Press, New York.

GRUZEWSKA, Z.
1932. Sur la coagulation du sang chez les Crustacés. C. R. Séances Soc. Biol. (Paris) 110: 920-922.

HAECKEL, E.
1857. Ueber die Gewebe des Flusskrebses. Arch. Anat. Physiol. Med. (1857): 469-568.
1862. Die Radiolarien, p. 104-106, Geo. Reimer, Berlin.

HALLIBURTON, W. E.
1885. On the blood of the decapod Crustacea. J. Physiol. (London) 6: 300-335.

HARDY, W. B.
1892. The blood corpuscles of the Crustacea, together with a suggestion as to the origin of the crustacean fibrin-ferment. J. Physiol. (London) 13: 165-190.

HEARING, V., AND S. H. VERNICK.
1967. Fine structure of the blood cells of the lobster, *Homarus americanus*. Chesapeake Sci. 8: 170-186.

HEDRICK, L. R.
1935. The life history and morphology of *Spiroxys contortus* (Rudolphi); Nematoda: Spiruridae. Trans. Am. Microsc. Soc. 54: 307-335.

HENSILL, J. S.
1948. Control of coagulation of decapod blood. Anat. Rec. 101: 736. (Abstr.)

HERRMANN, G., AND E. CANU.
1891. Sur un champignon parasite du Talitre. C. R. Séances Soc. Biol. (Paris) 43: 646-651.

HESS, E.
1937. A shell disease in lobsters *Homarus americanus* caused by chitinovorous bacteria. J. Biol. Board Can. 3: 358-362.

HIRSCH, J. G.
1959. Immunity to infectious diseases: review of some concepts of Metchnikoff. Bacteriol. Rev. 23: 48-60.
1965. Phagocytosis. Annu. Rev. Microbiol. 19: 339-350.

HIRSCH, J. G., AND Z. A. COHN.
1960. Degranulation of polymorphonuclear leucocytes following phagocytosis of microorganisms. J. Exp. Med. 112: 1005-1014.

HITCHNER, E. R., AND S. F. SNIESZKO.
1947. A study of a microorganism causing a bacterial disease of lobsters. J. Bacteriol. 54: 48. (Abstr.)

HUFF, C. G.
1940. Immunity in invertebrates. Physiol. Rev. 20: 68-88.

INMAN, O. L.
1927. A pathogenic luminescent bacterium. Biol. Bull. (Woods Hole) 53: 197-200.

IVERSEN, E. S., AND R. B. MANNING.
1959. A new microsporidian parasite from the pink shrimp (*Penaeus duorarum*). Trans. Am. Fish. Soc. 88: 130-132.

IVERSEN, E. S., AND N. N. VAN METER.
1964. A record of the microsporidian, *Thelohania duorara*, parasitizing the shrimp *Penaeus brasiliensis*. Bull. Mar. Sci. 14: 549-553.

JANOFF, A., AND E. HAWRYLKO.
1964. Lysosomal enzymes in invertebrate leucocytes. J. Cell. Physiol. 63: 267-271.

JENKIN, C. R., AND D. PALMER.
1960. Changes in the titre of serum opsonins and phagocytic properties of mouse peritoneal macrophages following injection of endotoxin. J. Exp. Med. 112: 419.

JIROVEC, O.
1932. Ueber eine Bakterieninfektion der *Daphnia longispina*. Arch. Protistenkd. 77: 175-181.
1936. Ueber einige in *Daphnia magna* parasitierende Mikrosporidien. Zool. Anz. 116: 136-142.

JOHNSON, P. T.
1968. An annotated bibliography of pathology in invertebrates other than insects. Burgess Publishing Co., Minneapolis, Minn. 322 p.

KOLLMANN, M.
1908. Recherches sur les leucocytes et le tissue lymphoïde des Invertébrés. Ann. Sci. Nat. Zool. Biol. Anim. 8: 1-240.
1921. Sur les premieres phases du développement des leucocytes des Crustacés. C. R. Séances Soc. Biol. (Paris) 84: 811-813.

KRANTZ, G. E., R. R. COLWELL, AND E. LOVELACE.
1969. *Vibrio parahaemolyticus* from the blue crab *Callinectes sapidus* in Chesapeake Bay. Science (Washington) 164: 1286-1287.

KRUSE, D. N.
1959a. Parasites of commercial shrimp. Assoc. Southeast. Biol. Bull. 6(2): 28.
1959b. A study of the taxonomy, morphology, incidence and biology of the parasites of the commercial shrimp, *Penaeus azteeus* Ives, *P. duorarum* Burkenroad and *P. setiferus* Linnaeus. Master's Thesis Fla. State Univ.

LANDY, M.
1960. Inactivation of endotoxin by humoral systems. Ann. N.Y. Acad. Sci. 88: 1273-1277.

LANDY, M., AND L. PILLEMER.
1956. Increased resistance to infection and accompanying alteration in properdin levels following administration of bacterial lipopolysaccharide. J. Exp. Med. 104: 383-409.

LANE, C. E., B. W. COURSEN, AND K. HINES.
1961. Biologically active peptides in *Physalia* toxin. Proc. Soc. Exp. Biol. Med. 107: 670-672.

LECHLER, E., AND R. GROSS.
1962. (Blut-)Gerinnung and Wundverschlub bie Invertebraten. Thromb. Diath. Haemorrh. 8: 355-391.

LÉGER, E., AND D. DUBOSCQ.
1908. L'evolution schizogonique de l'*Aggregata eberthi* (Labbé). Arch. Protistenkd. 12: 44-108.

LEVIN, J.
1967. Blood coagulation and endotoxin in invertebrates. Fed. Proc. 26: 1707-1712.

LÉVY, R.
1923. Sur la toxicité de la sacculine (*Sacculina*

carcini Thomp.) vis-a-vis du crabe (*Carcinus maenas* Penn.) et sur la recherche de réactions d'immunité chez ce dernier. Bull. Soc. Zool. Fr. 48: 291-294.

LIUZZO, J. A., A. F. NOVAK, AND J. R. ORTIGO.
1965. Physiological changes induced by gamma irradiation of bacteria from shrimp. J. Food Sci. 30: 710-713.

LOCHHEAD, J. H., AND M. S. LOCHHEAD.
1941. Studies on the blood and related tissues in *Artemia* (Crustacea, Anostraca). J. Morphol. 68: 593-632.

LOEB, L.
1903a. Über die Bedeutung der Blutkörperchen für die Blutgerinnung und die Entzündung einiger Arthropoden und über mechanische Einwirkungen auf das Protoplasma dieser Zellen. Virchow's Arch. Abt. a Pathol. Anat. 173: 35-112.
1903b. On the coagulation of the blood of some arthropods and on the influence of pressure and traction on the protoplasm of the blood cells of arthropods. Biol. Bull. (Woods Hole) 4: 301-318.
1904. Über die Koagulation des Blutes einiger Arthropoden. Beitr. Chem. Physiol. Pathol. 5: 191-207.
1907. Einige neuere Arbeiten über die Blutgerinnung bei Wirbellosen und bei Wirbeltieren. Biochem. Zentralbl. 6: 829-850; 889-912.
1910. Über die Blutgerinnung bei den Wirbellosen. Biochem. Z. 24: 478-495.

LÖWIT, M.
1889. Über die Beziehungen der weissen Blutkorperchen zur Blutgerinnung. Ziegler's Beitr. Pathol. Anat. 5: 469-520.

MACKANESS, G. B.
1962. Cellular resistance to infection. J. Exp. Med. 116: 381-406.

MACLEOD, C. M., AND L. E. CLUFF (editors).
1960. Non-specific resistance to infection. Bacteriol. Rev. 24: 1-200.

MANNSFIELD, W.
1942. Die Krebspest im Generalbezirk Lettland in den Jahren 1924-1938. Z. Fisch. 40: 395-417.

MANWELL, C., AND C. M. A. BAKER.
1963. Starch gel electrophoresis of sera from some marine arthropods: Studies on the heterogeneity of hemocyanin and on a ceruloplasmin-like protein. Comp. Biochem. Physiol. 8: 193-208.

MARREC, M.
1944. L'organe lymphocytogène des Crustacés Décapodes. Son activité cyclique. Bull. Inst. Océanogr. (Monaco) 41(867): 1-4.

MAYNARD, D. M.
Circulation and heart function. *In* T. H. Waterman (editor), The physiology of Crustacea, Vol. I, p. 161-226. Academic Press, New York.

MCDADE, J. E., AND M. R. TRIPP.
1967. Lysozyme in the hemolymph of the oyster, *Crassostrea virginica*. J. Invertebr. Pathol. 9: 531-535.

MCKAY, D., AND C. R. JENKIN.
1969. Immunity in invertebrates. II. Adaptive immunity in the crayfish (*Parachaeraps bicarinatus*). Immunology 17: 127-137.

MCKAY, D., C. R. JENKIN, AND D. ROWLEY.
1969. Immunity in the invertebrates. I. Studies on the naturally occurring haemagglutinins in the fluid from invertebrates. Aust. J. Exp. Biol. Med. Sci. 47: 125-134.

MENKIN, V.
1938. The role of inflammation in immunity. Physiol. Rev. 18: 366-418.
1949. Cellular injury in some invertebrates. Physiol. Zool. 22: 124-131.
1956. The biology of inflammation. Science (Washington) 123: 527-534.

METCHNIKOFF, E.
1884. Ueber eine Sprosspilzkrankheit der Daphnien. Arch. Pathol. Anat. 96: 177-195.
1893. Lectures on the comparative pathology of inflammation. Delivered at the Pasteur Institute in 1891. Kegan, Paul, Trench, Trübner and Co. Ltd., London. (Republished 1968 by Dover Publications, Inc., New York, 224 p.)
1905. Immunity in Infective Diseases (translated from the French by F. G. Binnie). Cambridge Univ. Press, London (orig.: Masson, Paris (1901)).

MICHAJŁOW, W.
1938. Über das Bedürfnis einer Vereinheitlichung der Forschungsmethoden, die sich auf die Copepoden als Zwischenwirte der Cestodon beziehen. Zool. Pol. 3: 15-22.

MILES, A. A.
1962. Mechanisms of immunity in bacterial infections. Recent Prog. Microbiol. 7: 399-409.

MORRISON, P. R., AND K. C. MORRISON.
1952. Bleeding and coagulation in some Bermudian Crustacea. Biol. Bull. (Woods Hole) 103: 395-406.

NELSTRUP, A. E., G. TAYLOR, AND P. COLLARD.
1968. Studies on phagocytosis. III. Antigen clearance studies in invertebrates and poikilothermic vertebrates. Immunology 14: 347-356.

NEWCOMBE, C. L., AND M. R. ROGERS.
1947. Studies of a fungus parasite that infects blue crab eggs. Turtox News 25(9): 1-7.

NOGUCHI, H.
1903. A study of immunization - hemolysins, agglutinins, precipitins and coagulins in cold blooded animals. Zwt. F. Bact. Parasitenk. Inf. 33: 353-360.

NOLD, R.
1921. Die Blutzellen von *Astacus*. Zool. Anz. 52: 277-285.

NOLF, P.
1909. Contribution à l'étude de la coagulation du

NOLF—Con.
sang (8e mèmoire). La coagulation chez les Crustacés. Arch. Int. Physiol. Biochem. 7: 411-461.

NUMANOI, H.
1938. On crustacean blood coagulation. Jap. J. Zool. 7: 613-641.

NYBELIN, O.
1935. Über die Ursache der Krebspest in Schweden. Fischereitg., Neudamm 38: 21.

OSAWA, S., AND E. YABUUBHI.
1963. Anticorps produits par le Homard americain, *Cambarus clarkii*. C. R. Séances Soc. Biol. (Paris) 156: 698-699.

PAPERMASTER, B. W., R. M. CONDIE, J. FINSTAD, AND R. A. GOOD.
1964. Evolution of the immune response. I. The phylogenetic development of adaptive immunological responsiveness in vertebrates. J. Exp. Med. 119: 105-130.

PARRY, G.
1960. Excretion. *In* T. H. Waterman (editor), The physiology of Crustacea, Vol. I, p. 341-366. Academic Press, New York.

PÉREZ, C.
1905a. Microsporidies parasites des crabes d'Arcachon. Note préliminaire. Trav. Lab. Soc. Sci. Arcachon 8: 15-36.
1905b. Sur une nouvelle glugéidée parasite du *Carcinus maenas*. C. R. Séances Soc. Biol. (Paris) 58: 146-151.
1907. Sur un cas d'envahissement de l'ovaire par *Thelohania maenadis*. C. R. Séances Soc. Biol. (Paris) 60: 1091-1092.

PHILLIPS, J. H.
1960. Antibodylike materials of marine invertebrates. Ann. N.Y. Acad. Sci. 90: 760-769.

PIXELL-GOODRICH, H. L.
1928. Reactions of *Gammarus* to injury and disease with notes on microsporidial and fungoid diseases. J. Cell. Sci. 72: 325-353.
1956. Crayfish epidemics. Parasitology 46: 480-483.

POISSON, R.
1930. Observations sur *Anophrys sarcophaga* Cohn (=*A. maggii* Cattaneo). Bull. Biol. Fr. Belg. 64: 288-331.

RABIN, H.
1965. Studies on gaffkemia, a bacterial disease of the American lobster, *Homarus americanus* (Milne-Edwards). J. Invertebr. Pathol. 7: 391-397.
1970a. Introduction. Symposium on Reticuloendothelial Aspects of Invertebrate Pathology. Res. J. Reticuloendothel. Soc. 7: 159-160.
1970b. Hemocytes, hemolymph, and defense reactions in crustaceans. Res. J. Reticuloendothel. Soc. 7: 195-207.

RABIN, H., AND J. T. HUGHES.
1968. Studies on host-parasite relationships in gaffkemia. J. Invertebr. Pathol. 10: 335-344.

READ, C. P.
1958. Status of behavioral and physiological "resistance." Rice Inst. Pam. 45: 36-54.

ROBINEAUX, J., AND J. FREDERIC.
1955. Contribution à l'étude des granulations neutrophiles des polynucléaires par la microcinématographie en contraste de phase. C. R. Séances Soc. Biol. (Paris) 149: 486-489.

ROGERS, D. E.
1960. Host mechanisms which act to remove bacteria from the blood stream. Bacteriol. Rev. 24: 50-65.

ROGERS-TALBERT, R.
1948. The fungus *Lagenidium callinectes* Coùch (1942) on eggs of the blue crab in Chesapeake Bay. Biol. Bull. (Woods Hole) 95: 214-228.

ROSEN, B.
1967. Shell disease of the blue crab *Callinectes sapidus*. J. Invertebr. Pathol. 9: 348-353.
1970. Shell disease of aquatic crustaceans. *In* S. F. Snieszko (editor), A symposium on diseases of fishes and shellfishes, p. 409-415. Am. Fish. Soc., Spec. Publ. 5.

ROSKAM, R.
1957. Gaffkaemia, a contagious disease in *Homarus vulgaris*. Int. Counc. Explor. Sea, Shellfish Comm., 4 p. [Mimeo.]

ROWLEY, D.
1956. Rapidly induced changes in the levels of non-specific immunity in laboratory animals. Br. J. Exp. Pathol. 37: 223-234.
1959. The role of opsonins in non-specific immunity. J. Exp. Med. 111: 137-144.
1960. Antibacterial systems of serum in relation to non-specific immunity to infection. Bacteriol. Rev. 24: 106-114.

RUHBERG, W.
1933. Ueber eine Hefeinfektion bei *Daphinia magna*. Arch. Protistenkd. 80: 72-100.

SAWYER, T. K.
1969. Preliminary study on the epizootiology and host-parasite relationship of *Paramoeba* sp. in the blue crab, *Callinectes sapidus*. Proc. Natl. Shellfish Assoc. 59: 60-64.

SAWYER, T. K., R. COX, AND M. HIGGINBOTTOM.
1970. Hemocyte values in healthy blue crabs, *Callinectes sapidus*, and crabs infected with the amoeba, *Paramoeba perniciosa*. J. Invertebr. Pathol. 15: 440-446.

SCHÄPERCLAUS, W.
1935. Die Ursache der pestartigen Krebssterben. Z. Fisch. 33: 343-366.

SCHIKORA, F.
1906. Die Krebspest. Fischereiztg., Neudamm 9: 529-532, 549-553, 561-566, 581-583.
1926. 50 Jahre Krebspest. Fischereiztg., Neudamm 29: 225-228, 250-253.

SCHNEIDER, T. A.
1951. Nutrition and resistance-susceptibility to infection. Am. J. Trop. Med. Hyg. 31: 174-182.

SCHULZ, F. N.
1925. Physiologie der Körpersäfte: Crustaceen. *In* H. Winterstein (editor), Handbuch der Vergleichenden Physiologie, Vol. I, p. 669-746. Fischer, Jena.

SCHWAB, G. E., AND P. R. REEVES.
1966. Comparison of bactericidal activity of different vertebrate sera. J. Bacteriol. 91: 106-112.

SINDERMANN, C. J.
1962. Serology of Atlantic clupeoid fishes. Am. Nat. 96: 225-231.
1963. Use of plant hemagglutinins in serological studies of clupeoid fishes. U.S. Fish Wildl. Serv., Fish. Bull. 63: 137-141.
1970. Principal diseases of marine fish and shellfish. Academic Press, New York, 369 p.

SINDERMANN, C. J., AND D. F. MAIRS.
1959. A major blood group system in Atlantic sea herring. Copeia 1959: 228-232.

SINDERMANN, C. J., AND A. ROSENFIELD.
1967. Principal diseases of commercially important marine bivalve Mollusca and Crustacea. U.S. Fish Wildl. Serv., Fish. Bull. 66: 335-385.

SKARNES, R. C., AND D. W. WATSON.
1957. Antimicrobial factors of normal tissues and fluids. Bacteriol. Rev. 21: 273-294.

SNIESZKO, S. F., AND C. C. TAYLOR.
1947. A bacterial disease of the lobster, *Homarus americanus*. Science (Washington) 105: 500.

SOGANDARES-BERNAL, F.
1962. Presumable microsporidiosis in the dwarf crayfishes *Cambarellus puer* Hobbs and *C. shufeldti* (Faxon) in Louisiana. J. Parasitol. 48: 493.

SORDI, M.
1958. Micosi dei Crostacei decapodi marini. Riv. Parassitol. 19: 131-137.

SPECTOR, W. G., AND D. A. WILLOUGHBY.
1963. The inflammatory response. Bacteriol. Rev. 27: 117-154.

SPRAGUE, V.
1950a. Notes on three microsporidian parasites of decapod Crustacea of Louisiana coastal waters. Occas. Pap. Mar. Lab., La. State Univ. 5: 1-8.
1950b. *Thelohania cambari* n. sp., a microsporidian parasite of North American crayfish. J. Parasitol. 36: 46. (Abstr.)
1965. *Nosema* sp. (Microsporida, Nosematidae) in the musculature of the crab. *Callinectes sapidus*. J. Protozool. 12: 66-70.
1966. Two new species of *Plistophora* (Microsporida, Nosematidae) in decapods, with particular reference to one in the blue crab. J. Protozool. 13: 196-199.

SPRAGUE, V., AND R. BECKETT.
1966. A disease of blue crabs (*Callinectes sapidus*) in Maryland and Virginia. J. Invertebr. Pathol. 8: 287-289.
1968. The nature of the etiological agent of "Gray Crab" disease. J. Invertebr. Pathol. 11: 503.

SPRAGUE, V., R. L. BECKETT, AND T. K. SAWYER.
1969. A new species of *Paramoeba* (Amoebida, Paramoebidae) parasitic in the crab *Callinectes sapidus*. J. Invertebr. Pathol. 14: 167-174.

STAUBER, L. A.
1961. Immunity in invertebrates with special reference to the oyster. Proc. Natl. Shellfish Assoc. 50: 7-20.

STETSON, C. A.
1961. Symposium on bacterial endotoxins. IV. Immunological aspects of the host reaction to endotoxins. Bacteriol. Rev. 25: 457-458.

STEWART, J. E., B. ARIE, B. M. ZWICKER, AND J. R. DINGLE.
1969. Gaffkemia, a bacterial disease of the lobster, *Homarus americanus*: Effects of the pathogen, *Gaffkya homari*, on the physiology of the host. Can. J. Microbiol. 15: 925-932.

STEWART, J. E., AND J. W. CORNICK.
1967. *In vitro* susceptibilities of the lobster pathogen *Gaffkya homari* to various disinfectants and antibiotics. J. Fish. Res. Board Can. 24: 2623-2626.

STEWART, J. E., J. W. CORNICK, AND J. R. DINGLE.
1967. An electronic method for counting lobster (*Homarus americanus* Milne Edwards) hemocytes and the influence of diet on hemocyte numbers and hemolymph proteins. Can. J. Zool. 45: 291-304.

STEWART, J. E., J. W. CORNICK, D. M. FOLEY, M. F. LI, AND C. M. BISHOP.
1967. Muscle weight relationship to serum proteins, hemocytes, and hepatopancreas in the lobster *Homarus americanus*. J. Fish. Res. Board Can. 24: 2339-2354.

STEWART, J. E., J. W. CORNICK, D. I. SPEARS, AND D. W. MCLEESE.
1966. Incidence of *Gaffkya homari* in natural lobster *Homarus americanus* populations of the Atlantic region of Canada. J. Fish. Res. Board Can. 23: 1325-1330.

STEWART, J. E., A. DOCKRILL, AND J. W. CORNICK.
1969. Influence of temperature on gaffkemia, a bacterial disease of the lobster, *Homarus americanus*. J. Fish. Res. Board Can. 26: 2503-2510.

STEWART, J. E., J. R. DINGLE, AND P. H. ODENSE.
1966. Constituents of the hemolymph of the lobster, *Homarus americanus* Milne Edwards. Can. J. Biochem. 44: 1447-1459.

STEWART, J. E., A. DOCKRILL, AND J. W. CORNICK.
1969. Effectiveness of the integument and gastric fluid as barriers against transmission of *Gaffkya homari* to the lobster *Homarus americanus*. J. Fish. Res. Board Can. 26: 1-14.

STEWART, J. E., AND D. M. FOLEY.
1969. A precipitin-like reaction of the hemolymph of the lobster, *Homarus americanus*. J. Fish. Res. Board Can. 26: 1392-1397.

STEWART, J. E., D. M. FOLEY, AND R. G. ACKMAN.
1969. Characteristics of *Gaffkya homari* the causa-

STEWART, FOLEY, AND ACKMAN—Con.
tive agent of the lobster disease, gaffkemia. J. Fish. Res. Board Can. 26: 1385-1389.

STEWART, J. E., AND J. F. MACDONALD.
1962. A report to the fishing industry regarding lobster disease (gaffkaemia). Fish. Res. Board Can., Technol. Sta., Halifax, New Ser. Circ. 9, 2 p.

STEWART, J. E., AND H. RABIN.
1970. Gaffkaemia, a bacterial disease of lobsters (genus *Homarus*). In S. F. Snieszko (editor), A symposium on diseases of fishes and shellfishes, p. 431-439. Am. Fish. Soc., Spec. Publ. 5.

STUTMAN, L., AND M. DOLLIVER.
1967. Coagulation mechanism in the hemolymph of *Gecarcinus lateralis*. Am. Zool. 7: 766.
1968. Mechanism of coagulation in *Gecarcinus lateralis*. Am. Zool. 8: 481-489.

SUTER, E.
1956. Interaction between phagocytes and pathogenic microorganisms. Bacteriol. Rev. 20: 94-132.

TAIT, J.
1908. A simple method of observing the agglutination of the blood cells of *Gammarus*. Q. J. Exp. Physiol. Cogn. Med. Sci. 1: 247-249.
1910a. Blood coagulation in the amphipod, *Gammarus*. J. Physiol. (London) 40: 41-46.
1910b. Crustacean blood coagulation as studied in the Arthrostaca. J. Exp. Physiol. Cogn. Med. Sci. 3: 1-20.
1910c. Types of crustacean blood coagulation. J. Mar. Biol. Assoc. (U.K.) 9: 191-198.
1917. XI-Body colour as affected by blood colour in Amphipods and Isopods, with some remarks on a bacterial infection of *Gammarus*. Proc. Roy. Physiogr. Soc. Lund 20: 161-162.
1918a. Capillary phenomena observed in blood cells: thigmocytes, phagocytosis, amoeboid movement, differential adhesiveness of corpuscles, emigration of leucocytes. Q. J. Exp. Physiol. Cogn. Med. Sci. 17: 1-33.
1918b. The blood of *Astacus*. Q. J. Exp. Physiol. Cogn. Med Sci. 8: 1-40.

TAIT, J., AND J. D. GUNN.
1918. The blood of *Astacus fluviatilis*: a study in crustacean blood with special reference to coagulation and phagocytosis. Q. J. Exp. Physiol. Cogn. Med. Sci. 12: 35-80.

TAYLOR, A. E., G. TAYLOR, AND P. COLLARD.
1964. Secondary immune response to bacteriophage T1 in the shore crab, *Carcinus maenas*. Nature (London) 203: 775.

TEAGUE, P. O., AND G. J. FRIOU.
1964. Lack of immunological responses by an invertebrate. Comp. Biochem. Physiol. 12: 471-478.

THÉODORIDÈS, J.
1961. Sur la distinction entre les grégarines des familles des Cephaloidophoridae et des Porosporidae, parasites de Crustacés Décapodes. C. R. Séances Soc. Biol. (Paris) 252: 3640-3642.
1962. Grégarines d'Invertébrés marins de la région de Banyuls. I. Eugrégarines parasites de Crustacés Décapodes. Vie Mileu 13: 95-122.

TONEY, M. C.
1958. Morphology of the blood cells of some Crustacea. Growth 22: 35-50.

TRIPP, M. R.
1966. Hemagglutinin in the blood of the oyster *Crassostrea virginica*. J. Invertebr. Pathol. 8: 478-484.
1969. General mechanisms and principles of invertebrate immunity. In G. J. Jackson, R. Herman, and I. Singer (editors), Immunity to parasitic animals, p. 111-128. Appleton Century Crofts, New York.

TUZET, O., AND R. ORMIERÈS.
1961. Sur quelques Grégarines parasites de Crustacés Décapodes. Ann. Sci. Nat. Zool. Biol. Anim. 3: 773-783.

TYLER, A.
1946. Natural haemagglutinins in the body fluids and seminal fluids of various invertebrates. Biol. Bull. (Woods Hole) 90: 213.

TYLER, A., AND C. B. METZ.
1945. Natural heteroagglutinins in the serum of the spiny lobster, *Panulirus interruptus*. I. Taxonomic range of activity, electrophoretic and immunizing properties. J. Exp. Zool. 100: 387-406.

TYLER, A., AND B. T. SCHEER.
1945. Natural heteroagglutinins in the serum of the spiny lobster, *Panulirus interruptus*. II. Chemical and antigenic relation to blood proteins. Biol. Bull. (Woods Hole) 89: 193-200.

UNESTAM, T.
1965. Studies on the crayfish plague *Aphanomyces astaci*. I. Some factors affecting growth *in vitro*. Physiol. Plant. 18: 483-505.

UZMANN, J. R., AND E. B. HAYNES.
1969. A mycotic gill disease of the pandalid shrimp, *Dicephalopandalus leptocerus* (Smith). J. Invertebr. Pathol. 12: 275-277.

VAGO, C.
1966. A virus disease in Crustacea. Nature (London) 209: 1290.

WATERMAN, T. H.
1960. Physiology of Crustacea. Vol. I, 670 p. Academic Press, New York.

WEINHEIMER, P. F., R. T. ACTON, S. SAWYER, AND E. E. EVANS.
1969. Specificity of the induced bactericidin of the West Indian spiny lobster, *Panulirus argus*. J. Bacteriol. 98: 947-948.

WEINHEIMER, P. F., E. E. EVANS, R. T. ACTON, AND B. PAINTER.
1969. Induced response of the lobster, *Panulirus argus*. Fed. Proc. 28: 752.

WEINHEIMER, P. F., E. E. EVANS, R. M. STROUD, R. T. ACTON, AND B. PAINTER.
1969. Comparative immunology; natural hemolytic system of the spiny lobster, *Panulirus argus*. Proc. Soc. Exp. Biol. Med. 130: 322-326.

WHITBY, J. L., J. G. MICHAEL, M. W. WOODS, AND M. LANDY.
1961. Symposium on bacterial endotoxins. II. Possible mechanisms whereby endotoxins evoke increased non-specific resistance to infection. Bacteriol. Rev. 25: 437-446.

WOOD, P. C.
1965a. A preliminary note on gaffkaemia investigations in England. Cons. Perm. Int. Explor. Mer. Rapp. P.-V. Réun. 156: 30-34.
1965b. Gaffkaemia, the blood disease of lobsters. J. Gen. Microbiol. 41: 28.

WOOD, P. J., AND L. P. VISENTIN.
1967. Histological and histochemical observations of the hemolymph cells in the crayfish, *Orconectes virilis*. J. Morphol. 123: 559-568.

WOOD, W. B., JR.
1953. Studies on the cellular immunology of acute bacterial infections. Harvey Lect. 47: 72-98.

WOOD, W. B., JR., M. R. SMITH, AND B. WATSON.
1946. Studies on the mechanism of recovery in pneumococcal pneumonia. IV. The mechanism of phagocytosis in the absence of antibody. J. Exp. Med. 84: 387-402.

WOODBURN, K. D., B. ELDRED, E. CLARK, R. F. HUTTON, AND R. M. INGLE.
1957. The live bait shrimp industry of the west coast of Florida (Cedar Key to Naples). Fla. State Board Conserv., Tech. Ser. 21: 1-31.

WRIGHT, A. E., AND S. R. DOUGLAS.
1903. An experimental investigation of the role of the blood fluids in connection with phagocytosis. Proc. Roy. Soc., Ser. B, Biol. Sci. 72: 357-370.

YASAKI, Y.
1927. Bacteriologic studies on bioluminescence. I. Cause of luminescence in the fresh water shrimp, *Xiphocaridina compressa* (De. haan). J. Infec. Dis. 40: 404-407.

YEAGER, J. F., AND O. E. TAUBER.
1935. On the hemolymph cell counts of some marine invertebrates. Biol. Bull. (Woods Hole) 69: 66-70.

ZUCKERKANDL, E.
1956. La variation au cours du cycle d'intermue des fractions proeiques de l'hemolymphe de *Maia squinado* separes par electrophorese. C. R. Séances Soc. Biol. (Paris) 150: 39-41.