PHYSIOLOGY OF THE BLUE CRAB *CALLINECTES SAPIDUS* RATHBUN DURING A MOLT

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INTRODUCTION

As a student in the laboratory of L. M. Passano (1960), I read many of the classic papers on the subject of blue crab molting although I, personally, did not work on it. Later, learning about gas exchange and metabolism in the blue crab (Mangum and Weiland 1975), the casual observations in those papers kept returning to my mind—molting should not be possible.

Long before this study, it was known that blue crabs are not very good anaerobes (Carpenter and Cargo 1957). Like many decapod crustaceans, they are more dependent on their oxygen (O₂) transport system than most other groups of animals. In the temperature range that prevails between the spring and the fall, the hemocyanin (Hc) in the blood of a blue crab transports more than 95% of the O₂ consumed by the tissues and almost none is carried in the free form. This is true because the crab is locked up in a gas-impermeable box that it uses as a skeleton, which does not exist in the same form outside of arthropods. When the O₂ transport system fails, which it sometimes does, the crab cannot sustain life for even an hour by an entirely O₂-independent metabolism.

Many of the casual observations in those old papers suggested that the system does fail during a molt, a time when the metabolic demands should be greater, not smaller, than usual. Specifically, the uptake of water, which lets the crab split the old exoskeleton, dilutes the Hc in the blood by about one-half. Our data show that the Hc levels fall far more than that, to about one-fifth of the intermolt value, which implicates a change in net synthesis or degradation. To compound the problem, a number of the factors that determine the amount of oxygen the blood transports also change. Acid-base balance and blood calcium (Ca²⁺) are perturbed; both hydrogen (H⁺) and Ca²⁺ combine with the Hc molecule and directly influence its O₂ binding. Shedding the old exoskeleton appears to require taxing motor activities and high levels of motor performance which, in turn, require input from anaerobic metabolism (McMahon 1981).

Additionally, a number of investigators believe that the ventilatory appendage cannot function because it is soft, which should inhibit O₂ uptake at the gill (e.g., Lewis and Haefner 1976). Both the exercise and the inhibition of ventilation should activate anaerobic glycolysis, which terminates in lactic acid. Lactate also combines with the Hc molecule and directly determines its O₂ binding (Truchot 1980).

If the system normally fueling metabolism does not work and if the alternative mode of metabolism is not sufficient to sustain life, then how is life possible during a molt? To find out, I persuaded several experts on various aspects of crustacean physiology to join me for a coordinated study of molting in the blue crab *Callinectes sapidus* Rathbun; the results summarized below were obtained by all of us. Because the processes of gas exchange and acid-base regulation are often intimately interwoven with those of mineral and water metabolism, our studies included those areas as well.

MATERIALS AND METHODS

Gas Exchange, Transport and Acid-Base Balance

The information in the literature on total O₂ uptake was contradictory so it was necessary to show that the blue crab behaves like other species, which it does. There was no clear increase in O₂ uptake during premolt, which had been reported for other species. But the rate clearly went up in early postmolt and then gradually returned to the intermolt value (Figure 1). At the tissue level an increase could be seen in the epidermis during premolt as well as postmolt, reflecting the onset of metabolic activity during manufacture of the new skeleton (Figure 1). However, the epidermis is such a small fraction of the total biomass that the change could
Figure 1. Changes in O$_2$ uptake in whole animals and isolated tissues during the intermolt cycle (24 to 26°C, 35 ppt, ambient PO$_2$ 100 to 159 mm Hg). (Data from Mangum et al. 1985).
not be detected in an intact animal. As shown by Mauro and Mangum (1982), total \( O_2 \) uptake was dominated by the locomotor muscle, which comprises about two-thirds of total biomass. In the present study, the interest was in the increase in \( O_2 \) uptake during the postmolt (Figure 1).

Blood \( PO_2 \) rises during premolt as the animal reduces its locomotor activity (Figure 2). At the same time blood \( pH \) rises, which is very important because it effectively loads the blood with \( HCO_3^- \), which will serve as a buffer against the metabolic acids produced later during exuviation. During this period there may be a small increase in ventilation, which would contribute to the rise in blood \( pH \) by blowing off more \( CO_2 \), but this is not shown in Figure 3 because it was not significant and it appeared to be too small to explain the change in full. During bursting blood pressure begins to rise as the animal takes up water (Figure 3). During actual exuviation blood pressure is 5.5 times that during internmol. It is this pressure that structurally substitutes for the loss of the hard exoskeleton and allows the animal to perform the muscular movements necessary to shed the exoskeleton. In essence the animal reverts to a more primitive form of support, the hydrostatic skeleton.

Measuring ventilation in crabs involves putting a mask over the ecurrent openings of the branchial chambers. The mask prevents exuviation because the sutures can not open; therefore, we were unable to measure ventilation during actual exuviation. Ventilation appeared to cease momentarily when the scaphognathite was being pulled out. We were able to measure ventilation at all other times. It was very clear that flow does not decrease much less cease during bursting (Figure 3). Blood \( PO_2 \) increases at the gill and decreases at the tissues (see Figure 2), which could be due to a decrease in blood flow thereby permitting longer equilibration times at both sites. Heart rate decreases at the time (deFur et al. 1985). Hemocyanin levels begin a sharp decline (Figure 2).

During exuviation blood \( PO_2 \) falls to very low levels, both at the gills and the tissues (Figure 2). Blood \( PCO_2 \) rises and \( pH \) falls from its premolt levels (Figure 2). However, because of premolt alkalosis, blood \( pH \) never drops below the internmol value, at least not as long as the animal survives. This is true even though \( PCO_2 \) is high and lactate begins to accumulate (Figure 2), which is very unusual and certainly a critical event in the success of a molt. This conclusion is underscored by what happens in animals that do not survive a molt. We made a number of measurements on animals that either did not shed or emerged with morphological aberrations of the gills and legs and died within several hours. In those animals lactate was very high; blood \( PO_2 \) and \( pH \) were lower than the internmol values, and \( pH \) fell on at least one occasion well below 7.0 (Table 1). These data clearly indicate that the immediate cause of death was in fact the failure of the respiratory system.

But what is responsible for an unsuccessful molt? It seems obvious that, if the osmoregulatory (see below) and cardiovascular systems are responsible for the pressure that permits exuviation, the failure to exuviate normally may be due to osmoregulatory or cardiovascular disease. This suggestion is strongly supported by observations on two animals that failed to emerge and died. In those two, blood pressure never rose to the levels shown in Figure 3, indicating that their osmoregulatory and cardiovascular systems were simply unable to generate the very high pressures required for exuviation.

In early postmolt the crab is weak. \( pH \) and \( Hc \) levels are at their lowest; lactate levels are at their highest (Figure 2). This is probably due to the lag between production and reoxidation of lactate rather than a high level of anaerobic metabolism during the postmolt. It is not true that the scaphognathite cannot work when it is soft, but it is true that it does not work as well. Ventilation is only one-half of the internmol rate (Figure 2), but blood \( PO_2 \) rises to levels much higher than internmol values while ventilation is still low (Figure 2). This finding gives us a clue to the answer to our original question. At the same time that the animal has reverted to a more primitive form of skeletal support, it has also reverted to a more primitive form of gas exchange, namely cutaneous.

The internmol carapace is effectively gas tight; however, the new cuticle is no less permeable to oxygen than any other tissue (Mangum et al. 1985). The epidermis, where the new cuticle is being formed, is well within the limiting diffusion distance for \( O_2 \) from the ambient medium. More importantly, so are the superficial blood spaces. The blood-medium diffusion distance across the general body surface of an early postmolt crab is less than twice as great as the distance across the gill of an internmol crab. The whole animal becomes a gill, so to speak.

Blood pressure begins to decline even though water uptake is continuing (Figure 3), which may seem to be paradoxical. However, this is not really surprising and it is almost certainly due to increased urine output. The basic mechanism of urine formation in crabs is filtration. Blood hydrostatic pressure, which forces water out through leaky epithelia such as renal membranes, is kept higher than colloid osmotic pressure. Colloid osmotic pressure, arising from the hydrophilic nature of plasma proteins such as Hcs, tends to suck water back in (Figure 4). In internmol animals the excess of hydrostatic over colloid osmotic pressure is very small, which is why \( Hc \) levels have to be so low. However, in postmolt animals with their hydrostatic skeletons and only one-fifth of their normal complement of \( Hc \), that margin increases by an order of magnitude. The net result must be increased urine flow.

Later in postmolt, when hardening is initiated and gas permeability begins to return to internmol levels, the animal begins to hyperventilate which continues throughout the postmolt (Figure 3). Blood lactate, \( pH \), and \( PCO_2 \) slowly return to normal so these factors should no longer perturb
Figure 2. Changes in blood respiratory and metabolic variables during intermolt cycle (21 to 26°C, 31 to 35 ppt, ambient PO$_2$ 145 to 159 mm Hg). (Data from Mangum et al. 1985.)
Figure 3. Changes in ventilatory and cardiovascular variables during intermolt cycle (21 to 26°C, 31 to 32 ppt, ambient PO₂ 145 to 159 mm Hg). (Data from deFur et al. 1985.)
TABLE 1.
Examples of blood respiratory and metabolic variables in animals that did not survive a molt (21 to 26°C, 31 to 35 ppt, ambient PO₂ 145 to 159 mm Hg).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
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<tbody>
<tr>
<td>pH</td>
<td>6.30 – 7.12</td>
</tr>
<tr>
<td>PCO₂ (mm Hg)</td>
<td>19.0</td>
</tr>
<tr>
<td>PO₂ (mm Hg)</td>
<td>0.5</td>
</tr>
<tr>
<td>Lactate (mM)</td>
<td>3.40</td>
</tr>
</tbody>
</table>

Figure 4. Relationship between blood hydrostatic pressure and colloid osmotic pressure. Width of arrows at top represents relative magnitudes of hydrostatic pressure. Number of circles represents the number of osmotically active colloids.
the O₂ transport system. However, Hc levels continue to remain low, which is somewhat surprising. In fact there should be no selection pressure for maintaining high Hc levels because blood PO₂ remains elevated, probably as a result of hyperventilation; the Hc molecule should not work under those conditions. The O₂ affinity of blue crab Hc under ionic conditions in an early postmolt animal is a little less than 10 mm Hg; however, tissue blood PO₂ is so high that the molecule would not unload—it would remain from 83 to 99% oxygenated (Figure 5).

Can the crab solve this problem? At the outset I said that blue crab Hc is allosterically modulated. However, the system is designed to be modulated during hypoxia by means of lactate production and Ca²⁺ dissolution (Truchot 1980, Mangum 1985). Here we are talking about “hyperoxia” for which the system is not designed. There is, however, another mechanism of adaptation that may operate during adaptation to low salinity, namely, a fundamental change in the Hc molecule (Mason et al. 1983). Hemocyanins are built of more subunits than needed for assembly of the molecule. If the respiratory properties of the subunits differ from one another, then the crab could modify the overall properties of the system by putting the molecule together with different proportions of the subunits. The ideal setting for such an adaptation would be a time of rapid change in net synthesis or degradation, as during molting. Despite the large changes in Hc levels during the molt cycle, however, there is no change in the subunit composition of the Hc molecule and none in the intrinsic properties of the molecule (Mangum et al. 1985).

**Intermediary Metabolism**

In the past 15 years or so it has been shown that many animals utilize pathways of anaerobic metabolism which are more efficient that the mammalian version of glycolysis in terms of energy production. This capability is often correlated with a high tolerance of hypoxia. We had no reason to suppose that intermolt crabs possess this capability but we had to be sure that the success of the molt was not due to the transient acquisition of it, and it was not. Not only did the activities of glycolytic enzyme systems remain unchanged but there was no sign of alternative end products.

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![Figure 5](image-url)  
*Figure 5. Relationship between O₂ binding of Hc and blood PO₂ in intermolt and postmolt crabs. Left member of each pair of arrows directed from abscissa designates value at tissues; right member designates value at gill. Vertical lines at far right indicate amount of HcO₂ delivered to tissues (data from Mangum et al. 1985).*
(Fields 1985). The crab is locked into classical glycolysis. However, there are two metabolic changes of interest. In the epidermis (but not in other tissues), hexokinase activity rises during premolt. This enzyme catalyzes the first step in the metabolism of free glucose, which could facilitate glycolysis. Secondly, during premolt the activities of pyruvate kinase and lactate dehydrogenase decrease in locomotor muscle (but not in other tissues). These enzymes are central to anaerobic pathways and the decreases are very likely responsible for the cessation of high levels of motor activity, which require input from anaerobic pathways.

**Water Uptake**

It is generally believed that water uptake during a molt occurs through the intestinal epithelium, a phenomenon that has been demonstrated directly in osmoconforming species such as the lobster (Mykles 1980), which lacks the ionic machinery necessary to actively absorb Na⁺ at the gill. Dr. W. A. Van Engel (pers. comm.) has shown that the blue crab inflates its gut with seawater during a molt, which almost certainly contributes to the rise in blood pressure. However, this process is probably better termed "imbibition" rather than "drinking," which implies transepithelial water movement. In the blue crab, a fairly strong osmoregulator, water uptake during a molt occurs primarily at the gill (Cantelmo 1976). The mechanism may be isosmotic fluid transport by the same enzymes that function during active salt uptake at low salinity. Even in isosmotic animals held at high salinity, clear increases in the activity of the branchial Na⁺ + K⁺ – ATPase coincide with the onset of water uptake and persist throughout postmolt (Figure 6). These enzymes transport Na⁺. For many years investigators have been searching for comparable enzymes that transport Cl⁻. A popular hypothesis has been an enzyme that can carry out a Cl⁻ – HCO₃⁻ exchange. However, no one has been able to find an enzyme with the properties that ideally it should have, namely, activation by both members of the postulated exchange. There is an enzyme that is activated by HCO₃⁻ and has a small and absolute requirement for Cl⁻. During premolt the activity of this enzyme appears to increase in the gills of the blue crab, but the trend is not shown in Figure 6 because it was significant at only one stage and it preceded by far the onset of water uptake.

**Calcification**

During a molt the activity of Na⁺ + K⁺ – ATPase increases in the epidermis (but not the gut) as well as in the gill, and the timing coincides with that of Ca deposition (Figure 6). Earlier workers reported that calcification was sensitive to ouabain, a specific inhibitor of this enzyme. The mechanism of action, however, remains obscure and the chronology is also consistent with the possibility that the increase is associated with water uptake across the cuticle and not calcification.

It is unlikely that the soft-crab industry could use ouabain to keep crabs soft. While a crab can live for a week in water containing 10⁻³ M ouabain, reflecting its impermeability, injection of the inhibitor into the blood causes immediate neurological damage resulting in impairment of ventilatory and cardiovascular functions followed by death (Mangum, deFur and Polites, unpublished observations). Incidentally, it also appears to be unlikely that crabs can be kept soft by injecting inhibitors of protein tanning. Twice daily injections of mimosine and kajic acid (final concentration 5 × 10⁻⁴ M), beginning at A₁ and continuing for two days had no perceptible effect on hardening, although the effect of gentisic acid was less clear. In all three cases mortality was heavy (Mangum, unpublished observations).

The mechanism of action of another enzyme, carbonic anhydrase (CA), is ostensibly more clear. This enzyme catalyzes the equilibrium reactions between molecular CO₂ and water and carbonic acid, which are believed to furnish the form of CO₃⁻ that will be precipitated:

\[
\text{CA} \\
\text{CO}_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-
\]

So it remains a puzzle that CA activity in the epidermis does not increase until postmolt, long after Ca deposition is initiated (Figure 6). The increase may, however, be responsible for the transition from low levels of calcification to maximal rates.

In osmoregulating crabs and also other animals the gills are specialized for different functions. The anterior gills, which have very thin epithelia, are the gas exchangers. The posterior gills, which have more tissue, are the salt pumps. The CA activity in both sets increases dramatically during the molt cycle, but at quite different stages. The gills, of course, are not calcified so increases in activity cannot be associated with that process. When an ion is taken up from the medium, electrochemical balance across the transporting epithelium must be maintained often by the extrusion of another ion of the same charge. Henry and Cameron (1983) have raised the possibility that during hyperosmotic regulation the branchial CA supplies the counterions for Na⁺ and Cl⁻ uptake in the form of H⁺ and HCO₃⁻. However, in isosmotic animals taking up water prior to a molt, the synchronization between the two enzymes is not very tight, and we are still left with the problem of finding a carrier for the postulated exchange of Cl⁻ for HCO₃⁻.

Additionally, there is an aspect of molting that presents a special problem for the hypothesis that CA functions to provide the counterions exchanged for the NaCl absorbed during isosmotic water uptake. For many years it has been known that most of the Ca laid down in the new skeleton must be taken up from the medium [see Sparkes and Greenaway (1985) for an interesting exception]. It is becoming increasingly clear that the HCO₃⁻ must also be taken up from the ambient medium (Dejours and
Figure 6. (A) Activities of \( \text{Na}^+ + \text{K}^+ \) ATPase and \( \text{HCO}_3^- \) ATPase in the posterior gills, gut and epidermis of the blue crab during intermolt cycle (data from Towe and Mangum 1985). (B) Activities of carbonic anhydrase in the anterior and posterior gills and in the epidermis (data from Henry and Kormanik 1985).
Beekenkamp 1978, Cameron [pers. comm.], and for the same reasons. Despite those increases in blood HCO₃⁻, there is not nearly enough inside the animal. So if HCO₃⁻ were extruded for the Cl⁻ taken up isosmotically, the gill would immediately have to turn around and take up the HCO₃⁻ again for calcification! That is probably not possible as I have stated it because the gill is ventilated at such a rate that the extruded HCO₃⁻ would be lost. It is conceivable that the extrusion-resorption takes place within the gill epithelium (Figure 7) although how the process could work at the molecular level is far from clear. We know that the Na⁺ + K⁺ - ATPases are located on the blood side of the gill epithelium and it is possible that the postulated anion pumps are as well. However, the very kinetics of the process would suggest that the same carrier cannot perform in both directions at once. All of these problems remain for future investigation.

Changes in Intracellular Free Amino Acids

Changes in the intracellular pool of free amino acids (FAA) are usually associated with volume readjustment during adaptation to a change in salinity. However, two previous investigations showed that the size of the pool changes during the molt cycle. In one study the change was attributed to a cycle of muscle fiber atrophy and reformation that occurs in the claw (Yamaoka and Skinner 1976; also Skinner, pers. comm.). Muscle proteins must be broken down to lower the volume of the claw to the point where the crab can get it out, and then the muscle proteins must be resynthesized so that the crab can use the claw again. These events are reflected in the changes of O₂ uptake seen in isolated claw muscle tissue (Figure 7). The claw, incidentally, is not functional at this time, which is not disadvantageous because the crab is not feeding and because a soft claw would not be of much use for defense. In another study a decrease in FAA was found in muscles that almost certainly do not atrophy (Duchateau et al. 1959), judging from their uninterrupted functional capabilities. This finding could conceivably be just a special form of volume readjustment following a salinity change. A hyperosmotic crab was taking up hypoosmotic water, which would swell the tissues. But in blue crabs taking up isosmotic water there are still decreases in the FAA pool in somatic muscles (Figure 8). The FAA could either be used in biosyntheses or simply excreted. In either case the reduction of the size of the FAA pool should lower tissue water content, which

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**Figure 7.** Model of gill cell showing complexity of the postulated movements of inorganic ions during a molt. Ions move passively into the branchial epithelium from the seawater. Na⁺ is absorbed in exchange for an unspecified monovalent cation and Cl⁻ perhaps for HCO₃⁻. HCO₃⁻ is also absorbed in exchange for an unspecified monovalent anion. The same exchanges take place from the gill cell to the blood by active transport. At least some of the HCO₃⁻ moved here is recycled.
Figure 8. Changes in free amino acids (FAA) in somatic muscle (data from Wheatly 1985) and ammonia excretion (Mangum, unpublished data) during intermolt cycle. For ammonia excretion, mean ± S.E. (N). Postmolt values adjusted for change in body weight due to water uptake. Arrows indicate significant (P < 0.01 according to Student's t test) changes, analyzed as paired observations on the same individuals (N = 2 to 9).
has in fact been observed (Wheatly 1985), and by implication the volume of the tissues. Assuming moderate values for blood flow and clearance, the increase in ammonia excretion (Figure 8) is more than enough to explain the fate of the amino group of the FAA, although the fate of the carbon skeleton remains unknown. Thus our data suggest that the loss of FAA from muscle serves to shrink the organs and to facilitate exuviation. Whether it also furnishes a keto acid for the metabolic processes associated with formation of the new skeleton cannot yet be decided.

The finding of isosmotic loss of FAA from tissues that were never swollen has interesting implications for the postulated mechanisms of extrusion. According to the hypothesis formulated by Pierce and Greenberg (1973), the gates in the cell membrane that normally prevent FAA loss pop open passively due to stretching in swollen cells, although an active process is required to close them again after volume readjustment. This simple model, which was formulated in the context of bivalve mollusk cells, does not provide for a passively generated force in unswollen cells, suggesting that an alternative process may occur in crustacean muscle, at least during a molt and possibly during adaptation to low salinity as well.

**REFERENCES CITED**


BLUE CRAB (CALLINECTES SAPIDUS RATHBUN) VIRUSES AND THE DISEASES THEY CAUSE

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INTRODUCTION

The first virus of a marine invertebrate was found about 20 years ago (Vago 1966). Since then, more than 40 viruses have been reported from a variety of marine invertebrates and many of them are from crustaceans, mainly decapods (Johnson 1983, 1984; Lightner and Redman 1985). The viruses of decapods can seldom be assigned with certainty to particular families because their biochemical and immunological characteristics are incompletely known. In development and morphology, the viruses are related to the various families listed in Table 1. Almost all of the decapod viruses discovered so far are from species of portunid crabs and penaeid shrimp, presumably reflecting the fact that these decapod groups have been most intensely studied. Viruses are important pathogens of cultured penaeid shrimp (Lightner 1983) and some species cause mortality in wild and captive populations of crabs (Johnson 1983, 1984). Seven, perhaps eight, viruses occur in the blue crab Callinectes sapidus Rathbun and some of them are associated with disease in their hosts.

DNA VIRUSES OF THE BLUE CRAB

Herpes-like Virus

Herpes-like virus (HLV) was found in crabs from Assawoman Bay, Delaware, and Chincoteague Bay, Virginia (Johnson 1976a, 1978). It has not been found elsewhere.

HLV infects hemocytes, occasionally hemopoietic cells, and probably connective-tissue cells and epithelial cells of the gill. Infected nuclei are greatly hypertrophied and either contain Feulgen-positive granules or are homogeneous and faintly to deeply Feulgen positive. Infected nuclei may contain large Feulgen-negative inclusions and similar inclusions regularly occur in the cytoplasm (Figure 1). The cytoplasm of severely infected cells is reduced to a thin rim, and release of virus is by lysis of the nucleus. Hemolymph of a terminally infected crab is filled with free virus and granular material probably derived from the breaking up of the cytoplasmic and nuclear inclusions. The central cylindrical nucleoid of HLV is surrounded by a toroid and the envelope of a fully developed virion consists of two membranes. The enveloped virion is hexagonal with a diameter of 185 to 214 nm (Figure 2). Entire development, including envelopment, takes place in the nucleus.

Prevalence of HLV in a natural population of juvenile blue crabs can be 13% (Johnson 1983). Infected crabs appear normal until shortly before death, when they become inactive and cease feeding. Hemolymph withdrawn from terminally infected crabs is an opaque, chalky white, and has lost the capacity to gel. Final diagnosis of HLV disease depends on finding typical infected cells in paraffin-embedded tissue sections, but reasonably firm diagnosis of terminal infections can be made on the basis of the hemolymph being chalky white and nongelling.

Death probably occurs because of hemocyte dysfunction and loss. Whether HLV-infected crabs ever recover is unknown. Crabs injected with hemolymph from moribund HLV-infected crabs can die in 30 to 40 days (Johnson 1978), but natural infections and some experimental ones may take much longer before causing death. Moderate to heavy HLV infections were found in juvenile crabs that had been kept in the laboratory for 50 days in separate containers with separate water supplies, showing that virus was present before capture, and some experimentally infected crabs were only lightly infected and still normal in appearance 65 days after injection (Johnson 1984). HLV caused an epizootic with a high mortality rate in juvenile crabs held in separate containers but with a common water supply, indicating that HLV disease is highly infectious by the water route.

Baculoviruses

Both of the blue crab baculoviruses are “nonocluded.” That is, the virions lie free in the nucleus rather than being “ocluded” within proteinaceous occlusion bodies that are often polyhedral. Nuclei infected with baculoviruses are hypertrophied, usually evenly Feulgen positive and often rimmed with strongly staining chromatin.

Baculovirus A (Baculo-A) infection is widespread along the Atlantic coast and its distribution in blue crabs may be general. Prevalence of the virus in blue crab populations usually varies from 4 to 20%, although 18 of 34 crabs (52%) were infected in one group taken from Chincoteague Bay, VA (Johnson 1976b, 1983). Baculo-A infects nuclei in the epithelium of the hepatopancreas. Infected nuclei are typically twice normal size and stain rather weakly by the Feulgen method (Figure 3). Even in heavy infections,
the virus appears not to affect the crabs, probably because it is almost always focal in nature and epithelial cells of the hepatopancreas are constantly being replaced by stem cells, which are not themselves infected. Development of Baculo-A is associated with intranuclear tubule-like structures which probably represent forming capsids (Figure 4). The enveloped virion is about 70 × 285 nm, and virions tend to form ordered arrays.

Infection with Baculo-A can be diagnosed with the light microscope by presence of hypertrophied nuclei in the epithelium of the hepatopancreas.

Baculovirus B (Baculo-B) of hemocytes occurs in crabs from Chesapeake Bay and its tributaries. Nuclei of hemopoietic cells and hemocytes are infected by Baculo-B. Infected nuclei may be completely homogeneous or rimmed with chromatin (Figure 5). Occasionally, there are hyperchromatic areas in the center of the nucleus. Nuclear hypertrophy is not as marked as in HLV (compare Figures 1 and 5), and there are no refractile nuclear or cytoplasmic inclusions. Usually, Baculo-B-infected nuclei are more strongly Feulgen positive than ones infected with either HLV or Baculo-A. Virions have tapered and rounded ends, so that the virion is slightly ovoid (Figure 6). Enveloped virions are approximately 100 × 335 nm. They have a tendency to form ordered arrays in the nucleoplasm, and their development is associated with intranuclear vesicles rather than long tubule-like structures as in Baculo-A. Diagnosis depends on finding characteristic hypertrophied nuclei in hemocytes by light microscopy.

The effect of Baculo-B on its host is unknown. Some of the crabs studied had naturally acquired infections, but others had been used in attempts to transmit various viruses (Johnson 1983). The latter may have had preexisting infections or the virus might have been accidentally transmitted to them. Some of the experimental crabs were sick, but because they were also infected with other viruses, the effect of Baculo-B could not be determined. Heavy infections result in loss and dysfunction of many hemocytes. One may suspect that the host is affected adversely in such cases.

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**RNA VIRUSES OF THE BLUE CRAB**

**Reo-like Virus**

Reo-like virus (RLV) is common in crabs from Chincoteague and Chesapeake bays, and is associated with fatal disease (Johnson and Bodammer 1975; Johnson 1983, 1984). Ectodermally and mesodermally derived tissues are infected, principally hemocytes, hemopoietic tissue and the glia of the nervous system. Epidermis, gill and bladder epithelia, endothelia of blood vessels, Y-organ, and various connective-tissue cells may also be infected. Infected hemocytes and hemopoietic tissue contain basophilic, Feulgen-negative, cytoplasmic inclusions that often are angulate but

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**TABLE 1.**

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<tr>
<th>Family</th>
<th>Host (Number of Viral Species)</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Herpesviridae</td>
<td>Callinectes sapidus (1)</td>
<td>Payen and Bonami 1979</td>
</tr>
<tr>
<td>ds DNA, enveloped, nucleus</td>
<td>Rhithropanopeus harrisii (1)</td>
<td>Johnsen 1976a</td>
</tr>
<tr>
<td>Baculoviridae</td>
<td>Callinectes sapidus (2)</td>
<td>Johnson 1976b, 1983</td>
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<td>Bazin et al. 1974</td>
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<tr>
<td></td>
<td>Carcinus mediterraneus (1)</td>
<td>Pappalaro and Bonami 1979</td>
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<tr>
<td></td>
<td>Penaeus spp. (3)</td>
<td>Couch 1974, Sano et al. 1981,</td>
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<td></td>
<td></td>
<td>Lightner and Redman 1981</td>
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<td>ss DNA, nonenveloped, nucleus</td>
<td></td>
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<tr>
<td>Reoviridae</td>
<td>Callinectes sapidus (1)</td>
<td>Johnson and Bodammer 1975</td>
</tr>
<tr>
<td>ds RNA (10–12 segments), nonenveloped, cytoplasm</td>
<td>Carcinus mediterraneus (1)</td>
<td>Bonami 1976</td>
</tr>
<tr>
<td></td>
<td>Macropipus depurator (1)</td>
<td>Vago 1966</td>
</tr>
<tr>
<td>Bunyaviridae</td>
<td>Carcinus maenas (1)</td>
<td>Bang 1971</td>
</tr>
<tr>
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<td>Bonami and Vago 1971, Zerbib et al. 1975</td>
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<td></td>
<td>Macropipus depurator (2)</td>
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<td>Callinectes sapidus (2 or 3)</td>
<td>Jahrmi 1977, Yudin and Clark 1978,</td>
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<td>Johnson and Farley 1980</td>
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<td>Chassard-Bouchaud and Hubert 1975</td>
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<td></td>
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<td>Bonami 1976</td>
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<td>Penaeus spp. (1)</td>
<td>Lightner et al. 1983</td>
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Figures 1 through 6. (1) Hemocytes infected with HLV. Arrow points to a normal nucleus. Bar = 10 μm. N, infected nucleus; i, cytoplasmic inclusion. Figures 3 and 5 are to the same scale. (2) Electron micrograph of HLV. Bar = 106 nm. Figures 4 and 6 are to the same scale. (3) Baculo-A infecting nuclei of the hepatopancreatic epithelium (arrows). (4) Electron micrograph of Baculo-A. Note tubule-like structures (arrows). (5) Hemocytes infected with Baculo-B. Arrow indicates a normal nucleus. N, infected nucleus. (6) Electron micrograph of Baculo-B. Note associated vesicles.
may be rounded. Major portions of the glia of the brain or thoracic ganglion, or both, may be necrotic and invaded by hemocytes. As infection progresses, crabs become sluggish, refuse to eat, and often suffer progressive paralysis. Hemolymph does not gel. When an infected crab is held out of water, the posterior pair of legs may tremble while the others hang limply. With electron microscopy, the cytoplasmic inclusions are seen to be paracrystalline arrays of virus particles (Figure 7). The virions of RLV are icosahedral and about 55 to 60 nm (Johnson 1977a, b).

A rhabdo-like virus (RhVA) was also present in tissues of crabs infected with RLV and examined by electron microscopy (Johnson 1983, 1984). This virus, which is described below, apparently acts synergistically with RLV to cause paralysis. Other viruses may occur in crabs infected with RLV, including another rhabdo-like virus (EHV) and the baculoviruses, Baculo-A and Baculo-B.

Injection of hemolymph infected with RLV+RhVA into healthy crabs causes disease that can kill some crabs, particularly pre- and postmolt animals, in as little as 3 to 4 days. RLV+RhVA probably enter through the gut epithelia in nature because these viruses can be transmitted orally by feeding of infected tissues. In this case, first deaths in intermolt crabs may not occur until 12 days after feeding (Johnson 1978, 1983). In the stressful environment of a shedding tank, the viruses possibly also enter through wounds or by other routes.

Prevalence of RLV+RhVA infection in natural populations is unknown. Crabs diagnosed as being infected usually had been held for a few to many days in the laboratory before exhibiting signs of infection, and transmission may have been occurring in the laboratory tanks.

Diagnosis of RLV+RhVA infection depends on demonstration of the viruses by electron microscopy of tissues of sick crabs that come from groups with signs of sluggishness, paralysis, nonseeding hemolymph, and with typical lesions in the nervous system and/or hemocytes and hemopoietic tissue. Electron microscopy need be performed only on one or two crabs in such groups for reasonably firm diagnosis of RLV+RhVA as cause of the morbidity or mortality, if bacterial disease has been ruled out as a major factor.

Rhabdo-like Virus A. The smallest rhabdo-like virus, Rhabdo-like virus A (RhVA), occurs in populations of blue crabs from the Atlantic and Gulf coasts and is probably ubiquitous in the blue crab. Jahromi (1977), Johnson (1978), and Yudin and Clark (1978) found RhVA in crabs stressed by culture conditions, infections with other viruses, and eyestalk ablation, respectively. RhVA is either bacilliform with rounded ends and 20 to 30 x 110 to 170 nm, or flexuous, the same diameter, and up to 600 nm in length.

Figure 7. Electron micrograph of cells jointly infected with RLV, RhVA, and EHV. Bar = 310 nm. E, mature extracellular virions of EHV associated with basal laminae; N, nucleus; R, RLV; r, RhVA; X, intracellular nucleocapsids of EHV.
(Figure 7). It buds into the endoplasmic reticulum, and infects many different tissues. It occurs regularly in glial cells of ganglia and the larger nerves, and is common in hemocytes, hemopoietic tissue, connective tissue, and various epithelia except those of the gut and antennal gland. It does not infect striated muscle and may not infect the Y-organ (Johnson 1978, 1983), but does infect the mandibular gland (Yudin and Clark 1978, 1979). RhVA occurred with one or more other viruses in crabs whose tissues were examined by electron microscopy by Johnson (1978, 1983). As stated earlier, sick crabs infected with RLV probably always have an associated infection with RhVA, and these two viruses are thought jointly responsible for the glial necrosis typical of the mixed infection.

RhVA cannot be identified by examination of tissues with the light microscope and proof of its presence in tissues rests upon examination with the electron microscope.

**Enveloped Helical Virus.** A second and larger rhabdov-like virus is the Enveloped Helical Virus (EHV) reported by Johnson and Farley (1980). EHV infects crabs in Chesapeake Bay and its tributaries. It also occurred in crabs from Chincoteague Bay and the eastern coast of Florida that had been used in various attempts to transmit viruses. The virions are either ovoid or somewhat rod-like. Ovoid forms are about 105 × 194 nm, and rod-like virions of the same diameter are up to 300 nm long. There are projections on the outer surface of the enveloping membrane. Mature virions form by budding through the plasma membrane and are found only extracellularly. They are often associated with basal lamina or lie between the basal lamina and the plasma membrane (Figure 7). Granular virogenic stromata in the cytoplasm of infected cells produce sinuous helical nucleocapsids (Figure 7). Virus was seen budding from hemocytes and hemopoietic-tissue cells, and was associated with the basal lamina of certain connective-tissue cells. Because presence of EHV (like that of RhVA) cannot be distinguished by examination of tissues with the light microscope and because it has been found only with other viruses, effect of EHV on the host cannot be appraised.

**Rhabdo-like Virus B.** Rhabdo-like Virus B (RhVB) is known only from the report by Yudin and Clark (1978), who discovered it extracellularly beneath the basal lamina of the mandibular organ. The ovoid virions were said to be 50 to 70 × 100 to 170 nm, and the enveloping membrane had surface projections. RhVB occurred in crabs from the Gulf of Mexico, was found in approximately 3% of 60 mandibular organs examined by electron microscopy and, in one case, was associated with RhVA. Crabs with RhVB infection appeared normal.

Because EHV and RhVB are strikingly similar in all but reported size, it is possible that they represent a single species of virus and that differences in measuring techniques may be responsible for the supposed differences in size.

**Chesapeake Bay Virus.** The picorna-like virus of the blue crab, Chesapeake Bay Virus (CBV), was discovered in a captive group of young crabs collected from Tangier Sound, Chesapeake Bay (Johnson 1978, 1983). It has not been seen by electron microscopy in tissues of wild crabs, but lesions suggestive of CBV disease are sometimes seen with the light microscope in tissues of crabs from other parts of Chesapeake Bay and from Assawoman Bay, DE.

CBV is associated mainly with tissues of ectodermal origin. It infects the cytoplasm of neurosecretory and other nervous cells (but not the glia), epidermis, gill and bladder epithelia, and epithelia of the fore- and hindgut. Hemopoietic tissue and hemocytes may be infected but many crabs with CBV do not have such involvement. Infection is often focal, with patently infected cells, recognizable with the light microscope, occurring in limited groups. Infected cells are usually hypertrophied and the cytoplasm is filled with Feulgen-negative, homogeneous material consisting almost entirely of virus (Figure 8). Although infection is often focal, the gill epithelium may be almost completely infected and destruction of the entire retina is common. The virions are approximately 30 nm in diameter, sometimes are in paracrystalline array, and may be associated with membranes.

![Figure 8. Electron micrograph of CBV. Bar = 310 nm.](image-url)
Preliminary diagnosis of CBV disease can be made on the basis of behavioral signs and presence in target tissues of characteristically hypertrophied cells with dense Feulgen-negative cytoplasm. Final diagnosis depends on demonstration of virus by electron microscopy.

**IMPLICATIONS OF VIRAL INFECTION TO SHEDDING OPERATIONS**

Couch (1974) and Couch and Courtney (1977) have shown experimentally that the stress of crowding or exposure to certain chemicals can increase prevalence and intensity of a viral disease in captive penaeid shrimp. Presumably, the same process could occur in crabs being held in the crowded environments of shedding tanks.

Viral infections in shedding-tank crabs could come from two sources: preexisting infection and infection acquired in the tank. Casual evidence suggests that viral disease can be epizootic in nature at certain times in certain crab populations. Premolt crabs in early or latent stages of infection, taken from a population undergoing an epizootic, would offer multiple sources of infection. The epizootic could be continued by transmission within the shedding tank, particularly among white-sign crabs (molt stage D1-D2), which might be expected to stay at least a week in the tanks before molting.

Viruses causing rapid death would appear most likely to be involved in shedding-tank mortalities. RLV+RhVA is a combination that fits this criterion. They kill rapidly and damage to nervous tissue may be the reason. In premolt crabs, such damage would be particularly grave because the events that take place during that stressful time are under nervous control. In an experimental series of crabs injected with RLV+RhVA, pre- and postmolt animals were the first to succumb to viral infection. Crabs that died on post-injection days 3-7 were either pre- or postmolt; deaths in intern molt crabs did not occur until post-injection day 11 (Johnson, unpublished data).

Necrosis in gills of the nervous system had occurred in dead and moribund crabs taken from two shedding-tank mortalities I studied. In the first case, RLV+RhVA, plus EHV, were found by electron microscopy in tissues of one of the crabs and presumably other animals with similar lesions were also virus infected. In the second mortality, electron microscopy was not performed, but lesions in the nervous system of affected crabs were similar to the above and, tentatively, are considered to have been due to infection with RLV+RhVA (Johnson, unpublished data).

Evidence, although fragmentary, suggests that viral infection may be a cause of shedding-tank mortality and warrants further investigation.

**REFERENCES CITED**


