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Gaffkemia, the Fatal Infection of Lobsters (Genus *Homarus*) Caused by *Aerococcus viridans* (var.) *homari*: A Review

JAMES E. STEWART

Abstract—A review of recent literature is given concerning the fatal septicemia in lobsters caused by the bacterium, Aerococcus viridans *(var.)* homari *(formerly* Gaffkya homari*). Decreasing salinity may increase or decrease the time to death in lobsters, depending upon whether or not the animals have been infected before or after the salinity change occurred. As the disease progresses, glycogen, ATP, glucose, lactic acid, and nonprotein nitrogen all decrease. Impairment of the major metabolic processes is suggested as the probable cause of death. A degree of host resistance can be induced by administration of appropriate vaccines.*

INTRODUCTION

Gaffkemia is a fatal bacterial disease which periodically causes heavy mortalities among American and European lobsters (*Homarus americanus* and *Homarus vulgaris*, respectively). The disease and the causative agent, *Aerococcus viridans* (var.) *homari* (formerly *Gaffkya homari*)[1], were described originally by Snieszko and Taylor (1947) and Hitchner and Snieszko (1947). The septicemic nature of the disease and the taxon assigned to the pathogen prompted the name "gaffkemia" (Roskam, 1957) for the disease. Severe losses from gaffkemia are experienced periodically in commercial lobster units. Two recent reviews (Stewart and Rabin, 1970; Sindermann, 1971) have dealt in depth with this disease. The background material from these articles is summarized below. The causative agent, *A. viridans* (var.) *homari*, is a gram positive, catalase negative, beta-hemolytic, tetrad forming coccus which appears to possess no exoenzymes (Snieszko and Taylor, 1947; Hitchner and Snieszko, 1947; Hucker, 1957; Stewart et al., 1969b). This lack of exoenzymes and consequent lack of invasive powers results in the bacterium being transmitted only through breaks or ruptures in the integument of the host which permit entry to the hemolymph. The exposure to *A. viridans* (var.) *homari* must come simultaneously with or soon after integumental rupture for the pathogen to circumvent the lobster's rapid and effective wound healing mechanism. Transmission does not occur when the lobster ingests infected material since the acidity of the gastric fluid (pH 5.0) is lethal to *A. viridans* (var.) *homari*. Despite the pathogen's lack of invasive powers, the development of epizootics is aided by the aggressive behavior of lobsters, which provides wounds for entry, and by the fact that normal lobsters have no apparent resistance to the pathogen after entry. Small numbers of a virulent strain of *A. viridans* (var.) *homari*, consisting of 10 or less per kilogram of the lobster's weight, injected into the hemolymph are sufficient to ensure a fatal infection. Injection, however, of large numbers (6×10^8/kg) does not accelerate the infection sufficiently to produce a significant decrease in time to death. There are no external signs of the disease with the exception that as the infection proceeds the lobsters become lethargic and progressively weaker until they die.

As the disease develops, the lobster suffers a massive decrease of circulating hemocytes, resulting in impairment of the clotting mechanism by the removal of the clot initiating factor contained in the hemocytes. The fibrinogen levels and other hemolymph proteins are not affected significantly. As a result of the loss of hemolymph clotting power, the risk of a fatal hemorrhage is introduced in the event of wounding.

[1]See Stewart and Arie (1974) for review of name change.

THE INFLUENCE OF ENVIRONMENTAL FACTORS

Variations in temperature and salinity have been examined and shown to have a marked effect on the disease. Since development of gaffkemia infections in lobsters is strictly temperature dependent, the disease remains largely dormant during the cold-water months. At 1°C the pathogen in vivo does not increase in numbers and the infection is subpatent until the temperature is increased. Then it flourishes and kills the host in a time span directly dependent upon the ambient temperature. The temperature influence on means for times to death (reported in days) were: 172 at 3°C, 84 at 5°C, 65 at 7°C, 28 at 10°C, 12 at 15°C, and 2 at 20°C. Additionally, the pathogen easily survives periods at 1°C, in vivo or in vitro, which are more than sufficient to carry the bacterium from one warm-water season to the next.

Salinity also has an influence on the course of the disease; the lobster, which is a relatively euryhaline animal, is a poor osmoregulator or conversely an "osmoconformer" (Dall, 1970).. Despite its osmoconformity, adaptations to salinity reductions occur slowly and require 75 h at 15°C for complete acclimation (Dall, 1970). It would be expected then that salinity change should in some way alter the pattern of the disease. The nature of the change, however, was quite unexpected (Stewart and Arie, 1973a). When lobsters were acclimated from the normal coastal salinity level of 31.8‰ to reduced salinity levels (26.1‰ and 21.5‰) prior to infection, the times to death upon infection were shortened in proportion to the salinity reduction (Fig. 1). Control animals exhibited no mortalities due to salinity changes. In contrast, when the salinity was reduced after infection an entirely different pattern developed. Reduction in salinity to 26.1‰ after

James E. Stewart is with the Department of the Environment, Fisheries and Marine Service, Halifax Laboratory, Halifax, Nova Scotia, Canada.

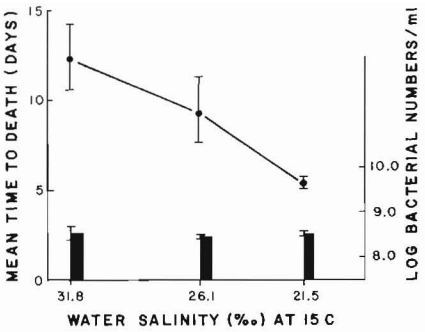

Figure 1.—Mean times to death (MTD) for lobsters infected with *A. viridans* (var.) *homari* and acclimated to water salinities of 31.8, 26.1 and 21.5‰ at 15°C prior to injection (——, MTD; bars indicate log bacterial numbers in lobsters). A total of 120 (60 infected and 60 control) animals were used in groups of 10 lobsters (4 groups at each salinity level). All groups were injected on the seventh day dated from the beginning of the salinity change. Each point represents the mean value plus standard error (SE) for a group of 20 infected lobsters. Uninfected control lobsters are not represented on this graph since none of these animals died (Stewart and Arie, 1973a).

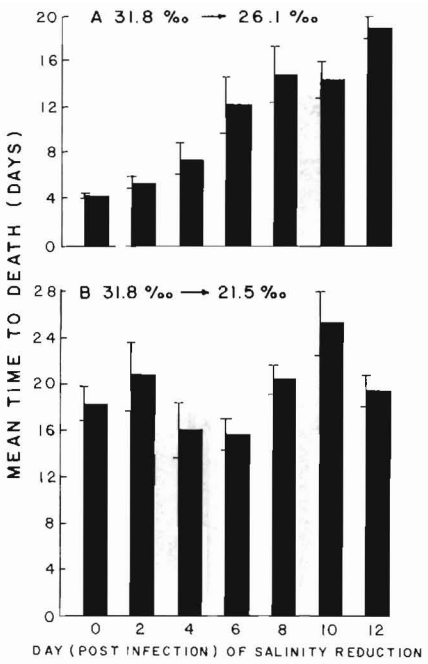

Figure 2.—Mean times to death for lobsters infected with *A. viridans* (var.) *homari* and subjected to water salinity reduction postinfection (over the course of 4 h) on the days designated. Columns represent mean values plus SE for groups of infected lobsters only, since none of the control animals died. (a) Reduction from normal 31.8 to 26.1‰. (b) Reduction of normal 31.8 to 21.5‰. Each vertical column represents a separate group of 10 lobsters plus SE (Stewart and Arie, 1973a).

infection initially decreased the times to death and then progressively increased the times to death (Fig. 2a), while reduction to salinity 21.5‰ after infection at any of the post-infection times increased the time to death (Fig. 2b) over that observed for infected lobsters kept throughout at salinity 31.8‰ (Fig. 1). An extensive series of experiments including sequential measurements of hemolymph nonprotein nitrogen, total carbohydrates, glucose, lactic acid, and serum osmolalities were performed during the course of the infections using both infected and healthy animals in an attempt to discover the basic reasons for the different responses (Stewart and Arie, 1973a). The growth of the pathogen in the infected animals subjected to the reduced salinities was examined, but it did not differ materially from that in infected animals kept at the normal salinity (Stewart and Arie, 1973a). Subjection of *A. viridans* (var.) *homari* to a much wider range of salinity values in vitro did not impair its growth (Stewart and Arie, 1973a). Although the data gained from measuring the various physiological parameters and the growth of *A. viridans* (var.) *homari* in relation to salinity change was interesting, particularly in terms of the responses of the noninfected control lobsters, none of this data nor a search of the literature suggested an explanation for the apparently contradictory results of the combined studies of salinity reductions and disease. Obviously the order in which the dual stresses of disease and salinity reduction are applied is a key factor in understanding the phenomenon. A point of departure for further studies might lie in a thorough examination of infected lobsters subjected to 26.1‰, where a decreased time to death occurs first, followed by a change in response resulting in increased times to death about the sixth postinfection day (Fig. 2).

PATHOGEN GROWTH AND BIOCHEMICAL CHANGES IN THE HOST

It has been concluded that death of lobsters infected with *A. viridans* (var.) *homari* is the result of an unsuccessful competition on the part of the lobsters for their own readily available energy reserves (Stewart et al., 1969a). The evidence for this is found in the pattern of development of the pathogen in vivo in the skeletal muscle, cardiac tissue, hepatopancreas and hemolymph (Fig. 3). Concomitant with the development of the pathogen is the decline of glycogen in the hepatopancreas (Fig. 4), the heart (Fig. 5), and the tail muscle (Fig. 6), and the loss of adenosine triphosphate (ATP) from these tissues (Figs. 7, 8, and 9). The ATP loss was most significant in the hepatopancreas. Parallel to these changes is the decline of the nonprotein nitrogen coincident with the disappearance of glucose and the decline of the lactic acid concentrations of the hemolymph (Fig. 10).

DISCUSSION

The development of the pathogen in vivo (Stewart and Arie, 1973b), the ability of the pathogen to utilize glucose, but not complex polysaccharides (Aaronson, 1956; Stewart and Cornick, 1972), its reliance on the nonprotein nitrogen (Stewart, Arie et al., 1969; Stewart, Foley, and Ackman, 1969) coupled with its inability to utilize proteins (Stewart, Arie et al, 1969; Stewart, Foley, and Ackman, 1969) indicate the following course of events. After entry into the hemolymph the pathogen is concentrated in the hepatopancreas and the heart, possibly as the result of phagocytic action. The subsequent development of bacteria in these two tissues is immediate and rapid, taking place at the expense of free glucose and nonprotein nitrogen. The latter is probably present in greater concentrations in the hepatopancreas than in the heart, which might account for the difference between the final bacterial numbers in these two tissues. As the bacterial numbers increase, especially in the hepatopancreas, the capacity of the phagocytes to transport the pathogen to and retain it in the hepatopancreas is lost through the increasing destruction of hemocytes, thereby permitting the pathogen to flourish in the hemolymph. This aspect of the interaction between the pathogen and the phagocytic mechanism is substantiated by the coincident exponential development of the pathogen in the hemolymph and the sharp decline in hemocyte numbers (Stewart, Arie et al., 1969). Glucose is the only fraction of the total carbohydrates in the hemolymph used by *A. viridans* (var.) *homari* (Stewart and Cornick, 1972; Stewart and Arie, 1973a) which the lobster can sustain at the expense of its glycogen. After exhaustion of the host's glycogen and subsequently glucose, the pathogen can utilize the limited supplies of lactic acid in vitro and apparently also in vivo (Stewart and Cornick, 1972; Stewart and Arie,

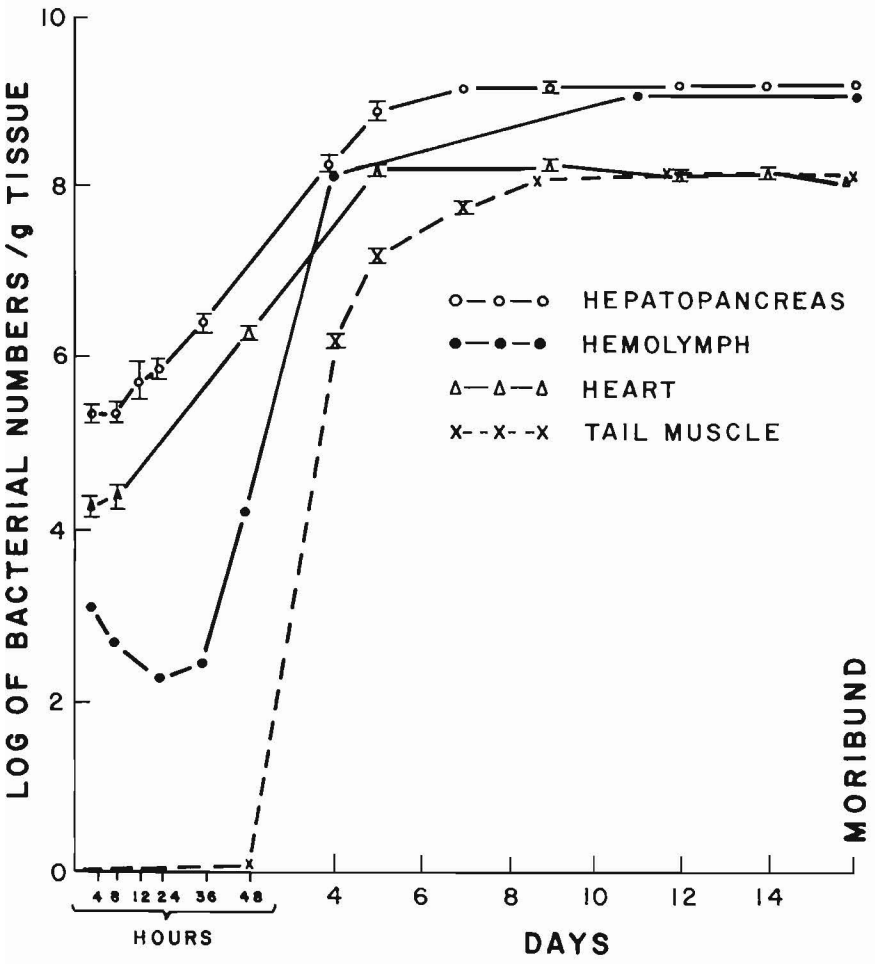

Figure 3.—Growth curves illustrating the development of *A. viridans* (var.) *homari* in the tissues of infected lobsters (Stewart and Arie, 1973b; Stewart, Arie et al., 1969).

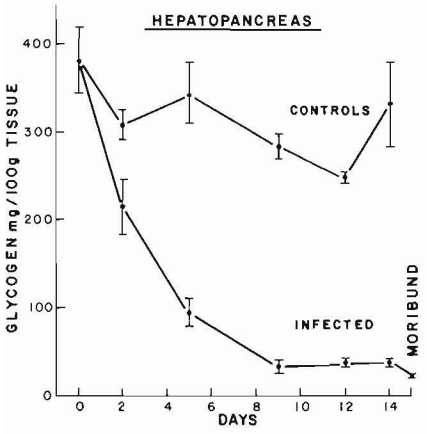

Figure 4.—Glycogen levels in the hepatopancreatic tissue of infected and control lobsters. Each point represents the mean value for five animals plus SE. The infected animals are those for which bacterial numbers are given in Figure 3 (Stewart and Arie, 1973b).

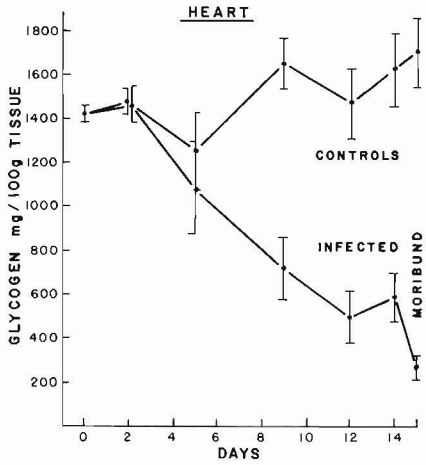

Figure 5.—Glycogen levels in the heart tissue of infected and control lobsters. The infected animals are those represented in Figure 3 (Stewart and Arie, 1973b). Each point plus SE represents a group of five lobsters.

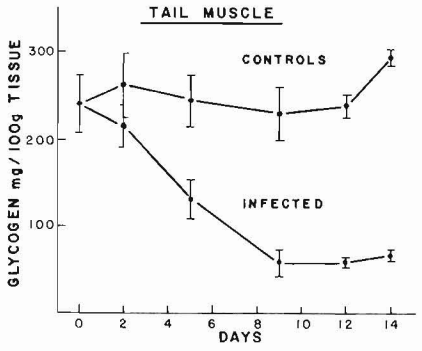

Figure 6.—Glycogen values in the tail muscle of the lobsters represented in Figure 3 (Stewart and Arie, 1973b).

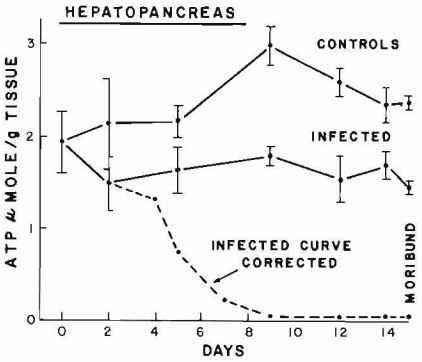

Figure 7.—ATP levels in the hepatopancreatic tissue of the lobsters represented in Figure 3 (Stewart and Arie, 1973b). The solid lines illustrate the actual measurements. The dashed line represents the corrected values obtained by subtraction of the bacterial ATP.

1973a). The drain upon the carbohydrate reserves in turn prevents the production of lobster hepatopancreatic ATP and suggests massive impairment of the vital hepatopancreatic functions of biosynthesis, detoxification, and repair (Stewart and Arie, 1973b). Lipid reserves remain unchanged throughout the course of the infection (Stewart, Arie et al., 1969), while hemolymph proteins, a ready reserve for the lobster (Stewart et al., 1967), are drawn upon only to a slight degree (Stewart, Arie et al., 1969). The possibility of replenishing the reserve materials from external sources is lost to the lobster by its refusal to feed after the onset of the infection. At the standard temperature of these studies, 15°C, lobsters will feed on the second day after being infected but refuse food thereafter (Stewart et al., 1972). The presumed reason for the refusal of food is the development of massive numbers of bacteria in the hepatopancreas, an organ which in crustaceans encompasses the function of liver and pancreas in vertebrates and also plays a prominent part in primary food absorption, a role fulfilled in vertebrates by the small intestine (Vonk, 1960). A soluble bacterial toxin would appear to be ruled out as a direct cause of death, since filter sterilized serum removed from heavily infected lobsters and then injected into healthy animals in amounts equal to more than 13% of the lobsters' body weights had no adverse

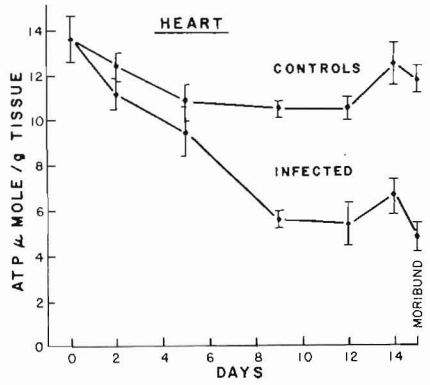

Figure 8.—ATP levels in the heart tissue of lobsters represented in Figure 3 (Stewart and Arie, 1973b). Bacterial ATP levels did not form a significant proportion of the total.

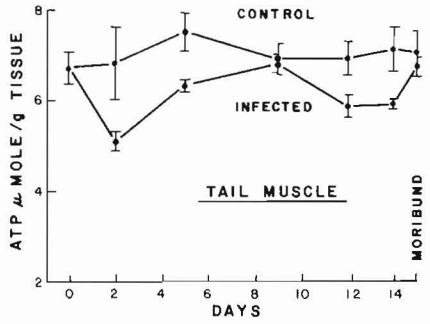

Figure 9.—ATP levels in the tail muscle tissue of lobsters represented in Figure 3 (Stewart and Arie, 1973b). Bacterial ATP levels did not form a significant proportion of the total.

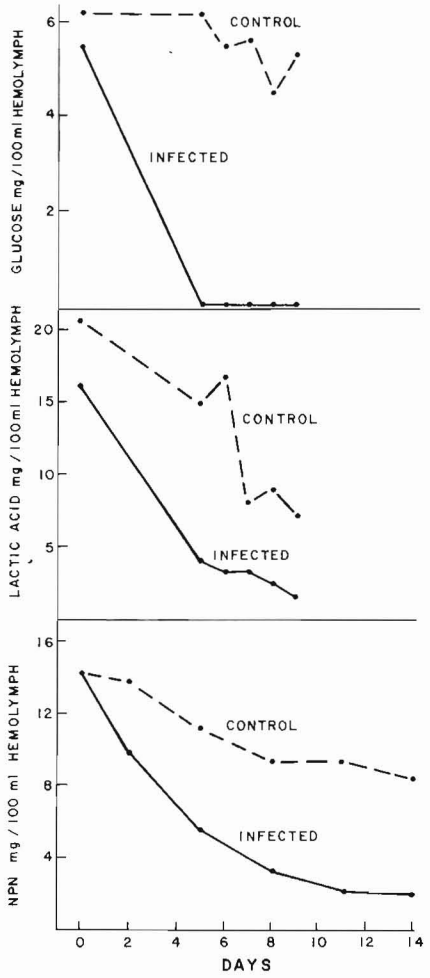

Figure 10.—Glucose, lactic acid and nonprotein nitrogen (NPN) changes in the hemolymph of infected and control lobsters (Stewart, Arie et al., 1969; Stewart and Arie, 1973a).

effect (Stewart and Arie, 1973b). Thus the extensive impairment of the central metabolic processes of the animal, which are carried out by the hepatopancreas, is suggested as the major cause of death in lobsters infected with *A. viridans* (var.) *homari*.

Resistance by lobsters to *A. viridans* (var.) *homari* infections has been observed only rarely (Stewart et al., 1966; Rabin and Hughes, 1968). It is possible, especially in the observations of Stewart et al. (1966), that the apparent resistance was actually due to a lack of virulence or a change in virulence of the pathogen. Under various influences, changes toward both increasing and decreasing virulence in *A. viridans* (var.) *homari* strains have been observed in this laboratory (unpublished results). Virulent strains of *A. viridans* (var.) *homari* are not affected by hemolymph agglutinins (Cornick and Stewart, 1968) or bactericidins (Stewart and Zwicker, 1972) and are able to overcome the effect of phagocytosis (Cornick and Stewart, 1968). A limited degree of resistance, however, has been observed upon injection of vaccines prepared from formalin-killed cells of avirulent strains of *A. viridans* (var.) *homari* (Stewart and Zwicker, 1974).

This resistance has taken the form of an increased time to death upon challenge with a standard suspension of a virulent strain of *A. viridans* (var.) *homari*, which is approximately double that recorded for animals challenged without being treated with the vaccine. The induced resistance has been shown to be quite separate from induced bactericidal activity (Stewart and Zwicker, 1974), a finding in keeping with results reported by McKay and Jenkin (1969) for induced resistance in the freshwater crayfish, *Parachaeraps bicarinatus*. The bactericidin in the serum of induced and noninduced lobsters, along with agglutinin activity and phagocytic action, however, does play a role in protecting lobsters against infection from microorganisms which otherwise could be as pathogenic as *A. viridans* (var.) *homari* (Cornick and Stewart, 1968; Stewart and Zwicker, 1972). The most recent studies on induction of resistance to *A. viridans* (var.) *homari* infections have resulted in the survival of challenged animals rather than mere increases in the time to death (Stewart and Zwicker, 1974b). These studies are being extended.

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