DEVELOPMENT OF BACTERINS AND VACCINES

for control of infectious diseases in fish

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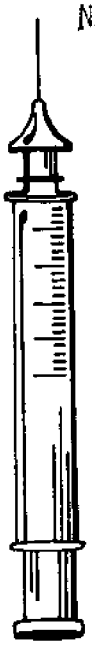

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INTRODUCTION by J. L. Fryer

This paper reviews the current status of immunization for control of certain infectious diseases in fish. Areas covered include (1) licensing and production of bacterins and vaccines;[1] (2) their use in fresh, salt, and warm water cultured fishes; (3) the testing and licensing of viral vaccines; and (4) the future of immunization for disease control in cultured fishes.

Duff (1942) reported the successful mass immunization of fish against *Aeromonas salmonicida*, indicating that vaccination for disease control on a production basis can be done. Unfortunately his experiments coincided with the availability of antimicrobial and chemotherapeutic drugs for veterinary use. This no doubt slowed progress in the development of immunization methods for disease control. Snieszko (1975) has listed the therapeutic substances used or tested for use in fish and a recent paper by Meyer et al. (1976) discusses the registration status of fishery chemicals. There are fewer drugs and chemicals available now for the routine treatment of fish diseases than there were ten years ago: certain of these compounds have been removed from use to comply with state and federal regulations. The potential problem of emergent drug-resistant strains of bacteria has reduced, or threatens, the usefulness of other antimicrobial substances.

These problems are in part responsible for the renewed interest in the immune response in fishes. The bacterial and

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[1]The terms bacterin and vaccine, as used in this paper, denote the following: (a) bacterin - immunogenic substances composed of either killed bacteria or products of bacteria; (b) vaccines - immunogenic substances composed of either live bacteria, live virus, or killed virus.

viral pathogens of commercially important food fishes have received the greatest emphasis. Bacterins have been prepared and tested against a variety of fish pathogens. *Aeromonas salmonicida* has received the most attention: numerous papers describe experiments on immunization against this bacterium (Krantz, Reddecliff, and Heist 1963; Klontz and Anderson 1970; Paterson and Fryer 1974). Schaperclaus (1972) described effective oral and parenteral immunization of carp against *Aeromonas hydrophila*. Bacterins have also been prepared against the enteric redmouth bacterium (Anderson and Nelson 1974; Anderson and Ross 1972). Perhaps the most successful and extensive testing has been on the bacterin prepared from *Vibrio anguillarum*, the causative agent of vibriosis. Successful immunization of fish has resulted from either injection or oral immunization (Fryer et al. 1976; Antipa 1976; Fryer et al. 1972; Harrell, Etlinger, and Hodgins 1975). Failures with immunization against *Vibrio* have also been noted, and one such case has been reported recently (Gunnels et al. 1976).

Development of viral vaccines has just begun, and much research remains before such preparations are available for routine use. Work thus far in the U.S. centers around the virus that causes infectious pancreatic necrosis (IPN) and infectious hematopoietic necrosis (IHN).

The perfection of suitable vaccination systems has turned out to be a difficult task. Six methods are either available or under investigation for antigen delivery: (1) injection; (2) oral administration; (3) vacuum infiltration; (4) the hyperosmotic technique; (5) direct addition to the water; and (6) spray method. Each appears to have its own peculiar difficulties, and no one method will serve all the requirements of the fish culture industry.

Initially, university and governmental laboratories began work on bacterin and vaccine development through small laboratory experiments and field trials. As part of this natural sequence of events, private companies have now emerged with the capital and expertise to develop these potential products and to license, manufacture, and market them for use in fish culture. Without private industry's interest in manufacturing these products, they might never become available for routine use.

BACTERIN AND VACCINE PRODUCTION by J.S.Rohovec

The United State Department of Agriculture (USDA-Veterinary Services), which places strict requirements and controls on biological firms, regulates producers of biologics for the fisheries industry. These regulations ensure that the user receives a safe and efficacious product.

Prior to full-scale production, the USDA must license both the establishment that produces the biologic and the product itself. Following is a brief description of some of the requirements a producer must meet to be licensed.

First, the production facility must be inspected and approved, and the producer must present acceptable procedures for making the bacterin. Following this outline of production, the producer must make three commercial quantities, or serials, which must be uncontaminated and, for killed preparations, free of any viable organisms. After completion of *in vitro* tests, the bacterin is administered to fish for *in vivo* tests, in which the product must demonstrate that it has no adverse effects on test animals, and that it is efficacious. Efficacy is tested by artificially challenging both nonimmunized and immunized groups of fish.

After satisfactory laboratory testing, the product is further examined through field trials that are designed primarily to prove the safety of the product. The unreliability of natural challenges makes efficacy difficult to demonstrate in the field. However, laboratory potency tests have been conducted on subgroups of fish that were immunized for field tests.

Results obtained *in vitro* and *in vivo* are submitted by the applicant company to the USDA for review. If approved by the regulatory agency, the bacterin is licensed and may be produced and marketed. All subsequent production lots of bacterin must also be subjected to the same rigorous laboratory testing, accomplished not only by the producing company but also by the government through its examination of samples. This extensive testing attempts to ensure that all bacterin produced is both safe and potent.

At present, a bacterin for the control of enteric redmouth and vibriosis are the only fish-immunizing agents that are licensed and commercially available. Through the research efforts of both private and public institutions, other efficacious

immunizing agents may be available in the near future.

BACTERINS AND VACCINES FOR COLD-WATER FISH
by A.J. Novotny and L.W.Harrell

The major obstacle to rearing salmonids in saltwater net pens has been bacterial disease--in particular, vibriosis caused by *V. anguillarum*. When crowded salmon are subjected to the various stresses that accompany their introduction to salt water, they often succumb to vibriosis or other bacterial diseases, which may be carried over from the freshwater phase of their life cycle (Novotny 1975).

Researchers in Japan (Hayashi et al. 1964) reported that bacterins injected into salt water-reared rainbow trout, *Salmo gairdneri*, prevented vibriosis. Later, Fryer et al. (1972) described the oral administration of a formalin-killed sonicate of *V. anguillarum* to protect fish from a natural *Vibrio* challenge.

During the past three years, the National Marine Fisheries Service (NMFS) research group at Manchester, Washington, has relied on intraperitoneal injection of bacterin to control vibriosis successfully in salmonids reared experimentally in salt water. Commercial growers, who vaccinated more than one million coho salmon *(Oncorynchus kisutch)* in Puget Sound during the past several years, have successfully used the technology for mass injection developed by NMFS.

A new serotype of *V. anguillarum* (designated 1669) has recently been described (Harrell et al. 1976). Bivalent bacterins made from this organism (1669) and the original Manchester isolate of *V. anguillarum* (775) have proven effective against both serotypes (unpublished data).

To avoid the two-week, post-immunization delay before introducing fish to salt water, NMFS has been using an effective bacterin-antibacterial preparation given by intraperitoneal injection. The vaccine delivers 675 µg heat-killed 775 *Vibrio* organism, 225 µg of 1669 *Vibrio* with 75 µg nitrofurazone and 95 µg oxytetracycline per 0.15 ml dose. However, the U.S. Food and Drug Administration (FDA) has not approved this preparation. Schaperclaus (1970) reported the successful use of this sound injection procedure to control disease in carp culture. FDA's clearance of this methodology would be highly desirable.

Amend and Fender (1976) have recently described a second delivery system, the hyperosmotic infiltration method, which utilizes changing osmotic gradients between the fish and the bacterin to carry the antigen into the animal. This method was recently tested on pink salmon *(O. gorbuscha)* fry that had an average weight of 1.8 gm. One lot of 727 fish were immunized with a bivalent preparation through the immersion method, and a control group of 574 pink salmon fry were treated with the same medium without killed bacteria. Forty days after introduction to salt water, 490 nonimmunized control salmon had died from vibriosis while only two of the immunized salmon were lost to this disease.

Vibrio bacterins to protect fish against vibriosis in salt water have been successful using several routes of administration. Which bacterin delivery system is most effective should become evident in the near future, since several researchers are currently investigating this subject.

BACTERINS AND VACCINES FOR WARMWATER FISH
by J.A. Plumb

Immunization has not been widely applied in warmwater fish culture, though interest in doing so is increasing. Recent experimentation in immunization against warmwater fish disease organisms has provided encouraging data on the applicability of immunoprophylaxis. The immune response of warmwater fish to soluble and insoluble antigens has been recently reviewed (Snieszko 1970; McGlamery et al. 1971; DiConza and Halliday 1971; Schachte and Mora 1973; Anderson 1974; Heartwell 1975 and Corbel 1975). Therefore, this discussion is confined to the experimental and practical applications of bacterins and vaccines to protect warmwater fish from disease.

Viruses

Some virus diseases of warmwater fish lend themselves to immunoprophylaxis. Channel catfish virus (CCV) is of greatest concern in the U.S., and CCV's ability to elicit an antibody response in large catfish has been demonstrated (Plumb 1973; Heartwell 1975). However, the disease is a problem mostly in juveniles: during the summer, CCV can infect channel catfish fry and fingerlings up to 3 to 4 months of age and 10 to 12 cm in length. Fijan (personal communication) demonstrated that fingerlings experimentally infected with CCV (by injection), which were held at 15°C for 27 days, were immune to reinfection. For

culture situations in which CCV infects fingerlings year after year, immunization is feasible depending on development of a suitable delivery method and availability of proper conditions.

In Europe, immunization of common carp against spring viremia of carp (SVC) is promising (Fijan 1976). Initially, carp surviving a natural SVC epizootic had antibodies detectable by indirect haemagglutination (Sulimanovic 1973), and carp that survived an experimental SVC infection resisted reinfection. Intraperitoneal injection of carp also provoked a solid and long-lasting protective immunity to SVC. Carp immunized by peroral application had weaker immunity, and only 50 percent of the fish had a long-lasting protective immunity. That some positive data are accumulating on immunity of warmwater fish to virus diseases is encouraging.

Bacteria

Bacterins have been used experimentally to protect warmwater fish against several bacterial organisms. The most common pathogens of channel catfish are *A. hydrophila* and *Flexibacter columnaris*. Each of these common waterborne bacteria have a number of specific antigens associated with different strains (serotypes), which creates problems in bacterin preparation and protective immunity (D. H. Lewis, personal communication; Schachte 1976). A bacterin prepared from one serotype may not necessarily protect against another serotype. Nevertheless, bacterins have been prepared from *A. hydrophila* and *F. columnaris* and used to immunize warmwater fish (Schachte 1976). Channel catfish were vaccinated by three methods (peroral, intramuscular injection, and immersion) and challenged by natural exposure in a pond. There were no significant differences in survival between the three routes of vaccination and the unvaccinated control groups; however, the injected group did produce the highest agglutination titers. Humoral antibody titers of individual fish were higher for *F. columnaris* than for *A. hydrophila*, indicating possible antigenic competitive inhibition of *A. hydrophila* by *F. columnaris* when the two antigens were administered simultaneously. In other tests, Schachte (1976) injected channel catfish with bacterins prepared from *A. hydrophila* and *F. columnaris* individually and in combination. Fish injected with the monovalent bacterin had highest titers for homologous antigen, but the group injected with the combined bacterins had an *F. columnaris* agglutination titer that was 16 times greater than the *A. hydrophila* titer. These data indicate that bacterins containing both organisms are not antigentically compatible.

Parasites

Ichthyophthirius multifilis is one of the most damaging parasites of warmwater fish and one of the most difficult to control. Becker and Allison (1964) and Areerat (1974) reported experimental immunization of catfish with "Ich." In the latter study, channel catfish 18 to 23 cm long were injected intraperitoneally with 0.5 ml of "Ich" vaccine prepared from ground trophozoites with and without Freund's adjuvant. Three weeks after vaccination, all immunized fish had humoral agglutination titers up to 1:2560 but agglutination was slightly stronger with serum from adjuvant-aided vaccine. Upon challenge, 100 percent mortality occurred in controls after seven days, while there were no deaths in vaccinated fish.

Practical Considerations

The practical application of immunoprophylaxis for warmwater fish diseases is yet to be demonstrated. Anderson (1974) cites the following factors that must be considered before a vaccine program is implemented:

1. The nature and severity of the disease.
2. The probability of exposure to infection.
3. The probability of fish contracting the disease.
4. The degree and duration of the protection conferred.
5. The frequency and severity of undesirable reactions to the vaccines.

These factors must be applied to each of the warmwater diseases for which immunization is considered. Certain channel catfish farms, where CCV is a recurring problem, would find a vaccine beneficial if a suitable means of vaccination is developed and if very young fish could be protectively immunized. In diseases to which larger fish are susceptible, such as SVC, *Aeromonas*, or *F. columnaris* infections, injection may be practical for vaccine application. A major problem in developing a bacterin for *A. hydrophila* or *F. columnaris* is the need for a poly-

valent vaccine that will protect the fish against all strains of the two organisms. No known method exists for growing the protozoan "Ich" *in vitro*; therefore, accumulation of sufficient antigen to immunize large numbers of fish is very time-consuming and impractical. Due to its effect on fish and the difficulties in controlling it, "Ich" should receive top priority for vaccine study.

VIRAL VACCINES by G.L. Tebbit

Viral vaccines for the control of fish diseases are also currently under development, and will probably be licensed and commercially available for use in the fisheries industry in the future. Both inactivated and live modified viral vaccines have drawn interest, and development of both is being initiated.

The use of live modified viral vaccines versus killed preparations in the fisheries industry has been much discussed. Development of live modified vaccines has been pursued for the following reasons: (1) attenuated viral vaccines that are used in human and veterinary medicine produce effective, long-lasting immunity; (2) an attenuated viral vaccine presumably produces local immunity that better protects the fish against infection encountered naturally; and (3) a live attenuated vaccine would require fewer virus particles than a killed preparation, and could thus be produced more economically. Although encouraging results have been obtained with a live modified IHN (Fryer et al. 1976) and IPN vaccine, considerably more development work is needed to license and make commercially available these live modified vaccines. Necessary back-passage experiments will determine the stability of a live modified virus and whether it is able to revert to a virulent form. If a live modified vaccine will be used for areas in which the disease is not endemic, a method of marking and distinguishing the vaccine virus from other naturally occurring strains may have to be developed. Potential problems of using a live virus in a public watershed will also have to be explored with the appropriate federal and state government agencies.

Those developing a viral vaccine for commercial use will have to examine thoroughly strain variances in antigenic composition among various regional isolates. This will probably be a greater problem with IPN than IHN. To compensate for these antigenic differences, vaccines may have to be developed that would consist only of regional strains for marketing only in those particular regions.

Licensing requirements for viral vaccines are similar to those for bacterins, which have been described previously. However, since the production of viral vaccines uses cell cultures, extensive quality control measures outlined by the USDA are undertaken to ensure sterility, purity, safety, and potency. Either primary cells or established cell lines can be used in producing viral vaccines. Primary cells must be routinely tested for contaminating microogranisms such as mycoplasma, bacteria, fungi, and adventitious agents. In addition to undergoing the above tests, established cell lines must be routinely characterized for modal number of chromosomes and tested for tumorigenicity and onocogenicity *in vivo*. All ingredients of animal origin, such as sera and trypsin used in the production of viral vaccines, must be tested for mycoplasma, bacteria, fungi, and adventitious viruses from the species of animal that was the source of ingredients.

FUTURE DEVELOPMENTS by D.F. Amend

Immunization has played a vital role in the control of infectious diseases. Smallpox was once the most dreaded disease known to man, but, because of the success of the vaccinia vaccine against it, the World Health Organization projected worldwide eradication of smallpox by 1976. Furthermore, immunization now controls most of the acute infectious diseases of human populations. Vaccine development for domestic livestock is equally impressive. Immunization against poultry diseases has made poultry farming a thriving industry; in fact, the poultry industry of 25 years ago is comparable to the fish industry of today. In the past, poultry farmers obtained surplus eggs to ensure adequate survivors for market, but immunization has eliminated the need for surplus eggs. The intensive rearing of poultry and other domestic animals is now an efficient, economical industry made possible by effective immunization.

Immunization of fish will most likely follow a similar pattern of development once vaccines become readily available. However, immunization of fish is a new concept, relatively untested on a production hatchery scale; therefore, several years may elapse before hatchery managers use vaccines widely. Within 10 years we will probably have effective vaccines for most of the infectious fish diseases, and

immunization of fish will be a common practice. This should make the rearing of fish more predictable and economical, and allow for expansion of fish farming. However, much more research and development must be conducted before this can occur.

Mass immunization techniques for fish need to be improved. Fish immunization has not been widely practiced because convenient methods for mass delivery of vaccines have been either unsatisfactory or unavailable. Needle injection is very effective, but impractical for large numbers of small fish. Oral immunization has not been very effective (Corbel 1975; Snieszko 1970; Klontz and Anderson 1970); however, Rohovec et al. (1975) demonstrated effective oral immunization of Pacific salmon against *V. anguillarum*. Newly developed techniques for hyperosmotic infiltration (Amend and Fender 1976; Fender and Amend 1976; Croy and Amend 1976), and the spray vaccination method (Gould 1977) show much promise. It is too early to predict which of these and other approaches to mass immunization will be most effective and least stressful for the fish.

Now that mass immunization techniques are being developed, commercial vaccine producers are obtaining licenses in compliance with federal regulations to ensure readily available sources of safe, effective, and potent vaccines. Wildlife Vaccines, Inc., and Tavolek Laboratories, Inc., are currently specializing in vaccine development for fish, and undoubtly more companies will enter the market as the field expands. Additional changes and improvements can be expected with increased competition between companies.

In the immediate future, most bacterins and vaccines will contain killed virulent microorganisms isolated from fish. Eventually, viable avirulent (attenuated) strains will be available for immunization, but much research will be required before they can be used safely. Can immunized fish become carriers and shed the organism? Can avirulent strains be distinguished from virulent strains when certifying fish for transportation? Are modified strains pathogenic to nontarget species? Can reversion to the virulent state occur after passage through the target or nontarget species? Can fish become immunotolerant and increase the shedding rate? These questions must be answered before avirulent vaccines can be used safely.

Other questions of immediate concern are: what is the duration of immunity, how does water temperature affect immunization, and what is the relationship between age and immunocompetence? Each of these parameters influence the efficacy of immunization. Furthermore, when we know more about the pathogens and the immune response in fish, better immunogens can be prepared. For example, bacterins and vaccines with proper delivery systems can be developed to favor specifically the cellular or humoral immune response. Also, specific immunogenic extracts may give better protection.

Most immunizing preparations of the future will be polyvalent, in which several strains, species, or serotypes of microorganisms may be administered in a single dose to provide broader protection. These combinations require screening for compatibility so that antigen competition does not reduce the efficacy of immunization. Adjuvants or adjuvant effects and the delivery systems that promote the best response will be used.

Although immunization of fish may become common, it will never replace good management practices. Immunized fish may become infected and diseased when stressed by poor environmental conditions or excessive handling. This has been true in other areas of animal medicine. Disease prevention should be the goal of hatchery managers - both good management and immunizaation will help.

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