

Cold-Blooded Vertebrate Immunity to Metazoa

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INTRODUCTION

There are very few reports on immunity of fish to metazoan parasites. The most recent and complete reviews of this neglected field are those by Bauer (1958, 1959). It is evident from these reviews that information on the presence of specific antibodies in fish to metazoan parasites is very scarce and fragmentary. This is so despite the well-established fact that fish, as other vertebrates, respond to parenteral introduction of antigens by production of specific antibodies. The only known exception is the hagfish *Eptatretus stouti* (Papermaster et al., 1962, 1964).

The metabolic rate of fish is temperature dependent (Lagler, et al., 1962). In regard to their optimum temperature, these creatures are conveniently, but

arbitrarily, divided into cold-water and warm-water fish. Salmonids and some other fish living away from equatorial environments belong to the first category. The optimum temperature for such fish is from 10°C to 15°C. The so-called pond fish and tropical fish thrive best at 20°C to 25°C. There is a close correlation between the temperature, the speed, and intensity with which fish produce antibodies. Cold-water fish produce antibodies more slowly than warm-water fish, despite the fact that the optimum temperature for their survival and metabolism is between 10°C to 15°C. Warm-water fish, if maintained at 20°C to 30°C, respond to immunization almost as strongly and as rapidly as warm-blooded vertebrates. At 5°C or below, there is no immunologic response in warm-water fishes (Nybelin, 1935; Snieszko et al., 1938; Bisset, 1947; Snieszko, 1954; Cushing, 1942; and Post, 1963). There are some antarctic marine fish which live in such cold-water and have such a slow metabolism that there are no erythrocytes in their blood (Ruud, 1965). It would be interesting to determine whether these fishes are capable of specific immunologic response.

Immune response in trout (a cold-water fish) was recently investigated by Krantz et al. (1964). At 12°C to 14°C the response was slow, reaching maximum titers about three months after injection. Use of adjuvants was needed to enhance the immunologic response. Likewise, when trout finally became immune, the decline and disappearance of the titer was very slow, requiring about a year. The most recent study on the immune response in carp (a warm-water fish) showed that there was no immunologic response within 30 days if fish were kept at or below 10°C to 12°C. At 15°C, the response was good (Fijan and Cvetnić, 1964, 1966).

The presence of specific antibodies in higher vertebrates is associated with serum globulins, and gamma-globulin in particular. There is some evidence indicating that antibodies in fish may be associated with other globulin fractions (Post, 1963; Engle et al., 1958).

In fish the protein contents of serum increases with age and varies with the season of the year. The seasonal variation in serum contents is more evident in fish living in an environment in which the temperature differences vary considerably between winter and summer. It has been reported by many investigators and reviewed by Riedmüller (1965) that pond fish become very susceptible to bacterial infections in the spring. At that time, following the stress of winter, fish may have significantly lowered serum proteins and erythrocytes. Electrophoresis of serum is now employed in Germany to separate fish with depleted blood protein from those which are normal, because depleted fish are much more susceptible to infections and therefore are less suitable for reproductive purposes. Available information on the proteins of fish blood and serum has recently been reviewed by Bouck (1963), Booke (1964a, b), and Post (1963).

Summerfelt (1966a, b) recently studied the question whether immune bodies are associated in fish with gamma-globulin and if gamma-globulin of fish serum can be considered as homologous with that of higher verte-

brates. *Trematodigmogonus* in hyperparasitism was associated with a quantity of antibodies indicated by golden serum globulin precipitated as those of similar type.

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brates. The author showed that gamma-globulin of the golden shiner, *Notemigonus crysoleucas*, is similar to that of humans. Gamma-globulin increased in hyperimmunized golden shiners. To prove the point, the immune serum was adsorbed with the specific antigen. This resulted in the reduction of the quantity of gamma-globulin to the same level as in the control fish. This indicated that the immune body was associated with gamma-globulin in golden shiner. There is lack of agreement regarding the presence of gamma-globulin in teleost fishes in data presented by Engle et al., (1958) and Summerfelt (1966a, b). My personal observations (Snieszko et al., 1966), as well as those of Post (1963), show that there is in trout serum a component similar to the gamma-globulin of warm-blooded vertebrates.

The conception of the term "immunity," particularly as it is often applied in parasitology of fish, is often much broader than that used by immunologists. This is clearly presented by Bauer (1959);

By immunity, the insusceptibility of the host to infection by an agent of a given disease is often meant. Today this overgeneralized definition, especially concerning immunity in parasitic diseases, is insufficient. In an interpretation like this, phenomena of specificity and insusceptibility towards the parasite are included, and are explained by certain anatomical and physiological peculiarities of the fish, etc.

According to Bauer (1959), "Orlov defines immunity as a specific insusceptibility, formed by the activation of specific protective processes in the host organism whose biochemical environment meets the parasite's challenge." Discussion of immunity to metazoan parasites in this review will be kept within the delineation as formulated by Orlov (1954).

The genetic aspects of immunity and resistance to infection, in their broad and narrow meanings, have been excellently reviewed by Hutt (1958, 1964) and Gowen (1960).

In 1958 and 1959 Bauer concluded that fish immunity to parasites, if used in its precise meaning, "has been left almost completely unstudied." This statement is, perhaps, somewhat too sweeping. It seems true, however, that descriptions of instances of immunity of fish to parasites are very inadequately documented to suit a contemporary immunologist.

EXTERNAL PARASITES

Trematoda. The best documented of the early observations on the acquired immunity of fish to monogenetic trematodes is that of marine fishes belonging to the genera of *Serranidae* and *Litjanidae* which often become heavily infested by *Benedenia (Epibdella) melleni* when kept in aquaria (Jahn and Kuhn, 1932). Up to 2,000 worms per fish were seen attached to eyes, gills, and nasal cavities. Such heavy infestations often killed the fish. Fish which

survived infestations harbored progressively fewer parasites. This has been explained as a gradual development of specific immunity by Nigrelli and Breder (1934), who reported that 57 species of marine fish kept in the New York City Aquarium were constantly exposed to infection and reinfection with *Benedenia melleni*. They differed greatly in susceptibility to infection and in the degree of severity of the disease. Many fish became resistant or immune to subsequent infections. Newly introduced fish were most intensely parasitized and often killed. Some infected fish developed partial immunity and some became completely immune. Some fish which appeared immune became lightly parasitized during acute epizootics but others continued to be susceptible. An apparently localized immunity was observed in the blunt-nosed shiner or horse fish *Vomer setapinnis* because reinfection did not occur in skin areas previously infected.

Detailed studies on the immunity of marine aquarium fishes to *Benedenia melleni* were performed by Nigrelli (1937). Passive immunization was carried out by injecting serum from immunized pompanos. The results were negative. Active immunization of fish was carried out by injecting an antigen consisting of ground fresh or dried parasites; the results were inconclusive. The best results were obtained by examination of the effect of fish mucus on the parasite.

In mucus from a naturally immune or resistant grouper, dogfish, or ray, parasites were moribund in 3 to 4 hours. Parasites remained alive for 18 to 24 hours in mucus from a round pompano which was susceptible. Parasites survived for 3 days in marine water. The cornea of the eye was most frequently and acutely infected, probably because fish eyes are not covered with mucus.

Glochidia. A glochidium is a larval stage of clamlike fresh-water mussels. When glochidia develop sufficiently, they are expelled from the brood pouch of the mussel. Glochidia of the majority of the species of mussels must pass through a parasitic stage during which they become attached by clamping their valves to the gills or fins of fish. In this process, a small bit of the fish tissue becomes enclosed within the glochidium. The tissue of the fish reacts by forming a cyst around the glochidium. In 10 to 20 days glochidia complete their parasitic stage and slough off. When infestation is heavy, consisting of from 600 to 1,200 glochidia per fingerling fish, heavy mortality may occur. A light infestation with glochidia is apparently harmless to fish (Davis, 1953).

Immunologic studies on fish infested by glochidia were carried out by Reuling (1919), who used the short and long nosed gar (*Lepisosteus osseus* and *L. platostomus*, respectively) in his studies. Following two successive and successful infections with glochidia, fish would reject glochidia in subsequent infections. Glochidia from the mussel *Lampsilis luteola* were introduced into sera from immune and control fish. Within two hours glochidia in the immune serum showed desquamation of cells of the mantle and of the abductor muscle. This proceeded until the glochidia were destroyed.

Similar results were obtained with glochidia from other species of mussels. Ring precipitin tests showed the presence of specific but weak antibodies. If

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artificial immunization with ground glochidia was compared with natural immunization, only the naturally immunized fish rejected glochidia. A limited sampling indicated that the acquired immunity may last from one year to another. The results of Reuling were confirmed by Arey (1932).

INTERNAL AND SYSTEMIC PARASITES

Digenetic trematodes. Observations reported by Todd (1929) are frequently given as examples of acquired immunity in fish infested with digenetic trematodes. This is a mistake since the author says, "no evidence of any difference in the action of the serum from normal and infested fishes was found. . . ."

Scheuring (1930) and Vogt (1938), as reviewed by Bauer (1958), fail to give convincing evidence that fish acquire specific immunity to digenetic trematodes. It seems more likely that the increased resistance in older fish was associated with age.

Cestoda. One of the few instances in which the presence of specific antibodies in fish infested with metazoan parasites was demonstrated *in vitro* was described by Molnár and Berczi (1965). Specific precipitins were demonstrated by agar gel diffusion in the serum of whitefish *Abramis brama* infested with the cestode *Ligula intestinalis*.

THE ROLE OF FISH MUCUS

Fish bodies are covered by a layer of mucus which is secreted by special cells (Lagler et al., 1962). Anyone who handles fish is well aware that removal of the mucus makes fish more susceptible to external infections. External disinfection of handled fish usually reduces the danger of bacterial and fungal infection. Serum of rabbits immunized with serum from bass (*Labrax morone L*) reacted specifically with mucus obtained from the body and gills of the same species of fish (O'Rourke, 1961). This author implied that specificity of fish mucus may explain the specificity of host-parasite relations in fish and he also suspected the presence of antibodies in the mucus.

Experimental evidence of the specific antiparasitic antibodies in fish mucus was furnished by Reuling (1919) and Nigrelli (1937) in their observations discussed elsewhere on the immunity of fish to glochidia and monogenetic trematodes.

FORMATION OF CYSTS

"When a parasite enters the fish, the latter develops a series of reactions directed to localizing the individual already present, to suppressing their

vital activities, and limiting further possible infections" (Bauer, 1959). It is also important for the parasite to protect itself from the defensive reactions of the host. Therefore, cysts around parasites in fish tissues are usually formed both by the host and the parasite. The most complete reviews on the cyst formation in fish are by Bauer (1958, 1959) and Reichenbach-Klinke (1954-1955). The latter author considered as a "cyst" the antiparasitic wall produced by the host, and as a "capsule" the wall produced by the parasite to protect it from the the defensive reaction of the host. In general use, however, both the walls produced by the host and the parasite are collectively called cysts. In his extensive observations, Reichenbach-Klinke examined cysts in four marine, 12 domestic fresh-water, and 36 species of aquarium fishes.

Some cysts are formed by the epithelium on the surface of the body or in the intestinal tract of fishes. Other cysts are formed by mesenchymatic cells. Lymphocytes, other leukocytes, histiocytes, fibroblasts, and cells of the connective tissue are involved in cyst formation. Cysts of mixed epithelial and mesenchymatic origin are also formed.

Typical epithelial cysts are caused by glochidia. Sometimes, if the valves of glochidia form a clamp around epithelium and some of the underlying mesenchymatic tissue, cysts of mixed origin may be formed. The mesenchymatic cysts are usually formed around metacercariae of digenetic trematodes. Metacercariae also contribute to the composition of the cysts by secreting substances from which a capsule is formed around the parasite.

When plerocercoids of cestodes invade fish tissues, very strong cysts are formed by the connective tissue of the host. When cysts are formed around the plerocercoids in trout liver, the initial cysts are made of lymphocytes and other leukocytes. Initially the connective tissue around the parasite is lysed. This is followed by formation of a spherical cyst by the interstitial tissue. When the proboscis of an acanthocephalan parasite penetrates the submucosa of the intestine, the capsule may protrude into the abdominal cavity. When fish tissues are invaded by certain nematodes, the cyst is formed exclusively by the host. It is assumed that as a result of an immune reaction of the host, the cuticle of the larvae is lysed first. This is followed by a gradual destruction of the contents of the cyst. In other cases, a delicate balance is established between the nematode and the host. The parasite slowly moves through the host tissues by a localized lytic action originated by the parasite.

In cases of attachment of monogenetic trematodes to the gills, proliferation of the gill epithelium continues until the parasite is removed from the source of food, which in this case is the blood of the fish. When the layer of epithelium is sufficiently thick, the parasite is starved and falls off (Putz and Hoffman, 1964).

Some metacercarial cysts in fish are colorless and others are black. The black color is caused by melanin. Reichenbach-Klinke suspected that the enzyme tyrosinase is furnished by the host.

The metacercarian cyst of *Posthodiplostomum minimum* in bluegills and other susceptible fresh-water fish is composed of the mucoid material secreted

by the parasite of origin. Both

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by the parasite, and a thin layer of the fibrous connective tissue of host origin. Both layers are clearly visible (Hoffman, 1958).

In case of *Neogogatea kentuckiensis*, the cercariae penetrate the skin rapidly and enter the musculature where they produce transient ecchymoses. If small fish are exposed to a high concentration of cercariae, they may be killed in a very short time. One or two days after invasion, refractory globules 60 μ to 60 μ in diameter appear in the parasite and grow larger. These granules appear to be cystogenous. The parasite also becomes surrounded by a cyst of host connective tissue. Degeneration of muscle fibers adjacent to the cyst also takes place (Hoffman and Dunbar, 1963).

HEMATOLOGIC OBSERVATIONS

Heavy infestation of fish with metazoan parasites probably causes anemia due to the debilitating effect of the parasites or to extensive hemorrhages. According to Bauer (1958), fish parasitized by helminths had a reduced number of lymphocytes, but monocytes, polymorphonuclear agranulocytes, and neutrophilic leukocytes increase in numbers. In carp infested with an external trematode parasite, *Dactylogyrus extensus*, similar hematologic changes were reported (Sadkowskaya, 1958).

AGE IMMUNITY

It is generally assumed that fish immunity to parasites increases with age. This is probably true because during their early months, or years, fish exposed to parasites become either more immune, resistant, or die. This is also supported by the common occurrence of heavy parasitic infestation causing extensive losses among fishes when they are exposed to parasites new to the environment or new to the fish population. According to Bauer (1959), increased age immunity is often nonspecific, resulting from ecologic changes of the fish as environment for the parasite. A good example of a nonspecific "age immunity" of fish to parasites is the whirling disease of salmonids caused by the sporozoan *Myxosoma cerebralis*. This parasite has specific affinity for the cartilage of young fish. When the cartilage ossifies in older fish they are still susceptible to infection, but not to a disease, since the ossified cartilage cannot be entered and damaged by the parasite (Hoffman et al., 1962).

In other examples of age-conditioned resistance, the older fish move to another ecologic environment and are no longer exposed to the parasite. Therefore, the decreased incidence of infection of older fish cannot be called "age immunity" unless it is associated with an increase of specific immunity resulting from the production of specific antibodies (Bauer, 1959).

No published reports were found on immunity of amphibians and reptiles to metazoan parasites. The most complete review of diseases and parasites of reptiles and amphibians is that of Reichenbach-Klinke and Elkan (1965). Walton collected bibliographies concerning parasites of amphibia (1964, 1966). None of the many listed titles indicated that information on immunity of amphibia to metazoan parasites was available.

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