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THE IMMUNE RESPONSE OF CHANNEL CATFISH

I. Basic responsiveness to soluble and viral antigens

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Abstract: Channel catfish *(Ictalurus punctatus)* were immunized with bovine serum albumin (BSA) or vesicular stomatitis virus (VSV). Groups of catfish, housed in cages in a pond, were immunized by intramuscular (IM) injection of single doses of BSA or VSV or 2 doses of BSA or VSV given 1 week apart. Other groups of catfish were immunized with 1 IM injection of BSA or VSV incorporated in Freund's complete adjuvant (FCA). The antibody responses were measured at weekly intervals. Passive hemagglutination was used for detection of anti-BSA antibodies, and serum-neutralization was used for detection of anti-VSV antibodies. A significant antibody response occurred in those catfish immunized with either BSA or VSV incorporated in FCA. One injection of BSA or VSV in physiologic saline induced little or no detectable antibody production. Two injections of BSA or VSV in physiologic saline induced a slight transcient antibody response between the third and fourth week post-injection.

INTRODUCTION

Good and Papermaster[6] and Ridgway et al.[9] have reviewed the literature concerning the capabilities of most lower vertebrates to synthesize humoral antibodies in response to antigenic stimulations. Snieszko[13] in a review of the literature relating to immunization of fish emphasized that optimum antibody response to an antigenic stimulation is dependent upon the optimum temperature for each species of fish. The immunologic and immunochemical nature of this antibody response of fish has been described on a phylogenic basis.[5,6,8,9] Various workers have studied the antibody response of the teleosts to soluble protein antigen.[4,12,15]

Clem and Sigel[4] reported that margates hyperimmunized with bovine serum albumin (BSA) produced high levels of specific antibody. Sigel et al.[12] reported that soluble BSA did not stimulate antibody production in snappers *(Lutjanus sp.)*, however, the fish did respond to alum precipitated BSA.

The antibody response of the margate to human influenza virus was studied by Sigel and Clem.[11] They found that multiple injections of antigen induced higher antibody titers than single injections of antigen. Incorporation of the influenza virus in Freund's complete adjuvant induced significantly higher antibody titers as compared with injections of soluble antigen. No attempt was made to follow the course of the antibody response.[11] Krantz et al.[7] protected trout against challenge infections of *Aeromonas salmonicida* by immunizing them with formalin-killed bacteria in a mineral oil adjuvant.

At this time, individual vaccination for disease prevention in large fish culture establishments is prohibitive for practical and economic reasons.[13] Additionally, the

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effectiveness of disease control programs based on oral immunization has not been proven conclusively.[13] This study was undertaken to provide initial information concerning the antibody response of channel catfish. These preliminary studies will provide a basis for further work on oral immunization.

MATERIALS AND METHODS

Experimental fish

A total of 720 channel catfish were equally divided into 12 groups and maintained by cage culture (3' x 3' x 3') in a 2½-acre closed experimental pond. These fish were estimated to be 18 months old and their weight range was 150-250g. They were fed Purina Trout Chow at 2-3% of their estimated biological weight 4 times weekly. Water temperature was continuously recorded and pertinent weather data were obtained.

Antigen preparation

Bovine serum albumin(1) (BSA) was dissolved in phosphate buffered saline (PBS), pH 7.2 at concentration of 20 and 40 mg/ml. The greater concentration of BSA (40 mg/ml) was mixed with equal volumes of Freund's complete adjuvant(2) (FCA). A stock of New Jersey strain of vesicular stomatitis virus(3) (VSV) was produced by subculturing on primary chicken embryo fibroblast cell cultures.

The stock virus contained 1 x 10^8 plaque forming units (pfu)/ml. Stock virus was divided into equal parts, one part diluted with an equal volume of PBS pH 7.2, the other mixed with an equal volume of Freund's complete adjuvant. Each immunizing dose of stock virus (0.5 ml) contained approximately 2.5 x 10^7 pfu.

Immunization of fish

Catfish were assigned randomly to groups and immunized as described in Table 1.

The groups of catfish housed in an individual cage were identified by clipping a different spine for each group.

All fish were immunized with 0.5 ml of the appropriate antigen, intramuscularly in the dorsal back muscles. Care was taken during the immunizing procedure to minimize stress on the fish. Booster injections of the antigens were given one week after the primary injection to the appropriate groups (Table 1).

Blood samples were obtained by venipuncture of the caudal sinus. Samples were collected prior to antigen administration and at weekly intervals post-immunization up to 7 weeks, a final sample being collected at week 15 post inoculation. The pre-immunization sample was collected from 70 fish; 10 pools were made of this blood. At each post-immunization bleeding 7 fish from each group were selected, bled, and the blood from each group pooled. The blood was allowed to clot overnight at 4 C, the serum decanted, clarified by centrifugation and stored at —20 C until needed.

Determination of antibody response. Detection of anti-bovine serum albumin antibodies

Anti-BSA antibody was detected using the passive hemagglutination technique as described by Boyden[2] and modified by Stavitsky.[14] In this study, the tannic acid treated sheep red blood cells were treated with 100ug BSA per ml of 2.5% suspension of cells. All serum samples were inactivated at 56 C for 30 minutes and absorbed with washed sheep red blood cells. Titrations were done in doubling dilutions starting with a 1:10 dilution. The end point was the last serum dilution giving complete agglutination.

(1) Nutritional Biochemicals Co., Cleveland, Ohio.
(2) Difco Co., Detroit, Michigan.
(3) Dr. J. Spalatin, University of Wisconsin, Madison, Wisconsin.

TABLE 1. Immunization scheme of 720 channel catfish randomly assigned to 12 experimental groups.

Cage No.	Group [a]	Antigen [b]	Vehicle
I	1	BSA[c]	PBS[d]
	2	BSA	PBS (Boosted)[e]
	3	none	PBS
II	4	BSA	FCA[f]
	5	none	FCA + PBS
	6	none	none
III	7	VSV[g]	PBS
	8	VSV	PBS (Boosted)
	9	none	PBS
IV	10	VSV	FCA
	11	none	FCA + PBS
	12	none	none

[a] Sixty catfish per group—each group identified by clipping of the spine.
[b] Intracellular injection, 0.5 ml.
[c] Ten milligrams bovine serum albumin—Nutritional Biochemicals Corp., Cleveland, Ohio.
[d] Phosphate buffered saline — pH 7.2.
[e] Reinjected 7 days following initial injection.
[f] Freund's complete adjuvant—Difco Co., Detroit, Michigan.
[g] Vesicular stomatitis virus (New Jersey strain).

Detection of anti-vesicular stomatitis virus antibodies

The beta-serum neutralization method (constant virus-decreasing serum method)[3] was used to detect anti-VSV antibodies in the catfish sera. Pooled serum samples were inactivated at 56 C for 30 minutes and diluted 1:5 with Hanks' Balanced Salt Solution (HBSS). Two-fold serial dilutions of the serums was made in HBSS. An equal volume of diluted virus (approximately 1000 pfu/ml) was added to each serum dilution and the mixture was incubated for 1 hour at 25 C. Virus-HBSS and horse serum - virus controls were prepared at this time and treated as above.

Preliminary work demonstrated that catfish serum at dilutions of less than 1:10 had a toxic effect on monolayers of chicken embryo fibroblasts. Additionally, heat stable viral inhibitors were detected. These effects were negated at a final serum solution of 1:10. Therefore, all serum neutralization tests were done beginning at serum dilutions of 1:10.

Primary chicken embryo fibroblasts (24 hr. monolayers) were inoculated (in triplicate) with 0.2 ml of each virus-serum dilution, virus-HBSS, and horse serum - virus mixtures respectively. To avoid bias, each plate was coded. As an additional control, plates of chicken embryo fibroblast monolayers were inoculated with catfish serum (dilution 1:10) to determine if the serum had any toxic effect on the cell cultures. All plates were incubated at 37 C in a humidified 5% CO_2 atmosphere for 1 hour. During this period, the plates were rotated gently several times to disperse the inoculum over the monolayers. After incubation, all plates were overlayed with a maintenance medium consisting of 1% Purified Agar,[4] Eagle's F-11,[5] 1% calf serum,

[4] Difco Co., Detroit, Michigan.
[5] Grand Island Biologicals, Grand Island, New York.

and antibiotics. The overlayed plates were incubated at 37 C (under 5% CO_2) for 30 hours at which time they were stained with a 1:5000 solution of neutral red. Three hours after staining, plaques were counted, the plates identified, and the results recorded. The 50% plaque reduction point was used as the end point for serum titration. Mid-dilution end points were interpolated by the Reed-Muench method.[3]

RESULTS

Response to bovine serum albumin immunization

The antibody responses of the catfish to BSA are summarized in Table 2 and Figure 1. A barely detectable antibody response was induced by a single injection of BSA (Group 1, Table 1 and 2, Figure 1). Two injections of BSA, a week apart, induced a minimal antibody response, with a peak titer of 1:20 at 4 weeks post-immunization (Group 2, Table 1 and 2, Figure 1). No antibody activity was noted in any of the control group serum (Groups 3, 5, and 6, Table 1). A significant antibody response was induced in Group 4 (Table 1) injected with the BSA incorporated in Freund's complete adjuvant (Figure 1, Table 2). In this group, there was a significant drop in antibody titer at weeks 5 post-immunization.

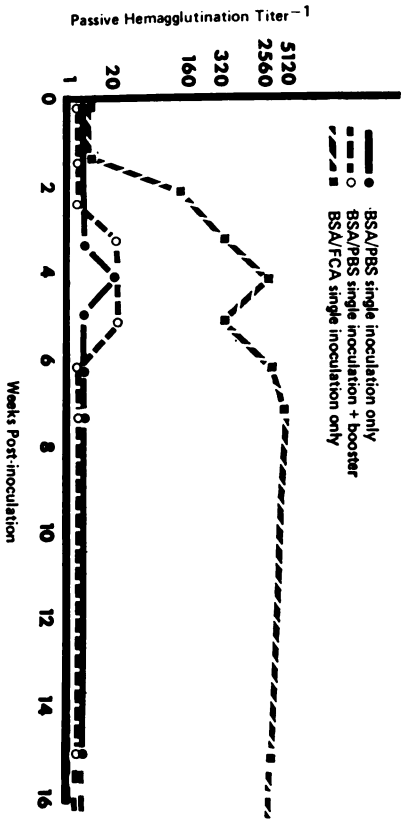

FIGURE 1. The antibody response of channel catfish to bovine serum albumin.

TABLE 2. Antibody response of channel catfish to bovine serum albumin determined by the passive hemagglutination test.

Group [a]		Weeks Post Inoculation 0	1	2	3	4	5	6	7	15
1	BSA[b]/PBS	*[c]	*	*	*	20[d]	*	*	*	*
2	BSA/PBS Boosted[e]	*	*	*	20[d]	20	20[d]	*	*	*
4	BSA/FCA[f]	*	*	160	320	2560	320	2560	5120	2560

a Control groups 3, 5, and 6 (Table 1) negative.
b Bovine serum albumin, 10 mg intramuscular injection.
c Asterisk means no detectable titer.
d Reaction at these dilutions was incomplete agglutination.
e Bovine serum albumin, 10 mg intramuscular injection; 2nd injection 1 week after initial injection.
f Bovine serum albumin, 10 mg mixed in Freund's complete adjuvant.

Response to vesicular stomatitis virus immunization

The responses of the catfish to immunization with VSV are summarized in Table 3 and Figure 2, 3, and 4. The 50% plaque reduction end points of all the control sera (Groups 9, 11, and 12, Table 1) were less than 1:20. On this basis, significant antibody response occurred only in Groups 8 and 10 (Table 3, Figures 3 and 4). Group 8 which received VSV (2 injections) had a peak titer at 3 weeks after initial injection (Figure 3). This titer dropped at week 4 after primary injection and then slowly declined. The greatest antibody response was induced in Group 10 injected with the VSV emulsified in Freund's complete adjuvant (Table 3, Figure 4). In this group, antibody was first detected at the 3rd week post-injection with a peak titer occurring at the 4th week post-immunization (Figure 4). There was a significant drop in titer at weeks 5 and 6 post-injection with a rise in titer at week 7 and a decline in titer to week 15 post-injection.

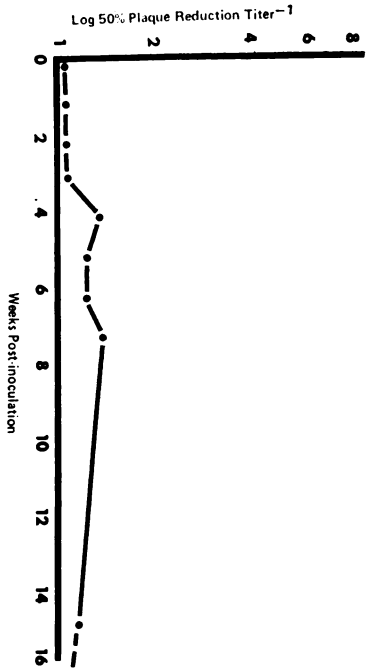

FIGURE 2. The antibody response of channel catfish to a single inoculation of vesicular stomatitis virus in saline.

TABLE 3. Antibody response of channel catfish to vesicular stomatitis virus measured by the serum neutralization 50% end point method.

Group		50% End Points[a] Week 0	1	2	3	4	5	6	7	15
7	VSV[b]/PBS	*[c]	*	*	*	20[d]	*	*	*	*
8	VSV/PBS Boosted[e]	*	*	*	640	71	40	25	20	20
9	PBS	*	*	*	*	*	*	*	*	*
10	VSV/FCA[f]	*	*	*	100	640	90	32	310	21
11	FCA/PBS	*	*	*	*	*	*	*	*	*
12	None	*	*	*	*	*	*	*	*	*

a Based upon six dilutions (base log 2), three replicates for each dilution.
b Vesicular stomatitis virus (New Jersey strain).
c Asterisk means a 50% end point of less than 1:20.
d End point calculations from the reciprocal of the dilutions.
e Boosted 1 week after initial immunization (week 0).
f Freund's complete adjuvant—Difco Co., Detroit, Michigan.

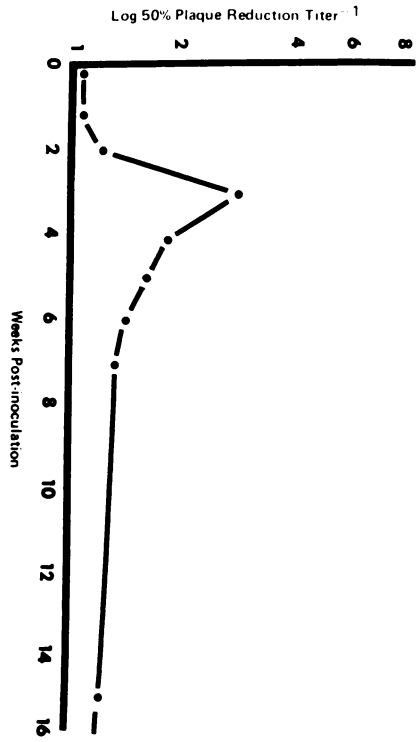

FIGURE 3. The antibody response of channel catfish to two inoculations of vesicular stomatitis virus in saline.

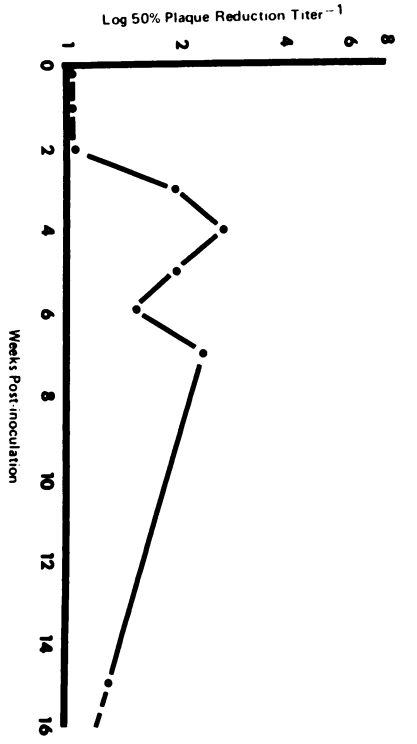

FIGURE 4. The antibody response of channel catfish to a single inoculation of vesicular stomatitis virus in Freund's Complete adjuvant.

DISCUSSION

The results of this study indicate that significant antibody titers to both a soluble protein antigen (BSA) and a viral antigen (VSV) can be induced in catfish if Freund's complete adjuvant is used to enhance the antigenicity of these particular antigens. It is apparent that single exposure to the antigens alone will not induce a significant antibody response, however, with the VSV a reasonable antibody response is induced if two injections of the antigen are given.

The findings in this study are in accord with the work reported by Sigel et al.[12] These workers found that soluble BSA given intravenously to snappers or intramuscularly to gars would not induce an antibody response. However, the intramuscular administration of alum-precipitated BSA did induce an antibody response in these fish. Trump[15] injected goldfish repeatedly with BSA and obtained a hyperimmune serum to this antigen. Ambrosius and Lehmann[1] reported that either aluminum hydroxide or Freund's adjuvant significantly increased the quantity of immunoglobulins produced in fish as compared to fish immunized without these adjuvants. They noted that the effect of adjuvants was more pronounced in the fish than it was in rabbits. Krantz et al.[7] observed that mineral oil adjuvants had a significant effect on the immune response of brown trout.

In both the antibody response to BSA and VSV, a significant drop in antibody titer occurred approximately at the 5th week post-injection. Since the antibody response of the group rather than individual fish was measured, the drop in antibody titer could be the reflection of differences in antibody responses of individuals within the group. At each sampling time the fish to be sampled were haphazardly selected since truly random selection is impossible without individual identification of the fish. If the drop in titer was the result of chance sampling of the populations the probability of getting low antibody producing fish at the same time in both groups is small. The fact that the observed drop in antibody titer was coincident in both groups of fish suggests that some common factor influenced both groups.

The time of drop in antibody titer coincided with an environmental change. During the 5th week of the experiment there was a heat wave; the water temperatures at this time ranged between 88 and 91 F, whereas in the preceding and succeeding weeks the water temperature fluctuated between 75 and 85 F. Additionally, during this time there was increased cloud cover and a significant rainfall. The optimum temperature for growth of catfish is between 73 and 84 F and growth drops off rapidly at higher temperatures.[10] The 3 to 6 degrees rise in water temperature combined with reduced oxygen tension as a result of reduction in photosynthesis of the algae due to the cloud cover and addition of silt from the surface runoff, could have provided sufficient environmental stress to significantly alter the metabolism of the catfish. This working hypothesis will have to be tested under laboratory conditions where the various environmental factors can be controlled.

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LITERATURE CITED

1. AMBROSIUS, H., and R. LEHMANN. 1965. Beitrage zur Immunbiologie poikilothermer Wirbeltiere. III. Der Einfluss von Ajuvanten auf die Antikorpwer-produktion. Acta biol. med. German., 14: 830.
2. BOYDEN, S. V. 1951. The absorption of proteins on erythrocytes treated with tannic acid and subsequent hemagglutination by anti-protein sera. J. Exp. Med., 93: 107.
3. CASALS, J. 1967. *In: Methods in Virology*. Vol. III. K. Maramorosch and H. Koprowski, editors. Academic Press, New York. 113-198.
4. CLEM, L. W., and M. M. SIGEL. 1963. Comparative immunochemical and immunological reactions in marine fishes with soluble, viral, and bacterial antigens. Federation Proc. 22: 1138.
5. CLEM, L. W., and P. A. SMALL. 1967. Phylogeny of immunoglobulin structure and function, I. Immunoglobulins of the lemon shark. J. Expt. Med. 125: 893.
6. GOOD, R. A., and B. W. PAPERMASTER. 1964. Ontogeny and phylogeny of adaptive immunity. Advan. Immunol. 4: 1.
7. KRANTZ, G. E., M. M. REDDECLIFF, and C. E. HEIST. 1963. Development of antibodies against *Aeromonas salmonicida* in trout. J. Immun. 91: 757.
8. PAPERMASTER, B. W., R. M. CONDIE, J. FINSTAD, and R. A. GOOD. 1964. Evolution of the immune response, I. The phylogenetic development of adaptive immunologic responsiveness in vertebrates. J. Expt. Med. 119: 105.

9. RIDGWAY, G. J., H. O. HODGINS, and G. W. KLONTZ. 1966. *In: Phylogeny of Immunity*. University of Florida Press, Gainesville, R. T. Smith, P. A. Meischer, and R. A. Good, editors.

10. SCHUMACHER, P. 1969. *Pond Construction, Water Quality, and Quantity for Catfish Farming*. Proc. Commercial Fish Farming Conference. Univ. of Georgia, Athens, Ga.

11. SIGEL, M. M., and L. W. CLEM. 1965. Antibody response of fish to viral antigens. Ann. N.Y. Acad. Sci. 126 (1): 662.

12. SIGEL, M. M., W. J. RUSSELL, and C. BRADSHAW. 1967. Deficient immunologic priming by a soluble antigen in Teleosts. Bact. Proc. M5.

13. SNIESZKO, S. F. 1970. Immunization of fishes: a review. J. Wildlife Dis., 6: 24.

14. STAVITSKY, A. B. 1954. Micromethods for the study of proteins and antibodies. I. Procedure and general applications of hemagglutination and hemagglutination-inhibition reactions with tannic acid and protein-treated red blood cells. J. Immun., 72: 360.

15. TRUMP, G. N. 1970. Goldfish immunoglobulins and antibodies to bovine serum albumin. J. Immun. 104: 1267.

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