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Methylmercury/Copper Effects on Hemosiderin: Possible Mechanism of Immune Suppression in Fish

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In recent years, considerable work has been concerned with the effects of sub-lethal doses of various pollutants on the immune response of various organisms. Lead (HEMPHILL et al. 1971), cadmium (JONES et al. 1971), DDT (WASSERMANN et al. 1971) and various pesticides (STREET and SHARMA 1975) have been implicated in decreasing immunity in mammals. SAROT and PERLMUTTER (1976) have shown that zinc will depress immunity in the zebrafish, and ROALES and PERLMUTTER (1977) have shown that copper and methylmercury, applied jointly and singly, decrease the immune response of the blue gourami (Trichogaster trichopterus Pallas) to viral and bacterial antigens.

The immune response of the blue gourami to viruses (YU et al. 1969) and bacteria (ROALES and PERLMUTTER 1975) has been recently studied. In addition, YU et al. (1970) have determined that immunity in the blue gourami is due chiefly to production of antibodies by the spleen. They suggested that the red pulp of the spleen might be the site of antibody production, although histological studies were not carried out. The normal morphology of the blue gourami spleen has been reported by YU et al. (1971), and mercury has been detected in the spleen of various fishes (SUZUKI et al. 1973).

In view of this evidence, the purpose of the present study was to determine if methylmercury and copper might produce any gross histological effects in the spleens of blue gouramis that might account for the decrease in immunity.

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MATERIALS AND METHODS

Commercially obtained blue gourami were used for all experiments, and were divided into various test groups. Group A (experimentals) consisted of two sub-groups. One sub-group was exposed to sublethal concentrations of methylmercury (8 fish), copper (8 fish) or methylmercury + copper (8 fish) and Infectious Pancreatic Necrosis (IPN) virus. The second sub-group was similar except that the fish were injected with Proteus vulgaris. The sublethal dosage was 10% of the 96 hour median tolerance limit (Tl_m) as reported by ROALES and PERLMUTTER (1974), or 9 ppb methylmercury (as methylmercuric chloride) and 9 ppb copper (as cupric chloride).

Group B (controls) was also sub-divided into two sub-groups. One sub-group was exposed to sodium chloride concentrations with a chloride ion concentration equal to that present in the methylmercury solutions (8 fish), copper solutions (8 fish) or methylmercury + copper solutions (8 fish) and IPN virus. The second sub-group was similar except that the fish were injected with P. vulgaris.

An additional number of control groups was also used: Group C (8 fish) was injected with Hank's Balanced Salt Solution (HBSS) which was used as a carrier for the viral and bacterial injections; Group D (8 fish) was subjected to sham injections, and Group E (8 fish) consisted of untreated, normal fish. These fish were all maintained in water containing chloride ion concentrations equal to that of the highest concentration in Group A.

The administration of IPN virus and P. vulgaris, preparation of immune sera, and procedures for assaying the immune response followed the techniques of ROALES and PERLMUTTER (1977). At the conclusion of the experiments, the spleens from the various groups were removed; placed in Helly's fixative for 4 hours, and then washed for 24 hours in distilled water. After washing, the spleens were dehydrated by passing the tissues through a graded butanol series. Following dehydration, the spleens were embedded in paraffin or Paraplast, and sectioned at 9 μ. The sections were then stained using the Toluidine Blue/Orange G/Eosin Y method of DOMINICI (1902).

In addition to the histological studies of Groups A - E, further studies were performed to determine the effects of exsanguination and/or anoxia following bleeding, since ZANJANI et al. (1969) had demonstrated that bleeding affected hemosiderin bodies in blue gourami. In these experiments, 8 fish each were bled and allowed to remain in covered containers for 1, 2, 4, 5 and 6 hours after exsanguination. The spleens from these fish were then examined histologically using the aforementioned procedures.

In order to quantitate the extent of any histo-

logical differences in all test groups, a total of ten slides, chosen at random, from the various experimental and control groups were observed at 100X, and any abnormalities, as evidenced by the break-up of the hemosiderin bodies, within a given randomly selected field recorded. The results were expressed as a ratio of the normal vs. the abnormal counts.

RESULTS AND DISCUSSION

The normal histology of untreated blue gourami spleens is shown in Figure 1. Fish injected with either IPN virus or P. vulgaris exhibited break-up of the hemo-

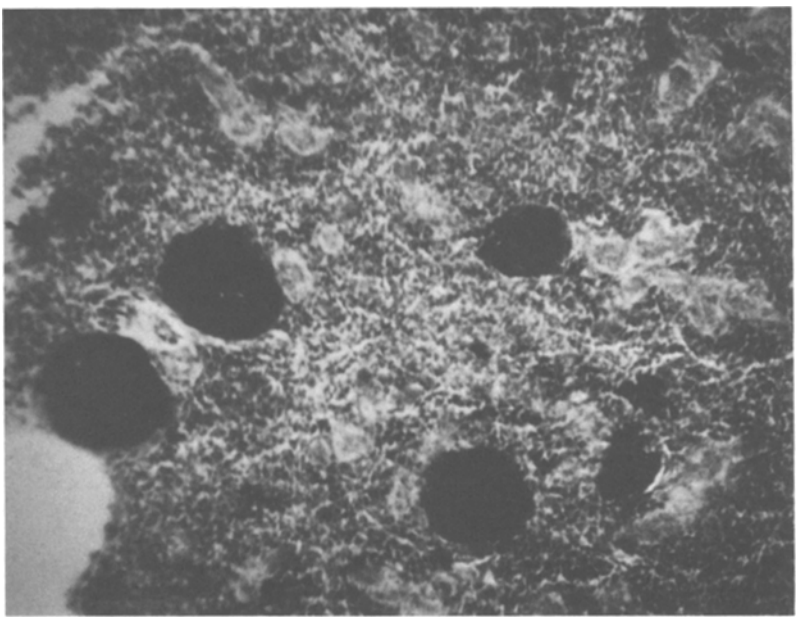

Figure 1. Photomicrograph of a normal blue gourami spleen showing, dark, round, and solid hemosiderin bodies. X 480

siderin (Figure 2) with the white pulp infiltrating the central portion of the hemosiderin bodies.

The results of the normal and abnormal hemosiderin counts are shown in Tables I and II. At one week after the challenge injection of antigens, a statistically significant decrease in the amount of abnormal hemosiderin present in Group A as compared with the controls (Group B) for that group was noted in two groups where no antibodies were detected (complete immunosuppression). Three groups: copper-treated/IPN-injected fish, methyl-

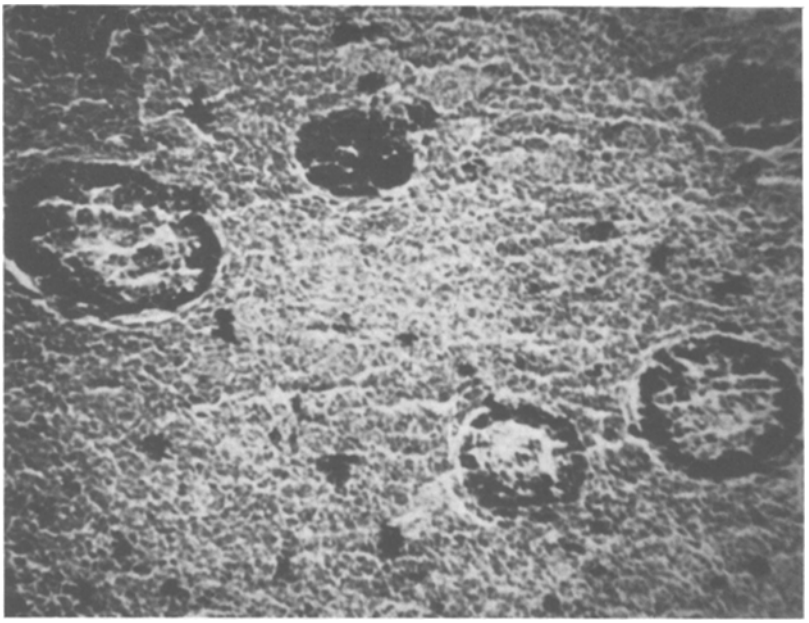

Figure 2. Photomicrograph of an antigen-treated blue gourami spleen showing infiltration of the hemosiderin by the white pulp. X 480

mercury + copper-treated/IPN-injected fish and methyl-mercury + copper-treated/P. vulgaris-injected fish, were exceptions to these findings. At two weeks after the challenge injection of antigens fish indicating complete immunosuppression also had statistically significant decreases in the amount of abnormal hemosiderin bodies.

Fish subjected to bleeding and anoxia exhibited no abnormal hemosiderin bodies as did Group D (sham injected). Groups C (HBSS injected) and E (untreated) both had small, but statistically insignificant, amounts of abnormal hemosiderin at two weeks and one week after the challenge injections, respectively.

In all experiments where fish were injected with antigens, the abnormal hemosiderin pattern resulted. Fish subjected to exsanguination and anoxia, sham injected, untreated and HBSS injected fish exhibited normal or slightly, but statistically insignificant, abnormal hemosiderin bodies.

The abnormal hemosiderin does not appear to be due to the toxicants, since fish exposed to sodium chloride solutions (controls) also exhibited abnormalities when exposed to the various antigens. This seems to indicate

TABLE I

HEMOSIDERIN ABNORMALITIES AFTER EXPOSURE OF BLUE GOURAMIS TO ANTIGENS AND TOXICANTS

WEEKS AFTER ANTIGEN CHALLENGE INJECTION	GROUP	PER CENT ABNORMAL HEMOSIDERIN [1,2] IPN VIRUS-TREATED FISH METHYL-MERCURY	IPN VIRUS-TREATED FISH COPPER	IPN VIRUS-TREATED FISH METHYL-MERCURY & COPPER	P.V. TREATED FISH METHYL-MERCURY	P.V. TREATED FISH COPPER	P.V. TREATED FISH METHYL-MERCURY & COPPER
1	A	55.8 ± 5.5 (16)	39.7 ± 7.1 (0)	2.8 ±2.0 (0)	12.7 ± 8.0 (0)	3.4 ±2.2 (0)	62.7 ± 8.6 (0)
	B	52.1 ±11.0 (32)	53.5 ±11.4 (16)	6.6 ±6.2 (48)	71.0 ±12.0 (128)	77.0 ±12.1 (64)	73.9 ± 9.1 (128)
	Significance of Difference of Abnormal. between A & B[3]	t=0.300 P=>0.7 <0.8	t=1.028 P=>0.3 <0.4	t=0.585 P=>0.5 <0.6	t=4.043 P=>0.001 <0.01	t=5.985 P=<0.001	t=0.887 P=>0.3 <0.4
2	A	23.5 ± 4.2 (0)	68.4 ± 4.8 (16)	14.7 ± 4.5 (8)	0.0 ± 0.0 (0)	23.2 ± 5.1 (0)	23.2 ± 1.5 (0)
	B	56.7 ± 7.0 (48)	59.4 ± 7.1 (64)	18.5 ± 5.8 (32)	32.0 ±12.0 (32)	96.3 ±12.9 (32)	96.3 ± 0.8 (32)
	Significance of Difference of Abnormal. between A & B[3]	t=4.069 P=>0.001 <0.01	t=1.050 P=>0.3 <0.4	t=0.518 P=>0.5 <0.6	t=2.667 P=>0.05 <0.1	t=5.270 P=<0.001	t=43.000 P=<0.001

1 Mean ± standard error

2 Numbers in parentheses refer to mean units of antibody

3 n = 9; p = 0.05 when t = 2.262

TABLE II

Result of spleen histology studies in control fish (Groups C, D, E)

WEEKS AFTER EXPOSURE TO SODIUM CHLORIDE SOLUTIONS	% ABNORMAL HEMOSIDERIN[1,2] GROUP C	GROUP D	GROUP E
1	0.0 ±0.0 (0)	0.0 ±0.0 (0)	1.1 ±1.1 (0)
2	3.3 ±3.3 (0)	0.0 ±0.0 (0)	0.0 ±0.0 (0)

[1] mean ± standard error

[2] numbers in parentheses refer to mean units of antibody

that the abnormalities in the hemosiderin bodies are due to the antigens. The abnormal hemosiderin seen in fish with no apparent antibody production, could be due to the production of antibodies at levels below those detected by the present assay system.

The fact that the abnormal hemosiderin bodies contain white pulp suggests that this phenomenon might be due to a proliferation of the white pulp into the hemosiderin bodies. This further suggests that the white pulp may be involved in antibody production in these fish. Further work is necessary to determine if this is indeed the case.

With the exception of the three groups previously mentioned in the results, in all experiments in which there was a suppression of the immune response there was a statistically significant lower percentage of abnormal hemosiderin as compared with their controls. The three exceptions to these findings all occurred at one week after the challenge injection of antigen, and involved methylmercury + copper or copper treatment. This phenomenon might be due to insufficient time for the toxicants to act, resulting in a proliferation of the white pulp into the hemosiderin as a result of antigenic stimulation. There appears to be no quantitative correlation between the amount of abnormal hemosiderin and the amount of antibody produced, since some cases in which a low amount of antibody was detected indicated a high percentage of hemosiderin abnormalities and vice versa.

Since the hemosiderin bodies represent stores of iron in fish spleens, it is possible that the abnormalities found in the present study were due to the release of iron necessary for RBC production as a result of bleeding or anoxia. However, ZANJANI et al. (1969) found no such abnormalities upon bleeding blue gouramis. Fish subjected to bleeding and/or anoxia only, in the present study, were found to possess no abnormalities. Thus, the abnormal hemosiderin pattern is not due to iron release, and a subsequent decrease in hemosiderin tissue.

In general, it appears that injection of antigen stimulates the proliferation of the white pulp of the spleen into the hemosiderin bodies, indicating that perhaps the white pulp is the source of the antibodies. Treatment with methylmercury, copper or methylmercury + copper appears to reduce this phenomenon as can especially be seen in cases where no antibodies are produced (complete immunosuppression). It would appear that methylmercury, copper and methylmercury + copper exert their immunosuppressive effects by preventing the proliferation of the white pulp of the spleen. However, the role of the white pulp in the production of antibodies remains unclear, and further work in this area is necessary to determine the exact cellular source of the antibodies.

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