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SUPPRESSION OF THE PRIMARY IMMUNE
RESPONSE IN RAINBOW TROUT, SALMO GAIRDNERI,
SUBLETHALLY IRRADIATED DURING EMBRYOGENESIS

by

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Eggs of rainbow trout were spawned artificially in the laboratory, fertilized, and immediately immersed in 0, 0.01, 0.1, 1.0, and 10.0 $\mu\text{Ci/ml}$ tritium (biological grade) contaminated spring water (pathogen free). Rearing through 20 days of embryogenesis at $10.5 \pm 0.2^\circ\text{C}$ was facilitated within a recirculation drip incubation system of 150 liter capacity. Exposure of the embryos to 0, 0.01, 0.1, 1.0, and 10.0 $\mu\text{Ci/ml}$ tritium resulted in an estimated total dose of 0, 0.048, 0.470, 4.550, and 40.348 rads.

At 20 days (eyed stage), the embryos were transferred to a single-pass incubation system maintained on thermally controlled, $10.5 \pm 0.2^\circ\text{C}$, dechlorinated sanitary water. Hatching occurred at 28.5 ± 0.5 days.

At 14 days post hatch, the fry were transferred to 10 ft x 2 ft x 1 ft fiberglass rearing troughs also maintained on thermally controlled dechlorinated sanitary water. Rearing temperature was adjusted to $20.0 \pm 0.3^\circ\text{C}$ at a rate of $2.0 \pm 0.5^\circ\text{C}$ per week.

A final transfer of the fingerlings to permanent 10 ft x 4 ft x 3 ft concrete rearing ponds also maintained on thermally controlled, $20 \pm 0.5^\circ\text{C}$ dechlorinated sanitary water was effected 3 months post hatch. Rearing thereafter was in accordance with standard hatchery practice.

At 5 months post hatch, control and irradiated test fish were administered intraperitoneally 0.1 cc of a heat killed antigen (1.8×10^8 cells per ml, Chondrococcus columnaris) in 25% Freund's incomplete adjuvant. A 0.1 cc sham vaccination containing saline and 25% Freund's incomplete adjuvant was similarly administered to another group of control fish. At 3 weeks post vaccination and at weekly intervals thereafter, a standard tube agglutination test for the specific antigen of vaccination

was performed on the serum from each control and irradiated test group.

Selected results of serum agglutination tests to assess the potential for suppressed immune competence in juvenile rainbow trout sublethally exposed to 0, 0.048, 0.470, 4.550, and 40.435 rads of tritium irradiation during embryogenesis are reported in Table I.

As in previous experiments, the percentages of fish with agglutinins and the magnitude of titers in both control and irradiated test groups increased following immunologic stimulation, Figure 1. However, control, 0.048, and 0.470 rad treatment groups demonstrated higher agglutinin titers suggesting a suppression of immune competence at the 4.550 and 40.348 rad levels. A chi-square test of independence of the titer distributions of each group verified this finding.

To determine the potential for qualitative and quantitative changes in serum components which would account for reduced immune competence in irradiated fish, polyacrylamide gel electrophoresis was performed. Additionally, total serum protein analyses were conducted.

Preliminary results with 5% acrylamide separation gel stained with 0.25% amido black 10 B demonstrated 31 bands as reported in Figure 2. Each band is numbered following decreasing mobility. The most rapidly migrating bands, 1-5, were found to be slightly more mobile than human serum albumins. All bands were generally well delimited. Mobilities did not change, but large dissimilarities in staining intensity were observed from one animal to another. A lowered total serum protein level did not influence the relative position on the number of bands and generally only affected stain intensity.

Inconstant bands were encountered in certain patterns. The main variations occurred in bands 29, 30, and 31. These bands were not observed

in non-immunized fish, either irradiated or control; however, the presence of these bands was found to consistently coincide with detection of antibody titers of at least 1:40 in nearly all immunized test fish, either irradiated or control.

It is presumptive that bands 29, 30, and 31 represented specific humoral antibody to the immunogen, Chondrococcus columnaris. It follows that in the greater proportion of fish irradiated at the 4.550 and 40.348 rad levels, bands 29, 30, and 31 were significantly reduced, and undoubtedly accounted for the lowered antibody titers in these test fish demonstrated by agglutination assay.

Total serum protein levels of all test fish throughout the test period remained the same; however, the Biuret technique here applied may not be sufficiently sensitive to detect the changes observed in bands 29, 30, and 31 by gel electrophoresis.

TABLE I. Serum Agglutinins to *Chondrococcus columnaris* in Juvenile Rainbow Trout, *Salmo gairdneri* Exposed to 0.048, 0.470, 4.550, and 40.348 rad of Tritium Irradiation During Embryogenesis. (Pond I, Compartment A-E).

Treatment	Weeks Post Vaccination	No. Fish	% Positive Titers	Range Positive Titers	Mean Titers
Control	0	6	0	-	0
	3	22	95	1:20 -40	1:28
	5	22	95	1:20 -640	1:196
	7	21	100	1:160-1280	1:1074
	9	20	100	1:640-1280	1:1024
	11	20	100	1:640-2560	1:1568
0.048 rad	0	10	0	-	0
	3	22	100	1:20 -40	1:29
	5	20	100	1:20 -320	1:117
	7	20	100	1:160-2560	1:1218
	9	20	100	1:320-1280	1:704
	11	20	100	1:640-2560	1:1120
0.470 rad	0	7	0	-	0
	3	25	76	1:20 -40	1:22
	5	24	100	1:20 -320	1:128
	7	22	100	1:320-1280	1:720
	9	20	100	1:640-1280	1:896
	11	20	100	1:320-2560	1:912
4.550 rad	0	8	0	-	0
	3	24	63	1:20 -40	1:18
	5	24	100	1:20 -640	1:132
	7	21	100	1:160-1280	1:720
	9	20	100	1:160-1280	1:584
	11	20	100	1:320-2560	1:816
40.348 rad	0	6	0	-	0
	3	24	88	1:20 -40	1:20
	5	21	100	1:20 -320	1:80
	7	21	100	1:80 -640	1:518
	9	20	100	1:160-640	1:504
	11	20	100	1:320-1280	1:704

Figure 1. The primary immune response to a single intraperitoneal injection of heat killed *Chondrococcus columnaris* antigen and 25% Freund's incomplete adjuvant in control, 0.048, 0.470, 4.550, and 40.348 rad treatment groups (Pond I, Compartment A-E).

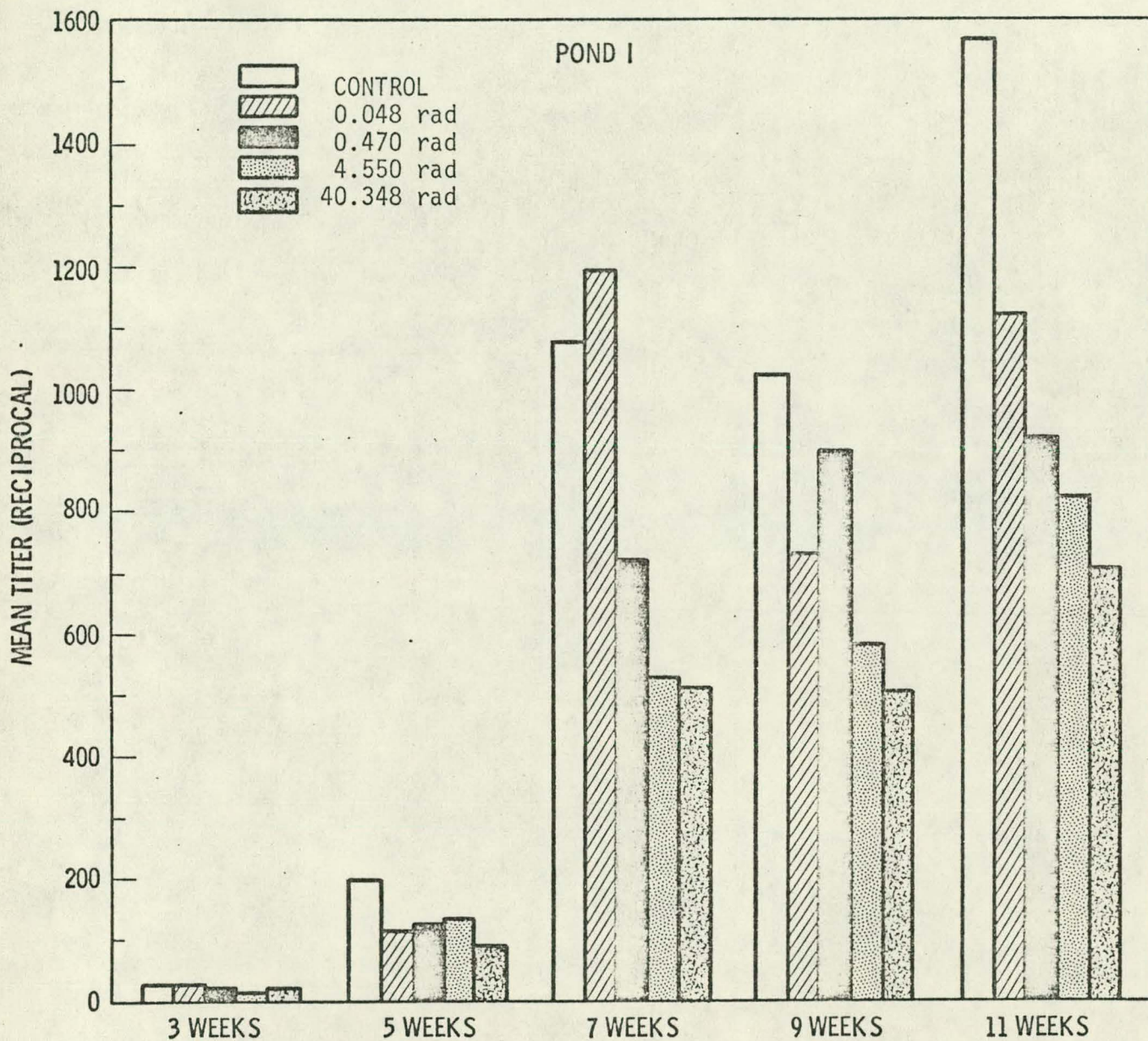


Figure 2. Electrophoretic pattern of rainbow trout serum proteins using 5% acrylamide separation gel. Bands are numbered following decreasing mobility. Principal inconstant bands are indicated by dashed lines. The pattern presented is representative for band thickness and relative mobility, but does not account precisely for relative concentration.

