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The Effects of Radium on Certain Goldfish Blood Constituents and Organs

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THE EFFECTS OF RADIUM ON CERTAIN GOLDFISH BLOOD CONSTITUENTS AND ORGANS

by

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INTRODUCTION

Background radioactivity from radium has long existed in ground and surface waters of the United States, but in the last 15 years radium has become a consequential unnatural contaminant of waters (Coulomb, 1955; Rone, 1952; Schlundt, 1910; Scott and Barker, 1956; Scott, 1961; and Tsivoglou et al., 1958). The onset of the atomic age initiated the increased processing of uranium ore for its uranium content. Radium is a daughter product and a by-product of uranium ore and its purification (Glasstone, 1958; and Tsivoglou et al., 1958). The uranium refinery wastes are released into streams in which all forms of aquatic life are exposed to the radioactivity of the radium contaminated waters.

The U. S. Public Health Service was interested in determining to what extent radium was incorporated and concentrated by the aquatic biota when exposed to the levels of contamination measured. These levels never exceeded 55 micromicrocuries per liter of water from the streams of concern in the Four Corners area of the United States (Arizona, Colorado, New Mexico, and Utah)(U. S. Geological Survey, 1959). They financed a segment of this study through their National Institutes of Health contract number RH-77 (C-1). The Atomic Energy Commission was interested in what subtle changes occur in aquatic biota subjected to the sublethal concentrations of radium found in the polluted waters. They supported a portion of this research through their contract number AT(11-1)-1023.

In order to fulfill the contractural obligations and obtain some indicative results definite limited objectives were established:

1. To determine what changes occur in the blood picture of fish

exposed to different levels of radium for different time periods.

2. To determine where the radium concentrates and to what extent in fish exposed to different levels of radium for different time periods.

Controlled conditions were extablished through laboratory experimentation. Goldfish were selected because they are convenient to get in large numbers as a homogeneous group. They are hardy and easy to handle, and they are a good representative of warm water fish.

The characteristics of the blood that were determined are:

a. Microhematocrits
b. White blood cell counts
c. Total plasma proteins
d. Differential smear ratios used to assess white blood cell composition.

This clinical study of the blood made it desirable to investigate radium concentration in certain organs. The selected tissues were:

a. Total internal organs
b. Backbone and opercular bones.

Consequently, a third objective was possible, though it was not fully achieved.

3. To determine if any justifiable correlation exists between radium concentration in a selected organ and the changes in the blood characteristics of fish exposed to different levels of radium for different time periods.

REVIEW OF LITERATURE

Radium, its chemistry, physics, and biology

Chemically, radium is a divalent element of Group II A with an atomic number of 88 and an atomic weight of 226.05 (Holmes, 1949). In the pure state it is a silvery white metal that easily tarnishes in air. The melting point is 960° C. and the boiling point is 1140° C. Radium is usually prepared in the solid carbonate or sulfate form or as a liquid in the very soluble bromide or chloride (Hodgman, 1949). Radium chloride was used for this study.

Radium is naturally radioactive and possesses a half life of 1620 years (Glasstone, 1958). It is a member of the Uranium Series.

The Uranium Series

Radioelement	Corresponding element	Symbol	Radiation	Half life
Uranium I	Uranium	U^{238}	Alpha	4.51×10^{9}yr
Uranium X_I	Thorium	Th^{234}	Beta	24.1 days
Uranium X_2	Protactinium	Pa^{234}	Beta	1.18 minutes
Uranium II	Uranium	U^{234}	Alpha	2.48×10^{5}yr
Ionium	Thorium	Th^{230}	Alpha	8.0×10^{4}yr
Radium	Radium	Ra^{226}	Alpha	1.62×10^{3} yr
Ra Emmanation	Radon	Rn^{222}	Alpha	3.82 days
(etc. through several steps and years to stable lead, Pb^{206})				

Source: Glasstone, 1958, p. 133

The alpha emitting radium is found in uranium ore at the ratio of one part radium to 2.8 million parts uranium. Uranium ore in this country is found in the mineral carnotite which has its parentage in igneous rock (Holmes, 1949).

One gram of radium is approximately equivalent to one curie of activity. One curie is defined as 3.7×10^{10} disintegrations per second. Deleterious radioactive activities are usually in the concentrations of microcuries

(10^{-6} curie) or micromicrocuries (10^{-12} curie). The maximum permissible body burden for humans is 0.1 microgram or microcurie (Fink, 1950; International Commission on Radiological Protection, 1959).

> The National Bureau of Standards in 1941 therefore set 0.1 microgram fixed in the body as the maximum safe level of radium content. This figure means a maximum over-all intake of one microgram as the safest limit, and certainly not more than five micrograms, inasmuch as permanent fixations of 0.1 to 10 percent of ingested radium have been reported with an average of about two percent. [Fink, 1950, p. 232]

The level of 0.1 microcurie per liter of water was the lowest activity to which goldfish were exposed, since both sublethal conditions and biological manifestations were desired.

Biological studies with radium have been well summarized by Fink (1950). After 5 days only 25-30 percent of ingested radium remained in the human body. Intraveneously administered radium is eliminated more slowly so 55-65 percent remained in the body after 5 days. Ninety percent of the excretion was via the feces. After the early high rate of elimination further discharge depended upon radium fixation which is influenced by the nutritional and metabolic state of the individual, plus the duration of exposure and the rate of absorption. Radium was quickly removed from the blood and stored in the soft tissues. After 48 hours only 0.1 percent of the dose remained in the blood. From the soft tissues all the radium was gradually transferred to the bone. It is here that over a period of years minute quantities of radium prove fatal.

Since the ingestion and intraveneous pathways are the most comparable routes of entry for radium chloride into fish and mammals, further remarks on elimination and retention are confined to such entry. Rapid early elimination from rats occurred about equally through the urine and feces (Fink, 1950). After 10 days 55 percent of the radium remained in the body, but it took 2 1/2 years to discharge 50 percent of the remainder.

Within 24 hours 50 percent of the dose (83 percent of the body content) was in the skeletal system. By the end of 10 days almost 55 percent of the dose (99 percent of the body content) was in the skeleton. The concentrations in soft tissues were low and became lower in time. After 1 days 10 percent of the dose was in soft tissues and over half of that was in the gastro-intestinal tract. The kidney and spleen showed the fastest uptake while the liver was slowest. By 10 days less than 1 percent of the body burden remained in the soft tissues. The blood has a very low retention rate. Blood contained 0.08 percent of the dose after 24 hours and this value dropped to 0.005 percent (0.01 percent of the body content) by 10 days.

In toxicity studies radium chloride was introduced into several mammals at repeated chronic doses through several weeks, while the responses were observed for several weeks more (Fink, 1950). The two standard manifestations were bone pathology and hematological abnormalities, mostly anemias, before death. Soft hemopoietic tissues also reflected radium damage through the loss of functional cells and atrophy. Widespread calcification of blood vessels and hemorrhage in the tissues were also noted. Sufficient LD_{50} data were not available, but the quantity of purified material needed in one dose to kill an average rat within a given time was:

Days to death	Microcuries per kilogram body weight
10	8,000
20	4,000
30	3,000
60	1,300
100	600
200	150
300	30

Source: Fink, 1950, p. 251 (a small portion of his Table 7.19)

The question of analogy between mammals (rats, rabbits, guinea pigs, or humans) and fish in the retention, distribution, and toxicology of

radium is still difficult to answer, but mammalian work does point out the trends and suggests possibilities of investigation. Therefore, the time periods of 5, 10, or 15 days were selected for this study with the preceeding remarks serving as guides.

Radiation and fish

The literature is resplendent with data on the subject of radiation and fish (Amano et al., 1956; Dunning, 1957; Foster and Davis, 1956; Mori and Saiki, 1958; Palange et al., 1954; Saiki et al., 1956; Seymour et al., 1958; Takase and Yamada, 1955; Tsivoglou et al., 1958). All of these studies were concerned with distribution and concentration of radioactive substances in fish living in waters used for nuclear tests, reactor coolers, or waste disposal. The possibility that contaminated fish may become human food prompted most early investigations. These findings produced several general observations and conclusions that relate to this study and are concerned with the actions of radioactive materials in fish.

Entry of radioactive substances into fish is dependent upon both the chemical nature of the element and the physiological characteristics of the organism. Each chemical element has its own selective behavior (Hiyama, 1956). Radioisotopes are selectively concentrated in the organs that need the element. The amount of radioactive material concentrated is inversely dependent on the amount of the identical stable ions in the water. Some of the other major factors that influence the uptake and concentration are:

a. Age, size, and metabolic processes of the organism.

b. Organisms need for the element.

c. Biological half life of the element.

d. Physical, chemical, and biological properties of the water, all of which are especially true circumstances for radium (Yamasaki, 1952).

Since radium is in the same family of elements as calcium and possesses the same affinity, it replaces calcium in skeletal tissue (Newman, 1955). Once fixed in the bone the weakly penetrating alpha radiation is absorbed with intense local damage, so radium is many thousands of times more harmful internally than externally (Downey, 1938).

The Japanese established an order of appearance and concentration into fish of fission products from nuclear tests (Amano et al., 1956; and Saiki et al., 1956). The first appearance was on the skin, followed secondly by blood and then concentration began. The sequence was:

a. First order -- liver, kidney, gall bladder, and heart.

b. Second order -- pyloric caeca, stomach, intestine, and gonads.

c. Third order -- skin, bone, and muscle.

When radioactive materials enter fish through food, the initial concentration (outside of the gastrointestinal tract) was highest in the blood with the kidney second and other internal organs third. The long continuous accumulation occurred in the liver, bone, integument, and muscle (Chipman, 1956; and Takase and Yamada, 1955).

Radioactive substances also enter the fish's body by way of the skin and gills (Chipman, 1956; and Yoshii et al., 1958). Chipman working with the little tunny, Euthynnus alletteratus, demonstrated that all fission products did penetrate the skin and deeper tissues from a lesser to a greater degree. Yoshii et al. (1957) used goldfish, Carassius auratus, to study the entry of radioactive materials when fish were just immersed in contaminated water for 69 hours. Their results are tabulated as follows:

	Results in radioactivity		
Isotope	Gills	Internal organs	Others
P^{32}	2.5	2.0	0.7
S^{35}	7.6	0.0	0.0
Ca^{45}	94.5	9.8	72.6
Sr^{89}	36.0	53.3	35.2

The relative radiosensitivity of the gills is noticeable. Nonfeeding brook trout absorbed calcium-45 from the water with the largest amount appearing in the gills (Lovelace and Podoliak, 1952). From this main path of entrance the calcium was distributed throughout the body by the blood stream.

Goldfish were similarly used by Hara and Yoshida (1960) to determine the uptake and areas of concentration of sodium-24 and potassium-42. The highest content in the body was equal to one half the concentration of each isotope in solution. Radioactive strontium-90 penetrated the body of carp directly from the water through the mouth, gills, and skin, and gradually concentrated in the bone (Danil'chenko, 1958). Regardless of high radioactivity of the water and high energy radiation of strontium-90 and yttrium-90, the fish kept in a radioactive solution of this substance had comparatively low radioactivity.

All these discoveries provided assurance that goldfish immersed in contaminated water for 5 to 15 days offered sufficient entry and exposure of the internal tissues to radium.

Radiation, radium, and blood

The actions of radiation and radium on blood were excellently summarized by Laurence Selling and Edwin C. Osgood in a chapter entitled "Action of benzol, roentgen rays, and radioactive substances on the blood and blood forming organs" in the Handbook of Hematology (Downey, 1938, p. 2691-2801). The following remarks are confined to circumstances and conditions pertinent to this study.

> The most important effect of customary therapeutic doses of irradiation on the blood elements [in humans] is to decrease the number of white cells[1], especially lymphocytes, so that leukopenia and lymphopenia may occur. Preceeding the decrease in the white count a transient increase

[1] white blood cell terms: leukocytes - all white blood cells, granulocytes - ones with granules, lymphocytes - nongranular ones.

> develops . . . very small doses of irradiation may permit a lymphocytosis . . . it irradiation causes the white count to reach its lowest point about six days later. . . . The fall in lymphocytes is greatest in the first 24 hours, but they continue to drop for about three days. These cells rise with the white count, but do so proportionately more slowly . . . the blood contains many degenerate white cells, especially in the first three days. . . . Important changes in the count of the red blood corpuscles and hemoglobin percentage do not occur as a result of mild or intensive therapeutic irradiation. Changes in the number of immature cells occur . . . [Downey, 1938, p. 2742-2743]

Changes in leukocytes following irradiation are indirect effects due to tissue responses rather than that of the circulating cells. Leukocytosis develops from small doses of x-rays. The percent of eosinophils and basiophils increased. The changes in white blood cell count and composition begin soon after irradiation.

Repeated small doses of x-rays caused a 50 percent drop in lymphocytes within 1 hour in rats. In several mammals the lymphocytes dropped to their minimum number by 48 hours, then showed a slight recovery and fall again before a return to normal. In humans that were given an injection of 50 milligrams of radium, the drop in white blood cells began in one-half to 6 hours, but returned to normal within 24 hours.

Generally speaking, the effects of radiation are first noticed in blood from one-half hour to 9 days after exposure with 35 hours the mean time lag. Recovery starts from 2 hours to 45 days after irradiation with 10 days as a mean (Albritton, 1952). These figures are broad, inclusive ones taken from several mammals and cover a multitude of experimental circumstances and cellular changes. But they do point out the relatively short period of time before a reaction to and recovery from radiation is noted in the blood.

The specific actions of alpha rays on blood and other organs are:

1. Aplasia of the bone marrow, commencing within 3 to 5 days and reaching a maximum at 9 to 10 days.

2. Leukopenia with a possible absence of leukocytes within 3 days. Small doses may cause an initial leukocytosis, which also occurs in some pathological conditions.

3. Anemia developing only after long periods of exposure to small doses or a long time after exposure to a large dose. (This delayed response is due to the long life of the erythrocyte in comparison to the short lived white cell.)

4. Negligible action on any of the mature circulating blood cells.

Some specific results of hematological studies on rats injected with radium chloride were reported by Fink (1950). Even though the dosages used were considerably higher than those in this study, the findings help point out possible changes to expect. Anemia developed after many weeks in animals injected with a single dose of 300 microcuries per kilogram of body weight. There was a drop within 2 days to 40-50 percent of the original leukocyte count in animals receiving 17 to 175 microcuries per kilogram. Rats injected with 51 microcuries per kilogram of body weight had a decline in lymphocytes in 24 hours and after 14 days these counts were 24 percent of control levels. Radium also caused changes in the hemapoietic system, liver, kidney, intestine, bone, testes, aorta, and coronary arteries. All experimental specimens died within a year regardless of the concentration of radium chloride injected. The higher the concentration, the sooner the animals died.

Recent reviews show that blood changes occur at fairly low exposure rates. The National Academy of Sciences (1956) concluded that blood counts in man were statistically altered, even at the maximum permissible exposure level by repeated low level exposure. There was a decrease in neutrophils and lymphocytes. Hollaender (1954) also concluded that a minimal exposure became important if repeated enough to become an accumulated dose. Definite hematological changes appeared in guinea pigs when exposed to 1.1[2] rads per

[3]A rad is defined as an energy absorption of 100 ergs per gram in any medium.

day for several days. The same was true with dosages of .11 roentgens of gamma rays (radium) per day for 1 month. Leukocyte reduction and macrocytic anemia occurred in mice and rabbits injected once with a dose of 0.1 to 0.2 micrograms of radium chloride. Even 0.02-0.03 micrograms per gram of body weight decreased the white blood cell count in these specimens (Altman and Dittmer, 1961). A single exposure of humans to 40 to 70 milliroentgens of x-rays produced a transient increase in leukocytes and lymphocytes after 24 hours (Urushiyama, 1960). The grade of reaction was not proportional to the quantity of irradiation. "In all probability, radiation quantities as low as 0.02-0.05 roentgen/day can, after a comparatively short time, give rise to blood changes in man." (Altman and Dittmer, 1961). This quote best summarizes all the previous remarks.

Exploration into blood plasma protein changes perpetrated by irradiation is of more current concern than the cellular manifestations, which were discussed. The two principal plasma protein fractions are albumins and globulins, both of which are synthesized by the liver (Everett, 1946; and Weil, 1959). Any liver injury retards protein regeneration and lowers plasma protein levels. All ailments that influence plasma protein concentrations cause a decline in albumins and an increase in globulins so the total amount of protein may remain the same (Wuhrmann and Wunderly, 1960). Therefore, when the total proteins are lowered, it indicates an abnormal decrease in albumins. Disruptions of the renal and hepatic metabolism cause a lowering of the total plasma proteins through the loss of functional cells. Prokopenko (1960) found that by screening the livers of rabbits from a whole body dose of radiation, there was no reduction in the albumin concentration and the synthesis of nonspecific gamma globulins.

Rhesus monkeys that received from 60 to 600 roentgens of x-radiation showed a decrease in serum albumin and an increase in gamma globulin

(Leone et al., 1959). A single 725 roentgen exposure to some other rhesus monkeys produced a decline in the serum albumin and gamma globulin in 1 to 14 days (Glenn, 1960). Total body irradiation to rats was followed by a reduction in the amount of albumins and globulins in the blood (Fischer et al., 1954). Both Kohn (1951) and Supple et al. (1951) also used rats to find that single exposures of 125 to 750 roentgens caused a decrease in total plasma proteins within 3 days that continued up to 10 days. The duration and degree of lowered protein content was dependent upon the dosage, but incomplete recovery was noticed by 20 days. The partial recovery may be helped because blood protein survival is 12 to 30 days and half of it is broken down and resynthesized in 10 days (Wuhrmann and Wunderly, 1960).

Even though all the previous remarks were confined to homoiothermous organisms, they did serve as guides in establishing the conditions, dosages, and time periods of this study.

A nominal amount of information was found on clinical manifestations of poikilothermous blood after irradiation. Bacteremia was associated with leukocytosis in plaice (a flatfish) following an 8,000 roentgen exposure (Preston, 1959). The fish died 45-51 days later. Hematological values were assessed on blood that was removed at death.

Table 1. Hematological values of the two fish exposed to 8,000 r compared with those of normal fish

Fish	Packed cells vol. %	Red blood cell count ($10^6/mm^3$)	Hemoglobin (gm/100ml)	Erythrocytes: leukocytes
Normal	21.8	2.02	5.66	40:1
Std. dev.	3.0	0.08	0.88	
45 days	10.4	0.81	3.63	1.2:1
51 days	6.5	0.49	3.22	1.2:1

Source: Preston, 1959, p. 833

Nakatani (1961) did some experiments on long term feeding of strontium-90 to trout and on termination some clinical blood analyses were conducted. The results were:

Table 1. Average values for hematology . . . of trout sacrificed at end of 21 weeks of isotope feeding

Categories	Treatment groups			
Treatment in uc/gm fish	0	.005	.05	.5*
Number of fish	4	2	3	3
Erythrocytes: 10^6 cells/mm^3	1.28	1.06	1.47	1.67
Hemoglobin: gm/100 ml	8.04	7.69	9.16	10.4
Packed cell volume in %	39	35	45	57
Leukocyte: 10^3 cell/mm^3	37.8	64.6	32.4	7.30

Only the last characteristic proved significant.
*Fed this ration daily for 21 weeks.

Source: Nakatani, 1961, p. 9

Frogs exposed to small doses of radium had a lower relative number of lymphocytes but no reduction in total white blood cells (Downey, 1938). Schjeide et al. (1953) did some studies on the frog tadpole, Rana catesbiania, using x-rays to give single doses of 100, 250, 500, and 1000 roentgens. All exposures caused a decrease in the number of circulating white cells. The decrease was slower in appearance after 100 roentgens than any of the others. The decline was fastest for lymphocytes and slowest for thrombocytes. There was no apparent restoration to normal within 25 days following irradiation.

The reasons for the pattern of blood cell changes in homoiotherms after irradiation are known, so an understanding of these may help elucidate the causes and happenings in poikilotherms. Radiation affects:

a. Blood forming organs, such as liver, spleen, and bone marrow. These are the sensitive tissues.

b. Tissues of the heart and blood vessels.

c. Composition of peripheral blood.

d. Structure of platelets so that they disintegrate, clutter capillaries, and cause blockage (Haddow, 1952).

The effects experienced in blood are not due to irradiation of mature cells, but rather the action of radiation in cutting off the supply of new cells by preventing their normal development in blood forming tissues. Therefore, the life span of various blood elements is the necessary consideration in observing changes due to radiation (Hollaender, 1954; and Spear, 1953).

After irradiation all blood cells decrease in numbers and these effects show for exposures of 25 rads or higher. The order in which the blood cells decrease is (from first to last) lymphocytes, granulocytes, reticulocytes, thrombocytes, and erythrocytes. This sequence of decline is also the order of life span from the shortest to the longest life. The short-lived lymphocytes and granulocytes (leukocytes) are the most radiosensitive of mammalian cells. If the exposure is only a single sublethal dose, there is a return to the normal number.

The order of recovery is slightly different than the order of decrease and it is reticulocytes, granulocytes, thrombocytes, erythrocytes, and lymphocytes. The lower the radiation exposure, the more rapid is the recovery. The erythrocytes and lymphocytes have the least power of recovery.

Radiation damage to blood forming tissues, as reflected by the blood cells, may be followed by:

a. Complete recovery of production and a return to status quo.

b. An "excessive repair" leading to an over-production of less mature cells.

c. Appearance of abnormal cells in circulation.

d. Permanent cessation of all new cell production.

e. Any combination of these, which emphasizes the possible complex responses (Spear, 1953).

The previous remarks in reference to homoiothermous blood and blood forming organs have been substantiated in part by several related studies on poikilothermous animals. Allen and his associates (1951a, 1951b, and 1953) worked on the effects of x-rays on the hemapoietic cells and tissues of tadpoles. Their research revealed that doses of 25 to 10,000 roentgens destroyed hemapoietic cells and the degree of destruction increased with the dose. The total amount of hemapoietic tissue decreased from all but the lightest doses. At 500 roentgens the mesonephroi hemapoietic cells manifested injury within 48 hours. The hemapoietic cells in the kidneys of chinook salmon declined in number within 2 weeks after a 500 roentgen dose of x-rays (Bonham et al., 1948). The temperature during the irradiation of tadpoles had no influence upon the rate of destruction of hemapoietic tissues (Allen et al., 1951a). Destruction of these cells and tissues took place at or near mitosis. Alpha rays caused the degeneration of potential dividing cells in considerable numbers, which compared with the results of relatively small doses of other radiation sources (Tansley et al., 1948).

Fish blood and bone

Accumulated research on fish blood extends over half a century, includes many species, involves a variety of isolated actions and reactions, and is reported in a multitude of journals and languages (Hunn, 1958). Only recently has consideration been given to blood changes in fish as a clinical method for diagnosis of fish ailments. Now the standard hematological techniques for humans are being adopted for fish and used to establish normal blood patterns (Hesser, 1960; Katz, 1950; Larsen and Snieszko, 1960; McCormick, 1960; Rice, 1961; Phillips, 1961; Schiffman, 1959;

and Smith et al., 1952). The general conclusion of these investigations is that there is extreme variability. The blood picture differs between species and within species according to age, size, diet, environmental conditions, and water temperature. Most studies require a control group of fish to serve for comparisons and then relative changes are recognizable. Good experimental design and consistent technique are necessary to insure detection of blood changes of small magnitudes.

Satisfactory clinical procedures for microhematocrits, blood cell counts, and differential smears were available and employed in this study (Hesser, 1960). Other investigators found the microhematocrit technique satisfactory for fish blood (Larsen and Snieszko, 1960; Schiffman, 1959; Snieszko, 1960 and 1961). Plasma protein as percent gram total protein was the fourth blood characteristic that was measured. It was assessed with a protometer (National Instrument Co., 1954). This instrument was suggested by Piper (1961) and proved to be a satisfactory, efficient, and consistent tool.

Information on hemopoietic tissues of fish is limited. The reference that contributed most heavily to this research was the "Studies on the origin, development, and seasonal variations in the blood cells of the perch, Perca flavescens." (Yokoyama, 1960). His research not only established what the hemapoietic tissues were, but also helped to classify them in a definite order of importance. That order is:

1. Mesonephric kidney - the major area
2. Heart
3. Spleen, liver, and pancreas.

The extensive review of literature (Yokoyama, 1960) unearthed findings that were confirmed by his results. Yuki (1958) further substantiated

this classification. The total internal organs from fish in this study were selected for radioassay determinations.

Bone was chosen because it is the site of radium deposition. The eventual arrival of all the radium into the bone has been fully confirmed by the reviews of literature and research reported by Downey (1938), Fink (1950), and the International Committee on Radiological Protection (1959). A general conclusion of most workers is that 50-55 percent of the dose to which the animals were subjected entered and remained in the body. All of it is eventually concentrated in the skeleton. Usually no marked variations in concentration were noted from one region of the skeleton to another. Therefore, the backbone and opercular bones were extracted for radioassay as representative of the total fish skeleton.

METHODS AND MATERIALS

Experimental design and research facilities

Since differences between fish were anticipated as a problem, the experiment was designed to minimize the biological variability. The basic experimental unit was a 1-gallon jar filled with water and containing three goldfish. The water was aerated through an air hose and stone. The goldfish ranged from 80-110 millimeters in length (body length) and 20-50 grams in weight. A hardware screen cloth on top of the jar was weighted down with a chunk of lead to keep the fish in the jar. Five jars were placed in the bottom of a 25-gallon fiberglass aquarium, which served as a statistical block. Each block was a replicate of its neighbor and there were up to 12 blocks, depending on the particular experiment. Each block contained five levels of radium chloride, one level to each jar (Figure 1). The concentrations were 0.0, 0.1, 0.2, 0.3, and 0.4 microcuries per liter, which meant that each respective jar received 0.0, 0.378, 0.756, 1.134, and 1.512 microcuries of radium chloride. The doses were randomly placed in the jars. The jars were color coded (from lowest to highest concentration: blue, brown, green, yellow, and red) and the jars randomized into the aquariums. When the jars were washed and used for a succeeding experiment, the residual effects were minimized.

The aquariums were set in a large water bath. Water was also placed in the aquariums to surround the jars and serve as a bathing medium for temperature control. The transfer of heat or cold through the water in the bath and aquariums was sufficient to sustain constant water temperatures

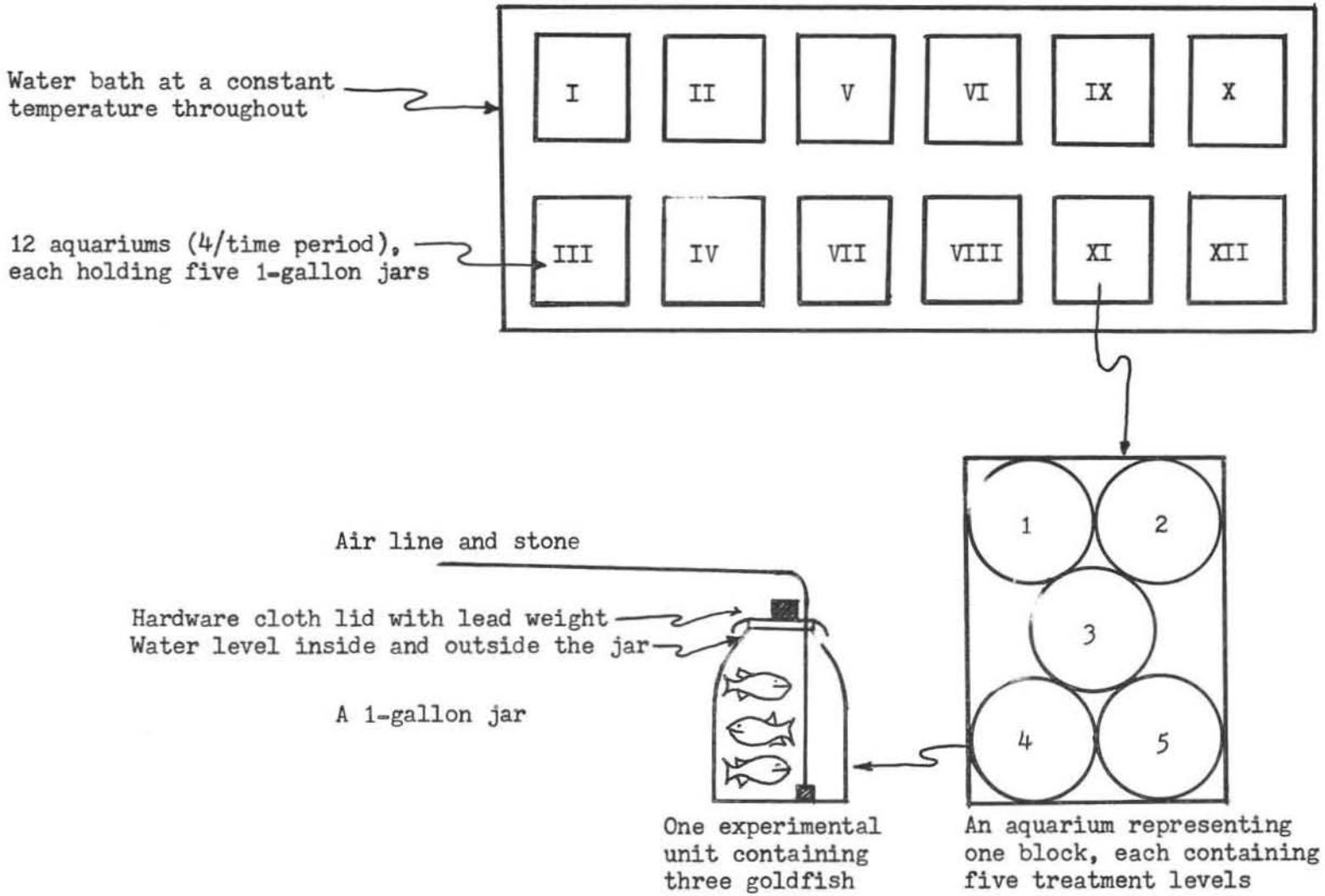

Figure 1. The physical plant for exposing goldfish to radium under controlled conditions.

in the jars throughout an experiment. The temperatures of 50°, 60°, and 70° F. were used. Cooler temperatures were maintained with a refrigeration unit that had its cooling coils in the water of the water bath. Warmer temperatures were achieved by placing several heating elements in the water bath. The temperature was checked with a constantly immersed maximum-minimum thermometer and a mercury pocket thermometer. The water in the bath was circulated with a Little Giant water pump, so homogenous mixing was maintained.

All these efforts made it possible to assume that the environment was constant, except for levels of radium, in each jar. Factors considered in this assumption included the temperature, air supply, light, space, competition, container, and surroundings. The second major assumption was that the fish were homogenous. This requirement was satisfied with the help of the Ozark Fisheries, Inc., of Stoutland, Missouri, who provided the fish free of charge. Factors considered in this assumption included source, size, age, growth rate, blood picture, and temperment.

With these two obligations satisfied, the experiment qualified best for a randomized complete block design (Cochran and Cox, 1957). Four replications of the five treatment levels were made at each of the time periods of 5, 10, or 15 days at any one temperature. The basic analysis of variance for any one fractional experiment was:

Source of variation	Degrees of freedom[a]
Blocks (replications)	3
Treatments	4
Error	12
Total	19

[a] A fractional experiment was composed of one temperature, one time period, five treatments, and four replications at each treatment level.

When three time periods (5, 10, and 15 days) at one temperature were

pooled for one analysis of variance, it was:

Source of variation	Degrees of freedom[a] Col. 1	Col. 2
Treatment	4	4
Within treatments	15	15
Time	2	1
Time x treatments	8	4
Error (b)	30	15
Total	59	39

[a]The degrees of freedom for Col. 1 were based upon a total experiment that contained one temperature, three time periods, five treatments, and four replications at each treatment level. The degrees of freedom for Col. 2 were based upon a total experiment of the same conditions as Col. 1 except there were only two time periods.

The same analysis of variance was used when the three temperatures (50°, 60°, and 70° F.) were pooled for one time period and analyzed. In this analysis of variance the temperature was substituted for time in the calculations.

Initiating and terminating an experiment

Once the physical facilities were arranged for an experiment, the water was added and brought to the temperature selected. The goldfish were starved 60-120 hours, depending on the succeeding experiment and its temperature. The fish were anesthetized with M.S. 222 (tricaine methane--sulphonate) in water, weighed on a Hansen Model 1440 dietetic scale, measured to the base of the tail, and added to the jars. Attempts were made to use fish within the 80-110 millimeter lengths and 20-50 grams range. All fish that superficially evidenced open sores or unhealthy conditions were rejected. The fish were allowed to acclimatize to their new conditions for 15-20 hours.

The radium chloride was in liquid stock solution, so it was diluted to the desired concentration and added to the water in the jars. The fish were retained under these conditions for 5, 10, or 15 days without

food or a change of water. The static environment was interrupted only by the entry of air and simulated day-night lighting period.

After the desired period of exposure, the fish were dip-netted out of the jars and placed in the anesthetic. This agent was utilized both times at a concentration of 1:7000 which provided complete narcosis within 8-12 minutes. At warmer temperatures less time was needed, while at colder temperatures the anesthetic took longer to be effective (Meister and Ritz, 1958; Sandoz, 1959). The fish was wiped dry and decaudated. The blood was collected in a spot plate. The blood from all three fish in a jar was collected in one operation, but kept separate for individual analyses. The three fish were wrapped together in cheese cloth and placed in formalin for preservation. Then they were transferred to alcohol for keeping until used later for radioassay determinations.

When all the fish were removed from the jars, the incoming air was shut off. The water was poured into a wash boiler on a hot plate, which evaporated the water and retained a concentrated radium residue. The jars were thoroughly washed in hot water and versene. Versene is a complexing agent containing EDTA salts (ethylenediamine-tetraacetic acid) that are effective in removing radioactive contamination. They were rinsed several times to help remove all traces of contamination. The air stones were rinsed and flushed in fresh water for 3 or more hours. The air stones were also color coded, similar to the jars, so residual effects were minimized by using the same air stones in the same concentrations in succeeding experiments. The water bath and aquariums were scrubbed and rinsed. After all these containers were cleaned and rinsed, they were once again placed into position for the next experiment.

Blood analysis

The blood was obtained from fish by cutting off the tail. The body end of the decaudated fish was stroked with a heparinized toothpick to prevent clotting and aid the flow of blood (Linn, 1962). If necessary, the spot plate which collected the blood was lightly coated with 10 percent heparin from a soaked cotton swab. The anticoagulate treatment was sufficient to prevent coagulation without harmful dilution and not too strong to cause hemoloysis. A 20-gram fish yielded approximately 0.25 milliliters of blood, while a 50-gram fish gave about 0.60 milliliters. The minimum amount was sufficient for the necessary blood tests, but it was easier to work with the larger amount. No further treatment of the blood was necessary before the tests.

The microhematocrits, white blood cells, and differential smears were processed according to Hesser (1960). The plasma proteins were treated as prescribed by the instrument's instructions (National Instrument Co., 1954). All characteristics were measured twice for each fish. The results for each characteristic were averaged then pooled with the related findings of the other two fish. This combination of values formed a composite average that became the figure used in the analysis of variance. Each characteristic of the blood was then computed in the analysis of variance as given previously.

The standard heparinized capillary tubes of 75 millimeters long x 1.2-1.4 millimeters in diameter were used for the microhematocrits. These tubes were filled with blood and the bottom sealed with Critoseal[3], a specially formulated vinyl plastic putty. The tubes were centrifuged in an International Micro-capillary Centrifuge at 11,500 rev/min for 4

[3]This registered trademark item is prepared by Biological Research, Inc., St. Louis 21, Missouri, and is available through most hospital supply outlets.

minutes. The final readings for percent packed red blood cells were made on a Critocap[4] Microhematocrit Tube Reader. These laboratory tools are standard items in human hematological work in which microhematocrits are an accepted method of clinical diagnosis (McGovern et al., 1955).

Upon completion of the microhematocrit reading, the capillary tube was broken at the junction of packed cells and plasma. The plasma was ejected upon the holding chamber of the protometer. The chamber was closed and the instrument directed at a light source. A graduated scale in the chamber makes it possible to look through the eyepiece and directly assess the protein content in gram percent total protein.

Differential smears were prepared by placing a drop of blood on one end of the standard 75 x 25 millimeter glass slide. A second slide was used to spread the blood to form a thin film over the slide. The slide was allowed to dry and then "fixed" with acetone free absolute methyl alcohol. The slides were stained using Hemal Stain Solutions I and II (Hemal Stain Co., Inc., date unknown). The ratio of lymphocytes to granulocytes was determined under oil immersion (97X) with a 15X eyepiece on the microscope. Usually, 100 to 200 white blood cells were differentiated per slide.

White blood cells were prepared for counting by the diluting pipette method. Accupettes[5] were utilized since these required half as much blood as the standard clinical pipettes. Whole blood was drawn up to the 0.5 mark of the pipette. Shaw's diluting fluid, Solution B (12.0 milligrams

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[4]This registered trademark item is prepared by Biological Research Inc., St. Louis 21, Missouri, and is available through most hospital supply outlets.

[5]This trademark item is available from Scientific Products, a division of American Hospital Supply Corp., with a general office in Evanston, Ill.

crystal violet, 3.8 grams sodium citrate, 0.4 milliliters formaldehyde, and 100.0 milliliters distilled water), was used to fill the pipette to the 11 mark. This step diluted the blood at a 1:20 ratio and stained the white blood cells blue. The pipette was placed in a Byron-Garrey pipette rotator to get the cells thoroughly mixed and stained, which took 10-12 minutes. The pipette was removed, some of the fluid expelled, and a drop then placed underneath the cover slip on a Spencer Bright Line Hemacytometer. The cells required 2 minutes to settle. The cells were counted with a microscope set at 10X eyepiece and 43X objective. The white blood cells were enumerated in the hemacytometer squares normally used for red blood cell counts. The number of cells counted was multiplied by 1000 to give the results in number of white blood cells per cubic millimeter of blood.

The factor of 1000 was calculated with the formula:

$$\frac{\text{Number of cells counted x dilution x 4000}}{\text{Number of small hemacytometer squares counted}} = \text{WBC/mm}^3$$

$$\text{So} \quad \frac{\text{number counted x 20 x 4000}}{80} = 1000 \text{ x number counted}$$

(American Optical, 1958).

A flow diagram of the blood analyses steps is given in Figure 2.

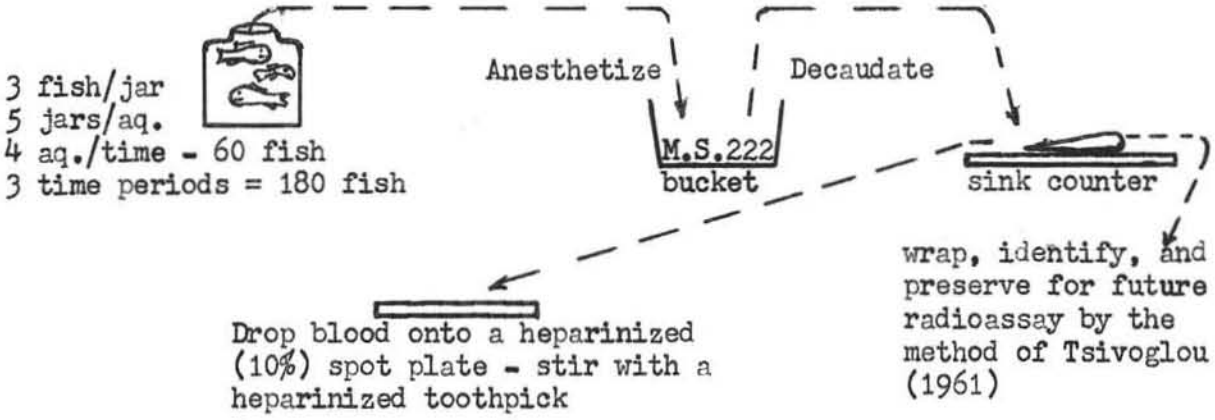
All the laboratory ware that was not discarded was cleaned with versene, 0.85 percent salt solution, and 50 percent alcohol and wiped dry with lens tissue or allowed to air dry.

Gross alpha determinations

Since radium analysis is a lengthy and involved procedure, gross alpha measurements were taken as an index to the uptake and concentration of radium in certain tissues of fish (Rushing, 1960; and Tsivoglou, 1961). The tissues selected were the total internal organs and the backbone and opercular bones. The first represented total piscine hemapoietic tissues,

FLOW DIAGRAM FOR BLOOD ANALYSIS

Abbreviated Presentation of Procedure



Thence to lab bench

One drop	One drop	One drop
Into MH cap. tube	Into WBC diluting pipette (accupette)	Onto slide for diff. smear
Seal with Crito-seal - into MH centrifuge	Color and dilute with Shaw's Sol. B	Spread and dry
Spin down-11,500 rpm for 4 min	Mix by rotator 10-15 min	Add Sol. A (abs. methyl ROH)
Onto Critocap rdr read and record in % vol. RBC	Onto hemacytometer (counting chamber)	Dry
Break tube at junction of cells and plasma-drop plasma onto plate of protometer	Under microscope count and record number of WBC	Into Hemal Stain Sol. I & II - 6 sec each to stain
Read and record plasma proteins in gram % total protein	(count x 1000 = WBC/mm^3)	Dry
		Determine ratios of WBC under oil (state as ratio)

A guide to some of the abbreviations

Aq. - aquarium
M.S.222 - tricaine methanesulphonate
MH - microhematocrit
cap. - capillary
rdr - reader
RBC - red blood cells
WBC - white blood cells
Sol. - solution
ROH - alcohol

Figure 2. An abbreviated flow diagram of the steps involved in analyzing blood from goldfish exposed to radium.

while the last two provided skeletal tissues (Yokoyama, 1960; and Fink, 1950).

Each set of three fish that was preserved after blood removal was taken out of the alcohol, opened, and rinsed. Each fish was dissected and the desired tissues extracted and separated into the two groups already mentioned. These groups of tissues were placed in separate crucibles (Coors No. 170, size 4). The bones from all three fish were removed and placed in the same crucible, so that there was one alpha determination for each experimental unit (the 1-gallon jar).

Once the tissues were in the crucibles, the necessary procedure for the dry ash method of gross alpha determination commenced (Tsivoglou, 1961). The steps were slightly modified and they were:

1. Dry at 90° C. overnight.
2. Determine dry weight.
3. Ignite at 600° C. for 12-15 hours.
4. Cool and determine ash weight.
5. Grind ash to a fine powder.
6. Weigh into three weights (50, 100, and 150 or 200 mg) onto separate planchets. This phase was necessary for the calculation of self-absorption in which the counts were plotted against the weights. The planchets were stainless steel cups, 1 1/4 inches in diameter by 3/32 inches in depth.
7. Add a small amount (approximately 0.75 ml) of reagent grade acetone to each planchet to distribute the solids.
8. Add 0.10-0.15 ml acetone-lucite to tack down the solids. Allow to dry.
9. Redry planchets at 90° C. for 5 hours and at least 12 hours before counting.
10. Weigh planchets with ash.
11. Count two successive 5 or 10 minute counts in an internal proportional counter.

The final results are given in counts per minute per known milligram ash weight.

The crucibles and planchets required pretreatment as follows:

1. Acetone rinse.
2. Mark sample number on the bottom with a wax crayon.
3. Ignite at 600° C. for 30-60 seconds.
4. Remove loose residue from the crayon.
5. Determine the weights.

The crucibles were coded so that the same tissues at similar levels of exposure were placed in the crucibles in succeeding analyses, thereby keeping residual effects to a minimum.

After the analyses were completed, the planchets were collected for discard. The excess ash in the crucibles was accumulated in a container for burial. The crucibles were soaked and washed in versene, rinsed in water, rinsed in 1:1 hydrochloric acid, rinsed in water, and air dried before reuse.

Water chemistry

In order to eliminate water changes as a source of environmentally induced blood anomalies, some basic water chemistry measurements were taken. Determinations were made for pH, carbon dioxide, dissolved oxygen and total nitrogen on the water from the control jars (no radium). The pH was assessed with a Hellige Pocket Comparator No. 605 H T using the Thymol Blue-B Color Disc No. 190-D. The Thymol Blue-B indicator solution is effective in the pH range of 8.0-9.6 (Hellige, Inc., 1950). The absence of free carbon dioxide was assumed when 10 drops of phenolphthalein indicator solution turned a 100 milliliter water sample pink (American Public Health Assoication, 1946). Dissolved oxygen was determined with the

sodium azide modification of the Winkler method and titrating in the final step with 0.025 N. sodium thiosulfate (American Public Health Association, 1946). The total nitrogen content was analyzed with the kjeldahl method by the Soils Laboratory at Utah State University.

RESULTS AND DISCUSSION

Fractional experiments

Blood analysis.--This category includes six experiments that were individually characterized by one temperature, one time period, and five treatment levels with four replications at each level (Table 1).

Table 1. The six fractional experiments and their experimental conditions[a]

Experiment number	Exposure to radium	
	In days	In temp. °F.
1	5	50
2	10	50
3	15	50
4	5	60
5	10	60
6	5	70

[a]All experiments received the same five treatment levels of 0.0, 0.1, 0.2, 0.3, and 0.4 uc. radium/liter of water.

Fish from each one of these experimental combinations had their blood analyzed for the microhematocrit, total plasma proteins, and white blood cell count values. The results for each of these characteristics were subjected to the standard analysis of variance for this type of design:

Source of variation	Degrees of freedom
Replications	3
Treatments	4
Error	12
Total	19

Source: Li, 1957.

The six experiments times three blood characteristics per experiment meant

that eighteen individual analyses of variance were calculated. Two additional analyses were conducted upon the results of the differential smear ratios obtained on blood that showed significant white blood cell count changes. All of the findings that tested significant at the 5 percent level of probability or less are so noted in Table 2.

Table 2. Results of the analysis of variance tests made on the data from the fractional experiments

Temperature °F	Character[a]	Time in days 5	10	15	Tests[b]
50	MH	N.S.[c]	N.S.	N.S.	
	PP	S 1%[c]	N.S.	N.S.	Linear
	WBC	N.S.	S 5%	N.S.	Tr.[e] 2-5 linear
	DS	-[d]	S 1%	-	Tr. 5 low vs. Tr. 1-4
60	MH	N.S.	N.S.		
	PP	N.S.	N.S.		
	WBC	S 1%	N.S.		Linear
	DS	S 1%	-		Linear
70	MH	S 5%			Tr. 1 low vs. Tr. 2-5
	PP	S 5%			Tr. 3 high vs. others
	WBC	N.S.			

[a] MH - microhematocrit, PP - plasma protein, WBC - white blood cell count, DS - differential smear ratio.
[b] The complete data and related statistical calculations are in Appendix B. These results are illustrated in the following sections.
[c] N.S. - not significant and S % - level of significance.
[d] Not determined.
[e] Tr. - treatment

All of these results are illustrated in three dimensional graphs in the four succeeding sections on the total experiments. In order to demonstrate these findings without duplication of graphical material, the

data are presented here in tabular form (Table 3).

Table 3. Fractional experiment results presented as the average of the means of the four replications at each treatment level for the characteristic tested. All these findings are illustrated in the following sections

Line no.	Character[a] and experiment	uc. radium/liter water 0.0	0.1	0.2	0.3	0.4	Manifestation
1	PP 50° F-5 days	4.38	4.11	4.01	3.94	3.44	Linear
2	PP 70° F-5 days	3.62	3.67	4.18	3.63	3.51	Tr.[b] 3 high vs. others
3	WBC 50° F-10 days	45.5	58.3	50.8	46.7	36.2	Tr. 2-5 linear
4	DS 50° F-10 days	7.13	7.68	8.20	7.78	20.60	Tr. 5 high vs. Tr. 1-4
5	WBC 60° F-5 days	34.9	34.6	56.3	52.9	76.0	Linear
6	DS 60° F-5 days	11.31	4.49	3.35	1.41	2.17	Linear
7	MH 70° F-5 days	31.1	37.4	39.3	39.1	37.6	Tr. 1 low vs. others

[a]PP - plasma proteins in percent grams of total protein in the plasma.
WBC - white blood cells in number of WBC in 1000s in a cubic millimeter of blood.
DS - differential smear in number of lymphocytes/granulocyte (WBC ratio).
MH - microhematocrits in percent volume packed red blood cells.
[b]Tr. - treatment.

The plasma proteins decreased as the treatment level increased at 50° F. (Table 3, line no. 1). This response was quite similar to that reported for mammals (Fischer et al., 1954; Glenn, 1960; Kohn, 1951; Leone et al., 1959; and Supplee et al., 1951). The change in constituents was probably quite similar, too, since carp, a near relative of the goldfish, possess albumin, alpha, beta 1, beta 2, and gamma globulin in their plasma

(Drilhon, 1953). The review of literature section showed that a lowering of total protein content meant an abnormal drop in albumin, while the gamma globulin increased, but not sufficient enough to compensate for the albumin loss.

The plasma protein concentrations in these goldfish ranged from 3.44-4.38 gram percent, which compared favorably with normal readings found in other fish:

Worker	Fish	Plasma protein in gram percent
Field et al. (1943)	Carp	3.25 - 4.75
	Trout	2.94 - 4.12
Phillips (1958)	Trout[a]	2.29 - 2.47
Lepkovsky (1929)	Goosefish	2.48 - 4.24
	Sea bullhead	6.30 - 7.66

[a]Brock and brown trout.

The plasma proteins were at a higher concentration at Treatment 3 than any of the others at 70° F. (Table 3, line no. 2). This response could possibly be due to an overcompensation of the organism to radium and the 5-day sample happened to catch the system in the period of over-compensation. Overcompensation of the cellular components is not rare and is reported by Spear (1953). Another possibility is that the Treatment 3 fish happened to get sampled during the period of biological adjustment. The blood at the higher treatment levels has already passed through its period of adjustment to radium contamination and has returned to normal. The fish at the lower two treatment levels have not started to adjust. Biological adjustment to similar toxicants is not unusual (Downey, 1938).

The white blood cell counts from fish in radium at 50° F. for 10 days were linear (Table 3, line no. 3). The slightly higher counts for Treatments 2 and 3 indicate a mild leukocytosis, while the lower reading at Treatment 5 revealed the leukopenia that eventually develops. These

apparently contradictory results were in harmony with the manifestations exhibited by mammals and discussed by Downey (1938) and Fink (1950). Since these organisms were in the process of biological adjustment to the presence of radium in their internal and external environments, due consideration must be given to the temperature, time of exposure, and treatment levels. All these factors influenced the rate of metabolism of the radium by the fish. The higher concentrations of radium perpetrated a response in fish similar to that of mammals such that leukopenia was quicker to appear.

The differential smear ratios for the blood of these fish substantiate the onset of leukopenia (Table 3, line no. 4). Treatment 5 caused a significant decrease in the granulocytes so that the ratio was greatly altered. Though there was a slight increase in white blood cell numbers at Treatments 2 and 3, there was no noticeable shift in composition.

The white blood cell counts for fish in 60° F water for 5 days (Table 3, line no. 5) showed a definite linear rise as the treatment level increased, which followed mammalian findings in which a mild leukocytosis is experienced (Downey, 1938; and Spear, 1953). The same results for fish were confirmed by their differential smear ratios (Table 3, line no. 6). There was a definite shift in the ratio, whereby the number of granulocytes increased to the point of almost equaling the number of circulating lymphocytes (Figure 3). There could have been a decrease in lymphocytes along with the increase in granulocytes, but the gain in granulocytes was the dominant change. These findings on the shift in white blood cell composition due to radium exposure reflect a pattern of response opposite to that of irradiated homoiothermous blood. Leukopenia in goldfish was caused by a drop in granulocyte numbers while leukocytosis was due to an

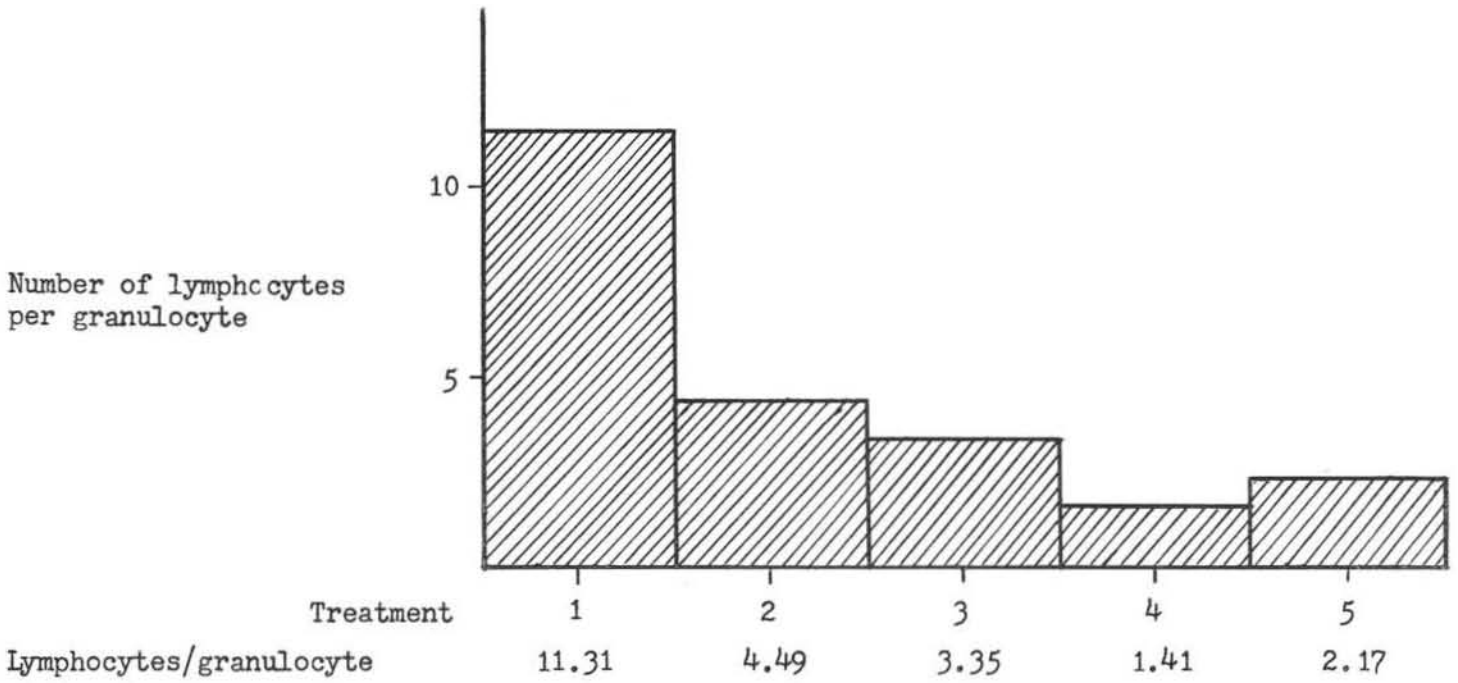


Figure 3. Number of lymphocytes per granulocyte (WBC composition ratio) in the blood of goldfish from the exposure conditions of 60° F., 5 days, and five levels of radium.

increase in granulocytes. Most research on homoiotherms reported that leukocytosis was contributed by lymphocytes while leukopenia is a reflection of a drop in absolute lymphocytes (Albritton, 1952; Altman and Dittmer, 1961; and Downey, 1938). Stearner (1950) did show that x-ray exposures of 600-900 roentgens produced the same sensitivities and responses in the leukocytes of frogs as was exhibited by mammals. The response was slower, possibly due to the lower metabolic rate of frogs. The same general conclusions can be applied to the results of these experiments on goldfish except for the shift in white blood cell composition.

Incidentally, the granulocytes were almost 100 percent basiophils in the exposed goldfish, which may have some significance for poikilothermous blood under these experimental conditions. Nothing was found in a survey of the literature to substantiate fully this response pattern as being typical of mammalian blood under radium contamination.

Few researchers have done much extensive work on the white blood cell counts in fish because it is a tedious time consuming process, and there is extreme variability between individuals and species:

Worker	Fish	White blood cells in 1000s/mm^3
Field et al. (1943)	Carp	3.245 - 4.290
	Trout	2.105 - 5.376
McCormick (1960)	Rainbow trout	2.50 -26.85
	Brown trout	4.65 -13.90
Yokoyama (1960)	Perch	16.00 -146.00

On the average, the results of the white blood cell enumeration in this study were somewhat higher than the counts just reported. This tendency was probably due both to the species and genetic strain of the fish used.

The microhematocrit readings at 70° F. were low for the control fish (Treatment 1) but higher and similar throughout the exposed fish (Table 3, line no. 7). Some workers on mammals and humans found that

small doses of alpha emitters did have a stimulating effect on red blood cell production, which was evidenced by an increase in circulating immature cells (Downey, 1938). The same explanation is probably sufficient for this phenomenon occurring in fish.

The microhematocrits from these goldfish averaged approximately 40.0 percent. Normal microhematocrits for other fish were determined by several workers, and give an indication as to what levels microhematocrit readings are found:

Worker	Fish	Microhematocrits in percent volume
McCormick (1960)	Trout[a]	21.0 - 44.0
Rice (1961)	Flounder	25.0 - 37.0
	Stripped bass	36.0 - 41.3
	Spanish mackerel	26.5 - 46.0
	Croaker	18.0 - 39.8
Schiffman (1959)	Rainbow trout	31.8 ± 1.39
Snieszko (1961)	Rainbow trout	47.8 ± 4.50
	Brook trout	50.7 ± 4.66
	Brown trout	39.5 ± 3.70
Field et al. (1943)	Carp (hematocrit)	21.0 - 40.0

[a]Brook and brown trout.

Most of the findings and remarks included in this section are best summarized by the following quotation:

> Both stimulation and destruction occur as a result of the action of alpha rays. There is some dispute as to whether the stimulation is direct or indirect . . . Against indirect stimulation is the fact that hyperplasia occurs in the hemapoietic organs before there is evidence of cell destruction. In favor of direct stimulation is the general law that all destructive agents used in sufficiently small quantities have a stimulating effect. But both factors may play a role. [Downey, 1938, p. 2782]

He further commented on the general opinion that small doses of roentgen rays stimulate cell activity while larger doses have a destructive effect. Radium had a purely destructive effect upon plasma proteins and even the literature failed to mention any possible stimulation to plasma protein production.

The results on goldfish blood indicate that the treatment levels of radium included a sufficient range of sublethal dosages to perpetrate manifestations comparable to those exhibited by mammals.

Bone analysis for radioactivity.--The radioactivity assay gave an indirect approximation of how much radium was taken up and concentrated by goldfish bone under various experimental conditions. The bone that was assayed was from fish exposed to the six environmental conditions listed in Table 1. These fish were decaudated and bled first for the blood analysis presented in the first part of this section. The radioassay results were based on gross alpha activity determinations on the assumption that all alpha emission from the bone was contributed by radium. In order to avoid duplication of illustrations the data are presented here in tabular form and in graphical form in the following total experiment sections. Since this work was essentially exploratory, much of the discussion and its conclusions must of necessity border on the speculative in order to offer suggestions for further reflection and research.

The findings for each of the six fractional experiments were eventually subjected to an analysis of variance similar to the one used for blood. The major exception was that only three replications were used instead of the four included in the design. The bone from all goldfish in all replications was assayed, but one of the four samples was always in aberration by a factor of 2-4 times the other three. Therefore, in order to provide good approximations of absolute values, the author chose to eliminate the aberrant figures from the data. Others associated with the project felt the decision was justified (Berger and Bohidar). (Private communication with Dr. R. L. Berger, physicist, and Dr. N. R. Bohidar, statistician, Utah State University.)

The first step was to treat all the data to Bartlett's Test of Homogeneity of Variance[6] and the F' test[6] (Snedecor, 1956). The test revealed that there was significant heterogeneity among the variances of the treatment means. This conclusion indicated that there were some differences among the treatments. When the data were separated into individual fractional experiments and tested with:

Source of variation	Degrees of freedom
Replications	2
Treatments	4
Error	8
Total	14

no differences between treatments were found. Such findings confirmed the presence of extreme variability among the replications. Another source of variation (other than the treatment itself) was present and it could not be detected with statistics.

If these extraordinary results were not artifacts, then two major reasons for such variability can be proposed. Despite the coding and cleaning of crucibles to prevent residual carry-over of contamination, the samples could have been contaminated. Secondly, the biological behavior of the goldfish in any given experimental unit (gallon jar) may have been sufficiently different to cause the variable uptake and deposition of radium.

Since the extreme variation within the data cannot be dampened or removed by statistics, it becomes necessary to present the material in an empirical manner. The averages of the replications are used in the tables and graphs to emphasize the possibilities of response patterns. The only statistically supported conclusion is that there is tremendous variability in the experimental data and further research would require many more samples.

[6]The complete data and table of results are in Appendix C.

The final results of the radioassay of the bone are contained in Table 4 with a suggested response pattern. The single answer for any set of three experimental conditions (temperature, time, and treatment) is the average of the three replications at that situation. An average figure gives a more useful absolute value. Since the radioassay technique determined the activity in counts per minute, a mathematical and graphical conversion to micromicrocuries per gram of ash was necessary (Tsivoglou, 1961, and Appendix D).

Table 4. Amount of radioactivity found in goldfish bone in micromicrocuries per gram ash weight

Line no.	Experimental conditions Temp. °F.	Time days	Treatment levels in uc. radium/liter H_2O 0.0	0.1	0.2	0.3	0.4	Response pattern[a]
1	50	5	112.4	964.6	2,451.3	3,230.1	3,610.6	linear
2	50	10	271.6	1,752.2	4,902.7	4,053.9	5,823.0	cubic
3	50	15	200.0	1,194.6	2,247.8	5,575.2	5,805.3	linear
4	60	5	5,716.8	12,752.2	10,017.6	13,292.0	11,212.3	quadratic
5	60	5	973.5	3,371.7	7,902.7	14,840.7	9,840.7	quadratic
6	70	5	1,469.0	2,123.9	3,097.3	2,451.3	3,796.5	cubic

[a]These patterns are illustrated in graphic form in the following total experiment sections.

The absorption and concentration of radium, as influenced by the environmental conditions of 50° F. and five days appears linear (Table 4, line no. 1). The more radium there was in the water the more the goldfish absorbed and collected in the bone. Rosenthal (1957) found the same reaction when using ornamental species (guppies, zebras, and white cloud mountain fish) in waters containing calcium-45 and strontium-90. The rate of uptake was linear in respect to the radioactivity in the water. Saurov

(1957) also drew the same conclusion when using strontium-90 in water holding bleak, roach, and carp.

The linear pattern was not complete at the temperature of 50° F. for 10 days (Table 4, line no. 2). The slight decline in the amount of radioactivity at treatment 4 interrupted an otherwise linear response of activity in bone to activity in water. It is difficult to speculate upon such a reaction, especially since a similar observation is recorded for 70° F. and 5 days (Table 4, line no. 6). Only two conjectures of what biologically might be happening are proposed for both of these circumstances. The latent period of response at treatment 4 under these experimental conditions was sufficiently different to retard the deposition of radium. Secondly, the bone itself was making an adjustment to the acceptance of radium. At the first two contaminated treatment levels the bone was still accepting radium without making any attempts to reject it. At treatment 4 the bone was in a period of rejection. By treatment 5 the bone had passed through its period of adjustment and had yielded to the presence of radium and was accepting it.

After 15 days at 50° F. the radioactivity in the bone increased linearly as the activity of the water became greater (Table 4, line no. 3). Such a response was the logical sequence to expect when using radium in equal increments.

The pattern of radium deposition in the bone at 60° F. was quite similar for both 5 and 10 days (Table 4, lines no. 4 and 4). The one exception that occurred was the out-of-place findings for the controls (no radium treatment) at 5 days. Though the real cause for such an aberrancy escapes detection, speculation can be made. For each major conjecture there is a rejoinder (Table 5).

Table 5. Speculations for and against the abnormal concentration of alpha radioactivity in the bones of nonexposed goldfish

	Possibilities why:	And why not:
1.	Radium put into the water with these fish or residual carry-over during experimentation.	Internal organs had no abnormal radioactivity (Appendix E). Equipment was color coded as a preventive measure.
2.	Fish became exposed after arrival and before use.	Same reason as above and they were only held for 5 days.
3.	Fish had been exposed before arrival.	All fish were from the same place and no other controls were as radioactive.
4.	Bone became contaminated in the fish in storage before assay.	Deposition of radium required active metabolic assistance.
5.	Bone sample, after removal from fish, became contaminated.	All four replicates displayed similar count results.
6.	Ashed samples became contaminated from crucibles or planchets.	Nothing that radioactive was ever in the crucibles or planchets, and the internal organs did not have such radioactivity.
7.	Deliberate contamination.	Self absorption counts and curves were too good.
8.	Errors made in counting, setting the voltage, or transcribing the figures.	Background count was .3 cpm while beta background at higher voltage is 55 cpm. Too many checks, double checks, and planchets in 2 days to be consistently erroneous.
9.	Calculations were erroneous.	All were double checked.

These major areas of error and their rebuttals are only suggestions because they do not solve the basic problem. Whether these unusual results were a combination of errors, an artifact, or some unconsidered possibility, a satisfactory explanation appeared lacking.

The quadratic picture of the data from the 60° F. experiments possessed a linear segment for the first four treatment levels. Three major reactions may transpire at treatment 5 to cause the drop in bone radioactivity. Aplasia of the bone marrow may occur in fish as in mammals, so the bone was unable to retain so much radium. Therefore, the treatment 5 level under these experimental conditions possessed some lethal qualities. The review of literature would support this reason as the most logical. A second possibility is that treatment 4 provided a saturation concentration for bone and treatment 5 presented too much radium, so it was rejected. Thirdly, the bone at this treatment level may have passed through its saturation peak and was in the process of eliminating radium.

The results listed on the last line of Table 4 were discussed earlier in this section along with the data of line no. 2.

The radioactivity in goldfish from this study never did approach the 50-55 percent of dose concentrated by mammalian bone. These results were quite comparable to the findings from similar work on other poikilotherms. Frog tadpoles in 1 week absorbed only 18 percent of the strontium-90 and 72 percent of the yttrium-90 (of which there is very little in a week) from water containing 0.125 microcuries strontium-90 per 500 milliliters (Lucas and Pickering, 1958). Studies on marine fish showed that 95 percent of the oral dose of strontium-89 is eliminated in 1 to 4 days (Hawaii Marine Laboratory, 1955). Neither the size of dose or repetitive feeding had any effect upon the internal distribution or percent of

retention after several days. All this research further illustrated the conclusion of Danil'chenko (1958) that despite the high radioactivity of water fish had low comparative radioactivity.

The exploratory work on the radioactivity of the internal organs of goldfish gave some erratic and confusing results. The lack of sufficient sample size forced a limitation of this phase of the research, though a pioneering effort was made. For these reasons the material was relegated to Appendix E, where it is accompanied by a brief discussion. The findings, as an indicator of what happens, were of sufficient interest to warrant inclusion, but not in the body of the dissertation. Some trends in time and temperature were noticeable, but the paucity of data made it difficult to elaborate.

Correlation between radium deposition and blood changes.--Correlation studies between the amount of radium deposited in the bone or internal organs and the peripheral blood changes were not attempted for three main reasons.

1. The bone in fish is not a hemapoietic tissue, so consequently, any relationship between it and blood is purely incidental (Yokoyama, 1960; and Yuki, 1958).

2. Even though the internal organs contain all the hemapoietic tissues, it is unjustified to correlate its radium content with blood changes because radium may be present in significant amounts in other than hemapoietic organs (Amano et al., 1956; Fink, 1950; Kawabata, 1955; and Saiki et al., 1956).

3. Neither the bone nor internal organ results provide sufficient significant data which can be associated with the blood data and subjected to the standard tests for correlation.

Water chemistry.--The water from the control jars (no radium treatment) was analyzed for dissolved oxygen, carbon dioxide, pH, and total nitrogen. The methods and materials are discussed in that chapter. The results were incorporated into Table 6.

Table 6. Results of the analyses of water from 24 gallon jars containing three goldfish each and no radium

Rep	Average fish Wt in g	Average fish Length in mm	Exposure Time days	Exposure Temp. °F.	Water chemistry[a] pH	CO_2 ppm	O_2 ppm	N_2 ppm
1	36.7	98.7	5	50	8.0	0	7.4	11.0
2	40.0	104.0	5	50	8.2	0	8.0	12.0
3	43.3	102.7	5	50	8.0	0	7.8	13.5
4	37.0	100.0	5	50	8.0	0	8.0	12.0
1	39.0	104.0	10	50	8.0	0	7.0	-
2	40.3	100.3	10	50	7.9	0	7.4	22.5
3	29.3	92.3	10	50	8.0	0	7.6	-
4	40.0	101.3	10	50	8.1	0	8.0	18.0
1	30.3	96.0	15	50	7.8	0	6.6	26.0
2	36.7	98.0	15	50	8.1	0	8.6	23.0
3	36.3	97.6	15	50	8.1	0	7.8	27.5
4	39.0	101.7	15	50	8.3	0	9.0	34.5
1	31.0	92.0	5	60	8.3	0	6.6	31.0
2	28.7	91.7	5	60	8.2	0	6.8	21.0
3	29.7	96.3	5	60	8.3	0	7.0	30.0
4	32.0	94.7	5	60	8.2	0	6.8	22.0
1	30.0	93.7	10	60	7.9	0	4.2	54.0
2	27.7	92.3	10	60	8.2	0	6.4	45.0
3	31.3	96.7	10	60	8.0	0	5.6	55.0
4	29.0	94.7	10	60	8.1	0	6.0	30.0
1	21.0	86.7	5	70	8.2	0	6.4	14.0
2	21.3	91.0	5	70	8.3	0	7.0	12.0
3	23.7	91.7	5	70	8.2	0	6.6	14.0
4	30.3	95.3	5	70	8.1	0	6.2	14.0

[a]Carbon dioxide, dissolved oxygen, and total nitrogen.

All of these factors were within the limits of tolerance for fish (Brown, 1957; California, 1952; Doudoroff and Katz, 1951; and Rounsfell and Everhart, 1953). The most interesting change in the water was the increase

in total nitrogen (N_2, last column) in respect to both time and temperature. At 50° F. the average nitrogen contents were 12.1, 20.3, and 27.8 ppm in 5, 10, and 15 days, respectively. Such a linear increase was to be expected in a static system where excretory products cannot be removed (Linn, 1955). At 5 days the average amounts of nitrogen were 12.1, 26.0, and 13.5 ppm at 50°, 60°, and 70° F. respectively. At 10 days the amounts were 20.3 and 46.0 ppm nitrogen for the waters of 50° and 60° F. If the concentration of total nitrogen at 50° and 60° F. for both 5 and 10 days was used as a measure of metabolic activity, then the metabolic response of the fish closely followed the Van't Hoff's rule or Q_{10} approximation (Giese, 1957; and Prosser and Brown, 1961).

Total experiment number one

Blood analysis.--The first major experiment studied the effects on the blood of the interaction between the five treatment levels of radium and the three time periods of 5, 10, and 15 days at 50° F. The results on each of the blood characteristics from all three of these fractional experiments were pooled and analyzed according to the analysis of variance[7].

Source of variation	Degrees of freedom
Treatment	4
Within treatment	15
Time	2
Time x treatments	8
Error (b)	30
Total	59

The significant findings of the analyses confirm what is visibly detectable in the three dimensional (3-D) figures used in these sections. The blood response values used in all the following three dimensional graphs are an average of the means of the four replications at any given combination of treatment level and time period.

[7]The complete data and statistical calculations are in Appendix B.

The 3-D graphs are constructed so the treatment levels are evenly distributed along the 60° axis. The treatment levels from 1 to 5 are 0.0, 0.1, 0.2, 0.3, and 0.4 microcuries of radium per liter of water. The time periods of 5, 10, and 15 days are spaced along the 30° axis. The same axis is used for the temperatures of 50°, 60°, and 70° F. when their effects are being illustrated. The response of the blood characteristic at any given combination of treatment and time (or temperature) is shown on the vertical axis with the appropriate 1 1/2 inches scale given beside each figure. All of these vertical lines are connected at the top to form the interaction surface.

Actually, Figure 4 was inserted to illustrate the lack of interaction. When the interaction surface was explored, no pronounced changes occurred at any combination of treatment and time. The blood characteristic response, as seen on the surface of this 3-D graph, was fairly even throughout.

Contrast Figure 5 with Figure 4 and the presence of interaction becomes apparent. Two important manifestations were noticeable. A linear decline in total plasma proteins in response to increasing treatment level increments resulted after 5 days of exposure. This particular response was discussed at greater length under the Fractional Experiments.

Secondly, the plasma proteins exhibited a return to normal by 15 days, even at the highest treatment level. Even though the goldfish remained continually immersed in a contaminated environment, the plasma protein level did return to normal. A return to normal was not totally unexpected since both Kohn (1951) and Supplee et al. (1951) mention some plasma protein recovery in relation to irradiated homoiotherms. Recovery or treatment must take place or else an extended period of reduced plasma proteins is fatal to the organism (Wuhrmann and Wunderly, 1960). Even

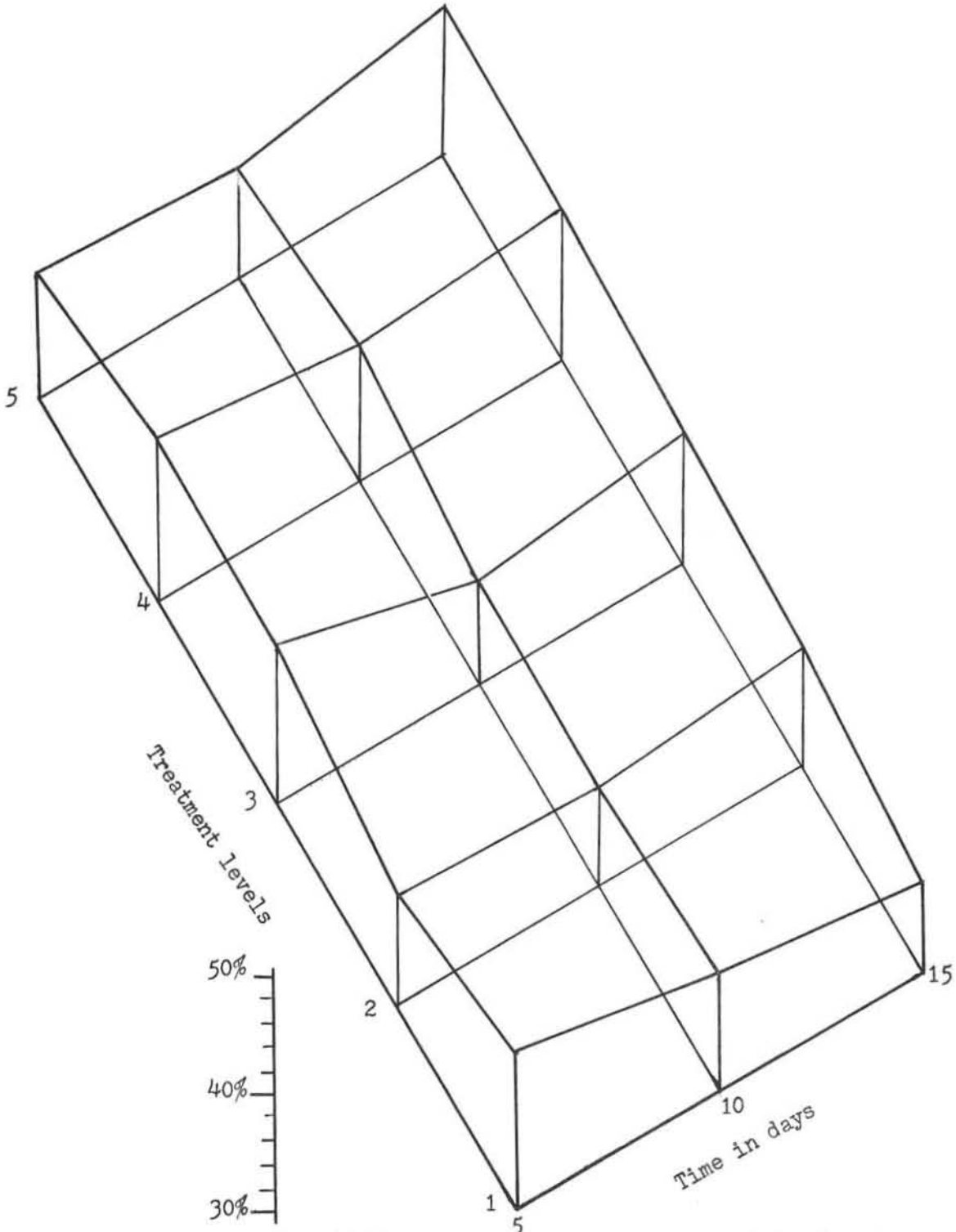


Figure 4. Exploration of the interaction surface formed by the micro-hematocrit response within goldfish exposed to five treatment levels of radium for three time periods at 50° F. The blood characteristic response is on the vertical scale which equals 20% per 1 1/2 inches and starts initially at a level of 30%.

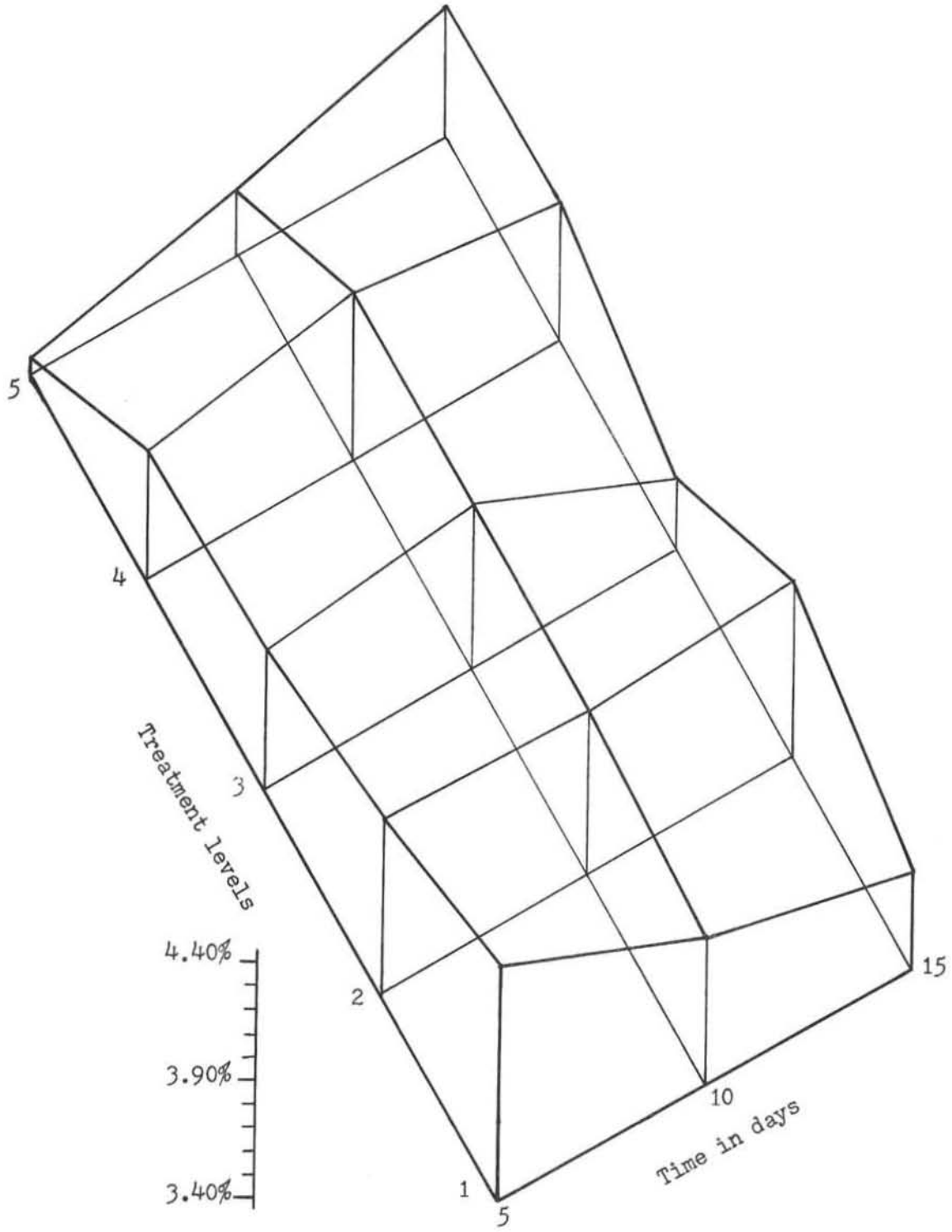


Figure 5. Exploration of the interaction surface formed by the plasma protein response within goldfish exposed to five treatment levels of radium for three time periods at 50° F. The blood characteristic response is on the vertical scale which equals 1.00% per 1 1/2 inches and starts initially at a level of 3.40%.

though the fish undoubtedly must continue to process radium through the body, the plasma protein producing area does compensate for its loss of producing power or functional cells.

The possibility that this response pattern was due to a plasma volume increase was easily discounted because that reaction takes 4 days or longer after radiation exposure (Altman and Dittmer, 1961). Supplee et al. (1951) also stated that before a plasma volume change there is a definite plasma protein concentration depression.

The slightly lower protein reading for treatment 3 at 15 days was not significantly different than the readings for the other four treatments at that time period.

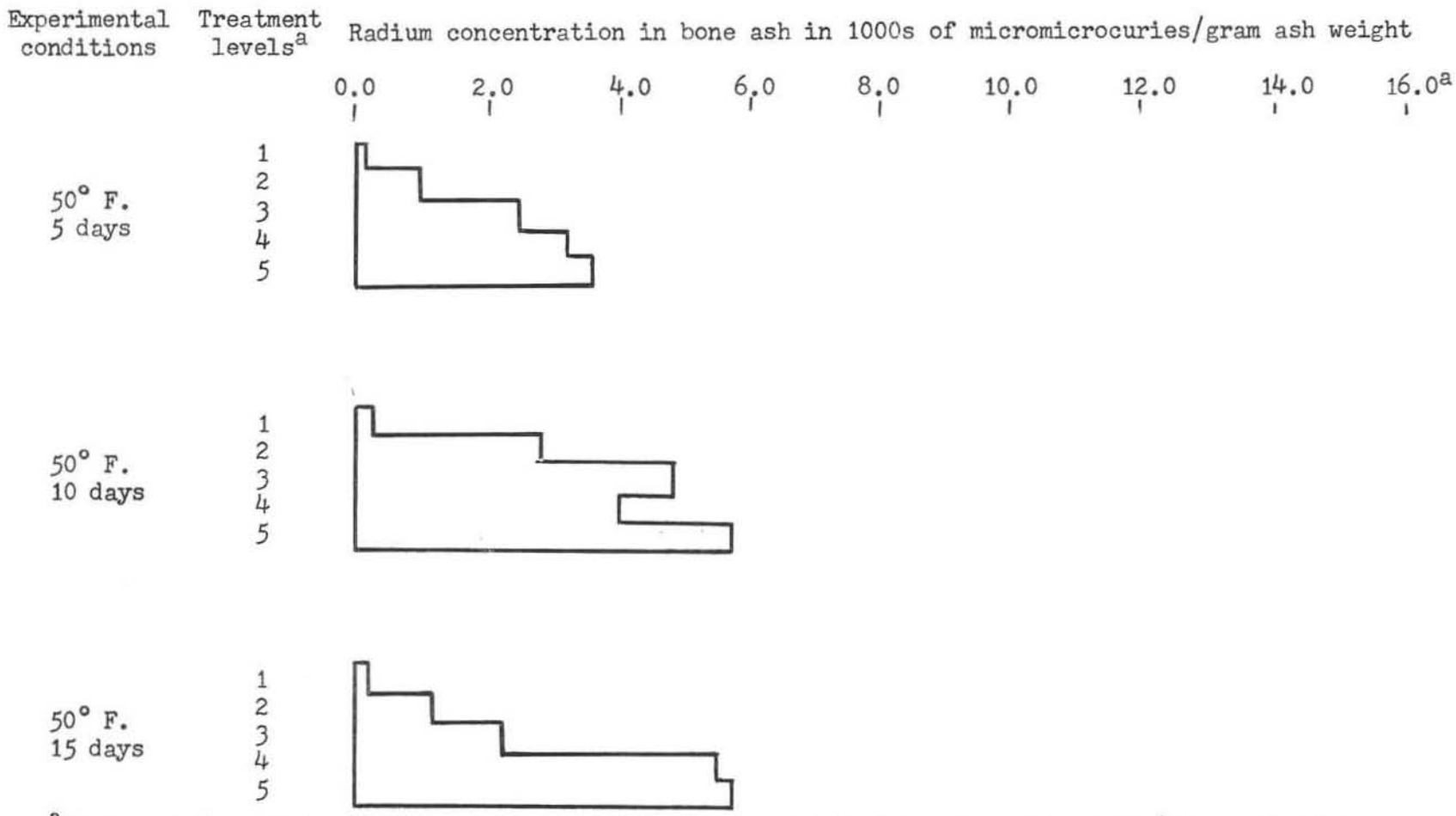
It must be concluded that time during and following exposure of fish to radium does play an important part. Time must be considered when attempting to detect any measurable initial changes in the total plasma proteins of goldfish.

Neither the white blood cell counts or microhematocrit readings from fish under these experimental conditions exhibited any important manifestations.

Bone analysis for radioactivity.--An empirical study of the interactions due to the environmental conditions of this major experiment and their effect on radium deposition in goldfish bone is possible with Table 7 and Figure 6.

Table 7. The effects of the interaction between treatment and time at 50° F. on the concentration of radium in goldfish bone

uuc. radium/ gram ash	Treatment levels in uc. radium/liter water				
	0.0	0.1	0.2	0.3	0.4
5 days	112.4	964.0	2,451.3	3,230.1	3,610.6
10 days	276.1	1,752.2	4,902.7	4,053.9	5,823.0
15 days	200.0	1,194.6	2,247.8	5,575.2	5,805.3



[a]Treatment levels from 1-5 are 0.0, 0.1, 0.2, 0.3, and 0.4 microcuries of radium/liter of water

Figure 6. Amount of radium found in bones of goldfish exposed to five treatment levels of radium for three time periods at 50° F.

A cursory review of the table and graph reveals several possible interaction combinations. Two noticeable ones were the radium concentrations in bone of fish from treatments 2 and 3 at 10 days. These two are out-of-line in respect to their counterparts at 5 and 15 days. A vague trend seemed noticeable in the jumble of unrelated responses recorded for higher treatment levels. The longer the period of exposure at these levels the greater was the concentration of radium in the bone. These indications were in agreement with the results obtained by Rosenthal (1957). The ornamental species of fish living in contact with calcium-45 and strontium-90 in the water for up to 20 days gained a greater radioactivity in time. The rate of uptake was linear in respect to time, which implied continual formation or exchange of mineral component of bone. Saurov (1957) also detected this trend when working with carp in water with strontium-90. This radioisotope became more concentrated in the bone through time. The same reaction was possible with radium entering goldfish, but the buildup in the bone slowed considerably after an early active period of deposition.

Even though these nonsystematic responses due to interaction were visibly discernable, there was no satisfactory way to confirm them since there was such variability in the experimental data. It is difficult to discuss these outcomes extensively or confidently.

Total experiment number two

Blood analysis.--The second major experiment studied the effects on the blood of the interaction between the five treatment levels of radium and the two time periods of 5 and 10 days at 60° F. The results on each of the blood characteristics from both of these fractional experiments were pooled and analyzed[8] according to:

[8]The complete data and statistical calculations are in Appendix B.

Source of variation	Degrees of freedom
Treatment	4
Within treatment	15
Time	1
Time x treatments	4
Error (b)	15
Total	39

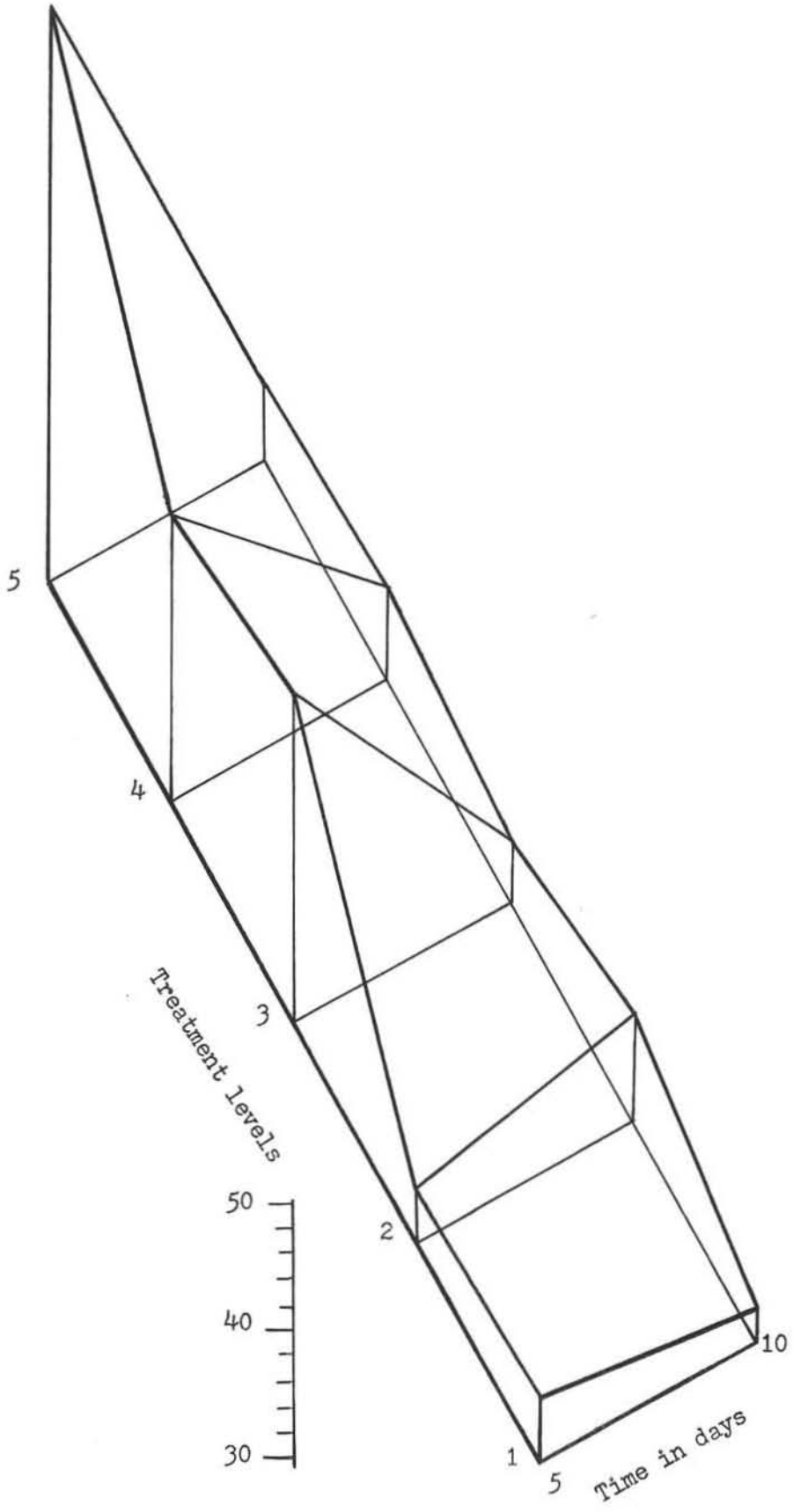
Only one blood characteristic showed any important alterations and the interrupted interaction surface of Figure 7 makes those changes obvious.

A linear increase in the number of white blood cells as the treatment level became greater resulted after exposure for 5 days and was discussed in detail under Fractional Experiments. By 10 days the white blood cell numbers returned to normal at all treatment levels. This return to normal within a few days followed the same pattern as that exhibited by mammals (Albritton, 1952; Altman and Dittmer, 1961; Downey, 1938; and Spear, 1953).

It was highly possible that between the sampling period of 5 and 10 days, the blood exhibited leukopenia the same as mammalian blood before stabilizing at normalness. Frogs exposed to x-radiation did show such a pattern (Patt and Swift, 1948). Shortly after irradiation frog blood experienced leukocytosis, followed by leukopenia, and then a return toward normal in time. These authors stated that the overt response of the frog, a poikilotherm, to x-radiation appeared quite similar in many respects to the reactions observed in mammals.

The same general conclusion can be made in relation to goldfish blood, particularly the white blood cells. A return to normalcy in number indicated that the leukocyte producing area of the kidney was capable of compensating for any initial damage done by radium, even though radium was continually processed through the kidney in elimination from the body.

Figure 7. Exploration of the interaction surface formed by the white blood cell response within goldfish exposed to five treatment levels of radium for two time periods at 60° F. The blood characteristic response is on the vertical scale which equals 20 (x 1000) cells per 1 1/2 inches and starts initially at a level of 30 (x 1000) cells.


5
4
3
2
1
Treatment levels
50
40
30
5
10
Time in days

The previous statement assumes that radium is considered a foreign substance to the goldfish system and the kidneys are performing their destined function of removing such materials (Copenhauer and Johnson, 1958; Norman, 1951; and Prosser and Brown, 1961).

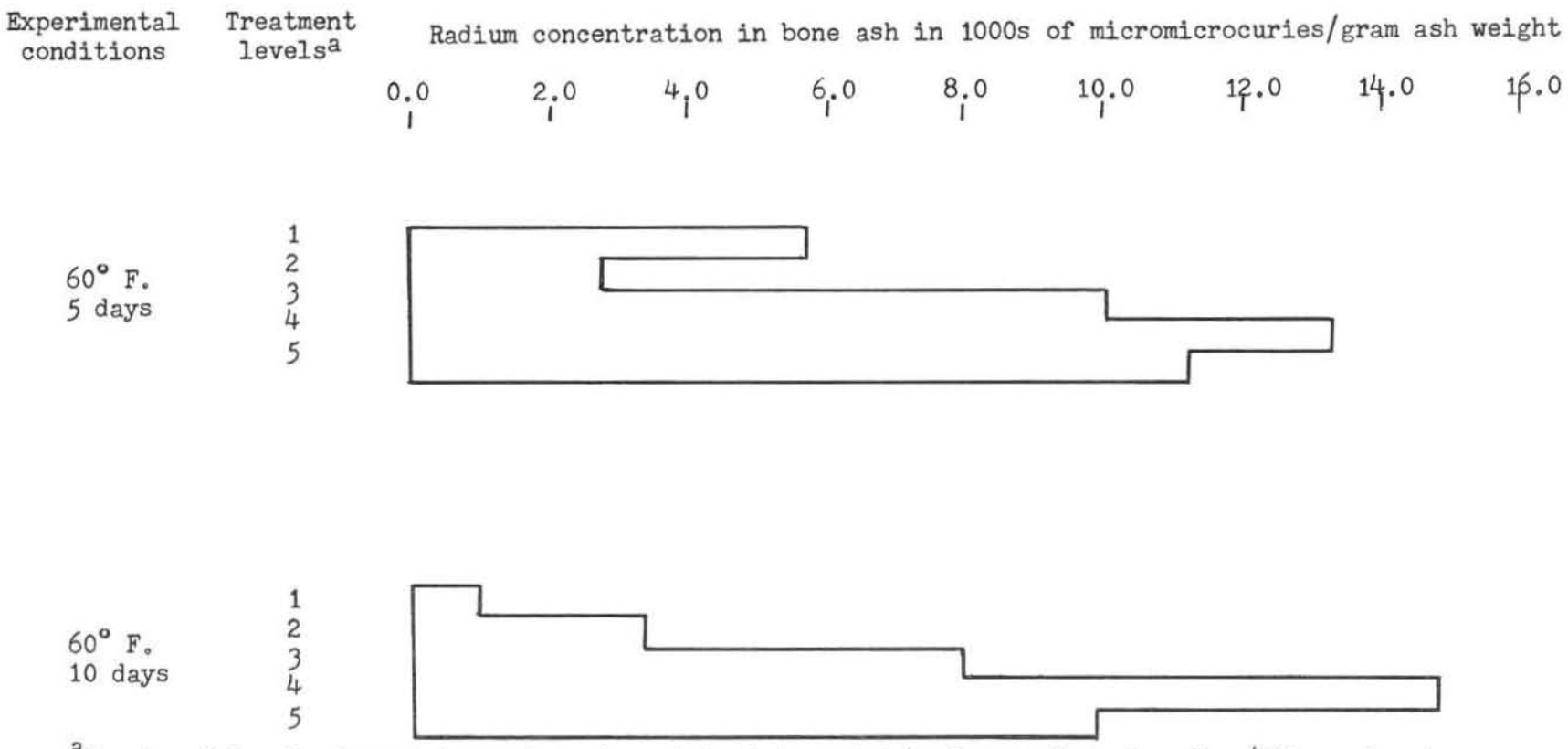
Again it must be concluded that time during and following exposure of fish to radium does play an important part. Time must be considered when attempting to detect any measurable initial changes in white blood cell numbers in goldfish. The conclusions of these experiments do not suggest what pattern of response would result during and after longer periods of exposure.

Bone analysis for radioactivity.--An empirical study of the interactions due to the environmental conditions of this major experiment and their effects on radium deposition in goldfish bone is possible with Table 8 and Figure 8.

Table 8. The effects of the interaction between treatment and time at 60° F. on the concentration of radium in goldfish bone

uuc. radium/ gram ash	Treatment levels in uc. radium/liter water				
	0.0	0.1	0.2	0.3	0.4
5 days	5,176.8?	2,752.2	10,017.6	13,292.0	11,212.3
10 days	973.5	3,371.7	7,902.7	14,840.7	9,840.7

Any interaction in this experiment was not easily detected, except for the aberrant figure at 5 days and Treatment 1, which was discussed in the section on Fractional Experiments. The systematic pattern of radium deposition into fish bone was the same through the upper four treatments in both time periods. The response through time escaped explanation, because two results at 10 days were higher and two were lower than at



[a]Treatment levels from 1-5 are 0.0, 0.1, 0.2, 0.3, and 0.4 microcuries of radium/liter of water

Figure 8. Amount of radium found in bones of goldfish exposed to five treatment levels of radium for two time periods at 60° F.

the same treatment at 5 days.

Total experiment number three

Blood analysis.--The third major experiment studied the effects on the blood of the interaction between the five treatment levels of radium and the three temperatures of 50°, 60°, and 70° F. at 5 days. The results on each of the blood characteristics from all three of these fractional experiments were pooled and analyzed[9]. The analysis of variance was the same as the one given in the section discussing Total Experiment Number One, except that the calculations were based on the numerical results at the given temperatures instead of time periods. The microhematocrits, total plasma proteins, and white blood cell counts evinced some interruption of their interaction surfaces as seen on the 3-D graphs of Figures 9, 10, and 11.

The only manifestation shown in Figure 9 was the low microhematocrit value for the controls at 70° F. Meanwhile the percentage of red blood cells from contaminated fish remained high and similar throughout their treatment levels. This phenomenon reflected an even amount of stimulation to red blood cell production by radium as mentioned in the Fractional Experiments discussion.

The depressing effects of Treatment 5 on the total plasma proteins was evident at all three temperatures (Figure 10). The overcompensation of plasma protein production at Treatment 3 and temperature 3 was easily seen and explained in more detail under Fractional Experiments. The linear pattern of decline in total proteins at 50° F. was also discussed previously. A more intense study of Figure 10 revealed a tendency for the plasma protein content to return toward a normal concentration at the

[9]The complete data and statistical calculations are in Appendix B.

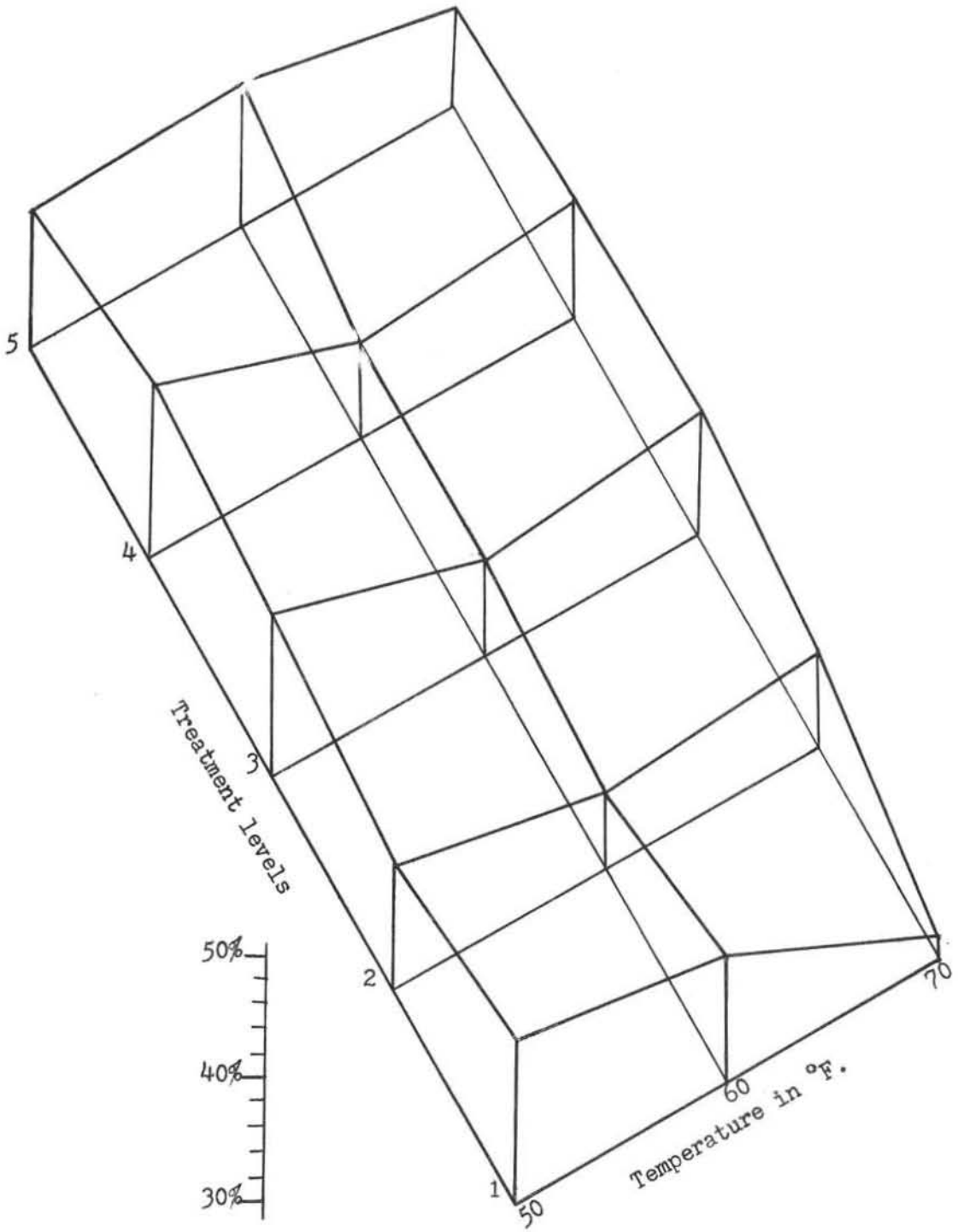


Figure 9. Exploration of the interaction surface formed by the micro-hematocrit response within goldfish exposed to five treatment levels of radium at three temperatures for 5 days. The blood characteristic response is on the vertical scale which equals 20% per 1 1/2 inches and starts initially at a level of 30%.

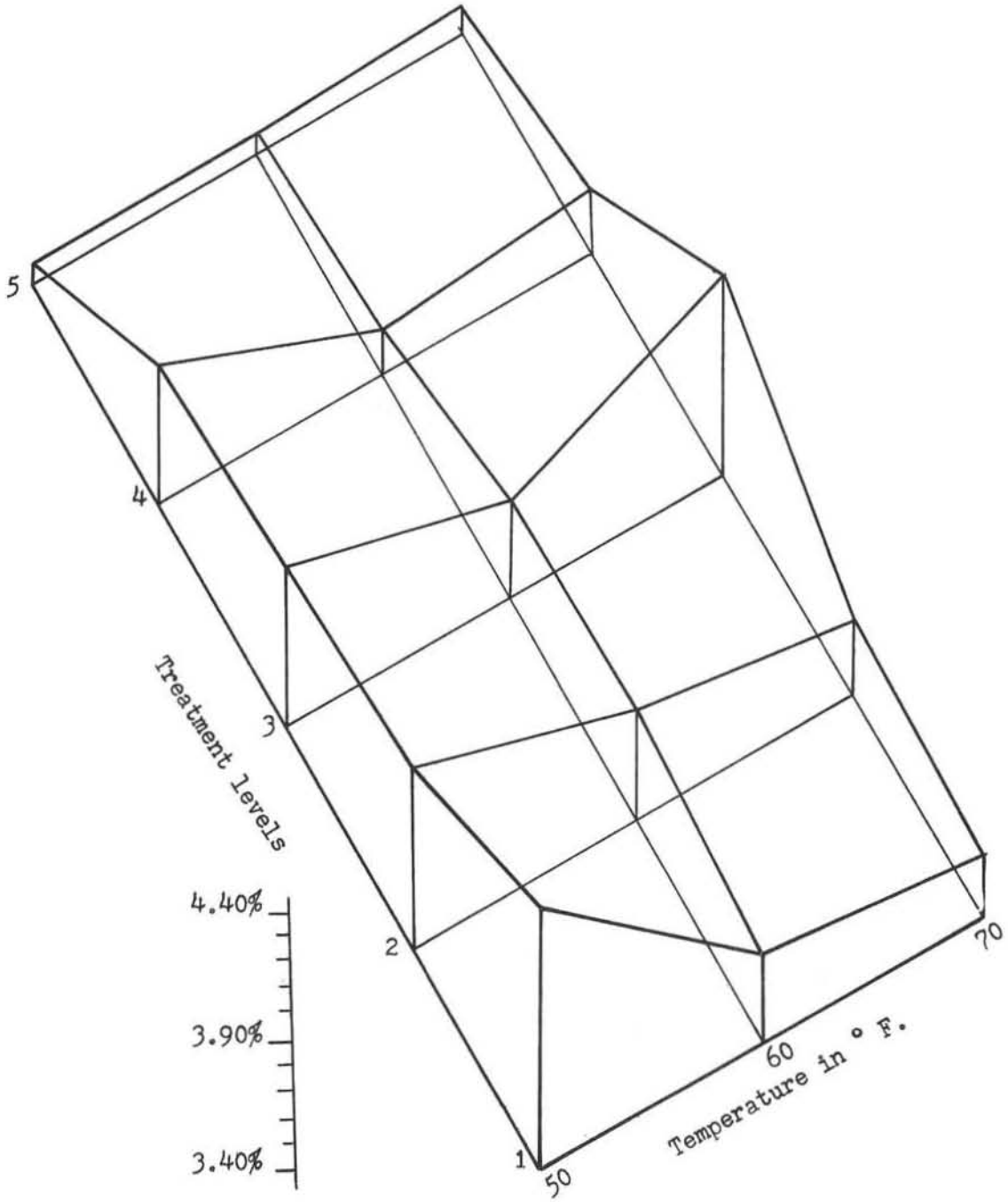
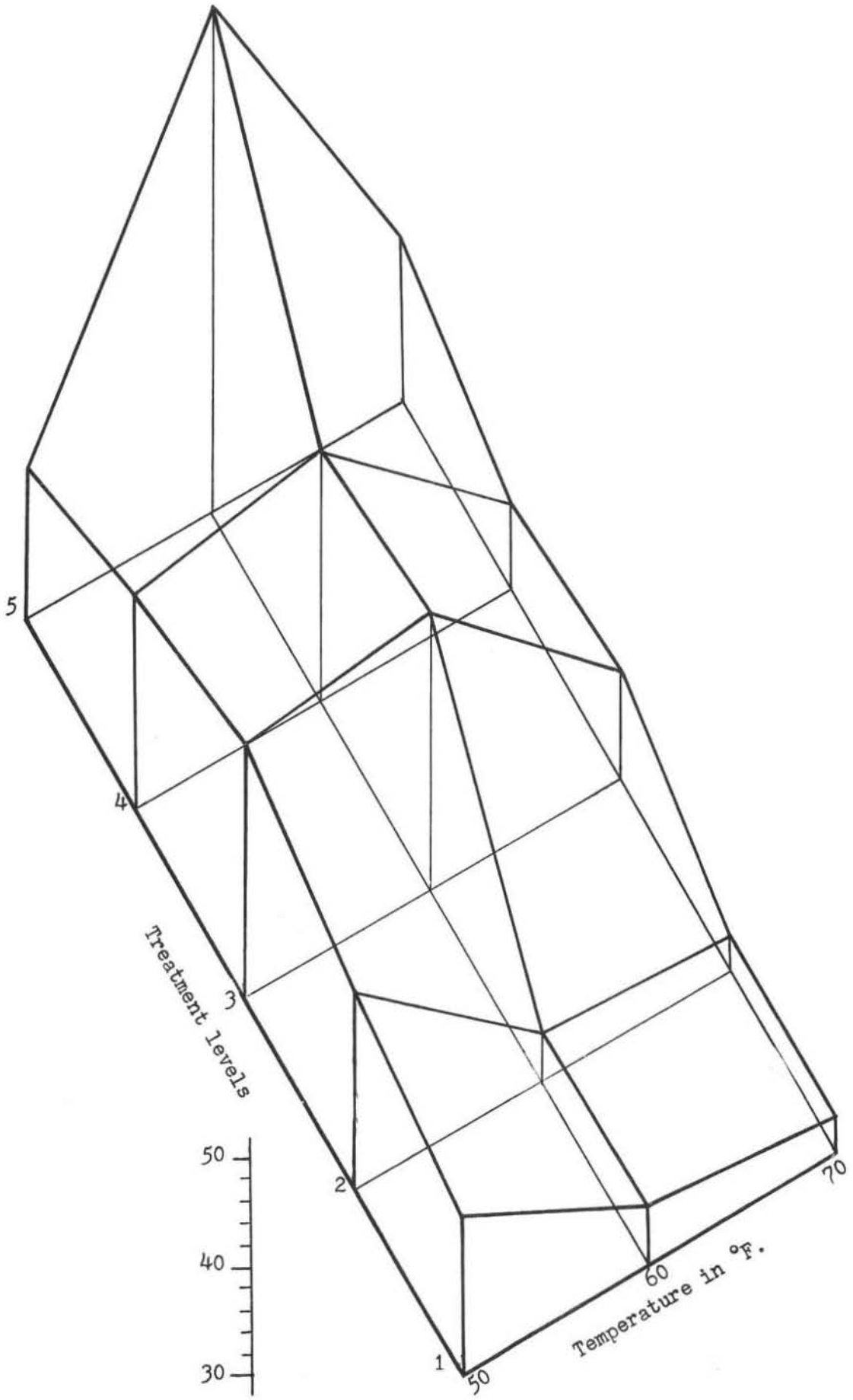


Figure 10. Exploration of the interaction surface formed by the plasma protein response within goldfish exposed to five treatment levels of radium at three temperatures for 5 days. The blood characteristic response is on the vertical scale which equals 1.00% per 1 1/2 inches and starts initially at a level of 3.40%.

Figure 11. Exploration of the interaction surface formed by the white blood cell response within goldfish exposed to five treatment levels of radium at three temperatures for 5 days. The blood characteristic response is on the vertical scale which equals 20 (x 1000) cells per 1 1/2 inches and starts initially at a level of 30 (x 1000) cells.


5
4
3
2
1
Treatment levels
50
40
30
50
60
70
Temperature in °F.

lower treatment levels in 60° and 70° F. waters. This pattern may imply that the blood had already experienced its plasma protein changes and it was on its way to normalness by the sampling time of 5 days.

The white blood cell counts in Figure 11 demonstrated the leukocytosis at 60° F. and 5 days that was discussed previously (Fractional Experiments). Meanwhile the white blood cells remained at a normal number through all treatment levels at both 50° and 70° F. A picture of this nature inferred that at 50° F. the blood has yet to show the effects of radium on the hemapoietic tissues. This contention was somewhat substantiated by the results of the leukocyte counts at 50° F. and 10 days. Under these experimental conditions a mild leukocytosis still existed at Treatments 2 and 3 while leukopenia was present at Treatment 5 (Fractional Experiments discussion and Figure 14). Despite the appearance that the treatments did cause a difference in the counts at 70° F. the data did not test significant. It is possible that the leukocyte production had already passed through a change due to radium exposure and the site of production was stabilizing at normalcy for 70° F.

The semblance of recovery of both the plasma proteins and white blood cells at the higher temperature implied a faster response to and recovery from radium poisoning. No doubt the higher temperatures perpetrated a faster metabolic processing of radium through the hemapoietic tissues and consequently there was an earlier onset of and adjustment to the damage and presence of radium. Higher environmental temperatures do raise the metabolic rate of fish as long as the temperatures are within the tolerance limits and these were (Brown, 1957; and Doudoroff et al., 1951).

Very few references were found in the literature covering the subject of interaction between irradiation and temperature as it influences the reactions of an exposed organism. Gros et al. (1958) used the goldfish,

Carassius carassius, to demonstrate that low temperature had a protective effect against ionizing radiation and low temperatures after x-radiation prolonged the survival time. Essentially the same reaction was found true with the newt, Triturus viridescens (O'Brien and Gojmerac, 1956). These authors felt that the temperature during irradiation was a radiosensitivity modifying factor. These previous conclusions did not concur with the results recorded for frogs (Patt and Swift, 1948). This work agreed that low temperatures after total body irradiation prolonged survival, but low body temperature during and/or the first 24 hours after a 1000-3000 roentgen dose did not influence the toxicity. The altered survival time was due to a decrease in the rate of development of radiation damage and not due to any appreciable recovery or lack of damage. If the lower temperature of an organism allows it to live longer because the onset of radiation damage is retarded, then other bodily manifestations would likewise react more slowly. Inversely, at higher temperatures the bodily responses to radiation damage appear and disappear sooner, which seems true in these experiments on goldfish.

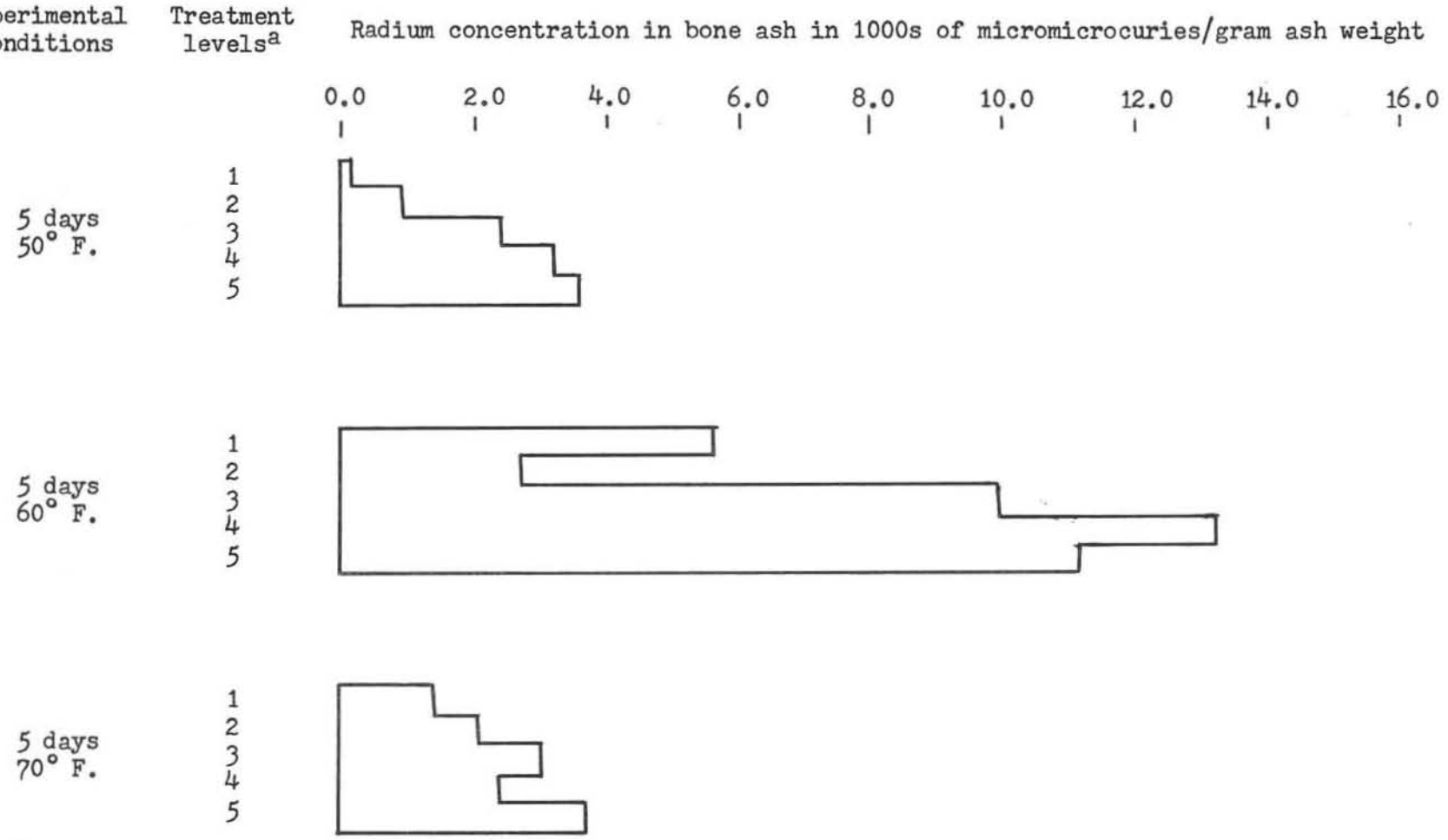
Therefore, it can be concluded that the temperature at which goldfish are exposed to radium does influence the rate of response and adjustment of blood forming organs to the presence of this contaminant. Temperature must be taken into consideration when attempting to measure any radium-caused changes that are clinically detected in the peripheral blood.

Bone analysis for radioactivity.--An empirical study of the interactions due to the environmental conditions of this major experiment and their effect on radium deposition in goldfish bone is possible with Table 9 and Figure 12. A cursory review of the table and graph reveals some possible interaction combinations, outside of the erratic Treatment

Table 9. The effects of the interaction between treatment and temperature at 5 days on the concentration of radium in goldfish bone

uuc. radium/ gram ash	Treatment levels in uc. radium/liter water				
	0.0	0.1	0.2	0.3	0.4
50° F.	112.4	964.6	2,451.3	3,230.1	3,610.6
60° F.	5,716.8?	2,752.2	10,017.3	13,292.0	11,212.3
70° F.	1,469.0	2,123.9	3,097.3	2,451.3	3,796.5

1 determinations. The most obvious interaction was the influence of the 60° F. temperature on the uptake and deposition of radium, especially at the three highest treatment levels. These relatively high amounts were in contrast with the contents detected for the same treatments at the other two temperatures. A somewhat related work evinced an accelerated uptake of cesium-147 and strontium-89 by fish as the water temperature became higher (Oak Ridge National Laboratory, 1953). The concentration of radioisotopes increased in the fish as the water temperature increased from 39° to 84° F. Within limits a similar conclusion seems possible for this research on radium and goldfish. The exception was that the goldfish under these environmental circumstances did not appear to have as great a metabolic activity at 70° F. as at 60° F. Therefore, less radium was concentrated at 70° F. If the activity of fish regulated the uptake and deposition of radium, then a 60° F. water temperature exerted the greatest influence upon the process under these conditions. An environment of 60° F. was closer to stimulating the maximum activity than 50° F. or 70° F. in this experiment. Though some kind of association between treatment and temperature did appear to exist, the variability of the data prevented statistical confirmation.



[a]Treatment levels from 1-5 are 0.0, 0.1, 0.2, 0.3, and 0.4 microcuries of radium/liter of water.

Figure 12. Amount of radium found in bones of goldfish exposed to five treatment levels of radium at three temperatures for 5 days.

Total experiment number four

Blood analysis.--The fourth major experiment studied the effects on the blood of the interaction between the five treatment levels of radium and the two temperatures of 50° and 60° F. at 10 days. The results on each of the blood characteristics from both of these fractional experiments were pooled and analyzed[10]. The analysis of variance was the same one as given in the Total Experiment Number Two section, except that the calculations were based on the numerical results at the given temperatures instead of time periods. Both the total plasma protein concentration and white blood cell numbers demonstrated some reaction as seen by the interruption of their interaction surfaces (Figures 13 and 14).

Even at 10 days the plasma proteins were lower in concentration at Treatment 5 than at the other four treatment levels (Figure 13). The sampling time of 10 days apparently caught these blood constituents still in the process of achieving a normal status. The protein content at 50° F. and Treatment 5 was not at a normal level, but a normal amount was found at the same treatment level at 60° F. So the temperature helped accelerate the replacement of plasma protein loss.

The 3-D graph for the leukocyte results (Figure 14) revealed the linear decline in numbers as the exposed treatment levels increased at 50° F. A mild leukocytosis was present at Treatments 2 and 3 while the anticipated leukopenia was produced by Treatment 5. These discoveries were further elaborated upon in the Fractional Experiments. At 60° F. the counts of white blood cells were relatively the same throughout the range of treatment levels. This picture indicated that these cells had

[10]The complete data and statistical calculations are in Appendix B.

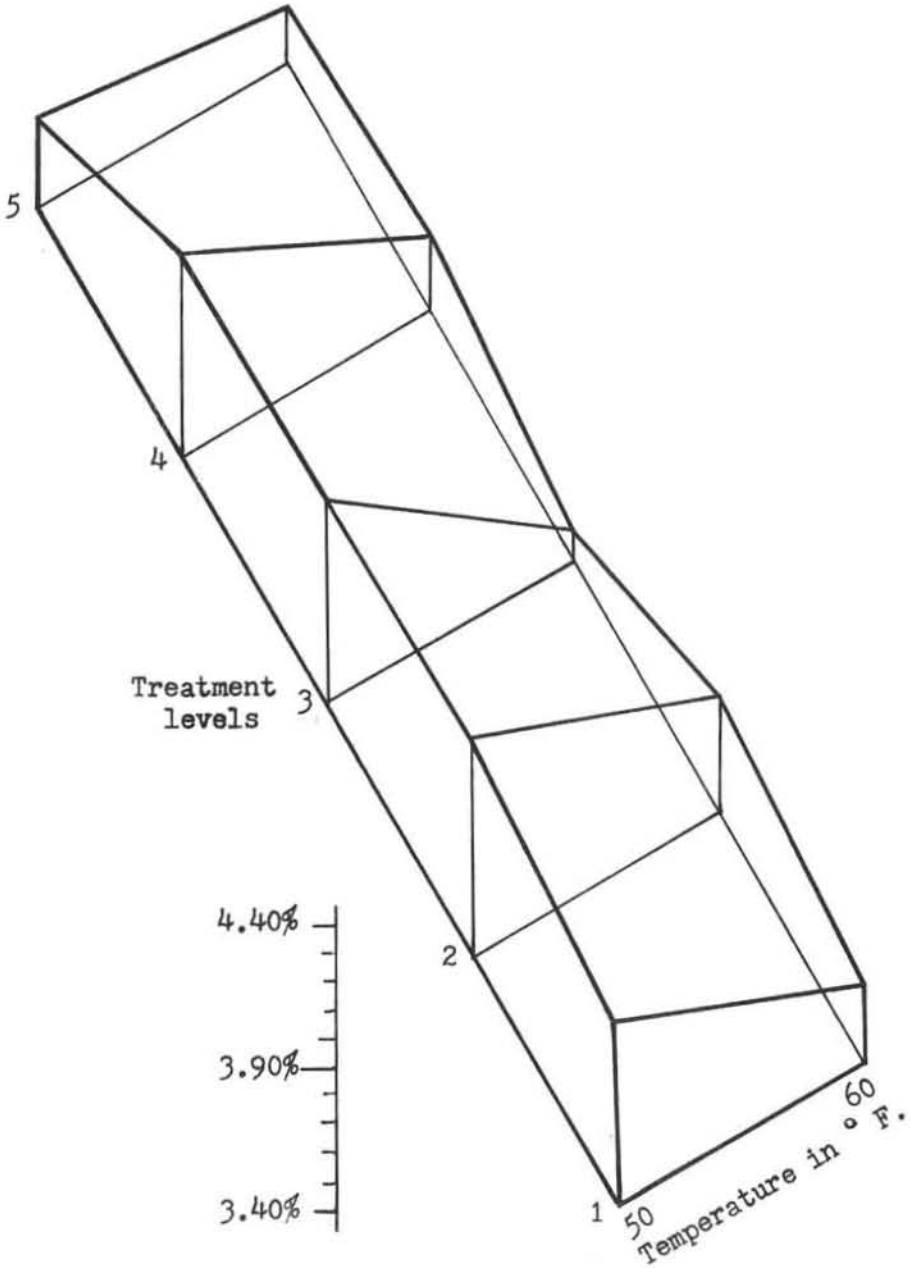


Figure 13. Exploration of the interaction surface formed by the plasma protein response within goldfish exposed to five treatment levels of radium at two temperatures for 10 days. The blood characteristic response is on the vertical scale which equals 1.00% per 1 1/2 inches and starts initially at a level of 3.40%.

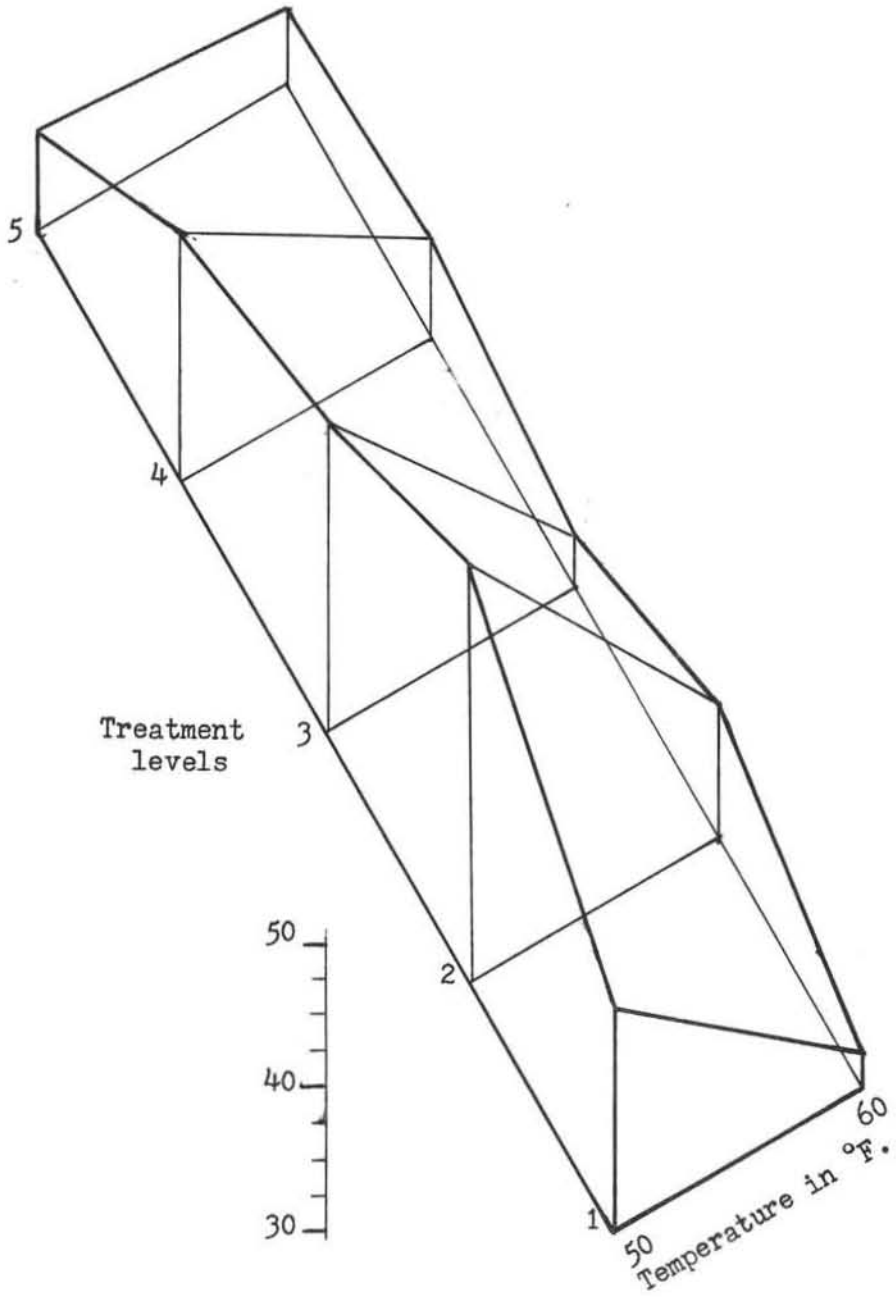


Figure 14. Exploration of the interaction surface formed by the white blood cell response within goldfish exposed to five treatment levels of radium at two temperatures for 10 days. The blood characteristic response is on the vertical scale which equals 20 (x 1000) cells per 1 1/2 inches and starts initially at a level of 30 (x 1000) cells.

passed through their change in numbers due to radium exposure and were achieving a normal status within 5 days at this temperature.

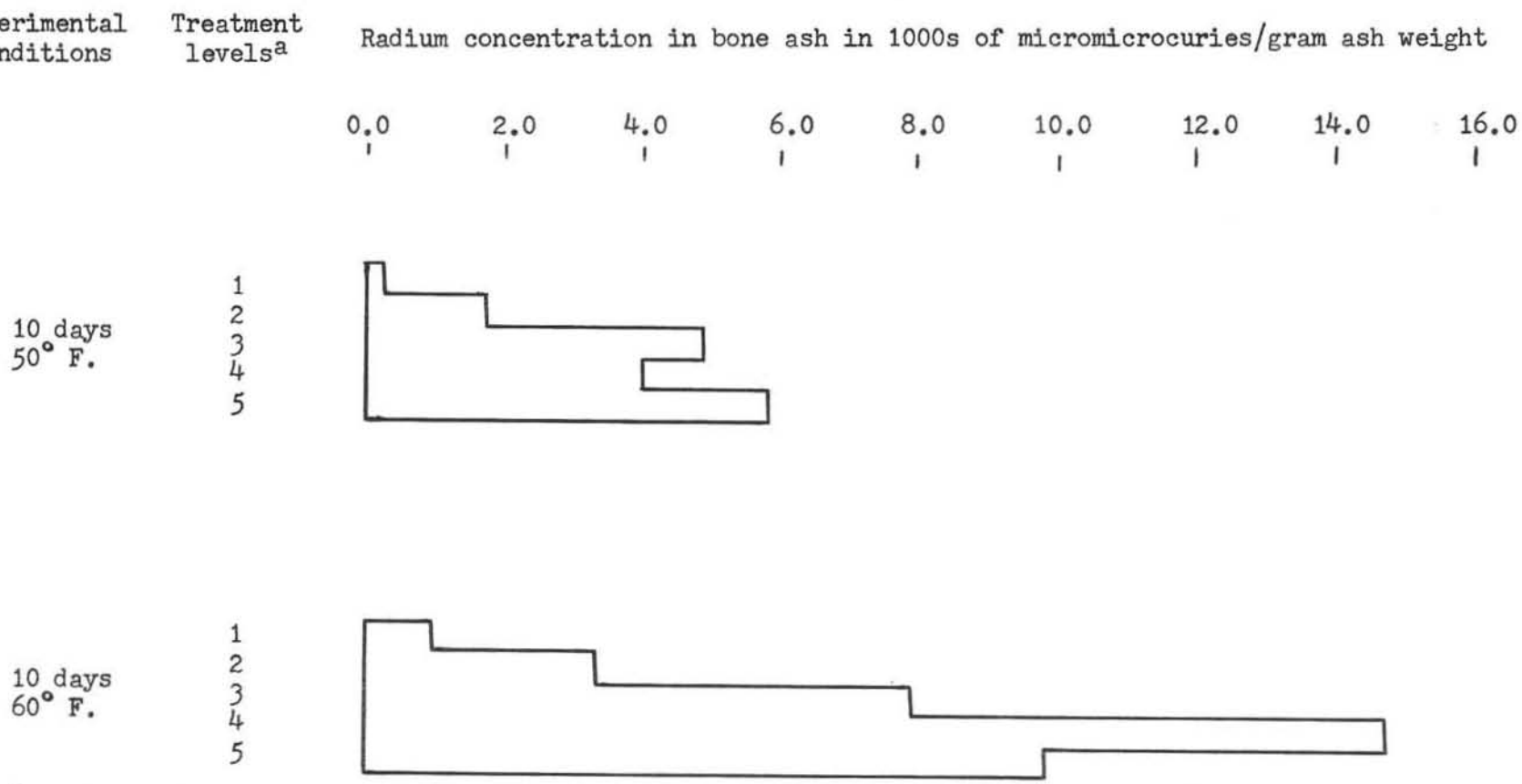
Once again it can be concluded that the higher temperatures accelerated the response and recovery of hemapoietic tissues to radium as its effect was measured in the peripheral blood of goldfish. Temperature must be taken into consideration when attempting to detect any of the initial changes caused by radium.

Bone analysis for radioactivity.--An empirical study of the interactions due to the environmental conditions of this major experiment and their effect on radium deposition in goldfish bone is possible with Table 10 and Figure 15.

Table 10. The effects of the interaction between treatment and temperature at 10 days on the concentration of radium in goldfish bone

uuc. radium/ gram ash	Treatment levels in uc. radium/liter water				
	0.0	0.1	0.2	0.3	0.4
50° F.	276.1	1,752.2	4,902.7	4,053.9	5,823.0
60° F.	973.5	3,371.7	7,902.7	14,840.7	9,840.7

Once again there appeared to be an association between temperature and treatment to influence a greater radioactivity in the bone at 60° F. The repetition of this interaction at 10 days added substantiation to the remarks about the similar phenomenon at 5 days and discussed in the previous section.



[a]Treatment levels from 1-5 are 0.0, 0.1, 0.2, 0.3, and 0.4 microcuries of radium/liter of water.

Figure 15. Amount of radium found in bones of goldfish exposed to five treatment levels of radium at two temperatures for 10 days.

SUMMARY AND CONCLUSIONS

Summary

Goldfish were exposed to radium by immersion under different controlled experimental conditions to study clinical blood changes and radium deposition in bone and internal organs (Table 11).

Table 11. Experimental conditions to which goldfish were subjected to study blood changes and radium deposition

Exposure conditions[a]			
Time in days	Water temperature in ° F.		
5	50	60	70
10	50	60	
15	50		

[a]Treatment levels are 0, .1, .2, .3, and .4 microcuries of radium/liter of water

The experiment was organized with a completely randomized block design containing five treatment levels, four replications at each level, and three time periods or temperatures. The overall objective was to develop some clinical method of detecting and measuring the degree of radioactive pollution by using changes within the fish as biological indicators.

After the predetermined exposure rates in time and temperature, the fish were removed, decaudated, and the blood extracted for analysis. The blood characteristics selected for study were the microhematocrits, total plasma proteins, white blood cell counts, and differential ratios (white blood cell composition). Everyone of the experimental combinations interacted with the radium treatments to influence changes in at least

one of the blood characteristics. Likewise, everyone of the blood characteristics showed at least one change sometime during the entire set of experiments. These detectable changes were statistically confirmed.

The fish were then dissected and the bone and internal organs removed and radioassayed. Both tissues were able to accumulate radium to varying degrees. The pattern of uptake and concentration was somewhat inconsistent. The experimental data were extremely variable, so that statistical tests could not be applied to help substantiate the empirical observations.

The following conclusions indicate that the individual life was not greatly impaired by radium, but these findings do not eliminate or elucidate any long range genetic damages to the population. Such hazards are possible and would require an entirely different set of conclusions.

Conclusions

1. Red blood cell production was stimulated in 5 days of exposure to radium at 70° F. The response to stimulation was the same at all levels of treatment. Red blood cell numbers, as measured by the microhematocrit technique, were not appreciably altered by radium under any of the other exposure conditions.
2. Total plasma protein concentrations decreased linearly with the increasing increments of radium following 5 days of exposure in 50° F. water. A return to normal was evinced by 15 days.
3. Other slight changes in total plasma protein content were detected after exposure conditions of 5 and 10 days at 60° F. and 5 days at 70° F. The major difference was a noticeable lowering of total proteins at the Treatment 5 level in comparison to the four lower levels.
4. The numbers of white blood cells increased linearly along with the increase in the radium content of the water following 5 days of exposure

at 60° F. There was a return to normal by 10 days.

5. After 10 days of exposure at 50° F., the white blood cell counts were high at the low treatment levels (0.1 and 0.2 microcuries radium/liter water) and low at the highest treatment (0.4 microcuries/liter). There was a return to normal by 15 days.

6. The change in white blood cell numbers under the previous conclusions was attributed to the granulocytes. They contributed to the increase and were absent in the decrease.

7. The radioactivity of the bone showed that goldfish quickly absorbed and concentrated radium from their environment with the 60° F. water temperature appearing to be the most influential factor.

   a. Time of exposure had a nominal influence.
   b. Higher concentrations of radium did not always contribute to an increasing deposition response, though higher levels of radioactivity tended to be found in fish from waters of greater radium concentrations.

8. Goldfish blood and bone reacted to these experiments in a manner similar to the responses recorded for other poikilotherms and homoiotherms exposed to corresponding conditions using other radioisotopes. The major exception is that the shift in white blood cell composition as the numbers changed was due to the granulocytes in goldfish rather than the lymphocytes as in homoiotherms.

   a. Radium did gain access to the internal tissues of goldfish where its influence was measured in the blood and bone.
   b. Changes in blood characteristics did occur at the levels of radium used.
   c. Blood changes did take place within the time periods of 5 to

15 days, and a return to normal pattern was exhibited during these time intervals.

d. The uptake and deposition of radium did follow a response pattern similar to those observed in other poikilotherms exposed to other radioisotopes except that after an initially high rate of uptake in the first 5 days, the accumulation of radium became minimal.

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APPENDIXES

Appendix A

Pictures of the experimental research and laboratory facilities

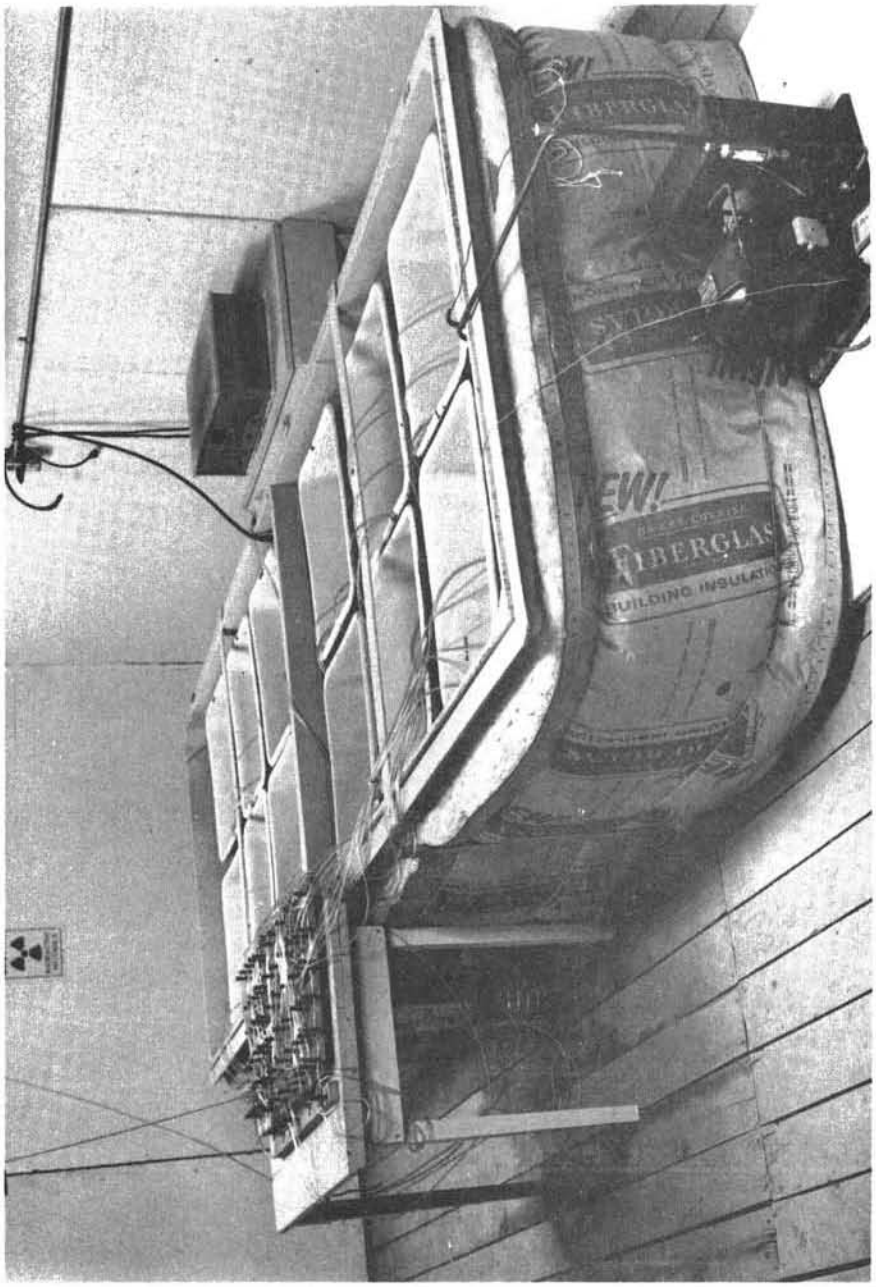

Figure 16. The entire experimental arrangement showing the large water bath which holds 12 aquariums, each classified as an experimental block. Within each block are five 1-gallon jars containing three goldfish each. The valves and hose represent the extensive air supply apparatus, which reaches each jar.

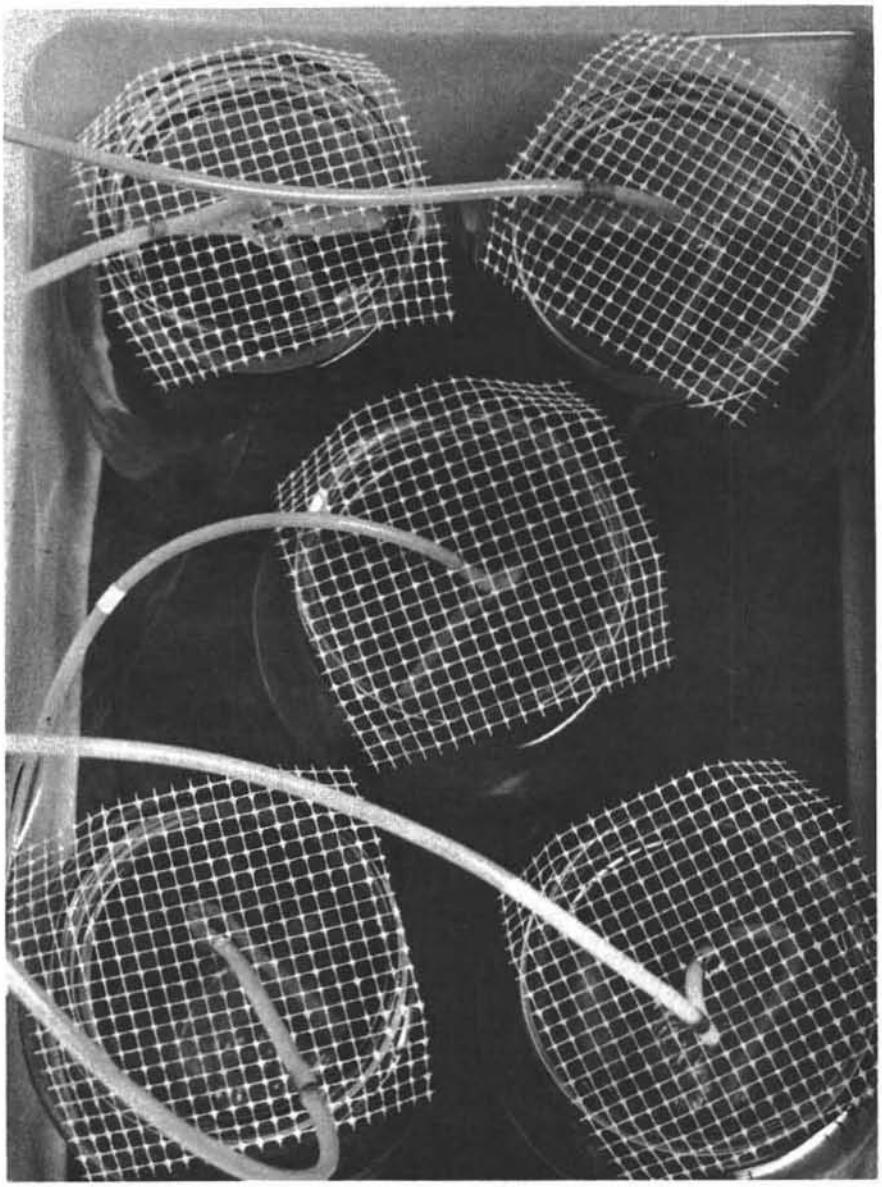

Figure 17. An experimental block containing the five 1-gallon jars, each holding three goldfish and a different level of radium. The other 11 blocks are similar.

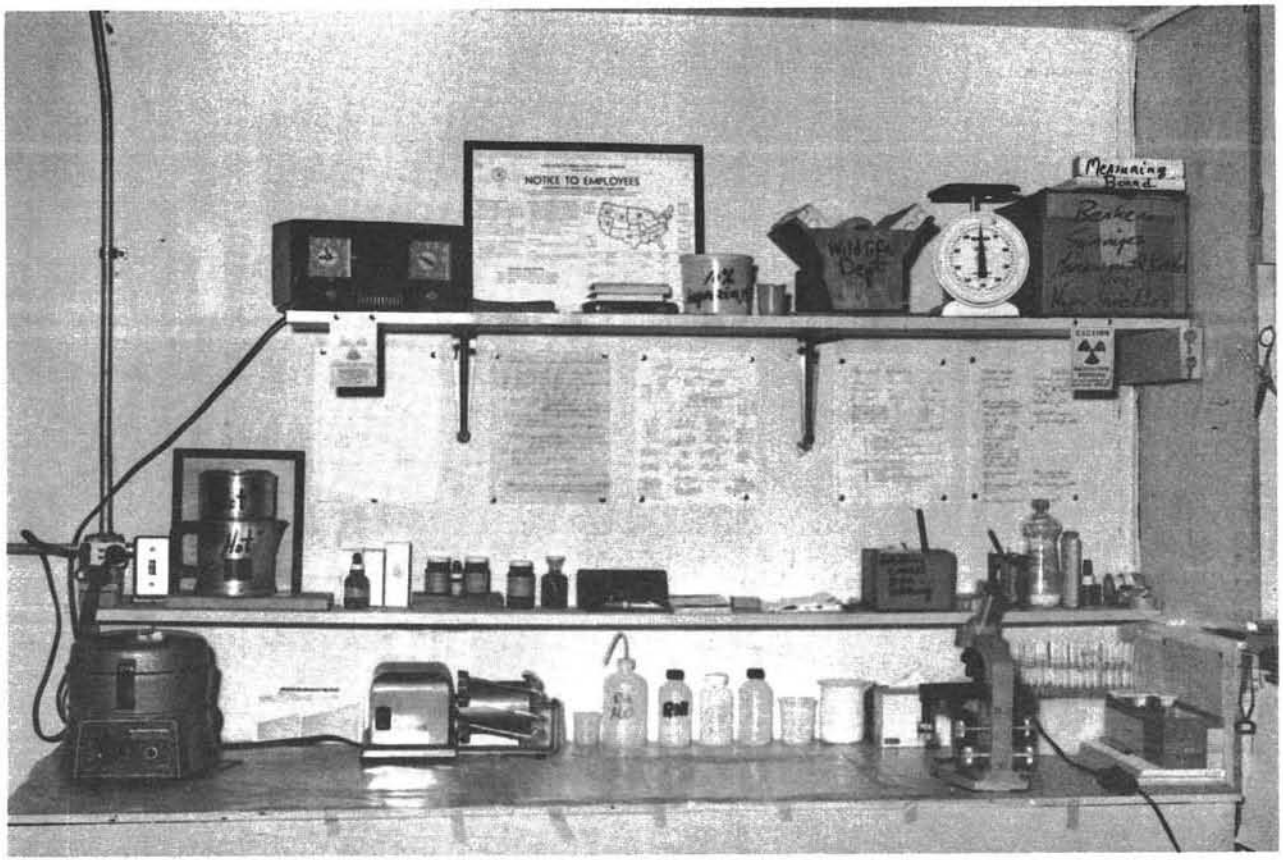

Figure 18. The laboratory bench where the blood from the fish was processed and analyzed.

Appendix B

Blood data and its significant statistical calculations

Table 12. Microhematocrit values in percent volume packed red blood cells from all the experiments. Each value represents the average of six determinations (two measurements on each of three fish). The average (Avg) of the replications is the figure used to plot the three dimensional (3-D) graphs in the text

Total expt.		One			Two		Three			Four	
Column no.		1	2	3	4	5	6	7	8	9	10
Time in days		5	10	15	5	10	5	5	5	10	10
Temp. in °F.		50	50	50	60	60	50	60	70	50	60
uc. radium/ liter water	Rep										
0.0	1	41.5	41.5	35.5	40.0	41.3	41.5	40.0	28.2	41.5	41.3
	2	46.0	41.0	38.3	37.0	44.7	46.0	37.0	28.2	41.0	44.7
	3	43.2	38.0	37.3	39.2	34.7	43.2	39.2	31.3	38.0	34.7
	4	41.2	39.0	39.7	42.7	37.8	41.2	42.7	36.7	39.0	37.8
	Avg	43.0	39.9	37.7	39.7	39.6	42.9	39.7	31.1	39.9	39.6
0.1	1	31.3	40.7	40.2	32.7	31.0	31.3	32.7	38.2	40.7	31.0
	2	39.3	32.0	32.8	37.2	40.2	39.3	37.2	36.3	32.0	40.2
	3	45.5	40.3	41.3	39.2	37.2	45.5	39.2	30.3	40.3	37.2
	4	44.3	39.5	43.7	33.0	40.2	44.3	33.0	44.7	39.5	40.2
	Avg	40.1	38.1	39.5	35.5	37.2	40.1	35.5	37.4	38.1	37.2
0.2	1	43.5	32.7	44.8	39.8	40.8	43.5	39.8	45.0	32.7	40.8
	2	36.8	43.0	44.8	39.3	38.0	36.8	39.3	38.3	43.0	38.0
	3	47.2	40.0	41.5	35.0	39.5	47.2	35.0	34.0	40.0	39.5
	4	44.7	40.0	35.0	39.7	42.5	44.7	39.7	40.0	40.0	42.5
	Avg	43.1	38.9	41.5	38.5	40.2	43.1	38.5	39.3	38.9	40.2
0.3	1	44.5	40.7	43.3	37.7	36.5	44.5	37.7	42.0	40.7	36.5
	2	37.0	35.5	39.7	39.5	39.0	37.0	39.5	35.7	35.5	39.0
	3	48.8	45.3	42.5	35.8	40.5	48.8	35.8	39.5	45.3	40.5
	4	43.2	43.0	43.2	39.8	41.5	43.2	39.8	39.0	43.0	41.5
	Avg	43.4	41.1	42.2	38.2	39.4	43.4	38.2	39.1	41.1	39.4
0.4	1	44.0	35.8	43.5	42.0	34.0	44.0	42.0	40.2	35.8	34.0
	2	40.0	39.2	45.5	41.2	43.8	40.0	41.2	39.3	39.2	43.8
	3	36.8	36.5	38.5	40.2	34.5	36.8	40.2	33.0	36.5	34.5
	4	40.8	43.8	42.4	42.2	39.7	40.8	42.2	37.7	43.8	39.7
	Avg	40.4	38.8	42.4	41.4	38.0	40.4	41.4	37.5	38.8	38.0

Table 13. The analysis of variance of the data from column number 8 in Table 12

Source of variation	Sum of squares	Degrees of freedom	Mean square	F test
Replication	116.31	3	38.77	
Treatment	179.16	4	44.79	3.57*[a]
Error	150.46	12	12.54	
Total	445.93	19		

*Significant at 5 percent probability.

[a]The significant mean was detected by using "Critical values of Duncan's new multiple range test" as described by H. L. Harter in Biometrics 14(4):671-685.

Table 14. Plasma protein values in percent gram total protein from all the experiments. Each value represents the average of six determinations (two measurements on each of three fish). The average (Avg) of the replications is the figure used to plot the three dimensional (3-D) graphs in the text

Total expt.		One			Two		Three			Four	
Column no.		1	2	3	4	5	6	7	8	9	10
Time in days		5	10	15	5	10	5	5	5	10	10
Temp. in °F.		50	50	50	60	60	50	60	70	50	60
uc. radium/ liter water	Rep										
0.0	1	4.43	3.92	3.45	3.90	3.68	4.43	3.90	3.68	3.92	3.68
	2	3.90	3.77	4.00	3.97	3.65	3.90	3.97	3.55	3.77	3.65
	3	4.65	3.98	3.97	3.37	3.40	4.65	3.37	3.38	3.98	3.40
	4	4.53	4.23	3.87	3.52	4.05	4.53	3.52	3.88	4.23	4.05
	Avg	4.38	3.98	3.82	3.69	3.70	4.38	3.69	3.62	3.98	3.70
0.1	1	3.83	4.57	3.92	3.62	3.80	3.83	3.62	3.68	4.57	3.80
	2	4.17	3.80	4.43	3.87	4.10	4.17	3.87	3.52	3.80	4.10
	3	4.18	4.12	4.17	4.03	3.68	4.18	4.03	3.77	4.12	3.68
	4	4.27	3.90	3.88	3.60	3.57	4.27	3.60	3.70	3.90	3.57
	Avg	4.11	4.10	4.10	3.78	3.79	4.11	3.78	3.67	4.10	3.79
0.2	1	4.02	3.93	3.57	3.58	3.43	4.02	3.58	4.40	3.93	3.43
	2	4.27	4.32	3.62	3.62	3.40	4.27	3.62	4.08	4.32	3.40
	3	4.00	4.02	3.77	3.63	3.55	4.00	3.63	3.83	4.02	3.55
	4	3.75	4.18	3.85	4.12	3.50	3.75	4.12	4.40	4.18	3.50
	Avg	4.01	4.11	3.70	3.74	3.47	4.01	3.74	4.18	4.11	3.47
0.3	1	4.02	4.05	4.02	3.43	4.02	4.02	3.43	3.77	4.05	4.02
	2	3.57	3.92	3.93	3.42	4.00	3.57	3.42	3.13	3.92	4.00
	3	4.28	4.20	3.95	3.35	3.10	4.28	3.35	3.95	4.20	3.10
	4	3.87	4.18	4.08	3.92	3.58	3.87	3.92	3.65	4.18	3.58
	Avg	3.94	4.09	4.00	3.53	3.68	3.94	3.53	3.63	4.09	3.68
0.4	1	3.57	3.65	3.97	3.52	3.65	3.57	3.52	3.57	3.65	3.65
	2	3.30	3.67	4.23	3.52	3.60	3.30	3.52	3.57	3.67	3.60
	3	3.30	4.15	3.68	3.48	3.33	3.30	3.48	3.70	4.15	3.33
	4	3.60	3.28	3.77	3.23	3.83	3.60	3.23	3.20	3.28	3.83
	Avg	3.44	3.69	3.91	3.44	3.60	3.44	3.44	3.51	3.69	3.60

Table 15. The analysis of variance of the data from column number 1 in Table 14

Source of variation	Sum of squares	Degrees of freedom	Mean square	F test
Replication	0.1499	3	0.04997	
Treatment	1.8692	4	0.46730	7.267**[a]
Error	0.7716	12	0.06430	
Total	2.7907	19		

**Significant at 1 percent probability.
[a]The linear response pattern was assessed by the method in Chapter 16; Li, J.C.R. 1957. Introduction to statistical inference. Edwards Brothers, Inc., Ann Arbor, Michigan. 553 p.

Table 16. The analysis of variance of the data from column number 8 in Table 14

Source of variation	Sum of squares	Degrees of freedom	Mean square	F test
Replication	0.1732	3	0.0577	
Treatment	1.0988	4	0.2747	4.48*[a]
Error	0.7359	12	0.0613	
Total	2.0079	19		

*Significant at 5 percent probability.
[a]The significant mean was detected by using "Critical values for Duncan's new multiple range test" as described by H. L. Harter in Biometrics 14 (4):671-685.

Table 17. The analysis of variance of the data from total experiment one (column numbers 1, 2, and 3) in Table 14

Source of variation	Sum of squares	Degrees of freedom	Mean square	F test
Treatment	1.3268	4	0.3317	7.929*
Within treatments	0.6275	15	0.0418	
Time	0.0823	2	0.0412	
Time x treatments	1.4294	8	0.1787	9.359*[a]
Error	0.5726	30	0.0191	
Total	4.0386	59		

*Significant at 5 percent probability.
[a]This interaction is illustrated in Figure 5 in the text.

Table 18. The analysis of variance of the data from total experiment three (column numbers 6, 7, and 8) in Table 14

Source of variation	Sum of squares	Degrees of freedom	Mean square	F test
Treatment	1.9103	4	0.4925	8.683**
Within treatments	0.8508	15	0.0567	
Temperature	1.2552	2	0.6276	10.350**
Temp. x treatments	1.3401	8	0.1675	2.763*[a]
Error	1.8187	30	0.0606	
Total	7.1751	59		

**Significant at 1 percent probability.
*Significant at 5 percent probability.
[a]This interaction is illustrated in Figure 10 in the text.

Table 19. The analysis of variance of the data from total experiment four (column numbers 9 and 10) in Table 14

Source of variation	Sum of squares	Degrees of freedom	Mean square	F test
Treatment	0.4034	4	0.1009	21.32**[a]
Within treatments	0.7094	15	0.0473	
Temperature	1.1972	1	1.1972	21.91**
Temp. x treatments	0.3322	4	0.0831	
Error	1.3521	15	0.0901	
Total	3.9943	39		

**Significant at 1 percent probability.
[a]This effect is illustrated in Figure 13 in the text.

Table 20. White blood cell values in 1000s/cubic millimeter of blood from all the experiments. Each value represents the average of six determinations (two measurements on each of three fish). The average (Avg) of the replications is the figure used to plot the three dimensional (3-D) graphs in the text

Total expt.		One			Two		Three			Four	
Column no.		1	2	3	4	5	6	7	8	9	10
Time in days		5	10	15	5	10	5	5	5	10	10
Temp. in °F.		50	50	50	60	60	50	60	70	50	60
uc. radium/ liter water	Rep										
0.0	1	46.8	42.3	29.8	38.2	38.0	46.8	38.2	35.3	42.3	38.0
	2	33.8	58.5	30.3	43.0	28.2	33.8	43.0	40.8	58.5	28.2
	3	43.5	42.8	39.5	25.0	32.0	43.5	25.0	25.5	42.8	32.0
	4	55.0	38.5	15.0	33.5	29.0	55.0	33.5	31.8	38.5	29.0
	Avg	44.8	45.5	28.7	34.9	31.8	44.8	34.9	33.4	45.5	31.8
0.1	1	58.3	61.5	51.0	33.0	42.0	58.3	33.0	35.8	61.5	42.0
	2	47.3	55.3	28.0	40.0	36.8	47.3	40.0	33.3	55.3	36.8
	3	35.8	60.2	33.2	21.0	34.8	35.8	21.0	25.7	60.2	34.8
	4	51.0	56.3	20.5	44.3	38.7	51.0	44.3	35.8	56.3	38.7
	Avg	48.1	58.3	33.2	34.6	38.1	48.1	34.6	32.7	58.3	38.1
0.2	1	46.3	41.8	38.0	56.2	29.0	46.3	56.2	30.7	41.8	29.0
	2	46.7	49.0	43.5	40.0	33.5	46.7	40.0	38.7	49.0	33.5
	3	57.3	52.0	19.7	65.8	37.7	57.3	65.8	42.5	52.0	37.7
	4	59.0	60.5	40.0	63.0	34.5	59.0	63.0	43.2	60.5	34.5
	Avg	52.3	50.8	35.3	56.3	33.7	52.3	56.3	38.8	50.8	33.7
0.3	1	45.7	56.3	35.8	51.8	44.8	45.7	51.8	44.0	56.3	44.8
	2	52.7	46.5	37.0	63.7	34.3	52.7	63.7	32.0	46.5	34.3
	3	51.7	35.5	15.0	52.3	36.0	51.7	52.3	29.2	35.5	36.0
	4	49.5	48.5	41.2	43.7	31.7	49.5	43.7	44.0	48.5	31.7
	Avg	49.9	46.7	32.3	52.9	36.7	49.9	52.9	37.3	46.7	36.7
0.4	1	48.8	38.3	36.0	96.3	34.0	48.8	96.3	45.2	38.3	34.0
	2	44.5	30.5	22.8	71.8	35.8	44.5	71.8	46.8	30.5	35.8
	3	40.3	37.0	47.0	68.3	39.0	40.3	68.3	45.0	37.0	39.0
	4	43.0	39.0	32.3	67.5	32.3	43.0	67.5	37.7	39.0	32.3
	Avg	44.2	36.2	34.5	76.0	35.3	44.2	76.0	43.7	36.2	35.3

Table 21. The analysis of variance of the data from column number 2 in Table 20

Source of variation	Sum of squares	Degrees of freedom	Mean square	F test
Replication	28.08	3	9.36	
Treatment	1,041.82	4	260.46	4.594*[a]
Error	680.32	12	56.69	
Total	1,750.22	19		

*Significant at 5 percent probability.
[a]The linear response pattern was assessed by the method in Chapter 16; Li, J.C.R. 1957. Introduction to statistical inference. Edwards Brothers, Inc., Ann Arbor, Michigan. 553 p.

Table 22. The analysis of variance of the data from column number 4 in Table 20

Source of variation	Sum of squares	Degrees of freedom	Mean square	F test
Replication	190.32	3	63.44	
Treatment	4,731.93	4	1,182.98	9.71**[a]
Error	1,461.86	12	121.82	
Total	6,384.11	19		

**Significant at 1 percent probability.
[a]The linear response pattern was assessed by the method in Chapter 16; Li, J.C.R. 1957. Introduction to statistical inference. Edwards Brothers, Inc., Ann Arbor, Michigan. 553 p.

Table 23. The analysis of variance of the data from total experiment two (column numbers 4 and 5) in Table 20

Source of variation	Sum of squares	Degrees of freedom	Mean square	F test
Treatment	2,430.9877	4	607.7460	9.045*
Within treatments	1,007.7977	15	67.1860	
Time	2,501.1425	1	2,501.1425	42.056**
Time x treatments	2,398.3913	4	599.5978	10.082*[a]
Error	892.0648	15	59.4710	
Total	9,230.3840	39		

* Significant at 5 percent probability.
**Significant at 1 percent probability.
[a]This interaction is illustrated in Figure 7 in the text.

Table 24. The analysis of variance of the data from total experiment three (column numbers 6, 7, and 8) in Table 20

Source of variation	Sum of squares	Degrees of freedom	Mean square	F test
Treatment	2,496.5257	4	624.13	6.252**a
Within treatments	1,497.4133	15	99.83	
Temperature	2,088.5402	2	1,044.27	9.56*
Temp. x treatments	841.1107	8	105.14	
Error	3,275.0525	30	109.17	
Total	10,198.6424	59		

**Significant at 1 percent probability.
*Significant at 5 percent probability.
[a]This effect is illustrated in Figure 11 in the text.

Table 25. The analysis of variance of the data from total experiment four (column numbers 9 and 10) in Table 20

Source of variation	Sum of squares	Degrees of freedom	Mean square	F test
Treatment	692.5570	4	173.139	4.63*
Within treatments	560.6580	15	37.377	
Temperature	1,540.0800	1	1,540.080	58.42**
Temp. x treatments	446.7530	4	111.688	4.24*a
Error	395.4270	15	26.362	
Total	3,635.4270	39		

*Significant at 5 percent probability.
**Significant at 1 percent probability.
[a]This interaction is illustrated in Figure 14 in the text.

Table 26. Differential smear ratio values in number of lymphocytes per granulocyte. These ratios were assessed only on blood that displayed significant changes in the white blood cell counts

Experimental conditions	Rep	Treatments in uc. radium/liter water 0.0	0.1	0.2	0.3	0.4
50° F.	1	7.13	7.13	7.55	9.30	32.33
10 days	2	7.55	8.09	7.55	6.41	17.86
	3	7.13	7.13	9.00	6.69	15.67
	4	6.69	8.35	8.71	8.71	16.54
	Avg	7.13	7.68	8.20	7.78	20.60
WBC count	Avg	45.5	58.3	50.8	46.7	36.2
60° F.	1	6.41	1.82	3.23	1.32	1.79
5 days	2	10.98	7.33	4.22	1.84	2.09
	3	8.85	5.89	2.28	1.33	2.11
	4	19.00	2.92	3.68	1.15	2.70
	Avg	11.31	4.49	3.35	1.41	2.17
WBC count	Avg	34.9	34.6	56.3	52.9	76.0

Table 27. The analysis of variance of the data from the first half of Table 26

Source of variation	Sum of squares	Degrees of freedom	Mean square	F test
Replication	39.9300	3	13.3100	
Treatment	535.2840	4	133.8210	10.32**[a]
Error	155.5357	12	12.9613	
Total	730.7497	19		

**Significant at 1 percent probability.
[a]The significant mean was detected by using "Critical values for Duncan's new multiple range test" as described by H. L. Harter in Biometrics 14 (4):671-685.

Table 28. The analysis of variance of the data from the last half of Table 26

Source of variation	Sum of squares	Degrees of freedom	Mean square	F test
Replication	26.1619	3	8.7206	
Treatment	250.5891	4	62.6473	8.79**[a]
Error	85.4914	12	7.1243	
Total	362.2424	19		

**Significant at 1 percent probability.
[a]The linear response pattern was assesed by the method in Chapter 16; Li, J.C.R. 1957. Introduction to statistical inference. Edwards Brothers, Inc., Ann Arbor, Michigan. 553 p.

Table 29. Bone radioactivity values in counts/minute from all the experiments. Each value is based upon the combined bone ash from all three fish in one experimental unit. The one replication (*) which showed aberrancy in each set of data was not included in the average (Avg) throughout the table[a]

Total expt.		One			Two		Three			Four	
Column no.		1	2	3	4	5	6	7	8	9	10
Time in days		5	10	15	5	10	5	5	5	10	10
Temp. in ° F.		50	50	50	60	60	50	60	70	50	60
uc. radium/ liter water	Rep										
0.0	1	13.6*	8.7	6.1	167.9	31.8	13.6*	167.9	52.5	8.7	31.8
	2	4.6	42.5*	8.5	188.6	34.4	4.6	188.6	61.3	42.5*	34.4
	3	4.6	13.0	16.5*	229.4*	104.6*	4.6	229.4*	123.0*	13.0	104.6*
	4	3.2	7.1	6.3	194.6	41.7	3.2	194.6	56.9	7.1	41.7
	Avg	4.1	9.6	7.0	183.7	36.0	4.1	183.7	56.9	9.6	36.0
0.1	1	43.3	135.9*	44.5	100.2	146.0	43.3	100.2	80.4	135.9*	146.0
	2	145.2*	75.6	52.8*	95.9	162.1	145.2*	95.9	129.3*	75.6	162.1
	3	28.1	61.8	50.3	121.9	133.0	28.1	121.9	85.7	61.8	133.0
	4	29.3	48.1	46.6	132.9*	196.0*	29.3	132.9*	91.0	48.1	196.0*
	Avg	33.6	61.8	47.1	106.0	147.0	33.6	106.0	85.7	61.8	147.0
0.2	1	124.7*	27.3*	77.4	325.8	296.0	124.7*	325.8	129.5	27.3*	296.0
	2	94.7	194.9	91.7	386.7	263.3	94.7	386.7	174.9*	194.9	263.3
	3	69.5	174.1	84.0	263.1	486.4*	77.6	263.1	132.1	174.1	486.4*
	4	86.6	147.4	153.0*	494.6*	279.5	86.6	494.6*	134.6	147.4	279.5
	Avg	83.6	172.1	84.4	325.2	279.5	86.3	325.2	132.1	172.1	279.5

Table 29. Continued

Total expt.		One			Two		Three			Four	
Column no.		1	2	3	4	5	6	7	8	9	10
Time in days		5	10	15	5	10	5	5	5	10	10
Temp. in ° F.		50	50	50	60	60	50	60	70	50	50
uc. radium/ liter water	Rep										
0.3	1	113.5	143.1	205.3	794.1*	263.7*	113.5	794.1*	100.0	143.1	263.7*
	2	18.2*	157.3	140.5*	473.4	500.9	18.2*	473.4	108.2	157.3	500.9
	3	128.6	373.9*	200.9	338.8	499.9	128.6	338.8	104.1	373.9*	499.9
	4	83.5	138.1	176.6	625.9	486.0	83.5	625.9	205.4*	138.1	486.0
	Avg	108.5	146.2	194.3	479.4	495.6	108.5	479.4	104.1	146.2	495.6
0.4	1	100.0	211.8	204.6	430.3	398.5*	100.0	430.3	151.3	211.8	398.5*
	2	79.9*	178.1	204.4	354.0	361.8	79.9*	354.0	146.8	178.1	361.8
	3	108.6	232.7	205.8	403.0	382.7	108.6	403.0	155.7	232.7	382.7
	4	115.9	138.4*	168.4*	691.8*	371.2	115.9	691.8*	192.9*	138.4*	371.2
	Avg	108.2	207.5	204.9	395.8	371.9	108.2	395.8	151.3	207.5	371.9

[a]The figures in the table are the counts/minute at approximately 50.0 milligrams ash weights. In order to convert from counts/minute to micromicrocuries of radioactivity per gram ash weight, counts are needed at approximately 100.0 and 150.0 or 200.0 milligrams ash weights. Since the data did not test significant, the additional counts/minute figures for heavier weights of ash have not been given. Figures are provided here so the reader can make some casual comparisons of relative radioactivities under the different experimental conditions. An illustration of the necessary figures and steps for the conversion of counts/minute to micromicrocuries of activity per gram ash weight is provided in Appendix D.

Table 30. Bartlett's test of homogeneity of variance of the data from Table 29 on the radioactivity of the bone ash[a]

pooled df	pooled ss	weighted msq	total ssq
40.000000	632993.47	15824.836	832604.40
unadj chi	adj factor	adj chi-sq	tabl chi-sq
21.265310	1.05000000	20.252676	.000000000
value f'	between gr df	calc df	sww msq
4.5102954*	4.000000000	17.912230	1.1116555

F 4,18 df = 2.6 (.05) Table 10.5.3[a]

[a]Calculations done on the IBM computer no. 1620 at Utah State Univ. following the techniques of section 10.20 in:
Snedecor, G. W. 1956. Statistical methods. 5th ed. The Iowa State College Press, Ames, Iowa. 534 p.
*Significant at the 5 percent probability.

NOTE: Since the total data tested significant to show that there was heterogeneity of variance, the data were then subjected to log transmission to achieve homogeneity of variance. When the data were separated into experimental units and tested, no significant differences were detectable among the treatments at the levels of confidence desired. This finding indicated extreme variability within the experimental data, no doubt due in a large measure to biological variability. Future research of this nature will require more samples and replications in order to achieve significant findings within the range of desired confidence.

Appendix D

Example of the conversion of counts per minute to micromicrocuries of radioactivity per gram ash weight

Table 31. Raw data in counts/minute of radioactivity in ashed bone from goldfish exposed to four levels of radium for 5 days at 50° F.[a] The average (Avg) values are used to construct Figure 19, in which the lines are fitted by eye

uc. radium/ liter water	0.1		0.2		0.3		0.4	
Rep	Wgts in mg.	Counts/ minute	Wgts in mg.	Counts/ minute	Wgts in mg.	Counts/ minute	Wgts in mg.	Counts/ minute
1	50.5	43.3	53.0	94.7	53.3	113.5	45.7	100.0
2	50.4	28.1	49.7	69.5	51.9	128.6	49.1	108.6
3	46.0	29.3	50.8	86.6	49.4	83.5	49.1	115.9
Avg	49.0	33.6	51.2	83.6	51.5	108.5	48.0	108.2
1	102.3	59.3	99.8	115.5	100.0	138.7	101.5	123.5
2	100.5	36.9	100.0	77.6	102.2	156.6	101.8	134.5
3	101.2	38.8	99.0	110.8	99.4	109.6	101.3	149.1
Avg	101.3	45.0	99.6	101.3	100.5	135.0	101.5	135.7
1	200.1	59.1	202.1	136.4	201.3	152.7	200.0	139.5
2	200.7	43.2	201.2	110.4	199.4	169.9	202.4	164.1
Avg	200.4	51.2	201.7	123.4	200.4	161.3	201.2	151.8

[a]The values from this experiment are used to illustrate the process of conversion to micromicrocuries of radioactivity per gram ash weight, as given by: Tsivoglous, E. C. 1961. Method for gross radioactivity analysis of environmental samples. Radiological Pollution Activities Unit, Robert A. Taft Sanitary Enginnering Center, Cincinnati, Ohio. 15 p.

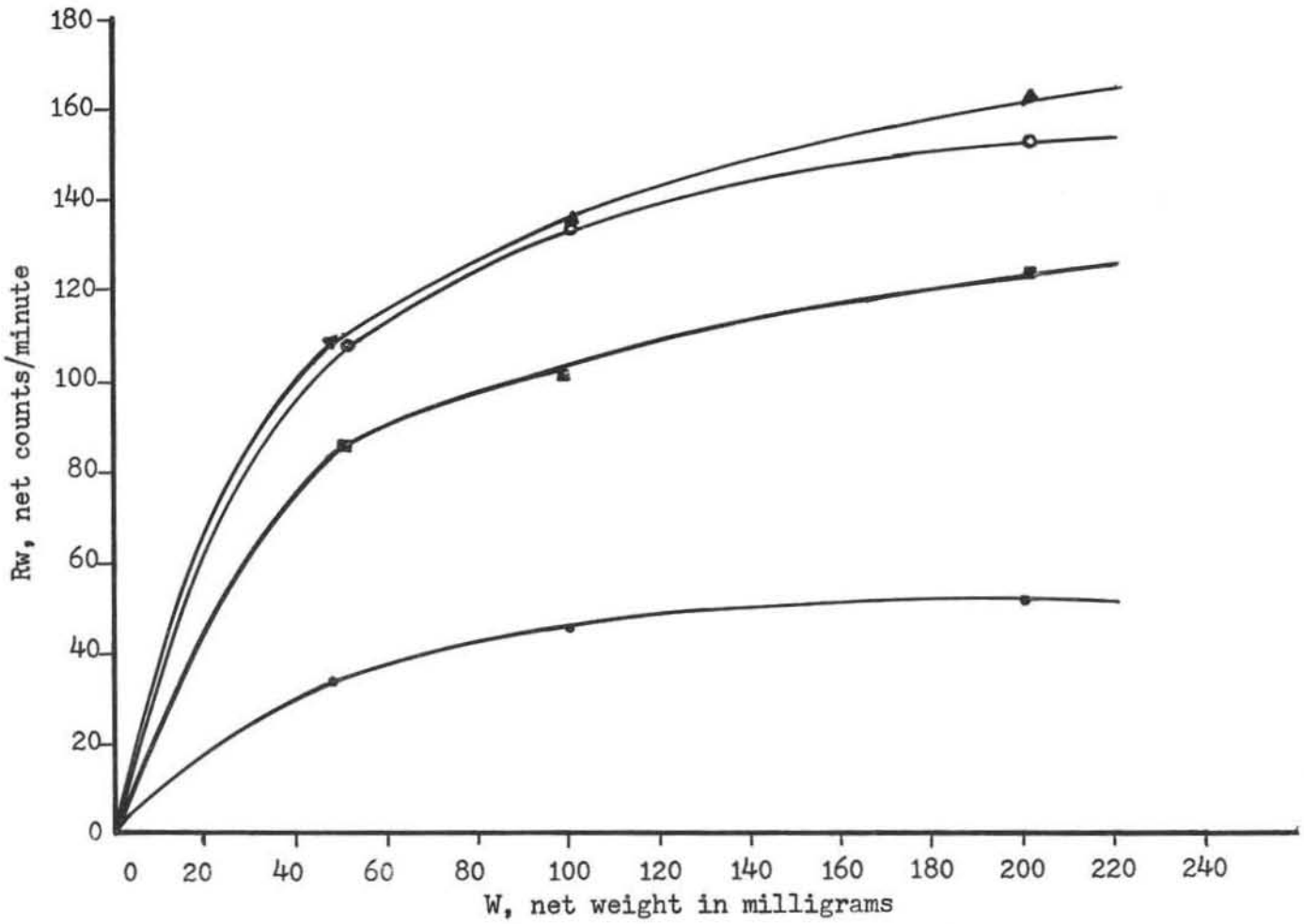

Figure 19. Arithmetic plot of the observed count rate (counts/minute) versus weight of bone ash counted. The lines are fitted by eye.

Table 32. Changes in the count rate as given in Figure 19 by determining the difference in the counts/minute between each 50 milligram interval. The differences are plotted on semilog graph paper at the midpoint of the weight intervals, as in Figure 20 where the lines are fitted by eye[a]

Tr.2[b] Wgts in mg.	Counts/ minute	Changes in c.p.m.		Corrected c.p.m.[c]	Tr.3 Wgts in mg.	Counts/ minute	Changes in c.p.m.		Corrected c.p.m.
		Act.	Cor.[d]				Act.	Cor.	
0	0			0	0	0			0
50	34	34	34	34	50	82	82	82	82
100	45	11	11	45	100	107	25	25	107
150	49	4	4	49	150	115	8	8	115
200	52	3	1	50	200	120	5	3	118
Tr. 4					Tr. 5				
0	0			0	0	0			0
50	105	105	105	105	50	115	115	115	115
100	135	30	30	135	100	146	31	32	147
150	145	10	9	144	150	158	12	9	156
200	150	5	3	147	200	165	7	3	159

[a] This step is used to determine the zero thickness count rate as if no self absorption took place.

[b] Treatments 2-5 are 0.1, 0.2, 0.3, and 0.4 uc. radium/liter water.

[c] Based on the corrected changes in counts per minute (c.p.m. Cor. in previous column), which are determined from the lines of Figure 20. These corrections are a means of adjusting for errors in laboratory procedures. Though minimal, they are the figures used in the remaining calculations.

[d] Actual and Corrected.

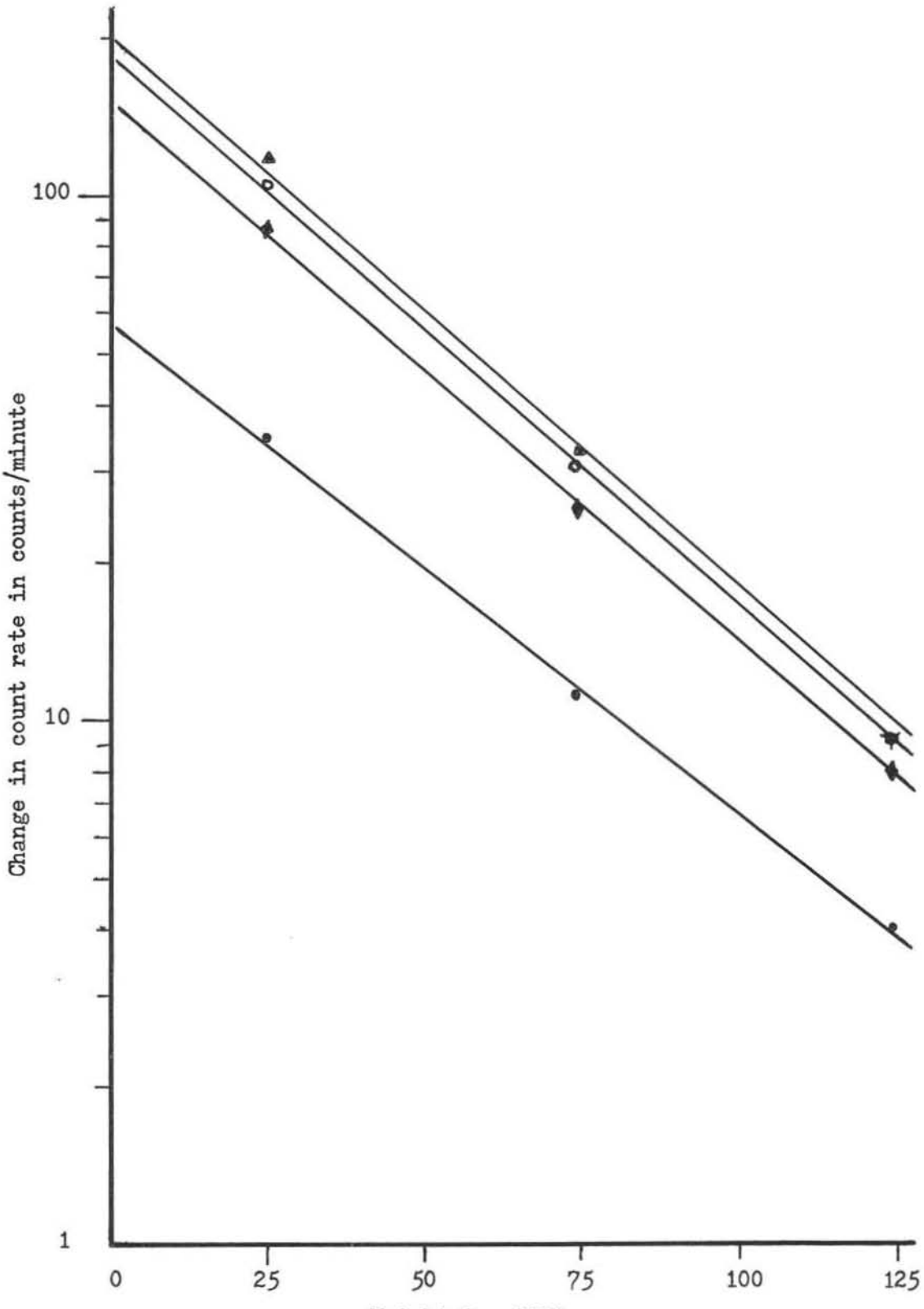

Figure 20. Semilog plot of the corrected change in count rate for equal intervals of sample weight versus the sample weight at the midpoint of the interval. The lines are fitted by eye.

Table 33. The calculations necessary to convert from counts/minute to micromicrocuries of radioactivity per gram bone ash using the corrected counts/minute from Table 32

Determination of C, the rate constant, in $mg.^{-1}$, where:

$$C = \frac{\ln (\text{1st difference in c.p.m./3rd difference in c.p.m.})}{\text{weight difference between 1st and 3rd counts (usually 100 mg.)}}$$

$$\text{Tr. 2} = \frac{\ln 34/4}{100} = \frac{\ln 8.50}{100} = \frac{2.14}{100} = 0.0214 = C_2$$

$$\text{Tr. 3} = \frac{\ln 82/8}{100} = \frac{\ln 10.25}{100} = \frac{2.33}{100} = 0.0233 = C_3$$

$$\text{Tr. 4} = \frac{\ln 105/9}{100} = \frac{\ln 11.67}{100} = \frac{2.46}{100} = 0.0246 = C_4$$

$$\text{Tr. 5} = \frac{\ln 115/9}{100} = \frac{\ln 12.78}{100} = \frac{2.55}{100} = 0.0255 = C_5$$

Determination of R_0, zero thickness count rate, in c.p.m./mg., where:

$$R_0 = \frac{CR_w}{1 - e^{-cw}}$$ where C = rate constant and R_w = corrected c.p.m. at weight, w

$$\text{Tr. 2} = \frac{.0214 \times 45}{1 - e^{-(.0214 \times 100)}} = \frac{0.96}{1 - e^{-2.14}} = \frac{0.96}{1 - .12} = 1.09$$

$$\text{Tr. 3} = \frac{.0233 \times 107}{1 - e^{-(.0233 \times 100)}} = \frac{2.49}{1 - e^{-2.33}} = \frac{2.49}{1 - .10} = 2.77$$

$$\text{Tr. 4} = \frac{.0246 \times 135}{1 - e^{-(.0246 \times 100)}} = \frac{3.32}{1 - e^{-2.46}} = \frac{3.32}{1 - .09} = 3.65$$

$$\text{Tr. 5} = \frac{.0255 \times 147}{1 - e^{-(.0255 \times 100)}} = \frac{3.75}{1 - e^{-2.55}} = \frac{3.75}{1 - .08} = 4.08$$

Determination of radioactivity in micromicrocuries/gram ash weight, where:

$$\text{uuc./gm.} = \frac{R_0 \times 1000}{2.22 \text{ cpm/uuc.} \times C \times B}$$

where C = 0.5, geometry of internal proportional counter and B = 1.02, alpha backscatter

Tr. 2 = 1.09 x 1000/1.13 = 964.6 uuc./gram ash weight

Tr. 3 = 2.77 x 1000/1.13 = 2,451.3 uuc./gram ash weight

Tr. 4 = 3.65 x 1000/1.13 = 3,230.1 uuc./gram ash weight

Tr. 5 = 4.08 x 1000/1.13 = 3,610.6 uuc./gram ash weight

Appendix E

Results of the radioassay of internal organs with a brief discussion

Table 34. Internal organs radioactivity values in micromicrocuries per gram ash weight from most the experiments. Each value is based upon the combined internal organs ash from 12 fish (3 fish x 4 jars per treatment level). The conversion from counts/minute to micromicrocuries per gram ash followed the techniques of Appendix D[a]

Experimental conditions		Treatment levels in uc. radium/liter water				
		0.0	0.1	0.2	0.3	0.4
Temp.	Time					
50° F.	5 days	53.1	124,449.0	133,851.8	38,028.8	88,515.3
	10 days	53.1	27,884.9	26,207.9	30,646.0	57,860.6
	15 days	53.1	3,650.4	37,904.0	17,778.8	17,674.8
60° F.	5 days	97.6	4,000.0	13,292.2	4,659.2	4,035.3
	10 days	97.6	2,685.8	5,433.6	10,119.4	8,707.9
70° F.	5 days	97.6	Samples destroyed in experimentation			

[a]The absence of a good correlated response pattern of radioactivity in internal organs to radioactivity of water is rather evident. This picture may be truly indicative of the state of flux the internal organs experience while fish are continually immersed in radioactive waters. The lack of consistency plus the variability of the results lends support to such a theory. It is difficult even to envision any type of trend, but two can be vaguely discerned.

Though no logical sequence of activity through treatments at any one time and temperature combination is present, a pattern seems established through time and between temperatures. There appears to be a loss of radioactivity through time at 50° F. The fish at 10 and 15 days have far less radioactivity than the 5 day samples. The radioactivity of fish exposed for 5 and 10 days at 60° F. is considerably less than at the same time periods for fish from 50° F. waters. Many more samples would be necessary in order to confirm either of these trends and give reasons for them.

The inconsistent insufficient data prevents further analysis and discussion.