ACCUMULATION AND ELIMINATION OF HEXAVALENT CHROMIUM IN RAINBOW TROUT

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By

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AN ABSTRACT

Submitted to the School of Science and Arts of Michigan State University of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

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ABSTRACT

Studies of fish toxicity resulting from inorganic metals and their salts have been confined largely to the determination of lethal doses. Little investigational work has been conducted on the effects of sub-lethal levels of such materials. In order to investigate the effects of sub-lethal levels of hexavalent chromium, rainbow trout were exposed to a solution of K_2CrO_4 containing 2.5 mg. Cr/l., part of which was in the form of Cr^{51}. Representative tissues were removed; their chromium content was determined by radiological methods. These studies revealed that hexavalent chromium was accumulated in quantities proportionally exceeding the aquarium water in spleen, liver, posterior gut, pyloric caeca and kidney. With the exception of the spleen, these organs were all capable of excretion, thus it was hypothesized that accumulation in these tissues might be correlated with excretion.

In another study, fish were exposed to 2.5 mg. Cr/l. for 12 days; then returned to clean water from which 4 or 5 fish were removed every 5 days. These fresh water elimination studies showed a rapid decrease of chromium from the blood, liver, stomach, pyloric caeca and posterior gut. These studies also indicated a retention of chromium in the spleen and kidney.

Three fish, given radiochromate via stomach tube, were returned to clean water for one day. Tissues from these fish were examined for Cr^{51}, indicating no appreciable chromium content in organs other than the stomach, pyloric caeca and posterior gut. Esophageally occluded fish exhibited an accumulation of chromium similar to that in non-occluded fish, when both were exposed to 2.5 mg. Cr/l. for 1 day. As a result of these observations, it was hypothesized that the major route of entry of hexavalent chromium was via the gills. Except in one fish, the blood concentration of chromium never surpassed that of the surrounding water, tending to indicate that hexavalent chromium probably crossed the gill membrane as a result of simple diffusion. A very low rate of increase in chromium concentration was noticed in blood, indicating either that the chromium combined into a complex when it entered the blood, or that nucleated fish erythrocytes behaved differently than do mammalian red blood cells.

On the basis of a rapid accumulation of chromium by the pyloric caeca, during the first few days of exposure, followed by a diminution of the rate of accumulation, it was hypothesized that the pyloric caeca, along with bile and kidneys, were a possible route of chromium excretion.

Based on information obtained, fish, caught in chromium contaminated streams not exceeding a concentration of 2.5 mg. Cr/l. of water, would probably not contain appreciable amounts of chromium in muscle tissue.

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To Ella and Bob

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INTRODUCTION

Although there is no known function of chromium in animals, it is present in trace amounts (Grushko, 1948; Van der Walt and Van der Merwe, 1938; Koch et al., 1956; Udy, 1956). Gray and Sterling (1950) reported mean values of chromium in human blood to be 20 µg. per cent for packed red blood cells and 14 µg. per cent for plasma. Similar data for other animals has not been found in the literature.

Chromium, atomic number 24, atomic weight 52.01, occurs in group VI of the Periodic Table, and although there are four naturally occurring isotopes (Cr^{50}, Cr^{52}, Cr^{53}, Cr^{54}), Cr^{52} is by far the most abundant. The following table presents a brief outline of some of the characteristics of the element (Hopkins, 1942).

Valence	Class	Reaction	Representative Salt	Ionic Nature
2	Chromous	Basic	$CrCl_2$	Cation
3	Chromic	Weakly Basic	$CrCl_3$	Cation
3	Chromite	Weakly Acidic	$NaCrO_2$	Anion
6	Chromate	Acidic	Na_2CrO_4	Anion
6	Dichromate	Acidic	$Na_2Cr_2O_7$	Anion

Generally speaking, hexavalent chromium exists in water in true solution, regardless of H^+ concentration or the presence of other ions. Trivalent chromium exists in water

in solution, as a complex, or as a colloidal or flocculent precipitate depending on the pH and dissolved substances.

Chromium compounds containing Cr^{51} have been used clinically in recent years. Beierwaltes et al. (1957) have reviewed procedures proposed for a reticulo-endothelial test, diagnostic test for hemolytic anemias, measurement of red cell volume and half-life, location and quantitation of intestinal bleeding and measurement of plasma volume, all of which utilize Cr^{51} in one form or another.

Gray and Sterling (1950), in a major contribution to chromium literature, showed that mammalian erythrocytes have a marked affinity for anionic chromate. They hypothesized that hexavalent chromium was bound or modified after gaining entry into the red cell. This binding was shown to be essentially irreversible. In addition, they reported that trivalent chromium was bound by albumin and this binding was also essentially irreversible.

Collins (1958), studying chromium excretion in the dog, ascertained that the major excretory route for intravenously injected chromium was via the urine. After a single intravenous injection, renal clearances of $CrCl_3$ decreased exponentially with time. Measurement of dialyzable Cr^{51} in plasma showed that the per cent of chromium not bound to plasma proteins also decreased exponentially with time. Glomerular filtration and tubular reabsorption were two mechanisms involved in the renal handling of unbound chromium. Renal function

was not impaired over a large range of plasma chromium concentrations. *In vivo* reduction of hexavalent chromium occurred. It was indicated that chromium was excreted at least in part in organic combination.

Chromium is of significance as a pollutant to streams, lakes and potable water supplies due to waste disposal by industry. Cr^{51} in low amounts is also present in the effluent of nuclear reactors along with Na^{24}, Cu^{64}, P^{32}, As^{76} and rare earths (Davis and Foster, 1958).

Studies of metal toxicity have been confined largely to the determination of lethal doses, with little investigational work being conducted on the effects of sub-lethal levels of a toxic substance.

In an effort to gain knowledge of possible routes of entry, methods of transport and routes of excretion in rainbow trout, a study of hexavalent chromium distribution patterns was undertaken, using 2.5 mg. Cr/l. Because chromium might be present in the environment of fish, a study of this type was felt to have value.

REVIEW OF LITERATURE

I. Chromium Toxicity Studies

Many papers dealing with the toxicity of both trivalent and hexavalent chromium have been reviewed by Doudoroff and Katz (1953). To date, most of the information has pertained to the determination of acutely toxic levels. Rushton (1922) observed that a 6 hour exposure to 100 mg./l. of $K_2Cr_2O_7$ proved fatal to trout within 12 hours after their return to fresh water. Oshima (1931) stated that the survival time of young eels in a solution of $KCr(SO_4)_2$ averaged 18.7 hours at a concentration of 5.2 mg. Cr/l. Anderson (1944) found that K_2CrO_4 immobilized Daphnia magna in 16 hours at a concentration of less than 0.6 mg./l. The toxicity of K_2CrO_4 and $K_2Cr_2O_7$ to yearling rainbow trout was evaluated by Grindley (1946). He concluded the lower limits of toxicity were slightly below 20 mg. Cr/l. Abegg (1950) studied the effects on bluegills of Na_2CrO_4 and $Na_2Cr_2O_7$. He expressed the 24 hour TLm (median tolerance limit) as 930 mg. of Na_2CrO_4/l. and 788 mg. of $Na_2Cr_2O_7$/l. Abegg also reported a slight but significant increase in the fluid content of muscle and a decrease in the blood specific gravity caused by exposure to $Na_2Cr_2O_7$. LeClerc and Devlaninck (1950), using trivalent chromium in the sulphate form, determined the minimum fatal

concentration for a 6 hour exposure period for minnows in distilled water to be near 40 mg. Cr/l.

Fromm and Schiffman (1958) determined the TLm for largemouth bass to be 195 mg. Cr/l. Under the conditions described, exposure to 94 mg. Cr/l. caused, initially, a slight but not highly significant increase in the oxygen consumption of bass, followed by a gradual decline to 27 per cent below normal after 68 hours exposure. Exposure to chromium caused severe pathological changes in the intestine immediately posterior to the pyloric caeca that in all probability completely destroyed its digestive function. The normal respiratory rhythm of the bass was not observably altered following exposure to chromium; however, coughing movements which occurred at fairly regular intervals were observed. No significant histological change in the gills of fish exposed to chromium were noted. In concentrations that were not acutely lethal, chromium apparently did not kill fish by interfering with the respiratory function of the gill.

Schiffman (1957) reported a 24 hour TLm for rainbow trout as being 100 mg. Cr/l. when using K_2CrO_4. Tracer experiments indicated that the spleen and gall bladder, including bile, accumulated chromium above the level of a section of the caudal peduncle, whereas there was no evidence that kidney or liver did. Weights of kidney, liver, spleen and total body water did not change when the fish

were exposed to K_2CrO_4. The hematocrits of both splenectomized and intact fish were significantly higher than control fish in tap water. This rise in hematocrit was due to an increase in cell number and cell volume. There was no evidence that the plasma or blood volume increased or decreased in fish exposed to K_2CrO_4. The presence of spleen was not necessary to increase the hematocrit under chromium stress. The hematocrit reached its maximum value at a concentration between 2 and 4 mg. Cr/l. Data indicated that the spleen may act as a limited buffer mechanism to sudden hemoconcentration.

Olson and Foster (1956a), studying the effects of low concentrations of $Na_2Cr_2O_7$ on young chinook salmon and rainbow trout, found that the eggs would hatch when placed in the highest concentration used (0.18 mg. Cr/l.). However, survival of the young salmon and trout was affected at 0.08 mg. Cr/l., and growth was possibly retarded in the low concentration of 0.013 mg. Cr/l. They reported the expected weight gain with time demonstrated a diminution as the chromium was increased. In a following study, Olson and Foster (1956b), reported further information regarding the toxicity of hexavalent chromium to chinook salmon. Chronic release of dichromate was slightly more toxic to aquatic life than the same quantity released intermittently. The dichromate appeared to have a greater toxic effect at 41°F. than at 50°F. However, no simple correlation between

temperature and toxicity was indicated. Davis and Foster (1958) conducted detailed studies of radioactivity uptake by different tissues of Columbia River fish, in relation to effluent from the Hanford, Washington reactor. Fish maintained in effluent from the Hanford reactors concentrated Na^{24} in tissues about 130 fold. Direct absorption of other dominant isotopes in the effluent, Cr^{51}, Cu^{64}, P^{32}, As^{76} and rare earths, appeared to be inconsequential. The contribution of food chains to the concentration of radioisotopes in aquatic animals was apparent. Fish collected down-river from the reactors were approximately 100 times as radioactive as laboratory fish that were exposed to equivalent mixtures of the effluent, but fed uncontaminated food.

Koenuma (1956) found that a $5x10^{-4}$ M. sea water solution of uranyl nitrate inhibited the formation of the fertilization membrane in Urechis eggs and led to polyspermy. These findings indicated that in determining the effects of toxic materials more than lethal effects should be considered.

The hardness of the experimental water used also plays an important part in determining lethal doses of some metals. Cairns (1957), using bluegills exposed to zinc, noted a 96 hour TLm to be 2.86 mg. Zn/l. at 18°C. and 1.93 mg. Zn/l. at 30°C. in soft water. In hard water, it was 10.13 mg. Zn/l. at 18°C. and 10.15 mg. Zn/l. at 30°C. This would indicate that temperature as well as hardness is an important factor in toxicity studies.

II. Tissue Uptake of Chromium

Visek et al. (1953), investigating the metabolism of chromium compounds, employed 200 rats sacrificed at intervals of up to 45 days after injection. They also correlated the electrophoretic behavior of each chromium compound present in serum with its tissue distribution. Ninety per cent of the injected $NaCr^{51}O_2$ was taken up by organs of the reticulo-endothelial system. The electrophoretic pattern showed it to be colloidal in serum. The liver and spleen retained 33 and 50 per cent respectively of their 4 day activity at the end of 42 days, whereas the lung showed only 10 per cent of its 4 day activity. After $Cr^{51}Cl_3$ injection, the activity was greatest in the liver, spleen and bone marrow, a reflection of reticulo-endothelial uptake. When $NaCr^{51}O_2$ was given, uptake of radioactivity by these tissues was somewhat less. The activity, once deposited, was removed very slowly. The concentration in the liver at 45 days was 35 per cent of its maximum value observed at 24 hours. All other tissues were practically constant throughout the 45 day period with the exception of blood and lungs. The blood retained practically no activity after the seventh day and the lungs at 45 days retained about 15 per cent of their maximum value, which was observed at 6 hours following injection. The spleen gained activity over the 42 day period. Orally administered $Cr^{51}Cl_3$ was almost totally excreted in the

feces at the end of 4 days. Less than 0.5 per cent of the dose was absorbed from the G.I. tract. Chromic chloride behaved as a colloid under these conditions. The electrophoretic pattern indicated that it was protein bound. Sodium chromate showed a major uptake by the liver, but less than that observed for $Cr^{51}Cl_3$ or $NaCr^{51}O_2$. Twenty-five per cent of the activity was observed in the liver 30 minutes after injection, while less than 1 per cent was present at the end of 42 days. Here again, the spleen seemed to gain activity with time. Skeletal tissues showed relatively small concentrations of chromium. The blood at 21 days had a concentration of approximately 1/5 the 30 minute value, while at 42 days it was practically zero. $Cr^{51}Cl_3$ in acetate (pH 5.5) and citrate (pH 4) buffers followed the same general pattern as non-buffered $Cr^{51}Cl_3$. There was however, a much larger urinary excretion when buffered $Cr^{51}Cl_3$ solutions were administered intravenously. The behavior of $Na_2Cr^{51}O_4$ was "probably ionic" under these conditions. The electrophoretic pattern indicated that it was ionic in serum and bound by hemoglobin in cells.

MacKenzie (1957) fed trivalent and hexavalent chromium to rats in drinking water for a period of one year. The chromium concentration in the water varied from 1 to 25 mg. Cr/l. as chromate ion. He reported no effect on water intake, food consumption or weight gain during the period of exposure. Also, no significant histological changes were

observed in the blood or tissues examined. There was an abrupt rise in retention in kidney, liver, femur and spleen at water concentrations above 5 mg. Cr/l. as chromate ion. Starved rats administered radiochromate via stomach tube absorbed approximately 5.5 per cent of the dose, while non-starved animals absorbed approximately 2.5 per cent. Blood, stomach, intestine, liver, kidney, urine and feces were analyzed. The highest activity in the blood occurred at 6 hours or earlier, whereas, the highest activity in the liver and kidney occurred at 24 hours. In a similar experiment, blood samples were taken 4 hours after administering Na_2CrO_4 or $CrCl_3$ via stomach tube. The starved animals receiving Na_2CrO_4 solution absorbed the most chromium, while the non-starved group given $CrCl_3$ absorbed the least. There was considerable reduction of the chromate ion in the digestive tract. After injecting Na_2CrO_4 and $CrCl_3$ directly into the intestine, much higher blood values were obtained than in the previous experiment, indicating that most of the chromate reduction occurred in the stomach.

III. Accumulation of Fission Products in Fish Tissues

Numerous studies have been conducted on uptake of nuclear fission products by fish. Boroughs et al. (1956a) conducted distribution studies of Sr^{89} in black skipjack, yellowfin tuna, and dolphins. They also conducted studies on the distribution of Yt^{91} in the marine fish Tilapia mossambica

(Boroughs et al., 1956b). Their most recent paper (Boroughs et al., 1957) discussed the uptake of Ca^{45} from artificial sea water containing the same calcium concentration as normal sea water. It was shown that marine fish can take up calcium directly from sea water and also that marine fishes will discriminate against strontium in favor of calcium. Rosenthal (1956) reported a considerable retention of Ca^{45} from water by guppies. Chipman (1956) placed pieces of skin separating two sea waters, one of which contained Sr^{89}, Cs^{137} or Ru^{106}. The diffusion of these isotopes through the tissues under the skin of iced fish was observed after a number of days contact with the skin surface. Cesium entered the fish through the skin very readily. After 4 days contact of the skin with the radioactive solutions, the activity from cesium of the deep muscle tissue was equal to that on the skin surface. On the other hand, after 8 days, the activity of the deep tissue was only 4 per cent of the skin surface activity for Sr^{89} and 1 per cent for Ru^{106}. Chipman et al. (1957) reported studies of metabolism of Zn^{65} by marine fishes. Zn^{65} was pipetted directly into the stomach of croakers, then they were placed in fresh sea water. Zn^{65} rapidly entered the blood stream and accumulated in the kidney, spleen and liver. There appeared to be a rapid loss from the internal organs and a slow and long continued accumulation, although still only a small amount of the administered dose, in the bone and muscle tissues. Welander (1957), studying radioactivity

in reef fishes of Belle Island, Eniwetok Atoll, collected 693 specimens. Skin, muscle, bone, liver and viscera were removed and examined for radioactivity. The greatest per-gram concentration of radioactive materials occurred in the alimentary tract with the liver, skin, bone and muscle having successively lesser amounts.

Following the hydrogen bomb tests at Bikini Atoll in the spring of 1954, radioactive fish began appearing on the Japanese markets. On March 1, 1954, the first of these fish arrived on the Fukuryu Maru No. 5, the ship on which the "Bikini ash" had fallen. On May 31, 1954, the Japanese Ministry of Health and Welfare established an arbitrary maximum permissible contamination of 100 c.p.m. when measured with a G-M tube placed 10 cm. from the skin of the fish. Fishing vessels returning from the general Bikini area were compelled to put in at certain harbors where the entire catch was subjected to scrutiny with a G-M counter. Following this decree, a great deal of scientific research was conducted to test the validity of the 100 c.p.m. maximum permissible level. This and other investigations by Japanese investigators noted below are presented in the two volume issue of the Japan Society for the Promotion of Science entitled "Research in the Effects and Influences of the Nuclear Bomb Test Explosions", 1956.

Saiki et al. (1956) reported radioactivity distributions in the tissues of various marine fish that were collected

from Japanese fish markets. The most radioactive portions were liver, kidney, spleen, pyloric caeca, stomach content, stomach and rectum. The radioactivity of muscles was reported to be low. Amano et al. (1956) reported similar results on studies made on fresh caught Pacific fishes taken by the government research vessel, R. V. Shunkotsu Maru. His group also attempted to chemically analyze the contaminants by separating them into five fractions following the procedures of qualitative chemical analysis. They stated that the major contaminant in white muscle was Zn^{65}. Kawabata (1956), calculating decay rates and absorption curves, along with chemical fractionation, determined the principal contaminant in white muscle to be Zn^{65} with other unidentified isotopes. Nagasawa et al. (1956) reported some of the unknown activity in white muscle to be due to Sr^{90}.

Yoshii (1956) conducted electron diffraction studies on the first vertebrae of Katsuwonus vagans. Only the patterns of $Ca_3(PO_4)_2$ and $CaCO_3$ were indicated. This same investigator conducted radioautographic studies that led to the following conclusions:

Stomach wall: In the tunic mucosa, radioactive substances are distributed from the mid-portion to the basal membrane, around lymphatic cavities and the vessels.

Intestinal wall: Radioactive substances are strongly localized in the walls of vessels and lymphatic cavities of the mucous membrane. They were also distributed along the

margins of muscle fibers.

Muscle: Radioactive substances were distributed along the margin of muscle fibers and the muscle bundles, and clearly on the dark zone of the striated muscles.

Skin: Most of the activity was concentrated in the connective tissue of the epithelium.

Testis: The activity was uniformly distributed around the circumference of the spermatocyte.

Gill: Activity was located on the gill filaments.

Liver: Radioactive substances were abundantly distributed in the liver cell strings.

Atlas: The deposition of radioactive substances was marked inside of the atlas and on the spinal cord.

Saeki and Sano (1956) placed goldfish in solutions of "Bikini ash". Tissue examination showed the gill, fins, and digestive tract, with contents, to have an activity exceeding that of the water in which the fish were placed. They reported skin and muscle as showing no activity. In fresh water experiments on fish previously exposed to the radioactive solution, it was reported that goldfish did not lose the absorbed radioactivity any faster than could be attributed to decay alone.

Hiyama et al. (1956), in laboratory studies, demonstrated that fresh water fish absorbed fission products faster than did salt water species. Inorganic contaminants placed directly in the stomach were almost unabsorbed, while contaminated

food was taken up more readily. (Note the food was contaminated Artemia).

Suyehiro et al. (1956) placed Japanese killfish in 10% physiological saline solution containing Sr^{90}. They reported that the uptake of this isotope was in proportion to the strength of the Sr^{90} solution. Goldfish were placed into water containing 10,000 c.p.m./l. Whole fish counts indicated an increasing accumulation trend till near the tenth day at which time the absorption rate tapered off. They also showed a correlation between the count of gills, liver and serum. In the carp, eel, goldfish, Labyrinth fish and conger eel, there was little difference in the distribution of radioactive material.

Ichikawa and Hiyama (1956), studying strontium, reported that a great part of the absorbed isotope was deposited on hard tissues such as scales, vertebrae, fin and gill, behaving in a similar manner to calcium.

Tomiyama et al. (1956a) conducted experiments to determine the route of calcium uptake from the surrounding water medium. Upon placing goldfish in water containing Ca^{45} and sacrificing, followed by counting separate tissues, it was noted that the gill exhibited the greatest activity, followed by the scales. The next series of experiments consisted of placing goldfish in small glass chambers. A rubber diaphragm isolated the anterior portion of the chamber and the fish from the posterior portion. The anterior portion of the

fish was then exposed to a concentration of 125 mg. Ca/l. The posterior portion was exposed to well water with no Ca^{45} ion. After an 18 hour exposure, the fish was removed and another placed in the apparatus, with the posterior portion being exposed to Ca^{45}. It was demonstrated that when the anterior portion only was exposed to calcium, a high uptake took place while very little uptake was observed when the posterior portion only of the fish was exposed. This group postulated that the gill is the most likely path for accumulation of calcium. Absorption of Ca^{45} via the water medium versus that taken up through food by goldfish was also compared by this group (Tomiyama et al., 1956b). Irrespective of different routes for Ca^{45} uptake, the deposition pattern was similar except in the intestine. This same group, Tomiyama et al. (1956c), studying marine fishes, noted a high concentration of Ca^{45} in the intestine, suggesting an uptake of radiocalcium by the intestine as well as the gill. The route of absorption of Sr^{90} was studied by placing carp in a diaphragm type of apparatus to separate the anterior from the posterior end of the fish and by occluding the esophagus (Tomiyama et al., 1956d). It was demonstrated that Sr^{90} was absorbed primarily through the gill, to a small extent through the skin, and not absorbed in a measureable amount through the digestive tract. Except for the muscle and gall bladder, activity was detected in tissues studied after as little as 30 minutes exposure. The deposition

pattern was similar to that of Ca^{45}. Sr^{90} administered intramuscularly (Tomiyama et al., 1956e) showed the same distribution as Sr^{90} taken up from the surrounding water. This group, Tomiyama et al. (1956f), also reported that $P^{32}O_4$ ion was readily absorbed by carp from the surrounding water. The absorbed P^{32} was distributed in the gill, caudal fin and scales similarly to Sr^{90} and Ca^{45}. In contrast to Sr^{90}, the blood, heart, liver and kidney were found to be high in P^{32}. The deposition pattern of P^{32} administered intramuscularly to carp was similar to the absorption of the isotope from the surrounding water (Tomiyama et al., 1956g).

Hibiya and Yagi (1956) exposed fish eggs of different species in various stages of development to different concentrations of ash taken from the deck of the Fukuryu-Maru. Japanese killfish eggs were killed by placing them into a concentration of ash that counted 62 c.p.m./cc.

Mikami et al. (1956) studied the effects of different concentrations of the radioactivity found in rainwater on the hatching success of zebra fish. When the activity was less than 4.2×10^{-4} μc./l., the activity found in the original rainwater, survival was not affected, but the mortality increased considerably when the medium exceeded 21×10^{-4} μc./l. Total mortality occurred when the concentration reached 150 to 210 $\times 10^{-4}$ μc./l.

Suyehiro and Hibiya (1956) injected Sr^{90} into goldfish and then removed three fish, for blood study, after varying

periods in fresh water. Blood was removed from the caudal artery. Slides were prepared using a variety of stains. A count was then made of all cells observed. A ratio obtained by dividing the number of erythrocytes into the number of leucocytes decreased with time after the first two days of injection, however, the ratios exhibited considerable variability.

METHODS

I. Accumulations of Hexavalent Chromium

Procurement and Holding Facilities

Rainbow trout, Salmo gairdnerii, obtained from the Michigan Department of Conservation Wolf Lake Hatchery, were used in this study. The fish were transported to the laboratory, in one hundred lot quantities in two double thickness plastic bags, each containing 50 fish.

Fish were held in four 100 liter glass aquaria under constant illumination at 14° ± 2°C. in a constant temperature room. No more than 35 fish were maintained in each aquarium. Aged tap water with the following properties was used: total hardness (Schwarzenback titration) 334 mg. of $CaCO_3$ per liter and pH of 8.5 to 8.8. The fish in each holding aquarium were transferred into clean, aged water once every three days. This coupled with aeration via a stone air-breaker and filtration of the water through a gravel, glass wool, charcoal filter made it possible to maintain the fish in a healthy condition. Fish in the holding aquaria were fed dried trout pellets, also obtained from the Michigan Department of Conservation. One half hour after feeding, excreta and uneaten pellets were siphoned off the bottom of the holding aquaria.

Experimental Aquaria

The experimental aquaria were kept under the same conditions as the holding facilities. The water volume in each experimental aquarium was established and maintained at 40 liters. These tanks were aerated and filtered through a gravel, glass wool, gravel complex. Schiffman (1957) determined that such a system would not remove dissolved chromium or hardness from the aquarium water.

The fish were exposed to approximately 2.5 mg./l. of chromium in the form of K_2CrO_4. Schiffman (1957) reported a rise in the hematocrit of fish exposed to potassium chromate solutions. He stated that "The hematocrit reached its maximum rise in value at a point between 2 and 4 mg. Cr/l." The 2.5 mg. Cr/l. concentration was believed to represent the near maximum tolerable level in which fish could live for extended periods of time.

A standard solution of K_2CrO_4 containing 10 mg. Cr/ml. was used in preparing the experimental aquaria. One millicurie of Cr^{51} was also added to the 40 liters of water in each aquarium. This isotope, supplied by the National Laboratory, Oak Ridge, Tennessee in the form of $CrCl_3$, was converted to hexavalent Na_2CrO_4 before adding to the aquaria. A sample of the aquaria water was then analyzed chemically by the Saltzman method in order to determine the chromium content more exactly. Both ash and non-ash procedures were run periodically in an effort to detect any valence change

of the chromium in the water, and data indicated that no change occurred.

Ten fish were placed in each experimental aquarium. These fish were removed at the end of the desired exposure period, the aquarium was allowed to stand for at least 24 hours, then 10 more fish were placed in the aquarium to be exposed for a subsequent period. Three aquaria were used in this manner. Fish were not fed while in the experimental aquaria; for this reason the study was to be conducted either until maximum uptake was established or starvation threatened to be an influencing factor in the results. The decision against feeding was based on the observation that feeding trout pellets tended to cloud the water. An increased biological oxygen demand (B.O.D.) coupled with decomposition of uneaten pellets or excreta could add to the toxicity of the water.

Tissues Studied

A preliminary study was conducted in an effort to establish chromium concentrating organs. Fish were exposed to hexavalent chromium containing Cr^{51}, and all accessible organs were removed, weighed and counted. The tissues studied included brain, gonads, urinary bladder, air bladder, heart, kidney, fin, liver, gill, eye, muscle, skin, stomach, pyloric caeca, posterior gut with contents, posterior gut washed, spleen, gall bladder with bile, blood, urine and

bone. The kidney, liver, blood, stomach, pyloric caeca, and posterior gut warranted further study as possible chromium concentrating organs. The posterior gut with contents was weighed and counted in the final experiment. Although initially the spleen showed little activity, Schiffman (1957) reported it as exhibiting a concentrating ability for chromium and it was retained for this reason. The gall bladder was not counted, since the rate of bile flow would have to be determined in order to quantitate any such information. Although it showed no significant activity, muscle tissue was counted routinely for purposes of comparison. A thorough study was made of muscle because of its use as a food source.

Preparation of Tissue

After exposure to chromium, the fish were removed singly, washed in running tap water and anesthesized with MS-222 (300 mg./l.) (tricaine methane sulfonate - Sandoz). When fully immobilized, the fish were blotted dry with a towel, weighed and measured. Blood was removed by heart puncture using a heparinized syringe fitted with a 27 gauge needle. The blood was expressed into an aluminum planchet and weighed. The remainder of the tissues were then removed in the following order: spleen, liver, gut, pyloric caeca, stomach, kidney and muscle. The total extraction process involved approximately 30 minutes per fish. With the exception of blood, all tissues were weighed using a Roller-

Smith balance having a maximum capacity of 500 mg. All weights refer to wet weights. After weighing, the tissues were placed in previously unused aluminum planchets. All samples were then dusted lightly with pyrethrum, as a deterrent to ants, and allowed to air dry before counting.

Counting Equipment

The counting equipment consisted of the following components: a two inch thallium treated sodium iodide scintillation detector assembly, model DS5, housed in a lead castle, model 3036, a radiation analyzer, model 1810, and a model 183 scaling unit, all manufactured by the Nuclear Instrument and Chemical Corporation of Chicago, Illinois. Use of the radiation analyzer enabled differentiation against all energy levels except that to which it was set. This instrument, with the window width set at 5 volts, was adjusted to produce a maximum count from a Cr^{51} standard. The efficiency of the counting equipment was maintained at a constant level during the total counting period. Using the Cr^{51} standard as a source, the equipment was first adjusted for peak counting efficiency. The standard was counted twice daily during the entire counting period. If any significant loss in efficiency occurred, the equipment was readjusted. All tissues were counted at constant geometry.

Feasibility of Counting Whole Tissues

Chromium-51 is a low energy gamma emitter (Comar, 1955).

As a result, the possibility of counted activity being affected by mass differences in the samples was investigated. Absorption of Cr^{51} gamma rays by water and lead was studied. In the study of absorption by water, a tall form, 100 ml. glass beaker was suspended by the flared lip from the top shelf of the lead castle. The Cr^{51} standard was placed underneath the beaker with a lead collimator placed between and a count was made. The beaker was then removed, weighed and 10 ml. of distilled water were pipetted into it; it was reweighed and counted again. This procedure was continued in 10 ml. steps until 100 ml. of water had been used as an absorber. The average inside diameter of the beaker was determined and the results were expressed graphically in Figure 1, as the log of activity versus absorber thickness in g./cm.^{2} of water. Absorption of gamma radiation by lead was studied. Varying thicknesses of the absorber material were placed between the Cr^{51} sample and the scintillator tube. These data were expressed graphically in Figure 2, as the log of activity versus absorber density in g./cm.^{2}.

Absorption of radiation by water is near that of tissue for the gamma energy of chromium, 0.32 Mev (Hine and Brownell, 1956). As a result, there would be little loss by counting whole tissue of the size extracted in this study. Four hundred mg./cm.^{2} of water absorbed approximately 3 per cent of the activity. This weight corresponded to some of the larger samples counted during the course of experimentation.

Figure 1. Absorption of 0.32 Mev gamma energy by water using a Cr^{51} source.

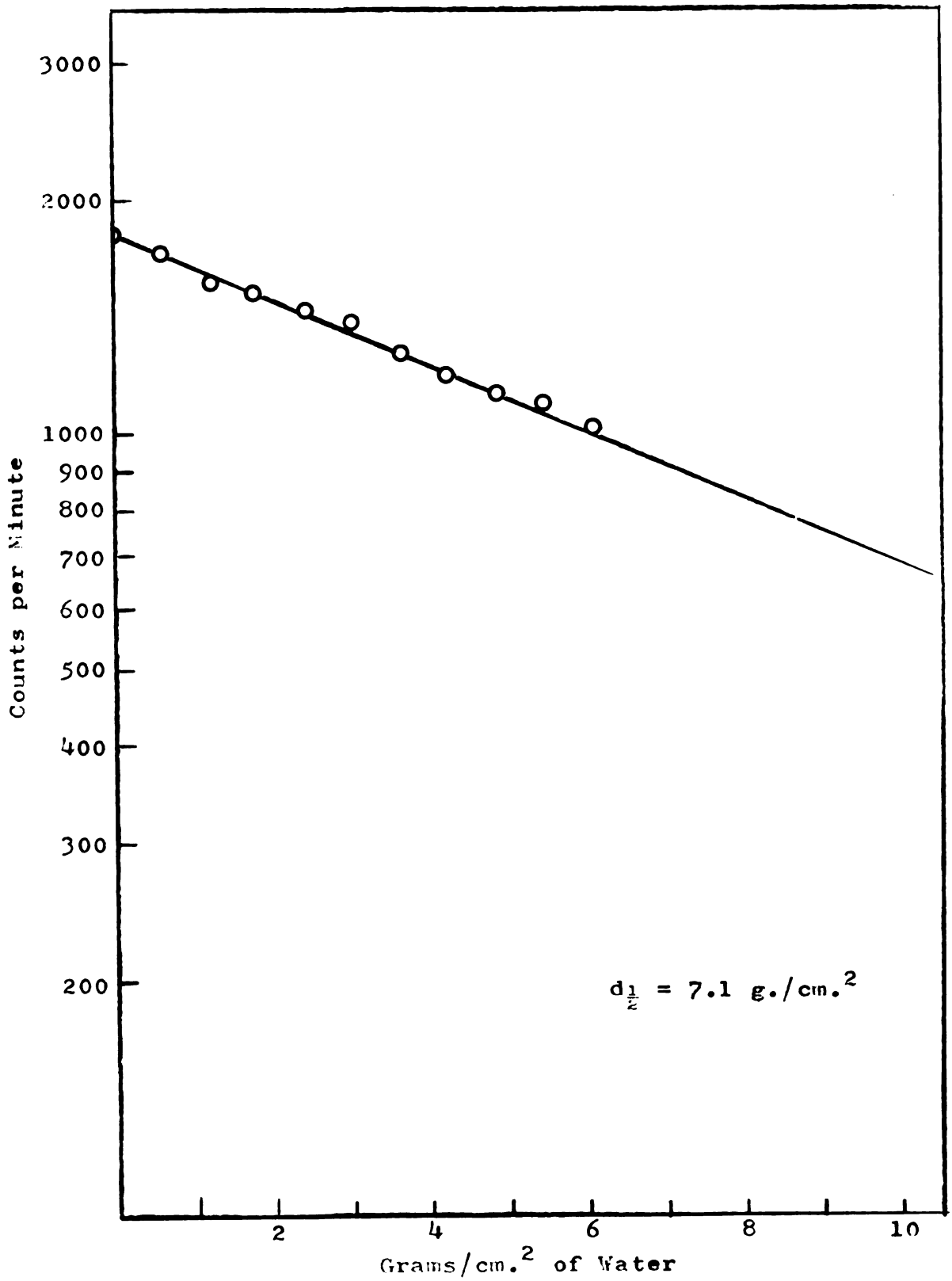
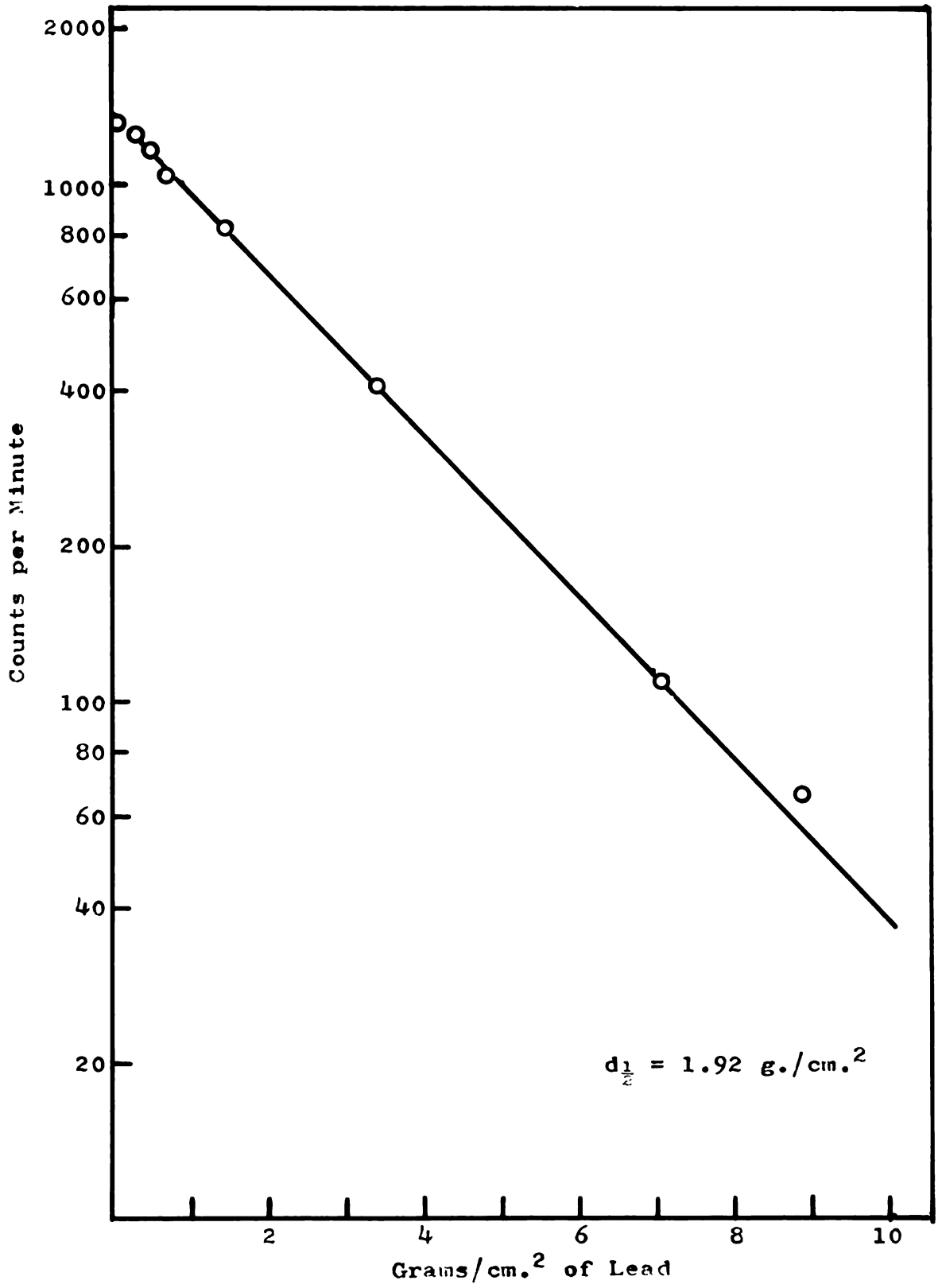


Figure 2. Absorption of 0.32 Mev gamma energy by lead using a Cr^{51} source.



Expression of Data

For standardization purposes, all data were expressed as μg. Cr/g. tissue. This was accomplished by counting a measured sample of the experimental aquarium water in approximately the same geometry as the tissues were counted. With the chromium content of the water having been previously determined, it was possible to express the water activity as being equivalent to a particular concentration of chromium.

$$\text{Activity of 1 } \mu\text{g. Cr} = \frac{\text{Activity of } H_2O \text{ minus background}}{(\text{ml. } H_2O \text{ counted})\ (\mu\text{g. Cr/ml.})}$$

The time of counting was recorded for each sample and proper decay corrections were introduced into all calculations.

II. Elimination of Hexavalent Chromium from Tissues upon Return of the Fish to Fresh Water

General procedures were the same, for this experiment, as were outlined in the chromium accumulation study. Fish were exposed to 2.5 mg./l. of $K_2Cr^{51}O_4$ for 12 days, after which time they were returned to non-chromated water. Four or five fish were sacrificed every 5 days during a 25 day period. This study was conducted to determine whether or not hexavalent chromium, once accumulated, could be eliminated readily by various organs. The removal and counting techniques were the same as those followed in the accumulation study.

III. Administration of Hexavalent Chromium Via Stomach Tube

Three fish were given, via stomach tube, .1 ml. of a solution containing 2.5 mg. $K_2Cr^{51}O_4$/1. They were then returned to normal aquarium water for one day, after which time they were sacrificed. Tissues were removed and counted following procedures described previously.

IV. Hexavalent Chromium Concentrations in Fish with Esophageal Occlusions

Small corks were adapted, for esophageal occlusion, by changing their shapes and attaching 2 small barbs to prevent their expulsion by the fish. With the aid of forceps, the cork was placed into the esophagus of an anesthetized fish. The fish was then placed into an aquarium containing 2.5 mg. Cr/1. for 1 day. Five fish were prepared in this manner. After 1 day of exposure, the fish were sacrificed. The procedures followed in the removal and counting of tissues were the same as those followed in the previous studies.

RESULTS

I. Accumulations of Hexavalent Chromium

Mean concentrations of chromium, in μg. Cr/g. tissue, were plotted versus exposure time in days. Open circles of Figures 3 through 9 represent these mean values. When amenable, linear regression lines $\hat{Y} = a + bX$, with the corresponding .95 two-tailed 100 P% confidence belts for the amount of chromium present in a fish after X days of exposure, were plotted. In Appendix A are found the data from which this series of graphs was constructed.

Blood (Figure 3)

Statistical treatment of the 85 individual observations on blood indicated the possibility of a linear regression during the time of study. The slope, 0.05, was significantly different from zero at the .95 limit of confidence ($r = 0.58$). With the exception of 1 fish, the chromium concentration in blood, although exhibiting considerable variability, never exceeded the concentration of the surrounding water.

Spleen (Figure 4)

The spleen exhibited an increased accumulation of chromium with time. This increase followed a linear regression

pattern, the slope of which was significantly different from zero, at the .95 level of confidence (b = 0.19, r = 0.77). Due to a very low R_S/R_B ratio (sample activity/background activity), mean values for the first 4 days, although included in Figure 4, were not statistically significant at a 5 per cent level of counting error. As a result, these particular values should not be considered as significant. The concentration of chromium in the spleen, as indicated by the mean regression line, exceeded that of the water medium at 14 days.

Liver (Figure 5)

Chromium concentration in the liver increased with time. The average concentration, as indicated by the mean regression line $\hat{Y} = -0.33 + 0.42X$, surpassed the average concentration of the aquarium water by the seventh day. Observations on the seventeenth and twenty-fourth days exhibited considerable variability.

Posterior Portion of Gut (Figure 6)

Chromium levels in the posterior gut rose rapidly during the first 4 days to an estimated level of 6 μg./g., after which they tended to taper off somewhat. This was plotted as a linear regression although, by inspection, a curving line, guided by the plotted mean values, might also have applied.

Pyloric Caeca (Figure 7)

This organ accumulated chromium rapidly, reaching a concentration above that of the aquarium water in slightly over 2 days time. This rapid uptake continued until about the tenth day, when the accumulation reached a value of approximately 12.5 μg. Cr/g. tissue. After 10 days exposure, the rate of accumulation leveled off considerably, reaching a value near 15.5 μg. at 24 days. This information was not plotted as a linear regression line, but rather as a tapering curve in Figure 7.

Stomach (Figure 8)

The stomach also exhibited a linear type of concentrating ability for chromium. The concentration of chromium in the stomach surpassed that of the aquarium water after 11 days exposure. The mean regression line derived from the formula $\hat{Y} = 0.24 + 0.20X$ is shown in Figure 8 ($r = 0.78$).

Kidney (Figure 9)

By the fifth day of exposure, the chromium level in the kidney exceeded that of the surrounding water. From the standpoint of radioactive analyses, kidney tissues were ideal in that they coupled a relatively high degree of activity with a very uniform degree of geometry. These organs presented the perplexing problem of a high degree of variability with time, coupled with relatively low standard errors for the daily mean values. Consequently, no fitted line was attempted.

Figure 3. Chromium Accumulation and Elimination.

Blood

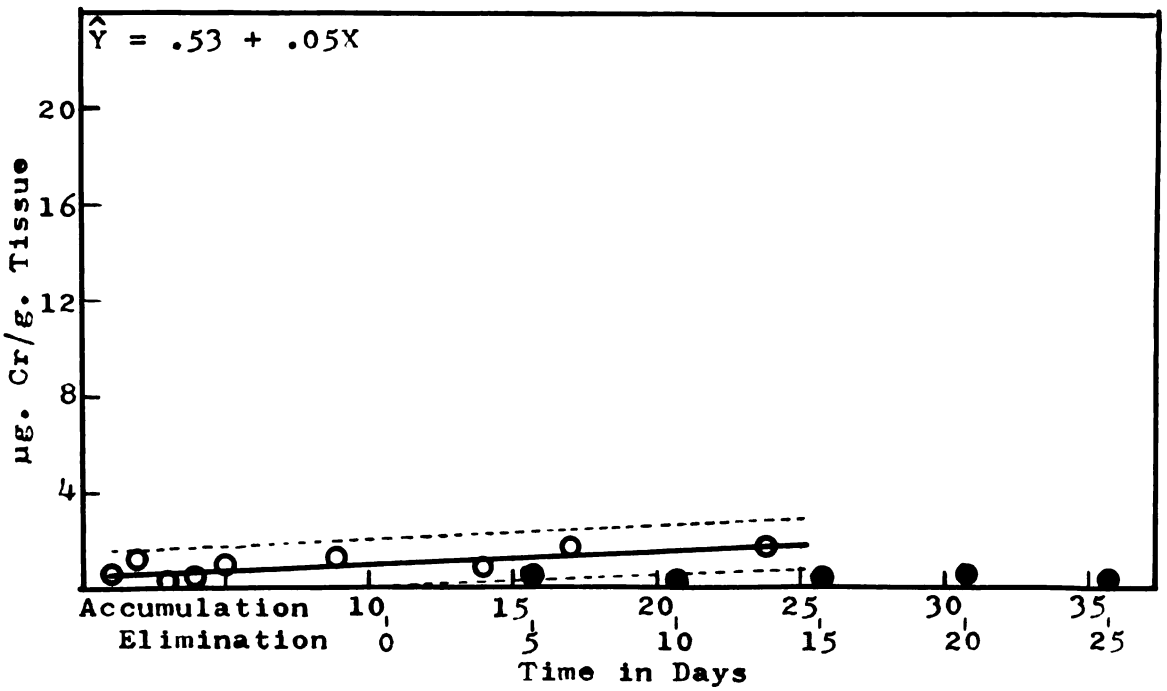


Figure 4. Chromium Accumulation and Elimination.

Spleen

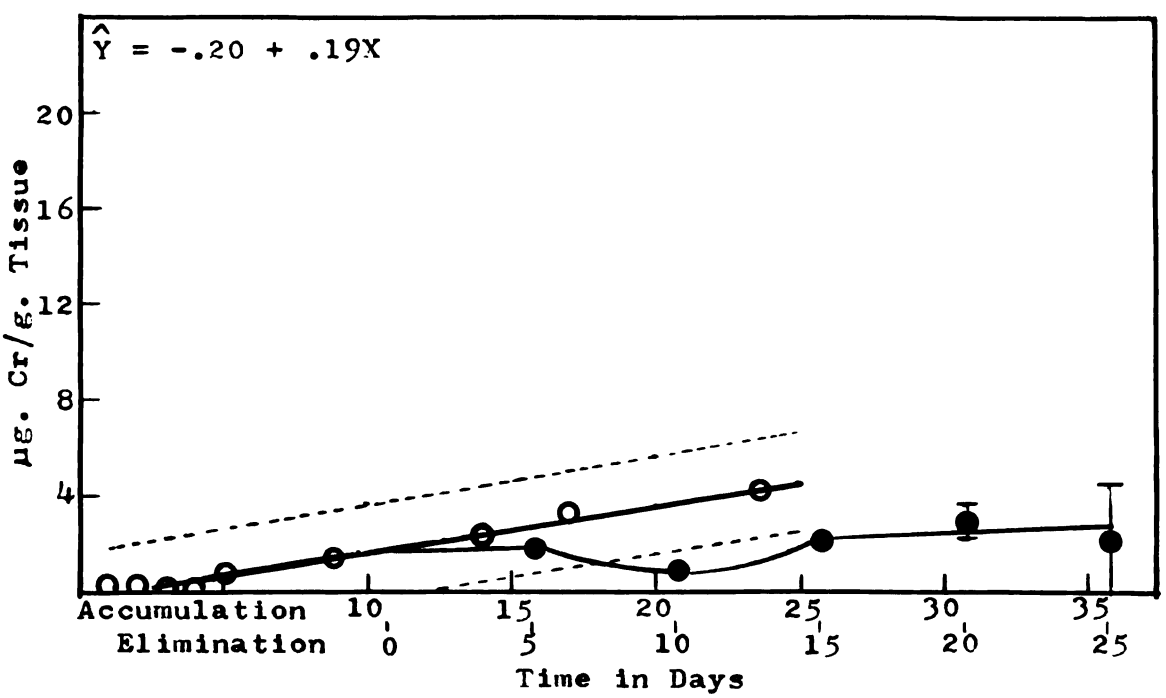


Figure 5. Chromium Accumulation and Elimination.

Liver

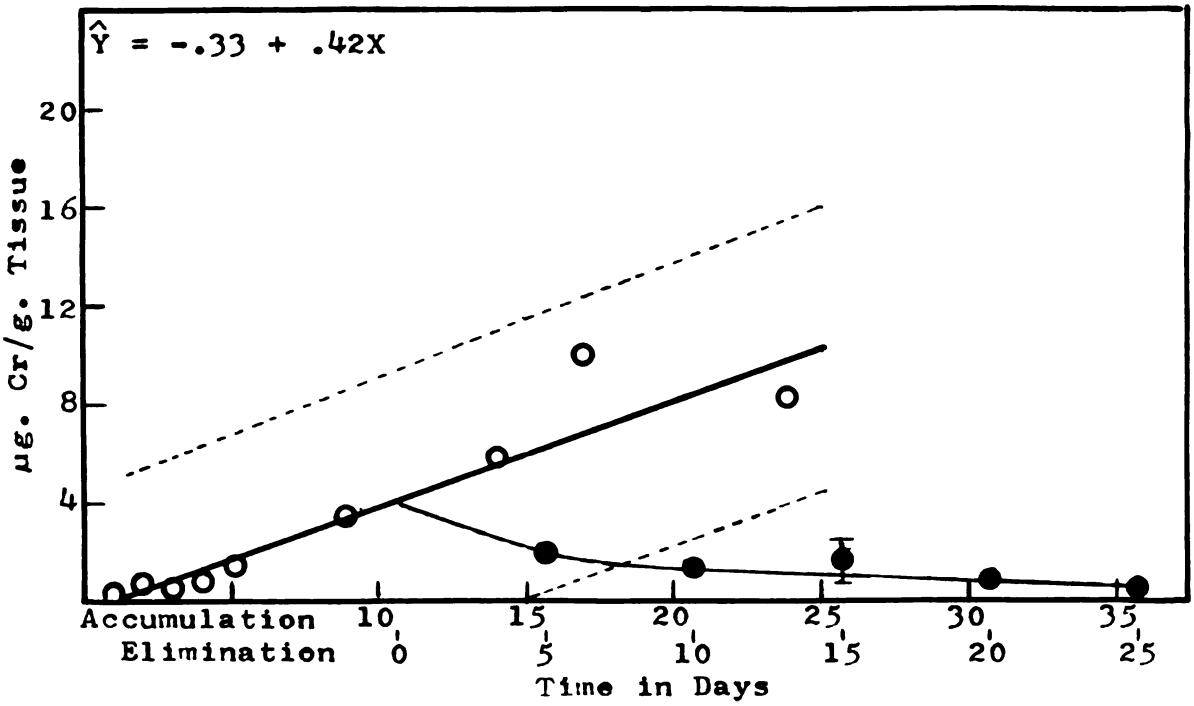


Figure 6. Chromium Accumulation and Elimination.

Posterior Gut

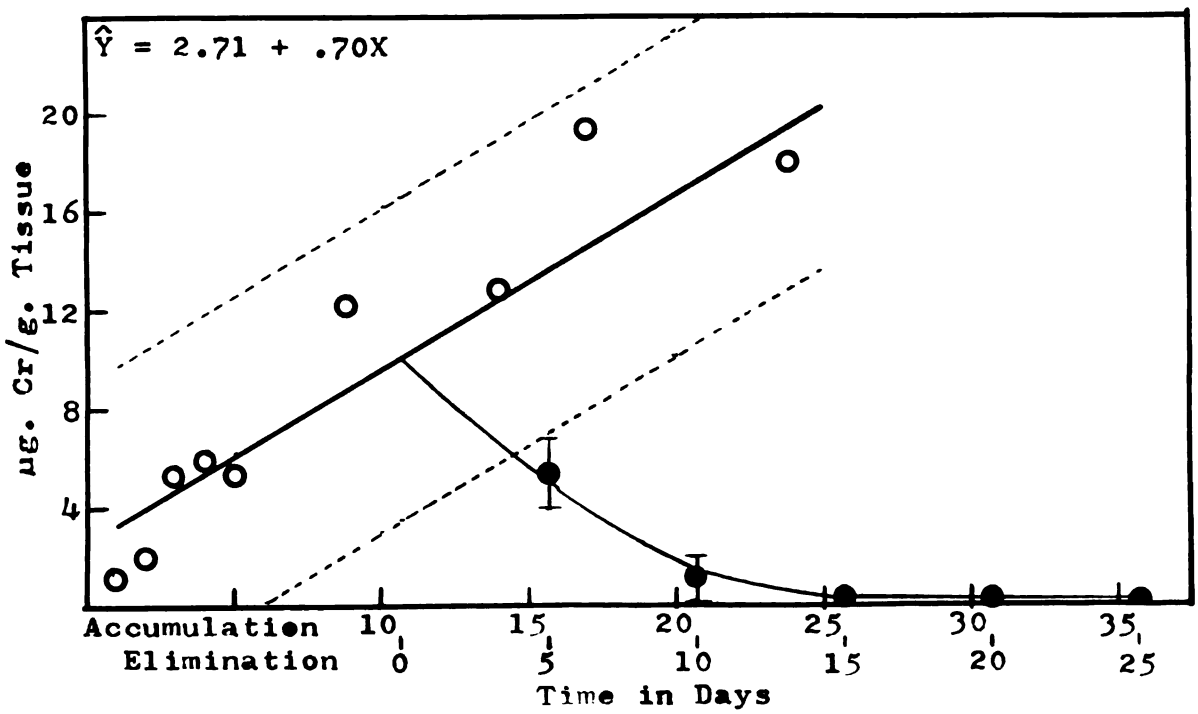


Figure 7. Chromium Accumulation and Elimination.

Pyloric Caeca

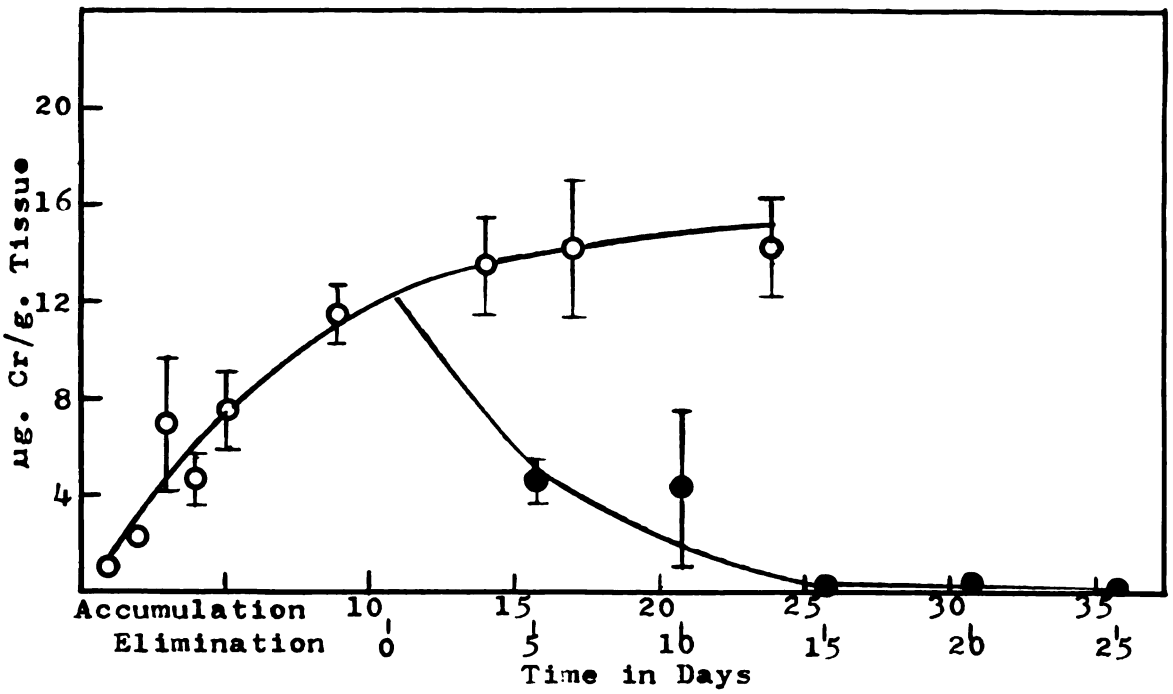


Figure 8. Chromium Accumulation and Elimination.

Stomach

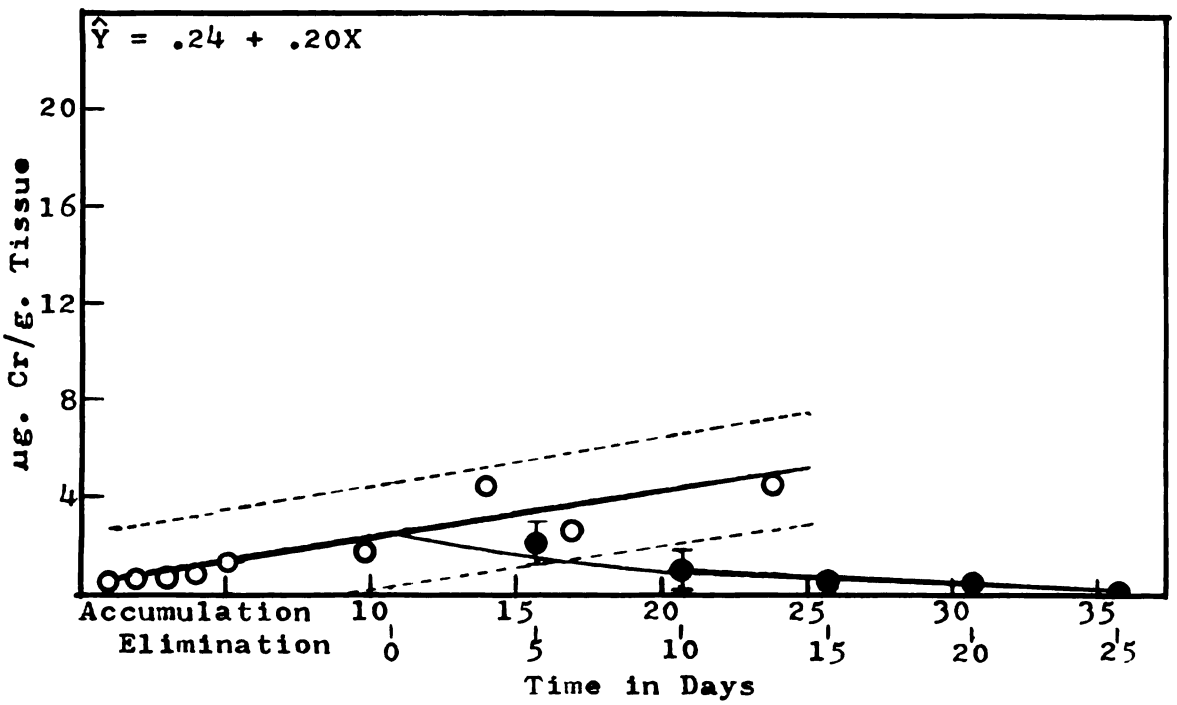
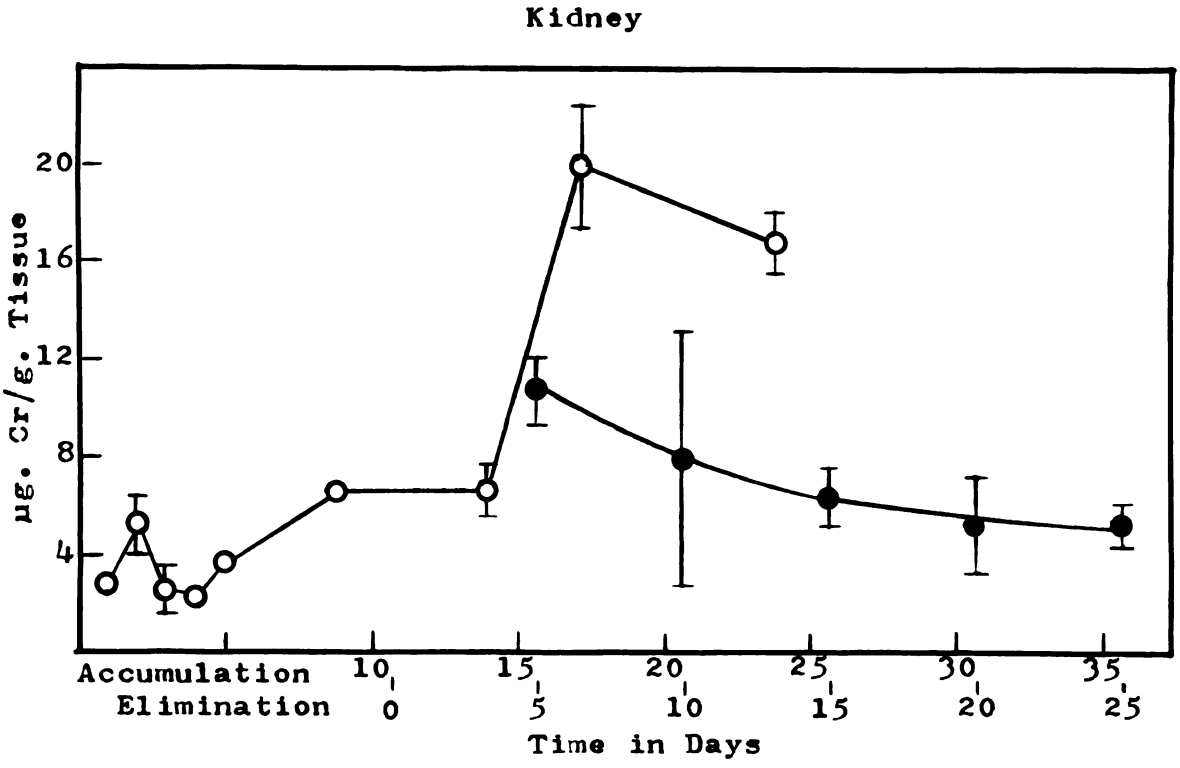


Figure 9. Chromium Accumulation and Elimination.



Muscle

It was concluded on the basis of 86 individual samples that muscle did not accumulate chromium in appreciable amounts during the course of this study. Due to a rather consistent count slightly above background, the concentration of chromium in µg./g. muscle was calculated. These data are presented in Appendix A, however, it should be stated that they are of questionable value, except to indicate that chromium does not accumulate in muscle to any appreciable extent.

II. Elimination of Hexavalent Chromium from Tissues upon Return of the Fish to Fresh Water

These data, along with the associated standard errors, are presented graphically in Figures 3 through 9 as shaded circles. Table 1, in which the per cent of graph estimated initial values is expressed, summarizes these results. The elimination of chromium was rapid in most tissues, the spleen and kidney being notable exceptions. The former lost little, if any, of its chromium content, even after the fish had been in fresh water for 25 days. Although the kidney retained only 50 per cent of its estimated initial activity at the end of 25 days, a smooth curve drawn through the mean values tended to become asymptotic, starting at 20 days, to the Y axis near a value of 5 µg. Cr/g. kidney.

TABLE 1

PER CENT OF TOTAL ACTIVITY REMAINING IN CHROMATE EXPOSED FISH AFTER RETURN TO CLEAN WATER

Days in Fresh Water	ORGAN						
	BLOOD	SPLEEN	LIVER	POST. GUT	PYLORIC CAECA	STOMACH	KIDNEY
5	46	88	40	49	34	80	102
10	28	39	29	11	32	42	73
15	37	99	38	3	2	22	60
20	43	136	17	3	3	20	51
25	25	95	10	2	1	5	50

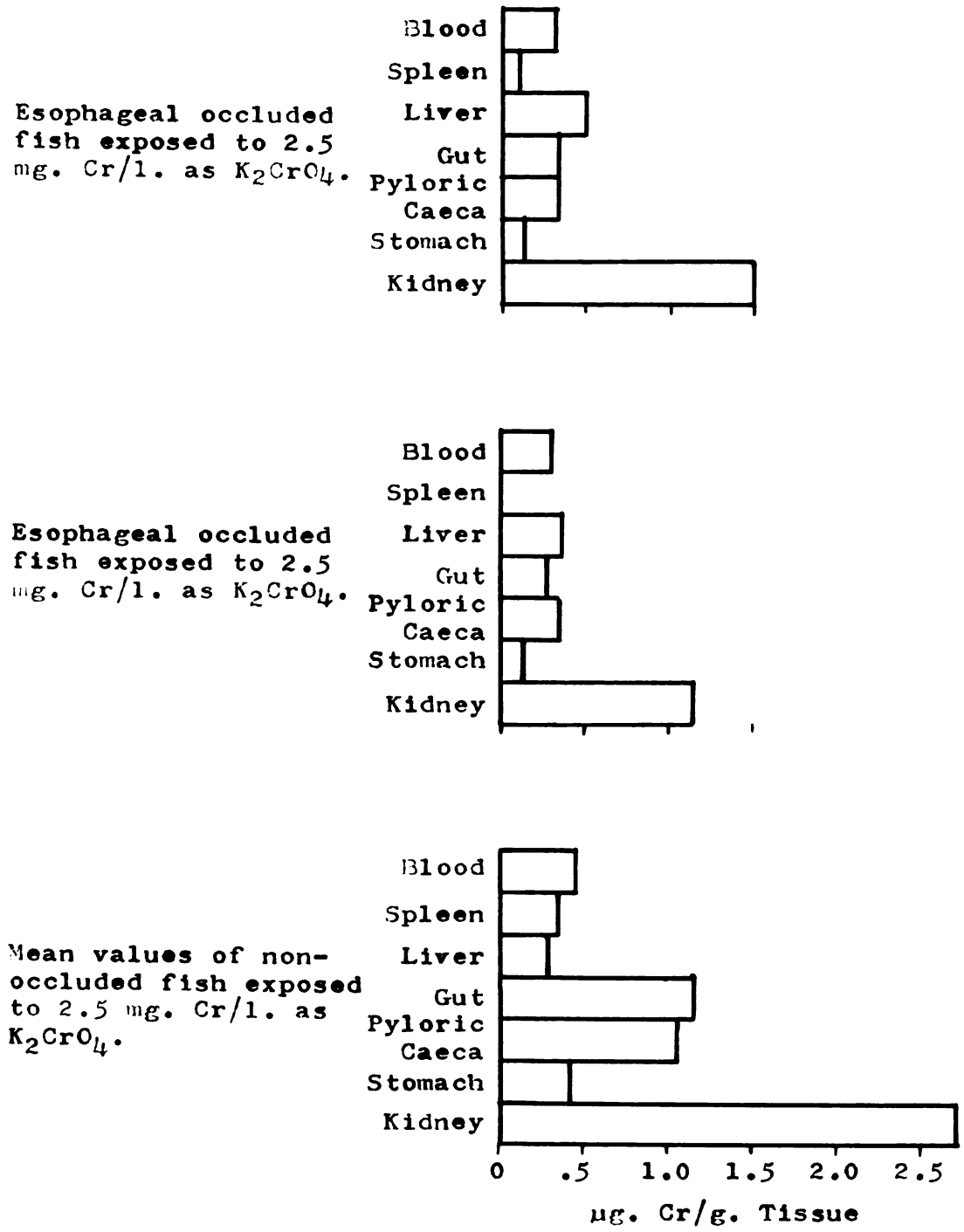
III. Elimination of Hexavalent Chromium Administered via Stomach Tube

Over one-half of the dose administered via stomach tube was eliminated within a 24 hour period, with no appreciable concentrations noted in any organs other than those of the digestive system. The greatest per cent administered dose of chromium, with the exception of the digestive system, was found in the liver (0.05 ± 0.02 per cent). This was followed by the kidney (0.04 ± 0.01 per cent) and blood (0.03 ± 0.01 per cent). The pertinent data are presented in Appendix C.

IV. Hexavalent Chromium Concentrations in Fish with Esophageal Occlusions

Although 5 fish were treated, the occlusion proved satisfactory in only 2. The 2 successful fish are graphed individually in Figure 10. The greatest activity was noted

Figure 10. Two Fish with Esophageal Occlusion Compared with Mean 24 Hour Values for Normal Fish.



in the kidney, followed by the liver. The gut, pyloric caeca and blood were all approximately the same value. These results compared favorably with normal fish exposed to hexavalent chromium over the same period of time.

DISCUSSION

I. Possible Entry Routes of Hexavalent Chromium

In fish, three avenues of entry are available to hexavalent chromium: absorption through the skin, ingestion into the stomach, or entry across the gill membrane.

The possibility of chromium gaining entrance via the skin would tend to be discredited, due to a consistently low muscle concentration of chromium, even when samples were taken adjacent to the skin surface.

Radiochromate, placed directly into the stomach, failed to be absorbed in significant amounts, thus tending to discredit the theory of uptake via the digestive system. Another factor opposing uptake via the digestive system is the theory that fresh water fishes do not drink appreciable amounts of water, there being a considerable osmotic force tending to drive water into the animal (Smith, 1951).

Tissue distributions attained by the esophageal occluded fish compared favorably with those of normal fish exposed to hexavalent chromium. With these observations in mind, it is postulated that the gill is the most likely path for the entrance of hexavalent chromium. Tomiyama *et al.* (1956) reported Ca^{45} uptake in goldfish, and Sr^{90} uptake in carp, as having taken place via the gills.

II. Transport of Hexavalent Chromium in the Fish

As previously mentioned, chromium levels in the blood rarely exceeded, and were for the most part, considerably lower than the level of the surrounding water. As a result of this concentration gradient, entry of chromium into the fish would be possible by diffusion across the gill membrane. The linear accumulation patterns with time would also support this supposition.

Gray and Sterling (1950), after injecting anionic Cr^{51} into dogs, noted that approximately 85 per cent of the activity was concentrated in the erythrocytes within 1 day. The lack of a substantial increase of activity with time, in fish, indicated either that chromium was tied up in a complex, and thus was not free to bind, or that the nucleated fish erythrocytes behaved differently than non-nucleated mammalian red blood cells. This latter point will be explored, by further research, in the near future.

Regardless of the method, it may be postulated that chromium is transported by the blood, from which it is removed by certain organs in the digestive, excretory and reticulo-endothelial systems.

III. Hexavalent Chromium Accumulation in Individual Organs

Fresh water elimination studies showed that blood, liver, stomach, pyloric caeca and posterior gut lost activity rapidly.

This would tend to indicate that hexavalent chromium did not bind to blood in appreciable amounts, that the liver could excrete chromium via bile, and that the stomach, pyloric caeca and gut could secrete chromium into the lumen, or that it was already in the lumen. It was noted that there was a substantial bile flow, along with a full gall bladder, in most of the fish dissected. Bile probably carries chromium into the lumen of the digestive system, to be eliminated via the anal route. The possibility of some chromium elimination via the gill also exists when the fish is placed in fresh water.

Kidney and spleen did not lose activity rapidly, upon return of the fish to fresh water. The spleen exhibited no significant decrease, even after the fish had been in fresh water for 25 days. Visek et al. (1953) reported an increase in spleen activity in rats with time, after a single injection of $Na_2Cr^{51}O_4$. Expressing their values as per cent of dose per gram of fresh tissue, they reported 0.91 $\pm$ 0.16 per cent at 4 days after injection, and 4.8 $\pm$ 1.6 per cent at 42 days after injection. This accumulation could be due possibly to phagocytosis where the chromium was then bound in the macrophages. The significant linear regression for blood, even though the slope was only +0.05 ($r = 0.58$, $N = 85$), indicates the probability of some erythrocytic binding of chromium. The delay in splenic uptake could well be explained by phagocytosis of aged or defective erythrocytes.

Kidney retention was an estimated 50 per cent of the 12 day accumulated value, after 25 days in fresh water, with indications of little further diminution. Collins (1958), studying excretion in the dog, postulated that glomerular filtration and tubular reabsorption were two methods involved in the renal handling of unbound chromium. If these methods applied to fish having glomerular kidneys, there is a possibility that bound chromium in the blood is bound to a small enough molecule, so as to pass into the renal tubule through Bowman's capsule, thence excreted. If these bonds were broken, however, then tubular reabsorption could return the free chromium into the blood stream. The irreversible binding in the kidney could be due to this free chromium entering the kidney cells and being unable to leave. The very slow increase in blood activity may be similarly explained by release of chromium from the kidney. Comar (1955) reported the kidney to be the critical organ for Cr^{51}, viz, that organ which receives radiation damage that results in the greatest insult to the total body. Visek et al. (1953) did not report as high a chromium retention, in the kidneys of rats, as observed in this study.

IV. Excretion of Hexavalent Chromium

In addition to the possibility of gill excretion of chromium by fish in fresh water, three possible routes of excretion exist: the bile, the kidney, and direct excretion

into the lumen of the digestive system. Without collection of bile, urine, and feces, these routes could not be quantitated. The role of bile and kidney have been previously discussed.

The fact that the pyloric caeca did not appear to follow a linear regression pattern in their accumulation of chromium, focused attention on this organ as a possible source of excretion of chromium from the body. The estimated slope of accumulation during the first 8 days was approximately +1.5. During the first few days, this organ was accumulating chromium at a more rapid rate, gram for gram, than any other tissue. The tapering of concentration increase with time, when plotted as mean values of µg. Cr/g. pyloric caeca versus the log of time, produced points that were capable of being graphed as a straight line. A plot of the reciprocal of time versus log of mean concentrations produced points, that with the exception of those for the first 3 days, could be connected into a straight line, producing a value of 21 µg./g. pyloric caeca when extrapolated to infinity. The half value of 10.5 µg. was attained at near 8 days. Although the possibility of a limiting mechanism in the pyloric caeca exists in reference to chromium, it would seem more plausible that this organ is behaving in an excretory manner. It would appear that the logarithmic increase with time represents chromium deposited in the lumina of the various blind pouches of the pyloric caeca. From here, the chromium could move out

into the main lumen, then into the gut, thus accounting for the lack of further increases. If the above hypothesis were true, the slope of the first few days should represent the actual excretory rate of the pyloric caeca. Fromm and Schiffman (1958) also reported a high chromium concentration in this area, resulting in severe pathological changes in the intestine immediately posterior to the pyloric caeca.

The question of chromium secretion directly into the lower gut must also be considered. If it were excreting chromium into the lumen, the gut would be expected to exhibit higher values of chromium than the pyloric caeca. An examination of the concentrations showed no statistical difference between the two, although the gut mean values did exceed those of the pyloric caeca somewhat in 6 out of 9 cases.

SUMMARY AND CONCLUSIONS

1. Hexavalent chromium was accumulated in quantities exceeding the aquarium water in spleen, liver, posterior gut, pyloric caeca, stomach and kidney. With the exception of the spleen, these organs are all capable of excretion; thus it is hypothesized that accumulation in these tissues may be correlated with excretion.

2. In the rainbow trout, the major route of entry, into the fish, of hexavalent chromium, is probably via the gills. Fish with esophageal occlusions demonstrated accumulations of chromium similar to those of normal fish in chromated water. Hexavalent chromium given to fish via stomach tube was not significantly accumulated by tissues other than the digestive system in 24 hours.

3. Hexavalent chromium probably crosses the gill membrane as a result of simple diffusion. Except for one fish, the blood concentration of chromium never surpassed that of the surrounding water.

4. Either this chromium combines into a complex when it enters the blood, or nucleated fish erythrocytes behave differently than do mammalian red blood cells. A very low rate of increase in concentration was noticed in blood. If erythrocytic binding were taking place, a high rate of increase in activity would be observed.

5. Fresh water elimination studies showed a rapid decrease of chromium from the blood, liver, stomach, pyloric caeca and posterior gut. These studies also indicated a retention of chromium in the spleen and kidney.

6. Possible sites of excretion of chromium from the body are liver (bile), kidney and pyloric caeca.

7. Based on information obtained, fish caught in chromium contaminated streams, not exceeding a concentration of 2.5 µg. Cr/ml. of water, would probably not contain appreciable amounts of chromium in muscle tissue.

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APPENDIXES

APPENDIX A

ACCUMULATION OF CHROMIUM IN μg./g. TISSUE WITH TIME

ACCUMULATION OF CHROMIUM WITH TIME
FOLLOWING EXPOSURE TO 2.5 mg. Cr/l.

BLOOD

	EXPOSURE TIME IN DAYS								
	1	2	3	4	5.2	8.7	14	17	23.8
µg. Cr/g. blood	0.26	0.93	0.25	0.65	0.82	1.45	0.48	1.42	1.74
	0.59	1.44	0.51	1.29	1.52	0.81	0.66	1.85	1.45
	0.20	0.72	0.66	0.73	1.08	1.13	1.25	1.81	2.42
	0.36	1.21	0.18	0.29	1.06	1.70	1.41	1.91	1.31
	0.76	1.54	0.07	0.14	0.69	1.01	0.53	2.02	1.97
	0.62	2.16	0.21	0.29	0.73	0.57	1.05		0.96
	0.58	1.24	0.25	0.37	1.44	3.28	0.70		2.47
	0.54	0.34	0.12	0.33	0.86	0.96	0.80		2.17
	0.24	0.54	0.36	0.52	1.04	1.43	0.46		
	0.21		0.26	0.87		0.97	1.38		
						0.91			
						0.48			
						1.98			
						1.19			
$\bar{X}$	0.44	1.12	0.29	0.55	1.02	1.28	0.87	1.80	1.81
s	±0.20	±0.56	±0.17	±0.35	±0.30	±0.70	±0.40	±0.22	±0.60
Std. Error	±0.06	±0.19	±0.05	±0.11	±0.09	±0.19	±0.13	±0.10	±0.21
Coeff. Var.	45	50	59	64	29	55	46	12	33

Data for plotting linear regression line
and .95 confidence interval:

$$\hat{Y} = .53 + .05X$$

$$r = .58$$

$$N = 85$$

$$s_{y \cdot x} = .5317$$

ACCUMULATION OF CHROMIUM WITH TIME
FOLLOWING EXPOSURE TO 2.5 mg. Cr/l.

SPLEEN

	EXPOSURE TIME IN DAYS								
	1	2	3	4	5.2	8.7	14	17	23.8
µg. Cr/g. spleen	0.34 0.31 0.06 0.71 0.03 0.46 0.51 0.02 0.99	0.03 0.30 0.42 0.89 0.44 0.42 0.22 0.21	0.43 0.46 0.34 0.03 0.67	0.34 0.06 0.99 0.25	0.79 0.94 0.67 0.81 0.21 0.69 1.14 1.33 0.86	0.74 2.02 0.56 0.91 1.47 2.34 1.80 2.29 1.32 0.35 0.21 5.19 1.28	0.13 1.26 4.37 2.28 0.69 2.50 3.51 0.95 6.81 1.74	1.30 6.68 2.91 3.42 3.86	5.03 2.13 3.73 5.03 5.96 3.53
$\bar{X}$	0.34	0.29	0.19	0.16	0.83	1.46	2.42	3.63	4.24
s	±0.04	±0.86	±0.26	±0.30	±0.30	±1.30	±2.00	±2.00	±1.37
Std. Error	±0.01	±0.27	±0.08	±0.15	±0.09	±0.35	±0.63	±0.89	±0.56
Coeff. Var.	12	296	137	188	36	89	83	55	32

Data for plotting linear regression line
and .95 confidence interval:

$$\hat{Y} = -.20 + .19X$$

$$r = .77$$

$$N = 84$$

$$s_{y \cdot x} = 1.06$$

ACCUMULATION OF CHROMIUM WITH TIME
FOLLOWING EXPOSURE TO 2.5 mg. Cr/l.

LIVER

	EXPOSURE TIME IN DAYS								
	1	2	3	4	5.2	8.7	14	17	23.8
µg. Cr/g. liver	0.10	0.25	0.30	0.93	1.59	4.28	2.39	3.02	17.98
	0.20	0.84	0.63	0.27	1.52	1.48	2.54	17.12	2.44
	0.16	0.90	0.68	0.76	1.38	10.79	2.54	9.20	5.65
	0.20	1.09	1.75	2.07	1.11	1.18	12.33	10.04	10.69
	0.65	1.51	0.24	0.20	0.57	1.93	2.26	10.18	9.90
	0.46	1.27	0.64	1.15	0.68	1.30	12.94		3.76
	0.40	0.25	0.69	0.36	3.79	2.60	3.19		6.50
	0.29	0.29	0.32	0.51	1.28	1.80	3.39		8.33
	0.15	0.16	0.12	0.69	0.82	5.53	3.98		
	0.32	0.43	0.26	1.14		2.55	12.62		
						1.01			
						1.76			
						7.48			
						3.99			
$\bar{X}$	0.29	0.70	0.56	0.81	1.42	3.40	5.82	9.91	8.16
s	±0.17	±0.49	±0.47	±0.60	±1.19	±2.80	±4.70	±5.00	±4.80
Std. Error	±0.05	±0.15	±0.15	±0.19	±0.38	±0.75	±1.49	±2.24	±1.70
Coeff. Var.	59	70	84	74	84	82	81	50	59

Data for plotting linear regression line
and .95 confidence interval:

$$\hat{Y} = -.33 + .42X$$

$$r = .72$$

$$N = 86$$

$$s_{y \cdot x} = 2.81$$

ACCUMULATION OF CHROMIUM WITH TIME
FOLLOWING EXPOSURE TO 2.5 mg. Cr/l.

POSTERIOR GUT

μg. Cr/g. posterior gut	EXPOSURE TIME IN DAYS								
	1	2	3	4	5.2	8.7	14	17	23.8
	0.18	0.52	1.33	7.81	10.00	24.27	8.76	5.65	27.01
	1.40	1.20	16.55	0.93	5.33	16.89	4.85	19.74	5.43
	0.52	0.94	9.53	24.48	5.80	7.76	8.18	26.02	6.58
	2.20	2.92	3.28	0.99	4.09	7.18	22.99	28.86	17.51
	1.91	1.84	1.95	7.43	4.48	6.07	5.83	17.47	25.62
	0.56	3.17	3.17	4.67	2.86	9.61	17.18		5.98
	0.64	1.98	11.77	1.26	13.35	4.96	9.33		10.65
	0.30	1.25	2.42	4.84	0.37	7.76	9.78		22.01
	3.64	5.27	1.72	3.81	3.97	12.34	12.66		
	0.21	0.42	1.67	3.25		23.71	29.23		
						6.90			
						11.56			
						17.84			
						13.79			
$\bar{X}$	1.16	1.95	5.34	5.95	5.38	12.19	12.88	19.55	18.10
s	±1.29	±1.49	±5.34	±6.90	±3.90	±6.30	±7.90	±9.00	±9.10
Std. Error	±0.41	±0.47	±1.69	±2.18	±1.23	±1.68	±2.50	±4.02	±3.22
Coeff. Var.	111	24	100	116	72	52	61	46	50

Data for plotting linear regression line
and .95 confidence interval:

$$\hat{Y} = 2.71 + .70X$$

$$r = .61$$

$$N = 86$$

$$s_{y \cdot x} = 3.32$$

ACCUMULATION OF CHROMIUM WITH TIME
FOLLOWING EXPOSURE TO 2.5 mg. Cr/l.

PYLORIC CAECA

µg. Cr/g. pyloric caeca	EXPOSURE TIME IN DAYS								
	1	2	3	4	5.2	8.7	14	17	23.8
	0.12	0.35	2.41	9.02	11.16	17.62	12.90	6.24	23.10
	0.64	1.56	16.22	0.84	7.51	16.54	4.57	17.81	4.74
	0.25	2.59	14.22	6.52	9.52	13.72	15.50	19.30	13.10
	2.25	3.63	3.53	2.72	6.63	4.26	27.26	21.45	15.42
	2.36	2.41	3.20	1.00	2.57	11.28	5.26	11.45	16.51
	0.62	3.41	10.07	8.82	4.18	12.02	13.94		10.70
	1.55	2.11	13.79	1.83	17.97	15.06	11.50		18.31
	0.97	0.94	3.35	4.90	0.38	13.98	13.34		20.31
	1.41	4.07	0.98	4.80	7.62	11.28	13.49		
	0.28	1.24	1.57	5.56		7.58	17.16		
						3.07			
						8.86			
						12.80			
						13.07			
$\bar{X}$	1.05	2.23	6.93	4.60	7.50	11.51	13.49	15.25	15.27
s	±0.82	±1.23	±5.96	±3.00	±5.20	±4.20	±6.30	±6.30	±5.80
Std. Error	±0.26	±0.39	±1.88	±0.95	±1.64	±1.12	±1.99	±2.83	±2.05
Coeff. Var.	78	17	74	65	69	36	47	41	38

ACCUMULATION OF CHROMIUM WITH TIME
FOLLOWING EXPOSURE TO 2.5 mg. Cr/l.

STOMACH

µg. Cr/g. stomach	EXPOSURE TIME IN DAYS								
	1	2	3	4	5.2	8.7	14	17	23.8
	0.14	0.25	0.33	1.00	0.97	2.37	4.29	2.32	5.57
	0.48	0.60	1.10	0.24	0.79	2.04	2.17	2.76	2.36
	0.23	0.61	0.99	1.31	1.07	1.11	2.66	2.63	4.29
	0.89	0.96	1.01	0.66	1.40	1.02	8.51	2.76	5.18
	0.58	0.64	0.91	0.45	1.49	1.30	2.67	3.03	5.55
	0.35	0.55	1.00	0.62	1.32	2.23	3.32		4.46
	0.51	0.35	0.47	0.41	1.73	1.68	6.48		4.20
	0.26	0.35	0.40	0.97	1.42	2.00	1.91		4.66
	0.57	0.63	1.10	1.02	1.19	2.04	2.89		
	0.16	1.21	0.38	1.42		2.02	9.21		
						1.40			
						1.65			
						2.65			
						1.81			
$\bar{X}$	0.42	0.62	0.77	0.81	1.26	1.81	4.41	2.70	4.53
s	±0.23	±0.28	±0.35	±0.40	±0.30	±0.48	±2.60	±0.30	±1.00
Std. Error	±0.07	±0.09	±0.11	±0.13	±0.09	±0.13	±0.82	±0.13	±0.35
Coeff. Var.	55	14	45	49	24	27	59	11	22

Data for plotting linear regression line
and .95 confidence interval:

$\hat{Y} = .24 + .20X$

$r = .78$

$N = 86$

$s_{y \cdot x} = 1.12$

ACCUMULATION OF CHROMIUM WITH TIME
FOLLOWING EXPOSURE TO 2.5 mg. Cr/l.

KIDNEY

	EXPOSURE TIME IN DAYS								
	1	2	3	4	5.2	8.7	14	17	23.8
µg. Cr/g. kidney	0.68	1.58	1.07	1.59	2.69	8.40	3.85	15.56	20.35
	2.43	8.98	9.59	1.81	5.67	5.56	11.83	27.55	15.83
	1.09	7.35	6.41	3.01	2.57	5.02	4.00	15.12	22.36
	3.33	7.78	3.25	2.10	4.72	6.29	11.20	16.59	19.48
	4.81	11.07	0.52	0.89	2.17	3.82	3.67	25.10	16.41
	3.74	8.64	1.24	2.10	2.23	7.42	9.73		10.87
	4.00	2.41	0.82	2.06	6.80	7.56	4.93		13.52
	2.88	1.35	0.57	1.36	3.53	3.15	4.02		15.48
	1.00	0.99	1.00	2.64	2.69	7.10	6.21		
	3.32	2.12	0.81	5.13		6.48	7.54		
						2.77			
						6.68			
						13.02			
						8.33			
$\bar{X}$	2.73	5.23	2.53	2.27	3.67	6.54	6.60	19.98	16.79
s	±1.40	±3.78	±3.08	±1.20	±1.50	±1.65	±3.00	±5.90	±3.70
Std. Error	±0.44	±1.20	±0.97	±0.38	±0.47	±0.44	±0.95	±2.64	±1.31
Coeff. Var.	51	23	122	53	41	25	45	30	22

ACCUMULATION OF CHROMIUM WITH TIME
FOLLOWING EXPOSURE TO 2.5 mg. Cr/l.

MUSCLE*

μg. Cr/g. muscle	EXPOSURE TIME IN DAYS								
	1	2	3	4	5.2	8.7	14	17	23.8
	-	-	0.09	-	0.13	0.18	-	0.14	0.10
	-	0.01	0.40	0.19	0.14	0.06	0.09	0.54	0.20
	-	0.08	0.06	0.03	0.08	0.15	0.40	0.25	0.04
	-	0.09	0.70	-	0.06	0.06	0.13	0.17	0.08
	0.07	0.02	-	-	0.28	0.21	0.17	0.37	0.37
	0.10	0.03	0.08	-	0.09	0.20	0.28		0.19
	0.06	-	-	-	0.48	0.57	0.24		0.18
	0.01	-	-	-	-	0.10	-		-
	0.09	-	-	-	-	0.02	0.01		
	-	0.04	-	0.06		0.17	0.10		
						-			
						-			
						0.21			
						0.33			
$\bar{X}$	0.03	0.03	0.13	0.03	0.14	0.16	0.14	0.29	0.14
s	±0.03	±0.01	±0.22	±0.01	±0.10	±0.10	±0.10	±0.15	-
Std. Error	±0.01	-	±0.07	-	±0.03	±0.03	±0.03	±0.07	-
Coeff. Var.	107	-	169	-	71	63	71	51	-

*Due to low counting ratios, none of the above values are statistically significant.

APPENDIX B

ELIMINATION OF CHROMIUM WITH TIME FOLLOWING RETURN OF FISH TO FRESH WATER AFTER 12 DAYS EXPOSURE TO 2.5 mg. Cr/l.

ELIMINATION OF CHROMIUM WITH TIME FOLLOWING RETURN OF FISH TO FRESH WATER AFTER 12 DAYS EXPOSURE TO 2.5 mg. Cr/l.

BLOOD

µg. Cr/g. blood	DAYS IN FRESH WATER					
	0*	5	10	15	20	25
	1.2	0.63	0.22	0.53	0.54	0.26
		0.43	0.34	0.26	0.44	0.36
		0.46	0.42	0.72	0.50	0.28
		0.36		0.25	0.60	0.31
		0.45				
$\bar{X}$		0.55	0.33	0.44	0.52	0.30
s		±0.44	±0.10	±0.23	±0.10	-
Std. Error		±0.19	±0.06	±0.12	±0.05	-
Coeff. Var.	-	79	30	52	19	-

SPLEEN

µg. Cr/g. spleen	DAYS IN FRESH WATER					
	0*	5	10	15	20	25
	2.1	1.96	1.41	1.23	1.20	1.17
		3.18	0.78	2.63	4.43	0.23
		1.96	0.26	2.91	3.37	5.82
		0.83		1.53	2.21	0.73
		1.33				
$\bar{X}$		1.85	0.82	2.08	2.80	1.99
s		±0.88	±0.58	±0.82	±1.40	±2.58
Std. Error		±0.39	±0.33	±0.41	±0.70	±1.29
Coeff. Var.		48	71	39	50	130

*Estimated Initial Value

ELIMINATION OF CHROMIUM WITH TIME FOLLOWING RETURN OF FISH TO FRESH WATER AFTER 12 DAYS EXPOSURE TO 2.5 mg. Cr/l.

LIVER

µg. Cr/g. liver	DAYS IN FRESH WATER					
	0*	5	10	15	20	25
	4.7	1.50	1.35	4.23	0.86	0.58
		2.11	1.99	1.12	0.90	0.52
		1.63	0.60	1.19	0.88	0.32
		2.36		0.60	0.55	0.50
		1.90				
$\bar{X}$		1.90	1.35	1.79	0.80	0.48
s		±0.35	±0.78	±1.65	±0.17	±0.10
Std. Error		±0.16	±0.45	±0.83	±0.08	±0.05
Coeff. Var.		18		92	21	21

POSTERIOR GUT

µg. Cr/g. gut	DAYS IN FRESH WATER					
	0*	5	10	15	20	25
	11.1	9.75	1.58	0.36	1.11	0.15
		3.11	1.24	0.40	0.09	0.16
		3.47	0.72	0.21	0.18	0.28
		8.22		0.46	0.11	0.50
		2.71				
$\bar{X}$		5.45	1.18	0.36	0.37	0.27
s		±3.28	±1.43	±0.21	±0.49	±0.17
Std. Error		±1.47	±0.83	±0.10	±0.24	±0.08
Coeff. Var.		60	120	58	132	63

*Estimated Initial Value

ELIMINATION OF CHROMIUM WITH TIME FOLLOWING RETURN OF FISH TO FRESH WATER AFTER 12 DAYS EXPOSURE TO 2.5 mg. Cr/l.

PYLORIC CAECA

µg. Cr/g. pyloric caeca	DAYS IN FRESH WATER					
	0*	5	10	15	20	25
	13.4	7.29	4.84	0.34	1.06	0.02
		2.72	6.53	0.20	0.21	0.14
		5.91	1.68	0.40	0.12	0.16
		2.86		0.39	0.24	0.23
		4.02				
$\bar{X}$		4.56	4.35	0.33	0.41	0.14
s		±1.99	±5.59	±0.19	±0.43	±0.10
Std. Error		±0.89	±3.23	±0.09	±0.21	±0.05
Coeff. Var.		44	129	58	105	71

STOMACH

µg. Cr/g. stomach	DAYS IN FRESH WATER					
	0*	5	10	15	20	25
	2.6	3.89	0.90	0.55	0.63	0.21
		0.77	1.46	0.62	0.34	0.17
		3.85	0.91	0.75	0.41	0.16
		1.10		0.30	1.04	0.57
		0.81				
$\bar{X}$		2.08	1.09	0.56	0.53	0.14
s		±1.63	±1.30	±0.34	±0.32	±0.20
Std. Error		±0.73	±0.75	±0.17	±0.16	±0.10
Coeff. Var.		78	119	61	60	143

*Estimated Initial Value

ELIMINATION OF CHROMIUM WITH TIME FOLLOWING RETURN OF FISH TO FRESH WATER AFTER 12 DAYS EXPOSURE TO 2.5 mg. Cr/l.

KIDNEY

µg. Cr/g. kidney	DAYS IN FRESH WATER					
	0*	5	10	15	20	25
	10.6	6.58	7.72	6.01	10.67	5.47
		12.94	7.10	10.42	5.15	3.41
		12.36	8.53	5.31	4.65	4.93
		8.07		3.65	0.99	7.46
		14.07				
$\bar{X}$		10.80	7.78	6.35	5.36	5.32
s		±3.25	±9.02	±2.89	±3.99	±1.67
Std. Error		±1.45	±5.21	±1.44	±1.99	±0.83
Coeff. Var.		30	115	46	74	31

MUSCLE

µg. Cr/g. muscle	DAYS IN FRESH WATER					
	0*	5	10	15	20	25
	-	0.02	0.04	0.17	0.65	0.03
		0.03	0.03	-	0.10	0.33
		0.05	0.03	-	0.18	0.84
		0.01		0.01	-	-
		-				
$\bar{X}$		0.02	0.03	0.05	0.23	0.30
s		±0.02	-	±0.08	±0.28	±0.39
Std. Error		±0.01	-	±0.04	±0.14	±0.19
Coeff. Var.		100	-	160	122	130

*Estimated Initial Value

APPENDIX C

DISTRIBUTION STUDIES OF RADIOCHROMATE ADMINISTERED TO RAINBOW TROUT VIA STOMACH TUBE AND DISTRIBUTION STUDIES OF CHROMIUM IN RAINBOW TROUT SUBJECTED TO ESOPHAGEAL OCCLUSION.

DISTRIBUTION STUDIES OF RADIOCHROMATE ADMINISTERED TO RAINBOW TROUT VIA STOMACH TUBE

Per cent of administered dose	ORGAN							
	BLOOD	SPLEEN	LIVER	POST. GUT	PYLORIC CAECA	STOMACH	KIDNEY	MUSCLE
	0.02	-	0.06	19.00	6.60	10.57	0.05	0.01
	0.04	0.01	0.07	36.03	10.46	1.33	0.04	-
	0.02	-	0.03	15.66	15.66	21.97	0.02	0.02
$\bar{X}$	0.03	0.01	0.05	23.56	10.91	11.29	0.04	0.01
s	±0.01	±0.01	±0.02	±10.95	±4.55	±10.34	±0.01	±0.01
Std. Error	-	-	±0.01	±6.33	±2.63	±5.98	-	-
Coeff. Var.	33	50	40	47	42	92	25	100

DISTRIBUTION STUDIES OF CHROMIUM IN RAINBOW TROUT SUBJECTED TO ESOPHAGEAL OCCLUSION

µg. Cr/g. organ	ORGAN							
	BLOOD	SPLEEN	LIVER	POST. GUT	PYLORIC CAECA	STOMACH	KIDNEY	MUSCLE
	0.33	0.09	0.50	0.32	0.32	0.12	1.49	-
	0.28	-	0.36	0.28	0.34	0.11	1.13	0.01

APPENDIX D

STATISTICAL FORMULAS

STATISTICAL FORMULAS*

Basic Conversion Formulas:

$$x = X - \bar{X}$$

$$\sum x^2 = \sum X^2 - \frac{(\sum X)^2}{n}$$

$$\sum y^2 = \sum Y^2 - \frac{(\sum Y)^2}{n}$$

$$\sum xy = \sum XY - \frac{(\sum X)\ (\sum Y)}{n}$$

Linear Regression Formulas:

$$\hat{Y} = a + bX \qquad \text{(Equation for a line)}$$

$$b = \frac{\sum xy}{\sum x^2} \qquad \text{(Slope of a line)}$$

$$a = \frac{\sum Y - b \sum X}{n}$$

$$r = \frac{\sum xy}{\sqrt{(\sum x^2)(\sum y^2)}} \qquad \text{(correlation coefficient)}$$

Linear Regression Confidence Limit Formulas:

$$s_{y \cdot x} = \sqrt{\frac{\sum y^2 - \frac{(\sum xy)^2}{\sum x^2}}{n - 2}}$$

$$s_Y = s_{y \cdot x} \sqrt{\frac{1}{n} + \frac{x^2}{\sum x^2} + 1}$$

$\hat{Y} - s_Y\, t_{\frac{1-P}{2}}$ = Upper two-tailed 100 P% confidence belt for amount of chromium present in a fish after X days of exposure.

$\hat{Y} - s_Y\, t_{\frac{1+P}{2}}$ = Lower two-tailed 100 P% confidence belt for amount of chromium present in a fish after X days of exposure.

*Statistical procedures were suggested by Dr. P. J. Clark, Zoology Dept., M.S.U.