EFFECTS OF INTRAPERITONEAL INJECTIONS OF CADMIUM CHLORIDE ON GONADS, LIVER AND KIDNEY OF GOLDFISH, CARASSIUS AURATUS (LINNAEUS)

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PREFACE

The objective of this study was to determine the effects of cadmium chloride injections on goldfish. The criteria used to make this determination were as follows: (1) changes in the gonadal body weight ratio, (2) cadmium residues in the gonads, liver, kidney and muscle, and (3) histopathological changes in the gonads, liver and kidney.

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INTRODUCTION

Heavy metals such as lead, mercury and zinc have received much attention as environmental pollutants because of recent findings of their deleterious effect on organisms. Although their presence in natural waters has been known for some time, recent discovery of these metals in aquatic organisms consumed by man, mostly fish, has led to real public concern. Of great importance but of much less general interest are the biological effects of these metals on aquatic eco-systems. Relatively large quantities of these heavy metals, which are all toxic to fish, are found in some natural waterways due to pollution. Thus, the accumulation of large residues of heavy metals in fish, occurrence of fish kills, and sublethal effects on fish has prompted further study on biological effects of heavy metals on fish.

Cadmium, considered a trace element, recently appeared in natural waters in increasing quantities from effluents of industrial processes including the manufacture of alloys, cadmium plating, vapor lamps, glassworks, storage batteries and zinc smelting (Frank, 1969).

Less than 1.0 part per billion (ppb) (lowest detectable level) cadmium is found in most rivers throughout the United States. Highest concentrations reported were 37 ppb in the South Platte River and 40 ppb in the Arkansas River (FWPCA Report, 1963). In streams below the outfalls of electroplating operations, however, values as high as 2,800 ppb were

reported (Frank, 1969). The same author reports 358 ppb in bottom samples of a lake 250 miles downstream from the source of contamination. Cadmium has also been detected in a variety of human foods including cereals, vegetables, fruits, beverages and dairy products. Cadmium values in foods ranged from 10 ppb in beverages to 380 ppb in fruits (Corneliussen, 1970). Higher values in fruits may be due to the cadmium found in the fungicides used on these products. Cadmium has been found in human blood, although no geographical patterns were discernible (Kubota, Lazar and Losee, 1968). Concentrations of cadmium were 5 ppb (lowest detectable level) in whole blood of donors in Lima, Ohio and El Paso, Texas to 141 ppb in Fargo, North Dakota.

Biological effects of heavy metals on fish are known only from short term toxicity studies to determine median tolerance limits (TL_m) (defined as the amount of a compound required to kill one-half of the test organisms in a given period of time, usually 24, 48 and 96 hours) to fish exposed to dilute aqueous solutions. Solutions of heavy metals suffocate fish by precipitating mucoproteins on gills which interferes with oxygen exchange (Belding, 1927). The quantity of a heavy metal required to kill fish is inversely proportional to the oxygen concentration of the water (Westfall, 1945) and solution pressure (Jones, 1939), and directly proportional to water hardness (Jones, 1938). Since the cause of death is due to precipitation of mucoproteins on the gills, the TL_m is changed directly with the number of fish per unit volume of water (Cairns and Scheier, 1957). There is also variation in tolerance due to species and age differences (Cairns and Scheier, 1965). In general, young fish are less tolerant than adults, larvae less tolerant than young fish and eggs more tolerant than adults. Crandall and Goodnight

(1963) found that holding fish in 2 to 5 ppm lead nitrate for four months inhibited gonadal development and occasionally produced gonadal degeneration.

Virtually all information on biological effects of cadmium on fish pertains to the TL_m. Anions in water responsible for water hardness precipitate cadmium and other heavy metals. Thus, the TL_m of various fish species for cadmium is dependent upon water hardness and values vary 10 to 100 fold from soft to hard water depending upon the species involved (Jones, 1939; Pickering and Henderson, 1966; and Doudoroff and Katz, 1953).

Pickering and Henderson (1966) studied the toxicity of several heavy metals, including cadmium, on fish in both soft (total hardness 20 mg/1) and hard (total hardness 360 mg/1) water. The 24 hour TL_m values of cadmium for fathead minnows were approximately 1 and 78 mg/1 in soft and hard water, respectively, and 7.8 and 88.6 mg/1, in soft and hard water, respectively, for green sunfish. In soft water, 24 hour TL_m values of cadmium for bluegills, goldfish, and guppies were 4.6, 3.5, and 3.4 mg/1, respectively. The 96 hour TL_m of cadmium for goldfish was 2.3 mg/1. Sublethal concentrations of cadmium in water can result in much higher cadmium values in fish tissues. The concentration of cadmium at which the average survival time of large sticklebacks (Gasterosteus aculeatus) was 1, 2, and 4 days was 7.0, 3.0, and 0.7 ppm, respectively (Jones, 1939). Thomas (1915) found that 6 ppm cadmium nitrate killed mummichogs (Fundulus heteroclitus) in 36 hours. Frank (1969) reported a range of concentration factors from 1 to 27 in the skin and muscle, and 7 to 72 in the gastro-intestinal tract and contents. Long term effects of cadmium on fish have not been investigated.

Parizek (1957) noted that single subcutaneous injections of cadmium chloride induced necrosis of testicular tissue in mammals. Damage to testicular tissue was irreversible, except when administered with concurrent high doses of zinc. An intraperitoneal injection of 7.5 mg cadmium chloride per kg of body weight (mg/kg) of the rat produced, within the first few hours, an increase in size and reddening of the testis, followed by development of a deep blue coloration, progressive necrosis of the tissue below the tunica albuginea, and finally complete disappearance of the testis. Testicular necrosis and atrophy in mammals were induced with injection of 5 to 10 mg/kg cadmium chloride (Chiquoine, 1964; Chiquoine and Suntzeff, 1965; Gunn, Clark, and Anderson, 1961, 1963, 1966; Gupta, Barnes, and Skelton, 1967; Kar, 1966; Mason et al., 1964; Meek, 1959; Niemi and Kormano, 1965; Parizek, 1960). Although cadmium injections resulted in testicular degeneration in rats, the interstitial cells were not damaged and full hormone production was unimpaired. Given a sufficient dose, proliferation of the germinal epithelium was rare (Allanson and Deansley, 1962).

The effects of cadmium on the mammalian testis was assumed to result from circulatory failure in the testes, causing a destruction of the tubules (Chiquoine, 1964; Niemi and Kormano, 1965; Waites and Setchell, 1966), or a direct and apparently specific toxic effect on the germinal epithelium (Parizek, 1960).

Rats are not the only animals susceptible to cadmium damage as similar results were observed in mice, rabbits, goats, and monkeys (Mason et al., 1964) although an effective does was not the same for all species. On the other hand, Chiquoine (1964) reported the frog, opossum, and rooster were insensitive to subcutaneous injections of cadmium

chloride. Johnson, Sigman, and Miller (1969) found that domestic fowl injected subcutaneously with cadmium chloride contained less cadmium in the testes 40 minutes after injection than did rats treated exactly the same. However, upon investigation of various cell components of fowl testis for cadmium, some cell fractions had higher concentrations than corresponding cell fractions of rats. Guthrie (1964) and Erickson and Pincus (1964) did not induce damage in testes of domestic fowl even when they used intratesticular injections. Kar (1965) successfully sterilized female guinea pigs with intraovarian injections of cadmium chloride.

Oral administration of cadmium to dogs revealed that it is highly concentrated in the liver and kidney (Decker, Hoppert, and Byerrum, 1956). Although most of the cadmium was excreted, a diet of 10 ppm per day for one year resulted in 80 ppm in the kidney and 40 ppm in the liver. Cotzias (1961) and Anwar (1960) found after cadmium injections that the liver of rats contained more total cadmium than the kidney because of the larger organ weight, but the kidney had a higher concentration of cadmium per unit weight of organ. Pathological effects of cadmium on organs, especially the kidney, liver, and lungs, was varied but there was some relationship between occurrence of sarcomas and tumors with experimental exposure to cadmium (Favino and Nazari, 1967).

The destructive effect of cadmium on mammalian testis suggests that occurrence of cadmium in the environment may have an effect on reproductive success and general well-being of fish. Reduced fecundity could alter population composition and damage the ecology of aquatic environments by selective elimination of the less tolerant species. Also, because fish are an important food of man, accumulation of cadmium in fish flesh should be examined becuase of potential transfer to man.

The present study describes effects of intraperitoneal injection of cadmium chloride on gonads, liver, and kidney of goldfish. Emphasis was placed on determining effects of specific quantities of the metal on various organs so that pathological effects could be related to long and short term exposures from single and multiple injections. The experimental procedures simulate conditions in which a fish might accumulate cadmium from a single or repeated exposure to sublethal concentrations in nature.

Goldfish were chosen as the test animal because of wide usage for other experiments, availability and survival under laboratory conditions. The present study reports on: (1) the LD_{50} of cadmium chloride for 24, 48, and 96 hours; (2) the effect of cadmium on the gonadal weight; (3) cadmium residues in the gonads, liver, kidney, and muscle, and (4) the histological effects of cadmium on gonads, liver, and kidney.

CHAPTER II

MATERIALS AND METHODS

Experimental Design

Experiment A

Experiment A was designed to determine the LD_{50} of cadmium to goldfish for 24, 48, and 96 hours for the purpose of finding a sublethal dose for use in later experiments. LD_{50} is reported as number of mg cadmium chloride administered to compare with cadmium studies done on mammals, and as mg of cadmium, given in parenthesis, for comparison with organ residue levels. The objectives of this experiment were accomplished by injecting groups of 10 fish each with: saline, 5 mg/kg (3.06), 10 mg/kg (6.13), 20 mg/kg (12.26), 40 mg/kg (24.52) cadmium chloride. Mortality, judged by no movement of the fish when touched, was recorded at 24, 48, and 96 hours and within the intervals. From this information LD_{50} determinations were established by graphical procedures (Standard Methods, 1965).

Experiment B

Based on the results of experiment A and cadmium concentrations known to produce testicular necrosis in mammals, experiment B was designed to determine if repeated sublethal injections of cadmium could be administered without killing the fish, and if such injections would

produce reduced gonadal weights. Eight fish were injected with 7.5 mg/kg (4.60) cadmium chloride each week for five weeks. One fish was sacrificed each week to determine gonadal weight expressed as a percentage of the body weight (GSI, or gonadal-somatic index).

Experiment C

This experiment was designed to determine if a single intraperitoneal injection of cadmium chloride caused a reduction in gonadal weight in a time period similar to mammals and if a difference in response to cadmium injection could be observed between fish with and without hormone injections. The use of hormone was an attempt to cause gonadal development at a desired time and to observe the results of cadmium administration on gonads in an active state. One group of 48 fish was injected with 7.5 mg/kg (4.60) cadmium chloride and an equal number with saline solution as controls (Figure 1). One-half of each of these groups were injected with human chorionic gonadotropin (HCG) each 10 days for 2 months. At each injection, six fish from each of the four groups were sacrificed, the GSI determined and tissues fixed for later histological examination.

Experiment D

This experiment was designed to repeat experiment C with a larger sample size, to extend the test period from two to approximately six months, and to collect gonadal tissue for histological examination. Three hundred goldfish were injected with 10 mg/kg (6.13) cadmium chloride and three hundred controls injected with saline solution (Figure 2). Ten uninjected fish were sacrificed as zero time controls.

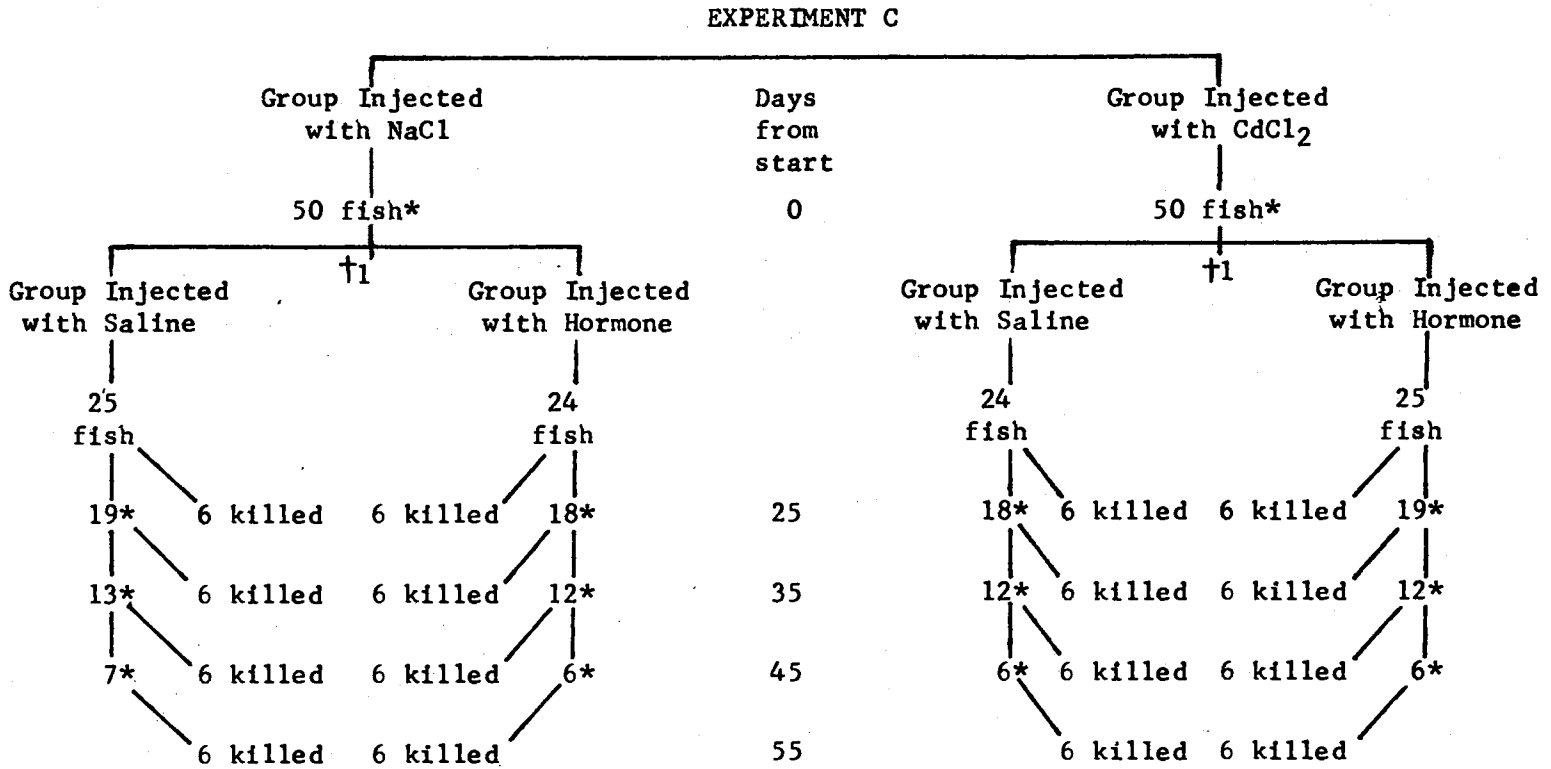
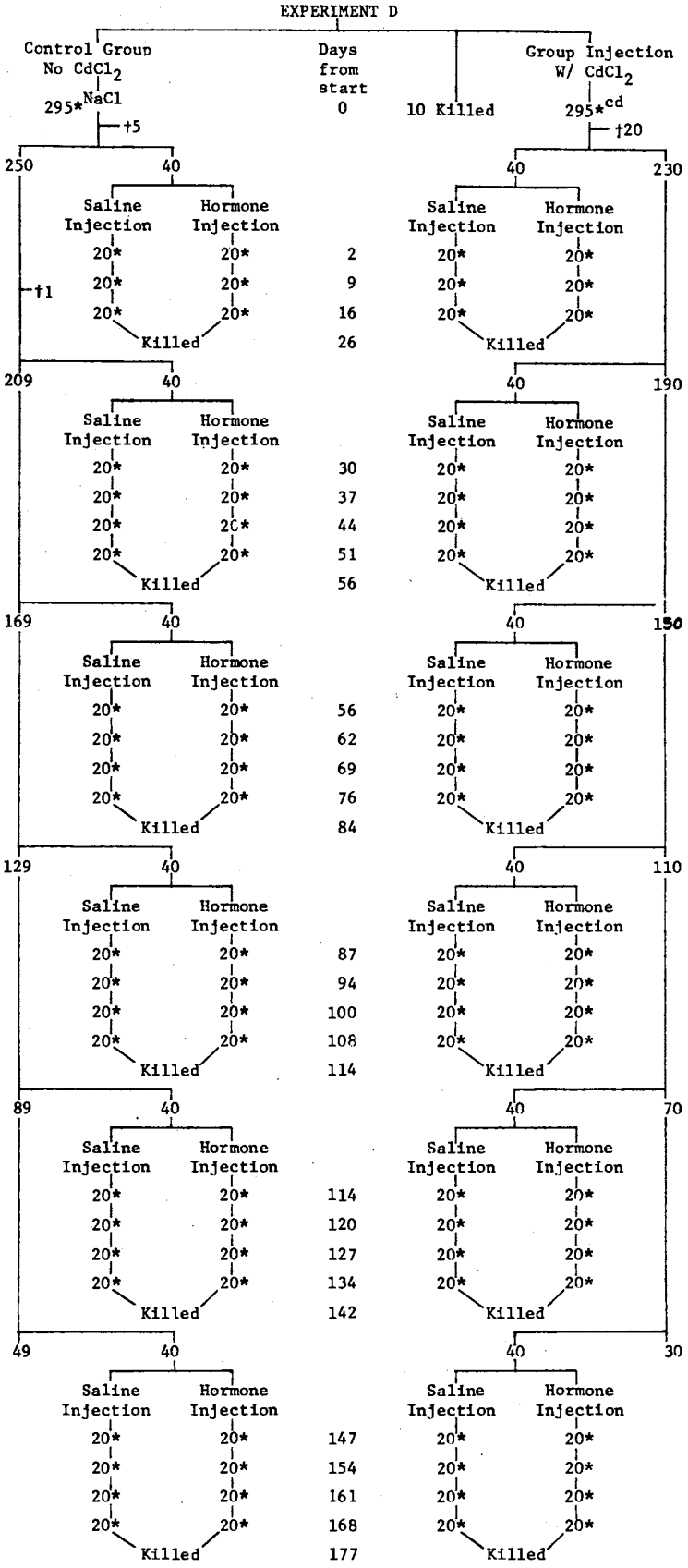


Figure 1. Design of Experiment C to Determine the Effect of a Single Injection of 7.5 mg/kg Cadmium Chloride on Gonadal Weight of Goldfish (* indicates injection of compound heading respective column.)

Figure 2. Design of Experiment D to Determine the Effect of a Single Injection of 10 mg/kg Cadmium Chloride on the Gonadal Weight of Goldfish Over a Four-Month Period (*indicates injection of material heading respective column)

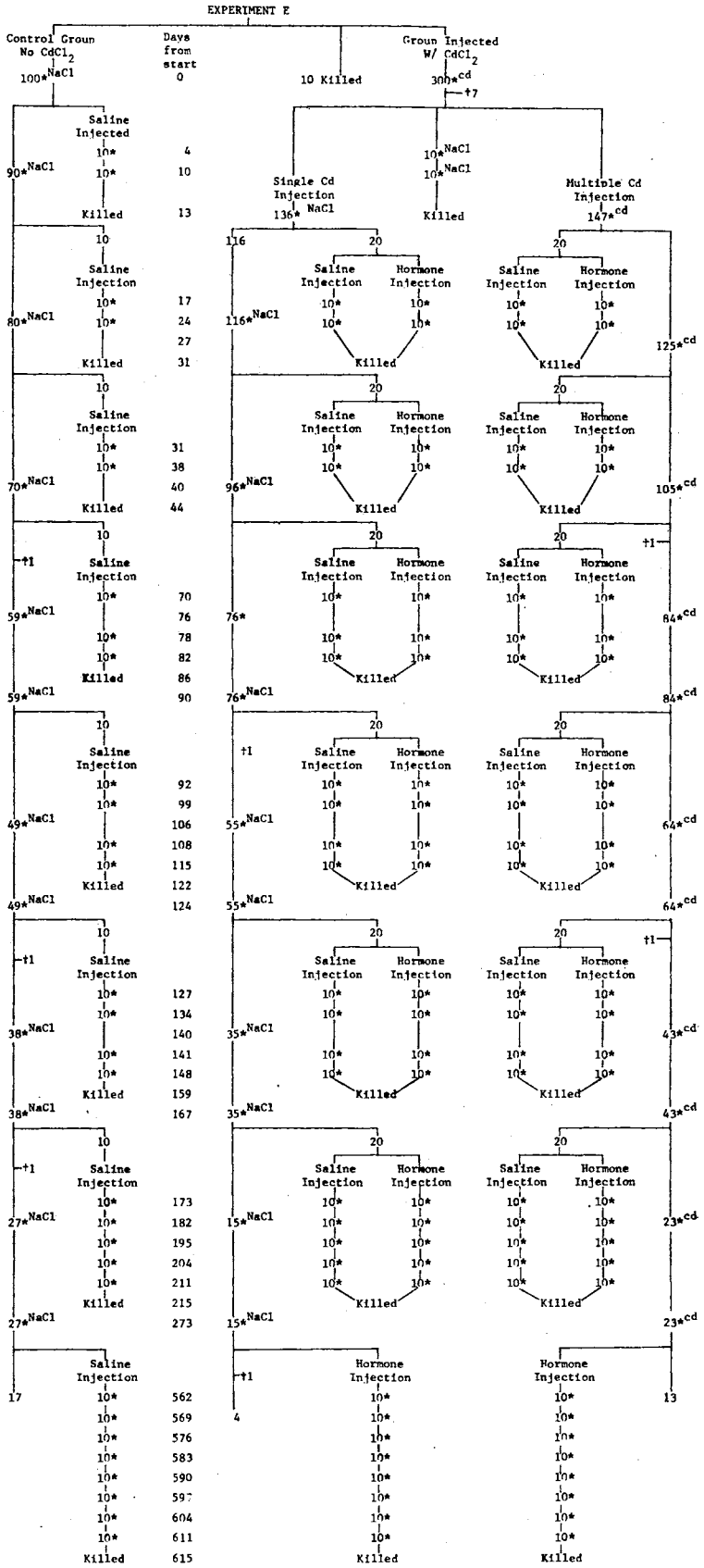

EXPERIMENT D
Control Group
No $CdCl_2$
295* NaCl
†5
Days from start
0
10 Killed
Group Injection
W/ $CdCl_2$
295* cd
†20
250
40
Saline Injection
Hormone Injection
20*
†1
2
9
16
26
Killed
209
30
37
44
51
56
169
56
62
69
76
84
129
87
94
100
108
114
89
114
120
127
134
142
49
147
154
161
168
177
230
190
150
110
70
30

Forty fish from each group were removed and one-half of these injected with 20 International Units (IU) of HCG. Injections of HCG were repeated each week on this set. The first set of 80 fish were sacrificed after 26 days, after which a second set of 80 fish from each group were removed and one-half were injected with 20 IU of HCG each week. This process was continued with one set being sacrificed and a new set injected with HCG at 26, 56, 84, 114, 136, and 186 days after the initial cadmium chloride injection. After injection with HCG, the fish were maintained in 37.6 liter aquaria, 26 to 50 days then sacrificed and necropsied, gonad weight taken and organs prepared for histological examination.

Experiment E

This experiment was designed to verify experiments C and D, determine the effects of repeated cadmium chloride injections on gonadal weight, determine the cadmium content of ovary, testis, liver, kidney, and muscle tissue, and observe histological changes in these tissues. Muscle tissue was not examined histologically. Three hundred fish were initially injected with 10 mg/kg (6.13) cadmium chloride. One-half of these received an additional 12 injections of the same dose approximately 20 days apart except for the final injection which was given after a lapse of three months. One hundred additional fish were used as controls and injected with saline. Sets of 40 fish from each tank of injected fish were removed, place in aquaria, and one-half of each set were injected with 20 IU of HCG at weekly intervals as were 10 control fish. At the end of 13, 31, 44, 86, 122, 159, 215, and 615 days (Figure 3) from the initial injection, the fish were sacrificed. The length of

Figure 3. Design of Experiment E to Determine the Effects of Single and Multiple Injections of 10 mg/kg Cadmium Chloride on Goldfish Over a Twenty-Month Period (*indicates injection of material heading respective column)


EXPERIMENT E
Control Groun No CdCl2
100* NaCl
Days from start
0
10 Killed
Groun Injected W/ CdCl2
300* cd
†7
Saline Injected
10*
4
10*
10
90* NaCl
Killed
13
10* NaCl
10* NaCl
Killed
Single Cd Injection
136* NaCl
Multiple Cd Injection
147* cd
10
Saline Injection
10*
17
10*
24
80* NaCl
27
Killed
31
116
20
Saline Injection
Hormone Injection
116* NaCl
125* cd
96* NaCl
105* cd
†1
76*
84* cd
59* NaCl
70
38
†1
35* NaCl
43* cd
27* NaCl
15* NaCl
23* cd
Hormone Injection
17
562
569
576
583
590
597
604
611
615
4
13

time fish were kept on HCG injections increased after the third set (commencing with the 92nd day) because of a failure to obtain significant gonadal development from hormone injection. After injection with HCG, the fish were maintained in 37.6 liter aquaria until they were sacrificed and necropsied for gonadal weight, and organs taken for analysis of cadmium and histological examination.

Methods

Conditions of Maintenance and Treatment of Goldfish

All goldfish (Commons) used in this study were obtained from a commercial source.[1] They were held in 610 liter wooden tanks measuring 0.55 X 0.60 X 1.80 meters. A constant flow of dechlorinated water, obtained by passage through an activated charcoal filter, was maintained to flush excess food and wastes. In the winter when ambient water temperatures were 5 - 10 C, temperatures in the tanks were maintained at 21 C, with thermostatically controlled 850 watt electric submersible heaters. In summer, when ambient water temperatures were 25 - 30 C, a water temperature of 18 C was maintained by thermostatically controlled pumping of water, cooled to 4 C with a submersible cooling unit, from 0.6 X 0.6 X 1.2 foot tank. The fish were fed small granule commercial trout chow on a maintenance diet of approximately 2% of the body weight daily. The feed contained approximately 0.7 ppm cadmium dry weight.

When gonadal development was being encouraged, groups of five fish were placed in 37.6 liter aquaria with water temperatures maintained at

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[1] Ozark Goldfish Inc., Stoutland, Missouri.

27 - 28 C with standard aquarium heaters, and were fed the same daily diet. To stimulate gonadal development, human chorionic gonadotropin (HCG) was administered. The powdered form was diluted with sterile water to give 200 (IU)/cc. The injection rate was 0.1 cc/fish/injection or approximately 1700 - 2500 IU/kg/injection. The number and time intervals between injections varied and will be discussed later. Aquaria were given continuous illumination throughout the stimulation period.

Cadmium was administered by intraperitoneal injection to obtain precise quantitation of the amount of cadmium given fish. Injections were made with a 1.0 cc tuberculin syringe with a 26 gauge needle. Cadmium was injected in the form of cadmium chloride solution, made from the salt, cadmium chloride, at the rate of 0.1 cc per fish per injection. The concentration of the cadmium chloride solution was adjusted to give the desired amount in mg of cadmium chloride per kg of body weight of the fish (mg/kg). Injections were administered intraperitoneally immediately posterior to one of the pelvic fins. For each injection, the fish were grouped by weight in one gram intervals and injected with an appropriate concentration of cadmium chloride solution in order to achieve the desired dose per kg of body weight. All fish were then released together in the wooden tanks.

Because there was no published LD_{50} level for $CdCl_2$ in fish, it was necessary to conduct preliminary experiments to determine if dosage levels of cadmium used in mammalian species could be used on the goldfish.

Procuring and Processing of Tissues for Analysis

Fish and tissues were handled minimally during necropsy to reduce

contamination. Fish were removed from the aquarium, blotted dry with paper towels, weighed to the nearest 0.1 gram and standard length measured to the nearest mm. Some difficulty was encountered locating the gonads, especially the testes, of fish in an undeveloped condition. They could usually be located in the posterior and dorsal part of the cavity although sometimes they were in a more lateral position. The kidney was paired, somewhat triangular shaped and located in the median and dorsal portion of the body cavity. The liver was diffuse, dispersed along the intestine and associated with the spleen. Intestine and spleen were carefully removed from liver samples by hand picking with forceps. Muscle samples were obtained by filleting the left side of the fish.

Gonadal condition, expressed as a relative index, the gonosomatic index (GSI), is the gonadal weight (the wet weight, prior to fixation, to the nearest 0.1 mg of both gonads), expressed as a per cent of the total weight of the fish. The GSI was calculated in experiments C, D, and E.

In experiment E, after weighing both gonads, one was fixed for histological sectioning and the other was placed in a 20 ml pyrex beaker for cadmium analysis. Liver and kidney of all fish in a group were pooled for an analysis of cadmium content. A small portion of the liver and kidney was used for histological sectioning. Muscle was analyzed for cadmium content, but not sectioned because small residues of cadmium which occurred in the muscle did not suggest the need for histological examination.

Histological preparation included fixation in Bouin's solution for a minimum of four days, dehydration to 70% isopropyl alcohol and

embedding in paraffin (Humason, 1967). The blocks were sectioned at 7.5 microns and affixed to glass slides with an albumin-glycerin solution, stained with Mallory's triple connective tissue stain, and hematoxylin and eosin using permount as a mounting medium. Four slides were made of each gonad. Two slides were made of each of three randomly selected kidney and liver tissue samples from each group.

Residue levels of cadmium in liver, kidney, muscle, and gonads were determined by atomic absorption spectrophotomety. Cadmium content of organs was accomplished by drying and ashing in 20 ml pyrex beakers. Dry weight was obtained by drying to constant weight ($\pm$0.1 mg) at 100 C in a drying oven. Dried samples were ashed in a muffle furnace at 550 to 575 C in the same beakers as they were dried. Loss of weight ($\pm$0.1 mg) on ignition was the ash weight. Cadmium in the ash was put into solution by dissolving it in 5 ml of 2 N nitric acid. Dial readings on an atomic absorption spectrophotometer were converted to ppm cadmium in the sample solution by expanding the per cent transmission scale to read 0 - 3 ppm cadmium using standards made from cadmium chloride where 1% transmission equaled 0.03 ppm. The ppm cadmium in the sample solution was converted to ppm cadmium wet weight, dry weight, and ash weight by the formula:

$$\frac{\text{ppm cadmium in solution} \times \text{ml of solution}}{\text{wet, dry, or ash weight (g)}} .$$

Reliable results were not obtained with the testis because of large and inconsistent variation in cadmium residue values. The amount of tissue yielded from some males was insufficient to weigh with enough precision to produce reliable results.

CHAPTER III

RESULTS AND DISCUSSION

Median Lethal Dose

The LD_{50} as determined in experiment A was 30.0, 23.0, and 20.0 mg/kg at 24, 48, and 96 hours, respectively (Figure 4). The maximum dose which did not produce mortality in 96 hours was 10 mg/kg. LD_{50} values of cadmium for fish were not found in the literature. Gross examination of the gonads gave no indication of damage caused by cadmium.

Intraperitoneal injections of 3.5 to 7.5 mg/kg body weight were sublethal to a variety of mammals (rats, mice, rabbits, hampsters, and guinea pigs), although a single subcutaneous injection of 7.5 mg/kg produced necrotic lesions in the testis (Parizek, 1957, 1960; Mason et al., 1964; Cameron and Foster, 1963; Gunn, Gould and Anderson, 1961; Kar and Das, 1960; Meek, 1959; Chiquione, 1964). The route of administration affects the lethal dose. Wilson, De Eds, and Cox (1941) reported cadmium administered subcutaneously and intravenously was lethal to rabbits at 18 and 5 mg/kg, respectively. They quote other authors as finding 250 ppm in food killed rats and perfusion of isolated frog hearts with 1 ppm caused cardiac arrest. However, none of these values are presented as LD_{50}'s.

A dosage of 7.5 mg/kg was used in experiment C on the basis of histopothological effects of this dosage on the mammalian testis and

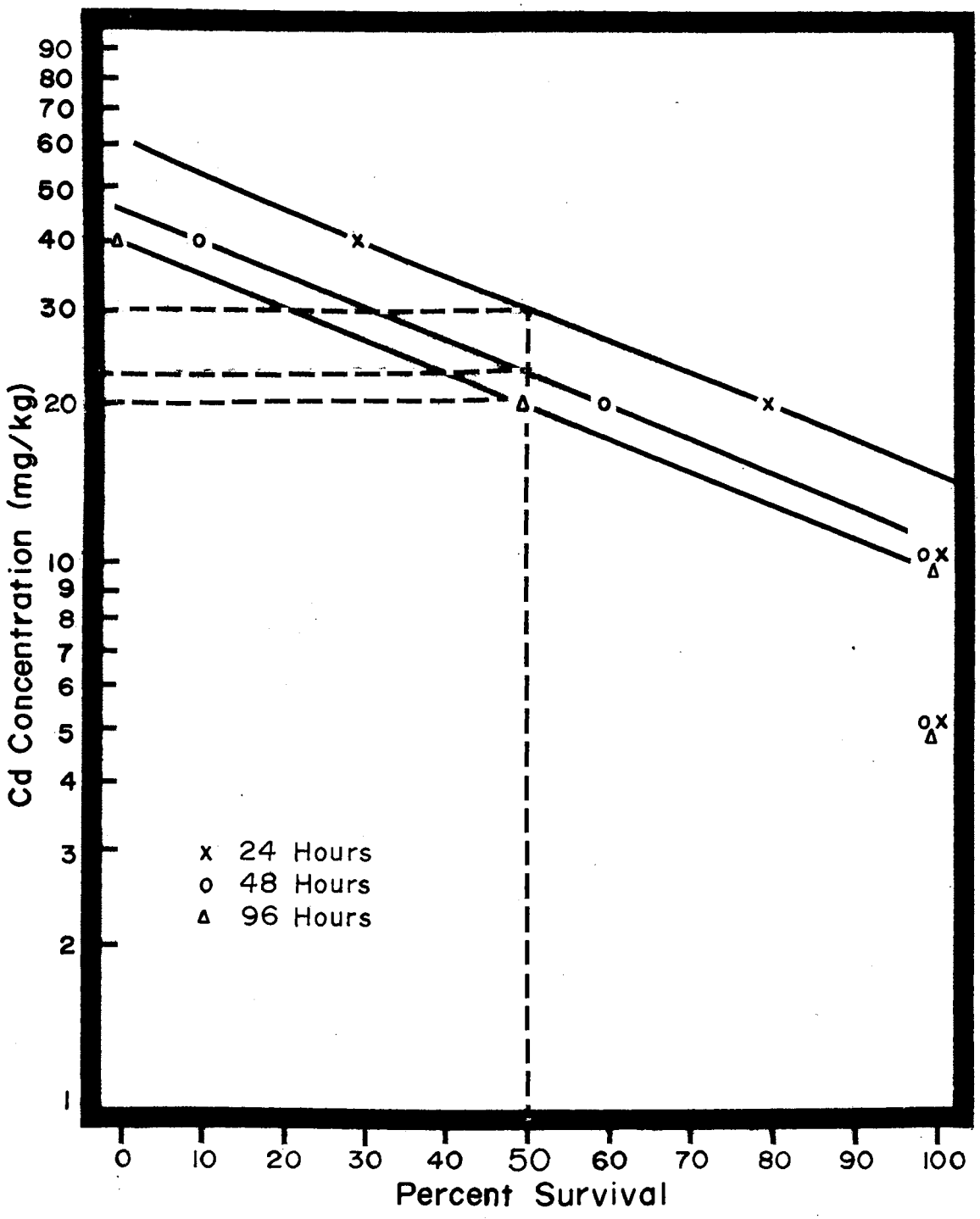

Figure 4. Cadmium Chloride LD_{50} for Goldfish at 24, 48 and 96 Hours. (Intersection of dashed lines with survival line (solid) indicates LD_{50} concentrations.)

knowledge that this dosage was considerably below the LD_{50} for 96 hours (20 mg/kg). However, the dosage was increased to 10 mg/kg in experiments D and E because a higher dosage would probably induce a greater effect. A dosage of 10 mg/kg allowed 100% survival in experiment A, but results from experiments D and E indicated <3% mortality over long periods.

Effects on Gonadal Weight

Experiment B

Because of variation and often only minor differences between groups, the GSI was used in this study only to indicate trends. The GSI was used only to determine if a consistent pattern of differences could be established between groups of fish receiving single and multiple cadmium injections, with and without hormone. It was not possible to determine the sex of the goldfish before they were killed which resulted in unequal sample size and often a very small sample size, especially with males. Those samples with only one or two individuals reduce the credibility of that GSI value. Low GSI values may have resulted either from inhibition due to injections or from stimulation and subsequent spawning. The action of HCG was very erratic and gonadal development was successfully achieved in control fish sporadically.

Experiment B was designed to determine if several times the dosage of an LD_{50} could be administered by repeated sublethal injections and to make preliminary observations on the effects on the gonads which may result from the anticipated accumulation of cadmium from multiple injections. A total of 37.5 mg/kg (23.0) cadmium chloride was injected into goldfish over a five-week period and one fish sacrificed each week

starting one week after the initial injection to determine the effects on gonadal weight. Goldfish survived a total dose of 37.5 mg/kg (23.0) cadmium chloride when administered over five weeks at levels of 7.5 kg/mg per injection. A single dose of 37.5 mg/kg would be expected to kill about 90% in 96 hours (Figure 4), and about 50% of the fish in five hours as observed in experiment A.

Smith, Smith, and McCall (1960) and Das and Das (1962) found that rabbits were insensitive to subcutaneous injection, but sensitive to intratesticular injections. Apparently, subcutaneous injections did not provide the critical level of testicular cadmium concentration obtained by direct organ injection and, thus, no damage resulted. Other authors have also produced necrosis in mammals with much lower doses using intratesticular compared with subcutaneous injections of cadmium (Kar and Kamboj, 1965; Chiquione and Suntzeff, 1965).

Experiment C

Experiment A indicated the maximum sublethal dosage and experiment B indicated that fish could tolerate much greater quantities of cadmium if administered by multiple sublethal injections. Experiment C was conducted to determine if changes in the mean GSI for a group of fish could be detected after a single treatment of 7.5 mg/kg (4.50) cadmium chloride and if a difference in effect of cadmium on the GSI would be observed in fish which had received injections of human chorionic gonadotropin (HCG) compared with the GSI of fish not given HCG.

Mean GSI of males in the group not given hormone or cadmium decreased from 2.8 to 1.7 between day 25 and 35, increased slightly from 1.7 to 2.2 between day 35 and 45, then increased again from 2.2 to 2.5

between day 45 and 55 (Table I). The over-all mean gonadal index of male controls given HCG (2.7 ± 0.8, 95% C.I.)[1] averaged slightly more than the controls without hormone (2.3 ± 1.7). The difference in the means of these two groups was not significant at .05(t_{25} = 1.600, $P_{.05}$ = 1.708). The variation in the response of the controls receiving HCG suggests a slight stimulatory response persisting 35 days, however, the increase is small and the GSI is essentially equal to the controls without HCG after 35 days.

At the end of 25 days, the mean GSI of males in both control groups was considerably higher (2.8 and 2.6 compared to 1.5 and 0.8) than either cadmium treated groups with and without HCG. At 35 days, a difference still existed, 3.1 for the control compared to 1.8 and 1.1 for the cadmium injected groups, except for 1.7 for the control group not receiving HCG. However, at 45 days, the values for all four groups were similar, ranging from 2.1 to 2.8. At 55 days, the GSI of the group receiving cadmium and hormone was 0.9% higher than the highest control.

The GSI of the fish injected with cadmium but not hormone increased from 1.5 to 2.4 from day 25 to 55. The GSI of fish receiving cadmium and HCG was only 0.8 on day 25, half of the lowest value (1.7) reported for either control group. The GSI of the cadmium plus HCG group increased throughout the period with the final value being fourfold greater than the initial one and highest of all groups at the termination of the experiment. Although the GSI of the group with cadmium but no HCG was only half of the value of the two controls at 25 days, it was approximately equal to them at 55 days.

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[1] All intervals presented are 95% confidence intervals.

TABLE I

MEAN GONOSOMATIC INDICES OF FISH FROM EXPERIMENT C AND D
(Numbers in parenthesis indicate number of fish)

	Time (Days)	Males W/ Cd		Males W/O Cd		Females W/ Cd		Females W/O Cd	
		W/O HCG	W/ HCG	W/O HCG	W/ HCG	W/O HCG	W/ HCG	W/O HCG	W/ HCG
Experiment C	25	1.5 (4)	0.8 (4)	2.8 (2)	2.6 (3)	5.2 (2)	3.1 (2)	8.3 (4)	7.1 (3)
	35	1.8 (5)	1.1 (3)	1.7 (4)	3.1 (3)	6.8 (1)	2.7 (3)	6.5 (2)	7.3 (3)
	45	2.3 (5)	2.1 (1)	2.2 (2)	2.8 (4)	---	2.7 (3)	3.4 (2)	5.4 (2)
	55	2.4 (4)	3.4 (3)	2.5 (4)	2.2 (5)	4.7 (2)	2.8 (2)	5.1 (1)	5.7 (1)
	$\bar{X}$	2.0	1.8	2.3	2.7	5.4	2.8	6.4	6.7
	N	(18)	(11)	(12)	(15)	(5)	(10)	(9)	(9)
Experiment D	0	---	---	2.0 (6)	---	---	---	2.7 (4)	---
	26	0.7 (5)	1.0 (5)	1.5 (15)	1.1 (8)	3.1 (1)	3.0 (5)	3.5 (5)	3.3 (12)
	56	1.2 (9)	0.3 (8)	1.0 (5)	1.5 (11)	2.4 (10)	2.6 (11)	3.2 (15)	3.0 (8)
	84	0.4 (3)	0.7 (10)	1.9 (13)	2.0 (9)	2.1 (2)	1.5 (10)	4.4 (7)	2.7 (10)
	114	1.1 (8)	1.4 (6)	2.4 (9)	0.8 (11)	4.0 (12)	3.2 (13)	5.6 (11)	3.9 (9)
	142	3.6 (9)	3.7 (4)	---	4.0 (4)	11.7 (8)	10.4 (3)	---	16.1 (2)
	177	1.9 (11)	1.4 (7)	1.2 (9)	3.2 (9)	7.3 (9)	6.7 (12)	7.8 (11)	9.8 (11)
	$\bar{X}$	1.7	1.1	1.7	1.8	5.7	4.1	4.2	5.3
	N	(45)	(40)	(57)	(52)	(42)	(54)	(53)	(52)

The over-all mean of the cadmium plus HCG group (1.8 ± 2.5) was lower than either control group or the cadmium group without HCG (2.0 ± 1.5). A comparison of over-all means of these groups indicates that there was a significant difference between the control group receiving hormone and the group receiving only cadmium ($t_{31} = 3.256$, $P_{.005} = 2.745$) as well as between the former group and the group receiving cadmium and hormone ($t_{24} = 2.535$, $P_{.01} = 2.492$). Differences in means (<.05) of all other groups were not significant. This would suggest that single intraperitoneal injections of cadmium reduced relative gonadal weight for a short period of time, but full recovery occurred by 55 days and that the HCG effect was slight but not significant. It is noted that the 35 and 45 day values of uninjected controls and fish receiving only cadmium were about equal; however, small sample size of individual sample dates indicate that over-all means more accurately suggest basic trends.

The GSI of female fish in both control groups, with and without HCG, averaged 3.6% higher than males but showed a similar pattern of variation in that the HCG injected fish had a higher over-all average GSI (6.7 ± 6.3) than the control group without hormone (6.4 ± 4.1). However, unlike males, GSI of females declined from day 25 to 45. The GSI of the control group receiving hormone was considerably lower on that day. The over-all mean GSI of the group with cadmium but without hormone (5.4 ± 4.0) was much lower than the control group without hormone. The over-all mean GSI of the cadmium group with hormone was 2.8 ± 5.6, or only half that of the cadmium injected group not receiving hormone. Cadmium administered to fish receiving HCG seemed to greatly

affect relative ovary weight as the GSI of this group was less than half of that of the control group.

A single injection of cadmium appeared to reduce the GSI of males for at least a month after injection, but the GSI recovered by day 55. Among females receiving cadmium injections, but not hormone, the GSI was conspicuously reduced after 25 days when compared to both control groups, followed by a brief recovery by day 35. In females, by day 55, the GSI of the cadmium injected group was lower but about equal to both control groups. Among both males and females, the GSI of the group injected with hormone and cadmium was lower than either of the two control groups or the group receiving cadmium but no hormone.

A comparison of the over-all means of these groups indicates that there was not a significant difference between the control groups with and without hormone ($t_{18} = 0.240$, $P_{.25} = 0.688$). No significant difference ($<.05$) was seen between either control group and the group receiving cadmium and no hormone. However, there was a highly significant difference ($>.005$) between the mean of the group receiving cadmium and hormone and all other groups. These results suggest that the injection of hormones slightly enhances the activation of both ovary and testis, although the difference is not significant. The injection of HCG and cadmium usually appears to enhance GSI reduction especially among females. The GSI activation may increase the uptake of or sensitivity to cadmium which may cause greater damage to the gonads than occurs in a less active gonad.

Experiment D

This experiment expanded observations on effects of cadmium as

studied in experiment C by using a larger sample size and by extending the observation period from 55 days to six months (Table I, p. 24).

The GSI of males not receiving hormone or cadmium averaged 1.6 and varied from 1.2 to 2.4 (Table I). The GSI of the hormone injected control group averaged 2.1 and varied from 0.8 at 114 days, about one-third the uninjected control value, to 4.0 at 142 days, almost twice the highest uninjected control value.

The GSI of males injected with cadmium but not HCG averaged 1.5, only slightly less than the control group not receiving HCG. The lowest GSI of the cadmium but no HCG group was 0.4 which occurred at 84 days. The highest GSI of this group was 3.6 nearly equal to the control group with hormone and 50% higher than the control group without HCG. The over-all GSI of males receiving cadmium and HCG was 1.1 ± 2.1, which was less than the cadmium but no HCG group (1.7 ± 2.6) and less than the control group with and without hormone (1.8 ± 2.4 and 1.7 ± 1.6, respectively). Fluctuations in the GSI of the cadmium plus HCG group was twelvefold, the lowest value (0.3) at 56 days, about one-third the lowest control value, and the highest value (3.7) at 142 days was nearly equal to the highest control group also receiving hormone. The GSI of the two control groups (i.e., no cadmium, with and without HCG) were consistently higher than either cadmium injected group, although all groups attained fairly high levels near the end of the experiment.

A significant difference ($>.01$) existed between the over-all mean of the HCG injected control group and all other groups. A similar condition was found in experiment C except in this case an even greater difference was demonstrated between control groups. All other groups showed no significant difference ($<.05$) in over-all means.

Effects of a single cadmium injection appeared to lessen after day 114, which is later than in experiment C where the GSI of males injected with cadmium equaled that of the controls by day 55. Since a highly significant difference in over-all means is seen between the HCG injected groups with and without cadmium, the inhibitory effect of cadmium is again demonstrated.

GSI of females varied considerably, but followed the same trends as the male groups. GSI of uninjected controls showed little change up to 56 days after the initial injection of cadmium, then increased through 177 days. Hormone injected controls showed a similar pattern except the rise did not begin until 114 days and reached a maximum at 142 days (also the highest mean GSI reached by any group.)

The group injected with cadmium and no hormone varied from 2.1 to 4.0 until 142 days, then increased sharply to 11.7 (threefold higher than the previous value). The GSI of 11.7 was higher than any level reached on this date by other groups except the hormone injected controls which was 16.1. Over-all GSI of cadmium plus HCG injected females averaged 4.1 ± 6.0, compared with 5.3 ± 7.7 and 4.2 ± 5.8 for the control groups with and without HCG, respectively, and 5.7 ± 9.7 for the cadmium but no HCG group. At 177 days, the GSI of all groups of females varied from 6.7 to 9.8, not greatly dissimilar but the two cadmium groups were smaller than the two controls.

A comparison of the over-all means of these groups indicated results similar to those found in males with significant differences ($>.05$) between the HCG injected control group and all other groups except the group injected with only cadmium. All other over-all group means showed no significant difference ($<.05$).

These results seem to indicate that a single cadmium injection without HCG contributed to an initial reduction of the GSI of both sexes but the effects largely disappeared by 177 days. This general trend is not significant when the over-all mean GSIs are compared for control versus cadmium injected fish without hormone injections. Difference in GSI of cadmium and control fish not receiving hormone which is not significant ($<.05$) is less than the difference in GSI of cadmium and control fish receiving hormone which is significant ($<.05$). The cadmium and hormone injected fish always had a much lower GSI than hormone injected controls. It is possible that the increased activity associated with hormone injections also increases uptake or sensitivity of the gonad to cadmium. In both experiments C and D, basic trends are reproduced which indicate that gonadal activity is a major factor influencing the effect of cadmium on the gonad.

Experiment E

This experiment was designed to provide further observations on the effects on GSI of a single cadmium injection, and a comparison of effects of multiple cadmium injections with single injections. There was not a control group without hormone. The GSI of hormone injected males without cadmium varied sevenfold from 0.3, day 86, to 2.2, day 13 (Table II). Although this group was given repeated HCG injections, there was wide variation in responsiveness. The over-all mean GSI of the control group with HCG was 1.3 ± 2.1, which was much higher than the male groups receiving single cadmium injection with and without HCG (0.4 ± 0.4 and 0.5 ± 0.7, respectively) or multiple cadmium injections with and without HCG (0.3 ± 0.3 and 0.3 ± 0.5, respectively).

TABLE II

MEAN GONOSOMATIC INDICES OF FISH FROM EXPERIMENT E
(Number in Parenthesis Indicate Number of Fish)

Time (Days)	Males					Females				
	Single Injection W/O HCG	Single Injection W/ HCG	Multiple Injection W/O HCG	Multiple Injection W/ HCG	Control	Single Injection W/O HCG	Single Injection W/ HCG	Multiple Injection W/O HCG	Multiple Injection W/ HCG	Control
0	---	---	---	---	1.7 (5)	---	---	---	---	6.5 (5)
13	0.7 (3)	---	---	---	2.2 (1)	2.2 (7)	---	---	---	2.7 (8)
31	0.7 (3)	0.4 (4)	0.3 (5)	0.4 (6)	0.4 (3)	2.5 (7)	2.0 (6)	1.7 (5)	1.8 (4)	2.1 (7)
44	0.3 (3)	0.4 (5)	0.6 (3)	0.6 (3)	1.3 (4)	1.8 (7)	2.8 (5)	2.0 (4)	1.7 (4)	2.1 (6)
86	0.2 (5)	0.3 (1)	0.3 (3)	0.2 (3)	0.3 (2)	2.3 (5)	3.0 (8)	2.2 (7)	2.0 (7)	2.2 (8)
122	0.3 (4)	0.6 (3)	0.3 (4)	0.2 (1)	0.3 (2)	2.0 (3)	1.6 (7)	2.0 (6)	2.0 (7)	2.0 (8)
159	0.9 (4)	0.5 (4)	0.3 (3)	0.3 (4)	0.5 (1)	2.0 (6)	2.5 (6)	2.0 (6)	1.5 (5)	5.1 (8)
215	0.4 (3)	0.3 (4)	0.4 (3)	0.3 (5)	1.7 (1)	2.5 (4)	3.0 (5)	3.2 (6)	1.5 (3)	5.8 (5)
615	---	0.5 (5)	---	0.2 (3)	0.4 (3)	---	4.9 (5)	---	2.9 (7)	3.4 (7)
$\bar{X}$	0.5	0.4	0.3	0.3	1.3	2.2	2.7	2.0	1.9	3.4

The GSI of males receiving HCG and a single injection of cadmium varied from 0.3, day 86, to 0.6, day 122. The over-all mean of this group (0.4) was only 40% of the controls with HCG (1.0). The GSI of the group receiving a single cadmium injection and no HCG varied from 0.2 (86 days) to 0.9 (159 days) which was slightly more than twice the values for other time periods. The GSI of males receiving HCG and multiple cadmium injections was consistently lower and varied from 0.2 to 0.6. The over-all mean GSI of this group averaged only 0.3 which was 30% of the control group with HCG. At intervals when gonadal development occurred in controls, the group with multiple cadmium had a very low GSI, often only one-fourth that of controls. Males receiving multiple cadmium injections but no HCG had an over-all mean GSI similar to the multiple injected group with HCG (0.3).

A significant difference in the over-all mean GSI existed between the controls and the group receiving a single cadmium injection with and without hormone ($t_{42} = 2.151$, $P_{.025} = 2.019$ and $t_{48} = 2.016$, $P_{.025} = 2.013$, respectively). An even greater significance is found between controls and the group receiving multiple cadmium injections with and without hormone ($t_{42} = 2.954$, $P_{.005} = 2.700$ and $t_{39} = 2.724$, $P_{.005} = 2.709$). The groups receiving a single cadmium injection with and without HCG were not significantly different from each other at the .05 level. However, the group receiving a single cadmium injection and no HCG was significantly different from the group receiving multiple cadmium injections with and without HCG ($t_{48} = 2.326$, $P_{.025} = 2.013$ and $t_{45} = 2.041$, $P_{.025} = 2.016$, respectively). The group receiving HCG and a single cadmium injection was not significantly different ($<.05$) from the group receiving multiple cadmium injections, but was

significantly different from the group receiving multiple cadmium injection plus HCG ($t_{42} = 1.786$, $P_{.05} = 1.672$). No significant difference occurred between the groups receiving multiple cadmium injections.

The results indicate inhibition of gonadal development only at time periods when gonadal development was produced in controls (44 and 215 days). The failure to produce a gonadal development in controls at other dates makes the results inconclusive. Small sample size also reduces the credibility of some GSI values. However, the over-all means support the general trend that the GSI of the single injected cadmium groups is significantly less than the control group and the GSI of multiple injected cadmium groups is significantly less than the singly injected cadmium groups and the difference between multiple injected cadmium groups and controls is highly significant.

The GSI of female controls ranged from 1.7, 13 days, to 5.8, 215 days, with an over-all mean of 3.4 ± 6.4. The GSI of females receiving HCG and a single injection of cadmium was lower than that of controls, ranging from 1.6 (122 days) to 4.9 (615 days) with an over-all mean of 2.7 ± 2.4. The GSI of fish receiving a single injection of cadmium and no HCG ranged from 1.8 (44 days) to 2.5 (215 days) with an over-all mean of 2.2 ± 1.5. Although the over-all mean GSI of both groups receiving a single cadmium injection is lower than controls, both groups exhibited the highest GSI at certain periods (86 days with HCG and 31 days without HCG).

The GSI of females receiving HCG and multiple cadmium injections were fairly consistent but were consistently lower than all groups ranging from 1.5 (159 and 215 days) to 2.9 (615 days) with an over-all mean GSI of 1.9 ± 1.6. This group never displayed a GSI higher than

that of the controls. The females receiving multiple cadmium injections and no HCG reacted similar to those also receiving HCG except a sharp rise in GSI was noted at 215 days. The range in GSI was from 1.7 (31 days) to 3.2 (215 days) with an over-all mean GSI of 2.0 $\pm$ 2.4. The GSI of all four cadmium injected groups remained near that of controls except 159 and 215 days, when they were much lower than the controls.

The over-all mean of controls was significantly different than that of the group receiving a single cadmium injection without HCG (t_{101} = 2.296, $P_{.025}$ = 2.007), but not significantly different (<.05) than that of the group with HCG. The control group was highly significantly different than the groups receiving multiple cadmium injections with and without HCG (t_{100} = 2.825, $P_{.005}$ = 2.647 and t_{96} = 2.432, $P_{.01}$ = 2.377, respectively). The group receiving a single cadmium injection and no HCG was not significantly different (<.05) than the group receiving multiple cadmium injections without hormone, but was significantly different than the group with hormone (t_{77} = 1.711, $P_{.05}$ = 1.667). The group receiving a single cadmium injection with HCG was significantly different than the groups receiving multiple cadmium injections with and without HCG (t_{80} = 2.631, $P_{.005}$ = 2.646 and t_{76} = 1.964, $P_{.05}$ = 1.668, respectively). The groups receiving multiple cadmium injections were not significantly different.

From the results recorded through 122 days, it would appear that cadmium had little or no effect on the GSI of female goldfish. However, the values at 159 and 215 days seem to indicate the opposite. By 615 days, it appears that all groups are able to reach fairly high GSI levels. At the time of necropsy, however, some ovaries were quite large, but appeared to contain only immature eggs, suggesting that

cadmium inhibits development of ova beyond immature stages. Comparison of over-all mean GSI indicates a significant difference (>.05) between controls and all cadmium injected groups except the singly injected group with HCG. There is also a significant difference (>.05) between single and multiple injected cadmium group except between both groups without HCG. These results closely parallel those found in males.

Trends suggested in experiment C and D were verified in experiment E: (1) the GSI of cadmium injected groups were usually lower than controls, (2) the over-all average GSI of cadmium injected groups were always lower than control groups (except females in experiment D), (3) the general over-all GSI of singly injected cadmium groups was lower than the GSI of controls and the GSI of multiple injected cadmium groups was lower than the GSI of single injected cadmium groups.

Cadmium Residues

The reduced GSI noted in experiments C and D paralleled damage observed in the gonads and kidney of mammals by other authors, and the indication of cadmium build up indicated by radioactive tracing studies following cadmium injections suggested the need for determination of cadmium residues in various organs. This portion of the current study was devoted to determination of cadmium residues in the gonads, liver, kidney, and muscle of goldfish injected with single and multiple doses of 10 mg/kg cadmium chloride over a period from 0 to 615 days.

Liver

The cadmium residues found in control fish ranged from 0.0 to 5.7

ppm (Table III). The group receiving a single cadmium injection and no HCG had cadmium residues ranging from 12.3 to 111.3 ppm with these values increasing from 74.9 ppm at 13 days to 111.3 ppm at 31 days then remaining around 100.0 ppm until 159 days when a steady drop was noted to 215 days (12.3 ppm) (Figure 5). The group receiving HCG and a single cadmium injection showed a similar pattern except a higher level was reached (139.6 ppm at 86 days) and the steady drop began at 122 days. The highest level reached by the group was 161.2 ppm at 615 days. This value appears to be out of line with the others. The group receiving multiple cadmium injections and no HCG had cadmium residues in the liver ranging from 87.5 ppm at 13 days to 241.7 ppm at 122 days (Figure 6). There was a steady increase throughout this period but the values then dropped 100 ppm at 159 and 215 days. The group receiving HCG and multiple cadmium injections ranged from 65.9 ppm at 13 days to 312.4 ppm at 122 days, also showing a steady rise to that point. The values then dropped to 223.8 ppm at 159 days, increased to 300 ppm at 215 days and finally was lowered to 165.4 ppm at 615 days, almost equal to the group receiving HCG and a single cadmium injection at the same date.

The consistent decrease in cadmium residues in the liver after discontinuation of cadmium injections in this study is in agreement with findings of Gunn and Gould (1957) who found liver tissue showed a decrease in cadmium content up to 20 days after injection. Others have found the cadmium levels in the liver of dogs after a single oral dose remained essentially constant (Decker, Byerrum, and Hoppert, 1957). In dogs, the liver contained the greatest total quantity of cadmium, due to its greater mass.

TABLE III

CADMIUM CONTENT (PPM) IN THE LIVER, KIDNEY, OVARY, TESTIS, AND MUSCLE OF GOLDFISH FROM EXPERIMENT E

Organ	Group	Time (Days)							
		13	31	44	86	122	159	215	615
Liver	Sing Injection	74.9	111.3	94.1	107.1	92.6	38.6	12.3	---
	Mult Injection	87.5	154.1	---	199.7	241.7	143.9	159.9	---
	Sing + HCG	105.3	103.5	---	139.6	71.6	34.9	33.1	161.2
	Mult + HCG	65.9	113.2	---	214.6	312.4	223.8	300.0	165.4
	Control	0.0	---	4.9	0.0	5.7	1.9	1.5	1.9
Kidney	Sing Injection	86.1	82.1	65.7	---	72.5	109.2	80.3	---
	Mult Injection	88.1	149.5	337.2	221.1	425.1	325.1	447.0	---
	Sing + HCG	108.0	84.7	154.1	---	113.1	93.9	118.8	366.4
	Mult + HCG	66.3	253.7	384.1	---	395.1	211.3	385.7	923.4
	Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	25.3
Ovary	Sing Injection	4.4	9.4	4.7	---	0.0	2.7	21.2	---
	Mult Injection	---	11.7	101.5	52.5	33.5	46.0	3.3	---
	Sing + HCG	---	0.0	14.2	20.5	5.3	29.8	1.8	7.3
	Mult + HCG	---	19.3	---	25.0	32.7	144.3	35.1	68.6
	Control	19.5	0.0	1.3	0.0	0.0	0.0	0.0	0.0

TABLE III (Continued)

Organ	Group	Time (Days)							
		13	31	44	86	122	159	215	615
Testis	Sing Injection	78.1	---	47.2	---	0.0	11.3	0.0	---
	Mult Injection	---	0.0	138.9	0.0	108.7	17.1	70.4	---
	Sing + HCG	---	---	0.0	---	0.0	---	0.0	21.8
	Mult + HCG	---	42.6	18.1	---	---	---	20.6	---
	Control	0.0	0.0	0.0	---	0.0	---	2.7	4.2
Muscle	Sing Injection	1.7	0.0	0.0	0.0	2.1	0.0	0.0	---
	Mult Injection	0.0	0.0	0.0	0.0	0.0	0.0	0.0	---
	Sing + HCG	2.1	0.0	1.5	2.1	0.0	0.0	0.0	1.3
	Mult + HCG	0.0	0.0	0.0	0.6	4.3	0.0	0.0	0.0
	Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

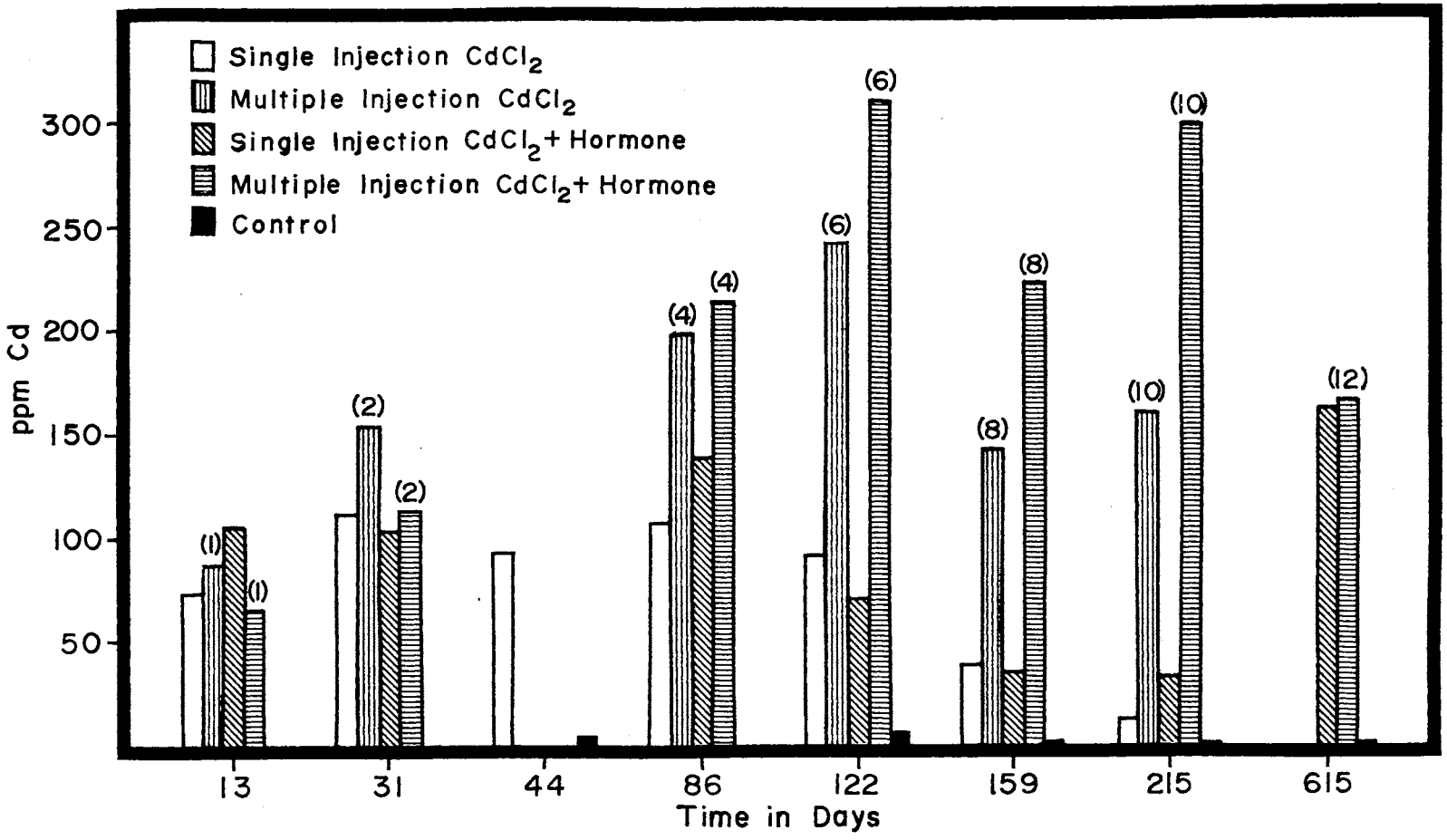


Figure 5. Cadmium Residues (ppm) in Liver of Goldfish Exposed to 0 - 120 mg/kg Cadmium Chloride From 13 to 615 days (Residues were determined only on days shown.)

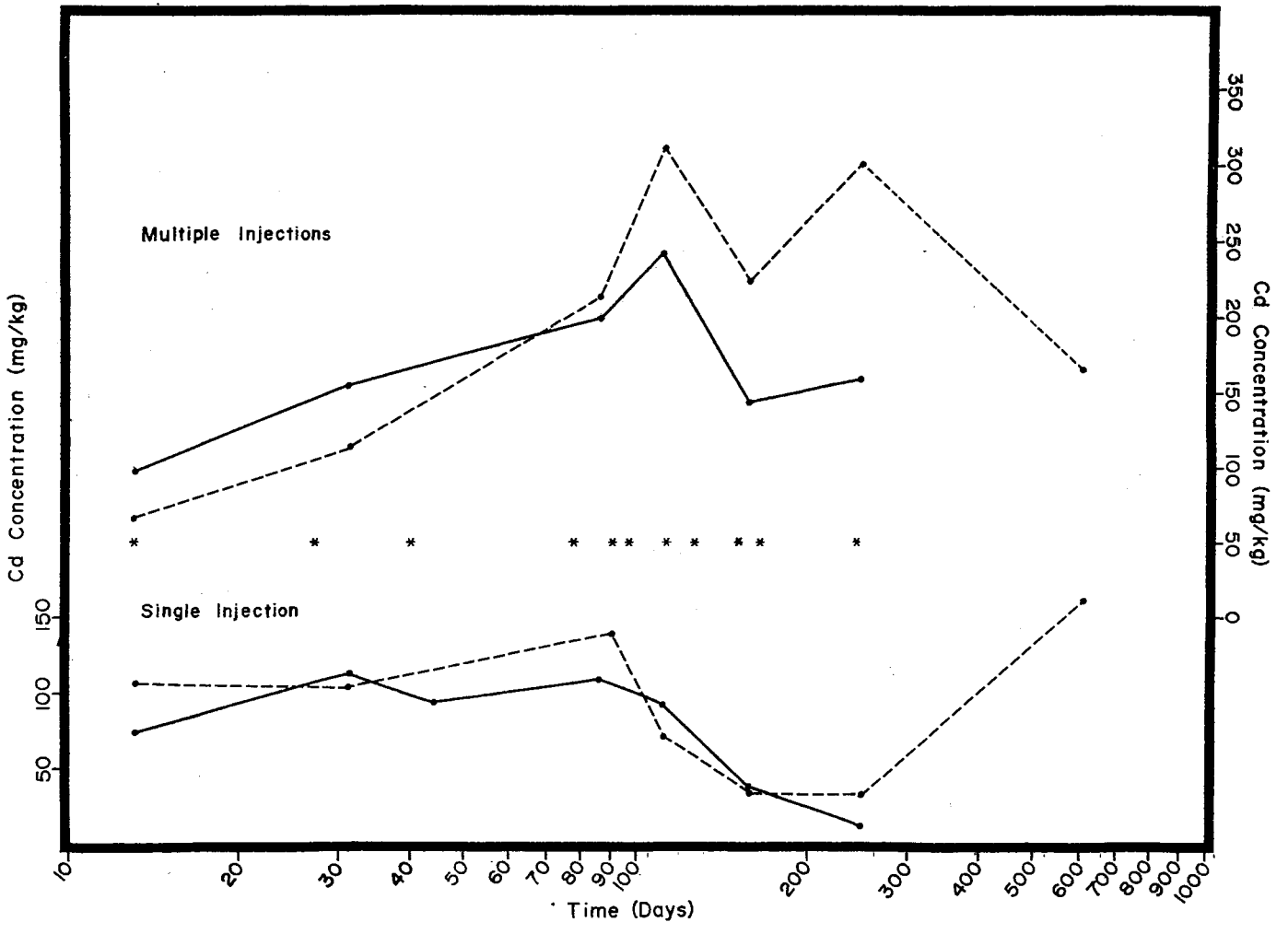


Figure 6. Comparison of Cadmium Residues (ppm) in the Liver of Goldfish Receiving Single Versus Multiple Injections in 10 mg/kg Cadmium Chloride (* injection dates; — fish W/ HCG; -- fish W/O HCG).

Kidney

The cadmium residue found in the kidney of control fish remained at 0.0 throughout the test period except at 615 days when it was 25.3 ppm. The group receiving a single cadmium injection and no hormone ranged from 65.7 ppm at 44 days to 109.2 ppm at 159 days (Figure 7). The cadmium residue following a single injection fluctuated, but no trend up or down was established. The group receiving HCG and a single injection of cadmium showed a similar consistent range of values although they were somewhat higher, 84.7 ppm at 31 days to 154.1 ppm at 44 days. The highest value of 366.4 ppm recorded at 615 days may be the result of a slow but steady increase in this organ over a long period of time. The group receiving multiple cadmium injections and no HCG had a residue value similar to those groups receiving a single injection at 13 days (88.1 ppm), but then showed a rapid steady increase to 337.2 ppm at 44 days (Figure 8). The remainder of the period had considerable fluctuation (>200 ppm), but the highest residue value for this group was recorded at 215 days (447.0 ppm). The group receiving multiple cadmium injections and HCG showed a similar pattern of increase from 66.3 ppm at 13 days to 395.1 ppm at 122 days. The residue values of this group remained above 200 ppm through the remainder of the period and reached 923.4 ppm at 615 days, probably due to steady accumulation over an extended period.

Radioactive cadmium tagging studies indicate a high concentration of cadmium in the mammalian kidney resulting from injection or feeding of cadmium compounds.

The dramatic build-up of cadmium residues in the kidney of goldfish and the consistent retention of high levels is in agreement with changes

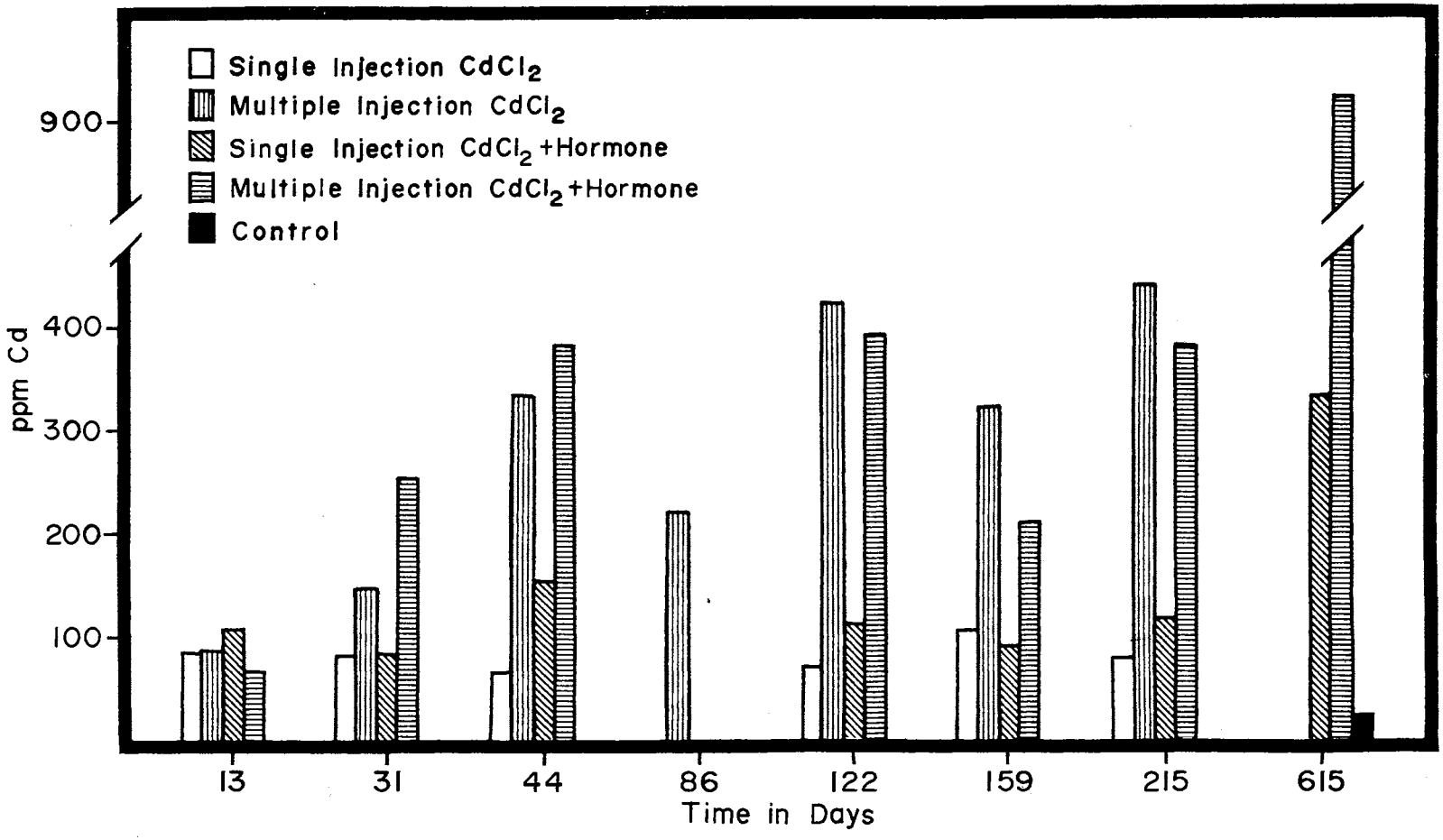


Figure 7. Cadmium Residues (ppm) in Kidney of Goldfish Exposed to 0 - 120 mg/kg Cadmium Chloride From 13 to 615 Days (Residues were determined only on days shown.)

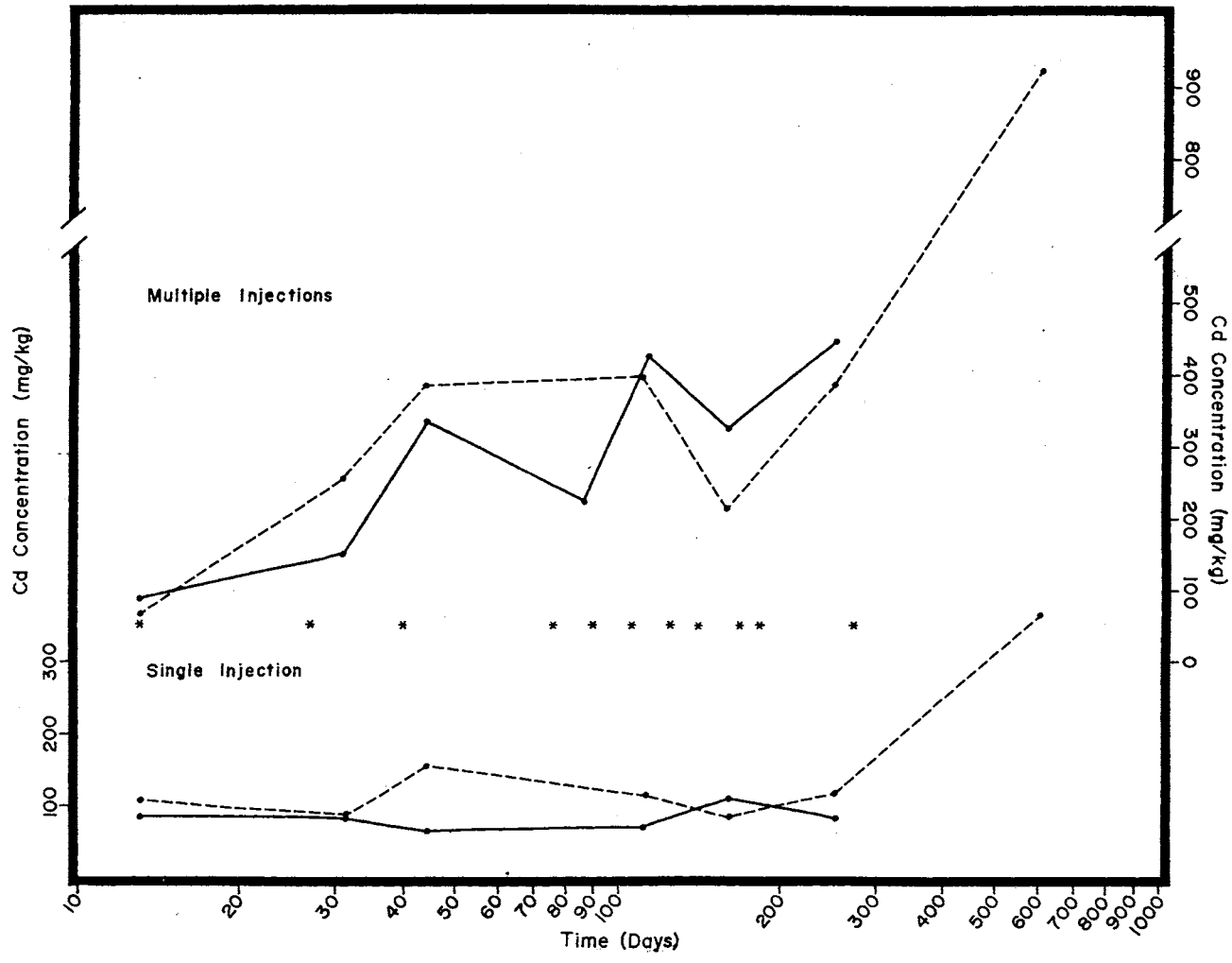


Figure 8. Comparison of Cadmium Residues (ppm) in the Goldfish Kidney Receiving Single Versus Multiple Injections of 10 mg/kg Cadmium Chloride (* injection dates; — fish W/ HCG; -- fish W/O HCG).

in cadmium levels in mammalian kidneys. High amounts of cadmium were found in the kidney of rats (Walsh and Burch, 1959; Decker, Byerrum, and Hoppert, 1957) and mice (Berlin and Ullberg, 1963) after a single cadmium injection. Gunn and Gould (1957) more specifically reported that the cadmium content of the kidney cortex increased sharply while the content of the medulla increased slowly. They also found the cadmium residue increased more slowly in young rats suggesting a relationship between cadmium retention and number of nephons present. Other authors reported accumulation of cadmium in the liver, kidney, intestine (Berlin and Ullberg, 1963) and adrenals (Walsh and Burch, 1959) after a single cadmium injection. Anwar, Hoppert, and Byerrum (1960), among others, point out that the kidney has the highest cadmium concentration per unit weight of organ although the liver has the greatest total amount of cadmium.

Only a small turnover of cadmium was indicated in mammals by Cotzias, Borg, and Selleck (1961). Lucis, Lynk, and Lucis (1969) found that after a single injection of cadmium chloride, the concentration of cadmium in blood cells, liver, and kidney increased for 336 hours. Walsh and Burch (1959) reported cadmium levels in the blood of rats receiving a single injection of cadmium decreased rapidly within 24 hours.

Gonads

Cadmium residues were found in the ovary of control fish only at 13 days (19.5 ppm) and 44 days (1.3 ppm) (Table III). The group receiving a single cadmium injection and no HCG had relatively low cadmium residues in the ovary, ranging from 0.0 ppm at 122 days to 21.2 ppm after

215 days. The cadmium residue in the ovary of the group receiving a single cadmium injection and HCG ranged from 0.0 ppm (31 days) to 29.8 ppm (159 days). The cadmium residues of both groups receiving a single injection showed minor fluctuations but no trend, up or down, was observed with time.

The groups receiving multiple cadmium injections had cadmium residues ranging from 3.3 ppm (215 days) to 101.5 ppm (44 days) in the group not receiving HCG, and 19.3 ppm (31 days) to 144.3 ppm (159 days) in the group receiving HCG. The group receiving multiple cadmium injections and no hormone had a maximum cadmium residue at 44 days followed by a continuous drop in cadmium through 215 days (Figure 9). However, the group receiving multiple cadmium injections and HCG had a progressive increase in cadmium residue through 159 days followed by a sharp drop, then a rise. It is not clear whether cadmium accumulates in the ovary, but both groups receiving multiple cadmium injections had consistently greater cadmium residues than groups receiving single injections even after 615 days (68.6 ppm and 7.3 ppm, respectively). This supports a hypothesis stated earlier (experiments C and D) that the greater decrease in GSI in fish receiving multiple compared to single cadmium injections was the result of greater uptake.

The inability to obtain a sufficient sample of testicular tissue resulted in many missing residue determinations and large variation in residue values obtained. Since the dry weight of the organ is the divisor for calculation of ppm, the very small dry weights obtained for testis caused large fluctuations in residues from a difference of a few tenths of a milligram in testis weight. Also, the testis could not be

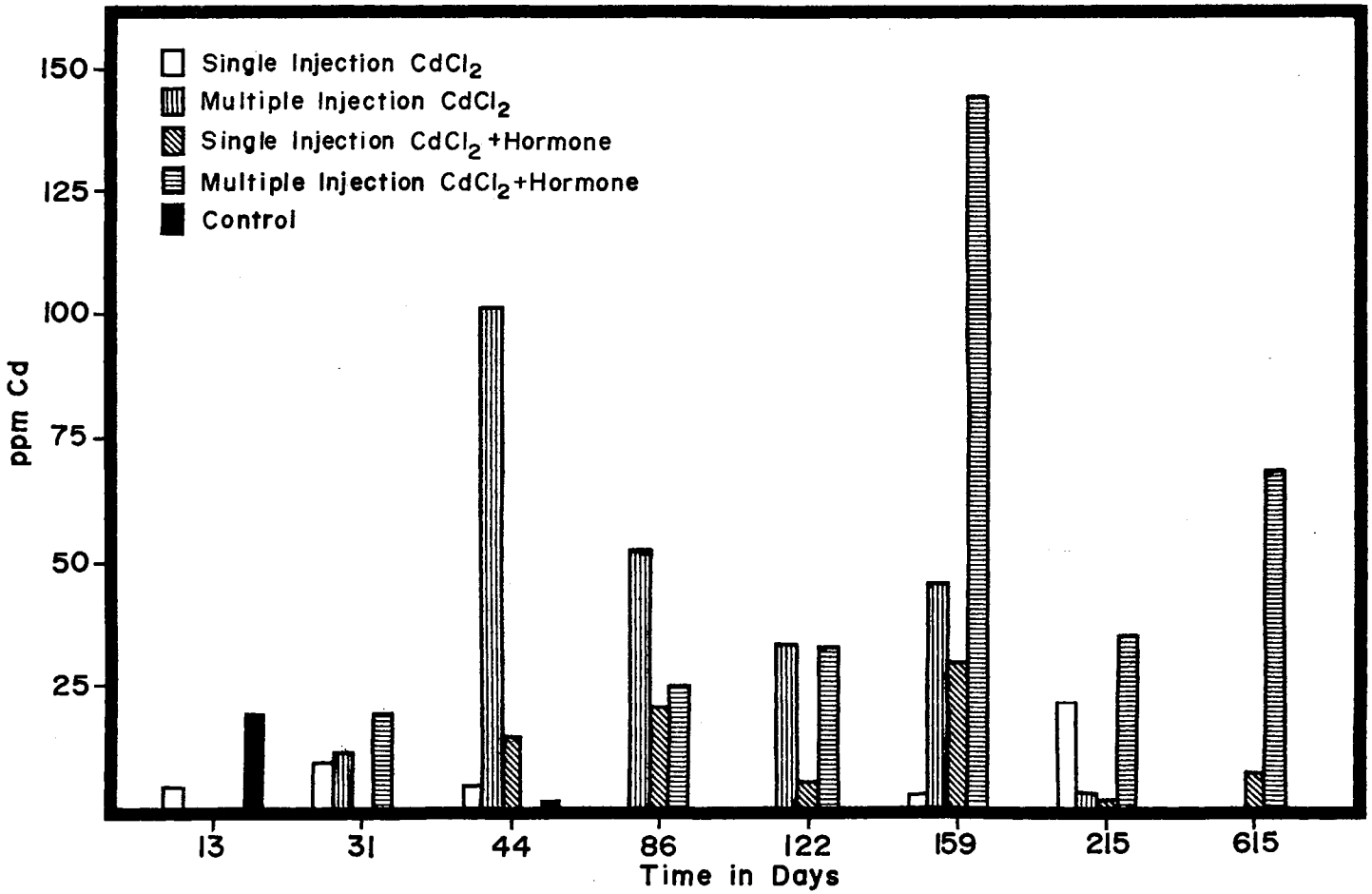


Figure 9. Cadmium Residues (ppm) in Ovary of Goldfish Exposed to 0 - 120 mg/kg Cadmium Chloride From 13 to 615 Days (Residues were determined only on days shown.)

found in many fish, especially those which had received multiple cadmium injections.

The sharp decrease in GSI of males receiving cadmium injections suggested a high concentration of cadmium would be found in the testis. However, this was not the case. Not enough residue was obtained in any group to establish trends, however, it is noted that the controls had no cadmium residue until 215 days (2.7 ppm) and 615 days (4.2 ppm) (Table III, p. 36). Groups receiving a single injection of cadmium had residues ranging from 0.0 ppm to 78.1 ppm while groups receiving multiple cadmium injections ranged from 0.0 ppm to 138.9 ppm. The only trend with time appeared to be a decrease in cadmium residue level in the group receiving multiple cadmium injections and no HCG (Figure 10). A greater frequency of high values (>70.0 ppm) was noted in the groups receiving multiple cadmium injections than in singley injected fish or controls. Cadmium was still detected (21.8 ppm) 615 days after a single injection. The testes were so small (i.e., nearly destroyed) by 615 days in fish receiving multiple cadmium injections that they could not be found. Since the testes of the multiple injected group could not be found, data is lacking in these groups when concentrations were probably maximum.

The high cadmium residue levels found in the liver and lower levels found in the testis are in agreement with those studies conducted on mammals. Berlin and Ullberg (1963) found significant amounts of cadmium in the interstitial cells of the testes. Parizek (1960) stated that, after a single cadmium injection, the liver contains 100 times as much cadmium as the testis. This ratio is somewhat higher than that found in goldfish which was about 15 times more cadmium found in the liver

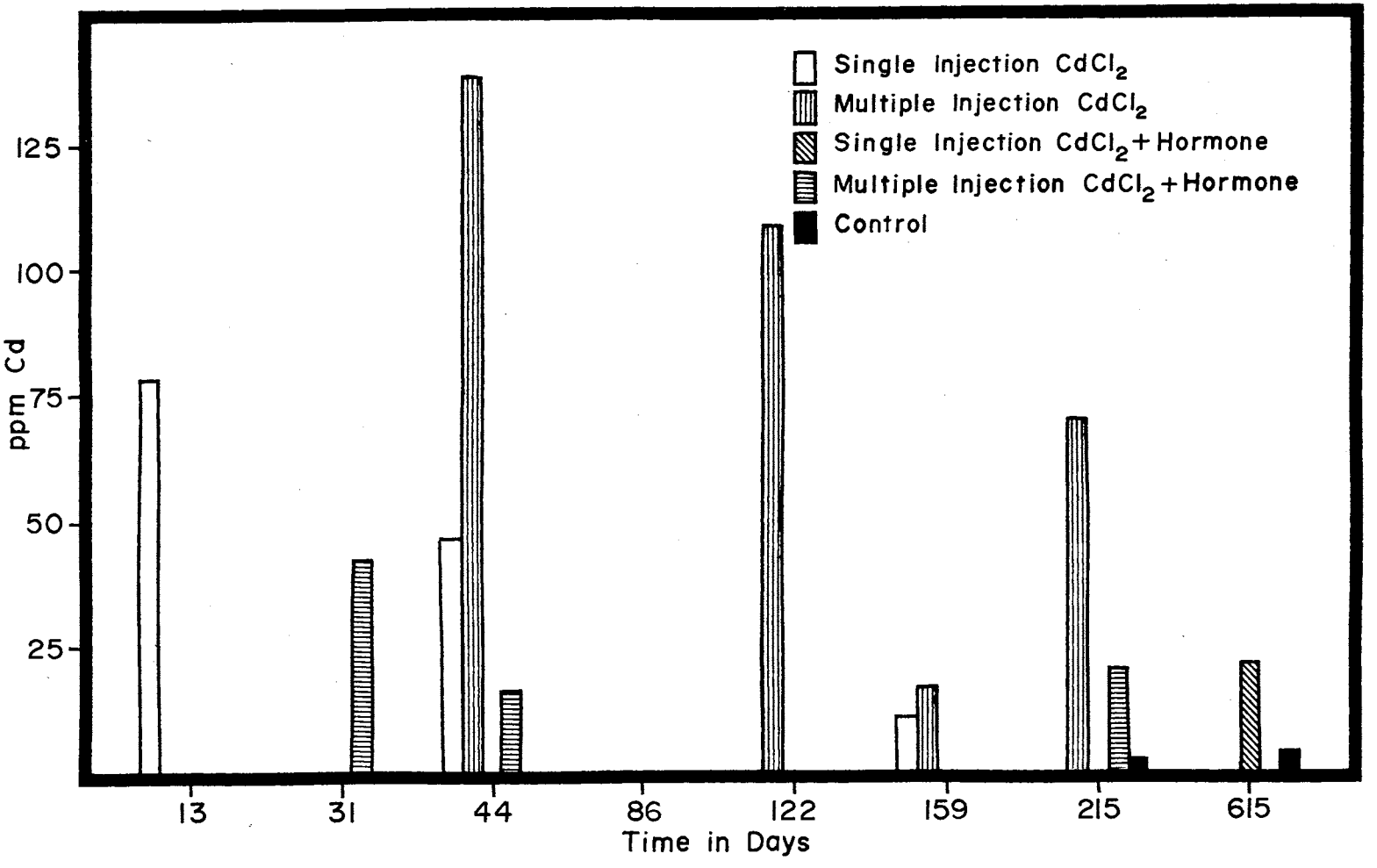


Figure 10. Cadmium Residues (ppm) in Testes of Goldfish Exposed to 0 - 120 mg/kg Cadmium Chloride From 13 to 615 Days (Residues were determined only on days shown.)

than in the testis. This ratio is only an estimate since the quantities present in both organs were not examined from the same fish.

Muscle

Cadmium was never found in the muscle of control fish and only rarely detected in muscle of fish receiving injections of cadmium. Highest cadmium levels in muscle of injected fish were 1.3 ppm 615 days after a single cadmium injection and 6.5 ppm over a year after termination of twelve cadmium injections.

It would be expected that two groups of fish receiving exactly the same quantity of cadmium would have very similar quantities in the organs. However, there was a considerable difference in the quantity of cadmium found in the liver and kidney of groups administered identical doses of cadmium. Usually, the group also receiving hormone had the highest quantity. Similar differences occurred in the other organs, but the differences were less pronounced. Differences in quantities of cadmium occurring in organs of fish receiving HCG and those not receiving HCG parallel the differences observed in GSI. A hypothesis that the increased metabolic activity produced by the hormone (or any number of other factors) caused a greater uptake of cadmium seems substantiated by residue analysis.

It should also be mentioned here that cadmium levels in the food used for these studies of about 0.7 ppm dry weight may account for cadmium residues found in controls.

Fish injected with cadmium had larger amounts of cadmium in the tissue compared to cadmium residues in the controls (Figures 5, 7, 9, 10, and Table III). The quantities of cadmium found in the organs

remained largely unchanged, except for liver, even more than 600 days after the single injection and more than one year after the last injection in the case of fish receiving multiple cadmium injections. Missing values in Table III were due to insufficient quantity of tissue available, levels below the minimum detectable levels, no tissue available (gonads) and accidental loss of material.

Histology

Observations were made on the histology of several organs to determine the extent of pathological changes occurring after single and repeated injections of 10 mg/kg cadmium chloride. Only the gross histological changes of the gonads, liver, and kidney tissue collected in experiment E are discussed.

Testes

Single and multiple injections of cadmium reduced the relative testis weight. A greater reduction occurred in males receiving HCG. Associated with the reduction in relative testis weight in experiment E were changes in spermatogenesis by the examination of histological sections. Spermatogenesis was divided into eight stages, determined by the degree of maturity of the germ cells (Ahsan, 1966). A summary of each developmental stage is given in Table IV and illustrated in Figures 11 and 12. Controls and cadmium injected fish were evaluated and an average computed for each group for each sample date.

A single intraperitoneal injection of 10 mg/kg cadmium chloride produced histological changes in 40% of the small adult male goldfish examined in experiment E. Groups receiving up to 12 cadmium injections

TABLE IV

DEVELOPMENTAL STAGES OF THE GOLDFISH TESTIS

Stage		Description
1	Early Resting	Consists primarily of resting primary spermatogonia
2	Late Immature	Slow mitotic activity, few secondary spermatogonia
3	Earl Maturing	Beginning of brisk spermatogenic activity with few mature sperm present
4	Late Maturing	Extensive spermatogenic activity, many mature sperm present
5	Early Mature	Last remnants of spermatogenic activity, some spermatocytes, lobules filled with sperm
6	Late Mature	Lobules bulding with sperm, no spermatogenic activity, sperm not in sperm ducts
7	Early Spent	Lobules still full but less bulging and sperm ducts also full of sperm
8	Late Spent	Lobules may contain a few remnant sperm, some sperm still in sperm ducts

Figure 11. Early Developmental Stages of the Normal Goldfish Testis (PG = primary germ cell, SG = spermatogonium, SC = spermatocyte, SP = sperm)

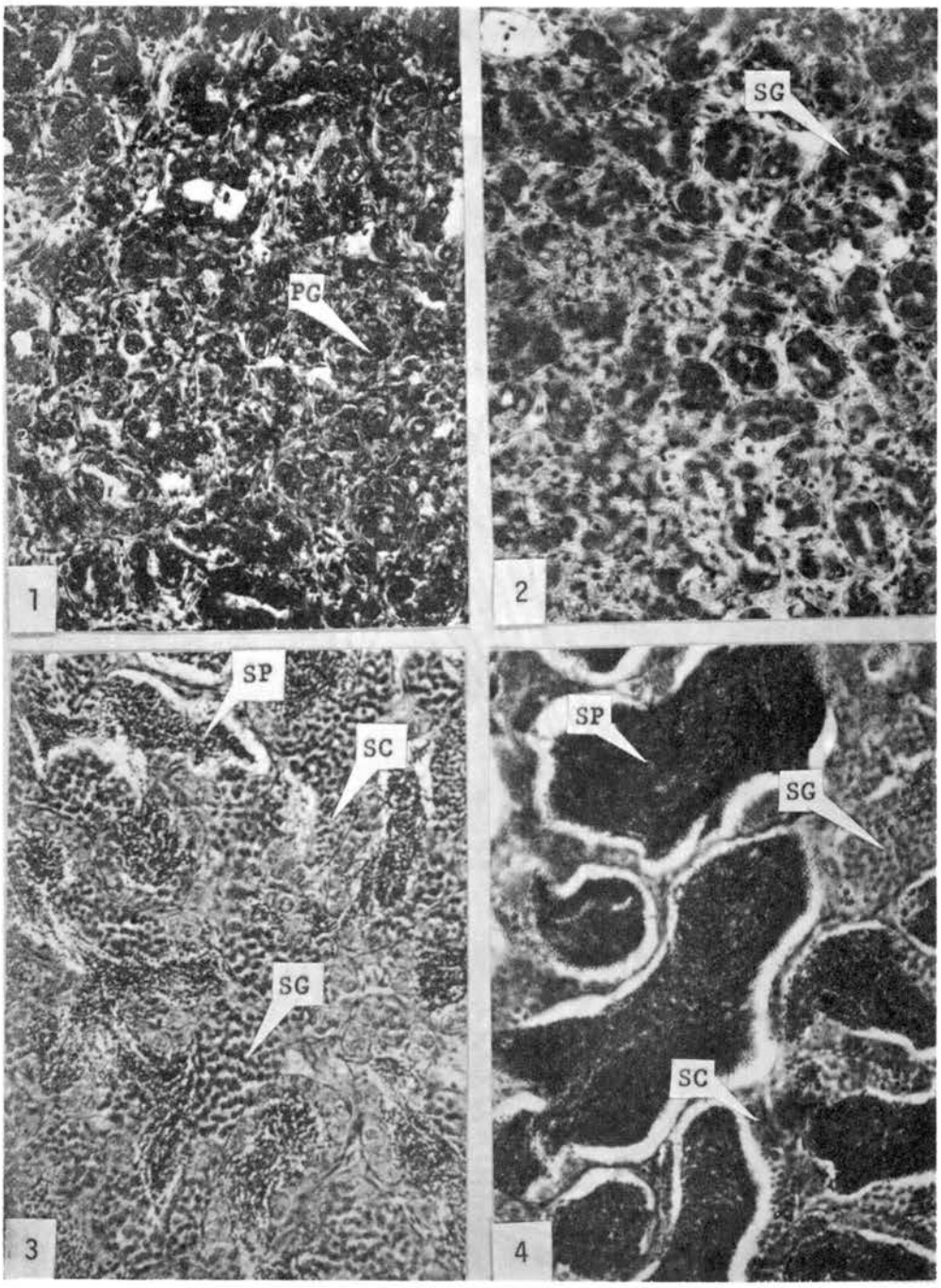
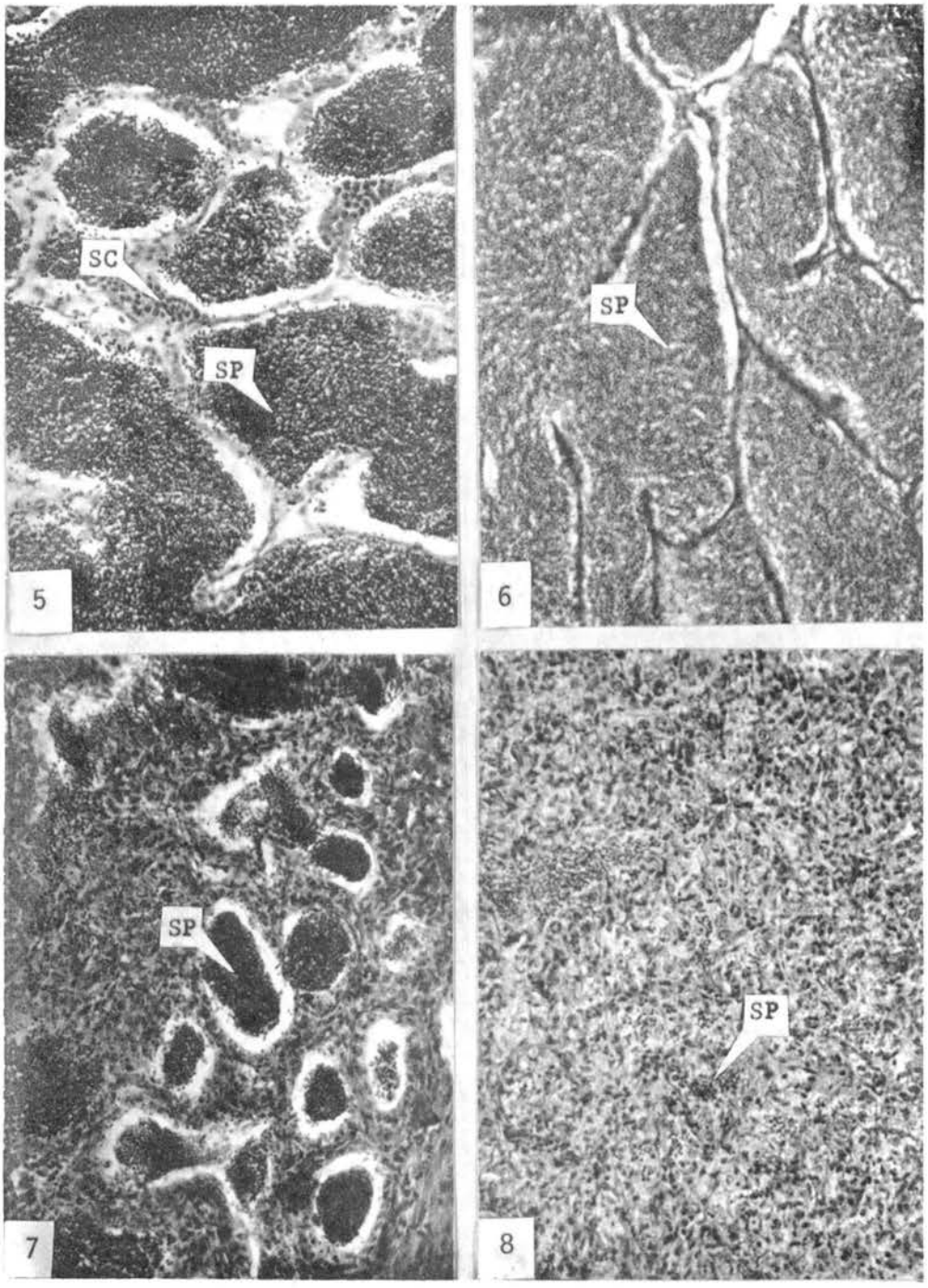

PG
1
SG
2
SP
SC
SG
3
SP
SG
SC
4

Figure 12. Late Developmental Stages of the Normal Goldfish Testis (SC = spermatocyte, SP = sperm)

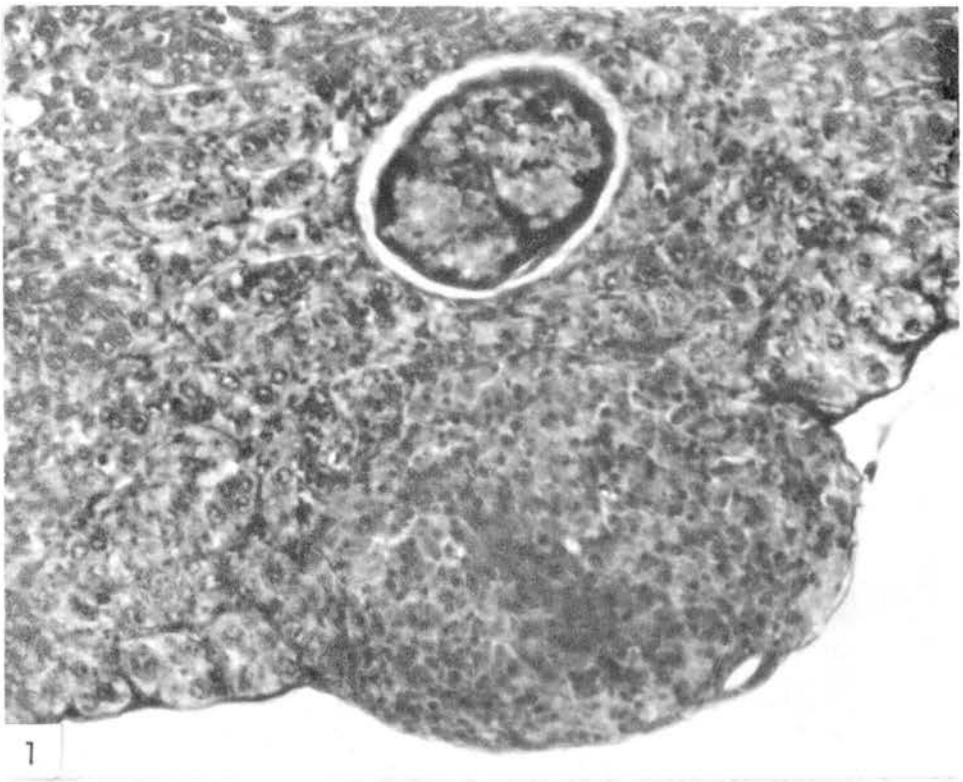

SC
SP
5
SP
6
SP
7
SP
8

had the same types of pathological changes as singly injected fish, but the frequency of occurrence and size of area affected increased as the number of cadmium injections increased. After five injections, nearly 100% of the fish were affected. The most obvious histopathological change was a sharp increase in the number of macrophages. The macrophages contained numerous small granules and appeared to be phagocytizing cellular debris. In severely damaged organs, these macrophages were aggregated to form small granulomas. Scattered among the macrophages were small mononuclear inflammatory cells. The granules were, as the remainder of the testis, not birefringent with polarized light, negative with Perl's stain testing for iron, and negative with sodium rhodizonate stain, which produces a colored precipitate at an acid pH in the presence of cadmium. A positive periodic acid-shiffs reaction indicated the presence of any of a group of compounds including glycogen, fibrins, and collagens in the macrophages. Another obvious pathological change was the occurrence of focal areas containing some macrophages and necrotic debris circumscribed by fibrous connective tissue (Figure 13 (a)). These areas often appeared to be completely disassociated from the surrounding tissue.

Often, the granulomas were seen at the surface of the testis forming a protruding growth (Figure 13 (1)). These granulomas contained numerous macrophages which appeared to be smaller than those found in the interior of the organ and many small mononuclear inflammatory cells.

Occasionally, the controls also had small focal accumulations of macrophages present, but usually they were diffusely distributed in small numbers throughout the organ. Degeneration of the germinal epithelium occurred after a single cadmium injection and after multiple

Figure 13. Photomicrograph of Testis From Goldfish Receiving: (1) Three Injections of 10 mg/kg Cadmium Chloride Showing Focal Granuloma Protruding From Surface of Organ, 315X; (2) Twelve Injections of 10 mg/kg Cadmium Chloride Showing Nearly Complete Destruction of the Organ and Only a Few Germ Cells, 315 X



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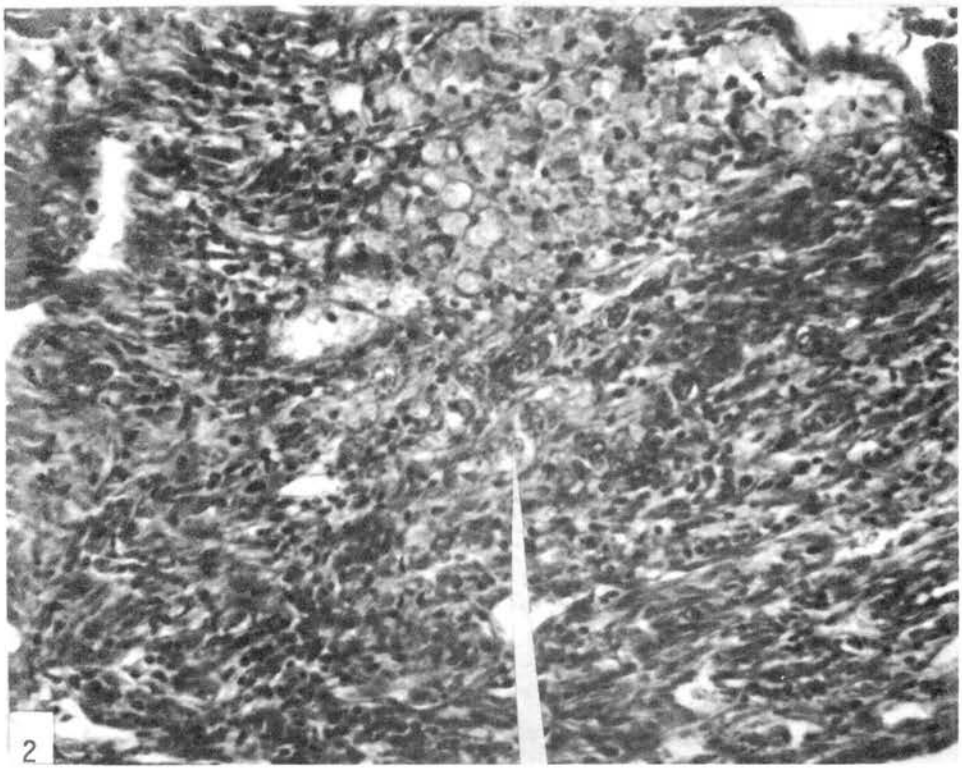

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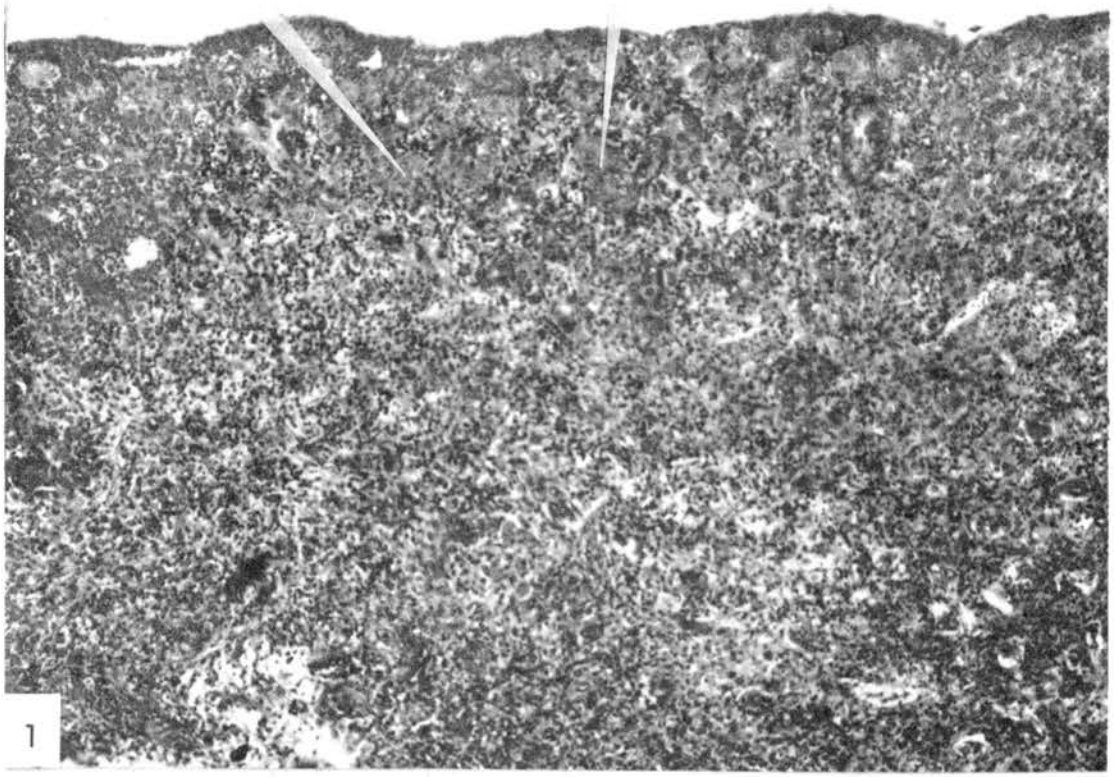
injections the entire seminiferous tubule was damaged. When damage was intense, primary germ cells appeared condensed, darkened, and finally separated from the basement membrane leaving only a shell of the seminiferous tubule (Figure 14 (2)). The nucleus and cytoplasm of the primary germ cells became darker and appeared to take up more Orange G, whereas normal tissue took up more acid fuchsin at this stage, indicating a change in the tissue from acidic to basic. The loss of primary germ cells resulted in a depletion of the number of seminiferous tubules (Figure 13 (2)), which were replaced with connective tissue and mononuclear inflammatory cells. This depletion occurred first in the interior of the organ leaving seminiferous tubules only at the periphery (Figure 14 (1)). After nine or more cadmium injections, the testicular tissue were difficult to recognize because of replacement of seminiferous tubules (Figure 14 (3)). These histopathological changes were never observed in testes undergoing spermatogenesis.

Comparison of the histopathological condition of fish sacrificed after receiving one or more cadmium injections showed damage in fish receiving multiple cadmium injections to be greater than in those receiving single injections which was greater than in controls. Especially obvious were the changes observed after six or seven injections. These differences were quantified by averaging the stages of development of males in each group of experiments D and E, although experiment D does not demonstrate these differences as well as experiment E. In the latter experiment, the mean stage of testis development of controls was higher than the cadmium treated groups at all time periods except 13, 44, and 159 days. At these time periods, the mean developmental stage of the testis was greatest in the group receiving only a single

Figure 14. Photomicrographs of Testis From Goldfish Receiving: (1) Six Injections of 10 mg/kg Cadmium Chloride Showing Partial Destruction of the Organ and Germ Cells Remain Only at Periphery, 200 X; (2) Six Injections of 10 mg/kg Cadmium Chloride Show Condensation and Separation of Germinal Cells From Connective Tissue Network, 315 X; (3) Twelve Injections of 10 mg/kg Cadmium Showing Almost Complete Destruction of the Germ Cells, 315 X



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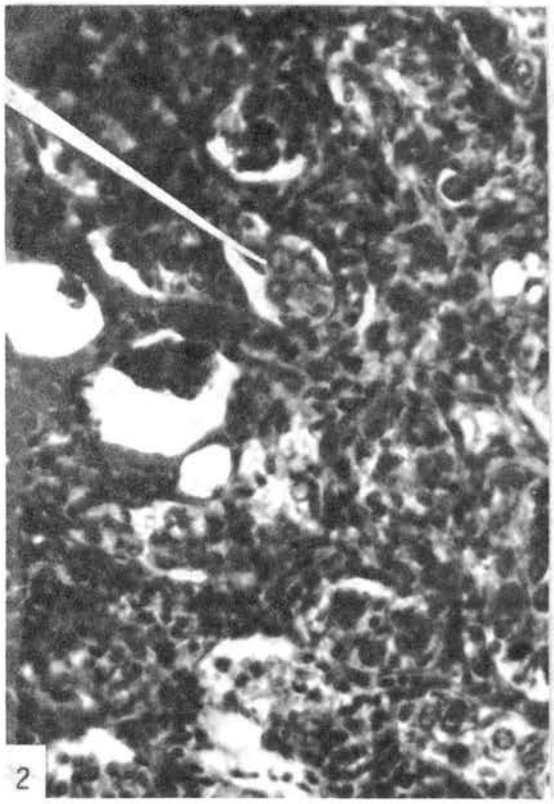

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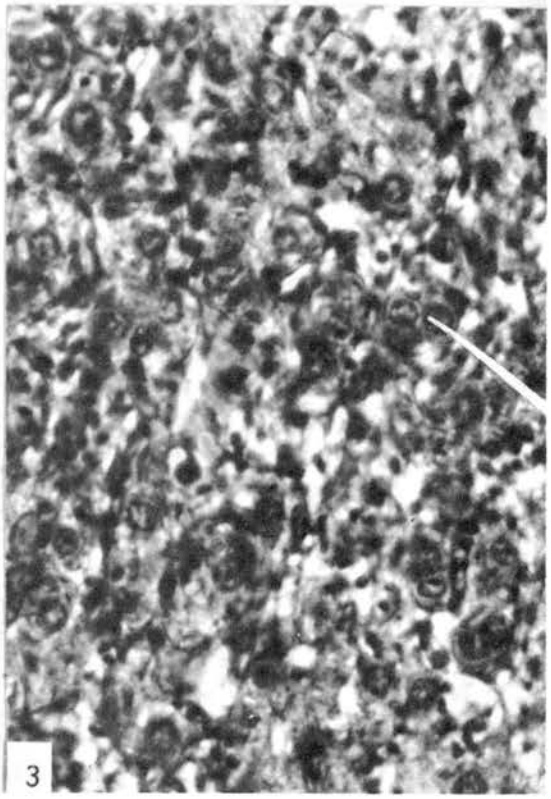


3

injection of cadmium. The other three groups receiving cadmium never had a mean stage of development higher than controls.

Fish receiving a single cadmium injection plus hormone had a lower mean stage of testicular development than singly injected fish without HCG (Table V). However, the lowest mean stage of development was found in fish receiving multiple cadmium injections after 44 days and only minor variations occurred between the group receiving hormone and that which did not. Very little difference occurred between control and cadmium injected groups in experiment D where the cadmium treated fish received only one injection. All mean stages of development were similar at the beginning of both experiments. The quantities of cadmium were somewhat higher in experiment D than in experiment E, especially for the group receiving both cadmium and hormone injections. Using the mean stage of testicular development as an index, testicular damage was greater after multiple compared with single injections.

Histopathological changes have been noted in mammalian testis after exposure to cadmium. Berlin and Ullberg (1963) found significant amounts of cadmium in the interstitial cells of the testis. Pronounced histological changes occurred in spermatogenic epithelium of rabbits 24 hours after a single subcutaneous injection (Cameron and Foster, 1963). Nuclei of spermatogonia, spermatocytes and spermatids were disrupted and a general disarrangement occurred in the regular succession of cells. A single injection of 1.1 mg/kg cadmium chloride in rats damaged the germinal epithelium and caused a complete denudation of seminiferous tubules except for an occasional Sertoli cell (Mason et al., 1964). The damage was said to be similar to that of Vitamin E deficiency in rats. Parizek (1960), who first reported distructive effects of cadmium on

TABLE V

MEAN STAGE OF GONADAL DEVELOPMENT OF MALE GOLDFISH IN EXPERIMENTS D AND E

Group	Experiment D Days						
	27	56	84	106	134	179	$\bar{X}^1$
Control	6.0 (3)	5.8 (5)	6.3 (4)	6.7 (3)	4.0 (2)	4.0 (2)	5.7 (19)
Control + HCG	5.5 (2)	6.4 (5)	6.7 (4)	5.7 (4)	---	7.5 (4)	6.5 (19)
Cadmium	7.0 (1)	6.0 (4)	3.5 (2)	5.3 (3)	5.3 (3)	5.4 (5)	5.4 (18)
Cadmium + HCG	6.3 (3)	7.0 (3)	4.7 (3)	6.0 (4)	4.0 (2)	6.5 (4)	5.9 (19)

Group	Experiment E Days								
	0	31	44	86	122	159	215	615	$\bar{X}^1$
Single Cadmium	5.5 (2)	7.3 (2)	7.5 (2)	3.4 (5)	4.5 (2)	4.0 (4)	2.9 (7)	---	5.3 (24)
Multiple Cadmium	4.5 (2)	7.0 (1)	4.0 (2)	0.0 (3)	1.3 (4)	2.0 (2)	0.0 (1)	---	2.2 (15)
Single + HCG	4.0 (2)	4.5 (2)	1.0 (2)	0.0 (1)	3.0 (2)	3.7 (3)	1.0 (3)	5.0 (1)	3.5 (17)
Multiple + HCG	---	6.0 (1)	3.3 (3)	3.0 (3)	0.0 (1)	1.0 (3)	0.3 (4)	0.0 (1)	1.8 (16)
Control	6.0 (2)	7.5 (2)	5.7 (3)	4.5 (2)	7.0 (3)	1.0 (1)	3.0 (5)	3.0 (2)	4.8 (20)

[1]Weighted mean of all samples; all numbers in parenthesis indicate sample size.

testicular tissue of rats, produced testicular necrosis in rats with a single subcutaneous injection of 3.7 mg/kg cadmium chloride. Regeneration of interstitial tissue occurred below the tunica albuginea and hormone production resumed, but the tubules did not regenerate. Even two years after a single subcutaneous injection of 1.2 mg/kg of cadmium chloride, Allanson and Deansley (1962) reported the germinal epithelium from the tubule was absent, apparently replaced by an amorphous mass of non-fatty material; however, these rats had regained androgenic activity by 266 days. Tubule regeneration could occur with doses of less than 1.2 mg/kg of cadmium chloride, but none occurred at larger dosages. At very low doses (0.45 mg/kg of cadmium chloride), damage to germinal epithelium was irregular and regeneration was the rule. Kar and Kamboj (1965) described the testis of the rat as completely necrotic and the seminiferous epithelium as a debris after a single injection of 2.5 mg/kg of cadmium chloride.

The mode of action of cadmium in the body has not been determined, however it has been suggested that cadmium can replace zinc in the body (Kar and Kamboj, 1965; Parizek, 1957, 1960; Gunn, Gould, and Anderson, 1963). These authors have also found cadmium damage can be prevented by simultaneous injection of large quantities (100 times) of zinc. Cotzias, Borg, and Selleck (1961) found a difference in accumulation of zinc and cadmium only in the nuclear fraction and suggested that cadmium can replace zinc but does not respond metabolically as zinc does. Johnson, Sigman, and Miller (1970) also report high quantities of cadmium in the nuclear fraction of the testis of rats injected with cadmium, but quantities were also high in the supernatant. In the fowl testis, quantities of cadmium were high only in the supernatant.

Some goldfish showed normal testicular development even after 5 to 12 injections of cadmium chloride. The refractivity of certain fish to cadmium may be an expression of the variability in gonadal development. Lofts and Murton (1966) reported that cadmium injections had little or no effect on the resting or regressive avian testis, but the germinal epithelium was affected when spermatogenic activity was high. If this is true in fish, it would explain the variable effect of cadmium on goldfish. As Lofts and Murton suggested, cadmium may only damage the gonads under conditions of spermatogenic activity. Fish would then be resistant to cadmium injections until the gonads began to develop. Development after cadmium injections were stopped would probably produce no testicular damage even though most of the cadmium was stored in other tissues. These conditions may have existed in experiment E because fish injected with cadmium were initially kept under conditions to prevent gonadal development and then after cadmium injections were terminated they were placed in conditions to promote gonadal development. The lack of damage in some testes even after repeated cadmium injections may be due to conditions of the experimental design, but it must be assumed that those testes that were damaged had undergone development even under the unfavorable conditions during the cadmium injections. Likewise, this would explain why some gonads were damaged by a single cadmium injection, if that injection was given during the developmental period of the testis.

It seems unlikely that the gonads are susceptible to cadmium only in an undeveloped condition because in this study fish were injected about every three weeks over a period of approximately seven months, and it is probable that at sometime during this period, the gonads of

all fish would have reached a resting stage and would have been susceptible to cadmium. However, variability in response would more likely be a result of vulnerability during an active rather than inactive condition. Fish were obtained at a time when the gonadal activity of many of them was high as indicated by the high GSI of the zero time controls. After their first injection, they were kept under conditions which may have inhibited development in those fish whose gonads were not developing. This condition may have enhanced resistance to the cadmium injection, while those undergoing gonadal development showed damage.

It was also observed that the sperm remaining in the spent testis of fish which had received multiple cadmium injections were stained with Orange G rather than acid fuchsin, indicating a cytochemical change from acid to basic.

Ovary

For the purposes of this study, ova development was divided into seven stages as done by Braekevelt and McMillan (1967) for the stickleback (Eucalia inconstans). The ovary was studied differently than the testis because developmental judgments were made on individual ova instead of the entire organ. The stage of development of 100 random ova from each fish was determined and the number of ova of each developmental stage reported as a per cent of the total. A brief description of these stages is given in Table VI and examples illustrated in Figure 15. The normal ovary of adult fish has stages 1, 2, and 3 throughout the year, but stages 4 to 7 were present only at spawning.

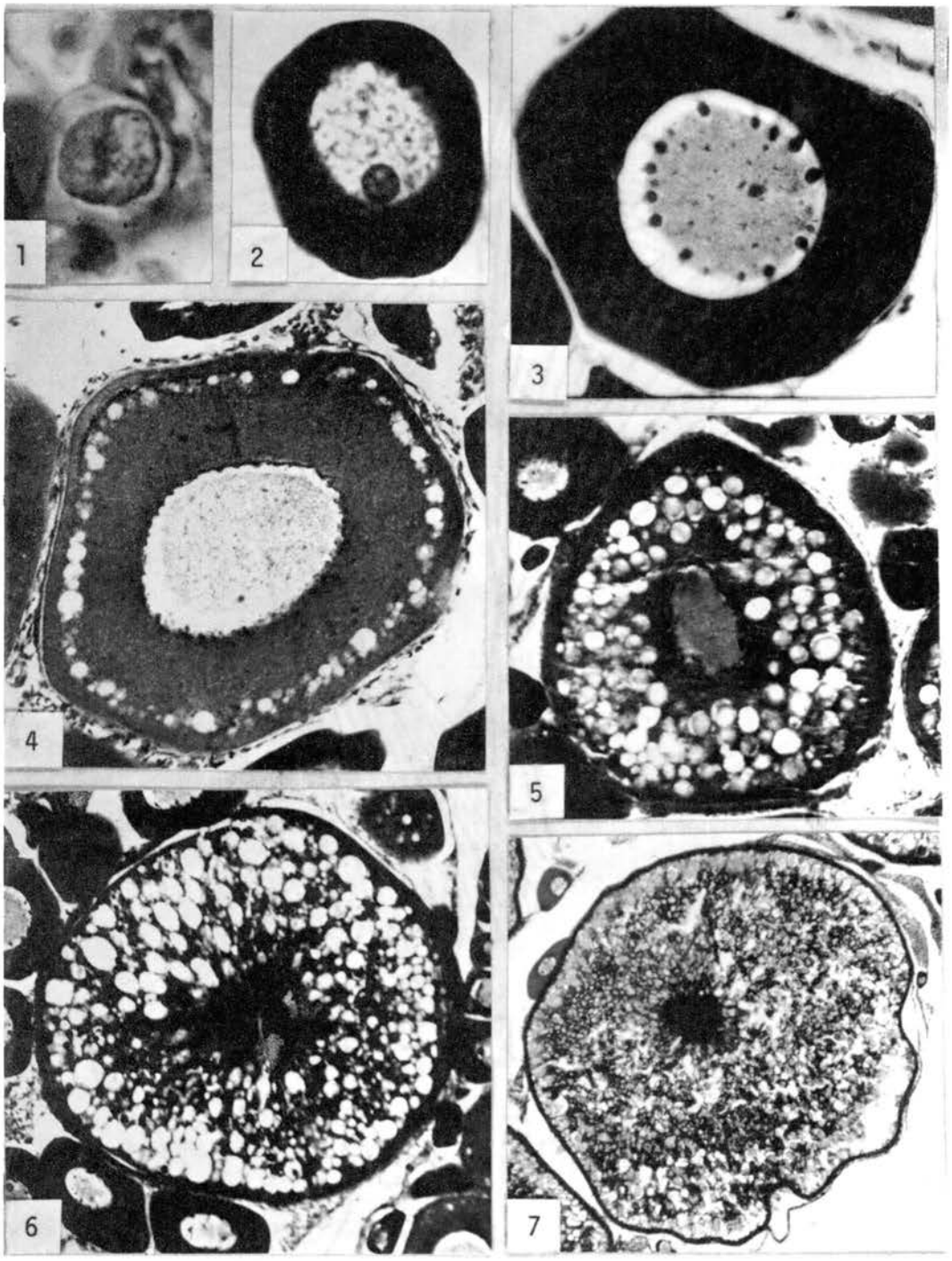
In experiment D, ova development was examined on six sample dates from 27 to 186 days. In all groups, controls and cadmium injected both

TABLE VI

DEVELOPMENT STAGES OF THE OVA OF GOLDFISH

Stage	Description			
	Nucleus	Nucleolus	Cytoplasm	Follicle
1	spherical, basophilic	single	transparent	none apparent
2	spherical, basophilic	1 - 2	homogeneous	none apparent
3	spherical, acidophilic	3 - 32	homogeneous to frothy	incomplete squamous layer
4	irregular, acidophilic	numerous >32	primary yolk on margin	single cuboidal layer
5	irregular, acidophilic	numerous	primary yolk fills cytoplasm	single cuboidal layer
6	located peripheral	numerous	secondary yolk on margin	single cuboidal layer
7	irregular peripheral	numerous	secondary yolk fills cytoplasm	single squamous

Figure 15. Developmental Stages of Normal Goldfish (Numbers correspond to egg stages in Table VI: (1) 1000 X, (2) 800 X, (3) 460 X, (4) 400 X, (5) 210 X, (6) 125 X, (7) 125 X)


1
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5
6
7

with and without HCG, stage 3 ova were most abundant. Stages 1 through 4 comprised 75 to 100% of all developmental stages. The more mature stages, i.e., 5, 6, and 7, were more abundant in each group on sample dates 103 to 186 and from 27 to 86 days. The greatest difference between control and cadmium treated groups with and without HCG occurred between days 27 and 86. At 56 days, the sum of frequency of occurrence of stages 5, 6, and 7 in both control groups was 6 and 5% compared with 0% for both cadmium treated groups. Controls showed development to stage 7 at each time period except 27 and 56 days, indicating at least some individuals were able to produce mature ova. Both groups receiving cadmium showed a lack of development of ova when compared to controls up to 86 days. By 103 days, some later stages of ova appear in all groups and this pattern continued through 186 days (Table VII). The effects of cadmium injections were temporary and the ovaries appeared to return to normal within approximately two months. The sum of the frequency of occurrence of the maturing stages for days 27 through 186 was lowest in the cadmium and HCG group.

Ova development was examined on nine occasions from 0 to 615 days. As in experiment D in all groups, controls, single, and multiple cadmium injected groups with and without HCG, stage 3 ova was most abundant (Table VIII). Stages 1 through 4 composed 89-100% of all developmental stages. The controls showed development to stage 7 ova at each time period after 44 days except at 122 days. There was a less frequent occurrence of stages 5, 6, and 7 in cadmium treated groups without HCG as compared with the control. The group receiving a single injection of cadmium and stimulated with hormone was more similar to the controls than any other group. The frequency of occurrence of maturing egg

TABLE VII

MEAN PERCENTAGE OF OVA AT EACH DEVELOPMENTAL STAGE IN EXPERIMENT D

Day	Treatment	Stage						
		1	2	3	4	5	6	7
27	Control	20	7	58	15	0	0	0
	Control + HCG	7	9	61	23	0	0	0
	10 mg/kg	2	7	66	18	7	0	0
	10 mg/kg + HCG	4	9	87	0	0	0	0
56	Control	10	9	72	7	2	0	0
	Control + HCG	3	9	62	17	7	1	2
	10 mg/kg	7	13	73	4	3	0	0
	10 mg/kg + HCG	5	11	79	4	1	0	0
86	Control	7	7	70	10	4	1	1
	Control + HCG	9	10	64	12	4	0	1
	10 mg/kg	8	9	81	1	0	0	0
	10 mg/kg + HCG	6	6	83	5	0	0	0
103	Control	7	11	77	3	0	0	2
	Control + HCG	5	8	61	16	1	1	8
	10 mg/kg	7	9	73	3	1	1	6
	10 mg/kg + HCG	7	10	69	10	2	2	0
134	Control	5	8	57	5	4	2	19
	Control + HCG	-	-	-	-	-	-	-
	10 mg/kg	6	10	69	6	1	0	8
	10 mg/kg + HCG	5	11	71	3	1	3	7
186	Control	10	10	67	4	3	1	5
	Control + HCG	7	11	81	0	0	0	1
	10 mg/kg	9	11	67	6	0	0	7
	10 mg/kg + HCG	9	9	74	4	1	0	3

TABLE VIII

MEAN PERCENTAGE OF OVA AT EACH DEVELOPMENTAL STAGE IN EXPERIMENT E

Day	Treatment	Stage						
		1	2	3	4	5	6	7
0	Control + HCG	1	3	74	9	3	1	7
13	Control + HCG	7	8	73	12	0	0	0
	10 mg/kg	12	9	74	5	0	0	0
31	Control + HCG	12	6	80	2	0	0	0
	10 mg/kg	3	4	91	2	0	0	0
	20 mg/kg	8	15	71	5	1	0	0
	10 mg/kg + HCG	4	7	88	1	0	0	0
	20 mg/kg + HCG	3	5	88	4	0	0	0
44	Control + HCG	13	15	66	6	0	0	0
	10 mg/kg	8	14	77	1	0	0	0
	30 mg/kg	29	14	53	8	0	0	0
	10 mg/kg + HCG	6	13	69	4	1	0	3
	30 mg/kg + HCG	10	15	71	5	0	0	0
86	Control + HCG	8	10	74	6	0	0	0
	10 mg/kg	8	9	76	5	2	0	0
	40 mg/kg	0	3	97	0	0	0	0
	10 mg/kg + HCG	0	2	82	0	3	0	3
	40 mg/kg + HCG	5	10	88	2	0	0	0
122	Control + HCG	8	10	80	2	0	0	0
	10 mg/kg	6	3	91	0	0	0	0
	60 mg/kg	4	8	88	0	0	0	0
	10 mg/kg + HCG	7	10	83	0	0	0	0
	60 mg/kg + HCG	9	8	79	4	0	0	0
159	Control + HCG	10	10	66	5	2	1	6
	10 mg/kg	4	5	90	2	0	0	0
	80 mg/kg	5	3	89	2	0	0	0
	10 mg/kg + HCG	7	5	85	3	0	0	0
	80 mg/kg + HCG	10	3	87	0	0	0	0
215	Control + HCG	7	7	70	5	4	2	5
	10 mg/kg	11	11	76	2	0	0	0
	100 mg/kg	7	5	84	2	0	0	2
	10 mg/kg + HCG	10	5	76	7	2	0	0
	100 mg/kg + HCG	26	5	69	0	0	0	0
615	Control + HCG	9	12	63	8	3	0	5
	10 mg/kg + HCG	4	8	65	12	2	0	9
	120 mg/kg + HCG	11	10	77	1	0	0	1

stages in the group receiving HCG and a single cadmium injection was much higher than either of the two cadmium treated groups not receiving HCG. This difference suggests the ability of the group receiving HCG and a single cadmium injection to respond normally after a loss of the effect of cadmium by 44 to 86 days. The frequency of occurrence of maturing egg stages in the group receiving multiple injections of cadmium and hormone was 0 at all sample dates except for 1% occurrence at 615 days.

The groups receiving multiple cadmium injections failed to develop ova beyond stage 4 with the following exceptions. Stage 5 ova were found in the group receiving only cadmium at 31 days and stage 7 ova at 215 days (one out of five fish) and 615 days (one out of eight fish).

The groups receiving multiple cadmium injections had approximately 10% more stage 3 ova than the controls. This is in part because the controls produced more of the mature ova. There is also considerable variation in the percentage of stage 1 and 2 ova. Average values for both stages usually were around 10%, but stage 1 ova varied from 0 to 29% (group receiving only multiple cadmium injections) with individual values up to 47% (the group receiving only multiple cadmium injections) where nests of ova appear throughout the ovary.

Also, only once did the mean number of stage 7 ova exceed 7% of the total number of ova in an ovary although in surface area the stage 7 ova occupied 70 to 80% of the ovary in the controls. Stage 7 ova often comprised 10 to 15% in individual and in at least one individual it was 28% (controls).

In general, this study indicated that the only histological change in the ovary was an inhibition of ova maturation. There was

occasionally a slight histological change in stain uptake by the ovary which was treated with cadmium but this was not consistent.

Little damage occurred to the ovary of mature rats after cadmium injections (Kar and Das, 1962). In prepubertal rats, the ovary was destroyed by subcutaneous injection of cadmium chloride but later regenerated (Kar, Das, and Karkum, 1959). However, Kar (1965) found destruction of the "germinal elements" 18 hours after intraovarian injections of guinea pigs with 1.25 mg/kg of cadmium chloride, and total loss of the histological integrity of the ovary by 15 to 90 days. This type of destruction was not found in the goldfish ovary. A sufficient level of cadmium should have been attained to produce this type of damage, as more than 100 ppm dry weight was occasionally found in goldfish ovaries, and 20 ppm dry weight was common.

Developmental stages of the testis and ovary were compared to evaluate the validity of the GSI as an index of gonadal maturity. Although the GSI is a widely used index, as a ratio, a body weight change may produce changes in the GSI even though the gonadal weight does not change. Crowding and social hierarchy in aquaria can lead to differential growth which could change the GSI of either dominant or subordinate fish. The mean and range of GSIs were determined for each stage of development of the ovary and testis of all groups as observed histologically. The stage of development was designated by the occurrence of the most advanced stages observed in the ovary. As indicated earlier, the ovaries of some fish were large but contained only immature eggs while others of similar or even smaller size contained mature eggs. A comparison of the means and ranges of the GSIs in female goldfish in experiments D and E (Table IX and Figure 16) showed an increase in the

TABLE IX

MEAN AND RANGE OF GSI OF MALE GOLDFISH AT EACH STAGE OF DEVELOPMENT AND FEMALE GOLDFISH AT DEVELOPMENTAL STAGES 3-7 IN EXPERIMENTS D AND E

Stage	Male			Female		
	Low	Mean	High	Low	Mean	High
			Experiment D			
1	0.06	0.21	0.33	----	----	----
2	----	0.35	----	----	----	----
3	----	1.46	----	0.89	2.26	4.25
4	3.05	3.68	5.01	1.72	2.95	4.08
5	0.58	3.04	5.88	2.84	4.08	5.29
6	0.65	1.84	4.92	4.53	5.59	6.65
7	0.29	1.18	3.71	1.72	9.75	21.26
8	0.14	0.52	1.11	----	----	----
			Experiment E			
1	0.08	0.29	0.55	----	----	----
2	0.12	0.23	0.35	----	----	----
3	0.34	1.35	3.07	0.98	1.90	3.21
4	0.43	1.87	3.77	0.80	2.00	4.01
5	0.79	1.47	2.15	2.24	2.89	3.53
6	0.25	0.73	1.26	3.25	3.67	4.09
7	0.27	0.61	1.71	1.69	8.62	19.70
8	0.20	0.39	1.08	----	----	----

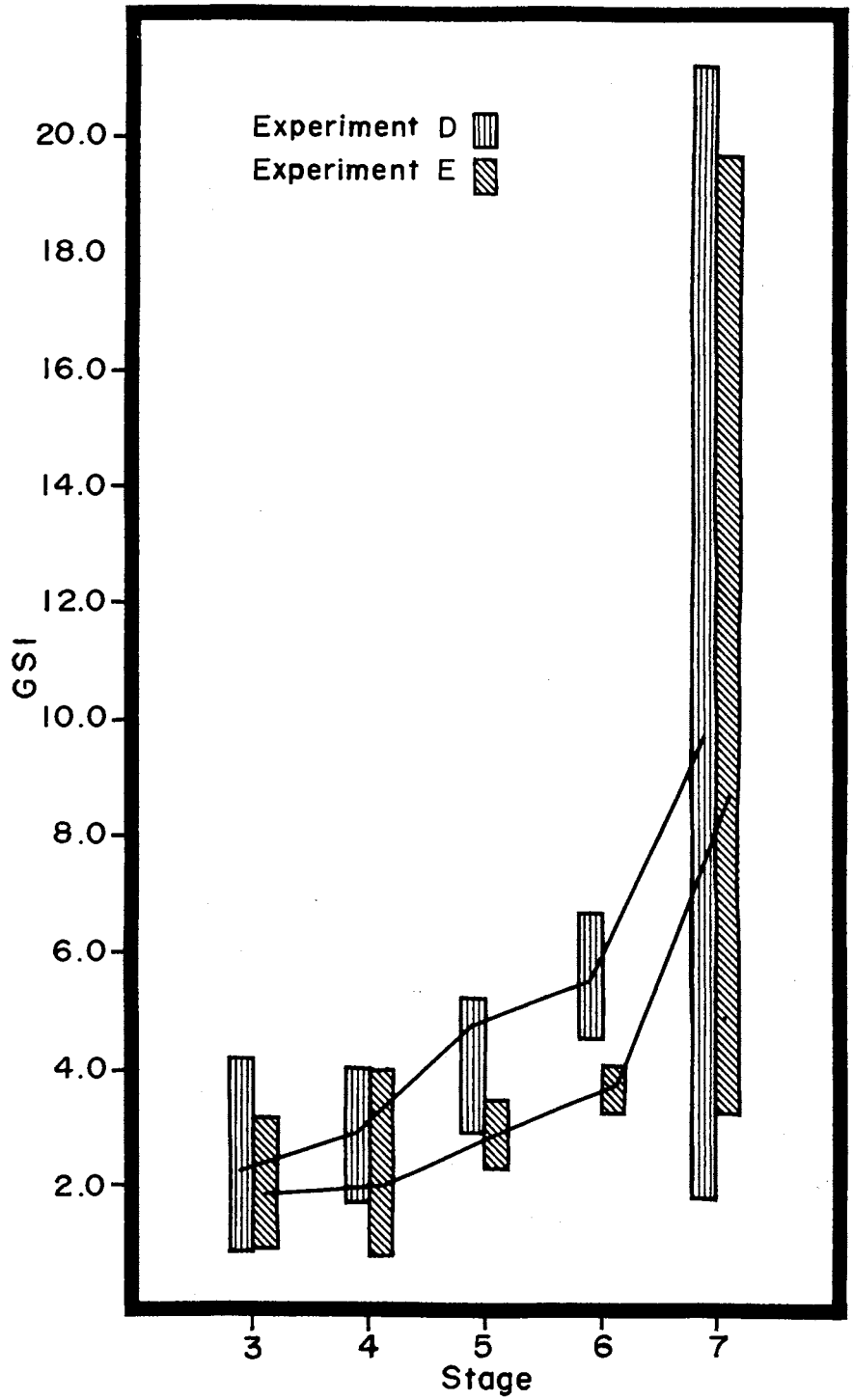


Figure 16. Mean and Range of Relative Gonadal Weight (GSI) at Each Stage of Development of Goldfish Ovaries for Experiments D and E (Solid lines connect means and vertical bars represent ranges.)

mean GSI through each stage of development, however, the range of GSIs was considerable at each stage and was especially high in fully developed or developing ovaries (stage 7). A similar situation existed for males except the maximum mean and range was reached at stage 4 (late maturing testis) and the range was large in all but very early and very late stages (Table IX and Figure 17).

Some values of the GSI of both males and females could fall into the range of more than one of the developmental stages. For example, a GSI for a male goldfish of 0.8 lies within the range of stages 3 to 8 in experiment D. Similarily, it is not possible to detect small undeveloped gonads from fully developed ones in stage 8 testis or stage 7 ovary. For example, in Figure 17, a testis may have a GSI of 0.3 and appear to be an undeveloped testis. One might even attribute this apparent lack of development to the treatment. However, this testis could be found in fish with stage 6, 7, or 8 testicular development which would indicate complete development and a spawning condition. A similar situation occurs in females which have a GSI range of 1.6 to 2.0 (Figure 16). Further, the spawned ovary was difficult to detect from the immature gonad simply from the GSI as shown by the sharp drop in the minimum GSI from stage 6 to stage 7 ovaries. Although these ovaries contained stage 7 eggs, the GSI was comparable to that of early egg stages. There was a considerable difference between experiment D and E, both in the mean (for both males and females always lower in E) and range of the GSI. In one case (stage 6 ovaries), the two ranges did not overlap; however, in that instance the sample size was small.

Although usually a useful tool, the GSI was misleading when used as an indicator of gonadal development of individual fish. It indicates

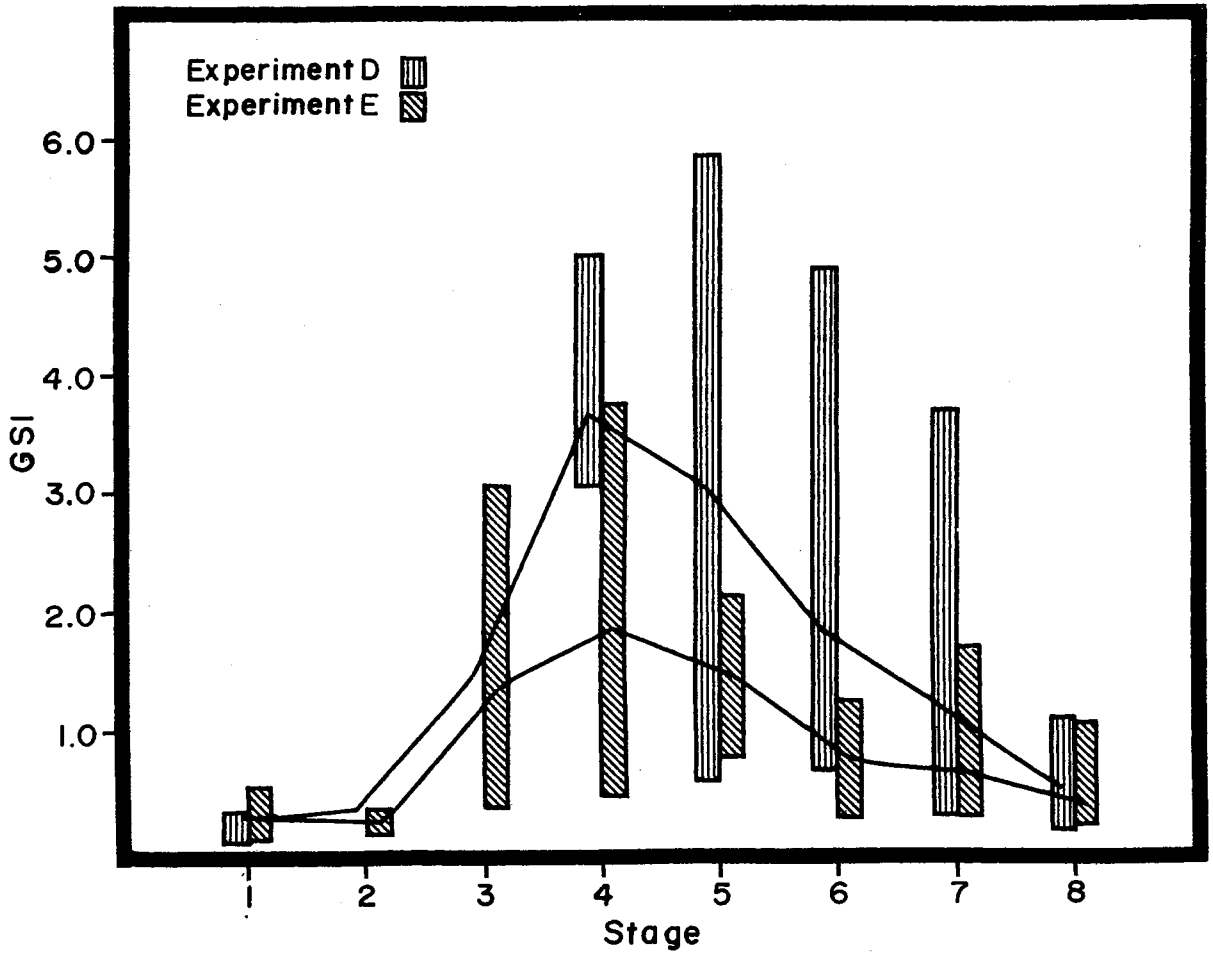


Figure 17. Mean and Range of Relative Gonadal Weight (GSI) at Each Stage of Development of Goldfish Testes for Experiments D and E (Solid lines connect means and vertical bars represent ranges.)

that a fish was in spawning condition if the value was high or that it was not in spawning condition, if it was very low. The gonadal condition for GSI values in the overlapping range was unclear. Therefore, the GSI would not be a useful index of the effects of cadmium on gonadal development of individual fish. However, histopathological changes did indicate that cadmium affected gonadal development.

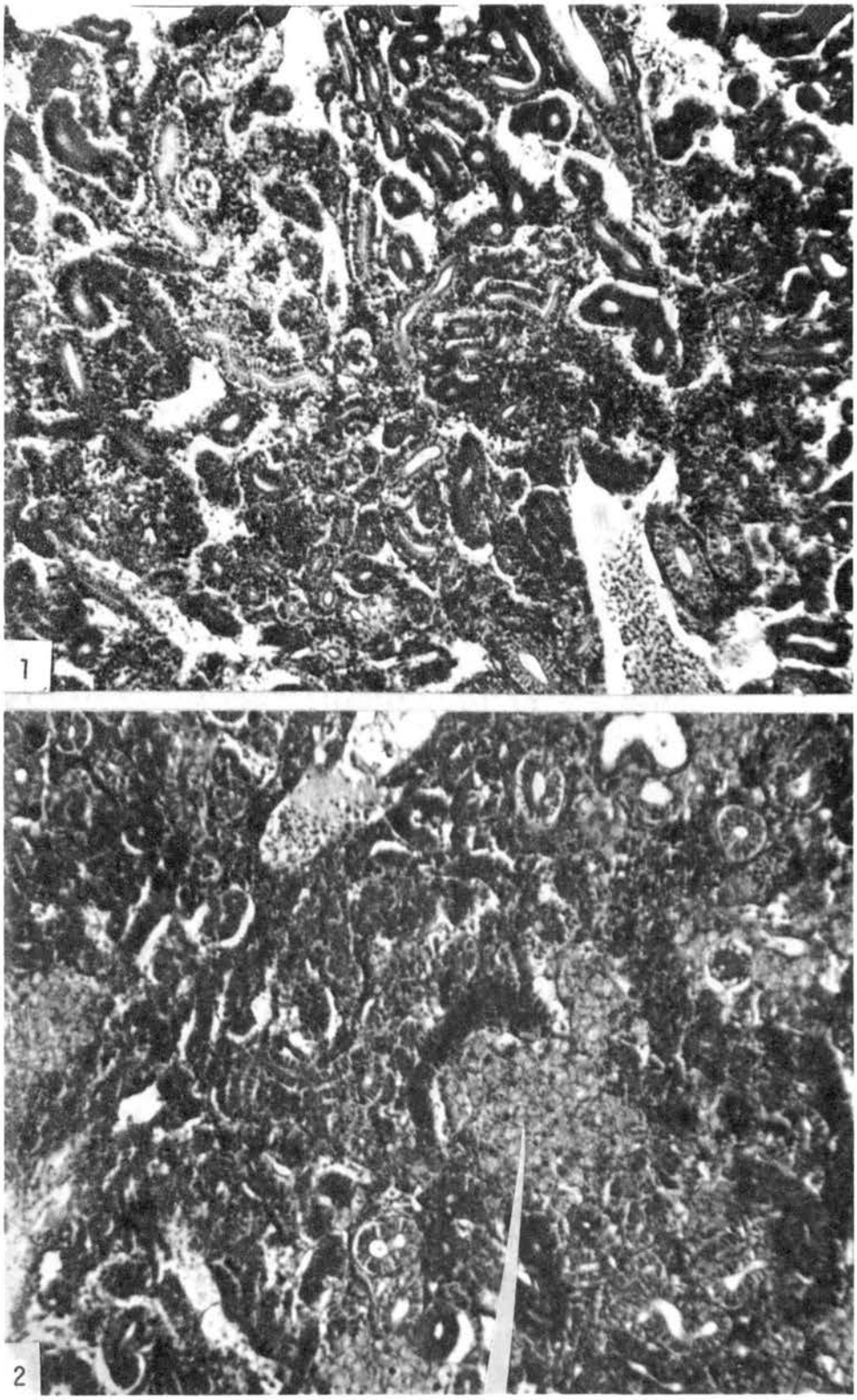
Kidney

According to Cleveland, Hickman, and Trump (1969), the kidney of normal freshwater fish consists of glomeruli, first and second proximal segments, intermediate and distal segments, collecting tubules and interstitial hematopoietic tissue. Most of these structures can be seen in any section through a goldfish kidney (Figure 18 (1)). The kidney of some of the control fish in this study also contained a few macrophages which were rarely aggregated into focal granulomas. The size, shape, and staining characteristics of these cells were very similar to the macrophages found in the testis.

A considerable degree of pathological change was noted in the kidney of cadmium treated fish. The greatest and most severe changes were noted after multiple injections but single injections also produced damage. Descriptions were limited to obvious histopathological changes.

The type of pathological changes in the kidney resulting from injections with cadmium were similar to those found in control fish, but frequency of occurrence and intensity of these effects were higher in the cadmium injected fish. The macrophages were more abundant in cadmium injected fish than in controls and were usually aggregated to form focal granulomas (Figure 18 (2)). Usually, the granulomas

Figure 18. Photomicrographs of Kidney From (1) Control Goldfish, (2) Goldfish Receiving Five Injections of 10 mg/kg $CdCl_2$ Showing Numerous Granulomas (Arrow), 315 X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occupied the area between the tubules, however, occasionally macrophages invaded the tubules (Figure 19 (1)).

Bonnell, Ross, and King (1959) reported renal lesions occurring after cadmium injections in rats. The authors found the intensity of renal damage related to the dose of cadmium and cadmium concentration in the kidney. The damage consisted of severe tubular atrophy. Because of similarity in size, shape, and characteristics of hematopoietic and mononuclear inflammatory tissue, it was not possible to distinguish the degree of inflammation in the kidney. Occasionally, renal granulomas were circumscribed with fibrous connective tissue as in the testis, however, the occurrence of granulomas in the kidney was much less frequent. On very rare occasions, large vacuoles appeared in the kidney tissue of fish receiving 10 or more injections of cadmium chloride (Figure 20 (1)). Vacuolar displacement of normal renal tissue probably caused a reduction in renal function. The relationship between the granulomas and the vacuoles is not clear, but the vacuoles may result from the loss of macrophages.

In order to determine the relationship between quantity of cadmium chloride injected and degree of damage in kidney (Figure 19 (2)), the damage of all kidneys was ranked as low, moderate, or heavy for fish receiving multiple cadmium injections (20 to 120 mg/kg) as compared with controls which showed no damage (Table X). The extent of tissue damage was classed as low if the damage area was less than 10% of the tissue, moderate if approximately 10 to 40%, and heavy if over 40%.

This comparison indicated that little damage occurred at low cadmium levels. Although it appears that the degree of damage increased with increasing dosage, it is also possible that once the degenerative

Figure 19. Photomicrographs of Kidney From Goldfish Receiving (1) Six Injections of 10 mg/kg $CdCl_2$ Showing Macrophages in the Kidney Tubule, 900 X, (2) Receiving Ten Injections of 10 mg/kg $CdCl_2$ Showing Extensive Masses of Macrophages, 315 X

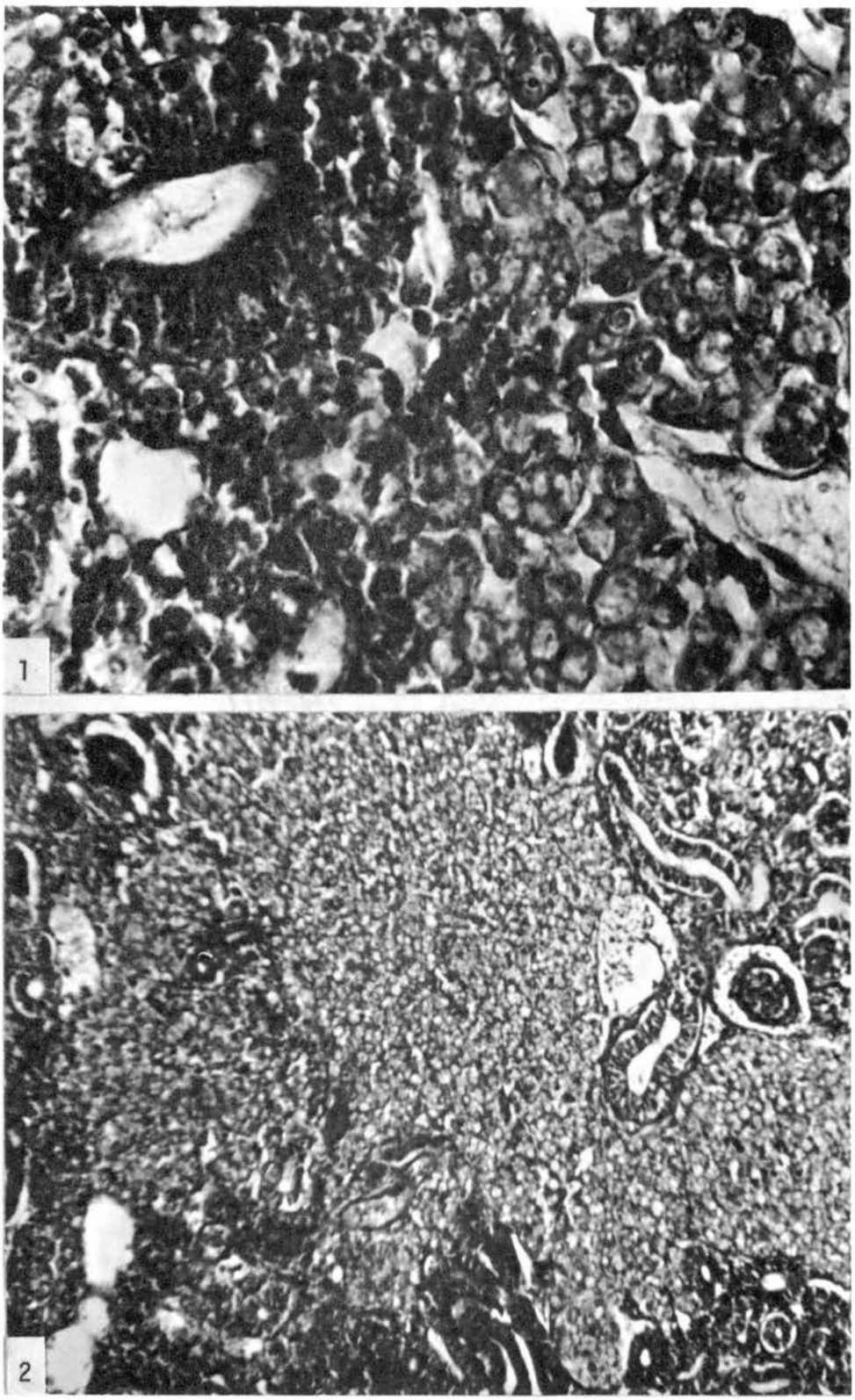
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Figure 20. Photomicrograph of Kidney From Goldfish Receiving Twelve Injections of 10 mg/kg $CdCl_2$ Showing Extensive Damage Including Large Vacuoles, 315 X, (2) Photomicrograph of Liver From Control Goldfish, 315 X

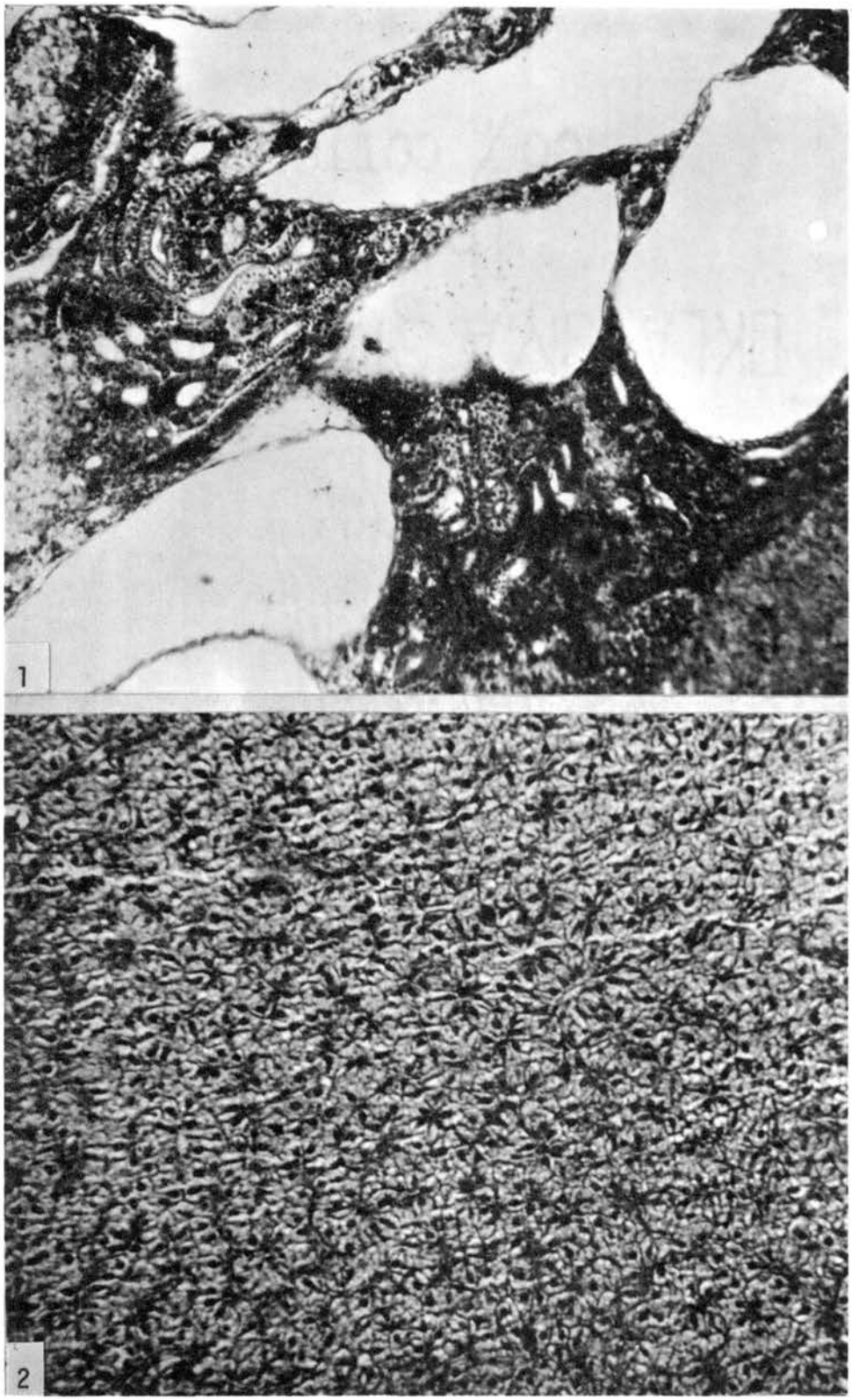
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TABLE X

PER CENT OF KIDNEYS SHOWING LOW, MODERATE, AND HEAVY DAMAGE IN EXCESS OF THAT FOUND IN CONTROLS AFTER CADMIUM CHLORIDE INJECTIONS

mg/kg $CdCl_2$ Injected	Control	Levels of Tissue Damage		
		Low	Moderate	Heavy
20	0	100	0	0
30	0	100	0	0
40	0	45	55	0
60	0	17	83	0
100	0	0	83	17
120	0	0	66	34

changes began they progressed from low levels of damage to moderate or heavy levels.

Selective staining of kidney tissue with Perl's, periodic acid-Shiffs and sodium rhodizonate stains produce the same results as in the testis, negative for iron and cadmium and positive for the group, glycogin, fibrin, and collagen.

Changes in metabolism, excretion, growth, homeostasis, and other physiological functions were not determined in this study. The osmoregulatory and hematopoietic activity of the kidney was probably affected as only a small area of normal tissue remained in the most severely affected fish. The hematopoietic function of kidney may be compensated by other organs.

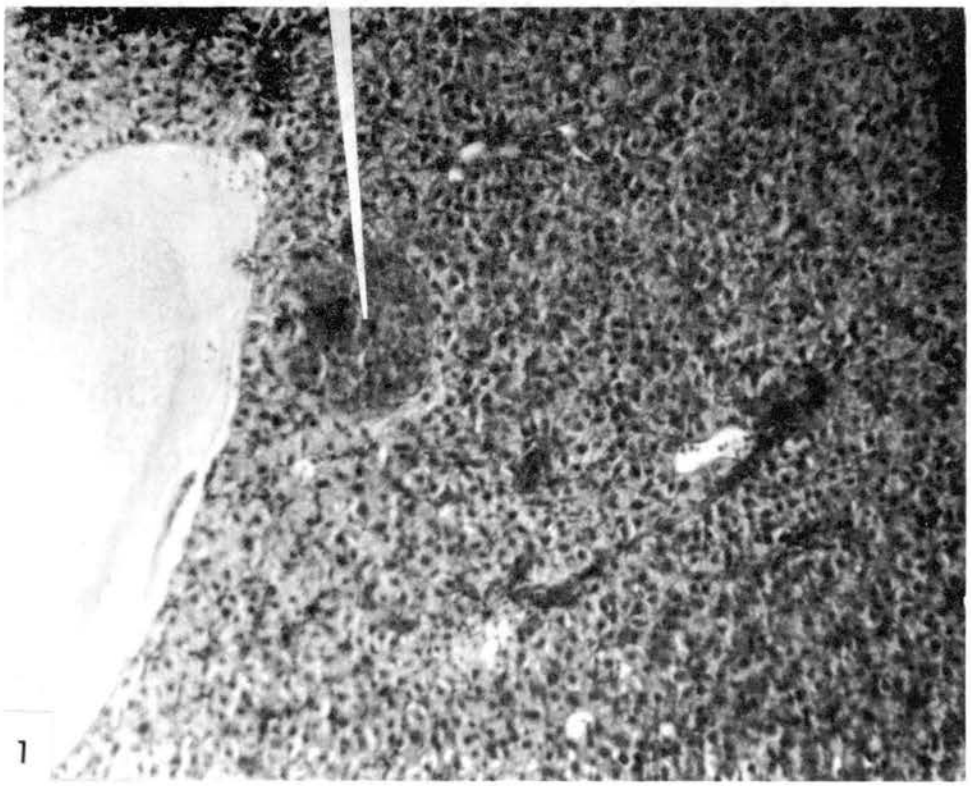
In spite of nearly total destruction of the kidney in some fish, no gross adverse effects were observed in behavior or general well being.

Liver

The liver of fish is composed of a mass of hepatic cells which are many sided with a centrally located nucleus and nucleolus. Occasionally, blood vessels are seen throughout the tissue (Figure 20 (2)).

Although the cadmium content in the liver reached high levels in injected fish, the liver often showed no adverse histological effects. Cadmium levels in the liver dropped after discontinuation of injections, suggesting elimination of the cadmium may have prevented damage. On some occasions, macrophages, usually forming focal granulomas, were visible and appeared to be identical with those found in the kidney (Figure 21). The granulomas only appeared in fish treated with multiple

Figure 21. Photomicrographs of Liver From Goldfish Receiving Six Injections of 10 mg/kg $CdCl_2$ Showing a Granuloma, 315 X



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injections of cadmium and never occupied more than a minor area of the tissue section.

The same selective stains (Perl's, periodic acid-Shiffs, and sodium rhodizonate) used on other organs indicated similar results, i.e., negative for iron and cadmium and positive for glycogen, fibrin, and collagen. The negative results obtained in all organs does not indicate the materials were not present because cadmium was found in high quantities in both the liver and kidney, but they simply were not detected by histochemical means. The nature and contents of the macrophages remains unknown and will require further study.

The reason for inconsistent damage in the liver is not clear, but damage did occur in some cases. The occurrence of damage in this organ as well as others may be related to the physiological condition of the organism at the time of treatment, but this hypothesis will also require further study.

In general, the histological changes in the testes, liver, and kidney were similar in that the macrophages aggregated to form focal granulomas. The decided increase in macrophages in treated fish as compared with controls strongly suggests damage resulting from cadmium injections. Specific changes in each organ such as sloughing off of germinal epithelium and replacement by fibroblasts in the testes and formation of large vacuoles in the kidney are further evidence of cadmium induced damage.

CHAPTER IV

SUMMARY AND CONCLUSIONS

Quantities of cadmium chloride ranging from 7.5 to 10.0 mg/kg in either single or repeated intraperitoneal injections did not alter the physical appearance or behavior of goldfish. However, examination of the GSI, tissue residues, and histological changes indicates numerous detrimental effects.

The LD_{50} of intraperitoneal injections of cadmium chloride for 24 hours was 30 ppm cadmium chloride, 23.0 ppm for 48 hours, and 20.0 ppm for 96 hours. Relatively small doses of cadmium (10 mg/kg) were lethal to some goldfish.

The gonosomatic index of males receiving a single injection of 10 mg/kg cadmium chloride was lower than controls for a period of 30 to 60 days, after which the GSI was similar to controls. Little difference in GSI was noted due to hormone injections, although GSI values for fish receiving both cadmium and hormone were usually lower than GSI values for groups receiving only cadmium injections or controls. Males receiving multiple injections of cadmium had GSI values lower than controls even after injections were discontinued. There was, however, considerable variation at all periods. The GSI values of female goldfish receiving a single injection of 10 mg/kg of cadmium chloride was only slightly affected. The GSI values of those fish receiving multiple injections appeared to be lowered slightly during the period of injections

but returned to normal one year after injections ceased. No consistent differences in the GSI of females was noted after injecting hormone compared to fish not receiving hormone. The use of the GSI as an indicator of gonadal condition on individual fish was only partially successful because of overlapping GSIs at each developmental stage of the gonads of both males and females. However, the over-all results were in general agreement with other criteria used.

Higher concentrations of cadmium were found in the kidney and liver than the muscle and gonads after a single cadmium chloride injection. The range in concentration of cadmium in the organs examined was: 12.3 ppm to 161.2 ppm in liver, 65.7 ppm to 366.4 ppm in kidney, 0.0 ppm to 29.8 ppm in ovary, 0.0 ppm to 78.1 ppm in testis, and 0.0 ppm to 2.1 ppm in muscle. During the test period in which fish received a total of 12 injections of 10 mg/kg cadmium chloride each, the cadmium residues were: 65.9 ppm to 312.4 ppm in liver, 66.3 ppm to 923.4 ppm in kidney, 3.3 ppm to 144.3 ppm in ovary, 0.0 ppm to 70.4 ppm in testes, and 0.0 ppm to 6.5 ppm in muscle.

Maximum cadmium residues appeared in the liver 90 days after a single injection, then the quantity of residue decreased in several samples, but the highest residues were found after 615 days. After multiple cadmium injections, residues of 200 to 300 ppm were reached by about 90 days and maintained to about 215 days, but dropped to 165.4 ppm by 615 days. Single injections of cadmium chloride produced cadmium residues of about 100 ppm in kidney, although 366.4 ppm were found after 615 days. Quantities of cadmium occurring in the kidney after multiple injections generally ranged from 300 to 400 ppm, but at 615 days the quantity was 923.4 ppm. Cadmium residues in the ovary fluctuated

greatly, but quantities of 5 to 15 ppm were common after a single cadmium chloride injection throughout the period, and cadmium residues of 20 to 40 ppm were common after multiple cadmium chloride injections. Quantities of cadmium in the testis were too variable to establish any pattern. Very little cadmium was found in muscle tissue, especially after single cadmium injections. The largest quantity was 6.5 ppm, found after multiple injections.

Some destruction of the germinal epithelium occurred in the testis after a single injection of 10 mg/kg of cadmium chloride. Occasionally, focal granulomas composed of macrophages and mononuclear inflammatory cells were noted. The testes of most fish were able to develop normally after single cadmium injections. Destruction of the germinal epithelium was much more severe after multiple cadmium injections. Destruction of the germinal epithelium was common and only a few fish were able to produce sperm. Those which developed normally showed no signs of damage anywhere, which may indicate that this organ is only vulnerable to cadmium at certain stages of development. Definite failure of development, compared with controls, occurred due to destruction of the organ.

The only histological change noted in the ovary was retardation of ova development. Some oocytes matured to the ripe condition (stage 7) in each group given injections of cadmium. However, development to mature ova was reduced immediately after a single injection of cadmium and reduced throughout the test period from multiple cadmium injections.

In the kidney, a slightly higher frequency of small focal granulomas was produced in singly injected fish than in the controls. Multiple cadmium injections produced extensive focal granulomas and smaller granulomas which were circumscribed with fibrous connective tissue. On

rare occasions, large vacuoles appeared in the tissue, leaving very little normal kidney tissue.

The liver showed no histological changes from a single cadmium injection, but some focal granulomas appeared after multiple cadmium injections. On rare occasion, the granulomas occupied considerable area of the liver and some hepatic cells appeared altered with apparent loss of nuclei.

Muscle tissue was not examined histologically due to very low cadmium residues.

The following conclusions can be drawn from this study:

(1) Repeated sublethal intraperitoneal doses of cadmium (10 mg/kg as $CdCl_2$) caused destruction of testis and kidney tissue with lesser effects on the ovary and liver.

(2) Quantities of cadmium over 400 ppm occurred in the kidney, the liver contained up to 300 ppm, and ovary contained less than 100 ppm.

(3) The liver had the highest total content of cadmium, but the kidney accumulated the greatest concentration.

(4) Cadmium was found in small amounts in the muscle of fish after multiple sublethal intraperitoneal injections.

(5) Histopathological damage to the testes was mainly limited to periods of active spermatogenesis.

The significance of this study lies in the comparative value of the effects of cadmium on mammals and knowledge of what cadmium can do to an aquatic vertebrate. The presence of cadmium in the aquatic environment could represent a hazard to the entire eco-system. The toxicity of

cadmium is highly variable from one species to another. It could kill plankton, thus destroying the food source of many fish, but it may accumulate in plankton and, thus, be transmitted through the food chain in small doses to fish. Evidence of the current study indicates that continuous exposure to sublethal doses would accumulate in several organs in the fish, cause destruction of the kidney and testis, and inhibition of ova production. Some long-term results of this type of action of cadmium on aquatic organisms could be (1) reduced fecundity among fish, which also could seriously alter not only the species involved but species composition; (2) destruction of vital organs could also render members of the species much more vulnerable to predation and, thus, alter predator-prey relationships; and (3) the presence of even small quantities of cadmium in the flesh of fish could transfer the cadmium danger to man. If the results of administration of cadmium to mammals and fish is an indication of the effects on man, the undesirability of additional cadmium in the environment is self-evident.

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