SOME EFFECTS OF CHRONIC CYANIDE POISONING ON THE GROWTH, RESPIRATION AND LIVER TISSUE OF RAINBOW TROUT

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ABSTRACT

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SOME EFFECTS OF CHRONIC CYANIDE POISONING ON THE GROWTH, RESPIRATION AND LIVER TISSUE OF RAINBOW TROUT.

Cyanide markedly affected growth and resting metabolic rate while causing liver necrobiosis in juvenile rainbow trout (Salmo gairdneri Richardson). This was revealed during two experiments performed in continuously renewed water at 12.5°C with fish fed a restricted artificial diet and exposed to assayed cyanide concentrations of 0.00, 0.01, 0.02, or 0.03 mg/l HCN for 18 days.

At 0.02 and 0.03 mg/l HCN growth was reduced by 40 to 95 percent after 18 days. At all concentrations cyanide caused a severe initial repression in specific growth rate followed by a highly significant increase which was, however, generally insufficient to compensate for the original repression.

Previous exposure to cyanide promoted a higher resting metabolic rate during the 6 days following exposure, the effect increasing with cyanide concentration.

At all concentrations tested, widespread cyanide-induced necrobiosis of hepatocytes was observed. Although more intense at higher cyanide concentrations, this necrobiosis was well established even at 0.01 mg/l HCN.

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INTRODUCTION

This laboratory project was initiated to study the effects of chronic cyanide poisoning on the growth of juvenile rainbow trout, Salmo gairdneri (Richardson), to measure their resting metabolic rate after the cessation of cyanide treatment, and to determine the extent of histopathological damage incurred in liver and gill tissues.

In Canada, particularly in the North, the mining industry represents a major source of cyanide release to surface waters. Alkaline cyanides are used as depressants during the concentration of ore by froth floatation, a process involving the segregation of minerals on the basis of their floatability in an aerated water column. Alkaline cyanides, in concentrations ranging from 5 to 250 g per metric ton of ore, are used as floatability depressants for sulphides of zinc, iron, copper and nickel (Anon., 1970). Bérubé and Gilbert (1971), working in the Northwest Territories, reported cyanide levels of 10 to 30 mg/l HCN in mine tailing ponds and levels of 0.10 mg/l HCN at the mouth of a river delivering mining effluent into Great Slave Lake. Cyanides are also widely used in the electroplating industry because of their unique ability to make soluble metallocyanide complexes of valuable heavy metals such as gold, silver, zinc, copper, or nickel, thus allowing better

electrolytic separation (Anon., 1970).

The acute toxicity of cyanide to aquatic organisms, particularly fish, has long been recognized and is hence well documented in the literature. LC50s, in mg/l as hydrocyanic acid (HCN), of 0.05 (Karsten, 1934), 0.09 (Burdick, et al., 1958), 0.104 (Burdick, et al., 1958), 0.15 (Doudoroff, et al., 1966) and 0.18 (Doudoroff, 1956) have been reported for brook trout (Salvelinus fontinalis Mitchill), brown trout (Salmo trutta Linnaeus), smallmouth bass (Micropterus dolomieui Lacépède), bluegills (Lepomis macrochirus Rafinesque) and fathead minnows (Pimephales promelas Rafinesque) respectively. Brown (1968) gives a 48-hour LC50 of 0.07 mg/l as HCN for rainbow trout held at 15°C in water with a pH of 7.7.

The acute toxicity to fish of simple alkaline cyanides and their metallo-complexes has been shown to result from the toxic action of molecular hydrocyanic acid (Doudoroff, 1956; Doudoroff, et al., 1966). While the acute responses of fish to cyanide, or for that matter to the majority of known toxicants, have been widely studied, chronic icthyotoxic effects have received relatively limited attention. It is becoming increasingly evident that simple acute toxicity testing is grossly inadequate for the establishment of sound water quality criteria aimed at the protection of fish populations over the entire range of their activities.

Iverson and Guthrie (1969), developing further the concept of "Scope for Activity" introduced by Fry in 1947, suggest that environmental stresses tend to be either additive or interactive and that the implications of their introduction into the environment cannot therefore be predicted on the basis of the evaluation of only one variable. When the only response to the single variable studied is lethality, the margins for error in conclusions concerning the environmental impact of a stressing factor are enormous. It is thus important that the effects of environmental stresses like cyanide on physiological functions of ecological importance, such as growth or respiration, be fully explored prior to the formulation of statements on the environmental impact of the stressing factor involved.

Several reports of the chronic effects of cyanide on fish appear in the literature. Neil (1957) demonstrated that chronic exposure of brook trout to cyanide concentrations of 0.01 to 0.05 mg/l as CN^- for up to 29 days markedly reduced their swimming stamina. The return of full swimming capacity following removal from cyanide was slow, with incomplete recovery after 20 days. Leduc (1966b) reported that the maximum sustained swimming speed of cichlids (Cichlasoma bimaculatum Linnaeus) was significantly reduced after a 24-day exposure to concentrations as

low as 0.04 mg/l HCN. Broderius (1970), working with coho salmon (Oncorhynchus kisutch Walbaum) that had been exposed to sublethal cyanide concentrations as low as 0.01 mg/l HCN, found that the fish showed an almost immediate and significant reduction in swimming time against water at constant velocity. This effect was shown to be of relatively long-lasting duration, with salmon that had been exposed to 0.01 mg/l HCN for 193 hours showing incomplete recovery after a 337-hour period in cyanide-free water. Speyer (1975) reported that rainbow trout which had been previously exposed to 0.02 mg/l HCN for 21 days exhibited a 75 percent decrease in swimming ability relative to controls.

The sublethal effects of cyanide poisoning on the osmoregulatory capacity of rainbow trout were studied by Chan (1971), who demonstrated that exposure of fish to cyanide levels of 0.01 to 0.03 mg/l HCN for 28 days in freshwater resulted in increases in plasma osmotic and plasma chloride concentrations, relative to controls, upon introduction of the test fish to 18.9 ppt saltwater. Reintroduction of the fish to freshwater induced a reduction of the plasma concentrations of the toxified fish to levels below that of the controls.

It is evident that the reproductive capabilities of fish can be seriously impaired by exposure to sublethal

cyanide concentrations. Leduc (1967) demonstrated that cyanide concentrations as low as 0.01 mg/l are capable of serious disruption of embryological development in the Atlantic salmon (Salmo salar Linnaeus). Ruby and Dixon (1974) reported that examination of the developing testicular germ cells of rainbow trout exposed to cyanide concentrations of 0.01 and 0.03 mg/l HCN revealed respective decreases in mitotic activity of 13 and 31.5 percent, relative to controls. It was also reported that at both concentrations cyanide acts as a strong mitotic inhibitor, preventing the maturation of developing germ cells past the metaphase stage.

Leduc (1966b), working with cichlids which were starved for 24 days in water containing concentrations of from 0.015 to 0.09 mg/l HCN, observed changes in the body weight and composition of the poisoned fish which indicated an acceleration in the utilization of body reserves and thus an increase in the maintenance food requirements of the toxified fish.

Leduc (1966b) also studied the effects of cyanide on the growth of juvenile coho salmon and cichlids fed unrestricted diets of earthworms and tubificid worms respectively. He found that coho salmon exposed for 24 days at 16°C in a flow-through system to cyanide concentrations of from 0.01 to 0.08 mg/l HCN showed significant reduction in growth

only at the highest cyanide concentration. He also noted that during the second half of the exposure period, the salmon exposed to 0.02 to 0.08 mg/l HCN grew faster than did the controls. The growth of cichlids exposed for 36 days at 25°C to cyanide concentrations ranging from 0.008 to 0.10 mg/l HCN revealed a response pattern similar to that of coho salmon, namely an early depression of growth followed by an increased growth rate by the end of the experiments.

Speyer (1975) exposed juvenile rainbow trout to 0.02 mg/l HCN for a 21-day period in continuously renewed water at 11°C and noted a response similar to that observed by Leduc. The fish underwent a significant reduction in growth during the first 10 days of exposure followed by an accelerated growth during the later 10 days.

In the experiment with cichlids Leduc (1966b) showed that the cyanide-toxified fish exhibited higher food consumption and a lower food conversion efficiency than did the controls. He speculated that had all the experimental fish been held on equal restricted rations the cyanide effect would have been more pronounced. With this in mind, it was decided to investigate the effects of chronic cyanide poisoning on the growth of juvenile rainbow trout held on a restricted diet.

Measurements of the oxygen consumption of an organism is a useful parameter for determination of the effects of a toxicant on the metabolic rate of that organism. Cairns (1966) considers it to be one of the two best methods for sublethal bioassay. Cairns and Scheier (1964) demonstrated a small but significant increase in the resting metabolic rate of pumpkinseed sunfish (Lepomis gibbosus Linnaeus) exposed to sublethal dieldrin poisoning. Waiwood and Johansen (1974) determined that exposure of the white sucker (Catostomus commersoni Lacépède) to 0.04 mg/l methoxychlor resulted in a two to three fold increase in the resting metabolic rate. In both of the above cases, the induced increases in resting metabolic rate represent a decrease in the scope for activity of the species tested.

While the fact that cyanide acts as a respiratory depressant on fish has been well documented (Jones, 1964. p. 83-96), the respiratory pattern of fish during the recovery period following the removal of the cyanide stress has not been examined. As was previously stated, Neil (1957) and Broderius (1970) noted that the return of full swimming capacity following removal from cyanide was slow, with incomplete recovery after up to 20 days in some cases. Considering this phenomenon, it was thought that it might prove worthwhile to determine if a similar time lag occurred in the recovery of normal respiratory activity

after the removal of cyanide stress.

While histology has been little used in the field of fish toxicology, it can prove very useful. The effects of chronic toxicity occur only after the internal organs have incurred significant levels of damage. Histological examination of tissue can yield useful information on chronic tissue damage before the effects become apparent at the organismal level. This approach has been taken by Hinton et al. (1973) in assessing the effects of methyl mercury on channel catfish (Ictalurus punctatus Rafinesque), by Skidmore and Tovell (1972) in evaluating the toxic effects of zinc sulphate on rainbow trout, and by Ruby and Dixon (1974) in evaluating the effects of cyanide on spermatogenesis in rainbow trout.

Considering the respiratory function of the gills and the many metabolic processes carried out by the liver, it was decided that an attempt to correlate damage in these tissues with the physiological reactions of respiration and growth in rainbow trout subjected to sublethal levels of cyanide would be a meaningful undertaking.

MATERIAL, APPARATUS AND METHODS.

MATERIAL

The rainbow trout (Salmo gairdneri Richardson) used during this study were obtained from La Pisciculture S. Elliot Enrg., St. Alexis-des-Monts, Maskinonge County, Quebec. The fish were transported in plastic bags containing a moderate amount of water and pressurized with oxygen. Because of summer growth in the hatchery, it was not possible to obtain fish of uniform size throughout this study, the mean weights of the fish in the first and second shipments being 2.85 g and 11.99 g respectively.

Upon arrival at the laboratory (Sir George Williams, Concordia) the fish were held in 80 l tanks at a density of about 100 individuals per tank. The tanks were supplied with a continuous water flow of 1.5 l/min at a constant temperature of 12.5 $\pm$ 0.5°C. The fish were fed daily at a level of approximately 2% of their weight with the same artificial diet as used during the experimental period. Less than 0.5% mortality occurred during transportation and the one week pre-experimental holding period.

APPARATUS

Water supply

The laboratory was supplied with City of Montreal water delivered to the experimental apparatus via plastic

(PVC) piping. The chlorine content of the water was reduced to less than 0.01 mg/l by means of an activated charcoal dechlorinator. The dissolved oxygen levels of the incoming water, monitored on alternate days, approximated 100% saturation throughout the experimental period. Other parameters of the water chemistry for the duration of the experiments are presented in Table 1.

Table 1. Chemical characteristics of the water used during the experimental period July 1973 to December 1973 (mean values from data provided by the City of Montreal).

Alkalinity $CaCO_3$ (mg/l)	Total hardness (mg/l)	CO_2 (mg/l)	pH
86.3	127.7	0.4	7.9

The temperature of the water supplied to the test tanks was regulated to 12.5 ± 0.5°C using one of two methods, both of which are represented schematically in Figure 1. During the summer months the water was cooled by means of an inline refrigeration unit (Model PC5HQ, Dunham-Bush of Canada Ltd.) supplemented by a portable cooling unit (Model PCC-1355A-2, Blue M-Electric Co.) immersed in the head tank.

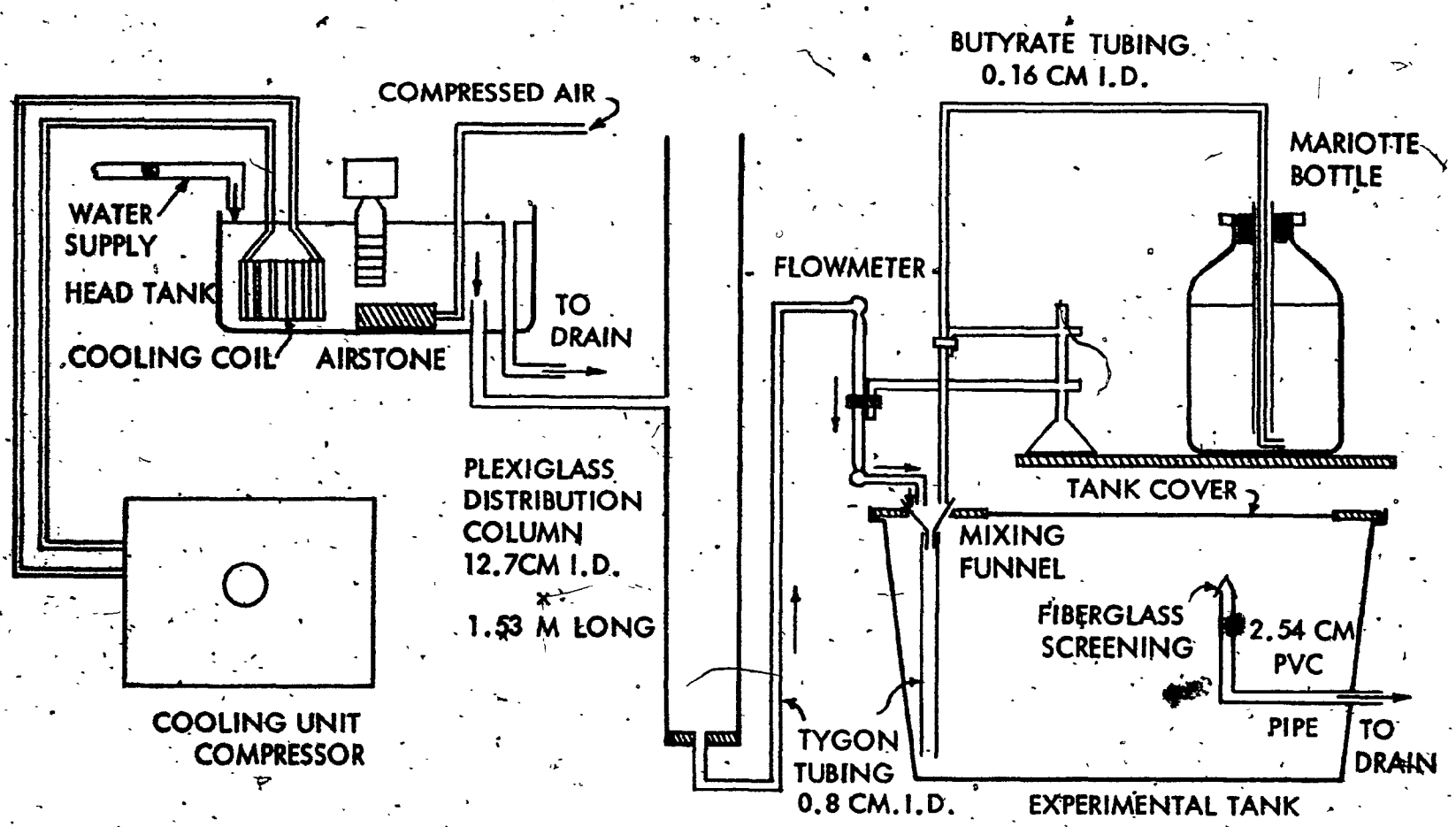

Figure 1. Schematic drawing of the experimental apparatus used for the continuous exposure of juvenile rainbow trout to sublethal concentrations of hydrogen cyanide, showing the heating and cooling systems, the direction of water flow, one experimental tank, and one Mariotte bottle for dispensing toxic solutions.

In winter the water was heated in the head tank by means of two eleven-hundred watt stainless steel immersion heaters (Portatemp, Model 14-X-6, Precision Scientific Co. Ltd.).

Test tanks

The experimental apparatus used to study the effects of cyanide on the growth of juvenile rainbow trout consisted of five polyethylene tanks (Rosedale Plastics, Montreal) measuring 68 cm long, 57.5 cm wide and 42 cm deep. The tanks, one of which is illustrated in Figure 2, were fitted with removable covers constructed of fiberglass mosquito screening on a Masonite pressed wood frame. Predictable flowmeters (Manostat Corp., New York, N.Y.) were used to regulate the flow of water to each tank to 500 ml/min, and each tank was fitted with a standpipe drain adjusted to produce a volume of 60 l in the tank. A sleeve of fiberglass screening was fixed to the top of each standpipe to prevent any dead fish from obstructing the water outflow. Mariotte bottles (Leduc, 1966a) were used to meter the cyanide stock solutions into the diluting water.

The entire five tank assembly, illustrated in Figure 3, was illuminated by evenly distributed 40-watt fluorescent lights (Model F-40-WW-Lifeline, Sylvania (Canada) Ltd.) controlled by a time-switch to provide a 12-hour photoperiod (9 to 21 hr.). In order to minimize the effects

Figure 2. Photograph of one experimental tank and one Mariotte bottle used for the continuous exposure of the experimental organisms to hydrogen cyanide.

Figure 3. Photograph of the five tank experimental assembly used for the continuous exposure of juvenile rainbow trout to sublethal concentrations of hydrogen cyanide.

external disturbance, the experimental apparatus was surrounded by a curtain of black plastic sheeting.

Respirometers

The respirometer used to measure the resting metabolic rate of rainbow trout after the cyanide exposure period is illustrated in Figure 4. It consisted of a series of 30 plexiglass tubes painted black to minimize disturbance. Figure 5, a diagram, shows one respirometer tube complete with tubing, stopcock, and sampling bottles (standard 300 ml B.O.D. bottles). Each respirometer was supplied with water from a head tank assembly similar to that shown in Figure 1.

The metabolic rate of each fish was calculated from the difference in dissolved oxygen concentration between the inlet and outlet sampling bottles, and the flow rate through the tube, which was maintained at approximately 20 ml/min.

METHODS

Diet preparation

The artificial diet used for this study was prepared according to the method of Kruzynski (1972), and consisted of beef liver, beef heart, and Ewos trout chow (F 169) in a ratio by weight of 2:1:1 respectively. The fresh liver and heart were homogenized in a blender and mixed with the

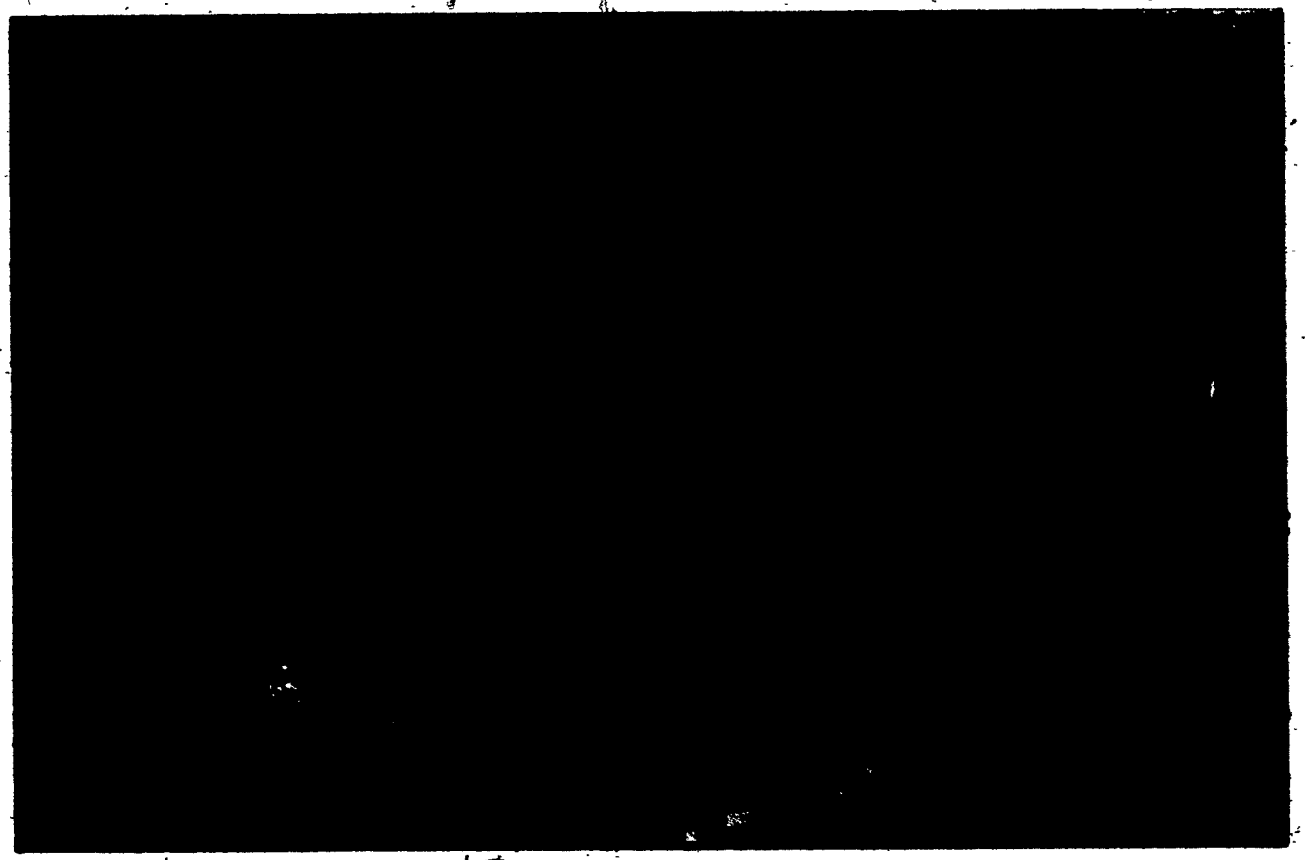

Figure 4. Photograph of the respirometer assembly used to monitor the respiration rates of juvenile rainbow trout following exposure to sublethal concentrations of hydrogen cyanide.

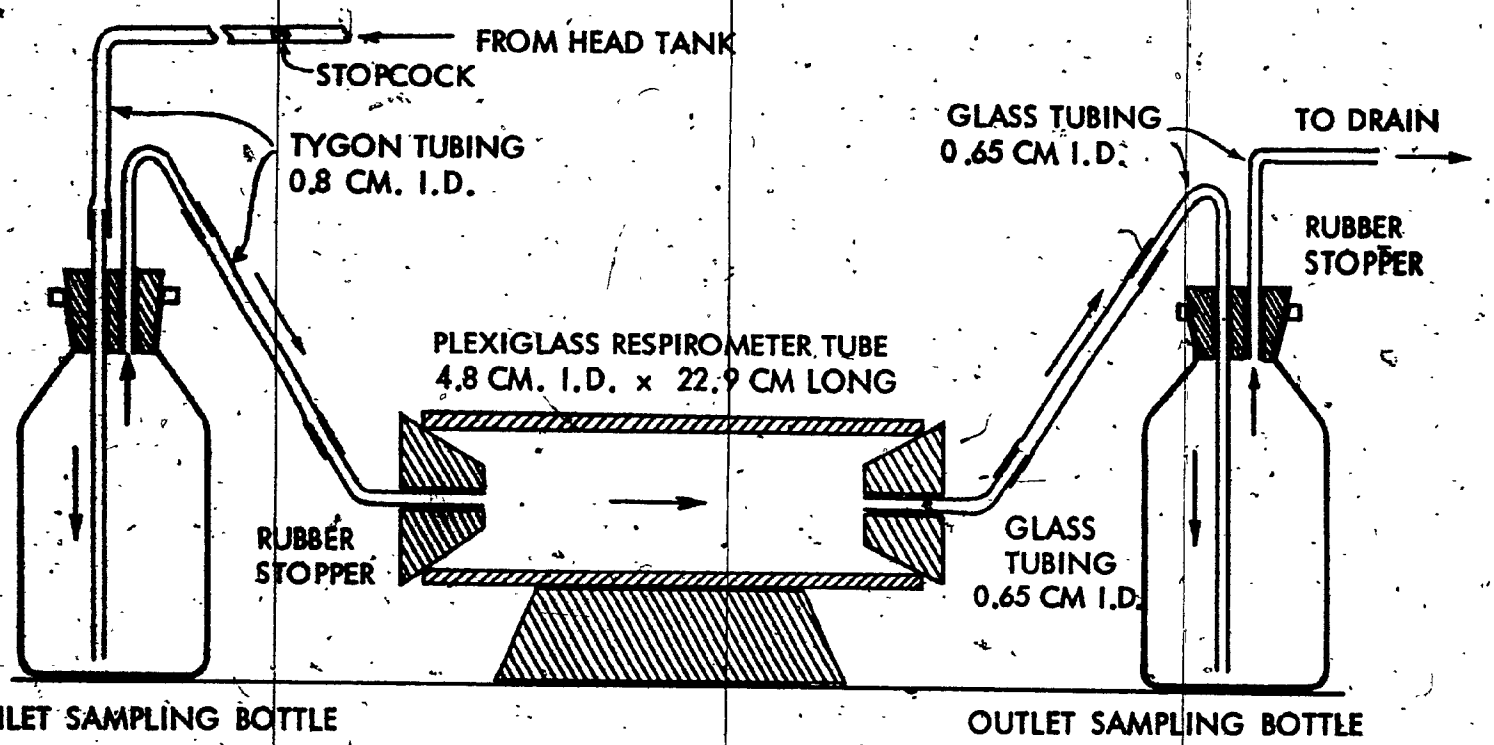

Figure 5: Schematic diagram of one flow-through respirometer showing the pattern of water flow through the system.

Ewos, after which the formulation was oven dried for six days at 60°C and then ground to a fine powder. The final diet was prepared by combining warm distilled water, the diet powder and gelatin in a ratio by weight 5:4:1 respectively. The mixture was spread on trays, allowed to set for one hour at room temperature, cut into pieces of desired size, and frozen until required for use.

The diet allotments for each experiment were analysed for water, protein, fat and ash content, and their composition is shown in Table 2. The water content, was determined by drying a 3.0 g sample for six days at 70°C. Dried samples of diet were used for the remaining three assays. The protein content was determined by the Biuret method (Gornall et al., 1949) after hydrolysis of a 1.0 gm aliquot with 1N NaOH for 24-hours at 37°C; the fat content by ether extraction with a Labconco Goldfisch Fat Extractor (Model 35003) using a 3.0 g sample subjected to a 4-hour reflux distillation; and the ash by incineration of a 1.0 gm sample at 1200°C.

Table 2. Analysis (based on dry weight) of the diet used during the study of the effects of cyanide on juvenile rainbow trout.

Experiment No.	Dry Matter (percent)	Protein (percent)	Fat (percent)	Ash (percent)
1	52.2	62.5	5.1	8.1
2	51.6	63.4	8.9	8.0

As indicated in Table 2, the diets used in each experiment were similar, with the exception that the fat content was almost twice as high in the second experiment as it was in the first. This is most likely attributable to a higher level of residual fat in the beef heart used during the preparation of the second diet lot.

Experimental design

This study consisted of two 18-day growth experiments, each followed by measurement of respiration rates and histopathological examination.

At the beginning of the first experiment, 180 rainbow trout of the desired size were selected and divided into six groups of 30 fish each. The fish were individually weighed in water to the nearest hundredth of a gram,

working under red fluorescent illumination (Model F-40-Red-Lifeline, Sylvania (Canada) Ltd.) to minimize disturbance.

Care was taken to maintain uniformity of total weight and size distribution for the fish in each of the six groups. The weight of the specimens in each group ranged between 2 and 5 g with a mean weight of approximately 3.1 g. Five of the groups were randomly distributed in the test tanks while the sixth was held in a 650 l refrigerated tank with the water renewed daily. This provided the fish with an amount of new water daily that was approximately equivalent to the 750 l/day supplied to the specimens in the flow-through test tanks.

Two days after segregation the fish were lightly anesthetized with 15 ppm MS222 (tricaine methane sulphonate) and individually marked using the liquid nitrogen method of Mighell (1969). They were returned to the test tanks for another 10-day acclimation period prior to the start of the cyanide exposure. During this time they were maintained under conditions identical to those which prevailed during the test period.

Before the start of the metering of cyanide all groups of fish were starved for 24 hours, then weighed by the method already described. The five test groups were returned to their respective tanks, while the fish from

the sixth group were sacrificed, oven dried for six days at 70°C and stored to provide pre-experimental values of the fat and water content.

On the morning of the next day the metering of the cyanide solutions, prepared after Leduc (1966b), was initiated to establish the desired cyanide concentrations of 0.01, 0.02, and 0.03 mg/l as HCN in the test tanks. The dilution water flow rate of 500 ml/min provided for a 99% replacement of the test water about every 9 hours, as calculated after Sprague (1973).

The fish were exposed to cyanide for 18 days, during which time they were fed on the artificial diet at a rate of 2% of their weight. This diet level was selected since it was the maximum allocation that the fish exposed to the highest level of the toxicant would readily consume. The fish were weighed on the tenth day of the exposure, at which time the feeding level was adjusted, and at the termination of the experiment. Routine experimental maintenance involved a daily cleaning of the tanks prior to feeding, as well as determination of cyanide concentration (Epstein, 1947) on alternate days. This allowed adjustment of the cyanide flow to maintain the desired concentrations in the tanks, although this was rarely necessary since the cyanide concentrations never varied by more than 1% of the predicted values.

At the end of the cyanide exposure period, and after the final weighing, eight fish from each group were returned to their tank, five for the respiration study and three for tissue sampling. The remainder were sacrificed, dried at 70°C for six days to determine water content, and stored for determination of fat content as previously described.

The five fish from each of the experimental groups to be used for the respiration study were randomly distributed to the respirometers and the flow rate through each tube was set at approximately 20 ml/min. Care was taken to remove all air bubbles from the system and to seal all joints against water loss since any leaks or airlocks would introduce inaccuracy into the results. The fish were allowed to recover from the transfer to the respirometers for 24 hours before the first reading was taken. Further readings were taken daily for five days at the same time on each day. Each reading involved measurement of the flow rate, removal and replacement of the outlet sampling bottle, and, finally, exchange of the inlet bottle. Three blank readings, from respirometers containing no fish, were also taken daily to determine if the respirometers themselves exhibited any oxygen demand, which they did not.

Dissolved oxygen concentrations in the sampling bottles were determined by an iodometric technique

involving a colorimetric assay of the iodine concentration. The dissolved oxygen was fixed with manganous sulphate solution, alkali-iodide-azide reagent and concentrated sulphuric acid using the method of Elliott (1963). The amount of dissolved oxygen present was then determined by reading the absorbance of the resulting iodine solution with a spectrophotometer (Bausch and Lomb, Spectronic 20) at 450 mμ as outlined by Oulman et al. (1956). The method was standardized for dissolved oxygen levels in the range of 2 to 11 mg/l against the azide modification of the Winkler titration (American Public Health Association, 1971).

Samples of gill and liver tissue were taken from the three fish in each group kept for histological examination. The tissues were immediately fixed in Bouin's solution, held for 48 hours, and then cleared with ten changes of 70% ethanol. They were infiltrated with paraffin (Tissuemat m.p. 56.6°C), sectioned into 9 mμ sections and stained with Harris's hematoxylin and Bowie's eosin.

The experimental design and environmental conditions for the second experiment were the same as those of the first, with the following exceptions. The number of fish in each group was increased from 30 to 33 and the specimens used were larger, their weights ranging between 9 g and 16 g with a mean weight for each group of approximately

12.8 g. The increased number of fish allowed for tissue sampling from 3 specimens at the midpoint of the exposure period as well as at the termination of the experiment. The feeding level was increased from 2% to 2.5% since the larger fish were capable of consuming more food at the highest level (0.03 mg/l) of cyanide.

RESULTS

During the acclimation periods of both the first and second experiments the fish readily adapted to the test tanks and accepted the experimental diet with no hesitation.

Upon introduction of the toxicant the fish held at 0.02 and 0.03 mg/l cyanide exhibited more activity as evidenced by rapid, highly random swimming. This changed after approximately two days to a more lethargic behaviour, particularly during feeding; although these fish still ate their entire ration, they took a much longer period of time, approximately one hour, than did the fish exposed to 0.01 mg/l cyanide or the controls, who consumed their ration within five minutes. The fish held at 0.02 and 0.03 mg/l became very dark, while at 0.01 mg/l the fish retained a light coloration similar to that of the controls.

One interesting characteristic of the specimens held at 0.02 and 0.03 mg/l HCN was the bright red coloration of the gills and the integument on the dorsal surface of the head. This was most probably due to the presence of cyanmethemoglobin in the blood (West, et al., 1966, p. 619).

Some mortality occurred during both experiments (see Table 3). In most cases mortality was minimal, except at 0.02 mg/l HCN in the first experiment when, on the fourth day of the cyanide exposure period, the water supply to the

Table 3. Incidence of mortality among control and cyanide toxified rainbow trout during 18-day experiments carried out in renewed water at 12.5°C.

Experiment No.	Cyanide Conc. (mg/l HCN)	Initial No. of Fish	Initial Mean Wet Weight (g)	Standard Deviation of Initial Mean Weight	No. of Mortalities During Experiments
1	0.0	30	3.25	0.91	3
	0.0	30	3.07	0.88	0
	0.01	30	3.15	0.91	6
	0.02	30	3.07	0.80	18 1/
	0.03	30	3.08	0.77	8
2	0.0	33	12.93	3.73	0
	0.0	33	12.46	3.56	0
	0.01	33	12.68	3.58	0
	0.02	33	12.92	3.95	2
	0.03	33	12.64	3.63	9

1/ See text for details

tank containing this group of fish was disrupted while the cyanide flow continued, resulting in a reduced dissolved oxygen concentration and a heightened cyanide concentration in the holding water; fifteen fish died as a result of this mishap. While the test was completed with the surviving fish, it was later decided to reject the data obtained, since, according to the criteria of Binns (1971), it could be biased.

Effects on growth

The effects of cyanide on the growth of juvenile rainbow trout during the 0 to 9 and 0 to 18-day periods of both experiments are shown in Table 4 which gives the means of the individual percent wet weight gains of the fish in each group. In Figure 6, eyefitted curves illustrate the growth over the 0 to 9 and 0 to 18-day exposure periods in each experiment.

As indicated by Figure 6, the results show that exposure to cyanide concentrations of 0.01, 0.02 and 0.03 mg/l HCN for an 18-day period causes a marked reduction in the wet weight gain of juvenile rainbow trout, and that most of this reduction in growth occurred during the first 9 days of exposure. During the 18-day growth period of experiment 1, the control fish exhibited a weight gain of about 17 percent, while the 0.01 mg/l cyanide-toxified

Table 4. Growth of control and cyanide toxified rainbow trout during 18-day experiments carried out in continuously renewed water at 12.5°C.

Experiment No.	Cyanide Conc. (mg/l HCN)	No. of Fish in Sample	Mean Wet Weight Change: 0 to 9 Days (Percent)	Standard Deviation of Mean Wet Weight Change: 0 to 9 Days	Mean Wet Weight Change: 0 to 18 Days (Percent)	Standard Deviation of Mean Wet Weight Change: 0 to 18 Days	't' Value for Comparison of the Growth Over 18 Days of Control 1 Versus Toxified Groups	't' Value for Comparison of the Growth Over 18 Days of Control 2 Versus Toxified Groups
1	0.0	27	6.67	5.41	18.14	10.16	-	-
	0.0	30	5.55	6.06	16.05	11.69	-	-
	0.01	24	0.29	6.49	10.19	13.43	2.46*	2.06*
	0.03	22	-6.88	6.84	0.40	14.37	5.05****	4.33****
2	0.0	30	10.08	3.07	23.16	6.31	-	-
	0.0	30	10.61	4.41	24.04	9.11	-	-
	0.01	30	8.82	6.15	23.08	12.40	0.03	-0.34
	0.02	28	4.09	6.54	14.35	11.31	3.72****	3.60****
	0.03	21	1.84	4.75	8.96	8.05	7.05****	6.10****

* 0.05>p>0.02; ** 0.02>p>0.01; *** 0.01>p>0.001; **** 0.001>p

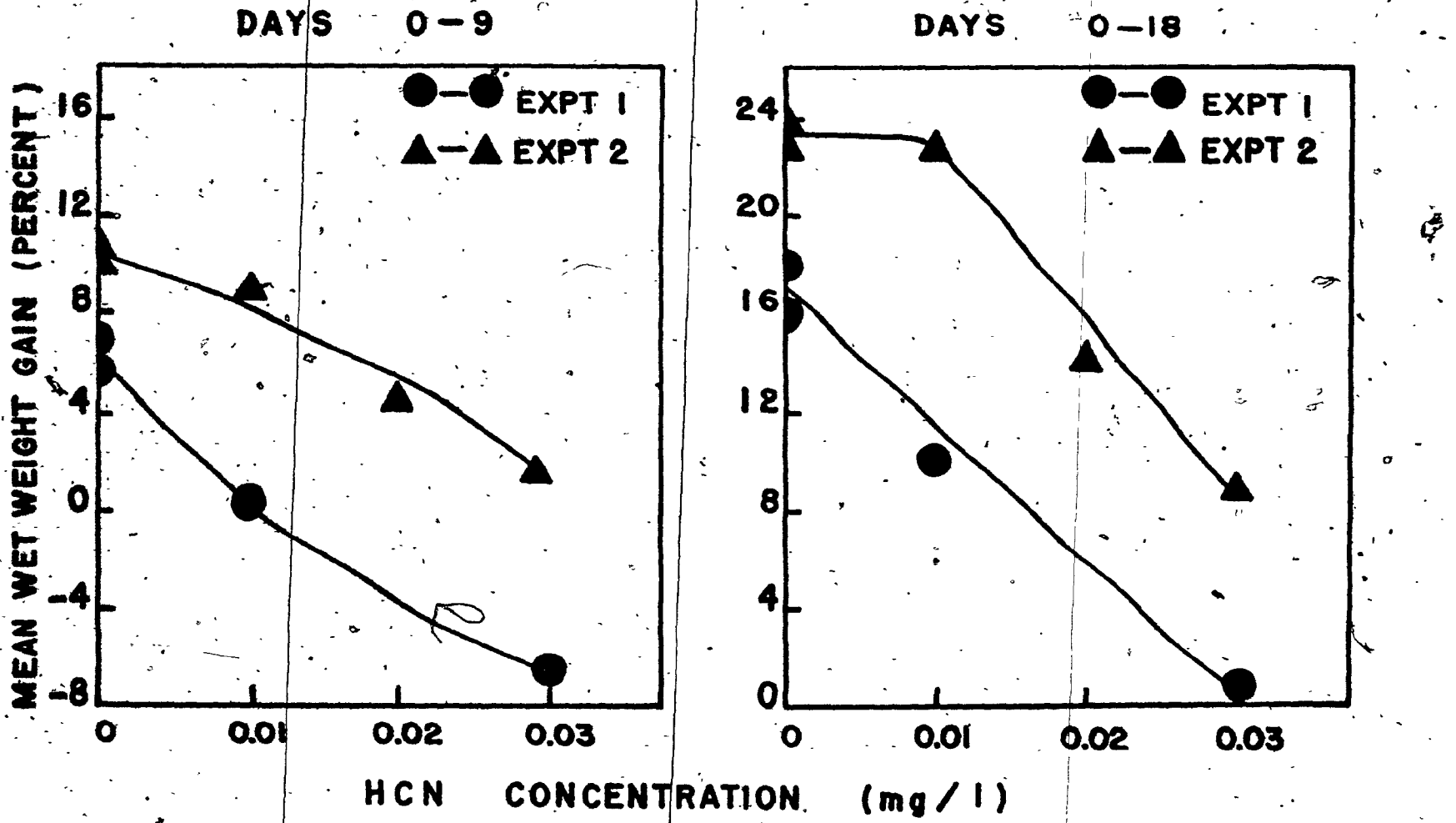

Figure 6. The relationship between the percent wet weight gain of juvenile rainbow trout and the concentration of cyanide to which they were exposed during 18-day growth experiments in continuously renewed water at 12.5°C.

fish gained approximately 10 percent. The fish exposed to 0.03 mg/l, however, showed virtually no growth. In experiment 2, the control fish gained 23.5 percent while the 0.01, 0.02 and 0.03 mg/l toxified groups showed relative wet weight gains of 23, 14.5, and 9 percent.

The mean percent wet weight gains of the controls in experiment 2 were higher than the control values obtained in experiment 1. This difference is readily attributable to the elevated feeding level, 2.5 instead of 2 percent of the wet weight per day, employed for the second experiment.

For both experiments, the mean wet weight gains for the cyanide-toxified groups were statistically compared to the control groups by means of Student's 't' tests. The analysis and levels of significance are given in Table 4. With the exception of the 0.01 mg/l cyanide-poisoned fish of experiment 2, the growth of all toxified groups was significantly reduced, relative to the controls, over the 18-day experimental period.

The specific growth rates of the various groups of fish during the first and second 9-day growth periods were calculated using the following equation (Brown, 1957, p. 365):

$$G = 100 \frac{\log_e Y_T - \log_e Y_t}{T - t}$$

where G is the specific growth rate in percent wet weight gain per unit of time and Y_T and Y_t are the recorded wet weights at times T and t, T being later than t.

The mean specific growth rates for each group of fish during periods 1 and 2 in both experiments were calculated from the individual rates of the fish within each group and are shown graphically in Figure 7. In all cases cyanide markedly increased specific growth rate from period 1 to period 2, the effect increasing with the concentration. Student's 't' tests were used to test the validity of the increases in specific growth rate from the first to the second period as shown by the toxified groups relative to the controls, and in all cases the increases were significant at least at the 95% level and in most cases at the 99.9% level (see Table 5). These increases in growth rate did not, however, fully compensate for the original repression of growth. It was only at 0.01 mg/l HCN in experiment 2 that the final wet weight gain was approximately equal to that of the controls (see Figure 6).

Effects on water content

The mean water content, expressed as percent of wet weight, for control and toxified fish at the termination of the 18-day experimental periods of experiments 1 and 2 are presented in Table 6. When the control water content values

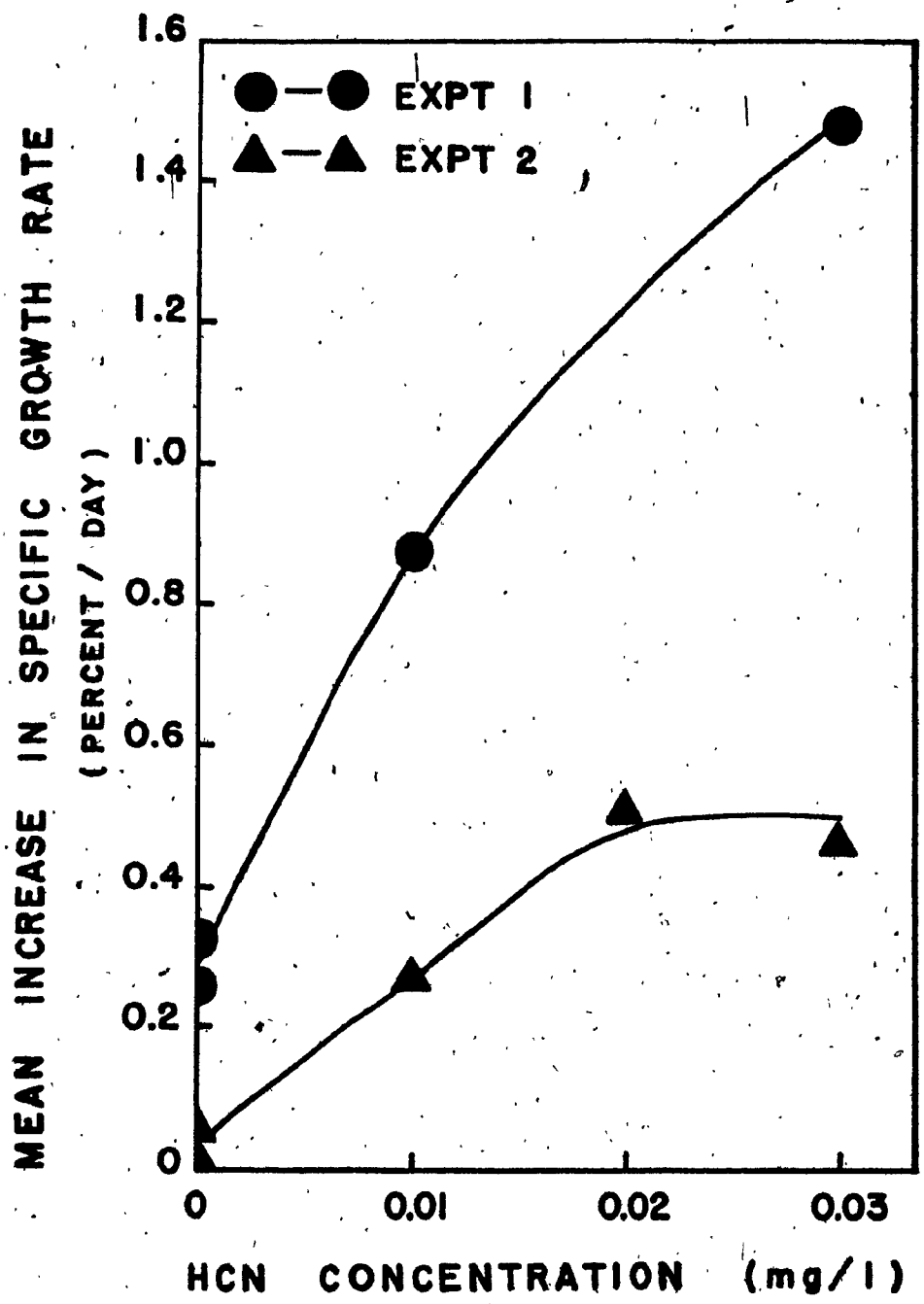

Figure 7. The relationship between the mean increase in specific growth rate from period 1 (days 0 to 9) to period 2 (days 10 to 18) of juvenile rainbow trout and the concentration of cyanide to which they were exposed during 18-day growth experiments in continuously renewed water at 12.5°C.

Table 5. Specific growth rates of rainbow trout exposed to various concentrations of cyanide during 18-day experiments carried out in continuously renewed water at 12.5°C.

Experiment No.	Cyanide Conc. (mg/l HCN)	No. of Fish in Sample	Mean Specific Growth Rate for Period 1: 0 to 9 Days (Percent/Day)	Standard Deviation of Mean Specific Growth Rate for Period 1	Mean Specific Growth Rate for Period 2: 10 to 18 Days (Percent/Day)	Standard Deviation of Mean Specific Growth Rate for Period 2	Mean Difference in Specific Growth Rates Between Periods 1 and 2 (Percent/Day)	Standard Deviation of Mean Difference	't' Value for Comparison of Mean Difference Between Control 1 and Toxified Groups	't' Value for Comparisons of Mean Difference Between Control 2 and Toxified Groups
1	0.0	27	0.78	0.57	0.99	0.56	0.29	0.69	-	-
	0.0	30	0.59	0.62	0.92	0.61	0.33	0.68	-	-
	0.01	24	0.01	0.73	0.89	0.79	0.88	0.73	3.12***	2.86***
	0.03	22	-0.82	0.83	0.67	1.09	1.49	1.15	4.63****	4.58****
2	0.0	30	1.06	0.31	1.12	0.35	0.06	0.39	-	-
	0.0	30	1.11	0.44	1.13	0.43	0.02	0.37	-	-
	0.01	30	0.92	0.64	1.20	0.52	0.28	0.36	2.27*	2.76***
	0.02	28	0.42	0.71	0.91	0.48	0.51	0.61	3.37***	3.73****
	0.03	21	0.19	0.53	0.66	0.44	0.47	0.52	3.22***	3.61****

* 0.05>p>0.02; ** 0.02>p>0.01; *** 0.01>p>0.001; **** 0.001>p

Table 6. Water content of rainbow trout exposed to various levels of cyanide during 18-day experiments carried out in continuously renewed water at 12.5°C.

Experiment No.	Cyanide Conc. (mg/l HCN)	No. of Fish in Sample	Mean Water Content as Percent of Wet Weight	Standard Deviation of Mean Water Content	't' Value for Comparison of Mean Water Content of Control 1 to Toxified Groups	't' Value for Comparison of Mean Water Content of Control 1 to Toxified Groups
1	0.0	30	80.34[1/]	0.95	-	-
	0.0	17	81.36	1.73	-	-
	0.0	20	80.42	1.26	-	-
	0.01	14	81.49	1.46	0.22	2.28*
	0.03	11	82.47	1.27	1.83	4.32****
2	0.0	30	77.55[1/]	0.81	-	-
	0.0	21	77.45	0.94	-	-
	0.0	21	77.44	1.29	-	-
	0.01	21	77.48	1.13	0.09	0.11
	0.02	18	77.94	1.41	1.32	1.16
	0.03	12	78.76	1.02	3.73****	3.07***

* 0.05>p>0.02; ** 0.02>p>0.01; *** 0.01>p>0.001; **** 0.001>p

1/ Pretest sample

for experiments 1 and 2 are compared, it is evident that the former values are substantially higher than the latter. In this respect it must be remembered that the fish in experiment 2 were considerably larger than those in experiment 1, and that as juvenile rainbow trout grow toward maturity their water content decreases (Love, 1970, p. 89).

The data indicates that exposure of juvenile rainbow trout to cyanide for an 18-day period produced a higher water content than in controls. The mean water contents of the various groups of cyanide-toxified fish were statistically compared to the control groups by means of Student's 't' tests. In the first experiment the water contents of the 0.01 and 0.03 mg/l HCN groups were significantly higher when compared to one control, but not when compared to the other. In the second experiment only the 0.03 mg/l cyanide group showed a water content that was significantly higher than that of the controls.

It should be noted that water content will bear an effect on the wet weight growth data presented under the previous heading. Since the mean water contents of all toxified groups were greater than those of the controls, expressing the growth data in terms of dry weight gain serves to magnify the disparity in growth between the control and cyanide-toxified fish (see Figure 8).

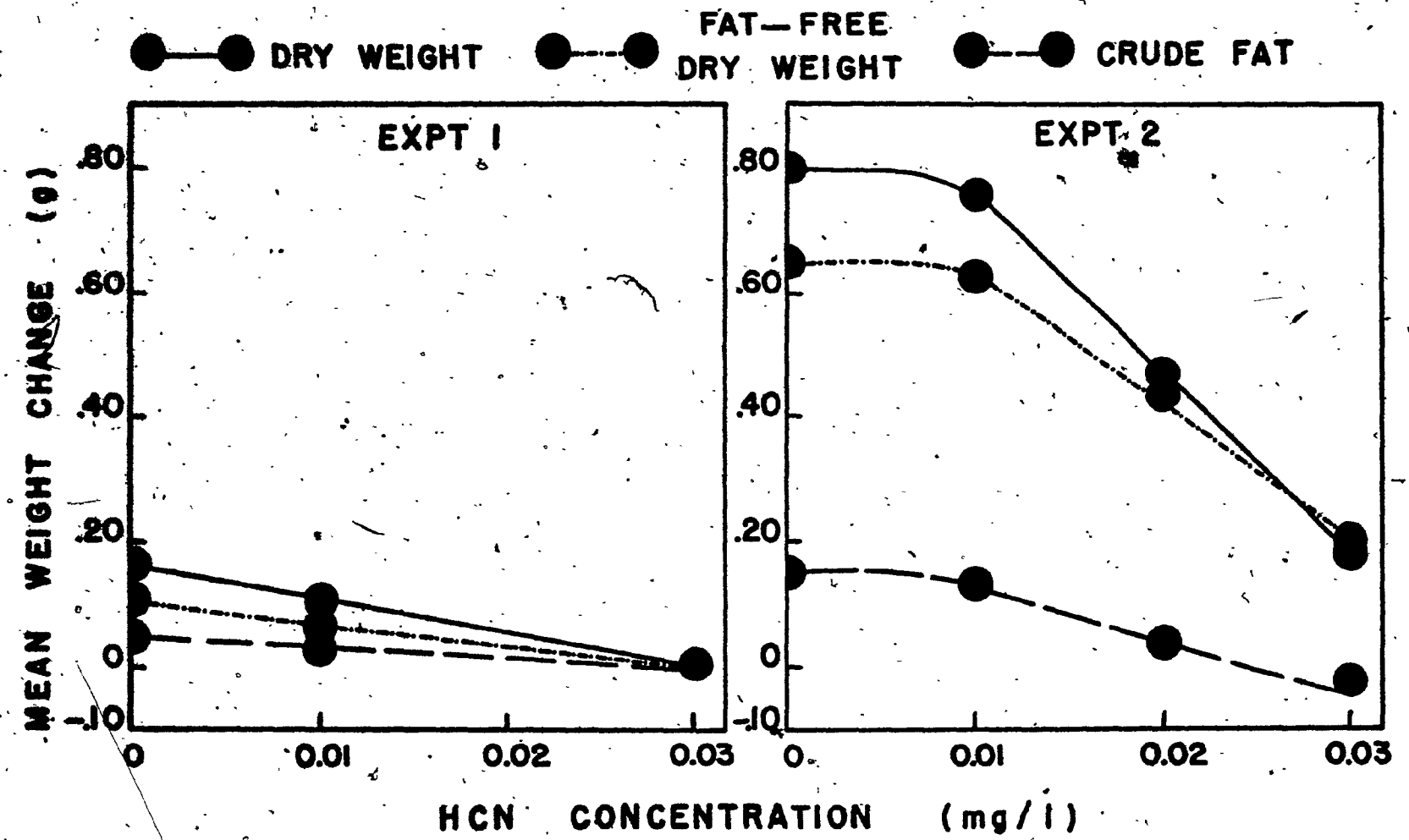

Figure 8. The relationships between the mean dry weight change, the mean fat-free dry weight change and the mean crude fat change shown by juvenile rainbow trout and the concentration of cyanide to which they were exposed during 18-day growth experiments in continuously renewed water at 12.5°C.

Effects on fat content

The mean fat content, as percent of dry weight, of the rainbow trout at the end of the 18-day growth periods of experiments 1 and 2 are presented in Table 7. Because of the small amount of tissue available for extraction from individual fish, each fat extraction was carried out on a pooled sample of three fish.

It should be noted that the elevated fat content of experiment 2 control fish relative to the control fish from experiment 1 can be attributed to the larger size of the organisms utilized in the second experiment, since the lipid content of fish increases with growth (Love, 1970, p. 91).

As indicated by the data in Table 7, an 18-day exposure to cyanide at concentrations of 0.02 and 0.03 mg/l HCN substantially lowered fat levels as compared to controls.

Subjection of the data to statistical analysis revealed significant reductions in fat content, relative to controls, for the 0.03 mg/l cyanide group of experiment 1 and for the 0.02 and 0.03 mg/l cyanide groups of experiment 2.

Table 7. Fat content of rainbow trout exposed to various levels of cyanide during 18-day experiments in continuously renewed water at 12.5°C.

Experiment No.	Cyanide Conc. (mg/l HCN)	No. of Samples Assayed (3 fish/ sample)	Mean Fat Content as Percent of Dry Weight	Standard Deviation of Mean Fat Content	't' Value for Comparison of Mean Fat Content of Control to Toxified Groups
1	0.0	4	12.20[1/]	0.52	-
	0.0	8	12.96	1.56	-
	0.01	4	12.19	0.39	0.97
	0.03	4	9.73	1.35	3.53***
2	0.0	4	18.25[1/]	1.93	-
	0.0	8	18.69	1.17	-
	0.01	4	18.61	1.56	0.09
	0.02	4	17.14	0.57	2.51*
	0.03	4	16.99	0.98	2.51*

* 0.05>p>0.02; ** 0.02>p>0.01; *** 0.01>p>0.001; **** 0.001>p

1/ Pretest sample

Figure 8 shows calculated values for the gains in dry weight, fat-free dry weight and crude fat exhibited by the control and cyanide-toxified fish during the 18-day growth periods of both experiments. In all cases the cyanide-toxified fish demonstrated at least some reduction in each of these three factors relative to the controls. At the end of the first experiment, the 0.03 mg/l HCN fish showed no gain in any of the three parameters, while at the termination of the second the mean of the 0.03 mg/l fish showed a small loss in fat content. During the second experiment, the fat-free dry weight gain represented almost the total dry weight gains of the 0.02 and 0.03 mg/l HCN toxified groups.

Effects on respiration during recovery from cyanide exposure

The mean daily respiration rates (resting metabolic rates) of the control and experimental rainbow trout during the 6-day periods following the end of the 18-day growth periods for experiments 1 and 2 are listed in Table 8. The general respiratory patterns are also illustrated in Figure 9.

During the six consecutive days following the end of the growth study for experiment 1, the mean respiration rate of the control fish gradually dropped from 0.36 mg O_2/g/hr to a steady level of 0.21 on the fifth day. The mean respiration rates of the fish which had been exposed to 0.01 and

Table 8. Respiration rates of control and previously cyanide-toxified juvenile rainbow trout during the 144-hour period following the termination of cyanide flow to the experimental groups.

Experiment No.	Cyanide Conc. (mg/l HCN)	No. of Fish in Sample	Mean Respiration Rates (mg O_2/g wet wt./hr)						Standard Deviation of Mean Respiration Rates					
			24 hours	48 hours	72 hours	96 hours	120 hours	144 hours	24 hours	48 hours	72 hours	96 hours	120 hours	144 hours
1	0.00	10	0.36	0.26	0.25	0.22	0.21	0.21	0.051	0.046	0.033	0.036	0.027	0.034
	0.01	5	0.24	0.26	0.32	0.34	0.26	0.26	0.053	0.034	0.046	0.046	0.036	0.036
	0.03	5	0.26	0.30	0.37	0.41	0.35	0.35	0.051	0.053	0.093	0.101	0.019	0.059
2	0.00	10	0.21	0.18	0.16	0.15	0.13	0.13	0.055	0.051	0.045	0.033	0.020	0.022
	0.01	5	0.21	0.17	0.15	0.14	0.13	0.13	0.058	0.064	0.039	0.033	0.015	0.019
	0.02	5	0.12	0.17	0.20	0.14	0.13	0.13	0.039	0.048	0.064	0.016	0.010	0.009
	0.03	5	0.11	0.16	0.21	0.17	0.15	0.15	0.022	0.043	0.033	0.026	0.018	0.018

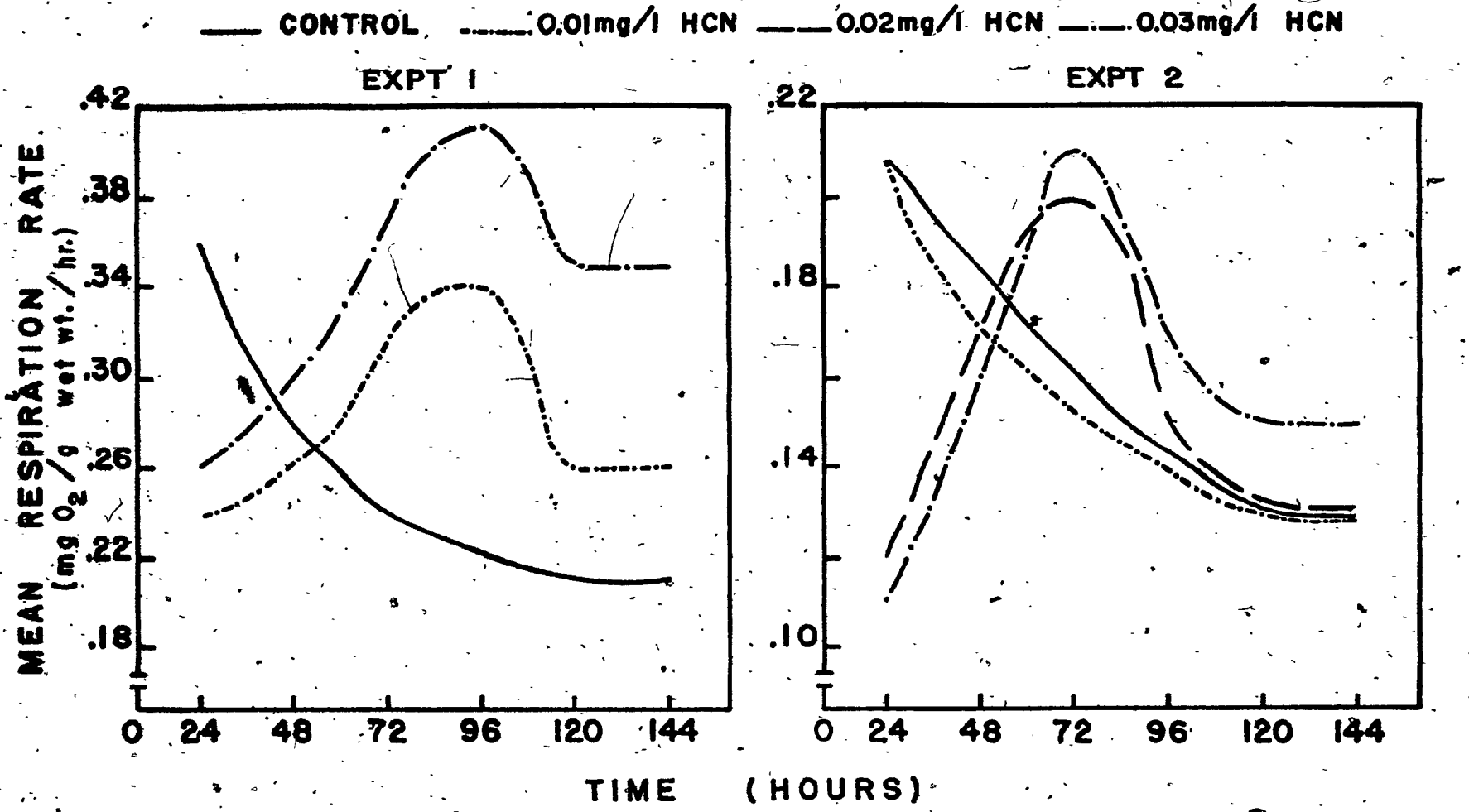

Figure 9. The respiratory patterns of juvenile rainbow trout during the 144-hour period following an 18-day exposure to various levels of cyanide.

0.03 mg/l HCN were initially lower than that of the controls, at 0.24, and 0.26 mg O_2/g/hr respectively. This was followed by a steady increase to respective peak levels of 0.34 and 0.41 on day 4. On days 5 and 6 the respiration rates of the cyanide exposed fish leveled off at values higher than the control, 0.26 for the 0.01 mg/l cyanide group and 0.35 for the 0.03 mg/l group.

The respiration patterns relative to the effects of cyanide obtained during experiment 2 are somewhat similar to those of the first; the overall respiration rates were, however, slightly lower than those of experiment 1. The fish used in experiment 2 were larger than those of experiment 1 and the metabolic rate of rainbow trout is known to decrease with increases in size (Phillips, 1972, p. 8).

As in experiment 1, the oxygen consumption of control fish dropped steadily from a high of 0.21 mg O_2/g/hr on day 1 to a constant level of 0.13 on days 5 and 6. Contrary to what happened in experiment 1, the 0.01 mg/l HCN toxified fish exhibited the same respiratory response, with only slight differences, as the control fish did. The 0.02 mg/l poisoned fish had an initial mean respiration rate of 0.12, substantially lower than that of the controls, which increased markedly to a high of 0.20 on day 3. This was followed by a decline to a steady rate of 0.13, equivalent to that of the controls, on days 5 and 6. The mean

respiration rate of the fish held at the highest cyanide level (0.03 mg/l) was initially similar to that of the 0.02 mg/l group, but slightly exceeded the level of that group from 72 hours onward until the termination of the test. On the whole, however, the fish previously poisoned at 0.02 and 0.03 mg/l HCN in experiment 2 gave a response very similar to that which occurred in experiment 1, although the differences from the control fish were smaller.

Histopathological effects of cyanide

Histological examination at the light level of liver tissue taken from control fish at the termination of the cyanide exposure period of the first experiment, as well as at the midpoint and termination of the second experiment, shows hepatic tissue in a normal healthy state (see Figures 10 and 11). The hepatocytes are arranged in a definite chord-like pattern around well defined sinusoids leading to a central vein. The nucleus of each hepatocyte is either spherical or slightly ovoid with regular surfaces and exhibits scattered chromatin granules and one or more nucleoli.

The examination of liver tissue from fish held at 0.01 and 0.03 mg/l HCN for 18 days during experiment 1, and from fish held at 0.01, 0.02, and 0.03 mg/l HCN for 9 and 18 days during experiment 2, revealed varying degrees

of necrobiosis, the degenerative process leading to cell death. In the second experiment it was observed that necrobiosis was already well established after 9 days of cyanide exposure and showed no further intensification after an additional 9 days of toxification. In view of this, only material taken from fish exposed to cyanide for the full 18-day period will be presented here. It should also be noted that at each sampling date tissue was taken from three fish in each of the test groups, and thoroughly examined by means of serial sections. The cyanide-induced histopathological effects described below and illustrated in Figures 10 to 15 for each of the cyanide concentrations tested are presented as being representative of the general conditions found throughout for each of those cyanide concentrations.

At 0.01 mg/l cyanide in both experiments the rainbow trout liver shows pycnosis (see Figure 12), a condition involving contraction of the nucleus and condensation of the chromatin into one or more heavily stained clumps.

At 0.02 mg/l (see Figure 13) cellular disorganization is evidenced by a breakdown in the chord-like arrangement of the hepatocytes (compare with Figure 10). In addition, cytolysis, which is manifested by an increase in cytoplasmic viscosity followed by swelling and bursting of the cytoplasmic membrane, occurs together with

karyolysis, a condition involving a loss of stainability by the nucleus and its chromatin.

The hepatic tissue taken from fish held at 0.03 mg/l (see Figures 14 and 15) exhibit an extensive breakdown in cellular organization as well as cytolysis and karyolysis.

While the symptoms of necrobiosis described above for each cyanide concentration were those which predominated at that concentration, the extent of tissue damage increased with the concentration of cyanide to which the fish had been exposed for 9 or 18 days. One of the most striking phenomenon evident from the histological data is the hepatic damage apparent in the fish exposed to 0.01 mg/l HCN during experiment 2. While this group of fish showed no poisoning symptoms when considering either their general behaviour, mean wet weight gain or respiration, there was extensive liver damage.

Examination, at the light microscope level, of gill tissue taken from the same fish from which liver tissue was examined revealed no apparent cyanide-induced histopathological damage.

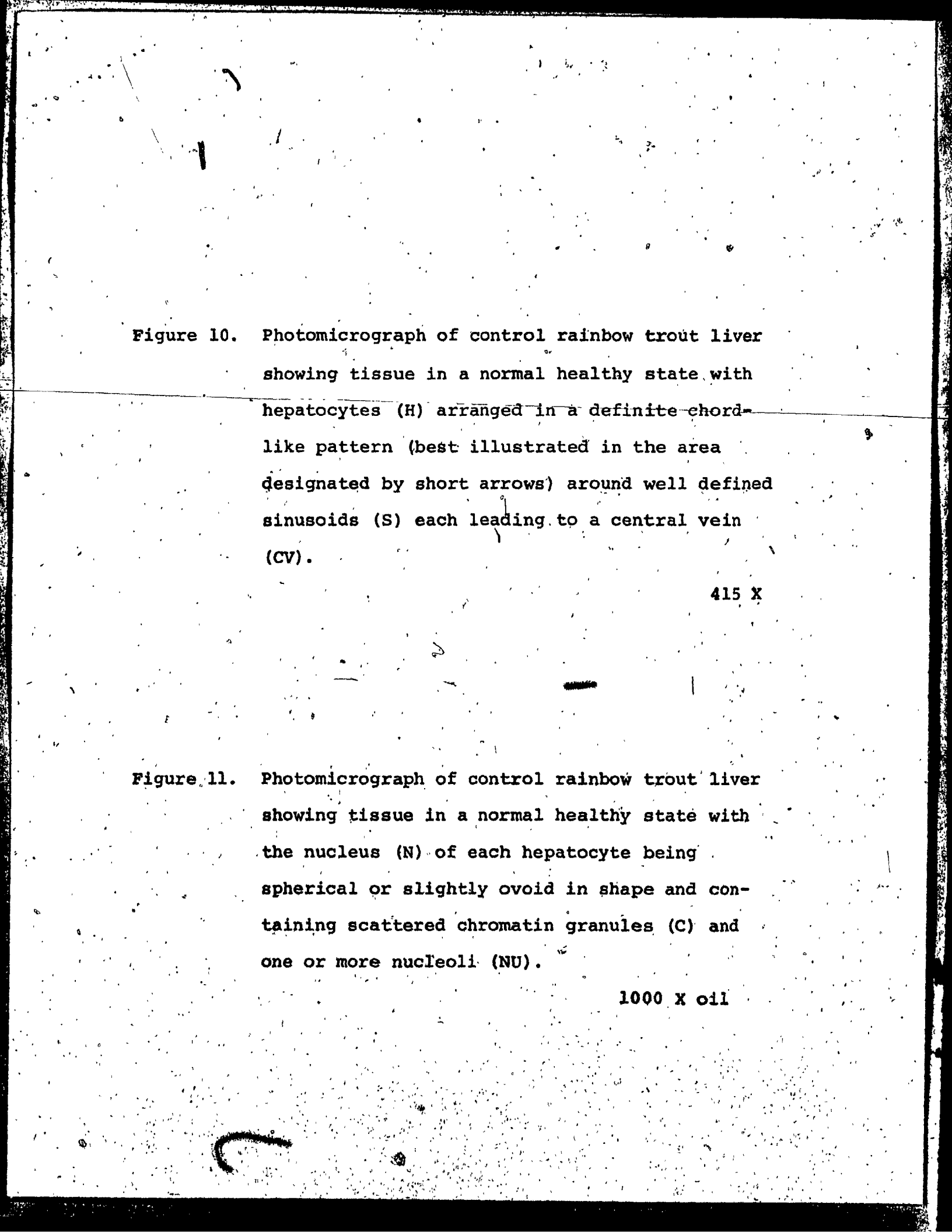

Figure 10. Photomicrograph of control rainbow trout liver showing tissue in a normal healthy state with hepatocytes (H) arranged in a definite chord-like pattern (best illustrated in the area designated by short arrows) around well defined sinusoids (S) each leading to a central vein (CV).

415 X

Figure 11. Photomicrograph of control rainbow trout liver showing tissue in a normal healthy state with the nucleus (N) of each hepatocyte being spherical or slightly ovoid in shape and containing scattered chromatin granules (C) and one or more nucleoli (NU).

1000 X oil

Figure 12. Photomicrograph of the liver from a rainbow trout which had been exposed to 0.01 mg/l HCN for 18 days in continuously renewed water at 12.5°C showing contraction of the nuclei (N) and condensation of the chromatin (CC) into heavily stained clumps.

1000 X oil

Figure 13. Photomicrograph of liver from a rainbow trout which had been exposed to 0.02 mg/l HCN for 18 days in continuously renewed water at 12.5°C showing a general loss of organization, the bursting of cytoplasmic membranes (BCM) and a loss of stainability by nuclei (LN).

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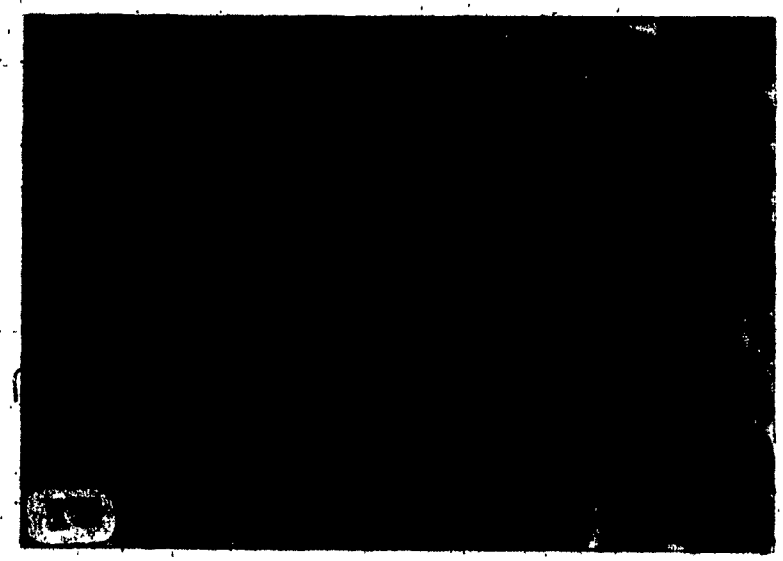
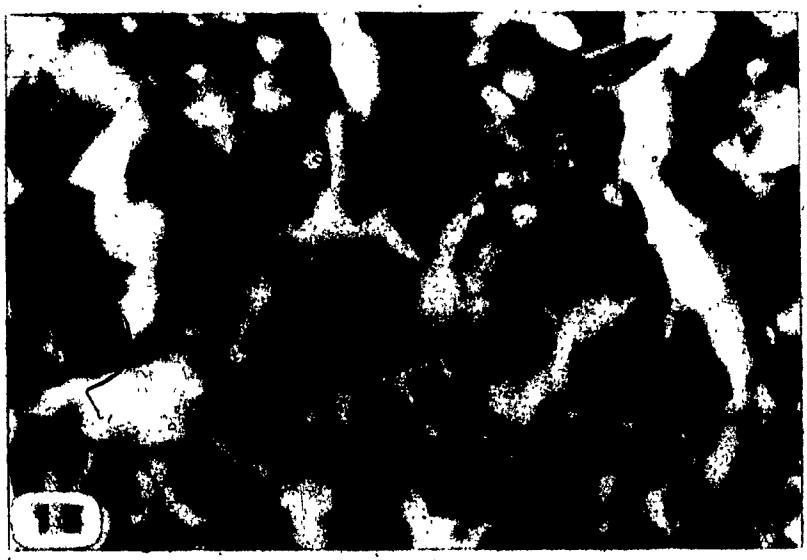


Figure 14. Photomicrograph of the liver from a rainbow trout which had been exposed to 0.03 mg/l HCN for 18 days in continuously renewed water at 12.5°C showing cellular disorganization as evidenced by a breakdown in the chord-like arrangement of the hepatocytes, a condition best illustrated in the area designated by arrows.

415X

Figure 15. Photomicrograph of the liver from a rainbow trout which had been exposed to 0.03 mg/l HCN for 18 days in continuously renewed water at 12.5°C showing the bursting of cytoplasmic membranes (BCM) and a loss of stainability by nuclei (LN)

1000 X oil



DISCUSSION

Physiological Implications

This study of the chronic effects of hydrocyanic acid poisoning on rainbow trout revealed that at all three concentrations tested, 0.01, 0.02 and 0.03 mg/l HCN, cyanide either adversely affected growth, altered respiration, or induced histopathological damage in the liver.

Growth

Over the entire 18-day growth period of each experiment chronic exposure of rainbow trout to cyanide caused significantly reduced growth at concentrations which were 15 to 45 percent of the 48-hour LC50 (0.07 mg/l HCN, Brown, 1968). In experiment 1, at rations of 2 percent of their body weight per day, the fish at 15 and 45 percent of the LC50 showed reductions in mean wet weight gain of 41 and 97 percent, whereas in the second experiment, at rations of 2.5 percent of their body weight per day, the fish at 15, 30 and 45 percent of the LC50 suffered reductions in mean wet weight gain of 2, 40 and 65 percent (see Figure 6).

Speyer (1975) observed a similar response with rainbow trout held at 0.02 mg/l HCN over a 21-day period and fed a daily ration of 2.5 percent of their body weight. On the other hand, juvenile coho salmon (Oncorhynchus kisutch Walbaum) exposed to concentrations varying from 0.01 to

0.08 mg/l HCN for 24 days at 16°C and fed an unrestricted diet of earthworms saw their growth reduced only at the highest concentration (80 percent of the 0.10 mg/l LC50) (Leduc, 1966b). Leduc (1966b) also measured the growth of chiclids (Cichlasoma bimaculatum Linnaeus) fed unrestricted diets of Tubificid worms during 36-day periods of exposure to cyanide concentrations ranging from 0.008 to 0.10 mg/l HCN at 25°C. At the end of the 36-day period only the fish held at 0.08 and 0.10 mg/l HCN (approximately 65 and 85 percent of the 0.12 mg/l HCN LC50) showed any appreciable reduction in growth relative to the controls.

In comparing our results and those obtained by Speyer (1975) with Leduc's (1966b) three important variables other than species difference must be considered: temperature; quality of food; and size of ration.

Temperature is a determining factor of both short-term and long-term bioassay results (Sprague, 1970). Although there has been extensive study of the effects of temperature on the various physiological parameters of fish, there is a dearth of information on the effects of temperature on response to toxicants, particularly at sublethal concentrations. While it has been shown (Sumner and Doudoroff, 1938; Anon., 1972) that the acute toxicity of cyanide to fish decreases with temperature, it is quite possible that the response would be reversed at sublethal

concentrations where cyanide could be more rapidly metabolized by fish held at a higher temperature, relative to those held at a lower temperature, due to the increases in metabolic rate which accompany increasing temperature. With regard to temperature effect at low cyanide concentrations, the only comparable growth studies are those of Leduc (1966b) and Speyer (1975). The extensive reduction in growth observed in this study and by Speyer may be attributed in part to the lower temperature relative to Leduc (1966b) who observed a much lesser effect on coho salmon tested at 16°C and on cichlids tested at 25°C.

Leduc (1966) fed his test organisms a natural diet while artificial rations were employed by Speyer (1975) and for the present study. Natural food could possibly enhance the resistance to poisoning and thus reduce the effects of HCN on growth. The proportions of protein, fat and carbohydrate in the diet could also affect the ability of fish to withstand cyanide poisoning. The respiratory quotient of fat is 0.71, of protein 0.80 and of carbohydrate 1.0 (Prosser and Brown, 1961, p. 161). This indicates that, when equal amounts are being considered, fat and protein require more oxygen for complete combustion than do carbohydrates. Since cyanide is a respiratory depressant, we may speculate that fish held on a diet rich in carbohydrate would be less affected by cyanide than those held on a ration with higher proportions of protein and fat. It

should also be noted that carbohydrates (glucose) would feed into the glycolytic pathway, thus supplying energy while bypassing the cyanide sensitive Krebs cycle. The natural diets employed by Leduc (1966b) undoubtedly differed from the artificial diets used here and by Speyer (1975) in the proportions of the various constituents, thus introducing a further variable into any comparison. Although the diets used during the two experiments of this study differed in the proportion of fat present (see Table 2) and hence, by difference, in the amount of carbohydrate, we doubt that the differences were large enough to result in the type of variation which could be expected in view of the above diet considerations.

Overall, Leduc (1966b) demonstrated that cyanide levels in the order of 65 to 85 percent of the LC50 were required to induce some reduction in growth of coho salmon and cichlids fed unrestricted diets. He noted that the cyanide-toxified cichlids exhibited a higher food consumption but a reduced food conversion efficiency, and speculated that had the fish been held on equal restricted diets the reduction in growth would have been much greater. The present study with restricted rations appears to indeed confirm this hypothesis, as does Speyer's (1975) work.

Warren (1971) defines the scope for growth of an organism as the difference between the energy content of the diet and the energetic cost of all activities other than growth under a particular set of environmental conditions. A stressing factor such as a toxicant can lead to a higher cost of maintenance with a concurrent reduction in the energy available for growth. On an unrestricted diet fish may compensate for a toxic effect by consuming more, and growth reduction will not be so severe as it would be if the fish were held on a restricted diet as was the case during this study. A trend of this nature was observed by Chapman (1965) with cichlids (Cichlasoma bimaculatum Linnaeus) held on either restricted or unrestricted diets while being exposed to sublethal concentrations of potassium pentachlorophenate. The poisoned fish held on unrestricted diets grew as well as the controls, while the growth of those held on restricted diets was severely repressed. Oladimeji and Leduc (1974) demonstrated that dietary methoxychlor increased the food maintenance requirements of brook trout (Salvelinus fontinalis Mitchill). It would appear that cyanide, like these other toxicants, acts as a metabolic stressing agent, increasing maintenance requirements and thus reducing the resources available for growth.

Relative to the controls, all cyanide exposed rainbow trout markedly increased their specific growth rate from the first to the second 9-day period of each experiment (see Figure 7), the effect being more pronounced during the first experiment (4 g fish) than during the second (12 g fish). Except during very early development the rate of acceleration of growth rate in fish decreases with age (Weatherley, 1972. p. 17). The greater increase in growth rate from the first to the second period shown by the 4 g control fish in experiment 1, compared to the 12 g control fish in experiment 2, indicates that the 4 g fish were in a stage of more rapid acceleration in growth rate. This would allow the 4 g cyanide-treated fish a greater range for rebound from toxicant stress and hence the more substantial increase shown by these fish from period 1 to period 2 relative to the 12 g toxified fish. In the present study we found that the accelerated growth during the second half of the cyanide exposure period did not fully compensate for the original repression. Only at 0.01 mg/l HCN in the second experiment did the growth of the toxified fish approximate that of the controls by the end of the 18-day exposure period (see Figure 6).

The rebound of growth after an initial repression by chronic cyanide poisoning seems to be a widespread phenomenon in various fish species. Leduc (1966b, 1967)

noted this response with juvenile coho salmon, cichlids and developing Atlantic salmon (Salmo salar Linnaeus) embryos, the response increasing with the cyanide concentration to which the fish were exposed. Speyer (1975) also observed this phenomenon in rainbow trout exposed to 0.02 mg/l HCN for 21 days at 11°C.

The delayed acceleration in growth rate exhibited by the cyanide-toxified fish is an indication of some acclimation or adaptation to the poison. While the nature of the mechanisms responsible for this acclimation are unknown, some explanation can be attempted. The primary pathway of cyanide detoxification in mammalian systems is by reaction with thiosulphate to form thiocyanate. This reaction is mediated by the liver enzyme rhodanese, with the thiocyanate being eliminated in the urine (Fruton and Simmonds, 1958. p. 796). Although it appears that to date rhodanese has not been isolated from the liver of any teleost fish, Achard and Binet (1934) showed that thiosulphate significantly prolonged the survival of carp (Cyprinus carpio Linnaeus) subjected to lethal cyanide concentrations, a phenomenon which tends to suggest the presence of a thiosulphate-rhodanese-thiocyanate system in fish. If this system is indeed present, it does not seem unrealistic to postulate a system whereby cyanide would induce an increase in rhodanese production and hence in the rate of cyanide detoxification.

Although hydrocyanic acid is readily absorbed in the blood stream via the gills, it does not react with hemoglobin but with methemoglobin to form the bright red nontoxic compound cyanomethemoglobin (Hewitt and Nicholas, 1963, p. 386-399). An organism could presumably increase its rate of cyanide detoxification by increasing the rate of methemoglobin production, thus to some extent freeing the cytochrome system of cyanide inhibition. Detoxification of cyanomethemoglobin would then proceed via the rhodanese system. During our study there was evidence of high levels of cyanomethemoglobin from the bright red appearance of the blood showing through the gills and the cranial area of rainbow trout exposed to 0.02 and 0.03 mg/l HCN.

Future research must be directed at determination of both the methemoglobin and rhodanese levels in fish subjected to cyanide stress in order to fully elucidate the methods of cyanide detoxification in fish.

Water and fat content

Water and fat contents of the control and cyanide-toxified fish were measured as further indication of the overall nutritional state of the fish. Over the 18-day experimental period cyanide induced higher water content and lower ether-extractable fat content in the toxified fish

than in the controls. Speyer (1975) noted a similar trend in 21-day growth experiments with rainbow trout exposed to 0.02 mg/l HCN. The increased water content of the toxified fish, as well as being indicative of the depleted nutritional state of the organisms, might also indicate a decrease in the osmoregulatory capacity of the fish, thus rendering them incapable of maintaining a normal water balance. This is consistent with the work of Chan (1971) who demonstrated that exposure to cyanide levels of from 0.01 to 0.03 mg/l HCN for 28 days seriously impaired osmoregulatory function in rainbow trout. Rao (1968), working with rainbow trout, found that in freshwater the cost of osmoregulation amounted to 20 percent of the active metabolic rate. When one considers the respiratory depressant activity of cyanide and the associated depletion of available energy as ATP, the evidence of impaired osmoregulatory capacity in cyanide poisoned fish is not unreasonable.

All cyanide exposed fish showed at least some reduction in fat content relative to controls, to the point where the fish exposed to 0.03 mg/l HCN in both experiments showed no gain in mean fat content relative to the mean of the pretest fish (see Figure 8). This response is consistent with both the scope for growth of Warren (1971) and with the cyanide-induced reductions in wet weight gain observed during this study. If the toxicant is impinging sufficiently

on the energy budget of the fish to result in a reduction in growth, one would indeed expect a reduction in biosynthesis of lipids with an unavoidable disturbance of physiological and biochemical functions, including the metabolism of compounds such as phospholipids and steroid hormones.

As indicated by Figure 8, cyanide-toxified fish were synthesizing fat-free dry matter (protein) in preference to lipids. It should be noted that the energy of biosynthesis of lipids is greater than that of protein (Florkin, 1960. p. 225-269) and hence protein could be more easily produced, on the basis of energetics, by the energy poor cyanide-toxified fish.

Metabolic Rate

During the 6-day period following the termination of the growth experiments the respiratory patterns of the cyanide-toxified fish differed substantially from those of the controls (see Figure 9). The initially elevated mean respiration rates of the control fish in experiments 1 and 2 gradually decreased to constant levels after 120 hours. Waiwood and Johansen (1974), who studied the effects of methoxychlor poisoning on white suckers (Catostomus commersoni Lacépède), noted a similar trend with their control fish although the respiration rates stabilized in a much shorter period of time (36 hours). This gradual decrease in the standard metabolic rate of control fish is most

likely attributable to either acclimation of the fish to the respirometers or to a transition from a feeding to a nonfeeding metabolic regime, or to both. Under stress of the type which fish might initially experience upon introduction into respirometers, organisms are known to release thyroxine into the bloodstream. Since thyroxine uncouples oxidative phosphorylation, this would result in an increased respiration rate (Goodman and Gillman, 1965. p. 1473). As the fish become more accustomed to their new environment there would be a decay of thyroxine levels and a corresponding decrease in oxygen consumption.

While the respiratory patterns of the control fish were the same in both experiments, the actual rates were higher in experiment 1 (4 g fish) than in experiment 2 (12 g fish). This difference can be largely attributed to the different sizes of the two groups of fish since the metabolic rate of fish is known to decrease as size increases (Phillips, 1972. p. 8).

With the exception of the fish exposed to 0.01 mg/l HCN during experiment 2, the respiration rates of all cyanide-toxified rainbow trout were initially lower than those of the controls, a response that was not unexpected when one considers that cyanide is known to act as a respiratory depressant in fish (Jones, 1964. p. 86-96), and that residual cyanide most likely remained in the fish for some

period of time after the cessation of toxification. The initial low respiration rates of the cyanide-toxified then increased steadily, peaking after 72 to 96 hours. This overcompensation of oxygen consumption during recovery from cyanide poisoning may be due to the oxidation of accumulated reduced metabolites. After peaking, and with the exception of the 0.02 mg/l HCN group from experiment 2, the standard metabolic rates of the cyanide-toxified fish stabilized at levels above those of the controls, a phenomenon which was also noted by Beadle (1931) in studies with the marine invertebrate Nereis diversicolor during the 50 hours following the termination of cyanide poisoning, and which is perhaps the most interesting aspect of these results. This effectively represents an increase in the resting metabolic rate of these fish for at least some period of time following the cessation of cyanide exposure. The physiological and/or biochemical significance of this phenomenon is not clear. There is an obvious long lasting effect of cyanide on respiration which is perhaps due to either endocrine disturbance or reduced oxidative phosphorylation or both. The elevated respiration rates of the cyanide-poisoned fish would most likely return to normal at some undetermined point in time. This parameter was not explored, however, since the tests were terminated after 6 days in order to avoid the onset of possible prolonged starvation effects. Future work should most definitely be

directed toward the study of the respiratory patterns of fish during recovery from chronic cyanide toxicity over more prolonged periods of time, preferably with a respirometer (Solomon and Brafield, 1972) whose design would allow feeding of the test organisms during the experimental period.

Histopathology

On the biochemical level cyanide toxicity is transitory since the HCN-Fe^{+++} porphyrin complex responsible for the inhibition of cytochrome oxidase is a reversible one. Cyanide can therefore be called a truly noncumulative protoplasmic poison (Hewitt and Nicholas, 1963. p. 386-399), and one would expect recovery to occur following a relatively short time lapse. If tissue damage follows cyanide exposure, however, then recovery will most likely take a much longer period of time.

Histological examination of all the tissue samples taken from cyanide-toxified fish revealed the presence of some degree of necrobiosis, the process of cellular degeneration (see Figures 10 through 15). Since the liver plays such an important role in intermediary metabolism, destruction of hepatocytes would have very serious consequences for the survival of toxified fish. One could postulate a progressive intensification of cyanide-induced liver damage through the inhibition of oxidative energy yielding reactions in the extremely mitochondria rich hepatocytes,

leading to degradation as a result of insufficient energy to maintain cellular integration.

Considering the numerous metabolic processes carried out by the liver, it is not surprising that the cyanide-toxified fish showed the substantial reductions in wet weight gain reported here. Not only would the ability of the liver to supply the products of intermediary metabolism necessary for growth be limited, but there would also be a decrease in the capacity of the liver to eliminate the elevated levels of waste products that would be derived during periods of high metabolic activity such as growth. It is interesting to note that while liver damage showed no apparent change from 9 to 18 days, the specific growth rate increased significantly during that period of time. This would tend to suggest that some hepatobiochemical alterations not evident at the histopathological level were occurring during this period of time, thus improving the ability of the fish to deal with cyanide.

When necrobiosis of hepatocytes does not destroy the entire cell population, the destroyed tissue can be regenerated by division of the remaining healthy hepatocytes. If, however, the cells are repeatedly destroyed while regeneration is still in progress, as would be the case if the organisms were exposed to a continuous or pulsating stress by cyanide, fibrosis and eventually cirrhosis, an irreversible

liver destruction, will result (Bloom and Fawcett, 1968. p. 600-601).

While the qualitative histopathology results presented here indicate the type, and to a limited extent the degree, of cyanide induced liver damage, obtaining quantitative expressions of damage should be the aim of future work. Quantitative histology would be more informative since it could be more readily correlated to parameters such as growth, respiration and swimming ability. Considering the extent of structural damage evident during this study, it is also evident that future work directed at the determination of the activity of selected hepatic enzymes could also prove informative.

The results for each of the three parameters tested, growth, respiration during recovery and histopathology, suggest a relationship between fish size and the magnitude of response to cyanide. In experiment 1, the rainbow trout used weighed approximately 4 g and exhibited deleterious effects on the three parameters tested at all cyanide concentrations used. In experiment 2, with fish weighing about 12 g, deleterious effects were observed for all parameters at 0.02 and 0.03 mg/l while at 0.01 mg/l HCN only necrobiosis of hepatocytes could be detected. This would tend to suggest that the larger fish were more resistant to the effects of cyanide toxicity. It should be noted, however,

that the 4 g fish were fed 2 percent of their wet body weight per day while the 12 g fish received 2.5 percent. At least as far as growth is concerned and according to Warren's (1971) scope for growth discussed earlier, the elevated feeding level could possibly account for the improved performance of the 12 g fish since they would have more energy resources at their disposal to counteract cyanide effects. It seems improbable, however, that the relatively small 0.5 percent spread in feeding levels between the two groups of fish could account for the whole difference between the degree of their response, and further research into the phenomenon of fish size and degree of cyanide response is required.

The increased resting metabolic rates of the cyanide-poisoned fish during recovery represent a decrease in the scope for activity (Fry, 1947) of these organisms after removal from cyanide, since there is a reduction in the difference between the minimum and maximum metabolic rates of the fish, and hence in the amount of energy available for activity above that required for the maintenance of life. The presence of hepatic damage at the end of the exposure periods also tends to suggest a reduction in physiological capabilities after the termination of exposure, since this damage would require at least some period of time for complete recovery. Both the increased resting

metabolic rate and the presence of hepatic damage tend to substantiate the swimming stamina results of Neil (1957) and Broderius (1970) who noted that the return of full swimming capacity following the termination of exposure of brook trout and coho salmon respectively to cyanide concentrations from 0.01 to 0.05 mg/l HCN required from 14 to 20 days even at the lowest cyanide concentration tested.

Ecological Significance

While our laboratory studies indicate that exposure of rainbow trout to sublethal cyanide poisoning induces reduced growth, elevated water content, reduced fat content, changes in respiration, and necrobiosis of hepatocytes, these results must be evaluated within the context of the release of cyanide into the aquatic environment. While our results were obtained in the rigorously controlled constant conditions of the laboratory, the effects of a stressing factor such as cyanide on fish must ultimately be interpreted within the multifactored dynamic system which constitutes the natural environment.

Growth is the determining factor in fish production and this study has shown that the scope for growth (Warren, 1971) of rainbow trout can be seriously impaired by low levels of cyanide. It has been suggested that fish could compensate for this cyanide-induced reduction in energy available for growth by consuming larger quantities of food.

This is highly unrealistic in nature where food is usually limited. In interpreting the growth results it should also be remembered that the experiments were carried out in tanks with no current where the fish had to exert relatively little effort in obtaining food. These activities would be more costly in a stream where the fish would have to swim against current as well as searching for and capturing food.

These increased energy expenditures could be further complicated in a natural aquatic system by variations in environmental factors such as temperature and dissolved oxygen. Our laboratory studies were carried out at 12.5 $\pm$ 0.5°C and at oxygen levels near saturation. The concentration of oxygen in natural waters varies due to natural and/or human influences, and any reduction would accentuate the respiratory depressant effects of cyanide since Cope (1961) showed that increased oxygen reduced the toxicity of cyanide to goldfish (Carassius auratus Linnaeus). For the same reasons the higher oxygen consumption exhibited by the toxified fish after cyanide exposure could place the fish under a greater stress than was observed in the laboratory.

Although the statistically significant increases in water content demonstrated by some groups of cyanide-toxified fish are small in absolute terms, they are most

probably significant in a physiological and ecological sense. It has been shown (Wood and Randall, 1972) that urine flow in rainbow trout markedly increases during periods of exercise. The increased stress of a cyanide-induced elevated water content could therefore increase the renal load during periods of high swimming activity and thus reduce performance. The statistically significant decrease in fat content demonstrated by the poisoned fish are also most likely ecologically significant. Depletion of fat reserves and the implied concurrent interference with lipid metabolism can have serious consequences for the survival of fish populations. As well as providing metabolic reserves for energy supply during periods of starvation, lipids are vital during reproduction. It has been shown that in the sockeye salmon (Oncorhynchus nerka Walbaum) 8 percent of the total lipid content of females is transferred to the ovaries during reproductive maturation (Love, 1970. p. 105). Severe depletion of lipid reserves would therefore impair reproductive capabilities or, failing this, tax the already depleted fat levels to such an extent that the females are left with insufficient reserves to survive periods of starvation. Newsome (1971), working with yellow perch (Perca fluviatilis flavescens Mitchill), attributed the selective mortality of females during the winter to insufficient fat reserves after translocation of lipids to the gonads.

A most interesting aspect of this study was the occurrance of hepatic necrobiosis in cyanide-toxified fish at concentrations as low as 0.01 mg/l HCN. Whether in the laboratory or in a natural environment the ability of fish to survive for an extended period of time would be jeopardized by hepatic damage. It can also be contended that, in light of the liver damage caused by cyanide, other physiological responses lose some of their significance since the condition of the liver clearly defines the disabled state of the organisms. Of all the parameters measured, histopathological examination of the liver proved to be one of the most sensitive and significant indicators of chronic cyanide poisoning in rainbow trout.

In determining the extent to which the observed effects are applicable in nature, one must consider the dynamics of cyanide, a highly labile compound which can certainly be expected to vary in both time and space in relationship to the toxicant source. These fluctuations could easily lead to a situation where the fish are exposed to varying toxic periods during their life cycle. Future research should most certainly be directed at evaluating the effects of pulsating cyanide exposure on the ecophysiology of fish. Alabaster, et al. (1972) suggest that not only should fluctuations in a single toxicant such as cyanide be considered, but also the implications of fluctuating multiple toxicity. Research on the nature of both

chemical and temporal interactions of various toxicants must be undertaken before reliable predictions of toxicity can be made.

It appears obvious that cyanide can markedly reduce the overall scope for activity of the individual fish (Fry, 1947) and hence the competitive ability and survival of the fish population as a whole (Iverson and Guthrie, 1969). In view of the results obtained under the laboratory conditions described here, particularly the development at 0.01 mg/l HCN of hepatic necrobiosis in fish that were apparently healthy in other respects, it is evident that the concentration of cyanide in receiving waters expected to support viable fish populations must be relatively low. Following the usual procedure for determining a maximum concentration, that of dividing the lowest concentration at which toxic effects are manifested by an application or safety factor of 10, the maximum concentration for cyanide in surface waters would be 0.001 mg/l HCN, whereas the United States Environmental Protection Agency (Anon., 1973. p. 189-190) sets the maximum concentration at 0.05 mg/l HCN.

SUMMARY

Two experiments were performed to determine the effects of chronic hydrocyanic acid poisoning on the growth, respiration during recovery and liver tissue of juvenile rainbow trout (Salmo gairdneri Richardson).

The fish were exposed to 0.00, 0.01, 0.02 and 0.03 mg/l HCN for 18 days in 60 l polyethylene tanks containing continuously renewed water at 12.5° ± 0.5°C, and were fed an artificial diet at a rate of 2 to 2.5 percent of their wet body weight per day.

Cyanide was seen to significantly reduce the mean wet weight gain of the 4 g trout exposed to 0.02 and 0.03 mg/l HCN during the first experiment and of the 12 g fish exposed to 0.02 and 0.03 mg/l HCN during the second. The mean specific growth rates of all groups of cyanide-toxified fish, which were initially depressed relative to the controls, markedly increased by the end of the experiments, suggesting some degree of acclimation to cyanide. This late recovery could not, however, fully overcome the initial reduction and only at 0.01 mg/l HCN in experiment 2 did the secondary increase in specific growth rate fully compensate, in terms of wet weight gain, for the original depression caused by cyanide.

By the end of the 18-day experiments a small though statistically significant higher percent water content was noted in fish exposed to 0.03 mg/l HCN, while a significantly lower fat gain was noted in those fish exposed to 0.02 and 0.03 mg/l HCN.

In some of the test groups cyanide produced higher metabolic rates during the 6-day recovery period following the termination of exposure to the toxicant. After an initial peak the respiration rates of the fish exposed to 0.01 and 0.03 mg/l HCN in experiment 1 and to 0.03 mg/l HCN in experiment 2 stabilized at rates higher than those of the controls.

Exposure of juvenile rainbow trout to all concentrations of cyanide tested during both experiments induced some degree of hepatic necrobiosis, the condition being well developed after 9 days and showing no appreciable change in either type nor degree by 18 days. The most interesting aspect of the induced necrobiosis was its appearance in trout exposed to 0.01 mg/l HCN in experiment 2, fish which appeared unaffected by cyanide in terms of the other parameters tested. As well as establishing the presence of a toxic effect at 0.01 mg/l HCN, this observation also indicates the high degree of sensitivity which can be obtained by the use of histopathological examination.

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