Biochemical Investigation on the Mode of Action of Cyanide in Rainbow Trout During Chronic Exposure

Pierre Raymond

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ABSTRACT

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The mode of action of cyanide in rainbow trout (Salmo gairdneri, Richardson) was investigated during 20-day exposure periods to 0.01, 0.02 and 0.03 $mg.L^{-1}$ HCN at 12°C in laboratory flow-through aquaria. The following parameters measured in 170 g and 30 g size group fish were: liver cytochrome oxidase activity, accumulation of thiocyanate in blood plasma, liver glycogen and hepatosomatic ratio.

The results indicate that rainbow trout of both size groups experienced a 60 to 80 percent reduction of their liver cytochrome oxidase activity within 24 h of exposure to all three concentrations of HCN. For both fish sizes examined, the control cytochrome oxidase activity differed, the larger fish having a greater activity per mg of protein. The minimal activity was attained in about 24 h of exposure and was almost identical in all fish exposed to any of the three cyanide concentrations.

Sublethal concentrations of cyanide significantly reduced liver glycogen levels with regard to length of exposure and cyanide concentrations. After 20 days, the liver of all the cyanide-exposed fish groups, with the exception of the 0.03 $mg.L^{-1}$ HCN group, had

returned to glycogen levels comparable to that of the controls.

Thiocyanate readily accumulated in cyanide-exposed trout. Compared with the controls, blood plasma thiocyanate levels in rainbow trout exposed to cyanide throughout the 20-day period were significantly increased both by cyanide concentration and duration of exposure.

The hepatosomatic index was reduced by cyanide exposure and response to cyanide was more precise at the highest cyanide concentration.

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INTRODUCTION

The present study was conducted to investigate the mode of action of cyanide in rainbow trout during chronic exposures to sublethal concentrations of the toxicant. To achieve this goal, the following parameters were examined: liver cytochrome oxidase activity, bioaccumulation of thiocyanate in blood plasma, liver glycogen, hematocrit and the hepatosomatic ratios.

Of all the various forms of cyanide found in the environment, it is the free cyanide HCN that is the most toxic. Its presence in natural waters is attributed to biological and antropogenic sources such as gold mining, milling industries, petroleum refineries etc. (Leduc, 1981). Although, there is an abundant literature on the acute and sublethal toxicity of cyanide to various freshwater organisms (see reviews by Doudoroff, 1976 and 1980; Leduc et al, 1982), little emphasis has been given to the investigation on the mode of action of cyanide during prolonged exposure to sublethal concentrations.

The mode of action of cyanide is generally attributed to the inhibition of cytochrome oxidase but there are other enzymatic reactions affected (Vennesland et al, 1981). This inhibition of cytochrome oxidase results in the blockage of the electron transport chain and associated ATP formation. To obviate this deprivation of ATP, anaerobic metabolism, through the glycolytic pathway and the pentose or phosphogluconate pathway is activated for the production of ATP (Solomonson, 1981).

The mechanism of cyanide toxicity has been widely described for mammals (Vennesland et al, 1981) but this is not the case for fish. For example, although the toxicity of cyanide is generally attributed

to the inhibition of cytochrome oxidase, only one report (Leduc, 1966b) on this effect at sublethal levels has been found in the aquatic toxicology literature. He reported no relation between the liver cytochrome oxidase activity and the cyanide concentrations to which cichlids (Cichlasoma bimaculatum) had been exposed.

It has been shown that prolonged exposure of fish to sublethal concentrations of cyanide had detrimental effects on osmoregulation (Leduc and Chan, 1975), reproduction (Ruby et al, 1979), growth and swimming performance (McCracken and Leduc, 1980; Kovacs and Leduc, 1982). It has also been shown that the toxicity of cyanide to guppies (Poecilia reticulata) is weight related (Anderson and Weber, 1975), the smaller fish being more resistant per gram of fish to cyanide.

Despite the deleterious effects of cyanide to fish, this toxicant, under particular conditions of exposure may induce growth enhancement and/or growth rebound (Leduc, 1966b; Speyer, 1975; Cheng, 1978; McCracken and Leduc, 1980; Dixon and Leduc, 1981; Kovacs and Leduc, 1982).

Similarly, Dixon and Sprague (1981) reported that pre-exposure of rainbow trout to 0.034 $mg.L^{-1}$ HCN for a 21-day period altered their sensitivity to acute cyanide poisoning which reached a maximum at 7 days but gradually returned to the control level within the next 14 days.

The growth rebound phenomenon as well as the apparent acclimation to cyanide was tentatively attributed to an increased activity of cytochrome oxidase and to a detoxification mechanism. Cyanide is detoxified into its less toxic form thiocyanate via the action of

rhodanese. Way (1981) and Westley (1981) give a very comprehensive discussion on the mode of action of this enzyme which was also identified in fish (Schievelbein et al, 1969; Sido and Koj, 1972). In addition to the role of rhodanese, binding of cyanide to molecules such as methemoglobin or dihydroxycobalamin (vitamin B12) has been shown to decrease the toxicity of cyanide (Way, 1981).

On the other hand, thiocyanate has been known for many years as an anti-thyroid drug because it decreases the ability of the thyroid gland to incorporate iodide (Wollman, 1956 and 1962; Wolff, 1964, Greer et al, 1966; Way 1981). A decline in gonadotropin hormone (GTH) and resulting retardation of gonadal development was observed in freshwater catfish (Heteropneustes fossilis Bloch) treated with thiocyanate. This reduction of GTH was attributed to the exhaustion of the thyroid gland from the production of thyroid hormone (TH) and associated to an increase in thyroid stimulating hormone (TSH) from the pituitary gland (negative feedback because of a lack of thyroid hormone) (Singh et al, 1977). Thiocyanate ion, which is very close to chloride ion in the lyotropic series, is also known to inhibit the gill chloride pump in teleosts (Epstein et al, 1973 and 1975, De Renzie, 1975) and branchial ATPase (Solomon et al, 1973 and 1975, Kerstetter and Kirschener, 1974; De Renzie and Bornancin, 1977; Ho and Chan, 1981).

It is regrettable that aquatic toxicologists have not better attempted to integrate the current knowledge on cyanide and thiocyanate toxicity since it appears that symptoms of sublethal toxicity of cyanide are more related to that of thiocyanate than to

cyanide itself. It was with this in mind that this study was initiated.

Consequently the following specific questions were addressed:

1) Do the growth rebound and increased resistance, previously observed during chronic exposures to sublethal levels of cyanide, result from an enhancement of cytochrome oxidase activity?

2) Is thiocyanate produced during exposure to cyanide and if so, does it accumulate in the fish?

3) Can changes in liver glycogen levels during cyanide poisoning be related to the growth rebound and acclimation to cyanide?

4) Is there a difference between fish size for the different responses of the preceeding physiological/biochemical paramaters?

To answer these questions, rainbow trout were exposed during 20-day periods to three sublethal concentrations of cyanide and sampled at time intervals for measurement of liver cytochrome oxidase activity, plasma thiocyanate accumulation, liver glycogen level, hepatosomatic ratio and hematocrit. Two fish size groups were also tested for size-related differential responses.

MATERIALS AND METHODS

Source and Treatment of Fish

Rainbow trout (Salmo gairdneri, Richardson) used in this study were purchased as required during the period of August 1981 to June 1982 from La Pisciculture desLandes, St-Félix de Kingsay, Cté de Drummond, Québec.

Upon arrival at the laboratory, the fish were held in 240L fiberglass oval-shaped tanks supplied with a continuous flow of water at a temperature of 12°C ± 1°C. The laboratory was supplied with City of Montreal water which was dechlorinated, thermally regulated, and delivered to tanks through plastic P.V.C. piping.

Fish were fed every second day approximatively 1.8% of their wet body weight with Purina Trout Chow, size 4P or 6P according to the fish size used during the bioassay. The fish were held under these conditions for a minimum period of two weeks prior to the beginning of the tests. During this acclimatization period, mortality never exceeded 0.5% (Table 1).

Experimental Design

Three series of experiments, were conducted at a temperature of 12°C during the months of October 1981, February and May 1982. In these experiments, the fish were exposed for a 20-day period to three different concentrations of cyanide, 0.01, 0.02 and 0.03 $mg.L^{-1}$ HCN with control fish kept under the same conditions but without cyanide. These cyanide concentrations were chosen because they are known to be sublethal levels at 12°C (Kovacs and Leduc, 1982).

In order to better diagnose the symptoms of the chronic cyanide poisoning, several parameters were measured namely: plasma thiocyanate and liver glycogen levels, liver cytochrome oxidase activity, the hepatosomatic index and hematocrit.

In experiment #1, liver glycogen and plasma thiocyanate were monitored in control and poisoned 165 g trout on day 0, 3, 6, 11 and 20. In subsequent experiments the influence of size of the fish was verified. For experiment #2 (February 1982) trout of 170 g were used whereas for the experiment #3 (May 1982) 30 g fish were tested. For both of these experiments liver cytochrome oxidase activity and plasma thiocyanate content were monitored as a function of length of exposure to cyanide and concentrations of cyanide by sampling fish on day 0, 5, 10, 15 and 20.

Fish were sampled as follows: On day 0 a total of five 170 g trout or six 30 g trout were randomly taken from the four tanks, whereas on other sampling days, the number of fish taken from each tank was five 170 g fish or six 30 g fish for a total of respectively 20 or 24 fish per sampling day.

Sampling techniques

Upon sampling, the fish were rapidly killed with a blow on the head, weighed, measured and the tail severed. Blood was immediately collected into heparinized centrifuge tubes. The plasma was separated from the whole blood by centrifugation for 15 min. at 3000 rpm with a IEC clinical centrifuge and stored on ice in 1.5 ml Eppendorf tubes for thiocyanate determination. With the 30 g fish, the blood of two or three fish had to be pooled to obtain a sufficient amount for

analysis.

The liver was excised from each fish, weighed, immediately frozen in liquid nitrogen and stored at -20°C for further analysis. These livers were used for the cytochrome oxidase activity assay or the glycogen content determination.

Conditions During Bioassays

All experiments were carried out in tanks similar to those used during the holding period. Prior to each experiment, the acclimatized fish were randomly distributed into four test tanks as described in Table 1 and kept for one week before the beginning of exposure to cyanide. During that period and for the duration of the experiment, the food ration was changed from a maintenance ration to a growth ration. The 160-170 g fish were fed daily with trout chow at a rate 1.1% of their wet body weight; the 30 g fish received a 1.9% ration (Leitritz, 1976). The amount of food given daily was adjusted according to the change in density after sampling and to the fish growth as determined from each sampling.

A continuous flow of water was supplied to the test tanks at a rate of 4 $L.min^{-1}$ allowing a 99% replacement in 5 hours (Sprague 1973). In all experiments, the stock solutions of cyanide were prepared from reagent KCN, dissolved in double distilled water and Mariotte bottles were used to meter the cyanide solutions in the tanks (Leduc, 1966a and b). On day 0, the calculated levels of cyanide concentrations were immediately obtained by the addition of known quantities of a cyanide solution directly to the tanks at the same time the flow of cyanide from the Mariotte bottles started.

The cyanide concentration in each test tank was monitored at least twice per experiment using the method of Lambert et al (1975). The cyanide concentrations never fluctuated by more than 10% of the predicted values.

Dissolved oxygen was measured in every tank at least twice per bioassay using a YSI model 54A oxygen meter, and was never found to be less than 80% saturation. The mean water temperature for each set of experiment ranged as described in Table 2 with an overall mean of 12.6°C S.D. 0.81°C. The overall maximum and minimum temperature for the three bioassays were 16°C and 11.2°C. All of the following parameters of the water quality, that are also found described in detail in Table 2, were obtained from l'usine de production d'eau potable de la Ville de Montréal. The pH averaged 7.87 while alkalinity and hardness averaged 83.3 and 127.7 $mg.L^{-1}$ as $CaCO_3$.

The illumination and photoperiod during acclimatization were identical to those used during the experiments. The tanks were equipped with fluorescent lights giving an illumination of 60-90 LUX measured at the water surface and a 12-h photoperiod regulated by a time switch.

No mortality occurred in any of the bioassays except once when two fish died after 8 days of exposure to 0.01 $mg.L^{-1}$ HCN during experiment #1 (October).

Biochemical Determinations

Plasma Thiocyanate

Thiocyanate analyses were performed within 24 h of sampling with the exception of the samples taken at day 0 which were analyzed

concurrently with those of day one. No significant losses of thiocyanate during storage should have happened since it has been shown by Ballantyne (1977) that storage of plasma at 0°C resulted in only slight fluctuations of thiocyanate levels during a period of up to 6 days.

The plasma was prepared for analysis as per Pettigrew and Fell (1972). The plasma was deproteinized by adding 9 volumes of 10% trichloroacetic acid per volume of plasma in centrifuge tubes which were then left to stand at room temperature for 10 min before centrifugation at 3000 rpm for 15 min. The samples were then immediately analyzed for SCN^- according to the technique used by Lambert et al (1975) modified as follows: one ml of the supernatant was mixed with one ml of 1 M phosphate buffer pH 7.5. Duplicate samples then received 0.05 ml of N-chlorosuccinimide-succinimide and 0.05 of pyridine-barbituric acid reagents. After incubation at 25°C for 12 min, the optical density was read at 575 nm using a Bausch and Lomb Spectronic 70 spectrophotometer.

Liver Cytochrome Oxidase

The activity of liver cytochrome oxidase was measured 48 h after sampling, but the livers removed on day 0 were analyzed with those sampled on day 1.

The livers were ground into a fine powder in a mortar filled with liquid nitrogen. Five ml per g of liver of a 0.02M phosphate buffer (pH 7.4) were added and the suspension kept on ice. The samples were then homogenized with a Sorvall micro homogenizer at a speed of 21,500 rpm for three min alternating homogenization and cooling in ice for 30

sec intervals. The remaining cell debris and nuclei were then precipitated by centrifugation at 2000 G for 15 min in a refrigerated IEC B-20A centrifuge. The supernatant was collected and diluted to the suitable concentration for the measurement of cytochrome oxidase activity according to the technique described by Smith (1955).

The reagent cytochrome C substrate was prepared (5 $mg.ml^{-1}$ from horse heart, 95% type III, Sigma Chemical Company) was prepared in a phosphate buffer (Yonetani, 1966) and stored at -20°C in 7ml vials for further uses. On the day of the analysis, two vials of cytochrome C were thawed, a minimal amount of solid $Na_2S_2O_4$ was added and nitrogen bubbled for 5 min to ensure a full reduction. The solution was then salted out as mentioned by Yonetani (1966). To prevent the formation of H_2O_2, a potential oxidizer of cytochrome C, the cytochrome C solution was again bubbled for 5 min with nitrogen, then one h with hydrogen and finally with nitrogen for 5 min. The buffered cytochrome C solution was then diluted to 90 uM in 0.01 M PO_4 buffer pH 7.0 (Smith 1955). All procedures for the preparation of cytochrome oxidase assays and the cytochrome C solutions were conducted in a cold room and all solutions and reagents kept on ice.

Cytochrome oxidase assays were carried out in duplicate by monitoring at room temperature the enzymatic oxidation of cytochrome C at 550nm. The measurements were taken from a PYE UNICAM SP B-100 ultraviolet spectrophotometer and recorded every 2 sec on a time lapse printer for further calculation of the cytochrome oxidase activity as the first order velocity constant (Smith, 1955). The total protein content of the assayed suspension was measured the next day by the Lowry method (Cooper, 1977).

A pilot study, carried out with 300 g rainbow trout exposed for 7 days to 0.03 $mg.L^{-1}$ HCN was conducted in order to verify the presence of cyanide-insentive respiration in the fish liver. The livers of control and treated fish were pooled respectively and their mitochondria extracted following the technique of Yonetani (1966). Respiration was then measured according to Chance and Williams (1955).

Glycogen Determinations

The liver samples kept for glycogen determinations were removed from the freezer at the end of the experiment and ground in liquid nitrogen to obtain a fine powder. The ground livers were then digested in 30% KOH and the glycogen precipitated with 95% alcohol according to the procedure described by Good et al (1933). The glycogen concentrations were determined according to the Montgomery (1957), phenol-sulfuric acid colorimetric procedure using a Bausch and Lomb Spectronic 70 spectrophotometer.

Hematocrit

The hematocrit was determined after 10 min of centrifugation of whole blood at 3000 rpm. Heparine rinsed Dade Wintrobe sedimentation tubes were used.

Hepatosomatic Index

The hepatosomatic index was calculated from the % ratio of the fresh liver weight over the fish body weight measured using a Sartorius 1364 MP6 digital scale with an accuracy of $\pm$ 0.01g.

Table. 1 Fish characteristics and mortality of rainbow trout (Salmo gairdneri) during three experiments to test the effect of chronic cyanide poisoning on various physiological and biochemical parameters.

Experiment # and date	Average length (cm) (± SD)	Average weight (g) (± SD)	Number of fish per aquarium	Sample size n	Mortality (# Fish) during acclimation	Mortality (# Fish) during test
#1 October 1981	24.9 (± 1.66)	163.33 (± 26.395)	27	5	3	2
#2 February 1982	26.7 (± 1.29)	168.51 (± 30.727)	27	5	0	0
#3 May 1982	15.8 (± 0.89)	32.28 (± 4.909)	32	6	0	0

[a]Size and weight measured from day 0 and 1.

Table 2. Physico-chemical characteristics of laboratory water during three experiments to test the effect of chronic cyanide poisoning on various physiological and biochemical parameters of rainbow trout (*Salmo gairdneri*).

Experiment # and date	Mean O_2 Conc. $mg.L^{-1}$	pH	Mean Temp. °C	Mean Alkalinity[a] $mg.L^{-1}$	Mean Hardness[a] $mg.L^{-1}$
#1 October 1981	8.7	7.9	13.0	81	121
#2 February 1982	8.6	7.8	11.7	89	134
#3 May 1982	9.5	7.9	13.2	86	128

[a]Measured as $CaCO_3$

RESULTS

Data Analysis

A three-way analysis of variance (mixed model, double nested) (Lindman, 1974) followed by a student-Newman-Keuls test for the posteriori comparison of means and the Sheffe test for the planned comparison of means were performed to determine the significance of changes in the cytochrome oxidase activity attained in control and cyanide-exposed fish, as the 20-day experiments progressed.

The changes in plasma thiocyanate level were tested in the same manner as for the cytochrome oxidase activity except for experiment #3 (May 1982) where a two-way analysis of variance was used since several blood samples had to be pooled for the thiocyanate determinations, thus eliminating the possibility of individual fish comparison.

The analysis of data for the liver glycogen, hematocrit and hepatosomatic index was achieved using the same statistical method as for the cytochrome oxidase activity except that there were no replicates.

In case of unequal sample size, subsamples were randomly chosen from original data in order to get equal sample size. The degree of significance was tested at a probability of 0.05 for the anova and the comparison of means by a priori test and at 0.01 for the a posteriori test.

Plasma Thiocyanate

The accumulation of thiocyanate in blood plasma was measured in three different experiments while rainbow trout were exposed to various cyanide concentrations for 20 days. The first experiment was carried out in October with 165 g fish, the second in February with 170 g fish and the third in May, with 30 g fish. Because of those differences in experimental conditions, the results presented in Table 3, 4 and 5 and graphically illustrated in Fig. 1, 2 and 3, will be analyzed separately.

The basal levels of blood plasma thiocyanate in control fish varied somewhat between the three experiments but no significant variation occurred within the experiments. Compared with the controls the blood plasma thiocyanate levels of cyanide-exposed rainbow trout significantly increased both with concentration and the duration of exposure (Fig. 1, 2 and 3). However, the pattern of thiocyanate accumulation varied among the three experiments.

By day 1, in experiment #1 and 2, there was a significantly higher thiocyanate level only at 0.03 $mg.L^{-1}$ HCN, (Figure 1 and 2) ($P = 0.05$). Between day 1 and 10, blood plasma thiocyanate increased in cyanide-exposed fish but the pattern differed among experiments. During experiment #1, the thiocyanate accumulation showed only a slight increase from day 1 to 11 but on that day, the levels in all cyanide-exposed fish were significantly higher than in the controls. In experiment #2, by day 5, the blood plasma thiocyanate levels in all cyanide-exposed fish were already significantly higher than in the controls and kept increasing with time beyond day 5. This trend was

more clearly defined in experiment #3 (see Fig. 3) where plasma thiocyanate levels increased both with the cyanide concentration and the duration of exposure from day 1 through day 10. Thus at day 10, all of the cyanide-exposed fish had significantly higher blood plasma thiocyanate levels than the controls.

During the second half of the experiments, the pattern of thiocyanate accumulation were quite different. In experiment #1, thiocyanate levels rose sharply in all cyanide-exposed fish between day 11 and 20. However, since no sample was taken at day 15 as in the other two experiments, the possibility of some levelling off at day 15 should not be overlooked. These levels of blood plasma thiocyanate attained on day 20 at 0.01, 0.02 and 0.03 $mg.L^{-1}$ HCN represent respective increments of 330, 372 and 447 percent above the average control levels. There appears to be a dose-response relationship in these levels at day 20 but the differences are not significant.

In experiment #2, performed in February, thiocyanate accumulation at 0.01 $mg.L^{-1}$ HCN levelled off at day 11 but at 0.02 $mg.L^{-1}$ HCN, thiocyanate showed a steady increase from day 11 to day 20. At 0.03 $mg.L^{-1}$ HCN thiocyanate reached a maximum on day 15 and then decreased towards day 20. At the end of the 20-day period, as in experiment #1, all of the cyanide-exposed fish exhibited blood plasma thiocyanate levels significantly higher than the controls. The final levels of thiocyanate at 0.01, 0.02 and 0.03 $mg.L^{-1}$ HCN were respectively 368, 713 and 511 percent higher than in the control fish. These blood plasma thiocyanate levels are significantly different from the controls and among themselves.

In experiment #3 (May), performed with 30 g fish, the trend was for a continuous increase of plasma thiocyanate up to day 10 or 15 then followed by a decline. So at 0.01 and 0.03 $mg.L^{-1}$ HCN, the maximum was attained at day 15 while at 0.02 it occurred at day 10. On day 20, the thiocyanate levels in fish previously exposed to 0.01, 0.02 and 0.03 $mg.L^{-1}$ HCN were respectively 202, 67 and 390 percent higher than the controls (Fig. 3).

In experiment #3, because of the smaller size of the fish, no individual data could be obtained but the blood plasma thiocyanate was measured from pooled samples. Consequently, it was impossible to determine any difference among fish groups at given cyanide concentration or length of exposure to cyanide. It was also impossible to estimate the 95% confidence limits for those fish groups (Table 5).

Finally, in order to verify the relationship between the size of the fish and thiocyanate bioaccumuation by the end of exposure to cyanide, a correlation analysis was performed on all individual pairs of data (fish weights vs thiocyanate increments). Individuals results were taken after day 20 from all three experiments. For experiment #3, the mean weight of the pooled fish were considered. This analysis is presented in Table 6 which indicates a highly significant ($P < 0.01$) positive correlation between fish weights and plasma thiocyanate gains in fish exposed to 0.01 and 0.02 $mg.L^{-1}$ HCN. The thiocyanate accumulation increased with the size of the fish. This correlation could not be established for fish previously exposed to 0.03 $mg.L^{-1}$ HCN.

Figure 1. Mean blood plasma thiocyanate levels and their 95% confidence limits in rainbow trout (165 g) exposed to cyanide for 20 days to various sublethal concentrations at 13°C in experiment #1

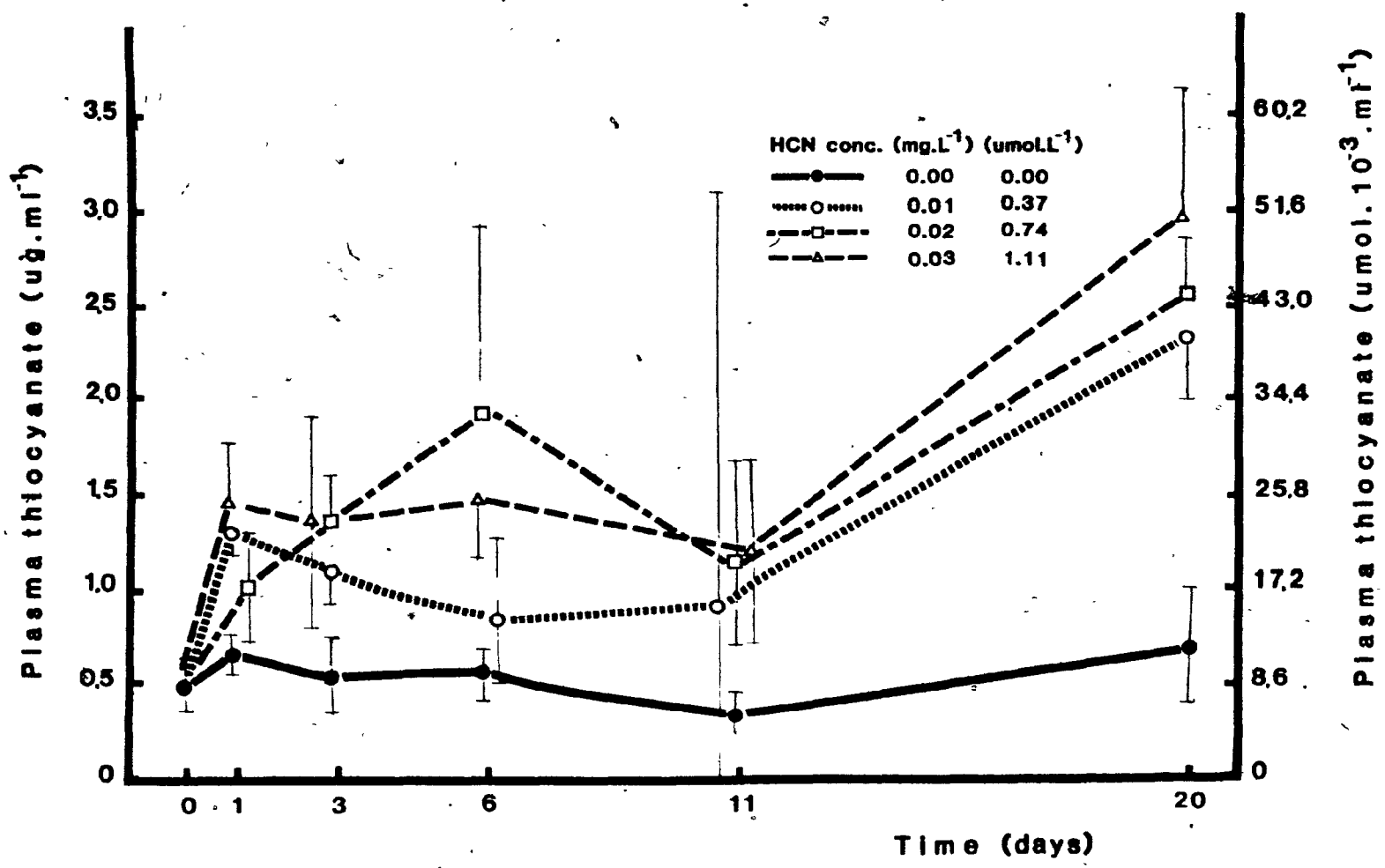

HCN conc. (mg.L-1) (umol.L-1)
0.00 0.00
0.01 0.37
0.02 0.74
0.03 1.11
Plasma thiocyanate (ug.ml-1)
Plasma thiocyanate (umol.10-3.ml-1)
Time (days)
0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
8.6
17.2
25.8
34.4
43.0
51.6
60.2
0
1
3
6
11
20

Figure 2. Mean blood plasma thiocyanate levels and their 95% confidence limits in rainbow trout (170 g) exposed to cyanide for 20 days to various sublethal concentrations at 12°C in experiment #2

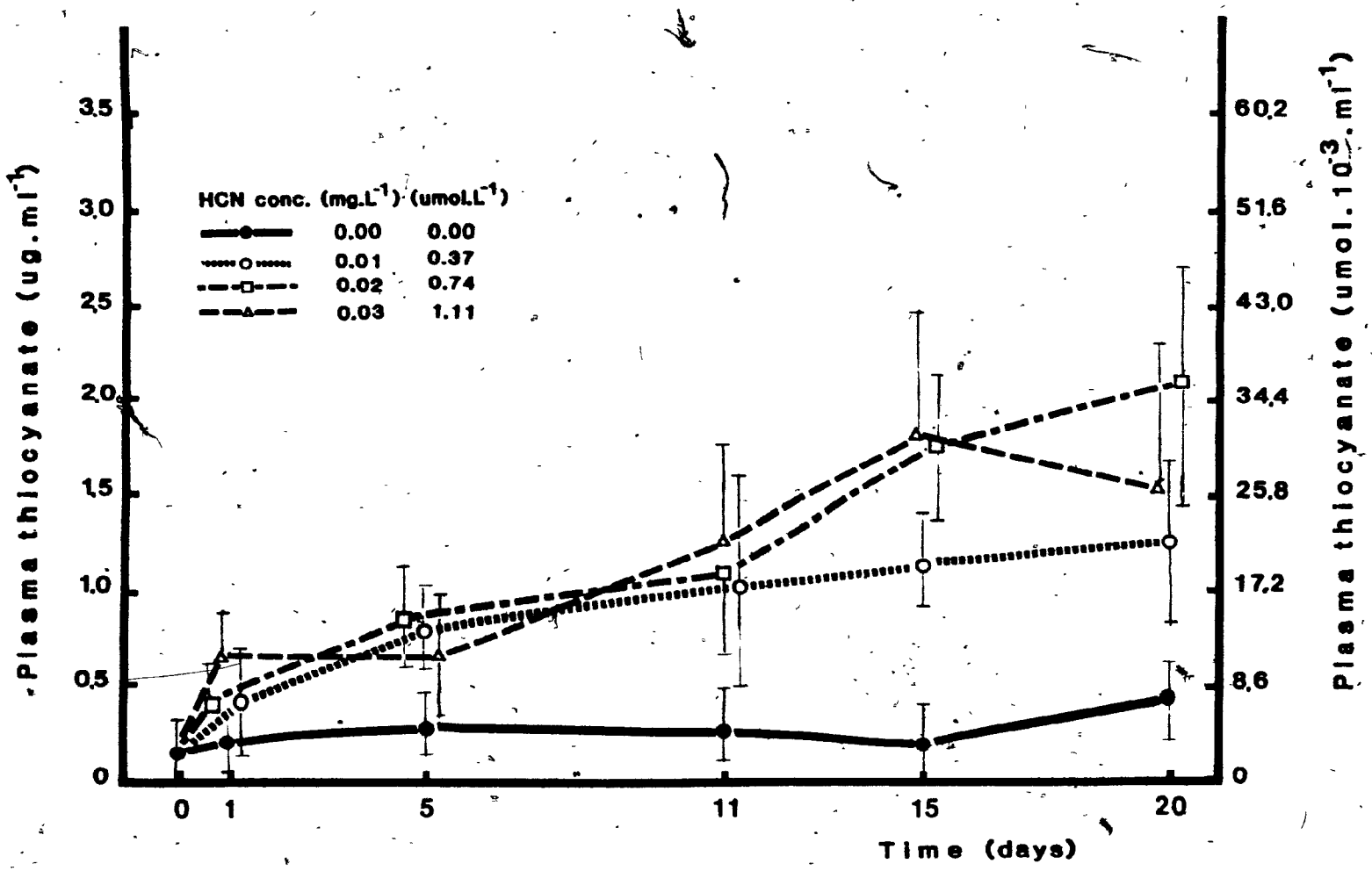

HCN conc. (mg.L-1) (umol.L-1)
0.00 0.00
0.01 0.37
0.02 0.74
0.03 1.11
Plasma thiocyanate (ug.ml-1)
Plasma thiocyanate (umol.10-3.ml-1)
Time (days)
0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
8.6
17.2
25.8
34.4
43.0
51.6
60.2
1
5
11
15
20

Figure 3. Mean blood plasma thiocyanate levels and their 95% confidence limits in rainbow trout (30 g) exposed to cyanide for 20 days to various sublethal concentrations at 13°C in experiment #3

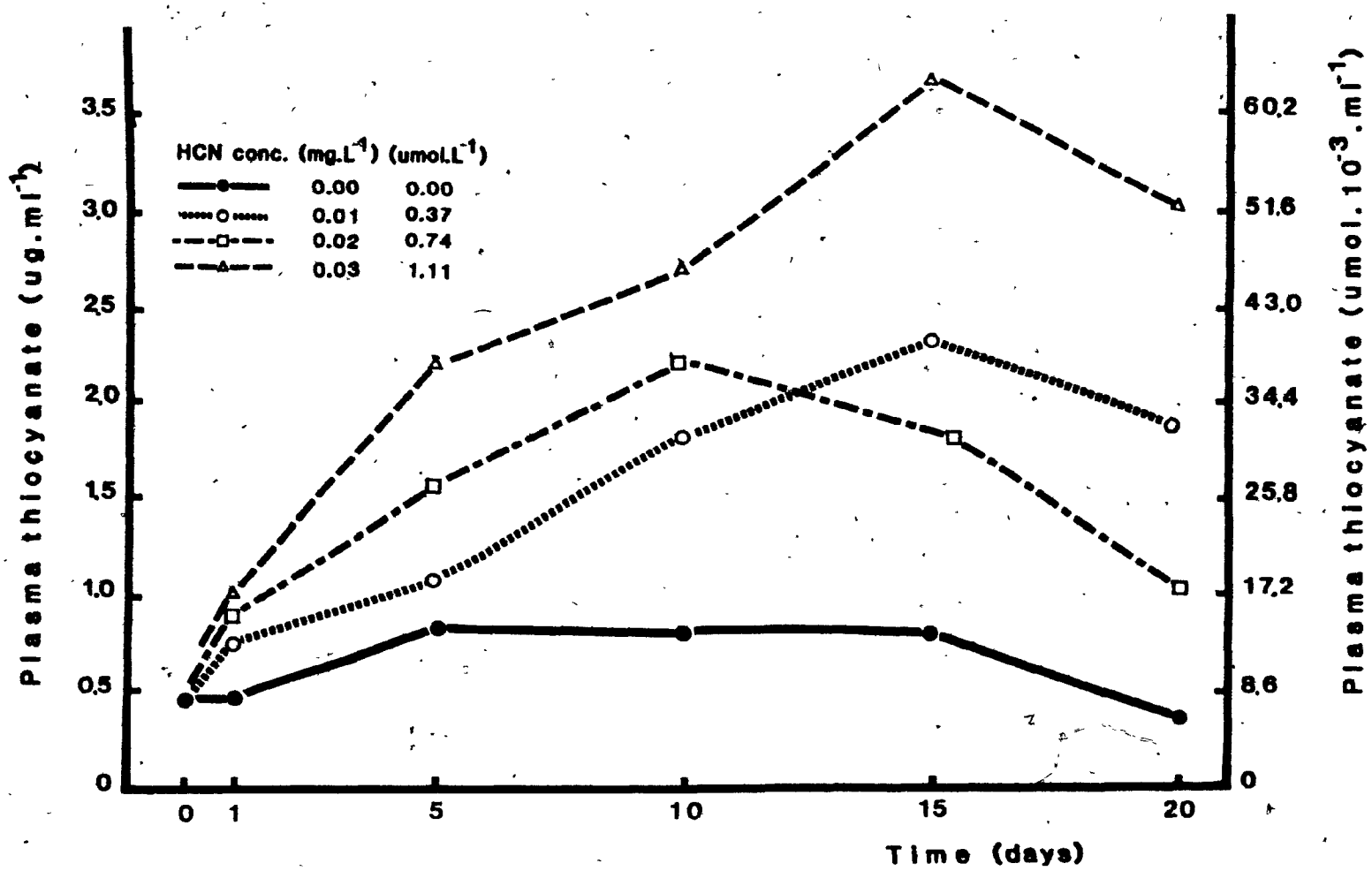

HCN conc. (mg.L-1) (umol.L-1)
0.00 0.00
0.01 0.37
0.02 0.74
0.03 1.11
Plasma thiocyanate (ug.ml-1)
Plasma thiocyanate (umol.10-3.ml-1)
Time (days)
0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
8.6
17.2
25.8
34.4
43.0
51.6
60.2
0
1
5
10
15
20

Table 3. Mean blood plasma thiocyanate levels and their 95% confidence limits in rainbow trout (165 g) exposed for 20 days to various sublethal cyanide concentrations at 13°C in experiment #1.

Day of exposure	HCN Conc. $mg.L^{-1}$	Sample size n	Mean Plasma SCN $ug.ml^{-1}$	(SD)	95% Confidence limit L_1	L_2
0	0.00	5	0.487	(0.1028)	0.3594	0.6150
1	0.00	5	0.651	(0.0806)	0.5506	0.7510
	0.01	5	1.278	(0.0967)	1.1582	1.3986
	0.02	4	0.998	(0.1942)	0.6892	1.3069
	0.03	4	1.421	(0.2002)	1.1026	1.7394
3	0.00	5	0.520	(0.1567)	0.3252	0.7148
	0.01	5	1.068	(0.1224)	0.9159	1.2201
	0.02	5	1.350	(0.1772)	1.1297	1.5703
	0.03	4	1.345	(0.3564)	0.7783	1.9117
6	0.00	3	0.540	(0.0529)	0.4086	0.6714
	0.01	4	0.840	(0.2627)	0.4223	1.2577
	0.02	4	1.893	(0.6463)	0.8649	2.9201
	0.03	4	1.450	(0.1883)	1.1506	1.7494
11	0.00	5	0.328	(0.0996)	0.2042	0.4518
	0.01	2	0.910	(0.2404)	-1.2507	3.0707
	0.02	4	1.113	(0.3420)	0.6688	1.6562
	0.03	5	1.172	(0.3973)	0.6780	1.6660
20	0.00	4	0.671	(0.2135)	0.3740	0.9674
	0.01	3	2.292	(0.1416)	1.9402	2.6423
	0.02	6	2.517	(0.2842)	2.2184	2.8146
	0.03	6	2.914	(0.6554)	2.2259	3.6012

Table 4. Mean blood plasma thiocyanate levels and their 95% confidence limits in rainbow trout (170 g) exposed for 20 days to various sublethal cyanide concentrations at 12°C in experiment #2.

Day of exposure	HCN Conc. $mg.L^{-1}$	Sample size n	Mean Plasma SCN $ug.ml^{-1}$	(SD)	95% Confidence limit L_1	L_2
0	0.00	4	0.134	(0.1278)	0.0697	0.3367
1	0.00	5	0.189	(0.1241)	0.0350	0.3436
	0.01	5	0.393	(0.2376)	0.0980	0.6888
	0.02	5	0.407	(0.1541)	0.2152	0.5984
	0.03	5	0.657	(0.2043)	0.4034	0.9113
5	0.00	5	0.282	(0.1295)	0.1207	0.4426
	0.01	5	0.798	(0.1864)	0.5666	1.0301
	0.02	5	0.856	(0.2186)	0.5845	1.1281
	0.03	5	0.651	(0.2562)	0.3298	0.9718
11	0.00	5	0.277	(0.1646)	0.0721	0.4814
	0.01	4	1.036	(0.3536)	0.4734	1.5977
	0.02	5	1.103	(0.3509)	0.6668	1.5393
	0.03	5	1.282	(0.3678)	0.8244	1.7391
15	0.00	5	0.202	(0.1636)	0.0015	0.4053
	0.01	5	1.162	(0.1967)	0.9178	1.4068
	0.02	5	1.742	(0.3048)	1.3626	2.1205
	0.03	5	1.793	(0.5296)	1.1346	2.4516
20	0.00	4	0.417	(0.1344)	0.2033	0.6306
	0.01	5	1.250	(0.3490)	0.8159	1.6837
	0.02	4	2.068	(0.3968)	1.4367	2.6984
	0.03	5	1.515	(0.6355)	0.7245	2.3048

Table 5. Mean blood plasma thiocyanate levels and their standard deviation in rainbow trout (30 g) exposed for 20 days to various sublethal cyanide concentrations at 13°C in experiment #3.

Day of exposure	HCN Conc. $mg.L^{-1}$	Number of observations	Mean Plasma SCN $ug.ml^{-1}$ (SD)
0	0.00	1	0.464 (-)
1	0.00	2	0.464 (0.0877)
	0.01	1	0.758 (-)
	0.02	3	0.878 (0.1664)
	0.03	2	0.980 (0.0170)
5	0.00	2	0.824 (0.0792)
	0.01	2	1.083 (0.4257)
	0.02	2	1.515 (0.2475)
	0.03	3	2.213 (0.4448)
10	0.00	2	0.788 (0.2376)
	0.01	2	1.792 (0.0113)
	0.02	3	2.208 (0.2253)
	0.03	3	2.670 (0.2474)
15	0.00	2	0.811 (0.3231)
	0.01	2	2.302 (0.0113)
	0.02	3	1.798 (0.4880)
	0.03	2	3.681 (0.0919)
20	0.00	3	0.323 (0.0210)
	0.01	3	1.851 (0.2102)
	0.02	3	1.026 (0.3417)
	0.03	3	3.004 (0.2868)

Table 6. Correlation and regression coefficient of the relationship between the percent increase of blood plasma thiocyanate and body weight of rainbow trout previously exposed to 0.01, 0.02, and 0.03 $mg.L^{-1}$ HCN for 20 days at 12-13°C during experiments #1, 2 and 3.

HCN Conc. $mg.L^{-1}$	Correlation coefficient	Sample size	Probability levels	Regression coefficient	Intercept
0.01	0.783	9	$P < .01$	1.30396	127.311
0.02	0.855	11	$P < .01$	3.22652	-68.078
0.03	0.115	12	$P > .05$	0.3524	404.65

Cytochrome Oxidase

The liver cytochrome oxidase activity was measured in rainbow trout exposed for 20-day periods to various sublethal concentrations of cyanide in experiments #2 and 3. The results are reported in Tables 7 and 8 and graphically illustrated in Figure 4 and 5.

During experiment #2 carried out with 170 g fish (see Fig 4), the controls showed significant variations ($P < .005$) between sampling days, obviously due to individual differences (Table 7). To facilitate the interpretation of the results, the arithmetic mean of the control values (0.0531 $sec^{-1}.mg^{-1}$ protein) was compared with the mean values of the cyanide-exposed trout. The cytochrome oxidase activity of all cyanide-exposed fish decreased on the average by 80% as early as day 1. This reduction was highly significant ($P < 0.001$) and remained constant throughout the experiment at an average level of 0.0104 $sec^{-1}.mg^{-1}$ protein (Fig. 4). There was, however no significant dose-response relationship, the level of activity being closely the same at the three cyanide concentrations.

Following a three-way ANOVA for this experiment, the sources of variation were calculated (Lindmen, 1974) and identified as follows: 61.6% of the variation resulted from the effect of cyanide and 26.7% was due to fish individual differences (Table 9). These results indicate that cyanide, at concentrations of 0.01, 0.02 and 0.03 $mg.L^{-1}$ HCN, caused a significant reduction of cytochrome oxidase activity within 24 h of exposure.

In experiment #3, carried out with 30 g fish, the cytochrome oxidase activity of the controls also fluctuated significantly with

time ($P < 0.005$) (Fig. 5) and the arithmetic mean of the control fish ($0.0272\ sec^{-1}.mg^{-1}$ protein) was compared to the cyanide exposed fish: The cytochrome oxidase activity of these fish was about half of that estimated in the larger fish of the previous experiment, In experiment #3, cyanide also significantly ($P < 0.001$) reduced cytochrome oxidase activity, but to a lesser extent than in the larger fish of experiment #2. Compared with the control arithmetic mean of cytochrome oxidase activity, all cyanide-exposed fish showed a drop of about 60%, which compared with experiment #2, appeared to be more gradual in time and dose-related; the level of inhibition increasing with cyanide concentration (Fig 5). From day 1 to day 5, the cytochrome oxidase activity at $0.01\ mg.L^{-1}$ HCN was significantly higher than that measured throughout the rest of the experiment. At $0.02\ mg.L^{-1}$ HCN, the activity measured on day 1 was the only one significantly higher than the activities observed subsequently. However, at $0.03\ mg.L^{-1}$ HCN, the cytochrome oxidase activity dropped immediately on day 1 and remained constant for the rest of the experiment at a mean value of $0.0124\ sec^{-1}.mg^{-1}$ protein which was not found significantly different of any value of all cyanide exposed fish after 10 days of treatment.

The sources of variation in experiment #3 were estimated as in experiment #2 and we found that 62% of the total variation was attributable to cyanide while 16% was to individual differences and 12% to analytical error (Table 10).

For both fish size groups (experiment #2 and 3), the minimal cytochrome oxidase activity of fish exposed to any of the three

cyanide concentrations was almost the same with 0.0104 $sec^{-1}.mg^{-1}$ protein in experiment #2 and 0.0107 $sec^{-1}.mg^{-1}$ protein in experiment #3.

Result of the pilot study indicated that fish exposed for 7 days to 0.03 $mg.L^{-1}$ HCN exhibited only 5% of cyanide-insensitive respiration in treated fish and control fish.

Glycogen Determination

Liver glycogen levels in control and cyanide-exposed trout were monitored over a 20-day period and the results are reported in Table 11. Due to coinciding fluctuation of liver glycogen levels in all fish groups during the tests, the data were normalized to the controls and the time course changes represented in Fig. 6 and Table 11. Liver glycogen levels significantly changed with time ($P < 0.001$) and also significantly varied with cyanide concentrations ($P < 0.01$) as determined by the 3-way ANOVA double nested model.

At 0.01 $mg.L^{-1}$ HCN, relative to the controls, there was no significant changes in liver glycogen levels with time except at day 6 when liver glycogen dropped significantly. At 0.02 and 0.03 $mg.L^{-1}$ HCN, there was a significant reduction of liver glycogen at day 3 which kept dropping through day 6 until day 11. On day 11, the liver glycogen level in trout exposed to 0.02 $mg.L^{-1}$ HCN began rising back throughout day 20 to a level comparable to controls, however, at 0.03 $mg.L^{-1}$ HCN, it levelled off through day 20 at a point significantly lower than other fish group.

Figure 4. Mean cytochrome oxidase activities ($\bar{K}$) and their 95% confidence limits in liver of rainbow trout (170 g) exposed for 20 days to various sublethal cyanide concentrations at 12°C in experiment #2

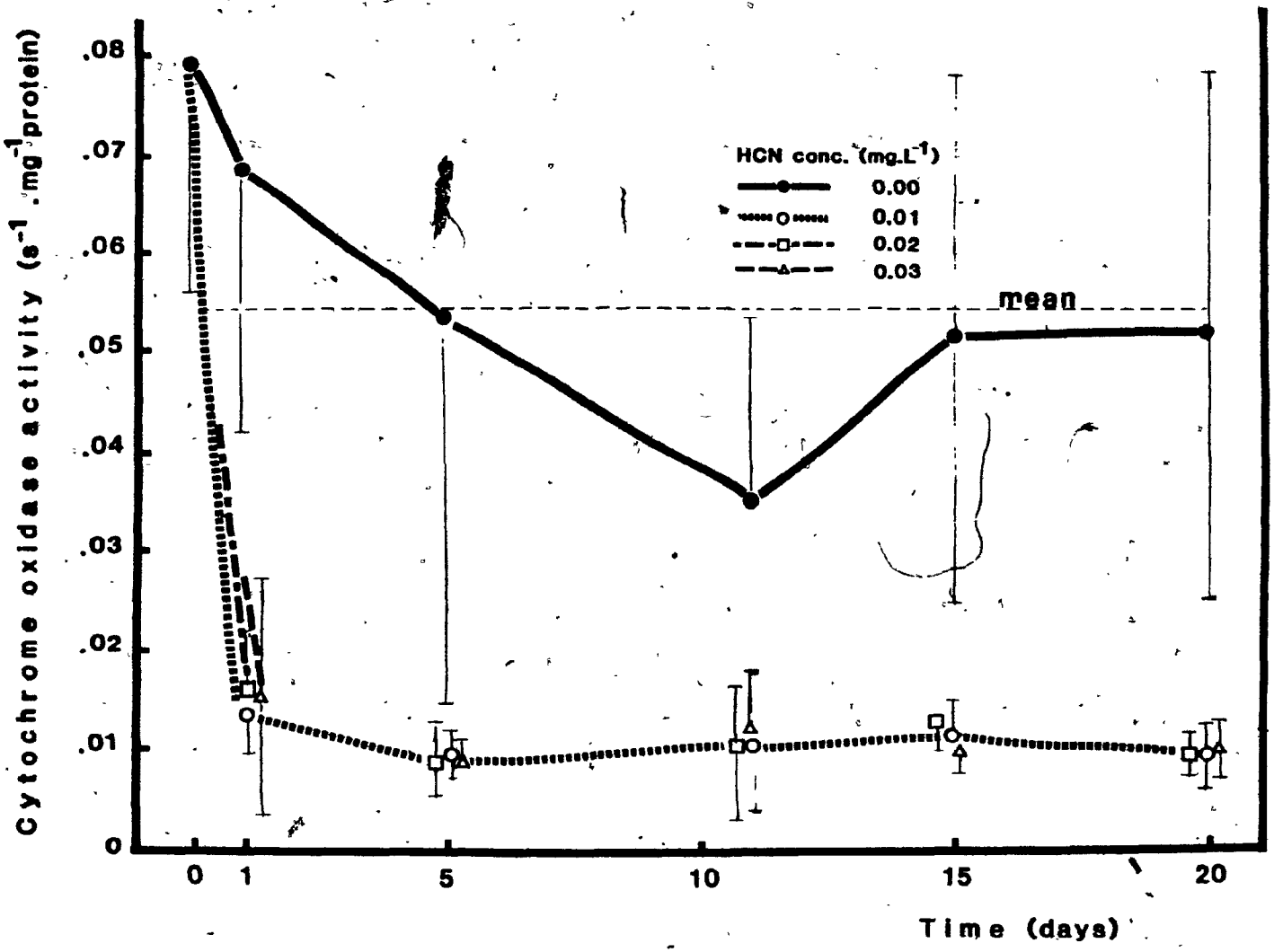

Cytochrome oxidase activity ($s^{-1}.mg^{-1}$ protein)
.08
.07
.06
.05
.04
.03
.02
.01
0
HCN conc. ($mg.L^{-1}$)
0.00
0.01
0.02
0.03
mean
0
1
5
10
15
20
Time (days)

Figure 5. Mean cytochrome oxidase activities ($\bar{K}$) and their 95% confidence limits in liver of rainbow trout (30 g) exposed for 20 days to various sublethal cyanide concentrations at 13°C in experiment #3

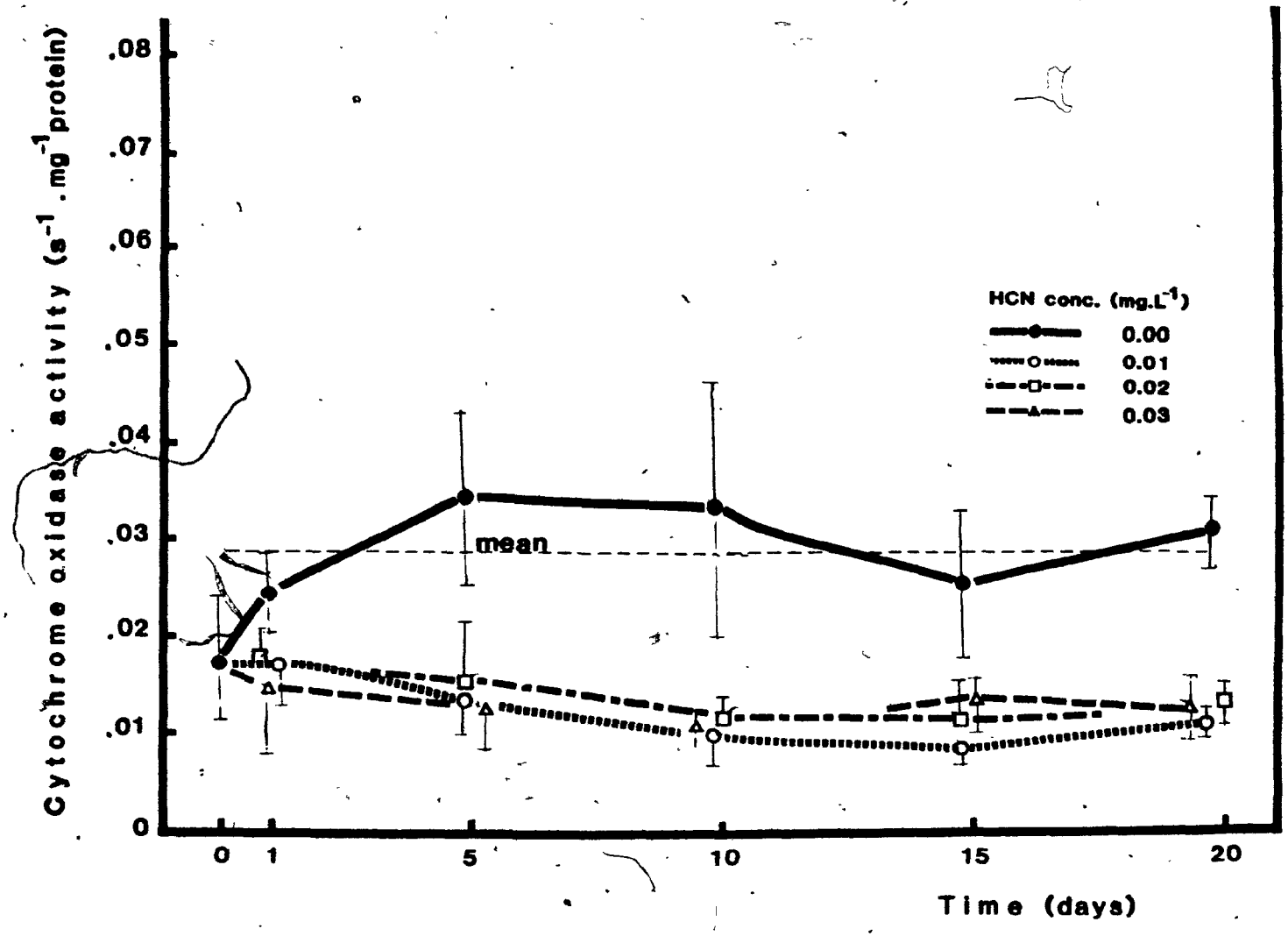

Cytochrome oxidase activity ($s^{-1}.mg^{-1}$ protein)
.08
.07
.06
.05
.04
.03
.02
.01
0
0
1
5
10
15
20
Time (days)
mean
HCN conc. ($mg.L^{-1}$)
0.00
0.01
0.02
0.03

Table 7. Mean cytochrome oxidase activities ($\overline{K}$) in liver, standard deviation and their 95% confidence limits in rainbow trout (170 g) exposed for 20 days to various sublethal cyanide concentrations at 12°C in experiment #2.

Day of exposure	HCN Conc. $mg.L^{-1}$	Sample size n	$\overline{K}$ $sec^{-1}.mg^{-1}$ protein (SD)	95% Confidence limit L_1	L_2
0	0.00	5	0.080 (0.0196)	0.0555	0.1043
1	0.00	5	0.068 (0.0213)	0.0416	0.0946
	0.01	5	0.014 (0.0034)	0.0098	0.0182
	0.02	5	0.016 (0.0051)	0.0089	0.0217
	0.03	5	0.015 (0.0094)	0.0033	0.0267
5	0.00	5	0.053 (0.0313)	0.0141	0.0920
	0.01	5	0.009 (0.0018)	0.0069	0.0114
	0.02	4	0.009 (0.0022)	0.0059	0.0128
	0.03	5	0.008 (0.0016)	0.0058	0.0098
11	0.00	5	0.035 (0.0144)	0.0167	0.0524
	0.01	5	0.010 (0.0026)	0.0071	0.0136
	0.02	5	0.009 (0.0046)	0.0030	0.0145
	0.03	5	0.010 (0.0051)	0.0038	0.0165
15	0.00	5	0.051 (0.0218)	0.0242	0.0783
	0.01	5	0.011 (0.0028)	0.0076	0.0147
	0.02	5	0.012 (0.0021)	0.0095	0.0146
	0.03	4	0.009 (0.0011)	0.0072	0.0106
20	0.00	5	0.039 (0.0117)	0.0248	0.0538
	0.01	5	0.009 (0.0031)	0.0055	0.0130
	0.02	5	0.009 (0.0024)	0.0063	0.0122
	0.03	5	0.009 (0.0032)	0.0064	0.0119

Table 8. Mean cytochrome oxidase activities ($\bar{K}$) in liver, standard deviation and their 95% confidence limits in rainbow trout (30 g) exposed for 20 days to various sublethal cyanide concentrations at 13°C in experiment #3.

Day of exposure	HCN Conc. $mg.L^{-1}$	Sample size n	$\bar{K}$ $sec^{-1}.mg^{-1}$ protein (SD)	95% Confidence limit L_1	L_2
0	0.00	5	0.017 (0.0052)	0.0108	0.0238
1	0.00	5	0.024 (0.0036)	0.0199	0.0288
	0.01	5	0.017 (0.0032)	0.0126	0.0207
	0.02	5	0.018 (0.0049)	0.0114	0.0236
	0.03	5	0.014 (0.0044)	0.0083	0.0193
5	0.00	5	0.034 (0.0074)	0.0249	0.0434
	0.01	5	0.013 (0.0024)	0.0096	0.0156
	0.02	5	0.015 (0.0052)	0.0086	0.0215
	0.03	5	0.012 (0.0031)	0.0076	0.0154
10	0.00	5	0.033 (0.0105)	0.0196	0.0458
	0.01	5	0.009 (0.0019)	0.0062	0.0110
	0.02	5	0.011 (0.0019)	0.0086	0.0134
	0.03	5	0.010 (0.0017)	0.0078	0.0122
15	0.00	5	0.025 (0.0059)	0.0176	0.0323
	0.01	5	0.008 (0.0013)	0.0064	0.0096
	0.02	5	0.011 (0.0031)	0.0073	0.0151
	0.03	5	0.013 (0.0025)	0.0095	0.0157
20	0.00	5	0.031 (0.0029)	0.0271	0.0344
	0.01	5	0.011 (0.0013)	0.0089	0.0122
	0.02	5	0.013 (0.0016)	0.0105	0.0146
	0.03	5	0.012 (0.0027)	0.0086	0.0154

Table 9. Estimation of variance components in liver cytochrome oxidase activity in rainbow trout (170 g) during experiment #2.

Factor	Variance Component	% of Total Variance
a	Length of Exposure	3.6
b	Cyanide concentration	61.6
ab	Interaction	4.1
c(ab)	Individual fish	26.7
w	Error within	3.8

Table 10. Estimation of variance components in liver cytochrome oxidase activity in rainbow trout (30 g) during experiment #3.

Factor	Variance Component	% of Total Variance
a	Length of Exposure	3
b	Cyanide concentration	62
ab	Interaction	6
c(ab)	Individual fish	16
w	Error within	12

Figure 6. Normalized liver glycogen levels in rainbow trout (165 g) exposed for 20 days to various sublethal cyanide concentrations at 13°C in experiment #1

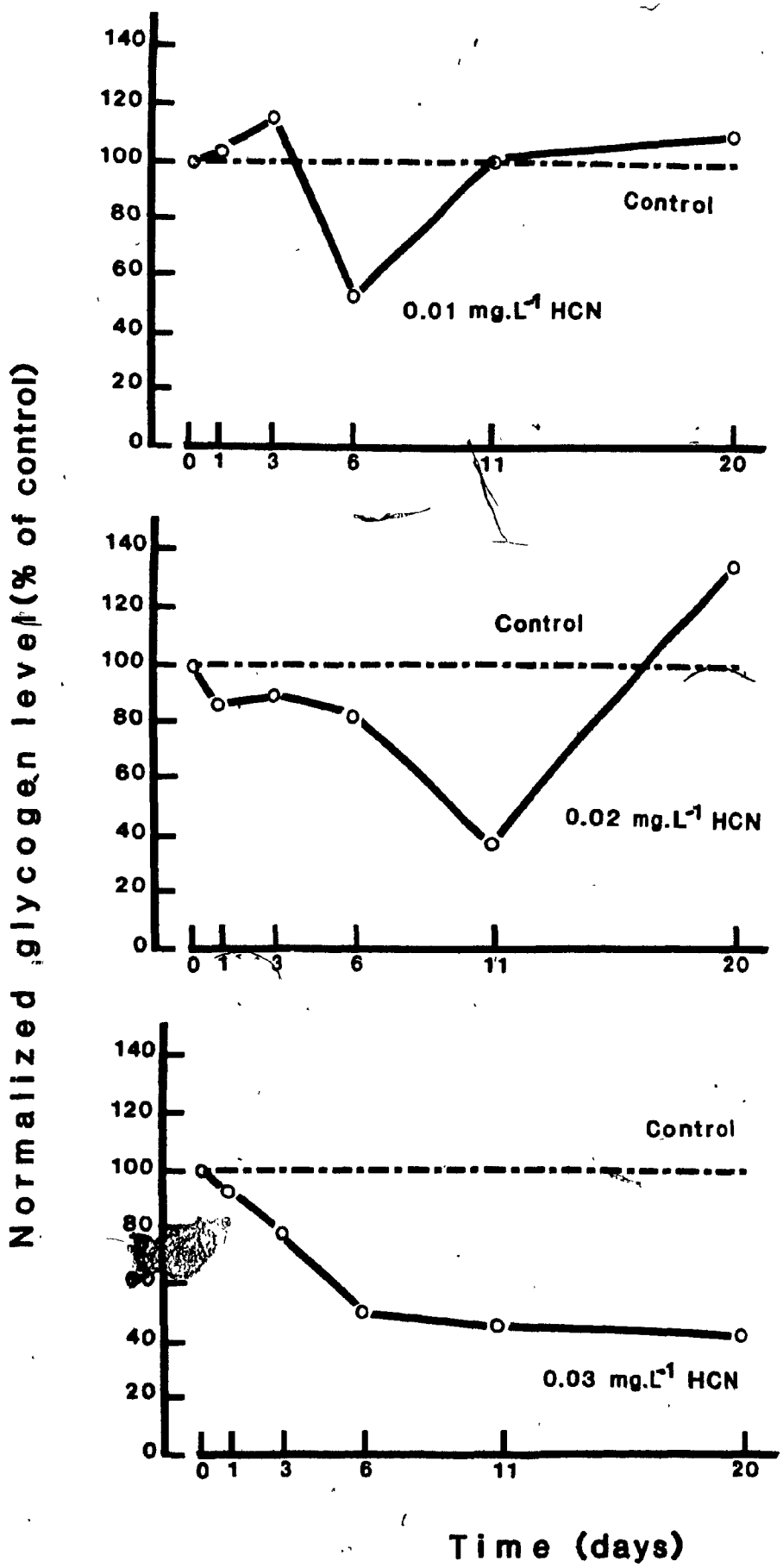

Normalized glycogen level (% of control)
140
120
100
80
60
40
20
0
Control
0.01 mg.L-1 HCN
0 1 3 6 11 20
Control
0.02 mg.L-1 HCN
Control
0.03 mg.L-1 HCN
Time (days)

Table 11. Mean glycogen levels in liver, standard deviation and their 95% confidence limits in rainbow trout (165 g)exposed for 20 days to various sublethal cyanide concentrations at 13°C in experiment #1.

Day of exposure	HCN Conc. $mg.L^{-1}$	Sample size n	Mean Glycogen Level $mg.g^{-1}$ protein (SD)	95% Confidence Limit L_1	L_2	Normalized data % Control	Mean Glycogen of Subsamples
0	0.00	5	60.49 (19.07)	30.17	90.81	100.0	66.5
1	0.00	5	113.32 (12.78)	97.43	129.21	100.0	112.3
	0.01	5	115.97 (40.79)	65.27	166.68	102.3	119.1
	0.02	5	96.63 (37.53)	49.98	143.29	85.3	113.1
	0.03	5	105.38 (28.19)	70.33	140.44	93.0	109.3
3	0.00	5	119.76 (13.07)	103.52	136.01	100.0	125.1
	0.01	5	138.97 (16.91)	117.94	160.00	115.6	140.7
	0.02	5	108.10 (29.14)	71.87	144.33	90.3	103.0
	0.03	4	92.01 (50.80)	11.24	172.79	76.8	92.0
6	0.00	4	123.68 (7.63)	111.55	135.81	100.0	123.7
	0.01	4	65.51 (7.65)	53.34	77.68	53.0	65.5
	0.02	4	101.45 (26.38)	59.52	143.39	82.0	101.5
	0.03	4	61.13 (12.22)	41.70	80.97	49.4	61.1
11	0.00	5	70.45 (68.57)	-14.80	155.70	100.0	69.7
	0.01	4	70.66 (41.11)	5.25	135.98	100.3	70.8
	0.02	4	26.58 (8.95)	12.35	40.81	37.7	26.6
	0.03	5	31.04 (22.23)	3.41	58.68	44.1	21.7
20	0.00	5	95.48 (56.79)	24.88	166.09	100.0	86.8
	0.01	3	105.18 (24.89)	43.39	166.97	110.2	98.0
	0.02	5	130.73 (48.30)	80.06	181.41	136.9	105.0
	0.03	5	39.87 (34.24)	- 8.82	88.56	41.8	45.8

Hepatosomatic Index

The hepatosomatic index (HSI) of fish exposed to various levels of cyanide for a 20-day period was measured during experiments #1, 2 and 3 and the data are shown in Table 12, 13 and 14 and graphically illustrated in Fig. 7, 8 and 9. As seen from these tables, the average hepatosomatic index of the 165 g control fish, experiment #1 and 30 g control fish, experiment #3 was 1.45 and 1.49 respectively whereas for the 170 g control fish, experiment #2, it was 1.04, a 40% difference which was statistically significant ($P < 0.001$).

During experiment #1, the hepatosomatic index varied significantly with time ($P < 0.01$) and cyanide concentrations ($P < 0.001$) (Fig. 7). Compared to controls, at 0.01 $mg.L^{-1}$ HCN the hepatosomatic index did not vary significantly. At 0.02 $mg.L^{-1}$ HCN, the HSI was significantly depressed at day 3 and 11 but rose back to the control level by day 20. However, at 0.03 $mg.L^{-1}$ HCN, the HSI decreased significantly at day 3 throughout day 11 and levelled off through day 20 at a value significantly lower than in other fish groups.

During experiment #2, the hepatosomatic index was also significantly affected by the cyanide exposure ($P < 0.05$) (Fig. 8). Compared with the controls, cyanide, at 0.01 $mg.L^{-1}$ HCN, significantly increased the HSI at day 1 and 15 while at 0.02 $mg.L^{-1}$ HCN the increase was only significant at day 5. At 0.03 $mg.L^{-1}$ HCN, the HSI was lower than in controls throughout the experiment but this difference was significant only at day 15.

As in experiments #1 and 2, the HSI measured during experiment #3

was also significantly ($P < .005$) affected by cyanide exposure. In comparison to controls, it appears from Figure 9 that 0.01 $mg.L^{-1}$ HCN had no effect on the HSI but that 0.02 and 0.03 $mg.L^{-1}$ HCN consistently depressed it. The difference were however, only significant on day 1 and 20 at 0.02 $mg.L^{-1}$ HCN and on day 10 and 20 at 0.03 $mg.L^{-1}$ HCN.

Our results indicate that cyanide decreased the hepatosomatic index of rainbow trout and that this effect is more evident at higher cyanide concentration.

Hematocrit

The effect of cyanide on the hematocrit of rainbow trout was measured during experiment #3 (30 g fish). No significant change due to cyanide treatments could be detected at any of the three concentrations tested. The 120 blood samples averaged an hematocrit of 0.277 with maximum and minimum values of 0.353 and 0.241.

Figure 7. Mean hepatosomatic index HSI and their 95% confidence limits in rainbow trout (165 g) exposed for 20 days to various sublethal cyanide concentrations at 13°C in experiment #1

Values without a common superscript are significantly different ($P < 0.01$)

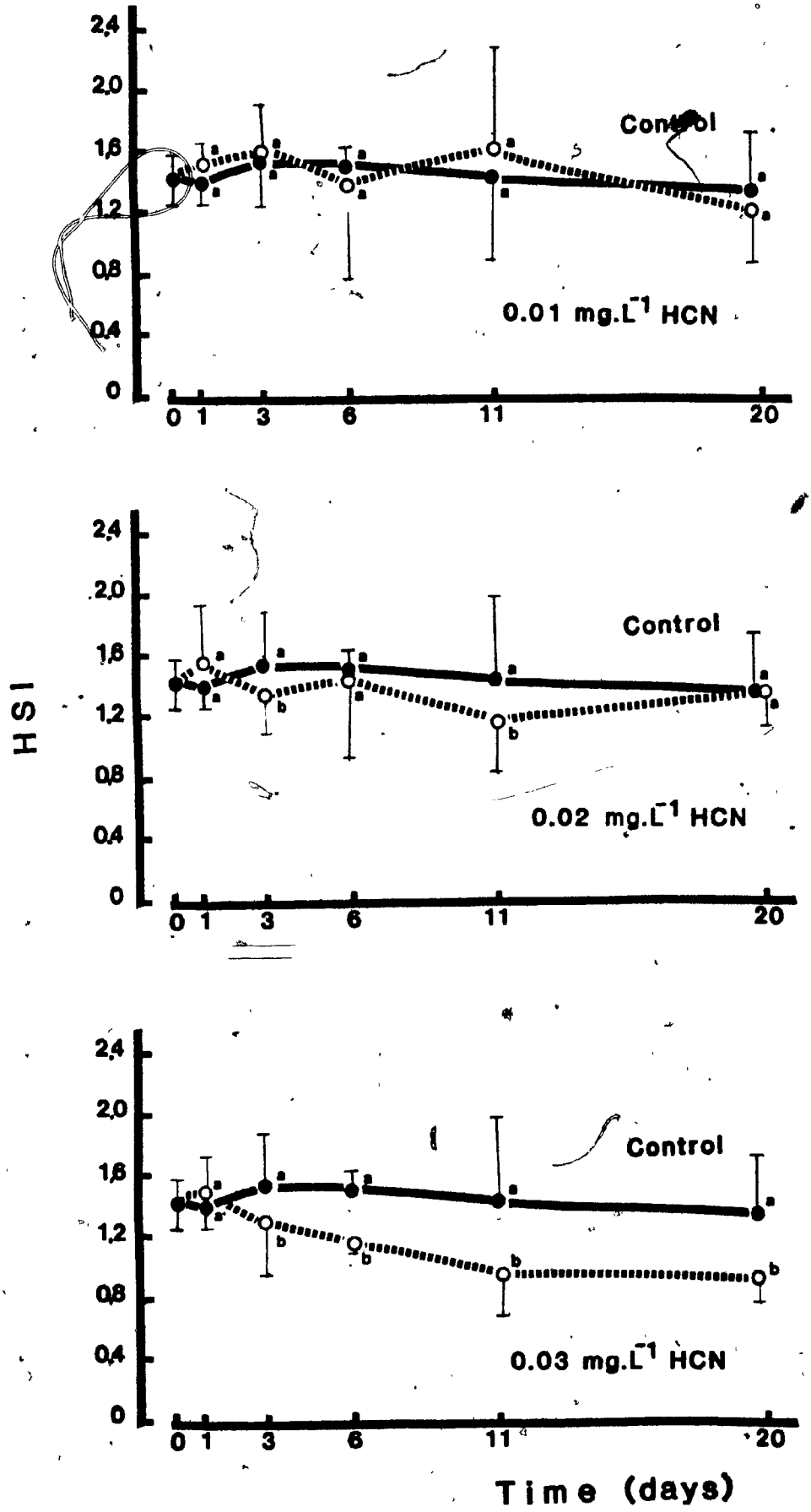

HSI
2,4
2,0
1,6
1,2
0,8
0,4
0
Control
0.01 mg.L⁻¹ HCN
0.02 mg.L⁻¹ HCN
0.03 mg.L⁻¹ HCN
0 1 3 6 11 20
Time (days)

Figure 8. Mean hepatosomatic index (HSI) and their 95% confidence limits in rainbow trout (170 g) exposed for 20 days at various sublethal cyanide concentrations at 12°C in experiment #2

Values without a common superscript are significantly different ($P < 0.01$)

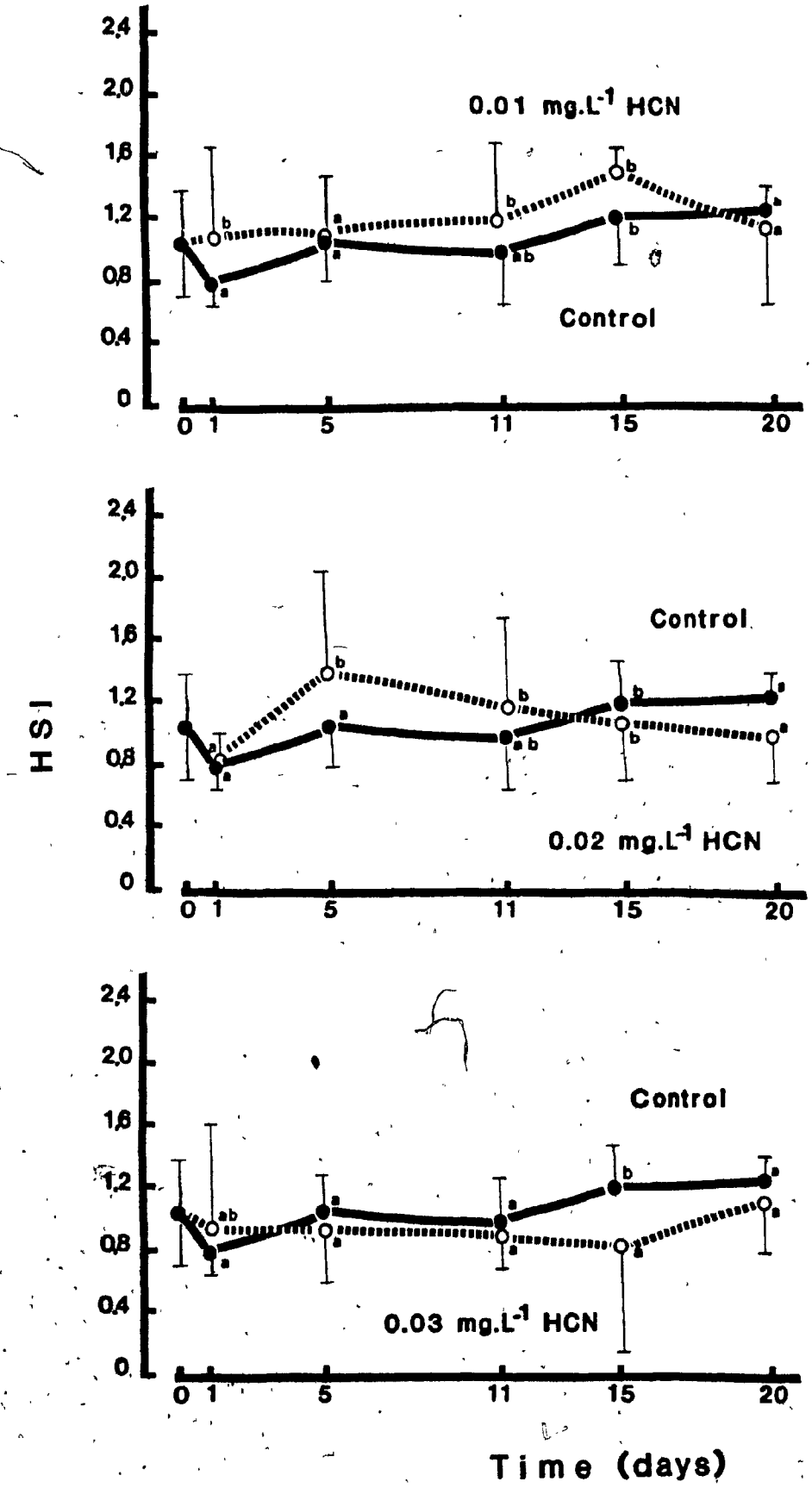

0.01 mg.L-1 HCN
Control
0.02 mg.L-1 HCN
Control
0.03 mg.L-1 HCN
Control
HSI
Time (days)
2,4
2,0
1,6
1,2
0,8
0,4
0
0 1 5 11 15 20

Figure 9. Mean hepatosomatic index (HSI) and their 95% confidence limits in rainbow trout (30 g) exposed for 20 days to various sublethal cyanide concentrations at 13°C in experiment #3

Values without a common superscript are significantly different ($P < 0.01$)

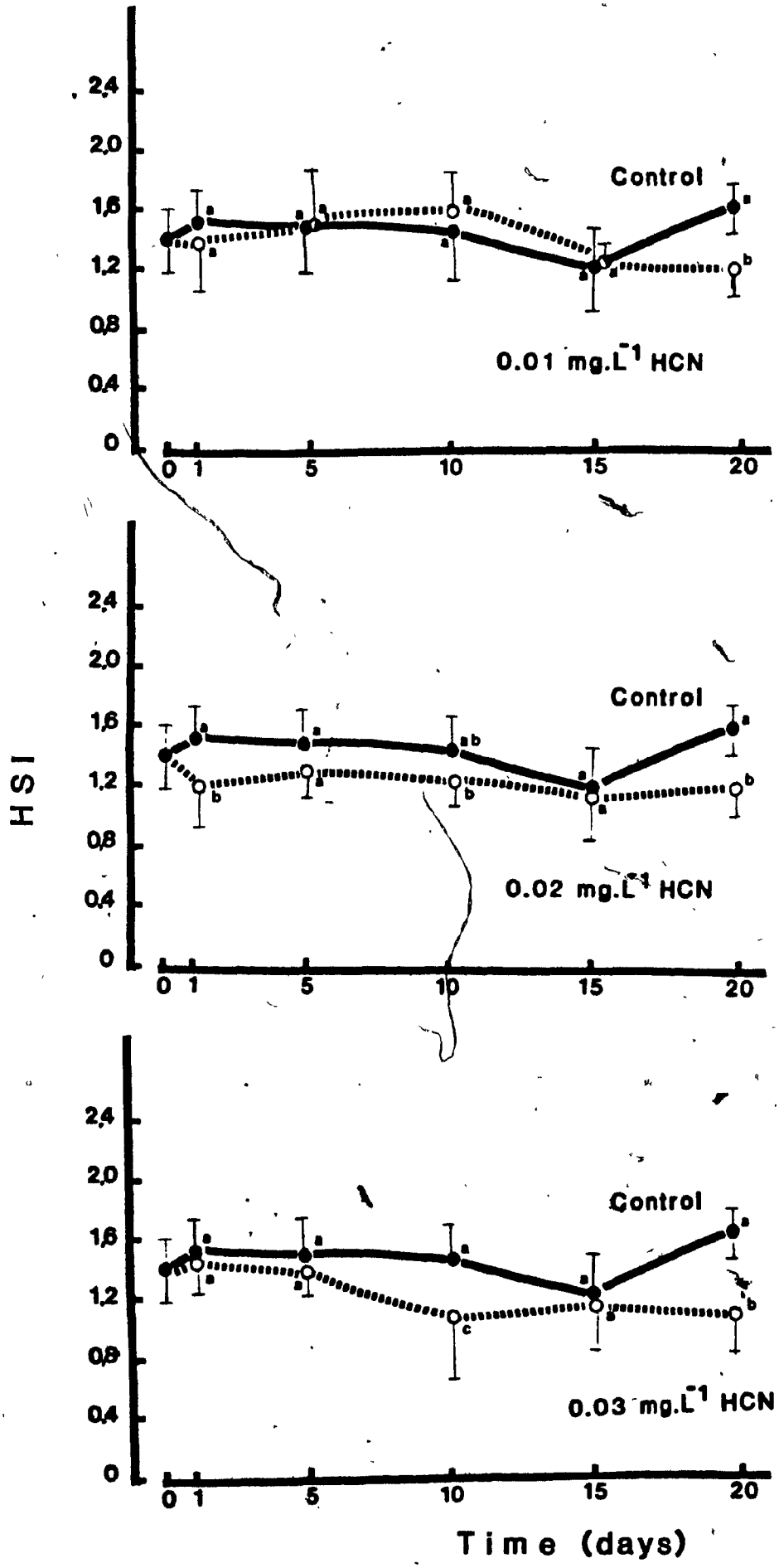

HSI
2,4
2,0
1,6
1,2
0,8
0,4
0
Control
0.01 mg.L⁻¹ HCN
0.02 mg.L⁻¹ HCN
0.03 mg.L⁻¹ HCN
0 1 5 10 15 20
Time (days)

Table 12. Mean hepatosomatic index (HSI) and their 95% confidence limits in rainbow trout (165 g) exposed for 20 days to various sublethal cyanide concentrations at 13°C in experiment #1.

Day of exposure	HCN Conc. $mg.L^{-1}$	Sample size n	Mean HSI (SD)	95% Confidence Limit L_1	L_2	Mean HSI of Subsample[a]
0	0.00	5	1.41 (0.148)	1.227	1.593	1.38
1	0.00	5	1.39 (0.113)	1.251	1.529	1.43
	0.01	5	1.52 (0.109)	1.384	1.656	1.54
	0.02	5	1.55 (0.334)	1.134	1.966	1.47
	0.03	5	1.46 (0.247)	1.155	1.765	1.42
3	0.00	5	1.55 (0.264)	1.222	1.878	1.58
	0.01	5	1.59 (0.289)	1.232	1.948	1.64
	0.02	5	1.33 (0.223)	1.052	1.608	1.31
	0.03	5	1.30 (0.270)	0.964	1.636	1.34
6	0.00	4	1.51 (0.064)	1.408	1.615	1.51
	0.01	4	1.39 (0.408)	0.740	2.040	1.39
	0.02	4	1.41 (0.324)	0.894	1.926	1.41
	0.03	4	1.15 (0.050)	1.070	1.230	1.15
11	0.00	5	1.42 (0.463)	0.845	1.995	1.45
	0.01	4	1.61 (0.435)	0.918	2.301	1.61
	0.02	4	0.96 (0.106)	0.791	1.129	0.96
	0.03	5	0.93 (0.219)	0.658	1.202	0.86
20	0.00	5	1.36 (0.302)	0.977	1.743	1.35
	0.01	3	1.20 (0.137)	0.860	1.540	1.20
	0.02	6	1.36 (0.231)	1.118	1.602	1.25
	0.03	6	0.89 (0.149)	0.733	1.047	0.89

[a] Subsample randomly chosen to bring all sample size to an equal number.

Table 13. Mean hepatosomatic index (HSI) and their 95% confidence limits in rainbow trout (170 g) exposed for 20 days to various sublethal cyanide concentrations at 12°C in experiment #2.

Day of exposure	[HCN] $mg.L^{-1}$	Sample size n	Mean HSI (SD)	95% Confidence limit L_1	L_2
0	0.00	5	1.05 (0.276)	0.707	1.395
1	0.00	5	0.78 (0.128)	0.636	0.942
	0.01	5	1.09 (0.493)	0.474	1.696
	0.02	5	0.81 (0.164)	0.602	1.008
	0.03	5	0.95 (0.521)	0.303	1.597
5	0.00	5	1.04 (0.189)	0.808	1.280
	0.01	5	1.12 (0.303)	0.740	1.496
	0.02	5	1.40 (0.569)	0.687	2.109
	0.03	5	0.93 (0.282)	0.580	1.280
11	0.00	5	0.97 (0.270)	0.632	1.304
	0.01	5	1.19 (0.398)	0.700	1.688
	0.02	5	1.17 (0.482)	0.570	1.770
	0.03	5	0.87 (0.167)	0.658	1.074
15	0.00	5	1.19 (0.245)	0.888	1.498
	0.01	5	1.48 (0.101)	1.356	1.606
	0.02	5	1.07 (0.319)	0.677	1.471
	0.03	5	0.79 (0.099)	0.666	0.910
20	0.00	5	1.22 (0.144)	1.042	1.398
	0.01	5	1.11 (0.396)	0.618	1.600
	0.02	5	0.97 (0.251)	0.655	1.277
	0.03	4	1.08 (0.201)	0.764	1.400

Table 14. Mean hepatosomatic index (HSI) and their 95% confidence limits in rainbow trout (30 g) exposed for 20 days to various sublethal cyanide concentrations at 12°C in experiment #3.

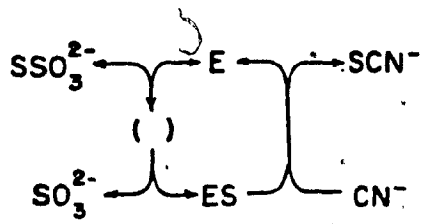
Day of exposure	HCN Conc. $mg.L^{-1}$	Sample size n	Mean HSI (SD)	95% Confidence Limit L_1	95% Confidence Limit L_2	Mean HSI of Subsample[a]
0	0.00	6	1.43 (0.213)	1.207	1.655	1.50
1	0.00	6	1.57 (0.214)	1.348	1.796	1.53
	0.01	6	1.43 (0.293)	1.121	1.739	1.43
	0.02	6	1.23 (0.235)	0.984	1.478	1.19
	0.03	5	1.48 (0.154)	1.288	1.672	1.48
5	0.00	6	1.51 (0.276)	1.219	1.801	1.31
	0.01	6	1.55 (0.202)	1.337	1.757	1.57
	0.02	6	1.35 (0.183)	1.161	1.547	1.41
	0.03	6	1.43 (0.193)	1.227	1.633	1.37
10	0.00	6	1.47 (0.270)	1.187	1.753	1.46
	0.01	5	1.64 (0.227)	1.355	1.921	1.64
	0.02	6	1.29 (0.135)	1.147	1.429	1.30
	0.03	6	1.11 (0.416)	0.668	1.542	0.97
15	0.00	5	1.26 (0.227)	0.977	1.543	1.26
	0.01	6	1.29 (0.122)	1.144	1.426	1.26
	0.02	6	1.19 (0.239)	0.942	1.446	1.21
	0.03	6	1.15 (0.276)	0.863	1.445	1.15
20	0.00	6	1.67 (0.147)	1.515	1.823	1.70
	0.01	6	1.25 (0.188)	1.057	1.453	1.28
	0.02	6	1.24 (0.139)	1.080	1.374	1.26
	0.03	5	1.11 (0.238)	0.815	1.403	1.11

[a] Subsample randomly chosen to bring all sample size to an equal number.

DISCUSSION

Toxicokinetics

The monitoring of blood plasma thiocyanate level in rainbow trout exposed to cyanide, allowed us to assess the absorption and the detoxification of cyanide in the fish. Most of the thiocyanate in the body arises from the detoxification of CN^- (Wolff, 1964) via the action of the enzyme rhodanese (thiosulfate: cyanide sulfur transferase, EC 2.8.1.1) (Westley, 1981; Way, 1981). The formal mechanism of rhodanese action is illustrated below:



E is the free enzyme; ES is the sulfur-substituted enzyme, (from Westley, 1981). This enzyme has also been identified in fish (Schievelbein et al, 1969; Sido and Koj, 1972). A base level of thioocyanate is commonly found in organisms because cyanide is ubiquitous in nature: it can originate from numerous precursors, namely cyanogenic glycosides, (Solomonson, 1981).

Our results indicate that there is an immediate accumulation of thiocyanate in blood plasma within 24 h of exposure to cyanide. No information could be found in the literature on the bioaccumulation of thiocyanate during a chronic exposure of fish to cyanide, however, our results agree well with findings observed in mammals (Vesey et al, 1976; Bourdoux et al, 1978; Barrett et al, 1978; Chandra et al, 1980) and birds (Davis, 1981).

The different patterns of accumulation of blood plasma thiocyanate observed in the present study (Fig. 1, 2 and 3) can possibly be attributed to variations: 1) in the rate of cyanide absorption via the gills, 2) the rate of thiocyanate formation by rhodanese and/or of the availability of the free form of CN^- to the enzyme rhodanese and 3) the rate of thiocyanate excretion. In other words, differences in the overall metabolic rate might well be responsible for the discrepancies observed in experiment #1, 2 and 3.

The accumulation of plasma thiocyanate was dose related and increased with the duration of exposure to cyanide up to day 10 during experiment #2 and 3. However, the rate of bioaccumulation appeared higher during experiment #3 carried out with 30 g fish compared to the larger fish (170 g) of experiment #2. The differences in the rate of accumulation of thiocyanate during exposure to cyanide in experiment #1, 2 and 3 may be explained by differences in fish body size and/or the season during which the fish were tested. Smaller fish having higher metabolic rates per unit weight (O'Hara, 1968; Anderson and Weber, 1975; Robinson et al, 1983) may absorb cyanide through the gills faster than larger fish. In addition, the rate of accumulation of thiocyanate seemed more rapid in the 30 g fish when comparing Fig. 2 and 3. After 10 or 15 days of cyanide exposure, levels of plasma thiocyanate in experiment #3 stopped increasing and showed a decline. However, during experiment #2 this decline was only observed at 0.03 $mg.L^{-1}$ HCN after 15 days of exposure to cyanide. Such a decline was not observed in Experiment #1 carried out with 165 g fish. As there was no observation taken at day 15 during this experiment, it might be

possible that the maximum thiocyanate level observed at day 20 was preceeded by an even higher thiocyanate level. The marked difference observed between the pattern of plasma thiocyanate accumulation in Experiment #1 (Fig. 1) and Experiment #2 and 3 (Fig. 2 and 3) may be explained by seasonal differences. Withey and Saunders (1973) and Beamish (1964) demonstrated that the oxygen consumption in fish varied greatly according to photoperiod or season, the oxygen consumption reaching a maximum during the period of reproduction. Rainbow trout, a spring spawner, for a constant temperature should have a higher metabolic rate in February, in comparison to the month of October, and thus should take up cyanide more rapidly. This is what we observe in Fig. 1 and 2 where fish in Experiment #1 (October) accumulate plasma thiocyanate more slowly than fish in Experiment #2 (February). In addition, fish in Experiment #1 exhibited a higher hepatosomatic index than those in Experiment #2 which can possibly compensate for the toxic effect of cyanide by supplying alternative energy sources such as glycolysis or other metabolic pathways. Fish tested in October would then appear more resistant to cyanide poisoning than those tested in February. Our results are consistent with the work of McGeachy (1984), who showed that rainbow trout tested in the fall exhibited a higher 96 h LC 50 value than fish tested in the winter.

According to Davis (1981) and Westley (1981), thiocyanate accumulation is limited not so much by its excretion as by the available sulfane sulfur which can be depleted by a continuous uptake of cyanide unless dietary sulfur overcompensates. Whether or not such

sulfur depletion occurred in our tested fish is not known but should be investigated since it has been shown that sulfur plays an important role in the tolerance of fish to cyanide poisoning (Achard, 1934).

In this study there was a sudden increase of thiocyanate in the blood plasma of trout exposed to any of the three cyanide concentrations tested followed by some leveling. Studies on chronic cyanide toxicity to man showed general patterns of thiocyanate accumulation similar to those observed here. Bourdoux et al (1978) demonstrated that 3 days of intensive cassava diet (a cyanogenic plant) significantly raised serum and urinary SCN^- over 10 $ug.ml^{-1}$ serum, fivefold the control thiocyanate level. Similarly, Chandra et al (1980) observed that blood and urine thiocyanate levels in cyanide-exposed workers of an electroplating factory was much higher than the control worker levels. They reported a 10 fold increase in the blood thiocyanate for cyanide-exposed workers, thiocyanate and cyanide levels averaging 0.46 $mg.100\ ml^{-1}$ and 10 $ug.100\ ml^{-1}$ respectively. Barrett et al (1978) observed the same type of effect when dietary cyanide significantly elevated blood thiocyanate levels in rats.

The bioaccumulation of plasma thiocyanate is supported by several characteristics of this ion. First, thiocyanate is an ion often used in the measurement of extracellular fluid volume because of its pseudohalogen characteristics, its stability and its similarity to chloride ions; it is reabsorbed almost completely by the kidney tubules (Guyton, 1976) of freshwater fishes (Hickman and Trump, 1969). Second, thiocyanate as with chloride ion is not actively excreted at

the gill level of freshwater fish, however its passive diffusion should be kept in mind, the gill being a major site of osmoregulation and excretion (Black, 1957).

It seems unlikely that the detoxification of cyanide could be limited by the thiocyanate excretion rate since thiocyanate levels never exceeded 3.68 $ug.ml^{-1}$ (63.4×10^{-3} $umol.ml^{-1}$) plasma in any of the three experiments even at the highest exposure levels (0.03 $mg.L^{-1}$ HCN or 1.11 $umol.L^{-1}$). This maximum plasma thiocyanate level observed in experiment #3 in 30 g fish after 15 days of exposure to 0.03 $mg.L^{-1}$ HCN is relatively low considering the level of 0.61 $ug.ml^{-1}$ plasma (10.5×10^{-3} $umol.ml^{-1}$) measured in control fish. In addition, Eales and Shostak (1983) showed that daily intravenous injection of 16 mg KSCN 100 g^{-1} killed all rainbow trout (mean body weight 145 g) within 48 h whereas no trout (mean body weight 433 g) died following daily injections of 1 mg KSCN 100 g^{-1} for 8 days. The plasma thiocyanate concentration possibly obtained with such injection would have been approximately 3.4 and 0.21 $mg.ml^{-1}$ plasma (58.2 and 3.6 $umol.ml^{-1}$) respectively, as calculated from the plasma volume.100 g^{-1} rainbow trout (Ninkinmaa et al, 1980). Thus, it appears that thiocyanate levels measured in our experiments are far less than the probable saturation levels encountered under lethal conditions.

Following the build up of thiocyanate in the blood plasma, a decline was observed after 15 days of exposure to cyanide (Fig. 2 and 3). The presence of renal tubular paranchymatous oedema as observed by Dixon and Sprague (1981) in rainbow trout (1 to 2 g) exposed to sublethal levels of cyanide for at least 14 days could possibly

explain the change in thiocyanate reabsorption. Also, the half-life of 3 to 5 days of thiocyanate in plasma as well as the possible sudden excretion at the gill and kidney level resulting from a mass action due to thiocyanate accumulation may corroborate the drop in plasma thiocyanate levels.

Thus it seems by comparing Fig. 2 and 3, that the larger fish (Experiment #2) were less susceptible to cyanide since the rate of plasma thiocyanate accumulation was less than those of the 30 g fish (Experiment #3). After 15 days of cyanide exposure the drop in plasma thiocyanate level, in experiment #2, only occurred for the 0.03 $mg.l^{-1}$ HCN. This seems to confirm an alteration in the bioaccumulation mechanism of plasma thiocyanate.

It seems though, that smaller fish are more sensitive to cyanide toxicity in comparison to larger fish and that fish tested in the winter are more susceptible to cyanide than fish tested in the fall. In addition, fish exposed to the highest cyanide concentration (0.03 $mg.L^{-1}$ HCN) appeared to be seriously impaired by cyanide since the relationship between plasma thiocyanate accumulation and body size (Table 6) was very poor in contrast to those exposed to lower levels of cyanide (0.01 and 0.02 $mg.L^{-1}$ HCN).

Following exposure to cyanide, fish readily accumulate thiocyanate, a detoxifying product of cyanide; the deleterious effects observed in fish exposed to cyanide cannot be attributed solely to cyanide, thiocyanate is also involved. The gill chloride transport inhibition by thiocyanate as well as the anti-thyroid potency of thiocyanate and its possible role in the growth rebound and growth

enhancement phenomena will be discussed in the section Physiological significance.

Toxicodynamics

Measurements of liver cytochrome oxidase activity and glycogen content, hepatosomatic index and blood hematocrit were taken as indication of the fish response to prolonged exposure to 0.01, 0.02 and 0.03 $mg.L^{-1}$ HCN at 12-13°C.

Cytochrome Oxidase

Liver cytochrome oxidase activity was monitored in rainbow trout of 170 g and 30 g during experiment #2 and 3. No explanation could be proposed concerning the difference in cytochrome oxidase activity between the two fish size groups or the large variations in activity (Fig. 4 and 5). However, Commoner (1940) suggested that the state of nutrition of the animal may account for the variations from sample to sample in the cyanide-sensitive respiration. The change in food ration, a week prior to the start of the experiment, may partly explain the marked discrepancies in cytochrome oxidase activity of the control from day 0 to day 1 in the experiments #2 and 3 (Fig. 4 and 5).

Cyanide readily inhibited cytochrome oxidase activity within 24 h of exposure to any of the three cyanide concentrations tested. The effect of cyanide on the respiratory electron transport chain is well described and Solomonson (1981) gives a good review on the topic. However, no information on the monitoring of cytochrome oxidase

activity in cyanide-exposed fish could be found in the literature. Our results agree with those of similar studies carried out with mammals *in vivo* and *in vitro*. Thus, Harry *et al* (1946) reported that an *in vitro* concentration of 3×10^{-7} M HCN (or 0.01 $mg.L^{-1}$ HCN) and 1×10^{-6} M (or 0.03 $mg.L^{-1}$ HCN) reduced the activity of brain cytochrome oxidase of rats by 55 and 65% respectively. Also, Nakamure *et al* (1977) reported an inhibition of mitochondrial respiration in rat liver homogenate suspension following the addition of sodium nitroprusside, a precursor of cyanide. Under these conditions, the oxygen uptake was inhibited by about 63% following the addition of 1 $mmol.L^{-1}$ of sodium nitroprusside which resulted in the production of 0.01 $mmol.L^{-1}$ of CN^- (or 0.28 $mg.L^{-1}CN^-$). Ballantyne *et al* (1972) reported that female rabbits killed by intramuscular injection of HCN at a dosage of 8 mg CN^- kg^{-1} or 0.31 $mmol.kg^{-1}$ exhibited cyanide concentrations in the liver of 20.9 $ug.100\ g^{-1}$ or 0.80 $umol.100\ g^{-1}$ of wet tissue and in the blood a concentration of 0.93 $ug.ml^{-1}$ or 3.5×10^{-2} $umol.ml^{-1}$); the liver cytochrome oxidase activity was inhibited by 72%. Thus even at high lethal cyanide concentration some activity remains in the cytochrome chain of the liver. These results are consistent with our results and the work of Commoner (1940). He estimated the relative rates of activity of the cyanide-sensitive respiration to about 90% of the maximum possible respiratory rate in most actively aerobic organisms. He also found that the cyanide-stable respiration is relatively small and constant, as compared with the activity of the cyanide-sensitive system.

In our study, the 170 g trout tested in experiment #2 experienced

an inhibition of liver cytochrome oxidase activity of about 80% within 24 h of exposure to any of the cyanide concentrations tested (See Fig. 4). The inhibition remained at that level for the rest of the 20-day exposure. For the 30 g trout (experiment #3) the inhibition was about 60% after 10 days of exposure to any of the three cyanide concentrations tested (Fig. 5). The apparent presence of a fraction of cytochrome oxidase enzyme free from the inhibition led us to verify the presence of cyanide-insensitive respiration. A pilot study on fish exposed for 7 days to 0.03 $mg.L^{-1}$ HCN exhibited approximately 5% of cyanide-insensitive respiration in treated and control fish. However the presence of a cyanide-stable respiration should not be ruled out on the basis of this pilot study. The constancy and levels of inhibited cytochrome oxidase activity encountered during experiment #2 and 3 (Fig. 4 and 5) agree with the cyanide-stable system proposed by Commoner (1940) which can be identified with flavoprotein. Palmer (1981) gives a review on this topic and mentions that cyanide-resistant oxygen uptake has been reported from animal but only investigated extensively in certain classes of protozoa, microorganisms and plants. On the other hand, according to Stannard and Horecker (1948) and Solomonson (1981), the inhibition of cytochrome oxidase by cyanide obeys the mass action law, one molecule of HCN inhibits one molecule of cytochrome oxidase enzyme. Cytochrome oxidase activity in fish is generally much higher than the corresponding activities of succinoxidase, NADH dehydrogenase and therefore, as in mammals, is not likely to be the rate limiting step in the electron transport chain (Smith, 1973a). Therefore, when

cyanide is detoxified by rhodanese into thiocyanate, its deleterious effect in the organisms may not attain a lethal level. Thus, cyanide-exposed fish might perform vital activity through cyanide-resistant respiration and/or remaining cytochrome oxidase activity, if present.

No increase in cytochrome oxidase activity was detected in the course of the experiment which could possibly explain the acclimation to cyanide observed by Dixon and Sprague (1981) or the growth rebound observed by Leduc (1966b), Speyer (1975) and McCracken and Leduc (1980), Dixon and Leduc (1981), or the return to the control glycogen level observed in this study (Fig. 6).

The extra release of energy to meet the growth rebound demand does not seem to occur through increased mitochondrial respiration. However, the extra energy supplied may arise through an other electron transport pathway, such as a cyanide-resistant respiration, not needing oxygen as an electron acceptor and/or through a configurational change in the structural composition of the mitochondrial membrane. Such change would conceivably induce a modification of substrate affinity of the membrane-bound enzyme and alter the enzyme activity. Such a change could not be detected with our measurement technique since the preparation of the homogenate implied the disruption of the mitochondrial membrane. However, as no detergents were used, the respiratory enzyme bound to the cell membrane were not uncoupled (Smith, 1973a).

An adaptation phenomenon possibly similar to the acclimation to cyanide and/or the growth stimulation is encountered in fish

acclimatized to cold temperatures. To maintain an adequate energy supply at low temperature, a fish may require enhanced electron transport capability by changing the lipids microenvironment of the respiratory enzyme associated to the membrane. This is achieved by incorporating higher unsaturation phospholipids into the membrane in cold acclimated fish (Shaklee et al, 1977; Caldwell, 1969; Irving et al, 1976). This, in turn, can alter the membrane associated enzyme activity by an increased permeability of the membrane or structural changes of the enzyme-protein membrane complex (Smith, 1973b). Smith (1973b) proposed that it is the translocation of ADP into the mitochondria, not the electron transport, that can be the limiting factor but Wilson and Erecinska (1979) proposed cytochrome oxidase as being the primary regulatory site of mitochondrial oxidative phosphorylation. All these indicate that a possible enhancement of cytochrome oxidase activity will not arise through a change in the cytochrome oxidase activity but rather through a qualitative change in the cytochrome oxidase environment.

Comparing the cytochrome oxidase activity (Fig. 4 and 5) and the blood plasma thiocyanate accumulation (Fig. 2 and 3) in the two fish size groups, it appears that the 30 g fish (Fig. 3) detoxified HCN much more rapidly than the 170 g fish (Fig. 2). This phenomenon may then account for the less pronounced inhibition by cyanide in liver cytochrome oxidase observed in 30 g fish (Fig. 5) from day 0 through day 10 compared with the 170 g fish where the inhibition was constant from day 1 through day 20.

The absence of a relationship between cyanide concentrations and

the inhibition of cytochrome oxidase acvitity may be linked to detoxification and/or to the presence of some cyanide in the liver even at the lowest exposure level tested. Assuming that the fish can rapidly transform HCN into thiocyanate and that the rhodanese-mediated reaction is not the limiting factor, the cyanide level in the fish will be kept minimal and so would its lethal inhibitory effect on the respiratory electron transport chain. However the important inhibition of liver cytochrome oxidase activity within 24 h of exposure seems to indicate the presence of cyanide in the liver at the start of the exposure and the constant cytochrome oxidase inhibition support a continuous cyanide uptake at the gill level.

Glycogen

The occurrence of a functionally anaerobic metabolism in trout treated with cyanide, demonstrated by the inhibition of liver cytochrome oxidase (Fig. 4 and 5), was further confirmed by the utilization of glycogen (Fig. 6), an immediate source of energy (Love, 1970).

The increase in liver glycogen content on day 1 and 3 for all fish groups, including controls (Table 11), may be explained by a change from a maintenance ration to the growth ration a week prior to the beginning of the bioassays. A similar response was observed, by McGeachy (1984). It is known that nutrition influences glycogen reserves (Hochachka and Sinclair, 1962). In the plaice (Pleuronectes platessa), the amount of glycogen inclusion is related to the peculiarities of fish feeding at a given period (Timashova, 1982). To

compensate for the effect of change in food ration, the results shown in Table 11 were normalized and illustrated in Fig. 6.

The depletion of liver glycogen for the cyanide-exposed fish is consistent with the results of Chaiken et al (1975), Shaffi and Prasad (1979) and Kovacs and Leduc (1982). In the first half of the bioassay (Fig. 6), owing to the blockage of ATP synthesis, the Pasteur effect became effective and glycolysis became the ATP generator. In the second half of cyanide exposure, the glycogen levels returned to normal suggesting the ATP synthesis through the cytochrome chain and/or through an other pathway had been established, except for the 0.03 $mg.L^{-1}$ HCN exposed fish group that was persistently affected. This recovery coincides with the recovery of the hepatosomatic index back to the control level (Fig. 7) observed during the same experiment (Exp. #1). Our results seem to corroborate the growth rebound and acclimation observed in trout exposed to sublethal cyanide concentrations (Leduc, 1966b; McCracken and Leduc, 1980; Dixon and Leduc, 1981; Speyer, 1975; Cheng, 1978; Dixon and Sprague, 1981; Kovacs and Leduc, 1982; Leduc et al, 1982).

However, at 0.03 $mg.L^{-1}$ HCN, no recovery was observed in the liver glycogen content or in the hepatosomatic index; such cyanide concentration could probably be too stressful for the fish to overcome the toxic effect of cyanide and be able to meet its energy requirement for purposes other than self-maintenance. Those fish exposed to 0.03 $mg.L^{-1}$ HCN manifested little activity and appetite at the time of feeding, compared to the other fish groups.

Hepatosomatic Index and Hematocrit

The hepatosomatic index (HSI) was measured in experiment #1, 2 and 3 during the months of October, February and May respectively. The differences in control fish HSI, between experiments, may be attributed to seasonal changes and partly to size differences. The smaller HSI of the 170 g control fish in experiment #2 (February) compared to the 165 g control fish of experiment #1 (October) is consistent with seasonal changes of HSI in rainbow trout; during the September-December period, the HSI of rainbow trout increased possibly due to pre-spawning induction of exogeneous yolk production (Van Bohemen et al, 1981, 1982). The higher HSI observed in the smaller fish (30 g) during experiment #3 (May), may be explained as being a characteristic of smaller trout. All HSI values reported for the control fish (Table 13, 14 and 15) compared favorably with reported values found in the literature (Schiffman and Fromm, 1959; Olivereau and Olivereau, 1979; Van Bohemen et al, 1981, 1982).

The effect of cyanide on the rainbow trout HSI differed within experiment #1, 2 and 3 respectively, and variation in HSI within each sampling group (Fig. 7, 8 and 9) was mainly attributed to individual differences.

By comparing the effect of cyanide on liver glygogen (Fig. 6) with the effect on HSI (Fig. 7), it appears that the reduction of HSI coincides with the depletion in liver glycogen. On the other hand, the slight increase in HSI for all fish groups from day 0 through day 3 probably resulted from the increased food ration one week prior to the start of the bioassay.

In general, it appears that fish exposed to cyanide, in October, experienced the greatest depletion of HSI probably due to greater reserves in their liver; only fish with a high HSI would be able to afford a decrease in their HSI as a response to cyanide exposure. Also the highest cyanide concentration tested (0.03 $mg.L^{-1}$ HCN) had the most depletive effect in all experiments.

Compared to controls, the hematocrit value of trout exposed to cyanide did not exhibit any significant variation. However, our trout hematocrit values compared favorably with those found in the literature (Schieffman and Fromm, 1959; Stevens, 1968; Houston and DeWilde, 1969; Cameron, 1970 and Nikinmaa et al, 1981).

Physiological Significance

Our results indicate that rainbow trout can detoxify cyanide into thiocyanate and accumulate it in the plasma up to 6 times the level found in the controls. Also they can live at concentrations of 0.01, 0.02 and 0.03 $mg.L^{-1}$ HCN with their liver cytochrome oxidase inhibited by 60 to 80%. Liver glycogen exhibited a tendency to decrease under cyanide exposure but at low cyanide levels a rebound back to that of the control level was observed after 11 days of exposure to 0.01 and 0.02 $mg.L^{-1}$ HCN (Fig. 6). In the same experiment, the hepatosomatic index response of rainbow trout exposed to cyanide coincided with that of glycogen levels (Fig. 7).

From the foregoing evidences, it appears that, as stated by Solomonson (1981), the metabolic response to cyanide will depend not only on its concentration, but also on a number of different factors

including: levels of vitamin B12, iodide, sulfur amino-acids and levels and distribution of detoxifying enzymes such as rhodanese.

Our results suggest the possibility of two kinds of sublethal responses: one at low sublethal levels and one at higher ones. In the former type of toxicity, trout would first show signs of response such as depressed HSI and liver glycogen (Fig. 6 and 7) but then after would manifest signs of recovery as these parameters rose back to the control level. However, at the highest cyanide concentration (0.03 $mg.L^{-1}$ HCN) tested in this study, there was no tendency of recovery, possibly because at such concentration, although sublethal, the deleterious effects of cyanide were too strong for the trout to overcome them.

At cyanide concentrations lower than 0.03 $mg.L^{-1}$ HCN, the major emphasis would be the effect of increased thiocyanate ion in addition to the direct effect of free cyanide on cytochrome oxidase and subsequent depletion of liver glycogen. The biotransformation of cyanide into thiocyanate via the enzyme rhodanese and its accumulation in rainbow trout exposed to low sublethal cyanide levels could possibly explain a number of symptoms previously attributed to cyanide alone.

Leduc and Chan (1975) demonstrated that cyanide affected both osmo and ionoregulation. After 260 h in salt water, plasma chloride was higher in previously cyanide-exposed rainbow trout, whereas upon return to freshwater the reverse occurred. The work of Kerstetter and Kirschner (1974) corroborates these results; they showed evidence that thioccyanate inhibits the active absorption of chloride by trout in

freshwater. Thiocyanate is also known to inhibit active excretion of chloride in gills of the eel (Anguilla anguilla) while in salt water, and chloride active absorption by Carassius auratus in freshwater (Epstein et al, 1973). Gills of teleosts are known to be a major site of ionic regulation, particularly in seawater (Solomon et al, 1973).

Thiocyanate is also a strong antithyroid agent since it competes with iodide in the active uptake of iodide by the thyroid gland in mammals (Wollman, 1956, 1962; Wolff, 1964; Greer et al, 1966; Kreutler et al, 1977; Bourdoux et al, 1978) and in fish (Singh et al, 1977; Eales and Shostak, 1983). However, slight antithyroid activity may induce growth in mammals (Tata, 1974). Specifically, slight hyperthyroidism causes enhancement of anabolic responses, whereas hypothyroidism and pronounced hyperthyroidism are inhibitory or catabolic. Thus the effects of thiocyanate, considering the major role played by the thyroid gland on the overall metabolism, are numerous.

With this in mind, the growth rebound and growth enhancement repeatedly observed in fish as well as the lower sensitivity in fish pre-exposed to low level of cyanide may be tentatively explained by the inhibition of thiocyanate on the thyroid gland. Let's first point out that Eales and Shostak (1983) reported that the injection of KSCN (0.12 mg or 1.2 umol and 1 mg or 10.3 umol.100 $g^{-1}.day^{-1}$) could enhance the production of thyroid hormones, whereas at the lowest thiocyanate dose (0.01 mg or 0.10 umol.100 $g^{-1}.day^{-1}$) they observed a decrease of T4 (thyroxine or tetraiodothyronine) in the plasma but not of T3 (triiodothyronine).

Under normal condition, T4 is produced in much larger quantitites than T3 but the later is 5 to 10 times more active (Lehninger, 1975). On the other hand the duration of the action of T4 is almost 4 times as long as that of T3 (Guyton, 1976). Thus, upon inhibition of the thyroid gland active iodide uptake by thiocyanate, we may expect that there is a reduction of the T4:T3 ratio due to a reduction of T4 synthesis.

We may then divide the action of thiocyanate into two ways according to concentrations as in the work of Eales and Shostak (1983). At very low cyanide concentration (e.g. 0.01 $mg.L^{-1}$ HCN), there are low thiocyanate levels produced since the two are closely related (Fig. 1, 2 and 3). Relative to T4, the synthesis of T3 would be enhanced and therefore the potency of thyroid hormone increased. Due to the role of thyroid hormones on growth stimulation, the growth enhancement at low cyanide concentration, observed by Negilski (1973), Leduc (1977), McCracken and Leduc (1980) and Kovacs and Leduc (1982) could possibly be due to such alterations of T4:T3 ratio. On the other hand at higher cyanide concentration (around 0.02 $mg.L^{-1}$ HCN) high thiocyanate level would first reduce the synthesis of thyroid hormones and growth rate.

However, after 10 days of exposure to cyanide, several authors observed a growth rebound coinciding with the higher production of T3 and T4 observed by Eales and Shostak (1983) after 8 days of thiocyanate treatment.

The growth rebound may possibly result from a rebound of the thyroid activity since we observed a decrease of plasma thiocyanate

around day 10 (Fig. 2 and 3). If the inhibitory effect on the thyroid gland is reduced, there could be a rapid uptake of the accumulated iodide from the blood with associated production of T4 and T3 hormones. This would result in an increased metabolic acvitity and growth and increase of protein synthesis for the second half of the 20-day exposure to cyanide. The change of sensitivity to cyanide, observed by Dixon and Sprague (1981), might possibly be explained following the same model, since in mammals, it is known that enzymes associated with the elotron transport chain and ATP synthesis decrease in hypothyroidism and increase in hyperthyroidism (Tepperman, 1980). In addition, Massey and Smith (1968) related the increased specific activity of oxidative enzyme system, observed when thyroxine was added to the tank water of brown trout (Salmo trutta fario L.), to an increased in growth rate. They also attributed the apparent loss in phosphorylation efficiency, commonly observed in such experiment, to a post-isolational lability of the mitochondrial membranes. A higher level of thyroid hormone causes change in the membrane which make it less stable. However, our results did not indicate any sign of enhancement of liver cytochrome oxidase activity (Fig. 4 and 5) that might supply the extra energy needed for the growth rebound or the recovery in liver glycogen level and hepatosomatic index. A change in the cytochrome oxidase environment and/or an alternative electron transport pathway should not be disregarded.

In comparison to mitochondria from control animals, those from animals receiving extra thyroxine exhibit an increased rate of electron transport but without decreasing the overall efficiency of

ATP synthesis (Metzler, 1977). Thus, part of the cellular response to thyroid hormone may be the alteration of inward transport of ADP in hypo and hyper functional thyroid states (Tepperman, 1980).

Also, upon action of thyroid hormone, the conversion efficiency of food to energy is affected (Tepperman, 1980). This characteristic of the thyroid gland may explain why Leduc (1966b) observed that cichlids fish (Cichlasoma bimaculatum L.) exposed to concentrations ranging from 0.008 to 0.08 $mg.L^{-1}$ HCN, although their food conversion efficiency was lessened, fed more actively than controls. In our study, a lower food consumption was observed mostly in fish exposed to 0.02 $mg.L^{-1}$ HCN while fish exposed to 0.03 $mg.L^{-1}$ HCN exhibited sluggish behavior. This peculiarity in fish behavior should not be neglected when examining physiological responses.

Fish exposed to low cyanide levels may sometimes grow faster but it is not necessarily an advantage since it is not known at what expense this extra growth can be attributed. One of the possible costs of this growth rebound or growth enhancement could be a retarded sexual maturation. Cheng (1978) reported that newly fertilized eggs of Ameican flagfish, Jordanella floridae, exposed to 0.065, 0.075 and 0.087 $mg.L^{-1}$ HCN from the time of fertilization until hatching displayed an enhanced growth but a delayed sexual maturity. Singh et al (1977) demonstrated that catfish, Heteroneustes fossilis Bloch, administrated with KSCN exhibited reduced thyroidal activity, accelerated thyrotropinsynthesis and lessened gonadotropin concentration with resulting retardation of gonadal activity. In addition Lundqvist (1980) showed that sexually immature female and

male baltic salmon parr, Salmo salar L., grew more rapidly than sexually maturing fish.

It seems doubtful that sublethal concentrations of cyanide could improve the overall performance of fish as it has been shown that swimming ability is reduced by concentrations below 0.01 $mg.L^{-1}$ HCN . (Leduc et al, 1982) thus indicating a serious metabolic impairment caused by cyanide such as the one on liver cytochrome oxidase activity demonstrated in this study.

The direct relationship between cyanide, thiocyanate and thyroid in fish has not yet been demonstrated and the model described above is limited by at least two points:

First, in the work of Eales and Shostak (1983), the concentration of thiocyanate injected daily in the fish in order to obtain a physiological reponse ranged from equal to ten fold the maximum plasma thiocyanate level measured in our study.

Secondly, thiocyanate levels used in these studies (Eales and Shostak, 1983; Singh et al, 1977), are nominal concentrations and injected at once daily while in our study thiocyanate in the plasma resulted in the detoxification of cyanide and thus appeared in the body rather slowly in comparison.

Nevertheless, this model on thiocyanate toxicity on thyroid function represents an interesting area for future research to elucidate chronic effects of sublethal concentrations of cyanide.

CONCLUSION

This study adds to the numerous articles on cyanide aquatic toxicology coming from laboratory studies carried out on fish that have never been subjected to cyanide, and held at fixed concentrations for the duration of the tests. These test conditions are certainly not representative of what is found in the environment, however, such investigation permits an estimation of the toxicity of cyanide in freshwater and a qualitative description of specific effects in fish.

We have shown in this study that cyanide could reduce, for the duration of the 20-day experiment, the liver cytochrome oxidase activity by 60 to 80% from that of the controls in rainbow trout tested in laboratory. We have also demonstrated the bioaccumulation of thiocyanate in plasma, a persistent diminution in liver glycogen level at the highest cyanide concentration and a decrease in hepatosomatic index in rainbow trout exposed to cyanide. Our results were discussed in view of observations on growth rebound, growth enhancement and cyanide acclimation in fish exposed to sublethal levels of cyanide as reported by several authors. We proposed a model of a direct relationship between cyanide, thiocyanate and the thyroid gland which, although based on an association of symptoms only, might explain the growth stimulation and the increase tolerance to cyanide.

Our results agree well with the concept of a 50% reduction in the relative performance index in fish exposed to 0.01 $mg.L^{-1}$ HCN described by Leduc (1977). Thus, as Leduc (1984) stated: "even though fish can survive indefinitely at 0.03 $mg.L^{-1}$ HCN in the laboratory,

the different physiological requirements necessary in nature to survive cannot be met". So, it appears that fish exposed to cyanide concentrations ranging from 0.01 to 0.03 $mg.L^{-1}$ HCN exhibit a marked reduction in active metabolism with a scope for activity being markedly reduced compared to that of hypothetical unstressed fish. Trout exposed to low cyanide level can live and perform any low demanding energy activty, however, such exposed fish are unable to perform activities such as swimming, fat biosynthesis, osmoregulation, reproduction and predation (Leduc, 1977) which require a burst of energy.

In this regard, the bioaccumulation of thiocyanate in plasma of cyanide-exposed fish might be of an ecotoxicological significance.

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