The Effect of Potassium Cyanide on Serum Protein of Snake Head, Ophicephalus argus(CANTOR)

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가물치 (*Ophicephalus argus* CANTOR)의 血淸蛋白質에 미치는 potassium cyanide 의 影響

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(Received Dec. 14, 1963)

摘 要

가물치 (*Ophicephalus argus* CANTOR) 2年生(168±4g) 107 마리를 對照群과 實驗群으로 나누고 實驗群에는 一時에 2.9mg/kg 體重의 potassium cyanide 을 背筋에 直接 注射하였다.

藥物 處理後 時間의 經過에 따라 나타나는 Albumin/Globulin(A/G) 比, 總血淸蛋白質量, hematocrit 比 및 肝臟 및 腎臟의 重量變化를 測定하였다.

藥物 處理 直後는 A/G 比, 總血淸蛋白質量 및 hematocrit 比는 各各 顯著하게 減少되었으나 많은 時間이 經過 함에따라 漸次 增加되어 228 時間以後는 거의 對照群의 그것과 가까운 結果를 얻었다. 그러나 肝臟과 腎臟의 重量은 藥物 處理 直後는 오히려 약간 增加되는 狀態였으나, 48 時間以後 急히 減少되었다가 漸次 亦是 對照群과 가까운 結果를 나타냈다. 以上의 結果로서 KCN 가 魚類의 呼吸 抑制作用 뿐만 아니라 蛋白質代謝에도 影響을 미치는 것으로 생각된다.

INTRODUCTION

Karston(1934) investigated the effect of cyanide on trout and Herbert and Merkens(1952) studied the toxicity of potassium cyanide to trout. In mammals also several toxications of cyanide were tested by Rabe and Burger(1936) and Moller(1935), and Common and Maw(1953) studied with avian serum protein.

Mayer and Heim(1960) reported the change in marine teliosts metabolism exposed to different environmental conditions. Carpenter(1927) and Jones(1935) studied the toxic action of heavy metal salts. Many studies were reported on the effect or the toxication of cyanide compounds on many kinds of lower animals(Reich, 1955; Potter, 1951; Commoner, 1940). Marget(1957) explained the mode of action and penetration of toxicants on fish. Also, Ellis(1937) and Mott(1951) presented the interference of blood circulation in the gill by any toxicants. According to Salter(1952), a part of red blood cells should be sacrificed by cyanide compound. Furthermore, Nam(1961) reported the results of paper-electrophoretic studies on blood serum proteins of amphibia.

Therefore, it is clear that cyanide compounds significantly influence many kinds of marine and fresh water fish respiration or other kinds of metabolism. But no one as yet has attempted to analyse the effect of cyanide compounds on the serum protein pattern in fresh water fish. The present study was undertaken to determine the albumin-globulin ratio, total protein of serum, hematocrit ratio, and kidney and liver weight changes of fresh water fish affected by potassium cyanide on snake fish, *Ophicephalus argus* CANTOR.

MATERIAL AND METHODS

This experiment was carried out from Sept. 5 to Oct. 28, 1963. The materials used were 107 male and female snake fish, 2 year-old, of body weight 168 ±4 g, obtained from fish ponds located in the vicinity of Seoul. The fish were cultured with tap water from the well nearby the laboratory for a week. Thereafter, the water was changed every 48 hours and continuous fresh air supplying was done by aeration pump. The fish were fed with small loaches two times daily, room temperature being kept at 17±2°C.

After a week of preculture, the fish were divided into two groups, control and treated(2.9 mg/kg body weight). Potassium cyanide was injected on dorsal muscle simultaneously. The fish were starved for 24 hours and solution used for toxicants was adjusted to pH 6.95. Blood was taken by direct heart disection of fish in 10 minutes, 48, 72, 96, 144, 216, 240, 264 and 288 hours, respectively, after potassium cyanide treatment, and in each stage 10-11 fish were used. Kidney and liver weights were measured by a chemical balance.

0.04 ml serum, separated by centrifuging with 3,000 rpm for 30 minutes, was then directly line-spotted on Toyo No. 51 A filterpaper with a micro-spotting tube. As soon as sampling was done, the electrophoresis was operated by Grassman-Hannig method with Veronal buffer, pH=8.6, Ω=0.05, 3.7 volts/cm and 0.2 mA/cm for 14 hours. After electrophoresis, the paper strips were stained with ethanolic bromophenol blue after dryness, optical density was determined by Toyo direct reading densitometer (Type 1) with wavelength of 540 mμ. Each fraction was identified by comparing with human serum protein fraction. Total serum protein of snake fish was determined by Folin Wu's and biuret's methods. Hematocrit ratio was determined by hemotocrit tube.

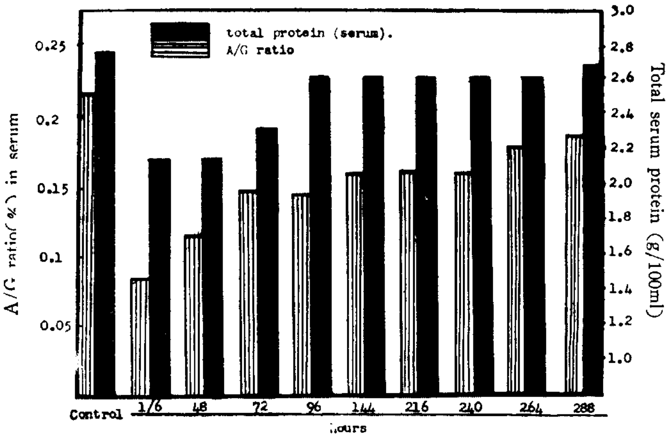

Fig. 1. Comparison of A/G ratio and total serum protein.

RESULTS

By this experiment the optical density revealed no significant effect on serum protein of snake fish under the same condition by sex. Albumin decreased a great deal after a chemical treatment, while β-globulin showed much increased conditions. Hence albumin-globulin ratio decreased, and peak revealed steep gradient (fig 1), but α-1 globulin decreased as albumin, and other globulin fraction showed no distinct change. As the time passed, the peak revealed gradual recovery to the original state; that is, albumin increased by and by after 48 hrs. and the peak already approached to the control after 288 hrs. On the other hand, β-globulin revealed continuous increase, but α-globulin showed comparatively medium decrease. The data are shown in table 1. Accordingly, albumin-globulin ratio gradually increased after 48 hrs. up to 288 hrs. of chemical treatment, and even after 288 hrs. A/G ratio revealed the state close to the control. Total protein revealed much more excessive diminution than control in

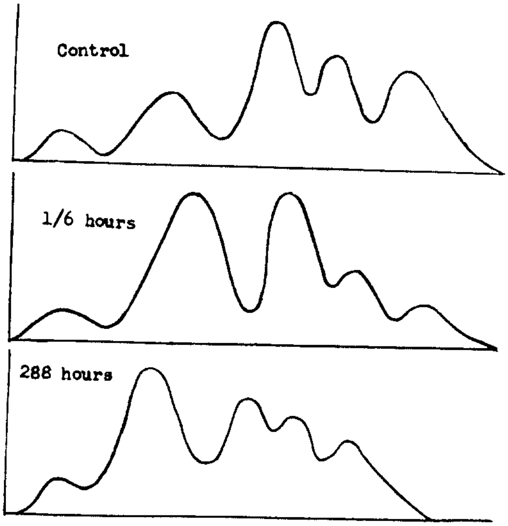

Fig. 2. Densitometer tracings of bromo-phenol blue-stained strips of serum protein of *Ophicephalus argus* after electrophoresis.

Table 1. Effect of potassium cyanide on serum protein of *Ophicephalus argus.*

Condition	No. of fish	Globulin(%) α_1	α_2	β	γ	Albumin %	A/G ratio	t test	total protein	t test
Control	10	24.24±2.53	32.42±4.61	15.92±5.46	6.35±1.41	21.09±3.16	0.21	—	2.8	—
10 min.	10	16.25±4.49	33.63±4.51	33.71±4.61	7.28±1.23	9.11±1.21	0.09	>p 0.01	2.3	>p 0.01
48 hrs.	10	14.91±2.25	32.70±4.46	32.71±4.20	7.29±0.91	12.32±1.17	0.12	>p 0.01	2.2	>p 0.01
72 hrs.	11	18.34±2.33	25.45±2.28	34.57±2.28	7.08±2.12	14.27±1.13	0.14	>p 0.01	2.4	>p 0.01
96 hrs.	11	25.50±2.22	27.40±3.12	24.64±5.12	7.09±1.41	14.26±2.33	0.14	>p 0.01	2.6	>p 0.01
144 hrs.	11	21.31±3.10	25.46±4.41	31.20±3.26	6.91±2.41	15.02±4.36	0.15	>p 0.01	2.6	>p 0.01
216 hrs.	11	21.01±3.11	25.76±2.23	31.01±2.21	7.12±2.23	15.13±3.17	0.15	>p 0.01	2.6	>p 0.01
240 hrs.	11	25.36±2.22	22.61±6.14	30.01±3.24	6.67±0.91	15.10±2.28	0.15	>p 0.01	2.2	>p 0.01
264 hrs.	11	21.93±3.15	19.41±2.27	34.91±2.22	7.01±2.18	16.65±1.65	0.16	>p 0.01	2.6	>p 0.01
288 hrs.	11	25.86±2.18	18.91±2.33	30.82±2.75	6.94±1.81	17.39±3.24	0.17	<p 0.01	2.7	<p 0.01

* Mean standard deviation

ten minutes, but, from time to time, it showed gradual increase up to 288 hrs. (table 1). To give accuracy for this table, t-test was managed(Croxton, 1956). By this test, it was known that all the data were highly significant, P>0.01 up to 264 hrs. Over 288 hrs. it showed no significance, P<0.01 and precise recovering state was recognizable. Hematocrit ratio also revealed much diminution than control right after potassium cyanide was injected but showed gradient increase up to 288 hrs. and recovering state was seen. This datum was shown in table 3. While liver and kidney weights, however, revealed no distinct difference soon after injection, liver weight alone was much less than control after 48 hrs. and gradual recovery was confirmed (table 2).

Table 2. Body weight ratio for liver and kidney of *Ophicephalus argus* for effects of potassium cyanide(2.9mg/kg) on organ weight.

Condition	No. of fish	Body weight (g)	Liver weight (g)	%	Kidney weight (g)	%
Control	10	187.42	6.2	3.31	1.3	0.68
10 min.	10	190.01	7.1	3.73	1.3	0.68
48 hrs.	10	184.13	3.5	1.90	0.7	0.38
72 hrs.	11	186.21	3.5	1.87	0.6	0.38
96 hrs.	11	186.21	3.5	1.87	0.7	0.37
144 hrs.	11	187.10	2.9	1.55	0.6	0.36
216 hrs.	11	187.15	4.2	2.25	0.7	0.37
240 hrs.	11	187.31	4.2	2.25	0.7	0.37
264 hrs.	11	186.26	4.0	2.15	0.7	0.53
288 hrs.	11	184.00	4.1	2.23	1.1	0.59

DISCUSSION

The above mentioned results confirmed that potassium cyanide is an influential chemical compound to A/G ratio, hematocrit ratio, total serum protein, and kidney and liver weights of snake fish. By Sollmann(1948), cyanide compounds clearly hinder the oxidative process of the tissues, which could be effected by blood circulation, especially in the aorta. When cyanide compounds penetrate into circulation, at least a part of red blood cells should be sacrificed to form a harmless cyan hemoglobin(Salter, 1952), and potassium cyanide acts as alkaline caustics, first makes cyan hemoglobin and naturally putrefaction occurs by fibrin ferment. In two weeks, the poison disappears during the putrefaction(Gettler and Baine, 1938). Sollman(1948) reported that oxygen consumption immediately increased, accompanying a severe drop of metabolism, as cyanide compounds were injected into animal tissues. By Ellis(1937), through capillaries, the blood circulation of fresh water fish was interferred by any toxic compound, and by Marget (1957), delicate tissues may be damaged and the excretory function of fresh water fish may be rendered fatal.

Table 3. Effect of potassium cyanide on blood of*Ophicephalus argus.* Haematocrit(% cells)

Condition	control	10 min.	48 hrs.	72 hrs.	96 hrs.	144 hrs.	216 hrs.	240 hrs.	264 hrs.	288 hrs.
Ratio	34.20	23.18	23.17	23.17	30.00	30.25	30.19	30.24	31.04	31.16

By these physiological abnormal facts, it might be suggested that any kind of interference can take place to circulation and to a practical function of liver by cyanide compounds. Naturally, any abnormality to the function of liver may well be said to have caused a reliable frustration to a pattern of serum protein and also to an amount of total protein in blood. Madden and Whippls(1940) made it even clearer when they said that synthesis of albumin and most globulin take place in the liver, and Abdel-Wahab *et al.* (1956) and William (1956) reported that a fall in albumin concentration may result from impaired synthesis or increased breakdown of protein by the liver or from specific elimination of this fraction *via* kidney. They also reported that a rise in globulin concentration may result from reticulcendothelial response or an accumulation of abnormal protein. Also by Sollman(1948), visceral and nervous degenerations were found when cyanide compounds were adopted and the glutathione level of the blood fell to half of the normal level. Therefore, it is considered that significant change from the normal range to subnormal value in amount of total serum protein, albumin-globulin ratio, hematocrit ratio, and organ weight might be due to many types of diseases, but little is known about the detailed physiological mechanism controlling the blood protein concentration or the origin and function of the various protein fractions on a complex pattern of change. Usuky(1959) reported that partial or perfect recovery of sea oyster gill was attained by prolonged washing with normal sea water, and the fish which is toxified with cyanide compounds may have much more chance to be recovered when their stomachs are full. By these facts, the recognized recovering condition may be explained by continuous refreshing air in water and sufficient feeding and water supply.

SUMMARY

Snake fish were injected with 2.9mg/kg-body-weight of potassium cyanide simultaneously on dorsal muscles

After the treatment the changes in A/G ratio, total serum protein, hematocrit ratio and liver and kidney weights were observed shortly after the treatment. A/G ratio, total serum protein and hematocrit ratio decreased distinctly. However, after 48 hrs. they showed gradual increase and, over 288 hrs. revealed to the state close to control.

On the other hand, liver and kidney weights revealed no distinct change right after the treatment and showed sudden decrease after 48 hrs. but gradually increased up to the state close to control. Therefore, potassium cyanide acts not only as an inhibitor of the respiratory function of fresh water fish, but also it may be considered that cyanide compounds effect on the protein metabolism.

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