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The Blood Acid-Base Changes in Acute Cyanide Poisoning in the Rat in Comparison with those in Acute Anoxic Anoxia
With Special Reference to the Relation with the Time to Death

Keiichi Yamamoto and Yoshiko Yamamoto

Department of Legal Medicine, Faculty of Medicine Kyoto University, Kyoto Japan

Summary. Rats were given orally various doses of NaCN (20,15,10, and 7 mg of CN per kg). When respiration stopped, the chest was opened and heart blood was taken anaerobically, and blood acid-base, lactate, and glucose determinations were made. As a control to chemical anoxia by cyanide, identical determinations were made in the rat acutely made anoxic by exposure to N_2.

The time to the cessation of respiration ranged 2,4 to 14 and 2.3 to 9.3 minutes in cyanide and N_2 experiments, respectively. The Po_2 at the time of the sampling was much higher in cyanide experiments than in N_2 experiments. The Pco_2 became lower as the time to the cessation of respiration became longer in cyanide group, and it was considered to diffusion of CO_2 into extravascular space. The hydrogen ion concentration, $[H^+]$, became higher with increasing length of the survival time in both groups. The $[H^+]$ was higher in the cyanide group than in the N_2 group in the rat with similar survival time. The increase in $[H^+]$ was due to the accumulation of lactate. Blood bicarbonate decreased with increasing length of the survival time.

The pattern of the acid-base changes in acute cyanide poisoning could be characterized by much higher Po_2 and greater inhibition of aerobic metabolism in comparison with those in acute anoxia by N_2.

Zusammenfassung. Ratten wurden peroral verschiedene Dosen von NaCN (20, 15, 10, und 7 mg von CN per kg) gegeben. Wenn die Atmung hielt, wurde die Brust geöffnet und Blut anärob vom Herz gewonnen. Blutgase, Milchsäure und Traubenzucker wurden gemessen. Zum Vergleich mit chemischer Anoxie von Cyanid, wurden gleiche Messungen an Ratten, die akut N_2 ausgesetzt wurden, durchgeführt.

Die Zeit bis zum Atemstillstand dauerte 2.4 zu 10 Minuten in Cyanid-vergifteten Ratten und 2.3 zu 9.3 Minuten in N_2 ausgesetzten Ratten. Der Partialdruck von O_2, Po_2, im Atemstillstand war viel höher in Cyanid-vergifteten Tieren als in N_2 ausgesetzten Ratten. Der Pco_2 wurde kleiner mit der Zeit zum Atemstillstand in Cyanidversuchen und das wird vermutlich auf Diffusion von CO_2 in Extravasal-

raum beruhen. Die Wasserstoffionkonzentration, $[H^+]$, wurde höher mit der Zeit bis zum Atemstillstand in beiden Gruppen und die Werte waren höher in Cyanid-vergifteten Ratten als in N_2 ausgesetzten Ratten mit gleichen Überlebenszeiten. Das Bikarbonation im Plasma, $[HCO_3^-]_p$, verminderte sich mit der Zeit in beiden Gruppen.

Die Veränderung von Säure-Basen Haushalt in akuter Cyanidvergiftung konnte durch viel höhere Po_2 und größere Hemmung von oxidativem Stoffwechsel charakterisiert werden, in Vergleich mit denen von akuter Anoxie von N_2.

Key words. Cyanide poisoning, Survival time. – Acute anoxic anoxia – Blood acid-base changes

Introduction

Cyanide is one of the most rapidly acting poisons. It combines with cytochrome oxidase and impairs the cell's capacity to utilize O_2, causing cytotoxic anoxia. The rapidity, with which toxic symptoms develop, depends on the doses of the ingested poison. High doses cause deaths within a few minutes from paralysis of the respiratory centers, and in small amounts cases have been reported in which death has been delayed for as long as several hours. The blood acid-base status is considered to change markedly with the lapse of the time to death, however, there has been little quantitative study on this subject.

In the present study, the relation of blood gases and lactate levels of the rat acutely poisoned with NaCN with the length of the survival time, which ranged 2.4 to 14 minutes, were examined in comparison with those in acute anoxia caused by exposure to N_2.

Materials and Methods

Male Wistar-strain rats each weighing about 200 g were used.

1. Acute Cyanide Experiments

NaCN was dissolved in distilled water and solutions containing *2,1.5,1,0.85, and 0.7 mg of cyanide ion per ml were prepared.* One ml per 100 g of body weight was orally given to the rat. *The highest concentration corresponds to the dose of 20 mg of CN/kg.* After administration the rat was put into an 36x36x10 cm transparent plastic box, the inside air of which communicates freely with room air, for observation of the behavior. Immediately after the respiration stopped ultimately, the chest was opened and blood sample was taken anaerobically by cardiac puncture from right heart into a heparinized syringe. The blood sample was stored in an ice bath until analysis.

2. Acute N_2 Exposure

After the rat was placed in a dessicator of about 1400 ml, the vessel was gassed with about 5000ml of pure N_2 from *polyvinylidene-chloride bags* by means of an aspirator. The rate of gassing was varied from experiment to experiment. As soon as the respiration stopped, the rat was taken out. The way, in which the blood sample was collected, was identical with that in the cyanide experiment.

3. Po_2, Pco_2, and pH

The Po_2, Pco_2, and pH were determined by means of a Combianalyzer U (Eschweiler). Prior analysis, the analyzer was checked with standard gases (O_2:CO_2:N_2 = 2.08:2.05:95.87 and 11.8: 5.93:82.27), and standard pH solutions (pH 6.838 and 7.382 at 37°C). The determination was made within 10 minutes after collection of the sample. The pH was converted into hydrogen ion

concentration, $[H^+]$. The plasma bicarbonate concentration, $[HCO_3^-]_p$, was calculated from the Pco_2 and pH according to the Henderson-Hasselbalch equation and in computation the pH of the whole blood was substituted for the plasma pH, [1].

4. Blood Glucose and Lactate

Determinations were made enzymatically, and in the case of glucose determination, a kit (Blood Sugar-GOD-Perid-Test, Boehringer-Mannheim) was used.

Results

1. The Time to the Cessation of Respiration [the Length of Survival Time (ST)]

In cyanide experiments the ST ranged 2.4 to 14 minutes and in acute N_2 exposure 2.3 to 9.3 minutes lapsed before the cessation of respiration. The ST became shorter with increasing NaCN doses (Table 1). When the chest was opened, the beating of the heart was observed in almost all animals.

2. The Pattern of the Behavior

The sequence of the toxic symptom was as follows: Following cyanide administration, the rat staggerd with a definite latent period, the length of which was dependent on the doses. The ataxia was followed shortly after by tonic convulsion, which was simultaneously accompanied by apnea. With the onset of clonic convulsion, weak respiration resumed. It became shallower, resulting in complete cessation. The similar pattern was observed in the rat acutely exposed to N_2.

3. Blood Gases, Lactate, and Glucose

Summarized data from both experiments are tabulated in Tables 2 and 3. In Table 4 is given the calculated values of the correlation coefficient between various parameters.
The length of ST and Po_2: In cyanide experiments the Po_2 at the cessation of respiration was within the ranges of 45 to 70 mmHg in the most animals and there was no correlation between the length of the ST and the Po_2. In acute N_2 experiments the Po_2 had fallen below 5 mmHg regardless of the length of the ST in the most cases.
The length of ST and Pco_2: The Pco_2 of the animal killed with NaCN became lower as the ST became longer (Fig. 1), and two parameters showed significant negative correlation. In N_2 experiments, no correlation was present.
The length of ST and $[H^+]$: In both experiments, the $[H^+]$ became higher with increasing length of the ST. As seen from Fig. 2, two regression lines were approximately parallel, and the line of cyanide lay above.

Table 1. The relation of the doses of NaCN (calculated as CN) with the time to the cessation of respiration

Doses (mg/kg)	No. of animals	Means (min)	Ranges (min)
7	6	11.5	9.3–14.0
8.5	1	10.0	
10	5	8.5	7.8–9.3
15	5	4.6	3.0–8.0
20	3	3.1	2.4–3.9

Table 2. Summarized data of acute cyanide poisoning in the rat. The results are arranged in the order of the lengths of the survival time

No.	Dose (mg/kg)	Survival time (min)	Po_2 (mmHg)	Pco_2 (mmHg)	$[H^+]$ (nM/1)	$[HCO_3^-]$ (mmol/1)	Glucose (mg/100ml)	Lactate (mg/100ml
1	20	2.4	39.4	76.0	71.6	25.4	95	40
2	20	2.9	58.0	67.8	81.3	19.9	123	48
3	15	3.0	58.8	69.0	75.0	22.0	114	47
4	15	3.1	49.5	66.2	84.1	18.8	132	55
5	15	3.8	53.9	84.0	90.2	22.3	122	58
6	20	3.9	53.0	71.5	101.2	16.9	146	64
7	15	5.0	19.8	88.0	106.4	19.8	180	63
8	10	7.8	62.3	65.0	134.9	11.5	295	69
9	15	8.0	65.0	67.0	107.2	14.9	242	67
10	10	8.3	74.8	60.0	93.3	15.4		66
11	10	8.5	61.8	60.3	114.8	12.6	288	38
12	10	8.8	62.0	63.8	123.0	12.4	264	82
13	10	9.3	66.5	69.0	125.0	13.2	325	84
14	7	9.3	46.0	73.9	157.8	11.2	298	74
15	7	9.8	63.0	52.0	102.3	12.1	192	71
16	7	9.8	46.2	74.0	151.4	11.7	238	87
17	8.5	10.0	50.9	70.8	138.0	12.3	323	68
18	7	11,0	57.0	54.0	131.8	9.8	294	62
19	7	13.0	54.7	44.2	107.9	9.8	212	68
20	7	14.0	66.2	44.0	117.5	9.0	165	73

Table 3. Summarized data of N_2 experiments. The results are arranged in the order of the lengths of the survival time

No.	Survival time (min)	Po_2 (mmHg)	Pco_2 (mmHg)	$[H^+]$ (nM/1)	$[HCO_3^-]_p$ (mmol/1)	Glucose (mg/100ml)	Lactate (mg/100ml)
1	2.3	5.8	52.5	48.3	26.2	123	27
2	2.3	0	51.0	55.7	21.9	144	36
3	2.6	7.4	52.5	51.3	24.5	98	30
4	2.8	1.0	60.6	52.5	27.6	105	27
5	2.8	6.0	64.0	57.5	26.6	117	32
6	2.9	5.0	52.2	49.2	25.4	123	27
7	3.4	7.0	67.0	55.0	29.2	186	37
8	3.8	0	76.3	69.2	26.4	134	34
9	4.3	0	43.3	59.6	17.4	149	57
10	4.5	5.0	48.0	48.5	23.7	160	29
11	5.1	0	50.5	53.7	22.5	138	39
12	5.7	2.0	72.0	66.1	26.1	210	50
13	5.7	0	63.5	81.3	18.7	235	50
14	6.6	3.0	70.0	63.8	26.2	224	44
15	6.8	6.0	70.0	79.4	21.1	166	59
16	6.9	0	54.0	79.4	16.3	179	58
17	7.7	6.0	90.0	95.5	22.5	176	55
18	8.3	0.5	48.3	74.1	15.6	193	62
19	8.6	4.5	69.0	91.2	18.1	203	66
20	9.3	3.0	69.5	114.8	14.5	300	65

Table 4. The relations between acid-base parameters. Twenty pairs were used for calculations except for the Glucose-Lactate pair in cyanide experiment

Combinations	Correlation Coefficient Cyanide N_2
Survival time-Pco_2	−0.68
Survival time-$[H^+]$	0.74
Survival time-Lactate	0.61
Lactate-$[H^+]$	0.68
Survival time-$[HCO_3^-]_p$	−0.92
Lactate-$[HCO_3^-]_p$	−0.63
$[H^+]$−$[HCO_3^-]_p$	−0.79
Glucose-Lactate	0.51

	0.39 (n.s.)
	0.87
	0.90
	0.83
	−0.69
	−0.80
	−0.66
	0.71

n.s.: Not significant.

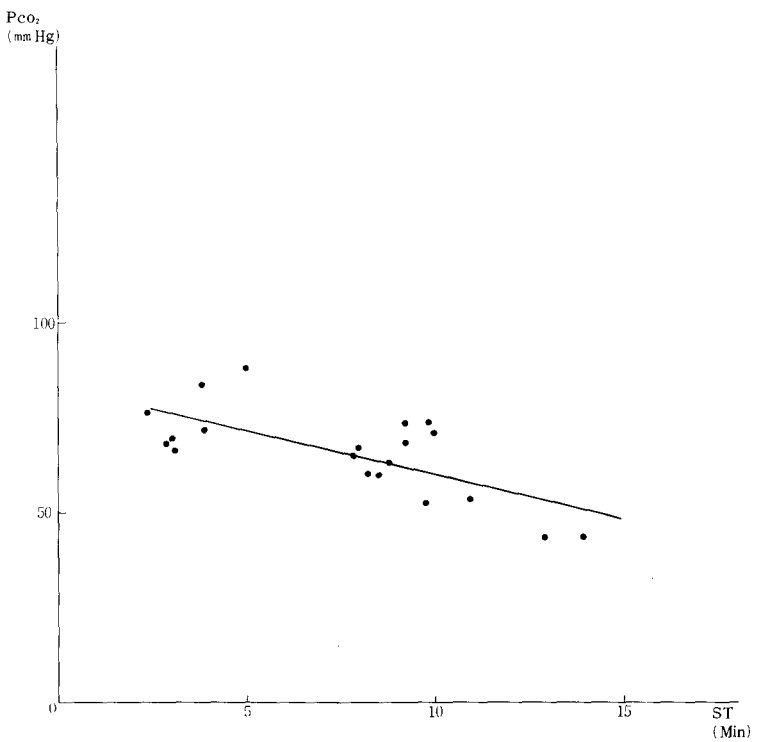

Fig. 1. Pco_2 and the time to the cessation of respiration in cyanide experiments. The Pco_2 of each animal at the cessation of respiration is plotted against the time taken by the animal to reach it. Solid line represents the fitted linear regression of the Pco_2 on the length of the survival time(ST)

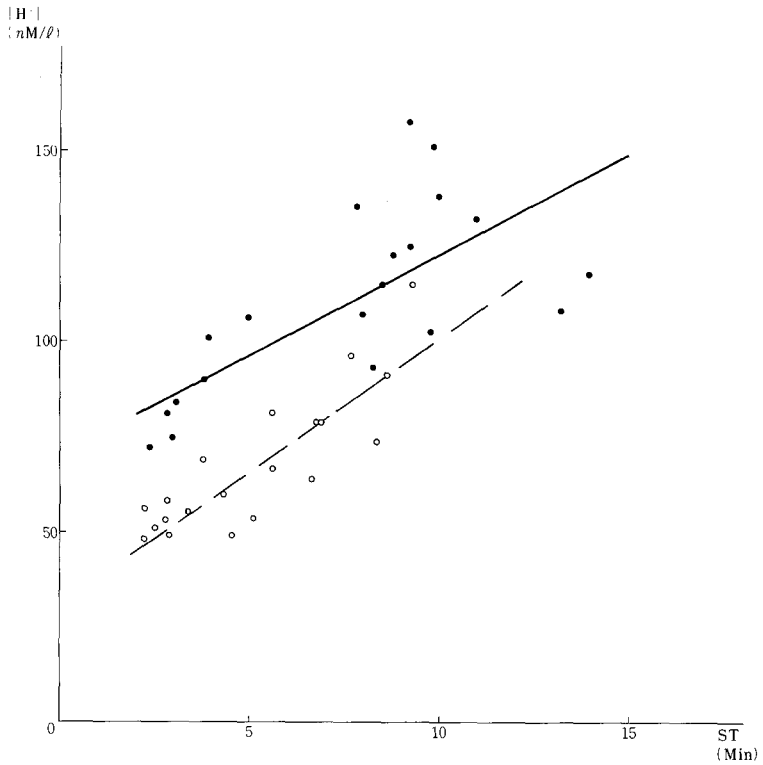

Fig. 2. The scatter diagram of $[H^+]$ and the length of the survival time. Ordinate indicates the $[H^+]$ at the time of the stop of respiration and on abscissal scale the time to the cessation of respiration is given. Closed and open circles indicate cyanide-and N_2 experiments, respectively. Solid and broken lines are the fitted linear regression of the $[H^+]$ on the length of the survival time in cyanide-and N_2 experiments, respectively

The length of ST and blood lactate: There were significant correlations between the two parameters in both groups (Fig. 3). The same was true for the combination of blood lactate and $[H^+]$, and the close similarity between the regression lines was observed (Fig. 4).

The length of ST and $[HCO_3^-]_p$: There were significant negative correlations between the two parameters in both groups. With increasing length of the ST profound metabolic acidosis developed. The two regression lines were parallel (Fig. 5).

Significant negative correlations were shown between the $[HCO_3^-]_p$ and the blood lactate in both groups (Fig. 6).

Blood lactate levels increased with glucose, the correlation having been significant (Table 4).

Discussion

In blood acid-base studies, arterial and/or venous sample reasonably should be used as samples, since there are definite differences between arterial and venous bloods with respect to acid-base parameters. In the present study on the rat without anaestesia the

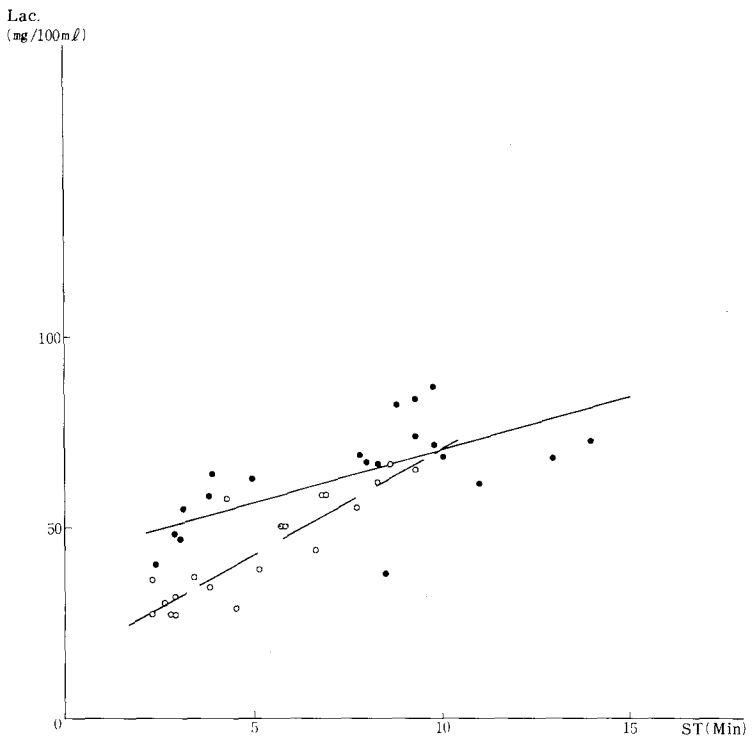

Fig. 3. The scatter diagram of lactate and the length of the survival time. The lactate of each animal at the cessation of respiration is plotted against the time taken by the animal to reach it. Closed and open circles indicate cyanide-and N_2 experiments, respectively. Solid and broken lines are the fitted linear regression of the lactate on the length of the survival time in cyanide-and N_2 experiments, respectively

heart blood was used as a sample. It is probable that the blood sample obtained by cardiac puncture has different proportions of arterial bloods from sample to sample and the use of the heart blood may seem improper. However, in the present study the heart still throbbed in almost all animals in both experiments at the time of sampling. The presence of the cardiac beating in the absence of respiration suggests the presence of the pulmonary circulation without gas exchange. Therefore, it was considered that there is not significant difference between arterial and venous bloods with respect to the blood acid-base parameters, and the use of the heart blood was considered to be justified in view of the above considerations.

It was considered that the deaths are due to the paralysis of the respiratory centers in both acute cyanide poisoning and N_2 exposure, however, the mechanism, by which anoxia is brought about, differs. In acute cyanide poisoning anoxia is caused by the combination of the cytochrome oxidase with the poison, in this case the utilization of O_2 by cells is prevented. The development of the toxic symptoms became rapider with increasing amounts of NaCN, the same is already reported by Schubert et al [2] in terms of the inhibition of liver cytochrome oxidase in the rodents. In N_2

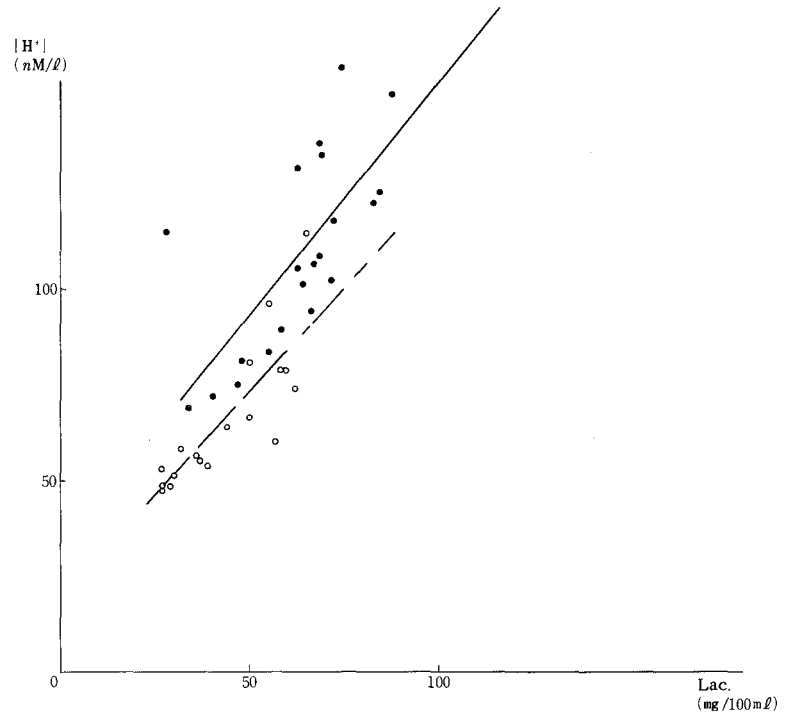

Fig. 4. The scatter diagram of the $[H^+]$ and lactate at the time of the cessation of respiration. Closed and open circles indicate cyanide-and N_2 experiments, respectively. Solid and broken lines are the fitted linear regression of the $[H^+]$ on the lactate in cyanide-and N_2 experiments, respectively

exposure, anoxia is brought about by the stoppage of the supply of O_2. The difference in the mechanism explains the difference in the Po_2 at the cessation of respiration between the both groups.

The aim of the present study was to detect any possible difference in the acid-base parameters between two types of anoxic deaths with similar survival times, in addition to the well-known difference in the Po_2. In both groups there was a significant correlation between the length of the ST and the $[H^+]$, the $[H^+]$ having been definitely higher in NaCN group than in N_2 group. The similar correlation has been observed by Godfrey [3], who acutely asphyxiated the fetal and newborn rabbits and examined the relation between the time to the last spontaneous gasp (L.S.G.) and the $[H^+]$ at the L.S.G. The correlation between these parameters are observed also in sub-acute anoxia, the ST of which ranged 50 to 70 minutes [unpublished data].

The main factor to increase the $[H^+]$ was considered to be accumulation of lactate, the end product of anaerobic glycolysis. There were significant positive correlations between these parameters in both experiments, like those between the ST and the $[H^+]$. The close similarity of the regression lines of the $[H^+]$ on blood lactate in both groups probably shows that the accumulation of the lactate raised the $[H^+]$. The bicarbonate plays an important role in buffering the non-carbonic acids such as lactic acid [4]. In the present study the $[HCO_3^-]_p$ decreased gradually with the lapse

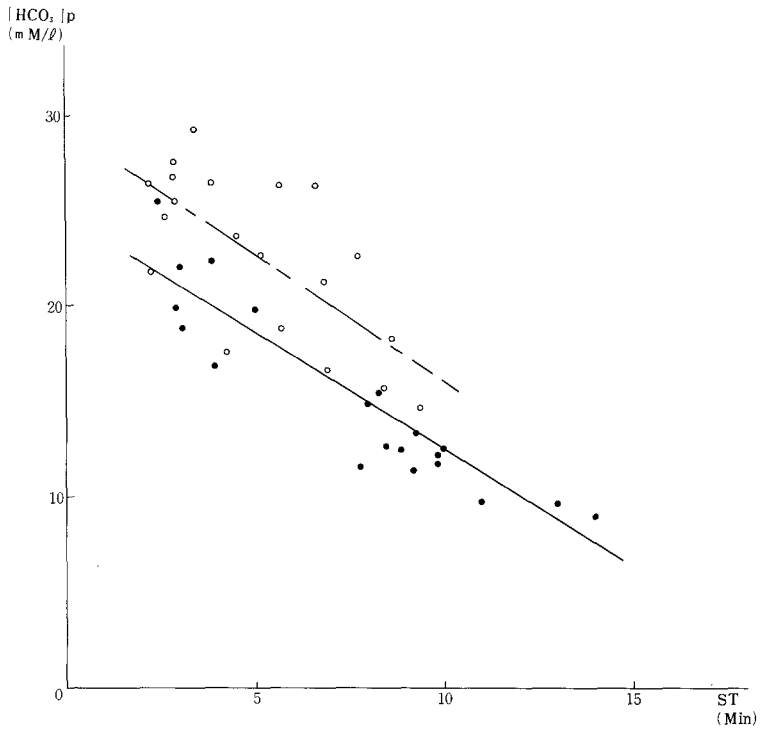

Fig. 5. The scatter diagram of $[HCO_3^-]_p$ and the length of the survival time. The $[HCO_3^-]_p$ of each animal at the cessation of respiration is plotted against the time taken by the animal to reach it. Closed and open circles indicate cyanide-and N_2 experiments, respectively. Solid and broken lines are the fitted linear regression of the $[HCO_3^-]_p$ on the length of the survival time in cyanide- and N_2 experiments, respectively

of the ST, and marked metabolic acidosis developed in the rat with longer ST. There was significant negative correlation between two parameters in both experiments (Fig. 5). Similar negative correlation existed between the lactate and the $[HCO_3^-]_p$. The normal value of $[HCO_3^-]_p$ of the rat, according to the published data [3,5,6], ranges 23 to 30 mmol/1. The values in the rat killed very acutely by NaCN was lower than the normal value and it was considered to show the very soon onset of the metabolic acidosis. On the other hand, in N_2 exposure the value in the rat with the similar ST was within the normal ranges.

The retention of CO_2 is considered the second possible factor to contribute to the increase in $[H^+]$. The balance of the production and the elimination of CO_2 is ingeniously maintained in the normal conditions. According to Godfrey [3], the Pco_2 at the L.S.G. increased as the time to the L.S.G. became longer. In the present study, there is not any significant correlation between the length of the ST and the Pco_2 in acute N_2 exposure, however, on the other hand, the significant negative correlation was observed in the rat acutely poisoned with cyanide, the Pco_2 at the stop of respiration became lower as the animal survived longer. At the apneic phase which developed in relatively early stage in high doses of cyanide, the retention of CO_2 probably occurred, because it is considered that in this stage the production of CO_2 is not completely diminished. The above consideration probably explains the slight degrees of

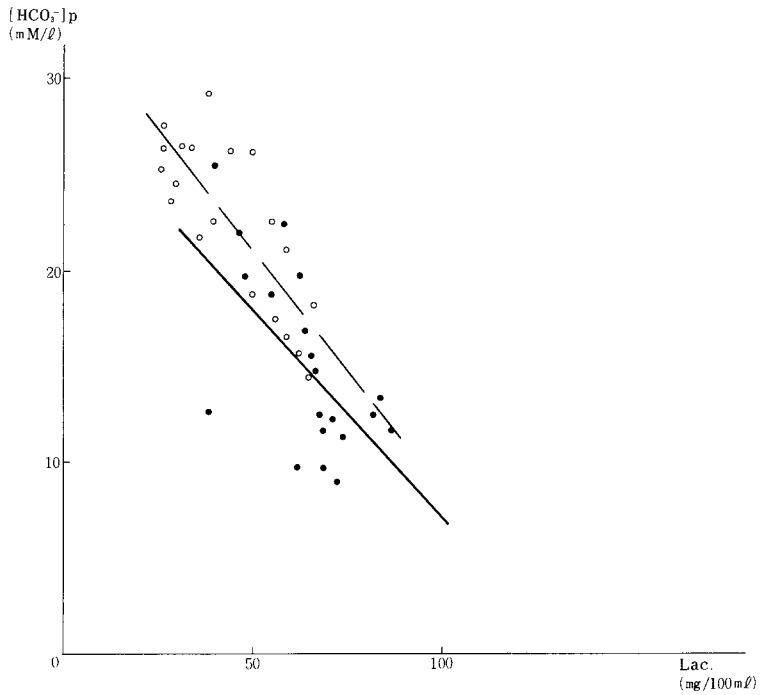

Fig. 6. The scatter diagram of the $[HCO_3^-]_p$ and lactate at the time of the cessation of respiration. Closed and open circles indicate cyanide-and N_2 experiments, respectively. Solid and broken lines are the fitted linear regression of the $[HCO_3^-]_p$ on the lactate in cyanide-and N_2 experiments, respectively

hypercapnia in the rat with shorter ST. With the course of the intoxication, within which CO_2 production falls and the elimination of it is inhibited, CO_2 probably diffuses into the extravascular space according to the pressure gradient, resulting in the decrease in the blood Pco_2. The lack of the significant correlation of the Pco_2 with the length of the ST in acute exposure to N_2 may be ascribed to too short a survival time in this group to allow the significant diffusion to take place.

In view of the above findings, the pattern of the acid-base changes in acute cyanide poisoning can be characterized by much higher Po_2 and greater inhibition of anaerobic metabolism in comparison with those of the acute anoxia by N_2.

Note added in Proof

In the present study it was considered that the eventual contamination of right heart blood to be sampled with left heart blood does not greatly affect the results. However, our recent cyanide intoxication studies on the rabbit under urethane anesthesia, in which right and left heart blood were collected separately, showed the significant difference in the Po_2 between the blood in two ventricles. Although the experimental conditions of the rabbit studies greatly differ from those of the rat studies, it can not be completely ruled out the possibility that there was actually a difference between the left and right heart blood in the blood gases of the rats.

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