# Cyanide and Thiocyanate Levels in Blood and Saliva of Healthy Adult Volunteers

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Cyanide and thiocyanate levels were determined by head space gas chromatography and the spectrophotometric König method respectively, for blood and salivary samples collected from 40 healthy adult volunteers. Blood cyanide (mean ± standard deviation: 0.27 ± 0.07 $\mu$M), plasma thiocyanate (111.2 ± 92.1 $\mu$M), salivary cyanide (0.66 ± 0.52 $\mu$M) and salivary thiocyanate (1655 ± 841 $\mu$M) levels were significantly higher in the group of 20 tobacco smokers than in the group of 20 nonsmokers (0.17 ± 0.04 $\mu$M, 33.5 ± 25.4 $\mu$M, 0.38 ± 0.26 $\mu$M, 542 ± 406 $\mu$M). Statistical correlations were observed between the blood cyanide and the salivary thiocyanate levels ($\gamma$ = 0.64), between the blood cyanide and the plasma thiocyanate levels ($\gamma$ = 0.54), and between the plasma thiocyanate and the salivary thiocyanate levels ($\gamma$ = 0.46). It is concluded that not only plasma and salivary thiocyanate levels but also blood cyanide level can be suitable indices for distinguishing smokers from nonsmokers.

**Key words** —— cyanide, thiocyanate, tobacco smoking, blood, saliva

## INTRODUCTION

Cyanide is a potent toxic agent which inhibits the activity of cytochrome oxidase.[1] Cyanide is incorporated into human bodies, through inhalation of hydrogen cyanide (HCN) gas produced in the process of pyrolysis or combustion of nitrogen-containing compounds,[2] through ingestion of cyanide salts for purposes of homicide or suicide,[3] of cyanogenic glycosides[4] as meals mainly in tropical countries, or of sodium nitroprusside as prescriptions.[5] Cyanide is metabolized to thiocyanate through sulfuration with thiosulfate by mitochondrial rhodanese.[6] In the physiological condition, blood cyanide is mainly distributed in erythrocytes,[7] tightly binding to met-hemoglobin. Blood thiocyanate is mainly distributed in serum and its presence is regarded as evidence of cyanide detoxification.[8] High levels of thiocyanate are also found in saliva, and the physiological role of salivary thiocyanate may be the antibacterial effect of hypothiocyanate which is produced by the action of salivary peroxidases from thiocyanate.[9]

The determination of fatal levels of blood cyanide (over 5 $\mu$M) is important in the diagnosis of acute cyanide intoxication.[10] In contrast, toxic levels of blood cyanide and serum thiocyanate are both important indicators in the diagnosis of chronic cyanide intoxication involving the exposure to HCN gas in industry,[11] involving the habitual digestion of cyanogenic glycosides[4] and in the diagnosis related to the tobacco amblyopias.[12] The determination of physiological levels of plasma thiocyanate[13] and salivary thiocyanate[14] have been used for distinguishing smokers from nonsmokers. An earlier report[15] indicated that plasma thiocyanate levels provide a more suitable index of the cyanide exposure than blood cyanide levels.

In the literature, the exact levels of normal blood cyanide in humans has not been settled yet. There is considerable variation in the reported values for the physiological blood cyanide levels in the literature.[11,15–20] This can be due to the methodology, which necessitates an acidification procedure to extract volatile HCN from blood, resulting in the artifactual production of a considerable amount of cyanide from thiocyanate.[21] This positive interference by thiocyanate, the blood concentration of which is much higher than cyanide,[15] would greatly affect the accuracy of the determination of physiological blood cyanide levels.

Head-space gas chromatography (HS-GC) is effective in the analysis of biological samples for volatile substances,[22] and has been used in our laboratory for the detection of blood cyanide.[21] A previous paper[23] indicated that the denaturation of oxyhemoglobin produced cyanide from thiocyanate, and

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that active oxygen scavenging reagents suppressed the artifactual cyanide production. For the blood samples of healthy adults, the cyanide levels measured in the absence of such a scavenger were mostly derived from the thiocyanate present in blood matrix.[24] The HS-GC method combined with the usage of ascorbic acid enabled the determination of physiological levels (sub $\mu$M) of blood cyanide without interference by thiocyanate.[24]

There has been no report in the literature on determining human cyanide and thiocyanate levels in both blood and saliva at the same time. Employing the above mentioned HS-GC cyanide assay and the modified spectrophotometric thiocyanate assay, we have measured cyanide and thiocyanate levels in blood and salivary samples collected from adult healthy tobacco smokers and nonsmokers, and statistically compared the determined levels in order to ascertain whether or not these levels are suitable for predicting tobacco smoking habit.

## MATERIALS AND METHODS

**Reagents** —— Cyanoline Blue (mixture of 1-phenyl-3-methyl-5-pyrazolone and 4,4'-bis(1-phenyl-3-methyl-5-pyrazolone)) was from Dojindo Laboratories (Kumamoto, Japan). All other chemicals used were of analytical grade. All aqueous solutions were prepared with deionized and distilled water.

**Sample Collection** —— Sample donors were employees of our institute, and at the sampling time they were all in good health. All the subjects were asked to fill in a questionnaire on their smoking habits. Ages ranged from 22 to 57 years. The subjects were classified into five groups as follows: (1) nonsmoker group ($n$ = 20), (2) smoker group I ($n$ = 1, smoking 1–5 cigarettes per day), (3) smoker group II ($n$ = 2, smoking 6–10 cigarettes per day), (4) smoker group III ($n$ = 3, smoking 11–20 cigarettes per day) and (5) smoker group IV ($n$ = 14, smoking more than 20 cigarettes per day). None of the nonsmoker group had been exposed to second-hand smoke in their working space. Blood samples were collected venipuncturally from the subjects into heparinized tubes at about A.M. 11:00, and at the same time, saliva were collected from the same subjects into plastic tubes. After the collection, samples were immediately sealed, stored at 4°C, and cyanide and thiocyanate were determined within 2 d.

**Cyanide Determination by Head-Space Gas Chromatography** —— Cyanide concentrations were determined by the HS-GC method, as described previously.[24] The 5890A gas chromatograph used (Yokogawa Analytical Systems, Tokyo, Japan) was equipped with a nitrogen phosphorus detector and a GS-Q column (30 m × 0.53 mm i.d., J&W Scientific, Folson, CA). The carrier-gas (helium) flow-rate was 4.7 ml/min. The injection port, detector and column oven were maintained at 200°C, 250°C and 140°C, respectively. The splitter ratio was adjusted to 3.5. HS equilibrium was performed in a screw cap septum vial [7 ml, Pierce (Rockford, IL)], to which were added 0.5 ml of blood or salivary sample, 0.03 ml of 1 M ascorbic acid and 0.27 ml of water. The vial was then sealed with a Tuf-Bond Disc (Pierce). Two hundred $\mu$l of 50% phosphoric acid was introduced through the disc using a glass syringe. The mixture was allowed to stand at 50°C for 30 min, and 0.5 ml of the gas phase in the sealed vial was injected into the GC using a glass syringe (Top, Tokyo, Japan) fitted with 0.50 × 25 mm needles (Terumo, Tokyo, Japan). A stock cyanide solution was prepared by dissolving potassium cyanide in 0.1 M sodium hydroxide (NaOH) solution, which had been standardized by titration with silver nitrate, and used as calibration standard after appropriate dilution with water.

**Spectrophotometric Thiocyanate Assay** —— Thiocyanate concentrations were determined by the König reaction[25] according to the previously described method[24] with slight modification. Three hundred $\mu$l of the plasma fraction centrifugally separated from the blood sample was mixed with 0.2 ml of 25% (w/v) trichloroacetic acid, and the trace amounts of plasma cyanide were completely eliminated by evaporation. Fifty $\mu$l of the resulting supernatant was then mixed with 15 $\mu$l of 1 M potassium hydrogen phosphate solution, 5 $\mu$l of 5 M NaOH solution and 10 $\mu$l of 6.25 mg/ml sodium $p$-toluenesulfonchloramide (chloramine T) solution in a ice bath. After 2 min of incubation, 0.12 ml of 0.27% (w/v) Cyanoline Blue solution in pyridine-water (1 : 5, v/v) was added to the mixture, and the whole was allowed to stand for 40 min at 25°C. The mixtures were poured into a 96-well polystyrene microtiter plate (Falcon 3077, Becton and Dickinson Co., Lincoln Park, NJ), and the absorbance was measured at 620 nm using a microplate reader (model Spectramax 250, Molecular Device Co., Menlo Park, CA). The concentration of the plasma thiocyanate could be measured up to 100 $\mu$M, and the samples giving thiocyanate levels higher than 100 $\mu$M were assayed again after appropriate dilu-

tion with water.

Forty $\mu$l of salivary sample diluted 25-fold with water was mixed with 20 $\mu$l of 1 M potassium phosphate buffer solution (pH 5.5) and 20 $\mu$l of 6.25 mg/ml chloramine T solution in ice. After 2 min of incubation, 0.14 ml of 0.27% (w/v) Cyanoline Blue solution in pyridine-water (1 : 5, v/v) was added to the mixture, and the whole was allowed to stand for 40 min at 25°C, and the absorbance at 620 nm was measured. The concentration of the thiocyanate could be measured up to 300 $\mu$M, and the samples giving thiocyanate levels higher than 300 $\mu$M were assayed again after appropriate dilution with water.

**Statistical Analysis** —— All the results were expressed as mean ± standard deviation. Statistical differences between the levels in the two groups were determined according to Student's *t* test. Correlation between the two levels was assessed by the Spearman test.

## RESULTS AND DISCUSSION

### Establishment of the Methods for the Determination of Cyanide and Thiocyanate

The HS-GC method using ascorbic acid enables us to determine the physiological blood cyanide levels down to 0.05 $\mu$M without interference by thiocyanate.[25] Under the same analytical conditions, the salivary cyanide could be quantified with the same detection limit (0.05 $\mu$M). This assay was not interfered with the thiocyanate because the addition of high levels of thiocyanate into saliva samples (final 5.0 mM) did not change the cyanide values. The recovery of cyanide was almost 100% because the slope of the straight calibration curve (cyanide concentration ranging from 0 to 5.0 $\mu$M) in saliva matrix was parallel to that in water. The intra-assay relative standard deviation was 6.0% ($n$ = 6) at 3.8 $\mu$M in the salivary samples.

The spectrophotometric method using König reaction converting thiocyanate to cyanogen chloride followed by color reaction with pyridine-pyrazorone reagent was adopted for the assay of the plasma thiocyanate after the deproteinization,[25] and the salivary thiocyanate without the deproteinization. For the optimization of the color reaction, Chloramine T concentration in the chlorination reaction mixture was raised 10-fold from 78 $\mu$g/ml[25] to 781 $\mu$g/ml to achieve quantitative chlorination reaction. For the salivary sample, the pH of the chlorination reaction was set at 5.5 instead of 7.0 to increase the color development. The recovery of thiocyanate was almost 100% because the slope of the straight calibration curve (in the thiocyanate concentration ranging from 0 to 300 $\mu$M) in saliva matrix was parallel to that in water. The intra-assay relative standard deviation was 6.7% ($n$ = 8) at 50 $\mu$M in the plasma samples and 4.7% ($n$ = 9) at 1000 $\mu$M in salivary samples. Because the salivary cyanide levels were very low compared to the thiocyanate levels, the positive interference by cyanide gave negligible effect on the salivary thiocyanate determination.

### Consideration of Toxico-Kinetics of Cyanide and Thiocyanate

We are assuming the following toxico-kinetics for cyanide and thiocyanate. The main route of cyanide intake is inhalation from the surrounding environment. The intake level is elevated, depending on the exposure situation such as tobacco smoke and automobile exhaust gas. Another route is the absorption from alimentary tracts. Blood cyanide level is in dynamic equilibrium, balanced by intake and elimination. The latter is composed of the evaporation and the metabolic conversion to thiocyanate as major routes and cystine conjugation to 2-aminothiazoline-4-carboxylic acid as minor route.[26] Serum thiocyanate is partly derived from alimentary absorption. Thiocyanate is included in many kinds of vegetables such as cabbages,[27] and eating such a vegetable is reported to increase the plasma thiocyanate level.[28] Serum thiocyanate level is in dynamic equilibrium, balanced by the production from cyanide metabolism, alimentary absorption and urinary excretion. Salivary thiocyanate may be derived from the physiological production, and also passive transfer responding to increased blood cyanide levels should be considered. It is reported that salivary thiocyanate levels are not affected by alimentary sources such as cyanogenic food.[29]

The ordinary levels of blood cyanide, serum thiocyanate, salivary cyanide and thiocyanate have, therefore, individual differences, which are influenced by their genetic traits, physiological conditions, diets, tobacco smoking habits and environmental factors. In this paper, interpersonal differences in the cyanide and thiocyanate levels were not investigated. Schulty *et al*. reported that the salivary thiocyanate levels showed much higher interpersonal differences (mean ± standard deviation, 830 ± 410 $\mu$M, $n$ = 25) than intrapersonal differences (760 ± 100 $\mu$M, $n$ = 15).[30] In addition, no definitive information could be obtained concerning the ali-

**Table 1.** Cyanide and Thiocyanate Levels ($\mu$M) in Blood and Salivary Samples Taken from Healthy Volunteers

| Subject number | Age | Gender[a)] | Smoking frequency[b)] | Cyanide in blood | Thiocyanate in plasma | Cyanide in saliva | Thiocyanate in saliva |
|---|---|---|---|---|---|---|---|
| 1 | 26 | M | S0 | 0.11 | 8.0 | 0.19 | 1630$^{\#\#}$ |
| 2 | 26 | M | S0 | 0.12 | 15.3 | 0.44 | 653 |
| 3 | 27 | F | S0 | 0.11 | 10.7 | 0.39 | 824 |
| 4 | 27 | M | S0 | 0.13 | 10.2 | 0.36 | 648 |
| 5 | 27 | M | S0 | 0.20 | 10.4 | 0.34 | 515 |
| 6 | 27 | F | S0 | 0.23 | 44.1 | 0.29 | 472 |
| 7 | 28 | M | S0 | 0.19 | 15.7 | 0.18 | 81 |
| 8 | 28 | M | S0 | 0.13 | 41.6 | 0.05 | 345 |
| 9 | 30 | M | S0 | 0.13 | 6.3 | 0.41 | 329 |
| 10 | 31 | M | S0 | 0.19 | 64.3 | 0.25 | 580 |
| 11 | 35 | M | S0 | 0.20 | 6.8 | 0.20 | 63 |
| 12 | 35 | M | S0 | 0.13 | 41.6 | 0.23 | 340 |
| 13 | 36 | M | S0 | 0.25$^{\#,c)}$ | 94.4$^{\#\#}$ | 0.39 | 1266$^{\#}$ |
| 14 | 42 | M | S0 | 0.17 | 67.6 | 0.73 | 423 |
| 15 | 43 | M | S0 | 0.18 | 59.6 | 1.20$^{\#\#}$ | 259 |
| 16 | 49 | M | S0 | 0.21 | 45.7 | 0.51 | 87 |
| 17 | 50 | M | S0 | 0.18 | 58.8 | 0.32 | 628 |
| 18 | 55 | M | S0 | 0.17 | 22.7 | 0.20 | 733 |
| 19 | 56 | M | S0 | 0.22 | 17.8 | 0.74 | 13 |
| 20 | 57 | M | S0 | 0.17 | 29.0 | 0.25 | 845 |
| 21 | 33 | F | S1 | 0.18 | 5.7 | 0.22 | 899 |
| 22 | 47 | M | S2 | 0.14 | 1.7 | 0.66 | 277 |
| 23 | 58 | M | S2 | 0.26$^{\#}$ | 107.7$^{\#\#}$ | 0.57 | 587 |
| 24 | 39 | M | S3 | 0.26$^{\#}$ | 13.1 | 0.13 | 2373$^{\#\#}$ |
| 25 | 41 | M | S3 | 0.29$^{\#\#,c)}$ | 119.8$^{\#\#}$ | 0.28 | 2104$^{\#\#}$ |
| 26 | 44 | M | S3 | 0.24$^{\#}$ | 95.0$^{\#\#}$ | 1.12$^{\#\#}$ | 1097 |
| 27 | 22 | M | S4 | 0.30$^{\#\#}$ | 134.0$^{\#\#}$ | 0.15 | 2123$^{\#\#}$ |
| 28 | 23 | M | S4 | 0.22 | 163.3$^{\#\#}$ | 0.23 | 801 |
| 29 | 26 | M | S4 | 0.19 | 34.2 | 0.32 | 662 |
| 30 | 33 | M | S4 | 0.31$^{\#\#}$ | 49.7 | 2.07$^{\#\#}$ | 1890$^{\#\#}$ |
| 31 | 34 | M | S4 | 0.30$^{\#\#}$ | 285.9$^{\#\#}$ | 1.62$^{\#\#}$ | 2566$^{\#\#}$ |
| 32 | 38 | M | S4 | 0.41$^{\#\#}$ | 23.5 | 0.39 | 2940$^{\#\#}$ |
| 33 | 41 | M | S4 | 0.36$^{\#\#}$ | 57.3 | 0.38 | 2070$^{\#\#}$ |
| 34 | 46 | M | S4 | 0.34$^{\#\#}$ | 260.6$^{\#\#}$ | 0.87$^{\#}$ | 624 |
| 35 | 49 | M | S4 | 0.37$^{\#\#}$ | 151.7$^{\#\#}$ | 0.52 | 1791$^{\#\#}$ |
| 36 | 50 | M | S4 | 0.23 | 80.4$^{\#}$ | 1.11$^{\#\#}$ | 2379$^{\#\#}$ |
| 37 | 50 | M | S4 | 0.27$^{\#\#}$ | 156.9$^{\#\#}$ | 1.04$^{\#\#}$ | 2620$^{\#\#}$ |
| 38 | 51 | M | S4 | 0.26$^{\#}$ | 290.0$^{\#\#}$ | 0.26 | 2799$^{\#\#}$ |
| 39 | 55 | M | S4 | 0.29$^{\#\#}$ | 182.9$^{\#\#}$ | 0.75 | 1273$^{\#}$ |
| 40 | 56 | M | S4 | 0.22 | 11.3 | 0.42 | 1224$^{\#}$ |

*a)* M: male, F: female. *b)* Number of cigarettes per day, S0: 0, S1: 1–5, S2: 6–10: S3: 11–20, S4: more than 21. *c)* Significantly higher than the average value of nonsmoker group ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$).

mentary intake and the environmental HCN exposure for the sample donors, and the only available information was their habits of tobacco smoking. As the salivary thiocyanate concentration is reported to follow a circadian pattern with increased levels occurring overnight,[30] sampling of saliva was conducted at late morning. Even though under such limited circumstances where we can not simply attribute the sources of cyanide and thiocyanate to the inhalation, it is worth comparing our data with the literature.

**Table 2.** Average Cyanide and Thiocyanate Levels ($\mu$M) in Blood and Salivary Samples Taken from Healthy Volunteers[a)]

| | Total | Nonsmoker | Smoker | 1–5[a)] | 6–10 | 11–20[a)] | 21 >[a)] |
|---|---|---|---|---|---|---|---|
| Number of subjects | 40 | 20 | 20 | 1 | 2 | 3 | 14 |
| Blood cyanide | 0.22 ± 0.08 | 0.17 ± 0.04 | 0.27 ± 0.07[b)] | 0.18 | 0.20 ± 0.06 | 0.26 ± 0.02[b)] | 0.29 ± 0.06[b)] |
| Plasma thiocyanate | 72.4 ± 77.4 | 33.5 ± 25.4 | 111.2 ± 92.1[b)] | 5.7 | 54.7 ± 53.0 | 76.0 ± 55.8 | 134.4 ± 96.3[b)] |
| Salivaly cyanide | 0.52 ± 0.42 | 0.38 ± 0.26 | 0.66 ± 0.52[c)] | 0.22 | 0.62 ± 0.05[b)] | 0.51 ± 0.54 | 0.72 ± 0.57[c)] |
| Salivary thiocyanate | 1098 ± 862 | 542 ± 406 | 1655 ± 841[b)] | 899 | 432 ± 155 | 1858 ± 672[b,d)] | 1840 ± 801[b,d)] |

*a)* Number of cigarettes smoked per day. *b)* Significantly higher ($p < 0.01$) compared to the nonsmoker group. *c)* Significantly higher ($p < 0.05$) compared to the nonsmoker group. *d)* Significantly higher ($p < 0.01$) compared to the smoker group who have smoked 6–10 cigarettes per day.

## Cyanide and Thiocyanate Levels in Blood Samples

Table 1 shows the ages of the subjects and the cyanide and thiocyanate levels in blood and salivary samples. No correlation was observed ($\gamma < 0.36$) between the age and any of the four determined levels in total subjects ($n = 40$), in the nonsmoker group ($n = 20$) or in smoker group ($n = 20$). The determined levels ranged from 0.11 to 0.41 $\mu$M for blood cyanide, and from 1.7 to 290 $\mu$M for plasma thiocyanate. As shown in Table 2, blood cyanide ($p < 0.01$) and plasma thiocyanate ($p < 0.01$) levels were significantly higher in the smoker group than in the nonsmoker group. Within the smoker group, only the heavy smoker groups (IV, having smoked more than 21 cigarettes per day) gave significantly higher levels in both determinants compared to the nonsmoker group. The moderate smoker group (III, having smoked 11–20 cigarettes per day) also gave significantly higher blood cyanide levels. As the literature shows a tendency for persons smoking more cigarettes to give higher serum thiocyanate[31)] levels, our results follow the same trend.

The reported blood cyanide levels of healthy donors show considerable variability among the literature. The main cause may be due to the methodological differences rather than the individual variation. An important problem is the artifactual formation of cyanide from thiocyanate under denaturing conditions in the presence of erythrocytes.[23)] Except for two reports,[15,20)] the literature[11,16–19)] indicate higher blood cyanide levels in smokers than nonsmokers. Our data for the nonsmokers ($0.17 \pm 0.04$ $\mu$M) and the smokers ($0.27 \pm 0.07$ $\mu$M) is quite consistent with Lundquist *et al.* ($0.13 \pm 0.08$ *vs.* $0.33 \pm 0.12$ $\mu$M[18)]) and similar to two other papers measuring the levels in red blood cells ($0.14 \pm 0.01$ *vs.* $0.35 \pm 0.08$ $\mu$M[17)] and $0.47 \pm 0.07$ *vs.* $0.71 \pm 0.11$ $\mu$M[19)]). Their cyanide assay methods were performed so as to suppress the artificial cyanide formation from thiocyanate. The other papers gave much higher cyanide levels (averages ranging from 0.5 to 2.9 $\mu$M in nonsmokers, from 0.6 to 6.8 $\mu$M in smokers),[11,15,16,20)] and this may be due to the positive interference by thiocyanate. The values ranging from 0.1 to 0.2 $\mu$M for nonsmoker and from 0.2 to 0.4 $\mu$M for smokers may be suitable values for normal blood cyanide levels.

All the literature indicate higher plasma thiocyanate levels in smokers than nonsmokers. Our data for the plasma thiocyanate levels in the nonsmokers ($33.5 \pm 25.4$ $\mu$M) and the smokers ($111.2 \pm 92.1$ $\mu$M) is consistent with the four papers ($46 \pm 17$ *vs.* $86 \pm 33$ $\mu$M[15)]; male $42 \pm 15$, female $46 \pm 18$ *vs.* $144 \pm 48$ $\mu$M[13)]; $33 \pm 26$ *vs.* $158 \pm 51$ $\mu$M[32)]; male $34 \pm 14$, female $34 \pm 14$ *vs.* male $60 \pm 21$, female $71 \pm 25$ for light smokers, male $87 \pm 28$, female $100 \pm 28$ $\mu$M for heavy smokers[31)]) and similar to the five papers ($36 \pm 9$ *vs.* $65 \pm 17$ $\mu$M[12)]; $31 \pm 29$ *vs.* $60 \pm 26$ $\mu$M[16)]; $57 \pm 22$ *vs.* $157 \pm 34$ $\mu$M[33)]; $20 \pm 7$ *vs.* $57 \pm 31$ $\mu$M[17)]; $53 \pm 27$ *vs.* $162 \pm 60$ $\mu$M[34)]). The other papers present rather low plasma thiocyanate levels (averages ranging from 5 to 12 $\mu$M for nonsmokers, from 13 to 74 $\mu$M for smokers).[11,20,35–38)] Considering the alimentary effect on serum thiocyanate levels,[9)] it is no wonder that remarkable variation is observed in serum thiocyanate levels among the literature.

## Cyanide and Thiocyanate Levels in Salivary Samples

As shown in Table 1, the determined levels ranged from 0.05 to 2.07 $\mu$M for salivary cyanide and from 13 to 2940 $\mu$M for salivary thiocyanate. As shown in Table 2, salivary cyanide ($p < 0.05$) and salivary thiocyanate ($p < 0.01$) levels were significantly higher in the smoker group than in the nonsmoker group. Within the smoker group, only the heavy smoker groups (IV, having smoked more than 21 cigarettes per day) gave significantly higher levels in both determinants compared to the nonsmoker group. The moderate smoker group (III, having smoked 11–20 cigarettes per day) and the light smoker group (II, 6–10 cigarettes per day) also gave significantly higher thiocyanate and cyanide levels,

**Table 3.** Correlation Coefficients of the Two Levels

| | Plasma thiocyanate | | Salivary cyanide | | Salivary thiocyanate | |
|---|---|---|---|---|---|---|
| Blood cyanide | Total | 0.54 | Total | 0.32 | Total | 0.64 |
| | Nonsmoker | 0.48 | Nonsmoker | 0.19 | Nonsmoker | 0.27 |
| | Smoker | 0.30 | Smoker | 0.13 | Smoker | 0.55 |
| Plasma thiocyanate | — | | Total | 0.34 | Total | 0.46 |
| | | | Nonsmoker | 0.28 | Nonsmoker | 0.09 |
| | | | Smoker | 0.21 | Smoker | 0.22 |
| Salivary cyanide | — | | — | | Total | 0.24 |
| | | | | | Nonsmoker | 0.26 |
| | | | | | Smoker | 0.11 |

respectively. As the literature show the tendency for persons smoking more cigarettes to give higher salivary thiocyanate[14] levels, our results follow the same trend.

The reported salivary thiocyanate levels also show considerable variability among the literature. Considering the circadian pattern in salivary thiocyanate level[30] and the considerable variation in the saliva collection procedures,[39] it is inadequate to compare the literature data unless saliva collection procedures are standardized. Nevertheless, the literature indicate higher levels in smokers than nonsmokers. Our data for the nonsmokers ($542 \pm 406$ $\mu$M) and the smokers ($1655 \pm 841$ $\mu$M) is quite consistent with two papers ($652 \pm 17$ *vs.* $1855 \pm 124$ $\mu$M[14]; $655 \pm 200$ *vs.* $1867 \pm 374$ $\mu$M[39]) and similar to other two papers (350 *vs.* 1300 $\mu$M[35]; 400 *vs.* $1095 \pm 205$ $\mu$M[37]). The other papers gave rather high levels (averages ranging from 1040 to 1700 $\mu$M for nonsmokers, from 3339 to 7100 $\mu$M for smokers).[28,33,40] The reasons for this discrepancy is not clear, but it may be attributed to the racial differences in salivary thiocyanate levels.

Because salivary cyanide exists in the protonated form ($pK_a = 9.3$, boiling point: 25.7°C), the possible volatile loss of cyanide should be considered. In addition, the high concentration of cyanide included in tobacco smoke may remain in mouth cavities of smokers, and the contamination into excreted saliva may artifactually increase the cyanide level. Therefore, saliva does not seem to be ideal nor a suitable sample. Nevertheless, salivary cyanide levels in the smokers were significantly higher than those in the nonsmokers (Table 2). Under more specified sampling conditions, salivary cyanide may be used as a biochemical parameter for predicting cyanide exposure.

## Correlation of Cyanide and Thiocyanate Levels in Blood and Saliva

As shown in Table 3, a positive correlation between the blood cyanide and the salivary thiocyanate levels was observed ($\gamma = 0.64$). Although there was no correlation in the nonsmokers, a correlation coefficient in the smokers was observed ($\gamma = 0.55$), and also the difference of both the blood cyanide and salivary thiocyanate levels between the nonsmoker group and the smoker group was significant (Table 2). The same result is drawn in the cases of the correlation between the blood cyanide and the plasma thiocyanate levels ($\gamma = 0.54$).

The correlation coefficient between the plasma thiocyanate levels and the corresponding salivary thiocyanate levels was low ($\gamma = 0.46$). This is possibly because no correlation ($\gamma < 0.22$) was observed between these two levels in the nonsmokers nor the smokers. Correlations between the salivary cyanide and the other levels also were not observed ($\gamma < 0.34$).

Although blood cyanide seems to reflect cyanide intake most directly, blood cyanide is metabolically unstable, and the difference between the levels of nonsmokers and those of smokers was not so large (ratio: about 1.6). Serum thiocyanate is metabolically stable, and gives rather high levels (about 20 $\mu$M), although its level is also strongly influenced by alimentary sources.[28] Salivary thiocyanate also gave extremely high level (1 mM), although the physiological relationship between absorbed cyanide and salivary thiocyanate is still not clear. It is reasonable that blood cyanide level was correlated most significantly with salivary thiocyanate level, which is not directly influenced by eating thiocyanate-rich food.

## Discrimination of Nonsmokers and Smokers

Table 1 shows the subjects whose determined levels were significantly higher than the average

values of the nonsmoker group. Judging by the blood cyanide levels, 14 smokers and one nonsmoker are recognized as showing high levels. Judging by the plasma thiocyanate levels, 12 smokers and one nonsmoker are recognized as showing high levels. Judging by the salivary cyanide levels, 6 smokers and one nonsmoker are recognized as showing high levels. Judging by the salivary thiocyanate levels, 13 smokers and two nonsmokers are recognized as showing high levels. One subject in the nonsmoker group (No. 13) gave significantly high blood cyanide, plasma thiocyanate and salivary thiocyanate levels. Three subjects (No. 21 and No. 22 in the light smoker group and No. 29 in the heavy smoker group) showed values not exceeding average values of the nonsmoker group. If the criterion to predict smoking habit by the determination of cyanide and thiocyanate levels in blood and saliva is set to fulfill at least two determinants showing significantly higher than those in the nonsmoker group, 15 smokers meet the positive criteria, and a false positive judgment is drawn for one nonsmoker.

Although blood cyanide, serum thiocyanate and salivary thiocyanate levels should hold individual variation, the correlation between the blood cyanide, the plasma thiocyanate and the salivary thiocyanate levels were observed, and also significant differences of these levels between the smoker and nonsmoker groups were seen. Our HS-GC method using ascorbic acid is simple and sensitive enough to measure the physiological level of blood cyanide without thiocyanate interference. Therefore, quantification of not only plasma thiocyanate and salivary thiocyanate but also blood cyanide should provide a more reliable prediction of cyanide exposure such as tobacco smoking.

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