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Concentration of Carbon Monoxide (CO) in Postmortem Human Tissues: Effect of Environmental CO Exposure*

ABSTRACT: We studied how carbon monoxide (CO) is distributed within the human body through quantitation of CO concentrations in postmortem tissue samples from fatalities including possible CO exposure. Stored, frozen tissues were diced, sonicated in water, and 0.01–8.0 mg wet weight (ww) tissues were incubated with sulfosalicylic acid in CO-purged, septum-sealed vials. CO released into the headspace was quantitated by reduction gas chromatography. Mean tissue CO concentrations (pmol/mg ww) from subjects diagnosed to have no known CO exposure (control, $N = 14$), died from fire ($N = 13$), and CO asphyxiation ($N = 7$), respectively, were: adipose (2;13;9), brain (3;13;65), muscle (15;97;297), heart (30;99;371), kidney (22;432;709, lung (54;690;2638), spleen (73;1366;3548), and blood (162;2238;5070). Carboxyhemoglobin concentrations were 1.4%, 25.2%, and 69.1% of total hemoglobin, respectively. We conclude that measurements of CO concentration in a variety of tissues can be used as markers for the degree of exogenous CO exposure and the identification of possible causes of death.

KEYWORDS: forensic science, cause of death, environmental conditions, forensic pathology, forensic science, gas chromatography, tissue carbon monoxide

Under normal physiologic conditions, the predominant source of endogenous carbon monoxide (CO) is the heme oxygenase-catalyzed degradation of heme, derived from the turnover of hemoglobin (Hb) and other hemoproteins (2). Other, usually less significant, endogenous CO sources are lipid peroxidation (3), photooxidation (4), bacterial heme metabolism (5), and cytochrome P450-mediated breakdown of halogenated hydrocarbons (6). CO metabolism to carbon dioxide (CO_2) has also been reported (7,8).

A far more significant source is the accidental or deliberate inhalation of environmental CO, which often leads to pathologic conditions and death. Acute inhalation of high concentrations of CO, first of all, will lead to increased levels of dissolved CO, and more slowly lead to the formation of carboxyhemoglobin (COHb). It is the non-Hb-bound CO that is thought to load the various organ tissues with CO and kill the organism via direct CO inhibition of tissue oxidative enzymes and not via oxygen deprivation alone (9,10). Once deposited into tissues, the CO is only slowly removed, via formation of COHb. In contrast, Sokal et al. (11) have observed that the severity of CO poisoning depends more on the duration of exposure than the CO levels inhaled as measured by COHb levels.

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The distribution of CO among tissues has been incompletely studied due to a lack of suitable methodologies (12). The degree of exposure to CO is most often assessed through measurements of COHb, most commonly with clinical spectrophotometric methods (10,13). Although since the 1960s, the use of the gas chromatographic (GC) method has become more widespread in forensic toxicology laboratories (14–17). However, the convenient, automated methods are limited to analysis of fairly intact blood even though some pretreatments, such as dithionite reduction, homogenization, and detergent treatment have been used to improve sample quality (10,15). Furthermore, analysis of aged, clotted, partially degraded, and/or heat-exposed postmortem blood is usually beyond the capability of these methods. Finally, there is considerable debate about the clinical value and the correlation between COHb levels and the clinical signs and symptoms of CO poisoning (10,18). However, usually blood is the only sample obtainable from live subjects. In contrast, in deceased subjects, blood and/or other tissues can be collected for analysis in order to learn if CO exposure contributed to the cause of death. In some situations, only solid organs may be available. We have recently described an accurate and sensitive GC method with a reduction gas detector for the determination of CO concentrations in tissues from native, heme-treated, and CO-exposed rodents (12). A major application of this method could be used for the investigation of fatalities due to accidental or deliberate CO poisoning in humans, and also for the study of CO distribution among organs and tissues in order to learn more about the biochemical and physiologic mechanism(s) of CO toxicity (19) and devise effective treatment strategies with oxygen and/or hyperbaric oxygen (20).

In this study, we report our findings on CO concentrations in frozen postmortem tissues from three Medical Examiner (ME)-determined categories of fatalities: trauma with no suspected CO exposure (controls), fire-related, and CO asphyxiation.

Methods

Human Subjects

Human tissue was obtained from the National Institute of Child Health and Human Development (NICHD) Brain and Tissues Bank for Developmental Disorders or received directly from the Office of the Chief ME as a consultation to determine CO concentrations in solid tissues.

Subjects were selected to represent the following three categories of CO exposure: (1) subjects who were the victims of fatal trauma, not involving CO exposure (controls); (2) subjects who died in fire-related accidents; and (3) subjects believed to have died from accidental or deliberate CO asphyxiation.

Tissue Preparation

Tissue aliquots (0.5–1.5 g) were sealed in 2 mL polypropylene freezer tubes at time of autopsy, stored at -80°C, for 1–120 days (25 ± 32 days) shipped on dry ice to Stanford University, and stored frozen at -20°C for 1 to 42 days (18 ± 17 days) before analysis.

For analysis, the tissues were defrosted in the sealed tubes at ambient temperature. Up to 100 mg aliquots were accurately weighed into 2 mL conical polypropylene microfuge tubes, diluted with nine weights of distilled water, and thoroughly diced with surgical scissors. The resulting 10% (w/w) tissue dilutions were then sonicated using one-sec pulses from a Microson ultrasonic cell disruptor (Model XL2000, Misonix, Farmingdale, NY) with a 1/8 in. diameter microtip probe, operated at 50% power (10 W) for no more than 15 sec to obtain homogeneous suspensions. The tubes were sealed and sonicates were analyzed immediately or kept in ice for short-term storage (<2 h) (12).

CO Analyzer

CO was quantitated by gas GC using a sensitive and accurate reduction gas analyzer (RGA, Model RGA2, Trace Analytical Inc., formerly of Menlo Park, CA, but presently marketed by Ametek Process Instruments, Newark, DE. Service and similar equipment is also available from Peak Laboratories, LLC, Mountain View, CA), which has been described in detail earlier (16,17,21). Before each analysis session, the instrument was calibrated with volumes (up to 1500 μL) of certified standard gas (10.82 ppm). The standard curve was linear to up to 100 pmol CO. Beyond this level, quantities of CO were calculated by interpolation of a gradually decreasing sensitivity factor, expressed as pmol/mV×s peak area as registered by a an integrating recorder (CR-3A, Shimadzu Scientific Instruments, Columbia, MD). The limit of detection of this type of instrument is approximately 0.5 pmol or 10 pL of CO or the amount of CO found in 0.01 μL of normal blood (21).

CO Quantitation

Because a very wide range of CO concentrations was anticipated in the various tissues, the volumes of sonicate used for analysis were varied between tissues in order to stay within the prepared standard curve range levels (0–750 pmol). Preliminary analysis of blood from each subject was used as index for the amount of sonicate to analyze. In summary, 1–40 μL volumes of sonicate were injected with gastight syringes in repeating dispensers (Hamilton, Reno, NV) through the septa of 2 mL clear borosilicate GC vials, placed in ice, containing 5 μL of 50% w/v sulfosalicylic acid in water, complemented with water to obtain a final volume of 60 μL, and purged with CO-free air. The CO in the vial headspace gas was quantitated with the RGA after ≥ 15 min reaction time. Amounts of CO found in the headspace were normalized to pmoles of CO/mg wet weight (ww) tissue.

COHb Quantitation

The determination of COHb by our GC method using the $K_3Fe(CN)_6$ CO-liberating reagent has been well described previously (13,16,17); whereas, the use of sulfosalicylic acid as protein precipitation/CO releasing agent was described more recently (3,12). CO concentrations in duplicate blood samples were determined as per other tissue analysis, described above using 1–2 μL blood from control subjects or 0.02 μL from CO-exposed subjects. Clotted blood samples were sonicated for ≤ 15 sec (12). Additionally, the total hemoglobin (tHb) concentration for each sample was determined using 4 μL blood for the manual cyanmethemoglobin method (Kit 525, Sigma-Aldrich, St. Louis, MO) (16). COHb concentrations were calculated in terms of %tHb bound to CO (or COHb) using the Hüfner factor of 1.368 nL CO that can maximally bind to 1 g of Hb (17). COHb determinations by the ME were also performed using GC (14). Two aliquots were prepared for each specimen. The first aliquot was sealed in a headspace vial. The remaining aliquot was saturated with CO using a tonometer and then transferred to a headspace vial. Potassium ferricyanide was added to each sample to release CO from the Hb. A sample of the vial headspace was injected into a GC (Model 6200, Agilent Technologies, Andover, MA) with a 5 A molecular sieve column. The CO was reduced to methane with a nickel catalyst, which was quantitated with a flame ionization detector (FID). A matrix blank and quantitative control were included in each batch. Percent CO saturation was calculated by comparing the CO content of the unsaturated to the saturated sample (14).

Calculations and Statistical Analysis

The results of individual subjects, grouped in the various categories, are summarized as mean ± SD (coefficient of variation, CV) and [range]. We routinely express CO concentrations in tissues as pmol CO/mg ww tissue. However, because the more commonly used forensic concentration units are sometimes reported in terms of mL or μL CO/100 g ww tissue (22), we also report some of our results in these units to facilitate comparisons. To this end, tissue concentrations in pmol of CO/mg ww tissue were multiplied by the factor 2.24 or 0.00224 to obtain mL or μL CO/100 g ww tissue, respectively. Statistical analysis between tissues was performed by ANOVA or Student's unpaired *t*-test. Linear regressions were performed using the method of least squares. For all comparisons, differences were deemed statistically significant when $p<0.05$.

Results

Table 1 lists, in the three CO exposure categories, the individual victim demographics such as #, age (years), gender, postmortem interval (PMI), ME notes and MB-determined COHb ($COHb_{ME}$), as well as the Stanford University-determined COHb ($COHb_{SU}$), tissue CO concentrations, and category of death (COD). Also, the mean ± SD, CV, minimum and maximum values, the number of organ tissues analyzed in each category are provided as well as statistical analysis between the tissues in each of the categories compared with those with no known exposure to fire or CO (controls).

The distribution of tissue CO concentrations, categorized by type of CO-exposure, is graphically displayed in Fig. 1. The

TABLE 1—*Individual subject parameters and organ tissue CO concentrations for the three fatality categories (non-CO involved fatality [control]; fire fatality; and CO asphyxiation fatality) as determined by the ME's office.*

Analysis	Victim	Age	Sex	PMI (h)	Notes	$COHb_{ME}$	Adipose	Brain	Muscle	Heart	Kidney	Lung	Spleen	Blood	COHb	Category of Death	tHb (g/dL)	Total Storage
1.12.01	#2	44	F	18	Normal		NA	1	10	16	12	8	26	56	0.5	C	17.3	3
1.12.01	#3	18	M	18	Normal		NA	2	26	82	34	71	NA	236	1.6	C	21.5	3
3.6.01	#6	68	M	24	Alcohol abuse		NA	3	18	27	29	172	193	231	2.8	C	12.3	54
3.6.01	#7	19	M	13	Gun shot wound to chest (homicide)		NA	4	8	21	4	30	50	153	1.7	C	13.4	54
3.6.01	#8	80	F	NA	Hx GI bleed		NA	2	6	9	10	6	10	22	0.4	C	9.3	53
3.6.01	#9	23	M	14.5	Stabbing victim (homicide)		NA	2	6	9	11	15	21	62	0.6	C	17.1	53
3.6.01	#10	26	M	16	Motor vehicle accident		2	5	20	67	33	188	67	523	4.6	C	17.0	49
3.6.01	#12	26	M	29	Gun shot wound to back (homicide)		0	2	10	14	6	8	18	50	0.8	C	0.2	46
3.7.01	#13	34	M	32	Hx drugs; stroke		3	5	23	49	59	60	213	289	1.5	C	21.1	47
3.7.01	#14	35	M	19	Collapse		4	3	36	40	42	61	173	113	1.1	C	13.8	46
3.7.01	#15	58	F	9	Hx of shortness of breath, diabetes		NA	2	10	8	10	13	26	31	0.4	C	11.2	44
3.12.01	#16	79	M	20	Hx cardiopulmonary disease, found in park, heart disease		3	2	8	16	7	27	19	41	0.6	C	0.3	48
3.12.01	#17	45	F	20	Hx alcohol abuse, high blood pressure		2	2	12	33	25	37	65	301	1.8	C	24.3	46
2.27.02	#33	27	M	20	Found in field	Normal	3	11	24	37	46	96	149	205	2.2	C		132
	Mean	**42**		**19**			**2**	**3**	**15**	**31**	**23**	**57**	**79**	**165**	**1**		**14**	**48**
	SD	**22**		**6**			**1**	**3**	**9**	**23**	**18**	**59**	**75**	**143**	**1**		**7**	**29**
	CV (%)	**52**		**32**			**44**	**78**	**60**	**74**	**75**	**104**	**94**	**87**	**79**		**54**	**61**
	Minimum	**18**		**9**			**0**	**1**	**6**	**8**	**4**	**6**	**10**	**22**	**0**		**0**	**3**
	Maximum	**80**		**32**			**4**	**11**	**36**	**82**	**59**	**188**	**213**	**523**	**5**		**24**	**132**
	n	**14**		**13**			**7**	**14**	**14**	**14**	**14**	**14**	**13**	**14**	**14**		**13**	**14**
1.12.01	#1	45	M	16	Fire victim	57	NA	3	26	90	667	148	1688	6586	101.5	F	4.6	3
3.6.01	#5	78	M	20	House fire; smoker	72	NA	5	116	225	444	1830	2504	1782	25.6	F	10.5	54
3.6.01	#11	46	F	18	Motor vehicle accident; auto fire	34	3.2	11	135	180	302	1021	433	2711	20.4	F	20.6	48
3.12.01	#18	54	M	21	House fire; smoker	35	5.1	17	6	61	726	1157	1770	1880	19.7	F	14.2	45
3.12.01	#20	59	F	10	House fire; smoker		NA	3	16	22	28	74	21	138	0.6	F	32.8	49
5.15.01	#22	77	M	23	Fire victim	46	68.9	17	84	80	426	194	1420	2654	28.3	F		77
8.15.01	#24	30	M	24	Gasoline tanker explosion	3	9.0	7	23	86	42	41	84	485	3.9	F	26.0	76
8.8.01	#26	49	F	NA	Motor vehicle accident; auto fire	11	1.6	6	2	59	30	369	95	317	9.9	F	9.6	22
8.15.01	#27	54	M	26	Plane crash/severe burn	Normal	NA	18	17	99	8	69	128	194	2.3	F	22.8	15
10.2.01	#29	22	M	7	Auto fire	Normal	1.5	5	50	33	32	50	35	362	3.9	F	19.6	32
8.8.01	#30	25	M	8	Accidental house fire	52	13.9	44	464	168	918	1734	4558	6160	48.3	F	18.0	24
10.2.01	#31	26	M	7	House fire	26	0.8	20	347	89	1565	1592	3660	3589	41.3	F	13.7	2
2.27.02	#34	36	F	12	Car accident/alcoholism/car fire	Normal	8.4	7	34	23	22	181	210	220	2.3	F		121
	Mean	**46**		**16**			**12**	**12**	**102**	**93**	**401**	**651**	**1277**	**2083**	**23.7**		**17.5**	**43.7**
	SD	**18**		**7**			**22**	**11**	**143**	**62**	**470**	**708**	**1514**	**2226**	**28.1**		**8.1**	**33.5**
	CV (%)	**40**		**44**			**173**	**91**	**141**	**67**	**117**	**109**	**119**	**107**	**118**		**46**	**77**
	Minimum	**22**		**7**			**1**	**3**	**2**	**22**	**8**	**41**	**21**	**138**	**0.6**		**4.6**	**2.0**
	Maximum	**78**		**26**			**69**	**44**	**464**	**225**	**1565**	**1830**	**4558**	**6586**	**101.5**		**32.8**	**121.0**
	n	**13**		**12**			**11**	**13**	**13**	**13**	**13**	**13**	**13**	**13**	**13**		**11**	**13**
	p	0.28		0.10	Versus control		0.101	0.007	0.025	0.002	0.007	0.005	0.007	0.005	0.007		0.127	0.350
1.12.01	#4	38	M	10	Suicide (CO inhalation)	80	NA	73	231	233	972	2821	3340	7309	54.1	S	30.8	3
5.15.01	#23	39	M	24	Depression, suicide CO inhalation	68	14.8	70	365	625	719	2151	4951	2933	31.2	S		63
8.8.01	#25	75	M	26	Depression, car CO inhalation	77	3.3	53	295	255	435	2940	2355	4968	84.1	S	11.5	24
2.27.02	#28	52	M	NA	Suicide/CO intoxication	59	35.4	22	88	413	890	2609	5158	3655	38.9	S		81
2.27.02	#37	54	M	72	Suicide/CO intoxication	53	3.6	57	31	592	901	99	4221	1536	16.4	S		19
10.24.02	#38	37	M	20	Suicide/CO intoxication	75	19.5	86	445	626	1331	2281	2363	7534	89.8	S		81
10.24.02	#39	46	M	19	Suicide/CO intoxication	81	75.4	145	401	947	949	5960	1800	8434	80.2	S		56
	Mean	49		29			25	72	265	527	885	2694	3455	5196	56		21	47
	SD	13		22			27	38	157	249	271	1730	1347	2625	29		14	31
	CV (%)	28		77			108	52	59	47	31	64	39	51	51		65	67

Minimum	37	10				3	22	31	233	435	99	1800	1536	16	12	3
Maximum	75	72				75	145	445	947	1331	5960	5158	8434	90	31	81
n	7	6				6	7	7	7	7	7	7	7	7	2	7
p	0.25	0.18	Versus control			0.048	0.001	0.003	0.001	0.000	0.003	0.000	0.001	0.001	0.291	0.453
p	0.36	0.08	Versus fire			0.150	0.001	0.012	0.000	0.003	0.004	0.001	0.005	0.009	0.296	0.415
Grand mean	45	20			13	21	100	157	345	827	1267	1934	21.3		15.9	46.3	
SD	19	12				21	32	144	224	448	1316	1680	2580	29.5		8.1	30.6
n	34	31				22	34	34	34	34	34	33	34	34		26	34

CO, carbon monoxide; PMI, postmortem interval; COHb, carboxyhemoglobin; Hx, history; tHB, total hemoglobin; CV, coefficient of variation; M, male; F, female.

dashed horizontal lines represent the mean ± SD of the height of the bars in the control (white), fire-related (stippled), and CO asphyxiation (black) categories, respectively. The distribution for all tissues, but the heart and possibly adipose, clearly indicate that some of the fire victims (#20, 24, 26, 27, 29, and 37) appear not to have inhaled significant amounts of CO to subsequently elevate their blood and most tissue CO concentrations. These victims should perhaps be included in the control group.

Alternatively, the fire-exposed category can be subdivided. Thus, when this category was reorganized into subcategories of no significant CO inhalation and CO inhalation before death (Table 2), the mean ± SD, CV, minimum and maximum values, for the former resembles that of the control category in Fig. 1 and Table 1, while the tissue CO values for the CO-inhaled subcategory became significantly more uniform as demonstrated by a much decreased CV and mean concentrations closer to those of the CO intoxication category.

The mean ± SD of the tissue CO concentrations (as pmol CO/mg ww tissue and μL CO/100 mL ww tissue) for each of the fatality categories studied are summarized in Table 3. These means were also normalized to the blood CO concentration (%).

When the mean tissue concentrations for the three main categories in Table 1 were expressed as a percent of the blood CO concentrations, the distribution of CO between the different organs becomes clear and appears to follow a similar pattern irrespective of the level of blood CO (Fig. 2).

Table 4 contains the results of blood COHb analysis with a research GC with an RGA and manual tHb assay and calculated CO saturation as used at Stanford University and a GC unit with methanizer with a FID and measured CO saturation determination used by the ME. The results from the different institutions frequently varied considerably, but in general, the $COHb_{ME}$ values were higher by 7 ± 24 (− 45% to 46% tHb).

Figure 3 displays the correlations between the GC-RGA-determined COHb values and the tissue CO concentrations and indicate what tissue(s) may be used as reliable substitutes for COHb measurements. A summary of these results is shown in Table 5.

Discussion

Death by fire often causes significant destruction to blood thereby making blood unavailable for toxicological studies. Therefore, this study was initiated to determine if solid organs could be substituted for blood with any degree of reliability to establish CO intoxication in postmortem tissue samples from fatalities due to fire, CO asphyxiation, and no known CO exposure. When the mean tissue concentrations for the three CO exposure categories in Table 1 were expressed as a % of the blood CO concentrations (not COHb), the distribution of CO between the different organs, as graphically illustrated in Fig. 2, appears to follow a similar pattern, irrespective of the level of blood CO, and presumably, CO exposure level (1-, 13-, and 31-fold of control levels, respectively).

The results in Table 3 indicate that, for most tissues, except perhaps adipose, there exists a definite and fairly strong relationship between COHb levels and the tissue CO concentrations. Furthermore, these results indicate that blood, spleen, lung, and kidney may be the most appropriate CO-exposure indicator tissues. In contrast, heart tissue CO concentrations appear to be of lesser diagnostic value, with less consistent CO concentrations and higher CVs. Our results most likely represent minimum tissue CO concentrations. Although blood samples, stored frozen or at 4°C in sealed tubes have been reported to be stable for several months,

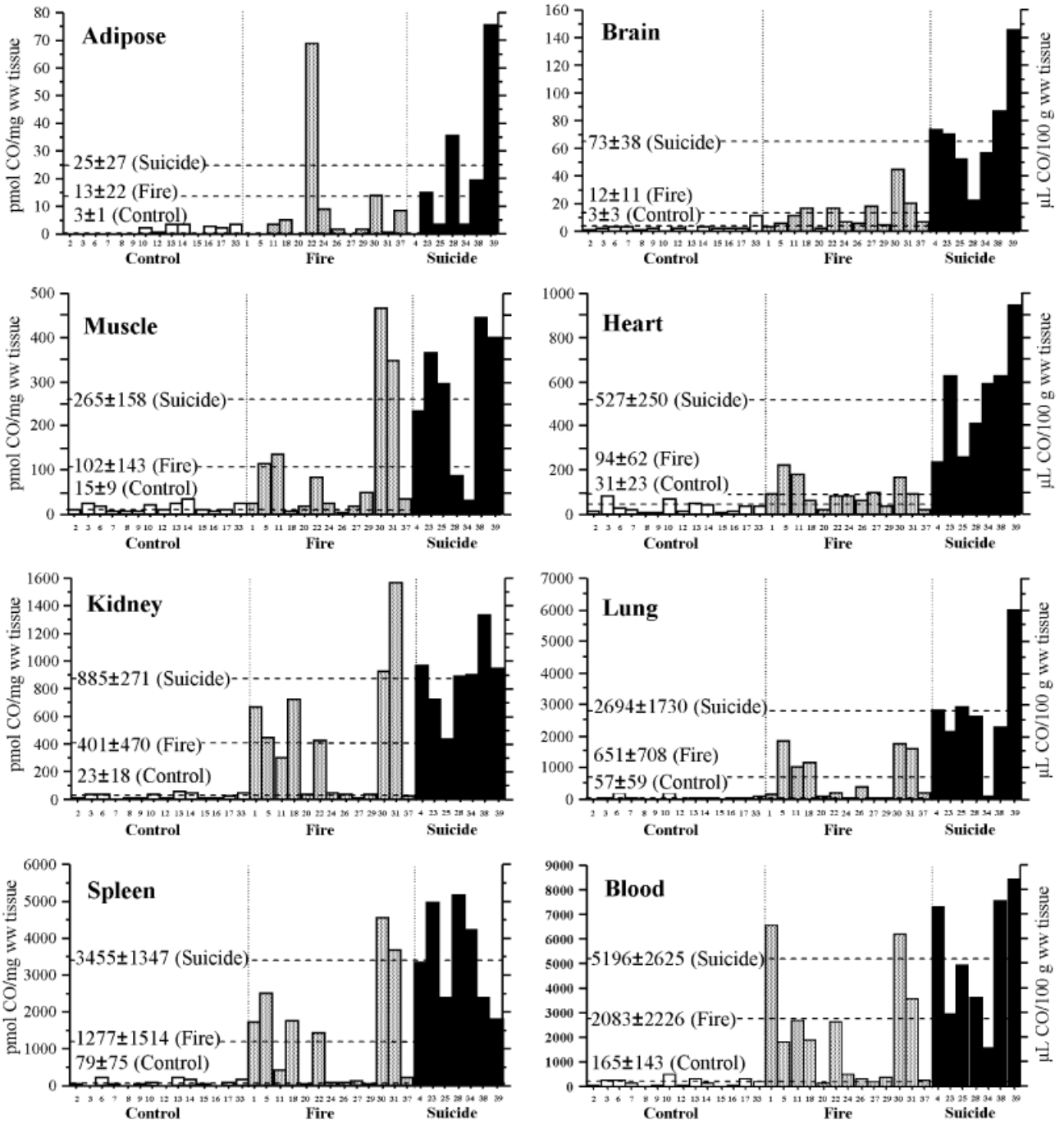

FIG. 1—*Carbon monoxide (CO) concentrations in autopsy tissues from three categories of fatalities as determined by the ME's office: victims of accidents not involving CO (control, white bars); fire victims (stippled bars); and CO asphyxiation victims (black bars). CO concentrations are presented in terms of pmol CO/mg ww tissue (left vertical axis) and µL CO/100 g ww tissue (right vertical axis. 1 pmol = 2.24 µL CO/100 g ww tissue). The dashed horizontal lines represent the mean CO concentration for each fatality category. The numerical values associated with each horizontal line represent the mean ± SD for each category.*

blood exposed to the atmosphere loses CO rapidly, and this loss is further exacerbated upon additional exposure to light and elevated temperatures (8,23). Whether this property of CO can be extrapolated to other tissues is not yet known. It seems reasonable, however, to assume that the loss of CO from ambient air-exposed tissues could be even more dramatic because it is well known that the binding of CO to myoglobin, catalase, cytochromes, and other hemoproteins is less tight than its binding to Hb. However, the loss of CO may be roughly proportional between tissues and thus the original CO relationships may be somewhat maintained, even though loss occurs during unrestricted and often unknown intensity of ambient environment exposure during the periods of: PMI and temperature, time during and after autopsy, sample collection, preparation for shipping, and sample preparation for analysis. It also needs to be realized that in some cases, tissue CO concentrations may have increased due to putrefaction.

Because our laboratory measures CO generated via several processes such as heme oxygenase activity (21), lipid peroxidation (3), photo-oxidation (4), and xenobiotic degradation (24), as well as tissue concentrations resulting from the operation of these

TABLE 2—*Summary of victims without exposure to fire or CO (controls), subdivision of tissue CO values from subjects involved in fire fatalities with no apparent CO inhalation versus subjects who clearly inhaled CO. Values are shown in pmol CO/100 g ww tissue.*

Subcategory	Pt #	Adipose	Brain	Muscle	Heart	Kidney	Lung	Spleen	Blood	COHb
No fire/no CO	Mean ± SD	2 ± 1	3 ± 3	15 ± 9	31 ± 23	23 ± 18	57 ± 59	79 ± 75	165 ± 143	1.5 ± 1.2
(control)	CV (%)	44	78	60	74	75	104	94	87	79
	Range	0–4	1–11	6–36	8–82	4–59	6–188	10–213	22–523	0.4–4.6
	n	10	14	14	14	14	14	14	14	14
Fire+no apparent	20	ND	2	16	22	28	74	21	138	0.58
CO inhalation	24	9	7	23	85	42	41	84	485	3.91
	26	2	6	2	59	30	369	95	317	9.87
	27	ND	18	17	99	8	69	128	194	2.28
	29	2	5	50	33	32	50	34	361	3.94
	34	8	7	34	23	22	181	210	220	2.3
	Mean ± SD	5 ± 4	7 ± 5	24 ± 16	54 ± 33	27 ± 11	131 ± 127	95 ± 69	286 ± 127	3.8 ± 3.2
	CV (%)	81	70	69	62	41	97	72	45	84
	Range	2–9	3–18	2–50	22–99	8–42	41–369	21–210	138–485	0.6–9.9
	n	4	6	6	6	6	6	6	6	6
Fire+CO inhalation	1	ND	3	25	90	667	148	1688	6586	101.5
	5	ND	5	116	225	444	1830	2504	1782	25.6
	11	ND	11	135	180	302	1021	433	2711	20.4
	18	5	16	6	61	726	1157	1770	1880	19.7
	22	69	17	84	80	426	194	1420	2654	28.3
	30	14	44	464	168	918	1734	4558	6160	48.3
	31	1	20	347	89	1565	1592	3660	3589	41.3
	Mean ± SD	18 ± 29	17 ± 14	168 ± 172	128 ± 63	721 ± 427	1097 ± 697	2290 ± 1409	3623 ± 1975	40.7 ± 28.8
	CV (%)	156	83	102	49	59	64	62	55	71
	Range	1–69	3–44	6–464	61–225	302–1565	148–1839	433–4558	1782–6586	19.7–101.5
	n	4	7	7	7	7	7	7	7	7
CO asphyxiation	Mean ± SD	25 ± 27	72 ± 38	265 ± 157	527 ± 249	885 ± 271	2694 ± 1730	3455 ± 1347	5196 ± 2625	56.4 ± 28.9
	CV (%)	108	52	59	47	31	64	39	51	51
	Range	3–75	22–145	31–445	233–947	435–1331	99–5960	1800–5158	1536–8434	16.4–89.8
	n	6	7	7	7	7	7	7	7	7

CV, coefficient of variation; ND, not determined.

processes (12), we express CO in terms of pmoles generated (or released) per mg ww tissue. Because in forensic applications, CO concentrations are expressed as mL or μL CO/100 g ww tissue, we have also expressed CO concentrations in these terms (Figs. 1 and 2 and Table 3) in order to facilitate comparison of our results to those reported earlier by others (22,23,25). For instance, the thorough studies by Wilks and Clark (22), more than half a century ago, using relatively simple, but elegant technology and nearly 1000 times the tissue weight as in the present study, nevertheless reported nearly equal CO concentrations for heart, lung, brain, and blood in control rats. However, they reported nearly 10-fold higher concentrations for muscle and kidney, in spite of the fact that human muscle has 5 to 6 times higher myoglobin than the rat.

Smokers are expected to be more likely to be the victims of CO asphyxiation than non-smokers, because these subjects already have elevated COHb, with associated tissue CO concentrations, of up to 10% (26). We do not have the social histories of these vic-

TABLE 3—*Mean ± SD (pmol CO/mg ww tissue) tissue CO concentrations [μL CO/100 g ww tissue*] and tissue CO concentrations normalized to that of blood (100%).*

Tissue	Control	Fire Only	Fire+CO	CO Asphyxiation
Adipose	3 ± 1	5 ± 4	18 ± 29	25 ± 27
	[6] (2%)	[11] (2%)	[41] (1%)	[57] (1%)
Brain	3 ± 3	7 ± 5	17 ± 14	72 ± 38
	[7] (2%)	[17] (3%)	[37] (1%)	[162] (1%)
Muscle	15 ± 9	24 ± 16	168 ± 172	265 ± 157
	[34] (9%)	[53] (8%)	[377] (5%)	[594] (5%)
Heart	31 ± 23	54 ± 33	128 ± 63	527 ± 249
	[68] (19%)	[120] (19%)	[286] (4%)	[1181] (10%)
Kidney	23 ± 18	27 ± 11	721 ± 427	885 ± 271
	[52] (14%)	[60] (9%)	[1615] (20%)	[1983] (17%)
Lung	57 ± 59	131 ± 127	1097 ± 697	2694 ± 1730
	[127] (34%)	[293] (46%)	[2457] (30%)	[6035] (52%)
Spleen	79 ± 75	95 ± 69	2290 ± 1409	3455 ± 1347
	[177] (48%)	[214] (33%)	[5131] (63%)	[7740] (67%)
Blood	165 ± 143	286 ± 127	3623 ± 1975	5196 ± 2625
	[370] (100%)	[640] (100%)	[8116] (100%)	[11638] (100%)
COHb	1.4 ± 1.2	3.8 ± 3.2	40.7 ± 28.8	56.4 ± 28.9

*Conversion Factor = pmol CO/mg ww tissue × 2.24 = μL CO/100 g ww tissue.

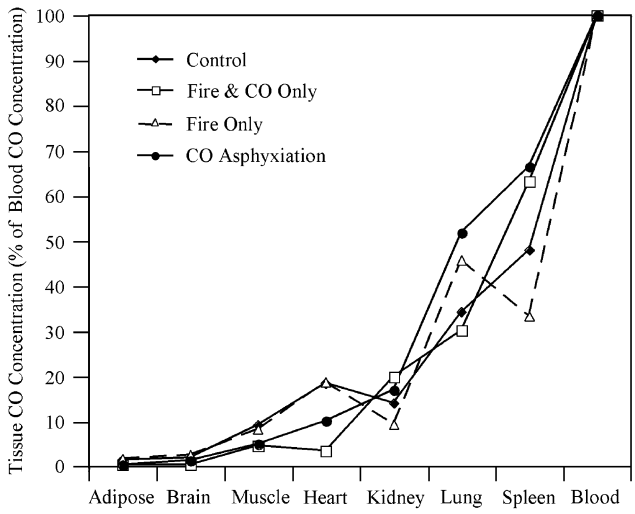

FIG. 2—*Tissue carbon monoxide (CO) concentrations for the three fatality categories, expressed as a percent of that found in blood (100%).*

TABLE 4—*Comparison of COHb values % saturation in blood obtained with different GC methods.*

Group	Victim #	$COHb_{ME}$	$COHb_{SU}$	$COHb_{ME}$–$COHb_{SU}$
	1	57	102	−45
	5	72	26	46
	11	34	20	14
	18	35	20	15
	20	(<12)	1	NA
	22	46	28	18
Fire and CO	24	3	4	−1
	26	11	10	1
	27	(<12)	2	NA
	29	(<12)	4	NA
	30	52	48	4
	31	26	41	−15
	34	(<12)	2	NA
	4	80	54	26
	23	68	31	37
	25	77	84	−7
Suicides	28	59	39	20
	37	53	16	37
	38	75	90	−15
	39	81	80	37
	Mean ± SD	52 ± 24	35 ± 32	9 ± 23
	CV (%)	47	91	273
	Range	3–81	1–102	−45–46
	n	16	20	16

Values in parentheses (<12% tHb) not included in the calculations.
NA, not available.

tims, but the control category's COHb values indicate that it is likely that victims #3, 6, 7, 10, 13, 14, 17, and 33 (8/14% or 57%) were smokers or had been otherwise exposed to CO, before death. Thus the control category's tissue CO values are not truly representative of normal controls, whose COHb usually ranges from 0.4%–0.8% tHb (26). The same applies to the other CO exposed categories. It is thus also likely that a significant number of the tissue CO values in the fire and CO asphyxiation categories include a smoking component. A habit of smoking also seems to be a likely source for the tissue CO concentrations in the fire with no apparent CO inhalation subcategory (Table 2). After all, it is well known that a significant number of fires are related to smoking. Finally, because of the wide range of CO exposures, it is not surprising that the victims in the fire-exposed category have the widest range of CO values, while most tissue values overlap in both the control and CO asphyxiation categories.

The CO asphyxiation category includes both unintended and deliberate exposure to CO from controlled combustion sources, such as internal combustion engines and gas-powered household devices. Even if death occurred due to CO toxicity, different tissue CO concentrations may be expected from acute (deliberate) and more or less chronic exposure. These processes may account for the relatively wide range in tissue CO concentrations in the CO-involved fire and CO asphyxiation categories and provide a means to differentiate between these two types of victims. Tissue values of CO asphyxiation victims #23 and 37 are puzzling in that they appear too low to be the main COD, even though they were diagnosed as suicide deaths. It is possible that other gases (CO_2, hydrogen cyanide, nitric oxide, etc.) generated by the CO source contributed to their deaths.

The CO concentration in liver tissue was not determined, because at the onset of the study, the frozen tissue was deemed to contain too much blood to be of any practical value as an uncontaminated tissue level. In retrospect, we should have also analyzed this major organ, irrespective of its blood content. In fact, the lung samples suffered from the same problem of often being massively contaminated with blood, which could not be removed from the once-frozen tissues samples.

Adipose and brain tissues contained the lowest amounts of CO. This may be due to a low solubility of CO in lipids, low levels of CO-binding heme compounds, or the lack of vascularization in these tissues. Only in the victims with CO asphyxiation was there a significant difference in brain CO concentrations from the other categories.

It is disturbing to note in Table 4 that the determination of COHb saturation in two different laboratories on a limited number of samples varied so significantly and widely (−45% to 46%). Although COHb saturation was measured by similar GC methods at Stanford University and the ME's office, the results varied frequently, with the $COHb_{ME}$ values being higher by a mean of 9 ± 24% tHb. The reasons for these differences may be severalfold. The $COHb_{ME}$ values were obtained with unfrozen, "fresh" blood samples, which had most likely lower MetHb levels than the frozen samples analyzed later at Stanford. Lewis et al. (27) reported recently that increased levels of MetHb yielded erroneously elevated COHb saturations. However, these results contradict our findings. Perhaps, the handling, freezing/thawing, and storage of the blood caused loss of CO. However, if this were true, one would expect a downward trend in COHb saturation between the ME and Stanford, but not the great variations that were observed. Even though the fundamental method of releasing CO with $K_3Fe(CN)_6$ and quantitating CO was by GC, the methods varied considerably in that SU measured CO with a RGA in which the CO after separation from other gases on the GC column, reacted with mercuric oxide (HgO) to liberate CO_2 and mercury gas, the absorbance of which was quantitated spectrophotometrically at 254 nm. The method used by the ME involved the separation of CO from other gases on the GC column, but reduced CO to methane with a methanizer and subsequently quantitated the methane with a FID. Also, Stanford calculated the %tHb saturation on the basis of tHb determination and application of the Hüfner factor, whereas the ME analytically determined the ratio of CO found in the untreated unknown samples and CO-saturated samples. It is not known that the different methods to determine COHb saturation should result in different values. However, the fact that the blood may have been exposed to elevated temperatures could also have been an important factor. Finally, perhaps the differences may be due to an accumulation of circumstances, such as the COHb determinations were performed in different locales, by different personnel, and widely different conditions of sample treatment, storage, etc. Although a comparison of COHb saturation measurement methods were not an objective of the present study, the discrepancies found warrant further investigation.

Toxic tissue CO concentrations are primarily a function of the concentration of CO in inhaled air and the duration of inhalation (28). These parameters are not known for the present category populations. Furthermore, it is safe to assume that none of the victims' bodies were at equilibrium with inhaled CO concentrations. However, the regression plots in Fig. 3 demonstrate relative strong relationships between COHb and tissues CO concentrations as indicated by *R*-values ranging from 0.48 to 0.94 and very low *p*-values (Table 5). The panel for blood shows a very close relationship from 0% to 40% tHb while at higher COHb levels the spread around the mean increases significantly. It is interesting to note that the spread for spleen is unusually large. Considering that this organ presumably contains much blood, one would have expected correlations similar to that of blood. Interestingly, when we

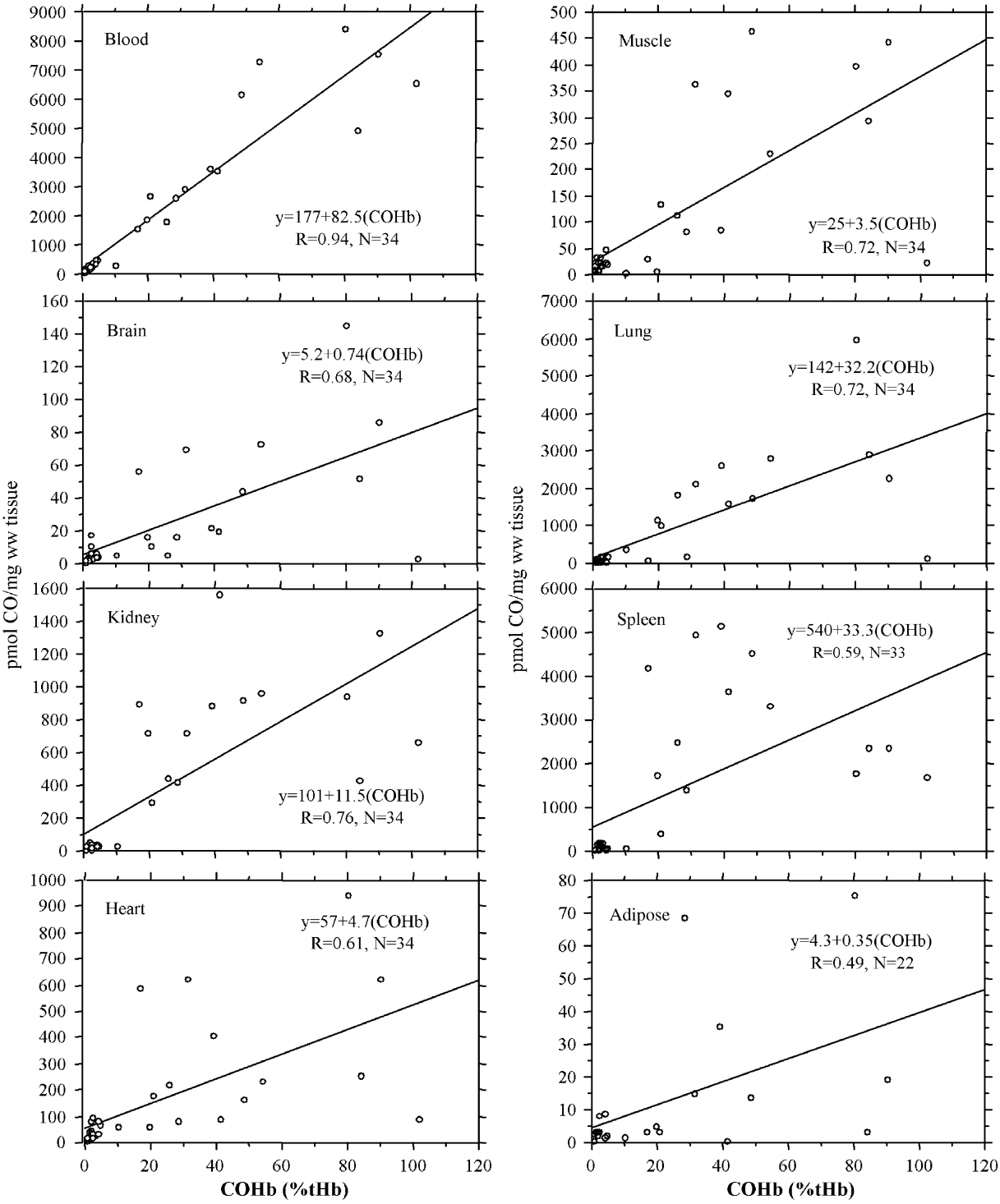

FIG. 3—*Regression analysis between gas chromatographic-reduction gas analyzer-determined carboxyhemoglobin values and carbon monoxide concentrations in the various tissues.*

performed regression analysis on the spleen data reported by Wu et al. (29), we obtained a considerably tighter correlation:

$$\text{Spleen CO (\% sat)} = 9.0 + 0.53\ \text{COHb(\% sat)};$$
$$R = 0.78;\ p < 0.0001$$

In conclusion, on the basis of the results of the present study, it appears that tissues, such as blood, muscle, brain, lung, and kidney are the most suitable index tissues for diagnosing death due lethal exposure to CO and may be used to differentiate fire only-related deaths from those due to CO inhalation before fire exposure. Spleen, heart, and adipose appear to be least suitable.

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TABLE 5—*Summary of regression analysis between GC-RGA-determined COHb values and CO concentrations in the various tissues.*

Tissue	Regression Equation	*R*-Value	*N*	*p*-Value
Blood	y = 177+82.5 (COHb)	0.94	34	<0.0001
Kidney	y = 101+11.4 (COHb)	0.76	34	<0.0001
Lung	y = 142+32.1 (COHb)	0.72	34	<0.0001
Muscle	y = 25+3.52 (COHb)	0.72	34	<0.0001
Brain	y = 5+0.74 (COHb)	0.68	34	<0.0001
Heart	y = 57+4.68 (COHb)	0.61	34	<0.0001
Spleen	y = 540+33.2 (COHb)	0.59	33	<0.0003
Adipose	y = 4+0.35 (COHb)	0.48	32	<0.0222

GC-RGA, gas chromatographic-reduction gas analyzer; COHb, carboxyhemoglobin; CO, carbon monoxide.

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