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Unconjugated Morphine in Blood by Radioimmunoassay and Gas Chromatography/Mass Spectrometry

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ABSTRACT: Morphine, the active metabolite of heroin, is rapidly inactivated by glucuronidation at the 3 carbon. Unconjugated (pharmacologically active) morphine was measured in postmortem blood by radioimmunoassay using an antibody-coated tube kit. The kit shows less than 0.2% cross-reactivity with codeine and morphine-glucuronide. Unconjugated morphine concentrations were confirmed by gas chromatography/mass spectrometry (GC/MS) using deuterated morphine as the internal standard. The blood was precipitated with 10% trichloroacetic acid (TCA) and concentrated hydrochloric acid (HCl), centrifuged, and decanted. The supernatant was then either diluted (unhydrolyzed) or heated to 100°C, 30 min (hydrolyzed), followed by a wash with 4:1 chloroform:isopropranol. The upper aqueous layer was then saturated with sodium bicarbonate ($NaHCO_3$) and extracted with 4:1 chloroform:isopropranol. The organic layer was evaporated, derivatized with trifluoroacetic anhydride (TFA), and analyzed by selected ion monitoring (SIM) GC/MS.

Comparison of the results for unconjugated morphine by radioimmunoassay and unhydrolyzed morphine by GC/MS gave a correlation coefficient of $r = 0.98$, $n = 100$. Unconjugated morphine ranged from 0 to 100% of total morphine with a mean of 42%, $n = 200$, for heroin or morphine involved deaths. Review of 56 putative rapid deaths gave a mean of 68% unconjugated morphine with a range of 26 to 100%. The ratio of unconjugated to total morphine was found to be stable in postmortem blood after more than a year of storage at room temperature, within the precision of the method.

KEYWORDS: toxicology, morphine, blood, radioimmunoassay, chemical analysis

Morphine is the active metabolite of heroin. Heroin (diacetylmorphine) is rapidly deacetylated in whole blood to 6-acetylmorphine (half-life 9 min), and 6-acetylmorphine is further hydrolyzed to morphine (half-life 38 min) [*1*]. Both heroin and 6-acetylmorphine have very little affinity for the opiate receptor; therefore, morphine probably accounts for all or most of the narcotic activity of heroin in the brain [*1*].

Morphine itself is rapidly inactivated by glucuronidation at the 3 carbon. This occurs in the liver and possibly at extra hepatic sites as well. The time course of morphine glucuronide formation has been studied for various routes of administration [*2-7*]. Between 1 and 2 h after injection, the morphine glucuronide concentration exceeds the concentration of morphine in the blood. Therefore, the ratio of unconjugated morphine to total morphine and

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morphine glucuronides in blood may provide information about the route and time of administration of the opiate [*8*].

Only the unconjugated morphine in the blood can cross the blood brain barrier and react with opiate receptors. Pare et al. [*9*] found a good correlation between unconjugated morphine and concentrations of morphine in the brain stem and thalamic areas associated with morphine receptors and morphine actions. Spiehler et al. [*10*] found poor correlation between total morphine (morphine and morphine metabolites) and brain regional morphine concentrations. This poor correlation suggests that the glucuronide metabolite does not cross the blood brain barrier.

In this study, morphine was measured in blood by a radioimmunoassay specific for unconjugated morphine and by electron-impact gas chromatography/mass spectrometry (GC/MS) [*11,12*]. Total morphine (morphine plus morphine glucuronides) was measured by GC/MS after acid hydrolysis [*13*]. The concentrations of unconjugated morphine and total morphine in heart blood taken at autopsy from 200 heroin- and morphine-involved deaths were reviewed to determine if metabolite to parent drug ratios could be related to the interval between administration of the opiate and death.

Methods

Blood samples were taken from the heart at autopsy and mixed with sodium fluoride and potassium oxalate. These samples were then stored at room temperature or refrigerated at 4°C until assayed. Tissue samples obtained at autopsy were frozen until assayed. Brain tissue was most often cerebral cortex. Blood samples from all coroner's cases submitted for toxicological examination were screened by radioimmunoassay[2] for total opiates: morphine, morphine glucuronide, codeine, and related compounds. Radioimmunoassay-screen positive blood samples, liver, and brain tissue were then assayed for morphine and morphine metabolites by the following procedures.

Radioimmunoassay for Morphine

Unconjugated morphine in blood was measured using the Coat-A-Count Morphine radioimmunoassay (Diagnostic Products Corp., Los Angeles). A 50-μL sample size was used. Blood was diluted 1 : 10 or greater with water as needed to bring the concentration within the range of the kit standards (1 to 100 ng/mL). Samples or standards and ^{125}I-labeled morphine were incubated for 1 h in antibody-coated tubes. After the liquid was throughly decanted, the tubes were counted in an gamma counter (Isodata, 20/20 Series) equipped with microcomputer data-handling capabilities. The standard curve was plotted as counts versus log of standard concentration, and the curve fitted point to point. Aqueous standards from the kit were adequate for quantitation.

Gas Chromatography/Mass Spectrometry

The GC/MS procedure in routine use in the laboratory at the beginning of this study [*14*] was inadequate for determination of unconjugated morphine in blood. The acid precipitate method of Brown [*13*] was modified for the measurement of both hydrolyzed and unhydrolyzed morphine as follows.

One millilitre of blood or one gram of tissue (2 g of 1 : 1 homogenate with water) or appropriate standards or quality control samples was mixed in a disposable culture tube (16 by 100 mm) with 1 mL of internal standard (1 μg/mL deuterated morphine and 1 μg/mL deuterated codeine). One millilitre of 10% trichloroacetic acid was added to each tube, and the

[2]Radioimmunoassay done by Abuscreen for Opiates, Hoffman LaRoche, Nutley, NJ.

tube contents were immediately and throughly vortexed. One millilitre of concentrated hydrochloric acid (HCl) was added to each tube, and the contents were again vortexed until they were well mixed. The tubes were then centrifuged in a countertop centrifuge for 5 min at 2000 $\times$ g. Sample proteins should form a compact plug at the bottom of the culture tube. The liquid supernatant was transferred from the culture tubes to a screw-top tempered glass culture tube (16 by 100 mm).

For determination of total morphine (morphine and morphine glucuronide), the samples were then hydrolyzed. These tubes were placed in boiling water for 30 min. The tubes were allowed to cool before proceeding to the next step. For determination of unhydrolyzed morphine (unconjugated morphine), the tubes were not heated.

Five millilitres of chloroform : isopropanol (4 : 1) were added to each tube. The tubes were capped with Teflon®-lined caps and shaken well. The tubes were then centrifuged and the upper aqueous layer was transferred to a second clean screw-cap tube containing about 0.5 g sodium bicarbonate and about 0.5 mL butylchloride : isopropanol (4 : 1). The acid liquid must be added slowly. The butylchloride is added to float on top of the reacting aqueous layer to reduce foaming. If necessary, more sodium bicarbonate was added to produce excess in the tube and ensure a saturated solution. Then 5 mL of chloroform : isopropanol (4 : 1) was added to each tube containing bicarbonate. The tubes were shaken well and more bicarbonate added if necessary so that the aqueous phase remained saturated. The tubes were centrifuged and the upper aqueous layer aspirated and discarded. The lower organic layer was transferred to disposable, pointed tubes by filtering through pasteur pipettes with a glass wool plug. The organic phase was then evaporated to dryness. Just before injection into the GC/MS, the residue was derivitized for 30 min with 200 μL chloroform and 200 μL trifluoroacetic anhydride (TFA) at room temperature. The residue was again taken to dryness and then dissolved in 50 μL chloroform. Five microlitres were injected onto a capillary column coated with OV17, on a GC/MS (Hewlett-Packard Model 5987) at 240°C. Masses 395 AMU (codeine-TFA), and 398 AMU (tri-deuterated codeine-TFA), 477 AMU (morphine-TFA), and 480 AMU (tri-deuterated morphine-TFA) were monitored. The ratio of morphine to deuterated morphine (internal standard) and the ratio of codeine to deuterated codeine (internal standard) were used for quantitation. Either peak height or peak areas were used to calculate the ratios.

Results

Comparison of radioimmunoassay results (Coat-A-Count morphine), for unconjugated morphine to the gas chromatograph/mass spectrometer results for unhydrolyzed morphine in blood gave a correlation coefficient of 0.987, $n = 100$ by linear regression. The best fit least squares line for the data was $y = 1.06x - 0.011$. The radioimmunoassay gave the same standard curve with spiked blood samples as with the urine-based kit standards. The recovery of the Coat-A-Count calibrator standards by the GC/MS procedure is shown in Table 1.

Aliquoits of postmortem blood from morphine involved cases were pooled and homogenized. This pool was repeatedly assayed to determine the intra- and inter-assay reproducibility of the radioimmunoassay and GC/MS procedures. The results for morphine are shown in Table 2.

The interassay reproducability (Table 2) was determined by serial analyses at least a week apart over three-months duration on refrigerated homogenized pooled postmortem blood. To determine the stability of the unconjugated to total morphine ratio in postmortem blood stored at room temperature, individual case blood samples were repeatedly reassayed by radioimmunoassay and GC/MS over the two years of the study. The results for one case are shown in Table 3. Within the reproducibility of the methods, the ratio appears constant. The stability of the ether glucuronide conjugate of morphine has been shown in many previous studies [*15*].

TABLE 1—*Recovery of DPC Coat-a-Count standards by GC/MS.*

Coat-a-Count Standard, ng	GC/MS, μg/mL
5	0.008
	0.006
10	0.013
	0.011
	0.012
25	0.029
	0.031
100	0.116
	0.114

TABLE 2—*Precision of procedures: morphine.*

Variable	Coat-a-Count Radioimmunoassay, μg/mL	GC/MS, Not Hydrolyzed, μg/mL	GC/MS, Hydrolyzed, μg/mL
Intra-assay Variation:			
Mean	0.537	0.429	0.874
Standard Deviation	0.073	0.015	0.032
n	31	21	21
Coefficient of variation	14%	3.5%	3.8%
Interassay Variation:			
Mean	0.443	0.441	0.868
Standard Deviation	0.038	0.025	0.048
n	9	10	12
Coefficient of variation	8.6%	5.7%	5.3%

TABLE 3—*Stability of unconjugated morphine/total morphine ratio in a single postmortem blood from a morphine-involved death.*

Date Analyzed, C84-415	Unconjugated Morphine, RIA	Unconjugated Morphine, GC/MS	Total Morphine, GC/MS	Ratio
5/24/84	. . .		1.00	. . .
8/27/84	0.47	. . .	. . .	0.47
10/17/84	. . .	0.56	. . .	. . .
10/19/84	0.45	0.50	. . .	. . .
11/12/84	0.40	0.53	1.25	0.46
12/29/84	. . .	0.60	. . .	. . .
1/02/85	. . .	0.48	. . .	. . .
2/26/85	. . .	0.45	. . .	. . .
3/06/85	0.50	0.37	. . .	. . .
6/21/85	0.41	0.56	1.0	0.49
12/13/85	0.43	. . .	. . .	. . .
1/16/86	0.44	. . .	. . .	. . .
1/23/86	0.43	. . .	. . .	. . .

This radioimmunoassay has been reported to have less than 0.2% cross-reactivity with codeine and morphine-3-glucuronide [*11,12*]. This appeared to be true for codeine overdose case samples tested (Table 4). However, codeine involved deaths were not included in the statistics described below for heroin and morphine involved deaths.

In the 200 cases of heroin and morphine involved deaths tested, the unconjugated morphine ranged from 0 to 100% of the total morphine. The mean was 42% unconjugated morphine with a standard deviation of 24% (Table 5). This is similar to the values reported by Reed [*16*]: for 55 cases the unhydrolyzed morphine ranged from 0 to 97% of the hydrolyzed morphine value with a mean of 38% and a standard deviation of 20%. The mean was recalculated for just those cases for which the investigation suggested that death had occurred rapidly based on circumstances in which the body was found (for example, syringe in arm, drug kit under body, tourniquet still on arm) or on statements of witnesses. A death occurring in less than 3 h after the deceased was said to have injected heroin was considered a "rapid" death following Garriott and Sturner [*17*]. The mean unconjugated morphine as a percent of total morphine in these 56 cases was 68% with a standard deviation of 18% and a range of 26 to 100%. In Table 5, these values are compared to those calculated from the data reported by Reed. In Table 6, the morphine and total morphine values for "rapid death" cases are presented. The results for the remaining cases are shown in Table 7. In these cases

TABLE 4—*Radioimmunoassay and GC/MS results in codeine overdose cases.*

Radioimmunoassay Coat-a-Count Unconjugated Morphine, μg/mL	GC/MS μg/mL			
	Unhydrolyzed		Hydrolyzed	
	Morphine	Codeine	Morphine	Codeine
0	0	1.6		1.6
0.02	0.02	3.50	0.03	3.70
0.03	0.02	1.75	0.10	5.1
0.04	0.04	0.39	0.24	0.66
0.08	0.08	2.56	0.18	4.20
0.09	0.05	3.75		3.98
0.13	0.15	0.45	0.37	0.85
0.33	0.32		0.07	0.66
0.39	0.28	0.90	0.60	1.00
0.59	0.54	1.11	1.30	1.20
0.59	0.65	1.20	1.30	1.20

TABLE 5—*Percent unconjugated morphine.*

Variable	Spiehler/Brown 1984-1986	Reed 1979
Overall:		
n	200	55
Mean	42%	38%
Standard Deviation	24%	20%
Range	0-100%	0-97%
Rapid Death:		
n	56	9
Mean	68%	51%
Standard Deviation	18%	20%
Range	26-100%	23-84%

TABLE 6—*Death less than 3 h after heroin/morphine injection: morphine concentrations, μg/mL.*[a]

Unconjugated	Total	%	Brain	Liver	Age, Years	Sex
1.65	2.2	75	1.1	5.5	33	M
0.89	0.94	88	0.68	1.3	34	F
0.75	0.90	82	0.28	2.5	28	M
0.73	1.10	66	0.86	5.5	28	M
0.68	1.3	52	3.4	1.0	18	M
0.64	0.69	93	0.22	1.0	37	M
0.54	1.3	41	1.4	1.4	31	M
0.52	0.55	95	0.34	. . .	22	M
0.51	0.95	53	1.0	3.2	22	F
0.51	0.83	60	0.37	1.4	30	M
0.50	0.77	65	0.30	3.4	20	M
0.50	0.83	60	0.37	1.4	30	M
0.46	1.0	46	0.80	2.9	22	F
0.46	0.63	72	0.32	. . .	40	M
0.44	0.59	73	0.23	1.22	32	M
0.44	0.88	50	0.71	1.0	29	M
0.44	0.90	48	0.35	1.5	35	M
0.41	0.62	66	0.37	1.42	35	M
0.41	0.46	88	0.45	2.0	44	M
0.40	0.73	55	0.55	1.08	34	M
0.41	0.66	61	0.68	2.25	45	M
0.40	0.76	53	0.53	1.6	31	M
0.40	0.56	71	0.28	1.26	35	M
0.40	0.50	79	0.03	1.8	24	M
0.39	0.76	53	0.53	1.6	31	M
0.38	0.44	83	1.0	1.4	36	M
0.38	0.82	45	0.43	1.0	32	M
0.36	0.93	39	0.32	1.42	37	M
0.36	0.43	83	0.30	0.86	36	M
0.35	0.68	57	0.44	2.5	32	M
0.35	0.45	71	0.26	. . .	30	M
0.32	0.46	70	0.14	0.92	38	M
0.31	0.72	44	0.46	1.22	27	F
0.28	0.28	100	0.32	0.78	33	M
0.28	0.4	70	0.3	1.4	21	M
0.28	0.31	90	0.25	0.69	44	M
0.26	0.38	68	0.39	1.1	28	M
0.26	0.52	50	0.3	0.68	35	M
0.26	0.42	63	0.18	1.2	30	M
0.24	0.62	39	. . .	. . .	25	M
0.24	0.47	51	0.23	0.59	40	M
0.24	0.31	73	0.10	. . .	30	M
0.23	0.27	85	. . .	. . .	29	M
0.22	0.26	85	0.11	. . .	18	M
0.22	0.40	55	0.17	0.72	26	M
0.22	0.45	48	0.18	. . .	29	M
0.21	0.53	40	0.21	1.5	30	M
0.21	0.21	100	. . .	. . .	33	M
0.20	0.32	63	0.30	2.0	43	M
0.20	0.40	50	0.25	0.92	32	M
0.20	0.23	85	0.21	0.48	43	M
0.18	0.30	61	0.50	0.93	36	M
0.18	0.52	35	0.28	1.0	20	M
0.15	0.26	57	0.33	1.1	41	M
0.10	0.10	99	. . .	. . .	21	M
0.08	0.30	26	0.12	0.41	38	M

[a] $x = 68$; $n = 56$; sd = 18.

TABLE 7—*Heroin/morphine-involved deaths (time of death longer than 3 h after dose or unknown): morphine concentrations (μg/mL).*

Unconjugated	Total	%	Brain	Liver	Age, Years	Sex	Cause of death
2.20	8.0	28	2.0	6.0	44	M	
1.10	3.09	35	0.56	2.32	29	M	homicide: gunshot wound
1.04	3.18	33	0.86	5.47	32	M	
0.89	2.0	45	1.0	4.2	37	F	
0.86	2.1	41	0.90	1.7	53	F	
0.80	1.6	50	. . .	. . .	41	M	homicide: trauma
0.72	2.9	25	. . .	. . .	59	F	
0.67	1.45	47	0.15	0.73	27	F	
0.66	1.1	60	0.27	1.3	63	M	
0.62	0.87	69	0.35	2.5	. . .		
0.56	3.4	15	. . .	. . .	49	F	suicide CO
0.53	1.0	55	0.06	2.2	43	M	
0.52	0.75	72	0.35	1.10	48	M	
0.52	2.3	22	0.24	5.7	54	M	suicide: overdose
0.50	0.77	65	0.30	3.4	20	M	
0.50	1.0	50	0.6	1.5	35	M	
0.50	1.1	45	0.4	2.05	41	M	
0.48	0.91	58	0.09	3.6	37	M	
0.44	0.91	49	0.61	3.6	37	M	
0.37	0.87	43	0.40	1.9	. . .		
0.34	0.70	48	0.22	0.44	35	M	
0.33	0.65	49	0.30	1.55	35	M	
0.33	0.81	41	0.34	2.37	31	M	suicide?
0.33	0.48	68	. . .	. . .	81	M	suicide: gunshot wound
0.33	0.64	55	. . .	. . .	54	M	cancer
0.30	1.0	30	2.5	0.73	34	M	suicide: gunshot wound
0.30	0.90	33	0.35	3.2	33	M	
0.30	0.73	41	0.43	2.8	20	F	
0.29	0.38	68	0.27	0.80	33	M	
0.29	0.64	46	. . .	. . .	54	M	cancer
0.28	1.36	20	0.16	0.79	0.5	M	homicide: trauma
0.28	0.45	57	0.16	0.68	36	M	
0.27	0.92	35	0.23	. . .	45	M	
0.26	0.73	37	0.32	1.8	28	M	
0.26	0.80	33	. . .	. . .	30	F	homicide: trauma
0.26	0.84	32	0.36	1.8	32	M	
0.25	0.60	42	0.29	0.98	36	M	
0.25	0.45	55	0.16	0.68	36	M	
0.24	1.2	20	0.18	0.90	34	M	
0.24	0.44	53	0.11	1.3	27	M	
0.23	0.55	41	0.27	1.6	. . .		
0.23	0.58	40	0.55	2.0	34	F	
0.22	0.85	29	0.45	1.3	34	M	
0.22	1.0	22	0.32	2.8	21	M	
0.22	0.40	54	0.13	0.76	22	M	
0.20	0.63	32	0.32	1.25	32	M	
0.20	0.50	40	0.45	1.2	27	M	
0.20	0.47	42	0.21	1.2	24	M	homicide: gunshot wound
0.19	0.87	22	0.17	1.1	38	M	
0.19	0.42	45	. . .	. . .	24	M	
0.18	0.80	23	. . .	. . .	67	F	
0.18	0.28	64	0.22	1.0	28	M	
0.18	0.46	40	0.20	0.73	29	M	
0.18	0.56	32	. . .	. . .	19	M	
0.18	0.62	28	. . .	. . .			
0.17	1.45	12	0.22	1.9	67	M	burns
0.17	0.4	43	0.04	1.36	23	M	

TABLE 7—*Continued.*

Unconjugated	Total	%	Brain	Liver	Age, Years	Sex	Cause of death
0.17	0.38	45	0.17	0.52	27	F	struck head
0.17	0.89	18	. . .	. . .	21	M	
0.17	0.32	53	0.17	0.40	30	M	
0.16	1.1	15	0.15	1.5	34	M	
0.16	0.59	28	0.16	1.68	25	M	heart disease
0.16	0.40	40	. . .	. . .	25	M	
0.16	0.37	45	. . .	. . .	28	M	
0.16	0.53	30	0.13	0.62	43	M	
0.15	1.03	15	0.27	1.66	32	M	
0.15	0.60	24	0.15	1.1	24	F	
0.14	0.59	26	0.17	1.1	37	M	suicide: overdose
0.14	0.69	20	. . .	. . .	23	M	gunshot wound
0.13	0.44	30	0.14	0.86	23	M	
0.13	0.30	40	0.16	0.56	24	F	
0.13	0.18	72	. . .	. . .	30	F	
0.13	0.73	18	0.12	0.87	24	F	
0.13	0.40	32	. . .	. . .			
0.12	0.37	35	. . .	. . .	57	M	
0.12	0.13	92	. . .	. . .	32	M	
0.12	0.27	44	0.17	1.3	27	M	
0.12	0.45	26	0.12	0.46	21	M	
0.12	0.16	81	0.13	0.4	33	M	
0.12	0.38	32	0.30	1.0	0	F	
0.11	0.56	21	. . .	. . .	36	M	
0.11	0.80	14	0.21	1.15	28	M	
0.11	0.95	12	. . .	. . .	20	M	
0.10	5.04	2	0.15	0.16	29	M	
0.10	0.86	12	. . .	. . .	66	M	
0.09	0.29	34	0	0.47	31	M	
0.09	0.16	56	. . .	. . .	50	F	heart disease
0.09	0.09	97	. . .	. . .	46	M	Rx liver failure
0.08	0.41	21	. . .	. . .	38	M	
0.08	0.46	20	0.10	0.56	29	M	
0.08	0.26	33	0.08	0.4	33	M	
0.08	0.95	9	0.19	0.86	24	M	
0.08	0.23	36	0.10	. . .	28	M	
0.08	0.59	14	0.20	1.0	24	M	homicide: stabbing
0.08	0.38	21	0.15	0.6	39	M	
0.08	0.30	26	0.12	0.41	38	M	
0.08	0.27	30	0.08	0.5	28	M	homicide: gunshot wound
0.07	0.23	33	. . .	. . .			traffic accident
0.07	0.16	48	. . .	. . .	35	M	
0.07	0.25	27	. . .	. . .	19	M	
0.06	0.47	13	0.17	0.83	33	M	
0.06	0.36	17	0.10	1.19	30	M	
0.06	0.24	23	0	0.10	34	M	
0.06	0.18	30	0.16	0.95			
0.06	0.10	55	. . .	. . .	29	M	suicide: gunshot wound
0.05	0.11	50	. . .	. . .	73	M	traffic accident
0.05	0.2	27	. . .	. . .	77	F	burns
0.05	0.4	15	0.07	0.34	62	M	gunshot wound
0.05	0.10	50	0.07	0.17	29	M	homicide: trauma
0.05	0.18	28	. . .	. . .	70	M	bronchopneumonia
0.05	0.18	27	. . .	. . .	72	M	
0.05	0.10	48	. . .	. . .	29	M	suicide: gunshot wound
0.05	0.20	24	0.57	0.75	35	M	
0.05	0.19	23	0.31	1.6	46	M	
0.04	0.48	9	0.10	0.39	32	M	

TABLE 7—*Continued.*

Unconjugated	Total	%	Brain	Liver	Age, Years	Sex	Cause of death
0.04	0.17	25	. . .	. . .	30	M	homicide: burns
0.04	0.16	25	. . .	. . .	0	M	stillborn
0.04	0.38	12	0.13	0.36	25	M	
0.04	0.13	32	. . .	. . .	35	M	
0.03	0.21	17	. . .	. . .	30	M	
0.03	0.15	23	. . .	. . .	72	F	traffic accident
0.03	0.53	6	0.08	0.30	28	M	
0.03	0.30	10	0.05	0.30	36	M	traffic accident
0.03	0.20	15	. . .	. . .	28	M	traffic accident
0.03	0.14	21	. . .	. . .	32	M	traffic accident
0.03	0.15	20	. . .	. . .	37	M	
0.02	qns		0.05	0.07	0	M	stillborn
0.02	0.15	17	. . .	. . .	21	M	traffic accident
0.02	0.13	20	0.07	0.19	37	M	homicide: gunshot wound
0.02	0.27	9	0.06	0.28	23	M	
0.02	0.21	11	0.24	0.14	16	M	
0.02	0.16	14	. . .	. . .		M	traffic accident
0.02	0.07	30	. . .	. . .	26	M	traffic accident
0.02	0.07	27	0.02	0.06			
0.02	0.08	25	. . .	. . .	19	M	traffic accident
0.02	0.10	13	. . .	. . .	.5	F	battered child
0.02	0.14	11	. . .	. . .	50	F	suicide: gunshot wound
0.02	0.04	40	. . .	. . .	52	M	suicide: hanging
0.01	0.23	6	0.05	0.48	27	M	
0.01	0.10	12	. . .	. . .	15	M	traffic accident
0.006	0.08	8	. . .	. . .	16	F	traffic accident
0.006	0.035	17	0	0.04	35	M	homicide: gunshot wound
0.005	0.06	8	. . .	. . .		M	
0.005	0.10	5	. . .	. . .	32	M	
0.004	0.04	10	. . .	. . .	85	F	
0.003	0.08	4	. . .	. . .	30	M	traffic accident
0.000	0.06		0.04	0.16		M	homicide

death occurred after greater than 3 h after morphine or heroin administration, or at unknown times. The distribution of percent unconjugated morphine in blood is shown in Fig. 1 for "rapid deaths," other heroin deaths, and for morphine given for medical reasons.

Discussion

In these cases, a death which occurs rapidly after injection of heroin (or morphine) is often characterized by a high percentage of unconjugated active morphine as well as high concentrations of total morphine. This is predicted by the pharmacokinetics of morphine and morphine metabolite formation published by Berkowitz [*3,4*], Brunk and Delle [*2*], and more recently by McDougal et al. [*5*], Svensson et al. [*6*], and Sawe et al. [*7,18*]. The blood morphine glucuronide concentrations would exceed the blood unconjugated morphine concentrations after 1 or 2 h or more of survival after heroin injection resulting in less than 50% unconjugated morphine. Although we did not find any case of someone who was known to have survived for a long time after heroin injection who maintained high blood concentrations of unconjugated morphine, this might be expected if the person was deficient in glucuronidating ability. Infants, for example, generally do not reach full adult glucuronidation ability until three years of age [*19*]. However, several cases were noted in which, although the

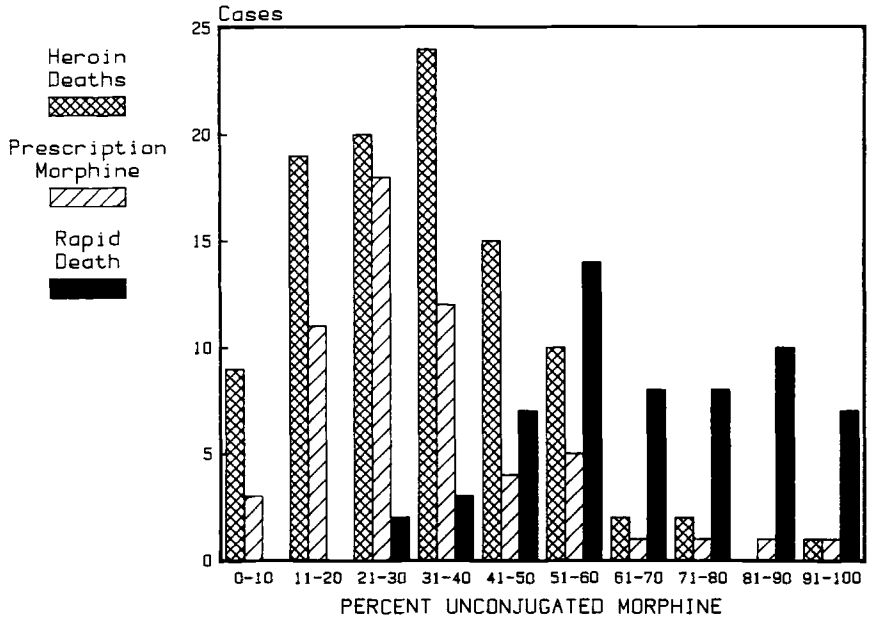

FIG. 1—*Unconjugated morphine as a percent of total morphine in postmortem blood in morphine involved deaths: for rapid deaths, deaths occurring in less than 3 h after injection; for other heroin involved deaths where death occurred after more than 3 h after administration or unknown; and for morphine associated deaths in which morphine was administered by prescription for therapeutic reasons.*

investigation suggested that the person died soon after the injection of heroin, the percent unconjugated morphine was much less than 50% of the total. This could be due to accumulation of the glucuronide metabolite. Baselt [*1*] comments that the data of Berkowitz suggest that the morphine glucuronide accumulates in blood. Sawe et al. [*18*] recently reported that the elimination half life of morphine-3-glucuronide was 4 h for normal volunteers and averaged 49.8 h for eleven uremic patients. Heroin users or patients treated with morphine who have renal disease or renal insufficiency as a result of hypoxia might have impaired ability to excrete morphine glucuronide and prolonged metabolite half-life. This would result in low ratios of parent drug to metabolite regardless of time since last dose.

Conclusion

In conclusion, the precepts of Stead [*8*] on the use of pharmacokinetic and drug metabolism data for the estimation of time after drug administration may be applicable to some cases of heroin involved deaths. High ratios (50 to 100%) of unconjugated morphine to total morphine (morphine and morphine glucuronides) suggest a rapid death after injection of opiate. Low ratios of unconjugated morphine to total morphine may be due to longer intervals between injection and death or to accumulation of morphine glucuronide in chronic use of opiates.

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References

[1] Baselt, R. C., *Disposition of Toxic Drugs and Chemicals in Man,* 2nd ed., Biomedical Press, Davis, CA, 1982.

[2] Bruck, S. F. and Delle, M. "Morphine Metabolism in Man," *Clinical Pharmacology and Therapeutics,* Vol. 16, 1974, pp. 51-57.

[3] Berkowitz, B. A., Ngai, S. H., Yang, J. C., Hempstead, J., and Spector, S., "The Disposition of Morphine in Surgical Patients," *Clinical Pharmacology and Therapeutics,* Vol. 17, No. 6, 1975, pp. 629-635.

[4] Berkowitz, B. A., "The Relationship of Pharmacokinetics to Pharmacological Activity: Morphine, Methadone and Naloxone," *Clinical Pharmacokinetics,* Vol. 1, 1976, pp. 219-230.

[5] McDougal, J. N., Butler, J. A., and Burks, T. F. "Morphine Distribution After Oral and Subcutaneous Administration" in *Proceedings,* Western Pharmacology Society, Vol. 24, 1981, pp. 331-334.

[6] Svensson, J.-O., Rane, A., Sawe, J., and Sjoqvist, F., "Determination of Morphine, Morphine-3-Glucuronide and Morphine-6-Glucuronide in Plasma and Urine Using Ion-Pair High-Performance Liquid Chromatography," *Journal of Chromatography,* Vol. 230, 1982, pp. 427-432.

[7] Sawe, J., Kager, L., Svensson, J.-O., and Rane, A., "Oral Morphine in Cancer Patients: In Vivo Kinetics and In Vitro Hepatic Glucuronidation," *British Journal of Clinical Pharmacology,* Vol. 19, 1985, pp. 495-501.

[8] Stead, A. H., "The Use of Pharmacokinetic and Drug Metabolism Data in Forensic Toxicology" in *Progress in Drug Metabolism,* Vol. 9, J. W. Bridges and C. F. Chasseaud, Eds., Taylor and Francis, London, 1985.

[9] Pare, E. M., Monforte, J. R., and Thibert, R. J., "Morphine Concentrations in Brain Tissue from Heroin-Associated Deaths," *Journal of Analytical Toxicology,* Vol. 8, No. 5, 1984, pp. 213-216.

[10] Spiehler, V. R., Cravey, R. H., Richards, R. G., and Elliott, H. W., "The Distribution of Morphine in the Brain in Fatal Cases Due to Intravenous Administration of Heroin," *Journal of Analytical Toxicology,* Vol. 2, No. 2, 1978, pp. 62-67.

[11] Matejczyk, R. J. and Kosinski, J. A., "Determination of Cross-reactant Drugs with a New Morphine Radioimmunoassay Procedure," *Journal of Forensic Sciences,* Vol. 30, No. 2, April 1985, pp. 677-680.

[12] Diagnostic Products Corp., Package Insert.

[13] Brown, R., "A Rapid and Efficient Blood/Urine Opiate Extraction Procedure," *Journal of Analytical Toxicology,* Vol. 9, No. 4, pp. 165-166.

[14] Pearce, D., Wiersema, S., Kuo, M., and Emery, C., "Simultaneous Determination of Morphine and Codeine in Blood by Use of Select Ion Monitoring Deuterated Internal Standards," *Clinical Toxicology,* Vol. 14, No. 2, 1979, pp. 161-168.

[15] Way, E. L. and Adler, T. K., "The Pharmacologic Implications of the Fate of Morphine and its Surrogates," *Pharmacological Reviews,* Vol. 12, 1960, pp. 383-446.

[16] Reed, D., "Comparison of Spectrofluorometric and GC/MS Procedures for the Quantitation of Morphine in Blood and Brain," *Clinical Toxicology,* Vol. 14, No. 2, 1979, pp. 169-180.

[17] Garriott, J. C. and Sturner, W. O., "Morphine Concentrations and Survival Periods in Acute Heroin Fatalities," *New England Journal of Medicine,* Vol. 289, 13 Dec. 1973, pp. 1276-1278.

[18] Sawe, J., Odar-Cederlof, I., Svensson, J., and Arner, B., "Kinetics of Morphine in Patients with Renal Failure" in *Proceedings,* 3rd World Conference on Clinical Pharmacology and Therapeutics, Stockholm, 1986.

[19] Warner, A., "Drug Use in the Neonate: Interrelationships of Pharmacokinetics, Toxicity and Biochemical Maturity," *Clinical Chemistry,* Vol. 32, No. 3, 1986, pp. 721-727.

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