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Skeletal Muscle as an Alternative Specimen for Alcohol and Drug Analysis

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ABSTRACT: In a random group of medical examiner cases, muscle tissue, as well as blood and vitreous humor, was analyzed for ethyl alcohol, and the results were compared. When the blood concentration was greater than 0.10 g/dL, the muscle to blood ratio was 1.00 or less (average 0.94), and when the blood concentration was less than 0.10 g/dL, this ratio was greater than 1.00 (average 1.48). The author proposes that this ratio is dependent upon the time course of absorption and distribution, as has been observed for vitreous humor, but with a more rapid equilibration.

Muscle tissue was also analyzed in another group of cases found to be positive for one or more drugs in blood. The concentrations of the drugs in muscle varied from none detected to 6.5 times those in blood and seemed to be dependent on the time course between ingestion and death, as well as on the nature of the drug. For most common basic drugs, the ratios were often near unity. Muscle is proposed as a useful alternative specimen to postmortem blood.

KEYWORDS: toxicology, musculoskeletal system, ethanol

The purpose of postmortem analysis for drugs and alcohol is ordinarily to determine as accurately as possible the concentration of the agents that existed in blood at the time of death, since blood and plasma levels are used to assess the effects and determine the likelihood of any drug toxicity.

In certain circumstances, such as decomposition, a valid blood concentration of drugs or alcohol cannot be determined or results are questionable. In addition, recent studies show that heart blood may have falsely elevated concentrations of certain drugs [*1,2*]. It is for this reason that alternative specimens must sometimes be chosen for analysis and evaluation of drug concentrations.

The conventional specimens used in most postmortem investigations other than blood are liver, kidney, brain, urine, gastric contents, bile, and vitreous humor. Gastric content and urine are considered to be of qualitative value only for drugs, since they are outside the body and concentrations of drugs in these specimens are not necessarily meaningful for determining toxicity. Liver and bile are collection and storage depots for some drugs and drug concentrations there may be many times those in blood, while kidney and brain may or may not closely reflect blood concentrations. An alternative specimen that would be expected to have alcohol and drug concentrations equivalent to those of blood would be useful in postmortem toxicology.

The present study presents data obtained by determining drug and alcohol concentrations in skeletal muscle and comparing these with blood concentrations.

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Methods

Blood was collected from the aorta by insertion of a large-gage needle attached to a 50-mL syringe. Two large (20-mL) red-stoppered Vacutainer tubes (untreated) and one 7-mL grey-stoppered Vacutainer tube (containing 14 mg of potassium oxalate and 17.5 mg of sodium fluoride) are routinely collected for toxicology purposes. Approximately 4 mL of vitreous humor was collected in a small red-stoppered Vacutainer (untreated). A section of about 100 g of thigh muscle was excised, placed in a plastic bag, and heat sealed. Blood and other fluids were maintained at 4°C until analysis, while tissues were maintained frozen at −20°C. The blood from the grey-stoppered tube was used for alcohol and narcotic/cocaine analysis, while the blood from the red-stoppered tubes was used for most other drug analyses.

Alcohol analyses were performed on blood, vitreous humor, and muscle tissue by use of the direct blood injection method of Jain [*3*] using a 1.3-m (4-ft) 0.2-mm inside diameter column packed with 0.2% Carbowax, 1500 on 60/89 Carbopack (Supelco, Inc., Bellefonte, Pennsylvania). Acidic and basic drugs were analyzed by previously published methods, but using capillary columns and, in some cases, using confirmation by gas chromatography/mass spectrometry (GC/MS) (Hewlett-Packard 5970 Series MSD) [*4,5*]. Muscle tissue was analyzed as a fluid after making a 1:4 homogenate with water by homogenizing the tissue in a Polytron microhomogenizer.

Morphine (free), codeine, cocaine, benzoylecgonine, and ecgonine methyl ester (EME) were measured by a modified procedure derived from that of Nakamura and Way [*6*]. Three millilitres of blood [buffered with 3.0 mL 40% potassium phosphate (K_2HPO_4)], containing 25 mL of 20 μg/mL nalorphine internal standard, are extracted with 10 mL ethyl acetate/isobutanol (9:1). This solution is back-extracted with 4.0 mL of 0.2*N* hydrochloric acid (HCl). After this phase is adjusted to pH 9.5, the drugs are reextracted into 10 mL of solvent, which is taken to dryness. Twenty-five millilitres of freshly prepared BSA [*N,O*-bis-(trimethylsilyl)-acetamide/ethyl acetate (1:1)] are added. After 3 min, 1 to 2 μL are injected into the GC/MS (Hewlett-Packard 5970 Series MSD). Three ions were monitored for all analytes, and quantitations were based on the base peak abundance, compared with standards extracted from blood with each run. Solvent blanks were injected following each case run.

Results

The alcohol concentrations in blood, muscle, and vitreous humor are shown in Table 1 (those with blood-alcohol concentrations [BACs] greater than 0.10 g/dL) and in Table 2 (those with BACs less than 0.1 g/dL). It soon became apparent that the muscle/blood ratios were characteristic for each BAC group. With only one exception, when the BACs were greater than 0.10 g/dL, the muscle to blood ratio was 1.00 or less (average, 0.94; range, 0.77 to 1.04). When the BAC was less than 0.10 g/dL, however, the muscle to blood ratio was greater than 1.00 (average 1.48, range 1.25 to 1.78).

Thus, for those cases in which the BAC was greater than 0.1 g/dL, the muscle concentrations were either the same or slightly lower than the BAC, while for those in which the BAC was less than 0.1 g/dL, the muscle alcohol concentrations were uniformly higher.

The vitreous alcohol concentrations were uniformly higher (with one exception) than the BACs in both groups, with an average vitreous/blood ratio of 1.26 ± 0.12 in the group with BACs greater than 0.1 g/dL, and 1.47 ± 0.36 in the group with BACs lower than 0.10 g/dL. In both groups, however, the muscle alcohol concentrations were very close to the corresponding blood values, with 7 of the 19 in Table 1 having the same value and 20/27 cases varying by 0.03% or less.

The maximum difference in the series was seen in one case (Case 6, Table 2), in which the muscle concentration was 0.16 g/dL, 78% higher than the corresponding blood

TABLE 1—*Blood/muscle/vitreous alcohol concentrations, in grams per decalitre; BAC > 0.10 g/dL.*

Case No.	Blood	Muscle	M/Bl[a]	Vitreous	V/Bl[b]
1	0.29	0.24	0.82	0.37	1.28
2	0.10 (am[c] 17)	0.09	0.90	0.13	1.30
3	0.14	0.14	1.00	0.18	1.29
4	0.14	0.14	1.00	0.19	1.36
5	0.11	0.11	1.00	0.16	1.45
6	0.18	0.15	0.83	0.15	0.83
7	0.29	0.25	0.86	0.35	1.21
8	0.18	0.18	1.00	0.25	1.39
9	0.27	0.25	0.93	0.31	1.15
10	0.15	0.15	1.00	0.18	1.20
11	0.10 (am 07)	0.10	1.00	0.12	1.20
12	0.12	0.10	0.83	0.15	1.25
13	0.19	0.15	0.79	0.20	1.05
14	0.23	0.24	1.04	0.28	1.22
15	0.22	0.22	1.00	0.25	1.14
16	0.22	0.17	0.77	0.28	1.27
17	0.21	0.20	0.95	0.27	1.29
18	0.12	0.14	1.16	0.22	1.83
19	0.21	0.18	0.86	0.25	1.19
			Average 0.94 ± 0.086		1.26 ± 0.12

[a]M/Bl = muscle/blood ratio.
[b]V/Bl = vitreous/blood ratio.
[c]am = antemortem blood specimen.

TABLE 2—*Blood/muscle/vitreous alcohol concentrations, in grams per decalitre; BAC < 0.10 g/dL.*

Case No.	Blood	Muscle	M/Bl[a]	Vitreous	V/Bl[b]
1	0.06	0.09	1.50	0.15	2.50
2	0.06	0.10	1.66	0.08	1.33
3	0.04	0.06	1.50	0.06	1.50
4	0.08 (am[c] 21)	0.12	1.50	0.14	1.75
5	0.06	0.08	1.33	0.06	1.00
6	0.09 (am 08)	0.16	1.78	0.14	1.56
7	0.04	0.05	1.25	0.03	0.75
8	0.03	0.04	1.33	0.04	1.33
			Average 1.48 ± 0.13		1.47 ± 0.36

[a]M/Bl = muscle/blood ratio.
[b]V/Bl = vitreous/blood ratio.
[c]am = antemortem blood specimen.

value, 0.09 g/dL. In this case, exsanguination had occurred and heroic resuscitative treatment with multiple transfusions occurred prior to death. In such cases, the muscle and vitreous alcohol concentrations are believed to reflect more accurately the "true" venous BAC before medical intervention.

The muscle and blood drug comparisons are shown in Tables 3 through 7. The drug cases were categorized into groups: Table 3, propoxyphene positive cases; Table 4, cases positive for tricyclic antidepressants; Table 5, cases positive for benzodiazepines; Table 6, cases positive for cocaine; and Table 7, cases positive for morphine.

For propoxyphene (Table 3), the muscle and blood concentrations were very similar. Muscle/blood ratios were close to 1.0 or higher in all cases, except in Case No. 6, a case

TABLE 3—*Blood and muscle drug concentrations propoxyphene.*

Case No.	Drug	Blood, mg/L	Muscle, mg/kg	M/Bl[a]	Cause of Death
1	Propoxyphene	0.29	0.24	0.8	head injury/accident
	Norpropoxyphene	0.73	0.47	0.6	
2	Propoxyphene	0.26	0.41	1.6	gunshot wound (GSW)/homicide
	Norpropoxyphene	0.47	0.30	0.6	
3	Propoxyphene	0.28	0.46	1.6	natural
	Norpropoxyphene	0.48	1.34	2.9	
	Desipramine	0.54	1.58	2.9	
4	Propoxyphene	1.25	3.61	2.9	mixed drug OD
	Norpropoxyphene	0.69	2.10	3.0	
	Verapamil	1.16	0.51	0.4	
	Norverapamil	0	0.27	. . .	
5	Propoxyphene	1.78	1.95	1.1	ASCVD[b]/natural
	Norpropoxyphene	2.40	4.35	1.8	
6	Propoxyphene	5.03	0.19	<0.1	acute propoxyphene
	Norpropoxyphene	6.81	2.71	0.4	
7	Propoxyphene	0.33	0.97	2.9	cirrhosis/hemorrhage
	Norpropoxyphene	0.23	0.27	1.2	
	Metoclopromide	0.48	2.22	4.6	
	Pethidine	0.10	0.39	3.9	
	Norpethidine	0.09	0.15	1.7	

[a]M/Bl = muscle/blood ratio.
[b]ASCVD = arteriosclerotic vascular disease.

TABLE 4—*Blood and muscle drug concentrations, tricyclic antidepressants.*

Case No.	Drug	Blood, mg/L	Muscle, mg/kg	M/Bl[a]	Cause of Death
1	Amitriptyline	1.90	2.36	1.2	amitriptyline OD/suicide
	Nortriptyline	0.76	0.66	0.9	
2	Amitriptyline	0.05	0	. . .	IV narcotism/accident
	Nortriptyline	0.03	0	. . .	
3	Imipramine	0.15	0.20	1.3	pulmonary embolism
	Desipramine	0.20	0.43	2.2	
4	Nortriptyline	1.81	0.75	0.4	undetermined
	Thioridazine	4.11	0.30	0.1	
5	Nortriptyline	2.54	3.79	1.5	acute OD/suicide
6	Nortriptyline	1.91	6.63	3.5	acute mixed OD/undetermined
	Chlorpromazine	1.77	0.98	0.6	
7	Thioridazine	2.94	3.10	1.1	multiple injuries

[a]M/Bl = muscle/blood ratio.

TABLE 5—*Blood and muscle drug concentrations, benzodiazepines.*

Case No.	Drug	Blood, mg/L	Muscle, mg/kg	M/Bl[a]	Cause of Death
1	Diazepam	0.25	0.14	0.6	acute IV narcotism
	Nordiazepam	0	0.09	. . .	
	Cocaine	0.29	0	. . .	
	EME	0.02	0	. . .	
	Morphine	0.09	0.32	3.6	
	Codeine	0.96	0.11	0.1	
2	Diazepam	0.10	0.18	1.8	acute IV narcotism
	Nordiazepam	0.71	0.40	0.6	
	Chlordiazepoxide	0.37	0.52	1.4	
	Morphine	0.70	0.12	0.17	
	Codeine	0.01	0	. . .	
3	Diazepam	2.67	0.40	0.2	acute IV narcotism
	Nordiazepam	0.92	0.32	0.4	
	Morphine	0.18	0	. . .	
	Codeine	0.02	0	. . .	
4	Diazepam	0.63	0.13	0.2	acute OD diltiazam
	Nordiazepam	0.02	0.03	1.5	
	Diltiazam	9.52	1.68	0.2	

[a]M/Bl = muscle/blood ratio.

involving acute overdose (OD) of propoxyphene, in which the muscle/blood ratio was less than 0.1. The average ratio in six cases of nonprimary propoxyphene overdose was 1.8, while the corresponding average for norpropoxyphene was 2.0. In the one case of acute propoxyphene overdose, the ratio was less than 0.1 and 0.4 for propoxyphene and norpropoxyphene, respectively. For tricyclic antidepressants (Table 4), muscle/blood ratios were generally higher (average ratio 1.2 for all cases). In contrast to propoxyphene, the highest muscle values were seen in the cases of acute overdose.

In four cases of benzodiazepine detection (Table 5) muscle/blood ratios varied considerably, from 0.2 to 3.6. The muscle/blood ratios of cocaine (Table 6) varied also from

TABLE 6—*Cocaine and metabolite concentrations in blood and muscle.*

Case No.	Drug	Blood, mg/L	Muscle, mg/kg	M/Bl[a]
1	Cocaine	0.24	0.22	0.9
	Benzoylecgonine	1.14	0.70	0.6
	EME	0.59	0.11	0.2
2	Cocaine	0.85	0.28	0.3
	Benzoylecgonine	1.16	0.81	0.7
	EME	2.10	0.07	0.03
3	Cocaine	0.04	0.26	6.5
	Benzoylecgonine	0.34	0.23	0.7
	EME	2.50	0.058	0.02
4	Cocaine	0.13	0.15	1.2
	Benzoylecgonine	0.88	0	0
	EME	0.88	0.05	0.06
5	Cocaine	0.10	0.06	0.6
	Benzoylecgonine	0.59	0	0
	EME	0.56	0.03	0.05
6	Cocaine	11.58	1.06	0.1
	Benzoylecgonine	6.18	0	0
	EME	4.9	0.11	0.2

[a]M/Bl = muscle/blood ratio.

TABLE 7—*Morphine and codeine concentrations in blood and muscle.*

Case No.	Drug	Blood, mg/L	Muscle, mg/kg	M/Bl[a]
1	Morphine	1.0	1.0	1.0
	Codeine	0.01	0.005	0.5
2	Codeine	0.37	0.017	<0.1
3	Morphine	0.23	0.16	0.7
	Codeine	0.19	0.003	0.02
4	Morphine	0.14	0.23	1.6
	Codeine	0.01	0.001	0.1
5	Morphine	0.88	0.09	0.1
	Codeine	0.04	0.002	0.05
6	Morphine	0.19	0.27	1.4
	Codeine	0.009	0.006	0.7
7	Morphine	0.09	0.32	3.6
	Codeine	0.96	0.11	0.1
	Cocaine	0.29	0	0
	Benzoylecgonine	0.02	0	0
	EME	0.02	0	0
8	Morphine	0.20	0.28	1.4
	Codeine	0.74	0.004	<0.01
	Cocaine	1.73	0.19	0.1
	Benzoylecgonine	4.45	0.63	0.1
	EME	0.82	0.028	0.34

[a]M/Bl = muscle/blood ratio.

0.1 to 6.5. On the other hand, benzoylecgonine and EME, the major metabolites of cocaine, exhibited consistently low ratios. In three cases, one even with a high blood cocaine level, no benzoylecgonine was detected in muscle.

In nine cases of morphine and codeine detection in heroin-related cases (Tables 7 and 5), the two drugs had average muscle/blood ratios of 1.4 and 0.2, respectively.

Discussion

Alcohol

In evaluation of postmortem alcohol concentrations, the heart blood has been shown to be generally equivalent to peripheral blood [*7*]. Therefore, one would expect that muscle alcohol concentrations would be present in a uniform ratio with blood. In this study, the muscle/blood alcohol distribution ratios fell nearly uniformly into two groups: those with BACs greater than 0.10 g/dL and those with BACs less than 0.10 g/dL. In the first group, the actual muscle alcohol concentrations were very close to those of blood, with an average of all cases of 0.94 ± 0.086. In eight of 19 cases (42%), the ratio was 1.00. The average vitreous humor/blood ratio in this group was 1.26 ± 0.12, which is virtually the same as the theoretical ratio (1.27) for these two specimens calculated from water content [*8*]. The group having BACs less than 0.10 g/dL had much higher muscle/blood ratios, averaging 1.48 ± 0.13, which is 57% higher than the corresponding ratio in the first group. In this group, the ratios were uniformly greater than 1.00, while the corresponding vitreous humor/blood ratios averaged 1.47 ± 0.36, almost identical to the muscle/blood ratio.

These observations are consistent with other studies of alcohol distribution into body compartments. In general, when the vitreous humor/blood ratio is greater than 1.27, the theoretical distribution ratio based on the water content of the two specimens, then gastrointestinal absorption and body distribution is complete and the individual is in the "postabsorptive phase" of alcohol distribution [*8,9*]. When the vitreous ratio is less than this value, the alcohol distribution is not complete, and the individual may still have been ingesting alcohol within 1 to 3 h of his death and has not reached a maximum alcohol concentration in the blood.

The time required for complete absorption (or maximum blood concentration) may be from 0.5 to 6 h but is on average 0.75 to 2 h, depending primarily on food consumption [*10*].

Thus, of the two groups of this study, the first group (Table 1), in general, is at or near the maximum blood concentration, or may still have been drinking alcohol at the time of death, while those with lower concentrations (Table 2) are generally past this stage, indicating complete distribution and declining BACs, with higher, lagging tissue concentrations. This is not to indicate that this will always be the case, however, since, obviously, if one has recently been drinking, distribution will not be complete and lower vitreous and muscle ratios will be expected whether the BAC is greater than or less than 0.10 g/dL (for example, Table 1, Cases 6 and 13; Table 2, Case 7).

The theoretical muscle/blood ratio for alcohol is 0.86 [*11*], based on the water content of both specimens. Experimentally, however, this ratio has been reported to be somewhat higher by various authors: 0.94 (>0.10 BAC) and 1.48 (<0.10 BAC) in this study, 0.97 in rats [*12*], 0.88 [*9*], and 0.90 [*13*].

What is most apparent, and of great forensic science value, is that the muscle seems to equilibrate with blood faster than the vitreous humor in the preabsorptive phase, and at equilibrium the ratio is very nearly equal to unity. In the postabsorptive phase, as in

most of the cases of Table 2, the muscle apparently lags in equilibrating with blood but is still very close to the blood concentration in most cases (one of eight cases varying by more than 50%, excluding Case 6). This observation may be at variance with that of Harger et al. [*11*], who reported that femoral muscle lagged about 4 h behind other tissues in alcohol equilibration in dogs.

One case deserves some special comment, due to the forensic significance of the alcohol findings: this was Case 6 (Table 2), having a BAC of 0.09 g/dL at death, a recent antemortem BAC of 0.08 g/dL, a muscle concentration of 0.16 g/dL, and a vitreous of 0.14 g/dL. In this case extensive resuscitation measures were applied prior to death, with fluids given. Intravenous fluid administration does not markedly change the BAC under normal circumstances. However, in fact, the antemortem or postmortem blood samples taken during or after these circumstances will often be artificially low, due to dilution of some vascular compartments. Presumably, this is because of the presence of shock (circulatory incompetence) and consequent lack of normal body distribution of the fluids being administered with remaining blood after severe hemorrhage. In Case 6, these circumstances applied and it would appear that the muscle alcohol concentration, due to its lag in equilibration with blood due to shock, is the more accurate representation of the BAC before fluid administration.

Drugs

In general, the results of the drug comparisons showed that most of the drugs detected in blood were also detected in muscle, with two instances of detection of drugs in muscle, but not in blood (norverapamil, Table 3, and nordiazepam, Table 5).

Moreover, the concentration ratio between the two specimens was usually near or greater than unity, suggesting that muscle might be a useful alternate specimen to blood or to confirm results found in blood.

The ratios might also be useful for ingestion time estimations, since the results indicate that some drugs follow a distribution pattern similar to that of alcohol, whereby blood and muscle tissue require a given time period to equilibrate after ingestion; likewise, a lag time exists during the time required for metabolism and disappearance from blood. The result in each case is a muscle/blood ratio greater than unity. Examples of these two circumstances may be illustrated by Cases 4 and 6 (Table 3), where propoxyphene has a muscle/blood ratio of 2.9 in Case 4, a probable chronic mixed drug case with a long interval between ingestion and death, and Case 6 has a corresponding ratio of less than 0.1, where death was sudden, due to a large overdose of propoxyphene.

The tricyclic antidepressants (Table 4) had a ratio of one or greater in almost all cases. From this limited number of cases, it might be useful to consider analysis of muscle for tricyclic antidepressant when artificially elevated blood levels may have arisen from postmortem release [*1*].

A limited number of cases with benzodiazepines detected (Table 5) indicated an equilibrium ratio of about 1.0, although, in two cases (Cases 3 and 4), muscle concentrations of diazepam were considerably lower than blood. Both of these cases involved acute overdose and possible rapid death.

An acute overdose of diltiazam (Table 5, Case 4), with a muscle/blood ratio of 0.2 is consistent with a rapid death after overdose with insufficient time for equilibration of the drug in muscle.

For cocaine (Table 6) and its metabolites (EME and benzoylecgonine), high muscle/blood ratios of near unity were found in most cases for cocaine, but much lower ones were found for the two metabolites. In Case 6, an acute cocaine overdose, muscle/blood ratios for cocaine, benzoylecgonine, and EME were 0.1, 0, and 0.2, respectively, con-

sistent with a rapid death and lack of time for tissue equilibration. A similar circumstance occurred in Case 8 (Table 7).

In Tables 7 and 5 (Cases 1, 2, and 3) are shown cases in which opiates were detected. It is believed that most of these cases involved intravenous heroin usage. In most of the cases in which morphine and codeine were detected, morphine was found in approximately equivalent concentration in muscle and blood (average muscle/blood ratio, 1.4), while codeine was found in very low relative concentrations in muscle (average muscle/blood ratio, 0.2). In a few cases, the lower morphine concentration in muscle (Table 7, Case 5; Table 5, Cases 2 and 3) may have indicated a rapid death with inadequate time for equilibration, while a much higher muscle/blood ratio (Case 7, Table 7) could indicate a longer time lapse since use of the drug.

In summary, most drugs detected in both blood and muscle tissue were found in approximately equivalent concentrations in the two specimens, except in cases with a presumed short survival time after drug use, in which the blood concentrations were much higher, and in a few cases with a presumed long-time interval between drug ingestion and death, when the muscle/blood ratios were greater than one. Exceptions were codeine (in association with morphine after heroin use) and the cocaine metabolites, benzoylecgonine and EME, which were always present in very much lower concentration in muscle tissue. The determination of this ratio for some drugs (such as propoxyphene and tricylic antidepressants) may be of interpretive value in estimating the time of drug ingestion relative to death and may aid in interpreting falsely elevated drug concentrations due to postmortem release [*1,2*].

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