TECHNICAL NOTE

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Comparative Study of Postmortem Barbiturates, Methadone, and Morphine in Vitreous Humor, Blood, and Tissue

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ABSTRACT: With the introduction of radioimmunoassay (RIA) techniques, it has become toxicologically possible to determine drug concentrations in postmortem vitreous humor. This study demonstrates and confirms this toxicological feasibility. In 49 medical examiner's drug related cases, postmortem tissue levels of morphine, barbiturates, and methadone were compared to the vitreous humor.

KEYWORDS: toxicology, vitreous humor, barbiturates, radioimmunoassay

The introduction of radioimmunoassay (RIA) has given greater feasibility to the toxicological analysis of vitreous humor [*1*]. This study illustrates the advantageous use of RIA in the determination of drug concentrations in postmortem vitreous humor. It presents the possibility of establishing a reliable means of predicting chronic or acute drug intoxication.

Forty-nine medical examiner's cases, which were determined to be drug related or drug induced deaths, were analyzed. Postmortem concentrations of barbiturates, methadone, and morphine that were recovered from blood, brain, liver, and kidney tissue were compared to the concentrations detected in vitreous humor via RIA analysis.

Materials and Methods

At autopsy vitreous humor specimens were collected and stored by the method of Sturner [*2*]. Biological tissues were simultaneously obtained. The determination of drugs in blood,

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brain, liver, and kidney was carried out by various accepted analytical methods including ultraviolet spectroscopy [3,4], gas-liquid chromatography [5], and RIA [6-8] in cases indicating morphine.

RIA standards were prepared in the following manner for all drugs that were to be analyzed in vitreous humor: stock standard was weighed as a free acid or base, diluted volumetrically to a concentration of 1 mg/mL, and subsequently diluted to a concentration of 10 μg/mL. Finally, 0.1 mL of 10 μg/mL was added to 3.9 mL of drug-free vitreous to give 250 ng/mL for a working stock standard. From that serial dilutions were made to give concentrations of the drug from 0 to 250 ng/mL.

In the cases of barbiturates, vitreous was diluted with drug-free vitreous to 1:100 in order for the samples to fall in the linear range of the calibrator curve. In certain cases the vitreous had to be diluted to 1:200 because of high tissue and eye fluid concentrations. Drug-free vitreous was also used in this dilution.

A concentration curve was prepared (in aqueous solution) for a comparison study of each of the drugs being analyzed. Using Abuscreen® radioimmunoassay kits (supplied by Roche Diagnostics), concentration curves of barbiturates, methadone, and morphine were prepared in aqueous solution for comparison studies and analysis. Following the protocol for the quantitation of tissue drug levels as described by Manning [9], analysis of the vitreous

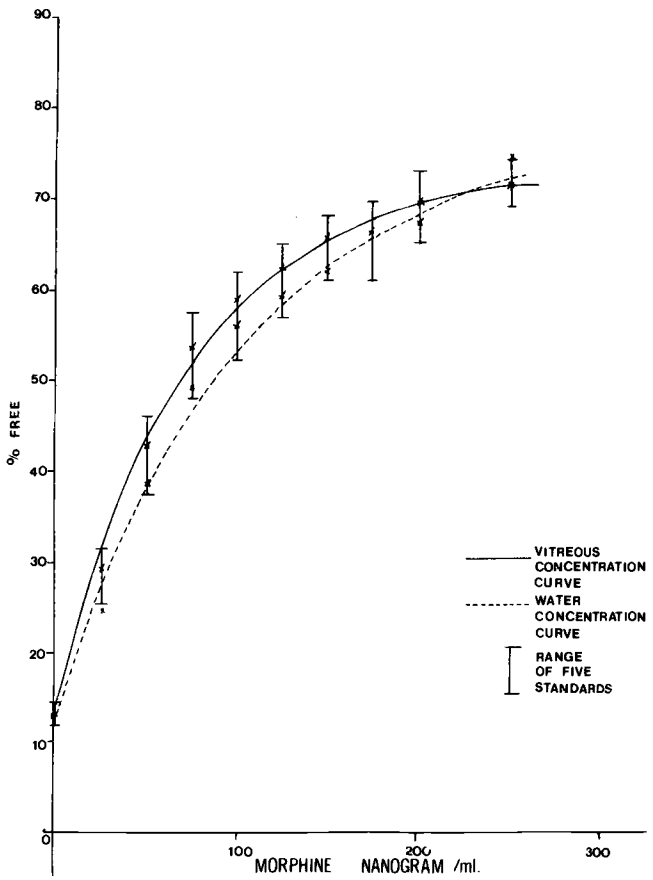

FIG. 1—*Comparison of vitreous and aqueous RIA standard curves for morphine.*

humor was performed. Standard curves for the various drugs were set up with percentage free versus concentration in nanograms per millilitres.

Results and Discussion

No significant differences can be noted between the aqueous and vitreous standard curve for morphine (Fig. 1) or methadone curves (Fig. 2). Barbiturates, on the other hand (Fig. 3) shows a difference between vitreous and aqueous curves.

Morphine

Data presented in Table 1 shows that in most cases where lethal morphine levels are present in blood and brain tissue, they are accompanied by elevated levels in vitreous humor. In only one case (12) was an elevated vitreous morphine level not accompanied by lethal blood and brain concentrations [*10*]. Further, in only one case (13) morphine was not detected in the vitreous humor. The cause of death in this particular case however, was certi-

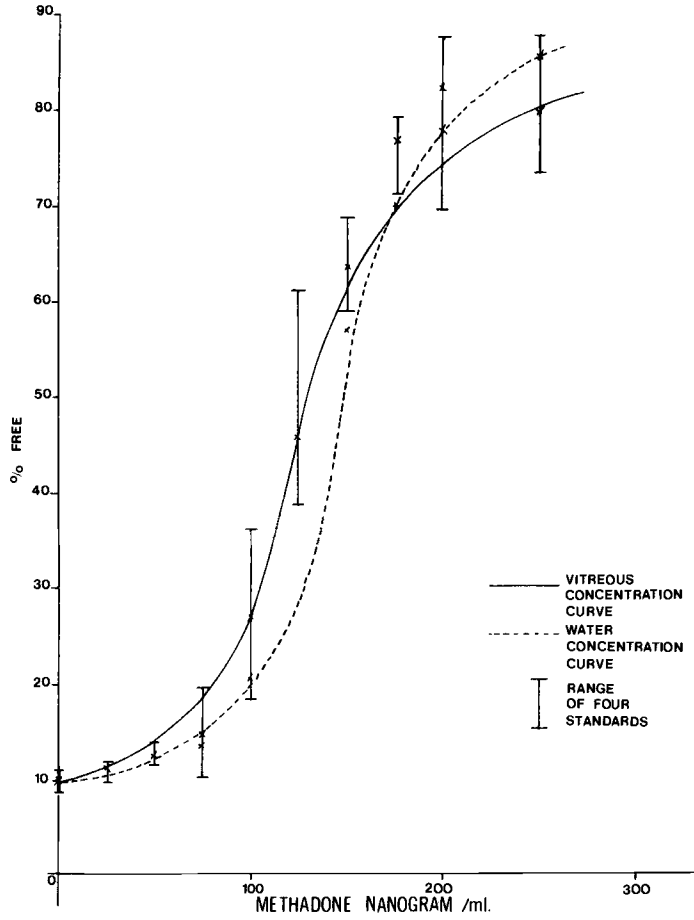

FIG. 2—*Comparison of vitreous and aqueous RIA standard curves for methadone.*

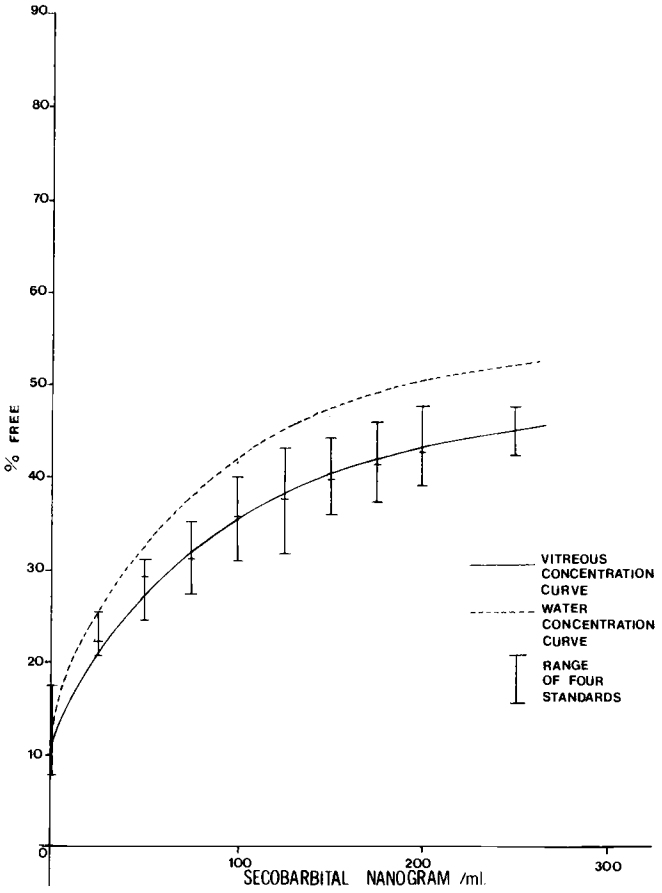

FIG. 3—*Comparison of vitreous and aqueous RIA standard curves for secobarbital.*

TABLE 1—*Morphine tissue concentrations using radioimmunoassay.*

Case	Vitreous, mg/L	Blood, mg/L	Brain, mg/kg	Liver, mg/kg	Kidney, mg/kg	Bile, mg/L	Urine, mg/L
1	0.04	0.60	0.50	0.85	1.10	1.50	...
2	0.14	1.90	0.30	3.80	0.85	0.10	17
3	0.12	4.20	0.78	2.60	3.80	0.38	156
4	0.03	0.15	0.10	0.10	0.15	...	...
5	0.10	3.50	1.00	1.70	3.00	0.2	...
6	0.11	1.00	1.00	2.40	3.50	3.00	...
7	0.05	0.80	0.60	1.80	2.00	9.00	20
8	0.08	0.20	0.05	0.25	0.25	0.40	...
9	0.03	not detected	0.05	0.10	0.10	1.75	25
10	0.08	0.60	0.33	0.73	0.25	0.05	11
11	0.03	0.05	0.05	0.05	0.05	0.20	15
12	0.08	0.25	0.05	0.75	1.15	0.65	...
13	not detected	1.70	0.20	4.50	7.00	0.95	...

fied as "acute opiate intoxication." This would indicate that death occurred before the morphine had a chance to equilibrate with the vitreous humor.

Barbiturates

As illustrated in Table 2, most cases where lethal barbiturate levels are present in blood and brain tissue, corresponding elevations are noted in vitreous levels. Of all the assays tested, it is apparent that the barbiturates show the best correlation between vitreous and blood and brain values. Since the vitreous values were approximately the same as the blood, it would appear that equilibration between blood and vitreous is rapid.

Methadone

Vitreous humor was assayed for methadone in twelve cases of known methadone overdose. Although methadone was detected in all cases, the levels were of questionable significance (as can be seen in Table 3). This would seem to lead to the conclusion that this method is not applicable to this compound for extracting meaningful data.

Conclusion

The data presented in this study agree with the observations of other researchers [*1,2,11,12*] regarding the concentrations of drugs in vitreous humor. Namely, drugs that are least affected by protein-binding, drugs with sufficient lipid solubility to transverse the blood-vitreous barrier, and drugs that are more water soluble, readily diffuse from the blood to the vitreous humor.

TABLE 2—*Barbiturate tissue concentrations using ultraviolet spectrophotometry* [3].

Case	Barbiturate	Vitreous, mg/L	Blood, mg/L	Brain, mg/kg	Liver, mg/kg	Kidney, mg/kg
14	amobarbital	8	6	11	19	10
15	amobarbital	26	28	24	50	30
16	pentobarbital	7	...	10	10	...
17	pentobarbital	8	3	4	8	7
18	pentobarbital	7	...	5	...	...
19	pentobarbital	8	13	5	8	13
20	pentobarbital	27	36	48	96	46
21	pentobarbital	7	4	5	10	7
22	pentobarbital	25	41	48	85	64
23	phenobarbital	8	15	18	17	15
24	phenobarbital	5	...	10	...	...
25	phenobarbital	2	4	...	...	...
26	phenobarbital	22	23	...	...	...
27	phenobarbital	4	...	5	...	...
28	phenobarbital	2	5	...	...	...
29	phenobarbital	8	25	...	...	...
30	phenobarbital	19	23	27	63	28
31	secobarbital	3	6	11	19	10
32	secobarbital	5	9	9	14	8
33	secobarbital	10	28	24	50	30
34	secobarbital	2	2	2	6	3
35	secobarbital	3	1	1	3	5
36	secobarbital	7	18	25	59	27
37	secobarbital	8	19	18	57	27

TABLE 3—*Methadone tissue concentrations using radioimmunoassay.*

Case	Vitreous, ng/L	Blood, mg/L	Brain, mg/kg	Liver, mg/kg[a]	Kidney, mg/kg	Bile, mg/L
38	25	...	...	1.2	...	...
39	25	...	...	3.2	...	...
40	25	...	...	2.5	...	...
41	52	...	...	5.5	...	...
42	25	...	...	1.4	...	...
43	25	...	...	2.1	...	...
44	35	...	...	3.2	...	...
45	50	...	...	4.0	...	...
46	82	1.0	2.0	6.0	8.0	7.0
47	50	1.4	0.4	1.2	2.6	5.5
48	25	...	...	3.0	...	...
49	37	...	...	2.6	...	...

[a] Methadone tissue concentration as determined by the gas chromatographic method of Robinson [5].

This study illustrates that, as a forensic science tool, RIA methodology provides an accurate and relatively direct and inexpensive method for analyzing drugs in vitreous humor. It further shows that RIA sensitivity allows the researcher to work with small quantities of limited biological substances and still obtain reliable results. This could definitely be a valuable method for the determination of chronic drug use as well as a situation of a one-time acute dose leading to death.

References

[1] Sturner, W. and Garriott, J. C., "Comparative Toxicology in Vitreous Humor and Blood," *Forensic Sciences*, Vol. 6, No. 1-2, Aug.-Sept. 1975, pp. 31-39.

[2] Sturner, W. and Coumbis, R., "The Quantitation of Ethyl Alcohol in Vitreous Humor and Blood by Gas Chromatograph," *American Journal of Clinical Pathology*, Vol. 46, No. 3, Sept. 1966, pp. 349-351.

[3] Goldbaum, L. R., "Determination of Barbiturates," *Analytical Chemistry*, Vol. 24, No. 10, Oct. 1952, p. 1604.

[4] Sunshine, I., "Ultraviolet Spectrophotometric Absorption Data," in *Handbook of Analytical Toxicology*, Vol. I, CRC Press, Cleveland, OH, 1969, pp. 814-851.

[5] Robinson, A. E. and Williams, F. M., "The Distribution of Methadone in Man," *Journal of Pharmacy and Pharmacology*, No. 23, May 1971, pp. 353-358.

[6] Spector, S. and Parker, C., "Morphine Radioimmunoassay," *Science*, Vol. 168, No. 3937, June 1970, pp. 1347-1348.

[7] Spector, S., "Quantitative Determination of Morphine in Serum by Radioimmunoassay," *Journal of Pharmacology and Experimental Therapeutics*, Vol. 178, No. 2, Aug. 1971, pp. 253-258.

[8] Coumbis, R. and Kaul, B., "Distribution of Morphine and Related Compounds in Human Tissue and Biological Fluids, Using Radioimmunoassay Techniques," *Journal of Forensic Sciences*, Vol. 19, No. 2, April 1974, pp. 307-312.

[9] Manning, T., Bidanset, J., Cohen, S., and Lukash, L., "Evaluation of The Abuscreen of Methadone," *Journal of Forensic Sciences*, Vol. 21, No. 1, Jan. 1976, pp. 112-120.

[10] Gottschalk, L. and Cravey, R., "Case Summaries of Psychoactive Drug-Involved Deaths," in *Toxicological & Pathological Studies On Psychoactive Drug-Involved Deaths*, Biomedical Publications, Davis, CA, 1980, pp. 301-362.

[11] Felby, S. and Olsen, J., "Comparative Studies of Post Mortem Barbiturates & Meprobamate in Vitreous Humor, Blood and Liver," *Journal of Forensic Sciences*, Vol. 14, No. 4, Oct. 1969, pp. 507-514.

[12] Sorensen, P. N., "The Penetration of Quinine, Salicyclic Acid, PAS, Salicyluric Acid, Barbital and Lithium Across the Vitreous Barrier of the Rabbit Eye," *Acta Pharmacologica et Toxicologica*, Vol. 29, 1971, pp. 194-208.

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