András Benkő,[1] *Ph.D.*

Toxicological Analysis of Amobarbital and Glutethimide from Bone Tissue

REFERENCE: Benkő, A., **"Toxicological Analysis of Amobarbital and Glutethimide from Bone Tissue,"** *Journal of Forensic Sciences*, JFSCA, Vol. 30, No. 3, July 1985, pp. 708-714.

ABSTRACT: Author examined cadaver organs and bone samples (sternum, rib) of drug poisoning cases. Following suitable procedures, active drug components (amobarbital, glutethimide, and so forth) were identified by gas chromatography/mass spectrometry (GC/MS). Based on results of quantitative GC analysis, relationships were sought between the active agent concentrations measured in the organs and the bone samples.

KEYWORDS: toxicology, amobarbital, glutethimide, chemical analysis, bone tissue, drug analysis, gas chromatography/mass spectrometry, gas chromatography

It is well known in classical toxicological analysis that certain toxic agents following a longer or shorter exposition can be detected from bone tissues or corneous matters after some time. Those are the compounds of lead, mercury, arsenic, and so forth [*1-5*]. Based upon recent scientific experiences, the practical postmortem toxicology raised an important question: how far can an organic compound, a pharmaceutical agent, penetrate into a bone tissue if the given compound gets into the living organism in toxic quantity?

Some publications drew our attention to the fact that the toxicological drug analysis is not hopeless even in border cases when except for the bone tissue no other test material is at disposal because of the putrefaction of the soft tissue [*6-15*]. With stimulation from known scientific works we try, with our experiments, to give an answer to the important question of what relation may be found between drug concentrations in organs and in bone tissue in poisoned people.

Method

We selected for our investigation cases where, according to preliminary data, suicide was committed with a drug. The deceased passed away without medical or hospital intervention and drug poisoning was already evident at the autopsy. Following our request, besides the standardly sent in cadaver organs we received bone remainings, that is, sternums and ribs as well. The organs of the ten cases selected were treated according to the Farago's [*16*] extraction process, while the bone samples underwent Harsányi's [*6*] extraction process modified especially for bone samples. Figure 1 shows the main steps of the extraction procedure.

Note that the spongiose with marrow and the compacta, these two parts of bone, were not isolated under our process. Recovery studies undertaken in our laboratory showed a recovery

Received for publication 14 June 1984; revised manuscript received 29 Oct. 1984; accepted for publication 2 Nov. 1984.

[1]Forensic toxicologist, National Institute of Forensic Toxicology, Budapest, Hungary.

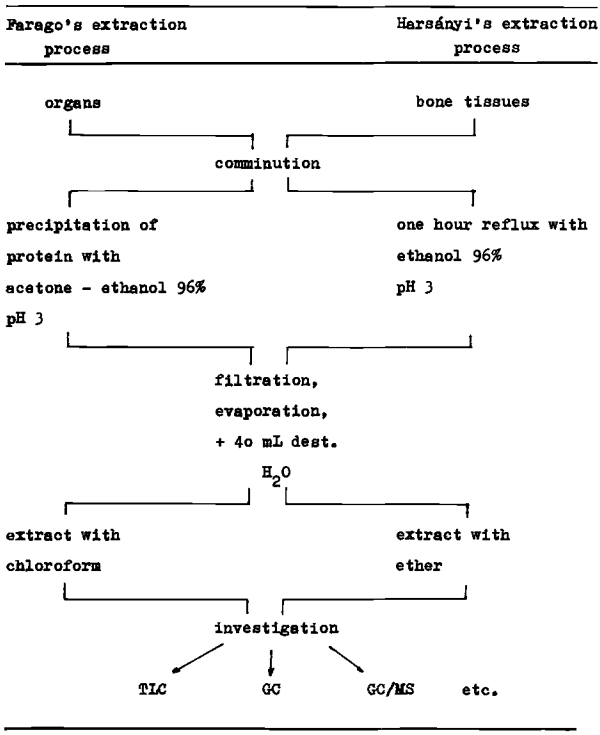

FIG. 1—*Isolation of drugs from organs and bone tissues.*

range of 57.5 ± 3.4% of amobarbital and glutethimide in cadaver organs with Farago's extraction process. These data are not found in Harsányi's extraction process, but we think that this is compensated for if we count on a recovery range of 60% in bone.

Qualitative Analysis

The qualitative analysis was performed with a HP 5985 gas chromatography/mass spectrometry (GC/MS) data system followed by the suitable isolation processes. The GC/MS parameters were as follows: A form—GC column was a 0.7-m by 2-mm inside diameter glass column packed with 2% OV-22 on Chromosorb W-HP, 100-120 mesh; column temperature, 180-250°C; programing rate, 10°C/min; injector temperature, 250°C. B form—GC column was a 2.0-m by 2-mm inside diameter stainless steel column packed with 5% SP 2100 on Supelcoport, 80-100 mesh; column temperature, 230°C, isothermal; injector temperature, 240°C.

In seven cases out of the examined ten, some amobarbital could be demonstrated. Parallel to it glutethimide was found in five cases. In one case also butobarbital and in two cases phenobartital were observed. In one of the two phenobarbital cases phenacetin and caffeine could be observed as well.

Quantitative Analysis

Quantitative analysis was performed only in those cases in which poisoning had been carried out with amobarbital and glutethimide as those permitted us to study any relations between them. The analysis was performed by a Perkin-Elmer Sigma 4B gas chromatograph.

The GC column was a 1.8-m by 2-mm inside diameter glass column packed with 3% OV-101 on Gaschrom Q, 80–100 mesh. GC conditions were as follows: column temperature, 210°C, isothermal; injector temperature, 250°C; and nitrogen carrier gas flow rate, 30 mL/min. A flame-ionization detector (FID) was used for the analysis. Retention time of amobarbital and glutethimide was 1.7 and 2.7 min. The quantitative analysis was made without internal standard.

The organ and bone concentrations of amobarbital and glutethimide are shown in Table 1. The results were averaged and the average values given in a column diagram (Fig. 2). Table 2 shows the average values as well as the corrected error ($\bar{x} \pm s_{n-1}$).

Discussion

Based on our results, calculations were made to investigate the relations of active agent concentrations measured in organs and bone samples.

The most conspicuous relation can be observed in Table 3 indicating the liver/blood quotient and the sternum concentration. This table shows clearly that the liver/blood quotient was found in all poison cases within a narrow band, in the case of amobarbital between 1.5 and 2.5, for glutethimide between 1.2 and 2.1. If, however, these values are shown in the function of sternum concentration, it is seen that the values can be divided into two groups according to individual active agents.

Examining the amobarbital values both on preliminary data and those of the autopsy reports it could be assumed that death must have followed in shorter time after taking the drug than in the case of the other group. It is obvious that the amount of drug getting into the sternum within a short time is relatively little. In case of an elongated death the measurable concentrations are higher. A similar relation can be observed also in connection with glutethimide.

The experiences gained in our experiments can be used, first of all, in the field of forensic toxicology, as the resistence of bone tissues to postmortem putrefaction enables us to prolong

TABLE 1—*Concentration values of amobarbital and glutethimide measured in individual cases.*

	Female, Sz. M.	Female, B. G.	Female, K. L.	Female, T. I.	Male, I. J.	Male, F. I.	Male, Sz. F.
				Age			
	85	49	42	76	33	56	57
			AMOBARBITAL, MG/KG				
Organs and bone samples							
Stomach	3874.0	60.1	163.2	82.5	103.5	1159.0	290.2
Intestine	118.9	50.2	78.4	...	129.2	2564.2	252.6
Liver	42.7	34.0	54.5	73.6	38.8	63.5	50.2
Kidney	37.4	33.8	39.3	39.4	35.2	34.5	57.5
Blood	28.2	16.2	26.6	36.5	25.1	26.1	28.7
Rib	6.9	17.6	13.0	22.0	18.3	6.0	8.3
Sternum	9.2	16.5	15.0	2.7	7.1	5.0	11.8
			GLUTETHIMIDE, MG/KG				
Stomach	2007.1	38.4	...	...	63.5	523.6	304.4
Intestine	32.8	23.7	...	...	43.9	492.8	165.2
Liver	29.4	26.1	...	...	28.5	44.6	35.7
Kidney	34.8	25.4	...	...	25.0	30.2	36.9
Blood	22.5	15.8	...	...	21.6	21.3	29.0
Rib	9.1	1.5	...	...	3.4	6.1	6.6
Sternum	8.1	4.9	...	...	4.3	4.4	13.0

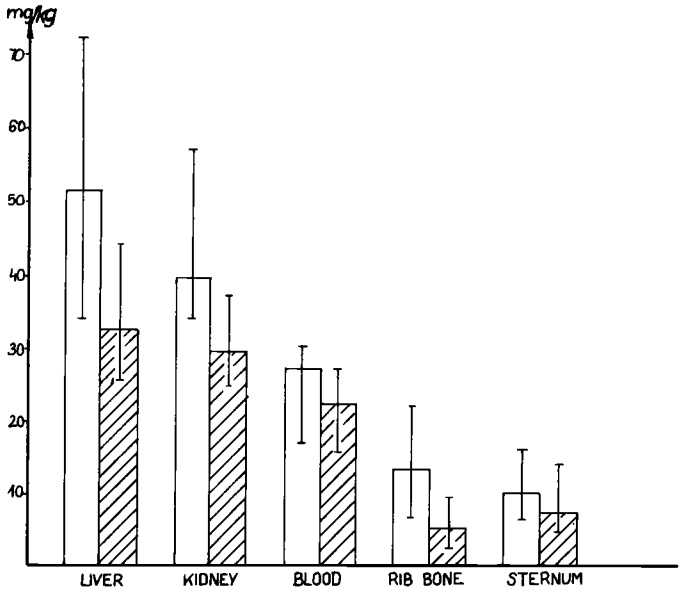

FIG. 2—*In the organs and bone samples of seven amobarbital and five glutethimide poisoned cases, the measured average drug concentration is in mg/kg. Also the highest and the lowest concentration values are figured on the column diagram. Shaded columns indicate the glutethimide values.*

TABLE 2—*Average ($\bar{x}$, mg/kg) of concentrations of amobarbital and glutethimide poisoning cases measured in organs and bone samples, as well as the corrected error (s_{n-1}) values.*

Organs and Bone Samples	Amobarbital $n = 7$	$\bar{x} \pm s_{n-1}$	Glutethimide $n = 5$
Liver	51.0 ± 14.0		32.9 ± 6.7
Kidney	39.6 ± 7.6		30.5 ± 4.8
Blood	26.8 + 5.5		22.0 ± 4.2
Rib	13.2 ± 5.8		5.3 ± 2.6
Sternum	9.6 + 4.7		6.9 ± 3.3

the period of investigation capability of toxicological analysis. Thus, even a year after death has set in we are able to give an opinion about the presence of a drug, possibly about the circumstances of death.

Finally, we should like to introduce a "typical case" that was investigated at the end of 1981.

Case Report

V. Cs., a 33-year-old woman, was treated in a neurological institute. Several times she wandered away. The remnants of her skeleton were found about a year and a half later in a big forest near the neurological institute. The possibility of suicide with drugs could be assumed as, before her disappearance, she tried to commit suicide with drugs several times.

The toxicological investigations identified 9.3-mg/kg amobarbital in the rib, 25.6 mg/kg in the sternum, and 9.2 mg/kg in the vertebra.

TABLE 3—*Numerical values of liver/blood quotients and concentrations measured in sternums.*

Serial Numbers and Names	Liver/Blood Quotients		Concentrations, mg/kg in Sternums	
	A[a]	G[a]	A	G
	ELONGATED EXITUS			
1. Sz. M.	1.51	1.30	9.2	8.1
2. B. G.	2.09	1.65	16.5	4.9
3. I. J.	1.54	1.31	7.1	4.3
4. Sz. F.	1.74	1.23	11.8	13.0
5. K. L.	2.04	...	15.0	...
	RAPID EXITUS			
6. F. I.	2.43	2.09	5.0	4.1
7. T.I.	2.01	...	2.7	...

[a]A = amobarbital, G = glutethimide.

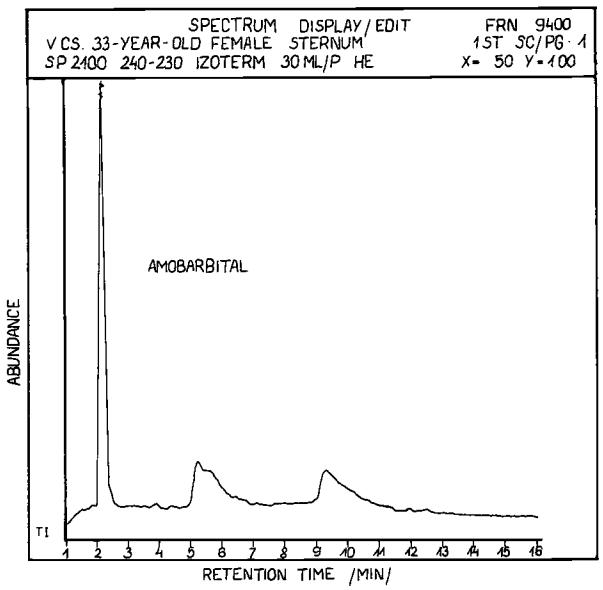

FIG. 3— *V. Cs., female 33 years old. GC/MS total ion chromatograms from sternum.*

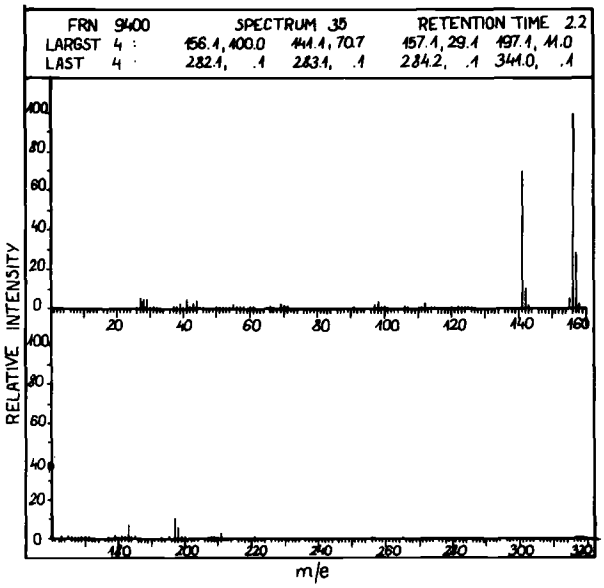

FIG. 4—*Electron impact (EI) mass spectrum of amobarbital from the sternum of V. Cs.*

Figure 3 shows the total ion chromatogram of the sternum of V. Cs. where the amobarbital peak appearing in the second minute is seen clearly—its mass spectrum is shown in Fig. 4. Further, the human fatty acid contaminations in the fifth-sixth and in the ninth-tenth minutes are shown.

Acknowledgment

At the completion of our work we wish to express our thanks to Dr. Antal Kőrösi, chemist, who gave us valuable help in the GC/MS examinations, as well as to Tamás Pozsgai, M.D., police medical officer, who put the investigation material requested at our disposal.

References

[1] Preuss, R., *Gadamers Lechrbuch der chemischen Toxikologie und Anleitung zur Ausmittelung der Gifte*. Vandechoeck und Ruprecht, Göttingen, 1979.
[2] Moeschlin, G., *Klinik und Therapie der Vergiftungen*, Georg Thieme Verlag, Stuttgart, 1972.
[3] Csiky, P., "Klinikai toxikológia," *Medicina*, Budapest, 1976.
[4] Knoll, J., "Gyógyszertan," *Medicina*, Budapest, 1982.
[5] Somogyi, E., "Igazságügyi orvostan," *Medicina*, Budapest, 1982.
[6] Harsányi, L. and Láng, Á., "Altatószerkimutatás csontszövetből," *Morphologiai és Igazságügyi Orvosi Szemle*, Vol. 19, No. 1, Jan. 1979, pp. 23-26.
[7] Terazawa, K. and Takatori, T., "Determination of Aminopyrine and Cyclobarbital from a Skeleton by Radiommunoassay," *Journal of Forensic Sciences*, Vol. 27, No. 4, Oct. 1982, pp. 844-847.
[8] Bellus, E. and Kenyeres, I., "A digitális mérgezésekről, hatósági boncolási anyag alapján," *Morphologiai és Igazságügyi Orvosi Szemle*. Vol. 22, No. 3, July 1982, pp. 195-197.
[9] Láng, Á. and Harsányi, L., "Lithium kimutatása csontszövetből," 7th Annual Meeting, Hungarian Society of Medical Forensic Sciences, Pécs, 24-26 Aug. 1983.
[10] Harsányi, L., Láng, A., and Farkas, Gy., "Schlaf mittelnachweis im Knochengewebe," *Archiv für Kriminologie*, Vol. 169, No. 5/6, May/June, 1982, pp. 149-154.
[11] Noguchi, T., Nakamura, G. R., and Griesmer, E. C., "Drug Analyses of Skeletonizing Remains," *Journal of Forensic Sciences*, Vol. 23, No. 2, July, 1978, pp. 490-492.

[12] Winek, C. L. and Esposito, F. M., "Comparative Study of Ethanol Levels on Blood versus Bone Marrow, Vitreous Humor, Bile and Urine," *Forensic Science International*, Vol. 17, No. 1, Jan./Feb. 1981, pp. 27-36.
[13] Winek, C. L., Pluskota, M., and Wahba, W. W., "Plasma Versus Bone Marrow Flurazepam Concentration in Rabbits," *Forensic Science International*, Vol. 19, No. 2, March/April 1982, pp. 155-163.
[14] King, D. L., "Plasma Versus Bone Marrow and Vitreous Humor Diazepam Concentrations in Rabbits," Master's thesis, Duquene University Graduate School of Arts and Sciences, Pittsburgh, 1982.
[15] Constantiono, A. G., "The Determination and Comparison of Postmortem Blood and Bone Marrow Pentobarbital Concentrations," Master's thesis, Duquene University Graduate School of Arts and Sciences, Pittsburgh, 1984.
[16] Farago, E., "Fatal, Suicidal Malathion Poisonings," *Archiv für Toxikologie*, Vol. 23, No. 1, July 1967, pp. 11-16.

Address requests for reprints or additional information to
András Benkő, Ph.D.
National Institute of Forensic Toxicology
1012 Budapest I. Győző u. 11.
Hungary