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Determination of Lead in Paired Samples of Human Blood and Synovial Fluid

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In spite of the numerous papers published on the toxicity of lead in mammals, little is known about its effects in synovial fluid and bone joints. Our literature search showed a lack of quantitative studies regarding the concentration of lead in human synovial fluid; in addition, normal values regarding the threshold for poisoning by lead in that fluid are unknown. The available literature published corresponds to samples of human wounds by lead bullets localized close to or in a joint (Machle 1940). Some of those papers dealing with lead-induced arthritis include symptoms of plumbism. They clearly demonstrate the ability of synovial fluid to dissolve lead and thereby make it available for systemic absorption (Dillman et al. 1979). The molecular mechanism whereby this process is performed is still unknown, although it would be of interest because of its possible relationship with joint pain, a common problem in patients with lead poisoning that so far has not been fully explained (Zuñiga-Charles et al. 1981). In a series of experiments with cattle, we found an average ratio of lead between synovial fluid and blood for paired observations of 4.2, although we have not found similar reports, and there is not sufficient information to make a total interpretation of these data.

The purpose of this study was to determine the concentration of lead in synovial fluid and blood of corpses and to establish a possible numerical relationship between those two variables.

MATERIALS AND METHODS

Experiments were performed on 40 human corpses of normal persons resident in Monterrey City, of both sexes (36 men and 4 women),

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weighing from 36-89 kg, deceased by accident on the streets. The samples were removed from corpses undergoing legal autopsy at the Autonomous University of Nuevo León.

Paired samples of synovial fluid and whole blood were collected from each body approximately 3 h (latent period) after demise. Synovial fluid from the knee joint and blood samples from the declivity spot of thoracic or cranial cavity were each withdrawn and transferred to heparinized tubes. In some samples, red blood cells were introduced in the process of arthrocentes.
Samples with an hemolysis of two crosses or lower were used (two crosses = 0.1 g % of hemoglobin). Synovial fluid contaminated with blood was centrifuged to remove cellular debris. The samples were immediately stored at -5°C until analyses. Measurements were made before 5 days after sampling.

Each defrosted sample of synovial fluid was placed in a 250 mL Kjeldahl flask for acid digestion (Villarreal-Treviño and Villegas-Navarro 1987; Villegas-Navarro et al. 1992). Blood and digested synovial fluid were treated with ammonium pyrrolidine dithiocarbamate and extracted with methylisobutyl ketone (Hessel 1968). Lead was determined in a Perkin Elmer (Model 370) atomic absorption spectrophotometer. The precision and accuracy of the determination of metal concentration including acid digestion and sample preparation, were estimated in synovial fluid by their recovery from seven spiked samples with lead nitrate and showed to be 91 $\pm$ 15 % (mean $\pm$ SD).

The Kolmogorov-Smirnov one-sample statistic test was used to show normal distribution of data (Daniel 1987). Parametric methods of correlation and regression with a probability level of $p < 0.05$ for significance were used.

Results are expressed as the direct values and the mean $\pm$ SD and the coefficient of variation (CV).

RESULTS AND DISSCUSSION

Table 1 shows the lead levels for paired samples of synovial fluid and blood; their ranges of concentration were 3.14 to 40.85 and 1.70 to 28.43 µg/dL respectively, and their statistic variability expressed as mean $\pm$ SD were 11.54 $\pm$ 7.93 µg/dL and 13.85 $\pm$ 7.35 µg/dL with CV of 68.70 and 53.00 % in the same order. The weight of collected samples was 5.67 $\pm$ 1.96 g with a CV of 13.8 % for synovial fluid and 10.9 $\pm$ 2.6 g with a CV of 11.1 for blood.

Table 1. Lead concentration values (μg/dL) for paired samples of synovial fluid and whole blood.

Experiment Number	Synovial Fluid	Whole Blood	Experiment Number	Synovial Fluid	Whole Blood
1	8.27	23.99	21	7.41	4.79
2	7.98	26.56	22	5.70	18.01
3	7.98	21.07	23	6.62	16.91
4	12.24	20.79	24	7.17	5.59
5	11.70	10.88	25	9.40	19.65
6	7.45	12.62	26	19.31	16.31
7	10.85	19.65	27	5.22	14.79
8	3.14	22.44	28	4.88	7.69
9	14.28	6.32	29	5.09	8.57
10	5.13	15.41	30	7.02	11.56
11	10.26	15.63	31	3.87	5.98
12	11.26	15.66	32	11.04	27.52
13	11.93	10.40	33	24.89	7.95
14	18.31	5.14	34	13.33	5.67
15	5.41	9.89	35	13.00	28.43
16	9.32	13.16	36	31.82	3.72
17	10.27	15.06	37	27.82	19.13
18	9.16	4.25	38	40.85	10.90
19	3.96	1.70	39	13.32	24.67
20	8.29	5.55	40	17.00	19.99

The linear, logarithmic and exponential correlation analyses between both biological fluids had the following parameters: slope 0.07, 0.33 and 0.05, the intercept 12.53, 13.10 and 8.63 and coefficient of correlation 0.065, 0.026 and 0.053 respectively, indicating that the two sets of data are not related ($p > 0.05$).

The values for blood lead levels are in agreement with those reported for normal Mexicans (Molina-Ballesteros et al. 1981). This shows that lead contamination in residents from Monterrey City, a highly industrialized zone, is not higher than the values accepted internationally as normal (Corey and Galvão 1989).

The mean value of blood lead determined in corpses was 13.85 μg/dL; for people in vivo was 13.60 μg/dL (Junco-Muñoz and Arrieta-Alcalde in press). Therefore, the corpses display normal lead concentrations. Furthermore, it could be inferred that a latency period does not change the lead concentration in blood.

We would like to draw the attention to the fact that lead level in the blood of human corpses is different for the bovine corpses 13.35 ± 7.35 and 3.54 ± 1.31 μg/dL ($p < 0.05$) (Villegas-Navarro et al. 1992) respectively, in spite that both populations were residing in Nuevo León State, and that the lead concentration in humans from rural areas was 11.51 μg/dL, indicating that there is not a significant difference in lead level in blood at 5% of error, for resident humans of urban or rural regions from Nuevo León State (Junco-Muñoz and Arrieta-Alcalde in press). Therefore the difference in blood lead concentration between humans and bovines can not be due to the residence place. Besides, it is a common observation that some laboratory animals, in spite of receiving high lead doses, their blood lead levels hardly induce clinical signs and reach levels as high as those in humans (Mitema et al. 1980). We do not know the cause of this difference in blood lead level but *in vivo* studies in dog, rabbits and rats showed this phenomenon (Fig. 1). In humans, Raghavan et al. (1980) reported the lead content in various fractions of red blood cell hemolysates; the most significant finding was a 10,000 MW lead-binding protein. It is possible that the concentration difference of blood lead between human and other species could be due to lead-binding proteins in erythrocytes, although a more detalled observations is needed.

The *in vivo* concentration of lead in synovial fluid in humans is not known, in this work, we show the presence of the metal in synovial fluid of corpses. According to relatives and forensic surgeons, the possibility of occupational lead exposure was discarded. Then, lead in synovial fluid could be due to chronic exposure to environmental lead. Whether postmortem lead concentration in synovial fluid is similar to the lead concentration *in vivo* is not known; therefore, a more detailed and *ex profeso* observations is needed. On the other hand, bullet wounds causing lead concentration as high as 1000 μg/dL of lead in synovial fluid have been reported in patients; some of them also have developed systemic clinical plumbism (Machle 1940; Slavin et al. 1988). Based on those reports and our data, it is feasible to believe that lead enters or leaves the joint fluid, but the mechanism by which this may happen remains still unsolved.

The errors introduced into the synovial fluid values by blood contamination does not nullify the average ratio between both fluids; only three samples of two crosses were used (*vide supra*) and, although the ratio for single paired samples could be changed, the average ratio can not be changed because the mean lead concentration in both fluids is similar.

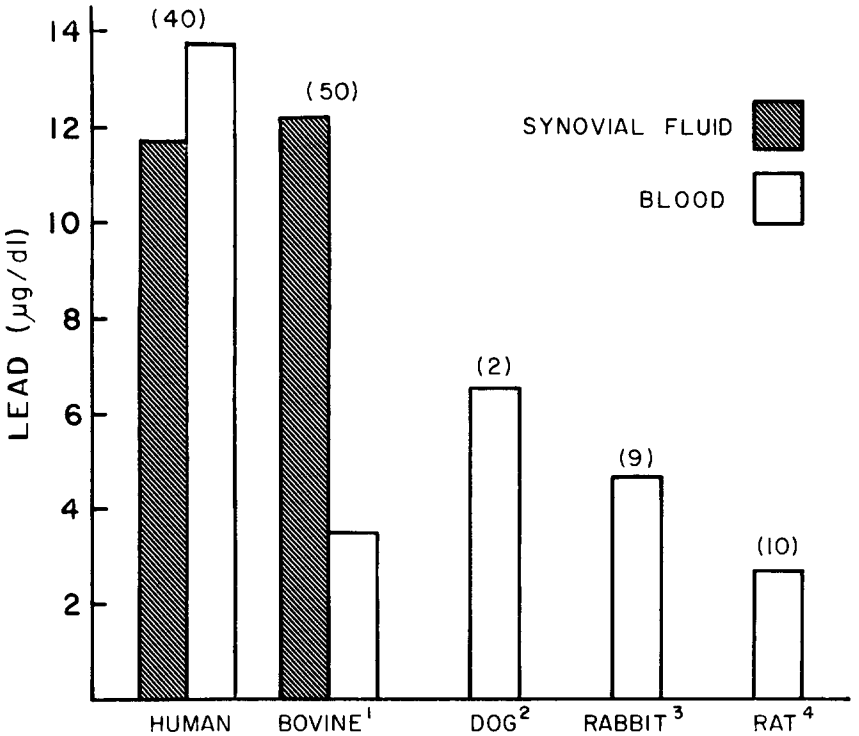

Figure 1. Blood and synovial fluid lead concentration of several domestic species. (N) = sample sizes. [1]Villegas-Navarro et al. 1992. [2]Mitema et al. 1980. [3]Villarreal-Treviño and Villegas-Navarro 1987. [4]Rossouw et al. 1990.

In spite of the fact that synovial fluid is to a large extent a practically acellular plasma dialysate and blood a tissue, there are no significant statistical differences between both fluids for paired observations ($p > 0.05$). Contrariwise, other trace metals such as copper, iron, zinc, gold, aluminum and landthanides, are inequally divided (Niedermeier et al. 1962; Esposito et al. 1986), and the distribution of other electrolytes is in agreement with the laws governing Donnan equilibrium (Gardner 1960). To our knowledge, the total inorganic phosphate has about the same concentration in synovial fluid and serum (Gardner 1960), and now we are reporting that total lead is also equally divided between the two fluids. We cannot offer any explanation for this effect at this time.

The correlation analysis between both biological fluids indicated that the two sets of data are neither linear, logarithmic nor exponentialy related ($r=0.043$; 0.026 and 0.053 respectively).

Similarly, there is no direct relationship between blood lead and clinical effects (Molina 1986; Cory-Slechta et al. 1983). However, it is clear that are myalgia and arthralgia the most frequently mentioned symptoms in patients with lead poisoning (Zuñiga-Charles et al. 1981) and subtle effects of lead on synovial fluid and joints are of major concern, and should be evaluated as a component of strategies for environmental lead toxicity.

Finally, in spite of the scattering of the values of lead in synovial fluid, the fit to a normal curve is promising ($p < 0.01$); however, the number of samples should be increased so that the results represent the population, and the range of "normal concentration" of lead in synovial fluid could be inferred.

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