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# Differential Diagnosis Between Vital and Postmortem Wounds: Ions as Markers

C. HERNÁNDEZ-CUETO, E. VILLANUEVA, A. LUNA

Department of Legal Medicine, Faculty of Medicine, 18071 Granada, Spain

## ÖLÜMDEN ÖNCE VE SONRA MEYDANA GELEN YARALARIN AYIRICI TANISI: İŞARET (MARKER) İYONLAR

### Özet

Adlî tıptaki önemli konulardan birisi de, cesetlerin üzerindeki yaraların ne zaman (ölümden önce veya sonra) meydana geldiğini tesbit edebilmektir. Bu işlem için, atomik absorpsiyon spektrofotometresi *(AAS)* kullanarak yara yerindeki Ca, Mg, Cu, Zn, Fe, Na, K iyonlarının değişimini inceledik. Deneylerimizde 56 domuzun kullanıldığı bu araştırmada, canlı hayvanlar yara oluşturulduktan değişik süreler sonrasında öldürüldüler; bu süreler 0, 5, 15, 30, 60 dakika , 3 ve 6 saat olarak uygulandı. Ayrıca öldürülen her domuzda, 10 dakika sonra postmortem yara meydana getirme işlemi de yapıldı. Çalışmanın sonucunda, kalsiyum iyonunun yara tipinin belirlenmesinde en uygun element olduğu görüldü. Bu teknik kolay, kesin ve yara tipinin belirlenmesinde yeterli bulduğu için önerilmektedir.

### Summary

We have studied the evolution of the ions of Ca, Mg, Cu, Zn, Fe, Na and K, with Atomic Absorption Spectrophotometry *(AAS)* to differentiate the vital and postmortem wounds which is very important in legal medicine. Fifty-six pigs were used in the experiments; every animal suffered one vital wound and one postmortem wound 10 min after death. The time elapsed between the moment of vital wounding and the moment of death was 0, 5, 15, 30 min and 1, 3, 6 h. At the end of the project, calcium ions was found a precious element in specification the type of wounds. We propose the technique which is easy, exact and efficient in estimating the origin of wounds.

**Keywords:** *Vital and postmortem wounds - Differential diagnosis - AAS - Ions*

*(*) This paper has been partially presented at the 10th Meeting of the I.A.F.S., Oxford, V.L., 1984.*

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## INTRODUCTION

Differential diagnosis between vital and postmortem wounds, and sometimes to establish the data, may be a transcendent and important problem for a practitioner forensic pathologist. Besides, in many times, to make this differential diagnostic may be fundamental, even the due, for explaining the death problem (1-3).

As has been published, many methods have been proposed to develop vitality markers: histamine, serotonin, enzymes, etc. (4-11).

In this work, we try to check the ability of a new group of biochemical markers: *the ions*. In a former work (12), we studied the behavior of some ions (calcium, magnesium, copper and zinc) with the same purpose. Similar studies were made by *Borrielo* and co-workers (13,14) with equivalent results, but testing only iron and zinc.

We have studied the levels of the ions calcium, magnesium, copper, zinc, iron, sodium and potassium at the edge of vital and postmortem incised wounds made in pig skin. Changes in levels were hoped according to the importance of the physiological role that ions plays in the biologic response of the tissues after wounding (12-15).

## MATERIAL AND METHODS

We have employed 56 domestic pigs (4 months old; average weight 100 kg) grouped in seven experimental series of eight animals each one. The only difference among the series was the time of evolution of the wounds (time elapsed between the moment of vital wounding and the moment of the death); these times were of 0, 5, 15 and 30 minutes and 1, 3 and 6 hours.

Every animal suffered one vital wound, with a time of evolution according to its serie, and also a postmortem wound ten minutes after death in an homolateral place of the back.

After washing and shaving, we obtained the wounds from the pig (a piece of sink including 12 cm around the cut), and, once fat free the tissue samples were divided in the following zones:

A : Control zone. Piece of skin without wounding, $B_1$ : Vital wound edge until 3 mm, $B_2$ : Vital wound edge from 3 mm, $C_1$ : Postmortem wound edge until 3 mm, $C_2$ : Postmortem wound edge from 3 mm. Perfectly identified and classified, the zones were frozen at -30° C until using.

Ion determinations were made by calcinating the different zones ( weight : 1 $\mu$ ) in an electiric oven at 500°C for 6 hours. Residues were washed with pure HCI *(Merck)* and dried out. Final residues were carefully diluted in 25 mL of HCI 3% (*Merck*) where were made the different determinations.

These determinations were made by Atomic Absorption Spectrophotometry *(Perkin Elmer, mod.560)* using hollow cathode lamps *(Perkin Elmer)* for Ca, Mg, Cu, Fe and Zn. Na and K were measured by emission. As control and calibration, we used standard solution for each element *(Carlo Erba)* diluted in HCI 3% (Table 1).

Statistical studies were carried out using *Student T-test* for coupled samples and linear regression tests.

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**Table 1.** Ions analytical conditions for flame AAS.

| Element | Wavelength (nm) | Mode | Slit (nm) | Fuel ($Kg/cm^2$) | Oxidant ($Kg/cm^2$) | Standard Conc. |
|---|---|---|---|---|---|---|
| Ca | 422.7 | Abs. | 0.7 | 40 | 55 | 0.10 mg %<br>0.20 mg % |
| Mg | 285.2 | Abs. | 0.7 | 40 | 20 | 50 µg %<br>100 µg % |
| Cu | 324.7 | Abs. | 0.7 | 40 | 20 | 50 µg %<br>100 µg % |
| Zn | 213.9 | Abs. | 0.7 | 40 | 20 | 50 µg %<br>100 µg % |
| Fe | 248.3 | Abs. | 0.2 | 40 | 20 | 50 µg %<br>100 µg % |
| Na | 589.0 | Em. | 0.2 | 40 | 20 | 0.024 mEq/L<br>0.030 mEq/L |
| K | 766.5 | Em. | 0.2 | 40 | 20 | 0.040 mEq/L<br>0.050 mEq/L |

Abs.: Absorption; Em.: Emission

## RESULTS AND DISCUSSION

Mean values (x) and standard deviations (SD) are expressed in Figures 1 to 7, including every marker in all the times of evolution.

Table 2 shows the results with statistical signification. The main problem, we have after studying the different results may be the great SD obtained in many ions, appearing difficulties to interpret the final results. It can be explained in many ways, although we think that the main reasons are: different origin of the animals, skin differences (pigmentation, number of hairs, thickness, etc.), bruises and traumatic antecedents, that were macroscopically unobservables. All of these factors do origin great SD mainly in the control zones (A).

The variation coefficients (VC) of the control zones (A) for the different ions are: Ca: 13.33%, Mg: 19.49%, Cu: 25.18 %, Zn : 16.20 %, Fe : 24.31 %, Na : 16.22 %, K: 17.58 %.

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As has previously been stated, these results express differences among animals, but not in the same animal itself. The is why we think that ions can perfectly be used as vitality markers because when we do test the difference in ion concentration between the vital ($B_1$, $B_2$) and postmortem ($C_1$, $C_2$) wound of the same animal, these differences do exist and are quite evidents. Nevertheless, these great SD limit ion's application to determine wound's data at all.

**Table 2.** Statistical signification for the ions in the different groups.

| Marker | Group | Statistical Signification |
|---|---|---|
| Ca | 1st ( 0 min ) | p <0.001 |
| | 2nd ( 5 min ) | p <0.001 |
| | 3rd ( 15 min ) | p <0.025 |
| | 4th ( 30 min ) | p <0.025 |
| | 5th ( 1 h ) | p <0.050 |
| | 6th ( 3 h ) | p <0.010 |
| | 7th ( 6 h ) | p <0.005 |
| Mg | 1st ( 0 min ) | p <0.001 |
| | 7th ( 6 h ) | p <0.010 |
| Cu | 1st ( 0 min ) | p <0.005 |
| | 2nd ( 5 min ) | p <0.001 |
| | 6th ( 3 h ) | p <0.025 |
| Zn | 1st ( 0 min ) | p <0.001 |
| | 2nd ( 5 min ) | p <0.001 |
| | 4th ( 30 min ) | p <0.025 |
| Fe | 1st ( 0 min ) | p <0.001 |
| | 2nd ( 5 min ) | p <0.001 |
| | 4th ( 30 min ) | p <0.001 |
| | 6th ( 3 h ) | p <0.001 |
| Na | 1st ( 0 min ) | p <0.001 |
| | 2nd ( 5 min ) | p <0.010 |
| | 6th ( 3 h ) | p <0.001 |
| K | 2nd ( 5 min ) | p <0.050 |
| | 4th ( 30 min ) | p <0.025 |
| | 5th ( 1 h ) | p <0.025 |
| | 6th ( 3 h ) | p <0.001 |
| | 7th ( 6 h ) | p <0.001 |

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In all the markers (Figs.1-7), vital wound ion levels ($B_1$, $B_2$) are clearly greater than the postmortem ones ($C_1$,$C_2$) and that the control zone (A). So, it is possible to demonstrate the vitality of a wound testing these elements, as we have statistically confirmed (Table 2).

We have detected statistically significative differences for calcium in all the series studied (Table 2). We think, it should be originated by its very important physiological role: histamine and serotonin release, lysosomal enzymes and phospholipase $A_2$ activation.

The behavior of the remainder ions is quite similar to the calcium one, although they don't have a statistical signification as perfect as the calcium one, Their physiological role, collaborating in many enzymatic reactions that are increased in tissue reparation after injury could perfectly justify their increase. Besides, blood cell deposition and accumulation, mononuclear destruction, etc., are phenomens that do increase ions in these places.

We have not found statistically significative differences in the values of the ions along their evolution. We think, it is due to the great number of factors that can influence the levels (hemorrhage magnitude, reaction of the tissue damaged, hypoxia and acidosis, etc.) that are not equivalent among all the animals. That is why we cannot use ions to determine the data of a wound.

To summarize, we do consider that the determination of the ions Ca, Mg, Cu, Zn, Fe, K and Na is a useful method to determine the differential diagnosis between vital and postmortem wounds. This is a quick and easy method with realiable results. Nevertheless, it is not useful to determine wound's data.

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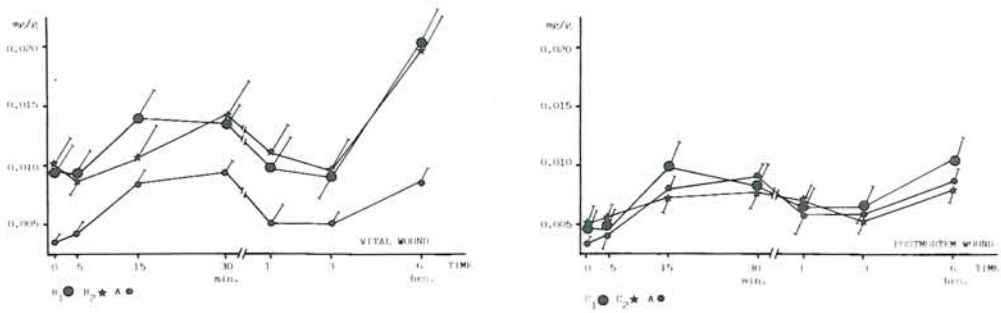


Fig. 1. Calcium evolution along the time.

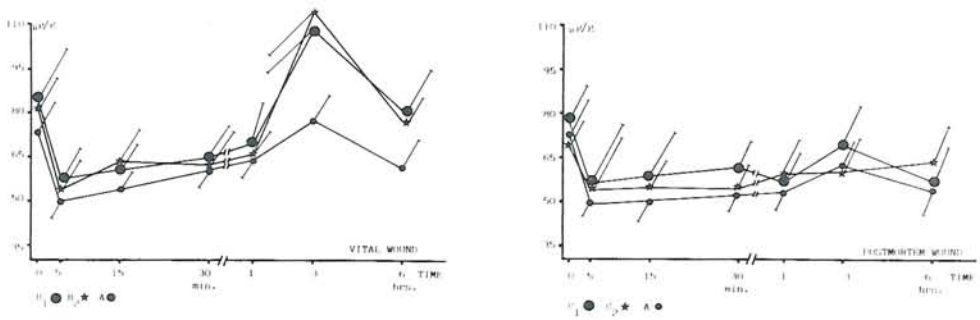


Fig. 2. Magnesium evolution along the time.

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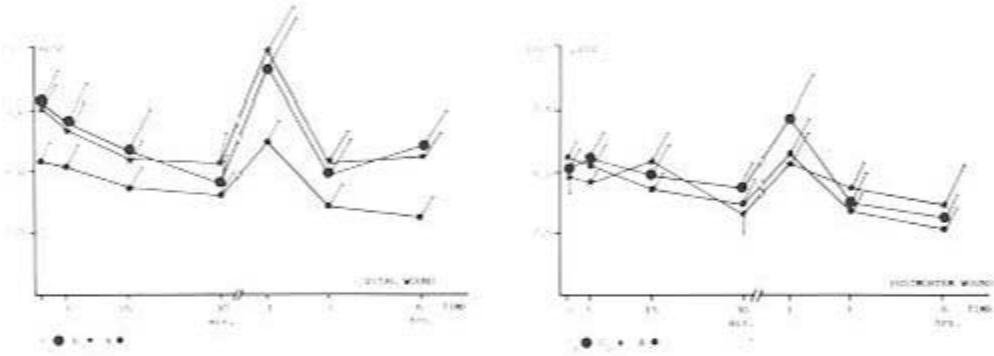

Fig. 3. Copper evolution along the time.

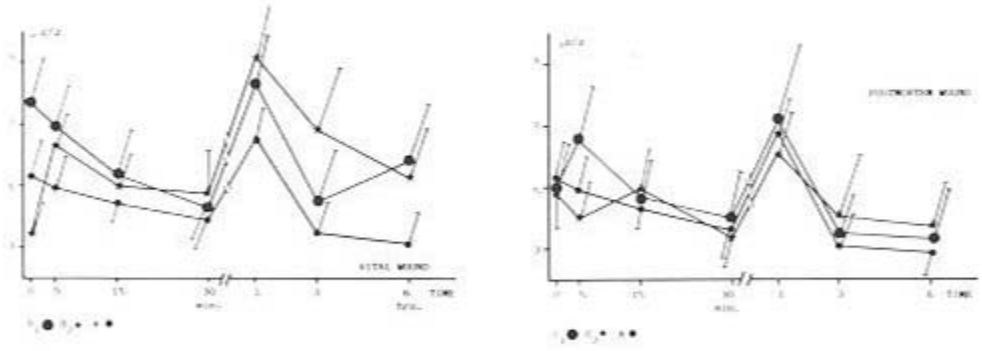

Fig. 4. Zinc evolution along the time.

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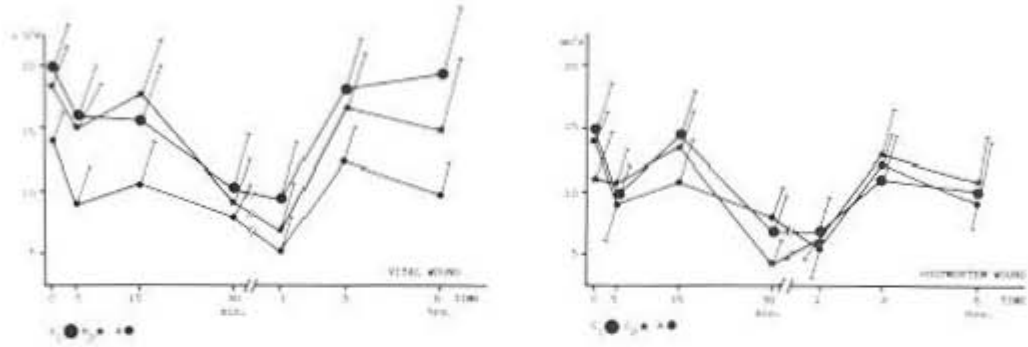

Fig. 5. Iron evolution along the time.

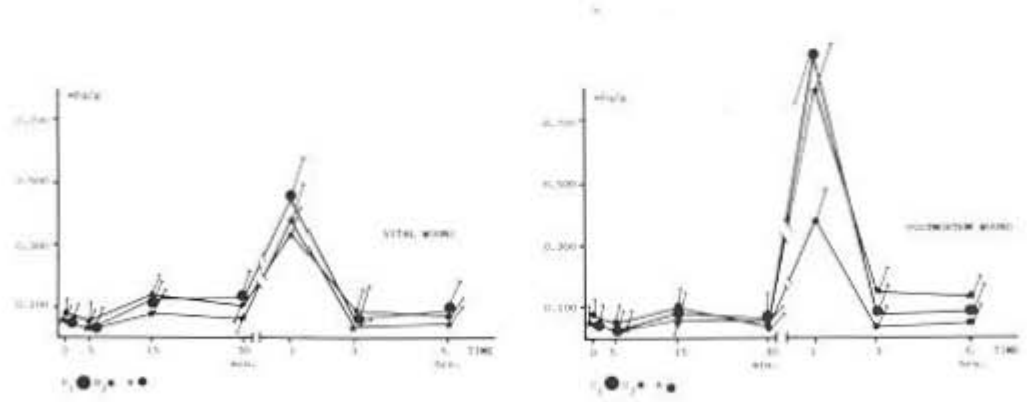

Fig. 6. Sodium evolution along the time.

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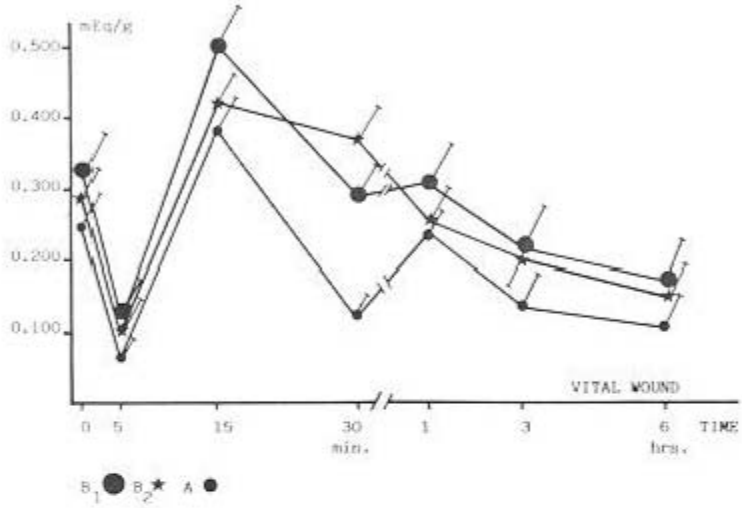


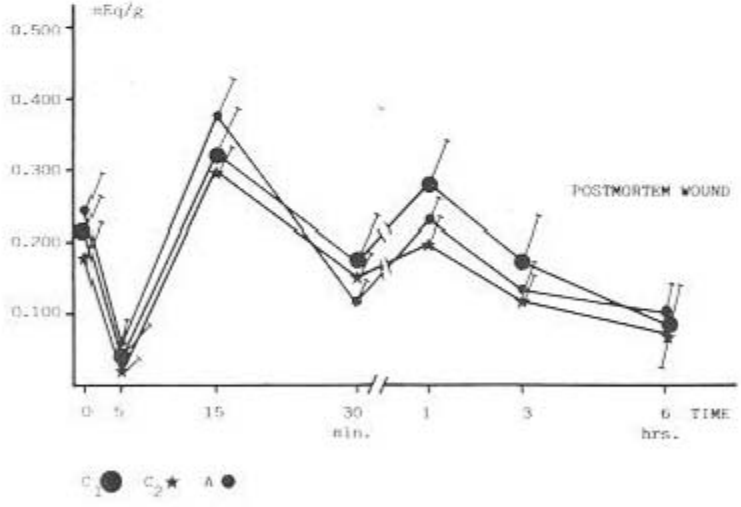


Fig. 7. Potassium evolution along the time

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**Reprints request to:**

Dr. C. Hernández-Cueto
Catedra de Medicina Legal
Facultad de Medicina
18071 Granada, Spain.