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Ions As Vital Reaction Markers
Comparative Study with Histamine and Serotonin

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İYONLARIN CANLILIK BELİRTECİ OLARAK KULLANIMI, HİSTAMİN VE SEROTONİN VERİLERİ İLE KARŞILAŞTIRILMASI

Özet

Adli tıptaki önemli konulardan birisi de, cesetlerin üzerindeki yaraların ne zaman (ölümden önce veya sonra) meydana geldiğini tesbit edebilmektir. Bu amaçla, domuz derisi üzerinde ölüm öncesi ve sonrası belirli zaman aralıklarında oluşturulan yaralarda kalsiyum, magnezyum, bakır ve çinko düzeyleri atomik soğurma spektrofotometrisi; histamin ve serotonin düzeyleri ise spektrofluorometri yöntemiyle izlendi.

4 aylık olan 10 domuzun kullanıldığı araştırmamızda, hayvanlar canlıyken sırt derisine cerrahi bistüri ile 6 insizyon yapıldı. Bu işlemi izleyen değişik süreler (0,10, 20, 30, 60 ve 120 dakika) içerisinde öldürülen hayvanlarda, aynı yöntemle, postmortem yaralar oluşturuldu. Lezyonlardan alınan 7-8 cm çapındaki deri parçaları -30°C'da dondurulduu. Elde ettiğimiz sonuçlar, histamin ve serotoninin canlılık belirteci olarak önemlerini doğrulamış, ayrıca, adı geçen iyonların belirtiminde kullanılan kolay, duyarlı ve özgül yöntemin yara yaşını saptamada taşıdığı önemi kanıtlamıştır.

Summary

We have studied the behaviour of the ions Ca, Mg, Cu, Zn and Fe with atomic absorption spectrophotometry (AAS) and the behaviour of histamine and serotonin by spectrofluorimetry in vital and postmortem wounds of different time of evolution made over pig skin.

Our results confirm the usefulness of histamine and serotonin as vitality markers and also confirm the great value of the ions studied with the same purpose because it is an easy, sensitive and high specific method.

Key words: *Ions - Histamine - Serotonin - Vital reaction*

INTRODUCTION

The application of enzymohistochemical and biochemical studies has supposed an important contribution to the differential diagnosis between vital and postmortem wounds. The study of the activity of some enzymes at the edge of wounds (alkaline phosphatase, esterase, aminopeptidase, etc.) mainly by *Raekallio* (1-3) and the study of some lysosomal enzymes such as cathepsins by ourselves (4-6) has been added to former investigations using histamine and serotonin (7-10). This kind of markers permit the detections of signs of vitality in wounds performed only few minutes before death.

Forensic practice need to employ these markers when wounds were made in moments closed to death; in these situations it is going to be difficult to differentiate the vital origin from the postmortem one only with macroscopic techniques. From some years ago we have been working in this field, looking for vitality markers of great sensibility and specificity. One of the markers that have been studied are some metallic ions (Ca, Mg, Cu, Zn, Fe) that showed the best results in former works (12,13). As we have also demonstrated their postmortem stability (6), these kind of markers can be specially usefull in those situations where the autopsy is performed in cadavers recovered some days after death.

At the same time we have determined the levels of the well known amines histamine and serotonin to compare their levels and evolution along the time with the ion's ones, following a previous work in the same kind of investigation from our Department (5). If ions show a correlation with histamine and/or serotonin, they could be useful not only to stablish if a wound was performed after or before the death, but also how many minutes before death was performed.

MATERIALS and METHODS

We have employed ten domestic pigs (age: 4 months; body weight: ~100 kg). Six vital incised wounds were made with a surgical blade scalpel to every animal in the back. Each wound has a different *"time of evolution"* (time elapsed between the moment of wounding and the moment of death). These times were of 0, 10, 20, 30, 60 and 120 minutes. The animals were sacrified by dellocation, washed with water and gently shaved; after shaving we made another identical wound (postmortem wound), in a homolateral place of the back. To obtain the samples we cut a piece of skin of 7-8 cm around the wound that was immediatly frozen at -30°C until using.

Before making determinations, each wound were divided as follows:

VITAL WOUNDS	POSTMORTEM WOUNDS
B_1 : edge of vital wound, until 3 mm	C_1 : edge of postmortem wound, until 3 mm
B_2 : edge of vital wound, from 3 mm	C_2 : edge of postmortem wound, from 3 mm
A : control zone: skin not injuried	

To determine ions we have employed a method formerly employed by ourselves (6,12,13). Once weighted, one gram of dried pig-skin without fat was calcinated in an electric oven at 500°C for 6 hours; ashes were dissolved in 3 ml of concentrated HCl and dried out in a heater and diluted in 25 ml of 3 % HCl, where were made the different determinations. The levels of Ca, Mg, Cu, Zn and Fe were measured by AAS with a Perkin-Elmer Spectrophotometer mod. 560 ; working conditions are expressed in Table I. Standard curves for control were made with standard solutions of each element (Carlo Erba, R) diluted in 3 % HCl.

Histamine was determined according to a classical and efficient method (14) based on the butanolic extraction of histamine; this butanolic layer is further mixed with NaOH and heptane to eliminate contaminants and closely-related products; finally, histamine is extracted with 0.1 N HCl and condensed with ortho-phtalaldehyde (OPT, Sigma, R) in acid medium, resulting a strong fluorescence that was measured with a Perkin-Elmer mod. MPF43A spectrofluorimeter (Exc: 360 nm; Em: 450 nm). Calculations were made by comparison of the results with standard curves of histamine (Sigma, R).

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To determine serotonin we have followed the method formerly employed with the same purposes by *Raekallio* et *al* (9), who made a modification of the original procedure (15). This method is based in the native fluorescence of serotonin in acid medium (pH: 2.5), that can be measured with a spectrofluorimeter (Exc: 295 nm; Em: 345 nm). Results were plotted against a standard curve made with serotonin (Sigma, R).

The statistical study of the results was carried out with a Student's-*t* test for coupled samples to compare differences between diverse zones. Additionally we made tests of linear regression and correlation to compare the evolution of histamine and serotonin levels with those of the different ions.

Table I. Working conditions for AAS.

Element	*Wavelenght (nm)*	*Slit (nm)*	*Fuel (kg/cm^2)*	*Oxidant $kg(cm^2)$*	*Standards*
Ca	422.7	0.7	40	55	0.10 mg %
					0.20 mg %
Mg	285.2	0.7	40	20	50 µg %
					100 µg %
Cu	324.7	0.7	40	20	50 µg %
					100 µg %
Zn	213.9	0.7	40	20	50 µg %
					100 µg %
Fe	248.3	0.7	40	20	50 µg %
					100 µg %

Table II. Markers with statistical signification between wounds edge and control zones.

Marker	*Time of Evolution*	*Signification*
Ca	0,10,60	$p < 0.01$
	20	$p < 0.10$
Mg	0,10,20,30	$p < 0.01$
Cu	0,10,20,30	$p < 0.01$
	120	$p < 0.10$
Zn	0,10,20,30	$p < 0.01$
Fe	0	$p < 0.01$
	60	$p < 0.05$
Histamine	All the series	$p < 0.01$
Serotonin	All the series	$p < 0.01$

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RESULTS

Our results are expressed in Figures 1 to 7 where are represented the levels obtained for each marker at the different time of evolution (0, 10, 20, 30, 60 and 120 min) in all the zones (A,B1,B2,C1 and C2).

Table II shows only the statistically significative results.

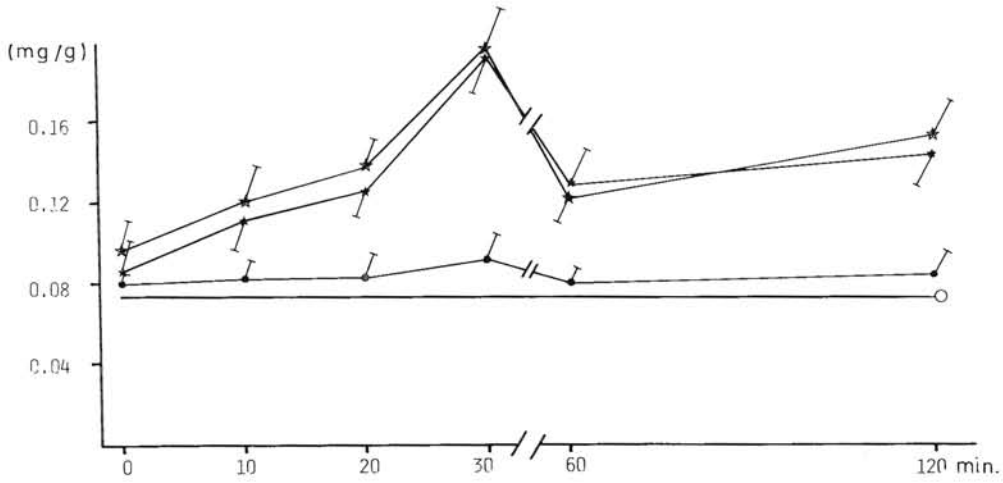

Figure 1. Evolution along the time of Ca levels in all the zones.
(★ B_1 ; ☆ B_2 ; ○ A ; ■ C_1 and C_2)

DISCUSSION

We have obtained statistically significative differences in all the times studied for histamine and serotonin. Their evolution along the time at the edge of vital wounds has been according to many previous authors (1,2,3,5,9,10,12). Ion's levels are really useful to differentiate vital from postmortem wounds.

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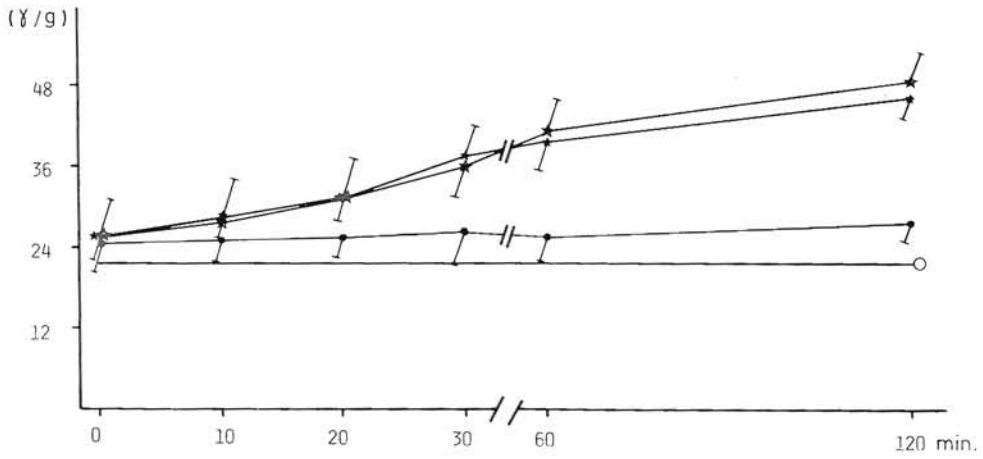

Figure 2. Evolution along the time of Mg levels in all the zones.
(★ B_1 ; ☆ B_2 ; ○ A ; ■ C_1 and C_2)

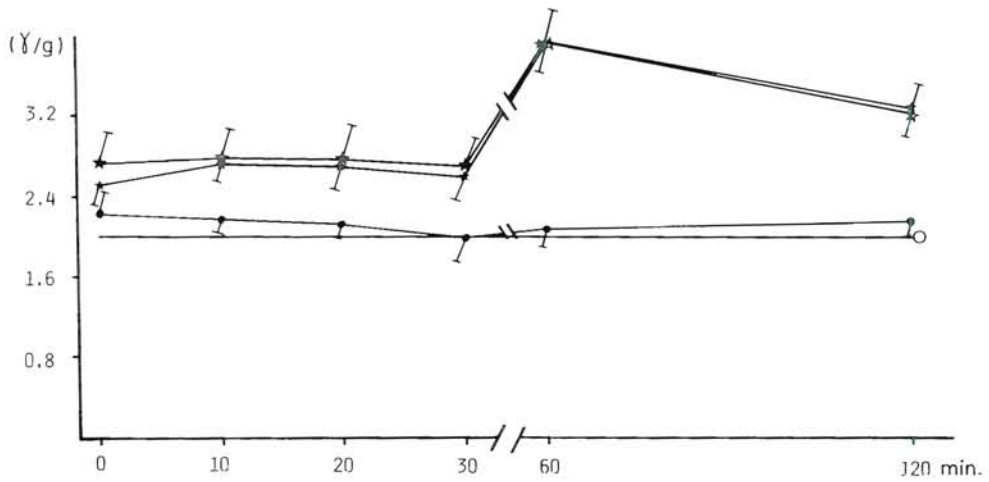

Figure 3. Evolution along the time of Cu levels in all the zones.
(★ B_1 ; ☆ B_2 ; ○ A ; ■ C_1 and C_2)

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The best results are obtained with Ca, but all the ions are useful (Table II). Nevertheless, the main problem we have is originated by the relatively high standard deviation (SD) that, although they are lower than in our previous works (6,12,13) they are still important. This high SD is due to the employ of different and not typical animals of laboratory; so, the biological variation is here specially increased, although we have to take account that this variation is going also to be observed in human samples and is going to be necessary everytime to compare a piece of wounded skin with a homolateral and not-wounded one (in our samples, zones A, C1 and C2).

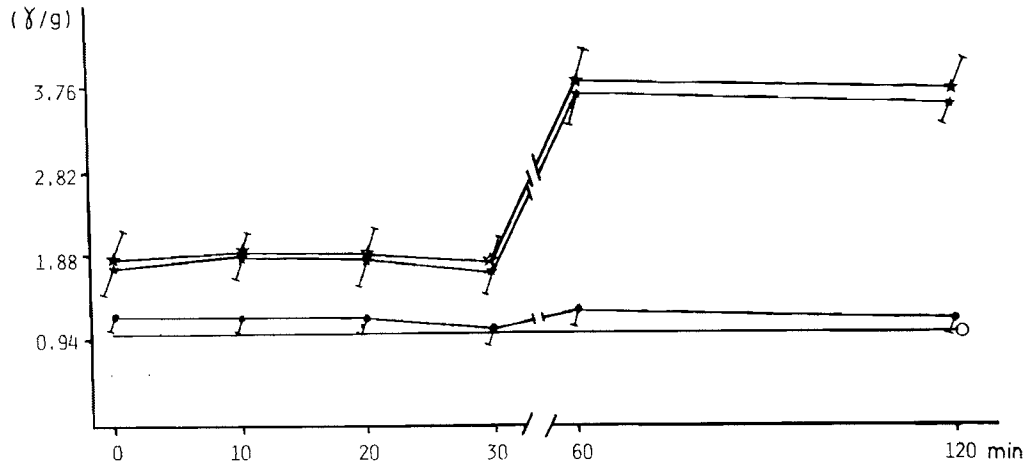

Figure 4. Evolution along the time of Zn levels in all the zones.
(★ B_1 ; ☆ B_2 ; ○ A ; ✱ C_1 and C_2)

The statistically significative increasing of the levels of the ions at the edge of the vital wounds (B1 and B2) in relation to the edge of the postmortem (C1 and C2) and the control zones (A) show their ability as markers of the vitality of the wound (Table II). They are involved in many physiological processes in the skin and also in the response of the tissues to injuries (acute inflammatory reaction) do perfectly justify their increasing.

Nevertheless, the no correlation between histamine and Ca, between serotonin andCa and among the different ions themselves or with histamine or serotonin, make us to doubt about the usefulness of these elements to determine the data of vital wounds.

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In summary, we can conclude that the study of the levels of Ca, Mg, Cu, Zn and Fe at the edge of the wounds is very useful to establish the differential diagnosis between vital and postmortem wounds. At the same time, the employ of histamine and serotonin as markers of vitality is very useful to establish -besides- the data of the vital wounds.

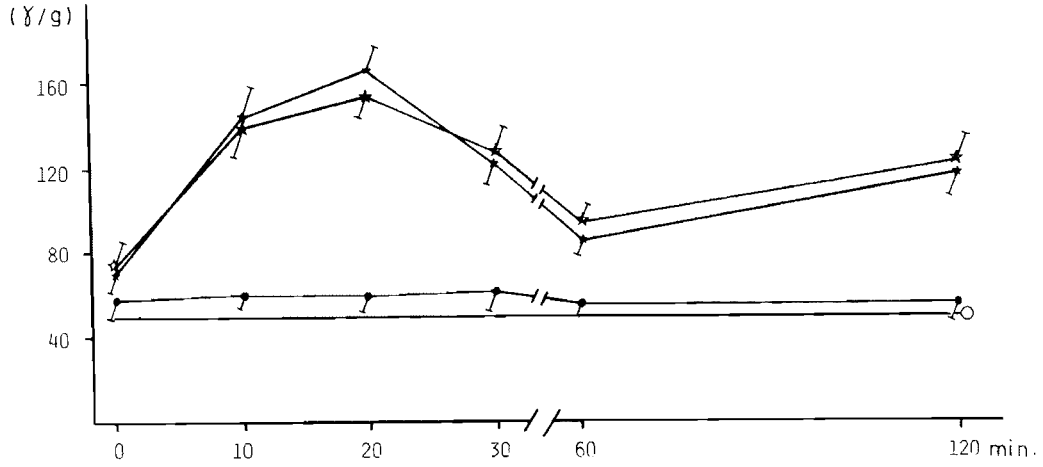

Figure 5. Evolution along the time of Fe levels in all the zones.
(★ B_1 ; ☆ B_2 ; ○ A ; ● C_1 and C_2)

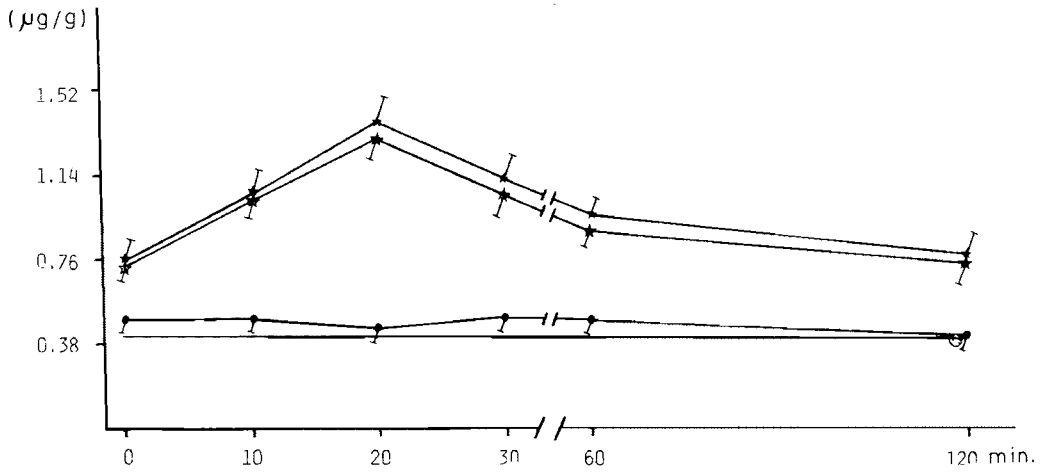

Figure 6. Evolution along the time of histamine levels in all the zones.
(★ B_1 ; ☆ B_2 ; ○ A ; ● C_1 and C_2)

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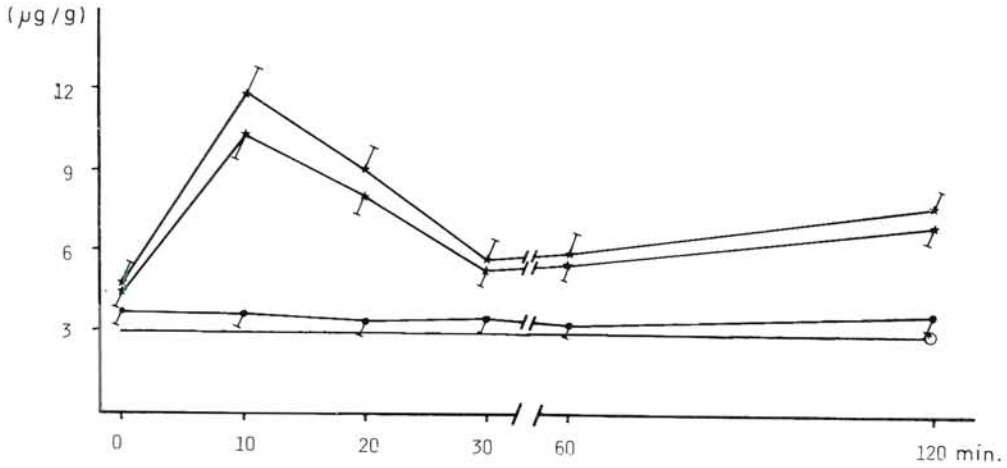

Figure 7. Evolution along the time of serotonin levels in all the zones.
(★ B_1 ; ☆ B_2 ; ○ A ; ■ C_1 and C_2)

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