Dept. of Biochemistry,
Faculty of Medicine, Assiut University,
Head od Dept. Prof. Dr. S.A. Ghaneimah.

RELATION BETWEEN THE TIME OF WOUND INDUCTION IN GUINEA PIG'S SKIN TISSUE AND SOME ELEMENTS AND ENZYMES

(With 2 Tables & 4 Figs.)

By
KARIMA M.H. EL-HUSSINY and SOAD M. ABDEL GHANY*

(Received at 26/8/1992)

العلاقه بين وقت الجروح المحدثه بجلد الخنزير الغيني وبعض العناصر والانزيمات

كريمة الحسيني ، سعاد عبدالغني

تم في هذه الدراسه بحث تأثير الجروح المحدثه على تركيز أيونات الكالسيوم والماغنسيوم والزنك والنحاس وكذلك نشاط انزيمي الفوسفاتاز القلوي والكولين استراز . وتمت دراسة هذه القياسات في عدد ٦٠ خنزيراً غينياً ، تم احداث جروح بها جميعاً في منطقة الفخذ . وتم تقسيمها حسب الوقت الذي تم فيه احداث الجروح قبل ذبح الحيوانات الى ستة مجموعات . تم ذبحها بعد كلا من نصف دقيقة ، ١٥ دقيقه ، ٣٠ دقيقه ، ٦٠ دقيقه ، ١٢٠ دقيقه ، ٢٤٠ دقيقه . وقد تم أخذ عينتان من جلد هذه الحيوانات .. واحده من منطقة الجرح (أ) وأخرى من المنطقه المحيطه به (ب) .. وتم استخدام هذه العينات في تقدير كل من تركيز العناصر والانزيمات ، بعد ذلك تم رسم منحنيات متوسطات تركيز العناصر ونشاط الانزيمات التي تم الحصول عليها في المنطقتين . وكذلك تم رسم نسبه الزنك الى الماغنسيوم والنحاس الى الماغنسيوم ، النحاس الى الكالسيوم والكالسيوم الى الماغنسيوم والزنك الى النحاس حيث نسبت جميعها الى الوقت . وقد أظهرت النتائج أن الحد الأعلى لمتوسطات تركيز عناصر الكالسيوم والزنك النحاس ونسبتهم الى الماغنسيوم يحدث عند ٦٠ دقيقه من احداث الجروح .. بينما يزيد نشاط الأنزيمات عند ٦٠ دقيقه ثم يصل الى ذروته عند ١٢٠ دقيقه من احداث الجرح بالاضافه لذلك لوحظ أن معظم هذه القياسات كانت مترابطه فيما بينها خلال فترة الدراسه وكانت أقصى زياده لمجموع تركيز العناصر الأربعه في النسيج عند ٦٠

* Dept. of Forensic Medicine & Toxicology, Fac. Med., El-Minia Univ.

Table (1) : Mean values ± S.E. of serum enzymatic activities of different breeds of rabbits

Statistical evaluation	serum alkaline phosphatase	serum aspartate amino-transferase	serum alanine mino-transferase
breed	(SALP) K.A.U./ dL	(SAST) I.U./ L	(SALT) I.U./ L
B	a 23.48±0.26	a 63.60±0.69	de 24.05 ±1.81
C	cd 18.83±0.47	a 63.70±1.20	d 24.75±0.80
N	d 17.49±0.20	a 59.70±2.03	a 47.65±1.00
B X C	d 17.06±0.48	b 33.00±0.67	c 29.60±0.99
C X B	bc 19.82±0.32	b 37.04±2.87	b 39.15±1.36
N X B	b 21.11±0.64	b 37.50±1.25	ef 20.73±1.05
N X C	e 13.06±0.30	c 31.90±0.56	f 20.35±1.20
C X N	f 15.09±0.74	c 28.60±1.09	f 19.35±1.37

Table(2):Mean values of some organic and inorganic constituents in serum of different breeds of rabbits.

statistical evaluation	total protein	albumin	globulin	calcium	inorganic phosphorus	iron
breed	g %	ga %	g %	mg %	mg %	mg%
B	c 4.50 ±0.12	c 2.49 ±0.15	b 2.01 ±0.09	a 13.86 ±4.03	a 9.84 ±0.38	a 187.5 ±26.35
C	a 6.10 ±0.29	b 3.40 ±0.17	a 2.70 ±0.23	b 12.07 ±0.11	b 6.57 ±0.28	b 152.48 ±35.78
N	ab 5.92 ±0.37	b 3.35 ±0.27	b 2.57 ±0.23	b 12.18 ±0.11	c 5.45 ±0.16	bc 141.30 ±28.69
BXC	c 4.96 ±0.19	b 3.50 ±0.12	c 1.46 ±0.11	b 11.47 ±0.29	c 5.23 ±0.17	a 185.60 ±26.74
CXB	c 4.92 ±0.12	c 2.90 ±0.09	b 2.02 ±0.09	b 11.65 ±0.29	d 4.60 ±0.06	bc 125.97 ±16.84
NXB	b 5.40 ±0.15	bc 3.00 ±0.24	ab 2.40 ±0.18	b 12.03 ±0.33	d 4.57 ±0.15	a 185.30 ±65.14
NXC	bc 5.30 ±0.36	b 3.24 ±0.23	ab 2.06 ±0.14	b 11.61 ±0.07	c 5.85 ±0.10	c 115.02 ±21.05
CXN	a 6.35 ±0.14	a 4.15 ±0.30	b 2.20 ±0.06	b 11.65 ±0.12	d 4.31 ±0.09	bc 127.74 ±18.39

B= Baladi = Californian N= New Zealand

The same letter mean significant while different letter mean non significat

دقيقة مما يثبت أيضا الترابط بينها . ولذا فان هذه المنحنيات التي تم الحصول عليها من تحليل أنسجة البشره المصابه بالجرح يمكن أن تلعب دوراً مفيداً في تحديد وقت حدوثها .

SUMMARY

In the present study, the effect of skin induced wounds on tissue calcium (Ca), magnesium (Mg), zinc (Zn) & copper (Cu) concentrations as well as alkaline phosphatase and cholinesterase activities was investigated. Sixty guinea pigs with induced injuries at the gluteus areas were used. They were divided into six groups according to the time of injury prior to slaughter, including 0.5, 15, 30, 60, 120 & 240 minutes. Postmortem-skin tissue excision in (A) and around (B) the injured site was performed. Two portions from each specimen were taken, one for determination of the elements while the other is for determination of the activity of the enzymes. The mean concentrations of both the elements & enzymes in the two zones of the skin tissue homogenate in correlation with the time of wound induction were graphed. The ratios of the element mean concentrations of Zn/Mg, Cu/Mg, Cu/Ca, Ca/Mg, Zn/Ca & Zn/Cu versus time were also graphed. The results showed that the maximal increase in Ca, Zn & Cu mean concentrations & their ratios to Mg occurs at 60 minutes after wounding, while the two enzymes showed an increase at 60 min. then reach its maximum at 120 min. Moreover, it has been observed that these parameters were significantly intercorrelated with each other all over the period of the study. This is evidenced by the maximal increase of the sum of the four elements concentrations/g tissue at 60 min. These curves achieved by analysing wounded skin tissues may play a useful role in estimating their time.

INTRODUCTION

The timing of wounds is one of the cardinal problems of forensic medicine. It has been of increasing importance due to crime as well as traffic and industrial accidents increase. Several procedures have been used for

wound timing including detection of cellular infiltration, determination of enzymes, histamine and serotonin levels (TENDESCHI, et al. 1977). Unfortunately, cellular infiltration (the most reliable histological criterion) becomes well marked only 4–8 hours after wounding (RAEKALLIO, 1972). This latent phase is far too delayed to satisfy the demands of a medicolegal examination.

However, TENDESCHI, et al. (1977) found that the maximal increase in the free histamine content occurs within 20–30 minutes after wounding, while serotonin is demonstrable still earlier. Moreover, MAENO, el al. (1990) determined histamine and polyamines simultaneously in injured rat skin in order to estimate the age of wounds.

This study was designed to estimate the elements: Ca, Mg, Zn and Cu concentrations as well as alkaline phosphatase and cholinesterase activities in wounded skin in correlation with the time of injury. In this investigation two independent procedures (elements and enzyme determination) were used to find suitable biochemical panels for determination of wound age.

MATERIAL and METHODS

Guinea pigs were chosen in this study, because of their high compatibility with human when skin is concerned (ABATON, 1976 and HUNT, 1980).

Sixty guinea pigs with a mean weight ± SD of (420 ± 16) grams were used and divided into six groups (ten at each group).

Local anaesthesia with ethylchloride was applied to the gluteus area of the animals, then full skin–depth incisions were made, 2–cm long after hair shaving. Animals were slaughtered: after 0.5 minute, (min.) (the first group), after 15 min., (the second group), after 30 min., (the third group), after 60 min., (the fourth group), after 120 min., (the fifth group) and the last one after 240 min. from the injury. Skin specimens at the gluteus were removed from all animals. Each specimen was segmented into two zones, the traumatic (A) and the peripheral (B) at distance of 2 cm from the traumatic area. Fat was removed and 4 grams of each specimen were weighed. Each specimen was divided into 2 portions after being washed with distilled water. The first portions were immersed in a solution containing 2 ml of HNO & 2 ml of HC10 . After 72 hours in this solution, the tissues were completely

dissolved (MARGALIOTH, et al. 1983) the clear solution was aspirated to the Shimadzu Atomic absorption/Flame Emission spectrophotometer Model A-360-02. The concentrations of the elements (Ca, Mg, Zn and Cu) in the tissues were measured in Ug or mg/g wet tissue.

The second portions of the specimenss were rapidly frozen at -70 and then subjected to the enzyme analysis. Tissues were homogenized in TED buffer formed of 10 mM tris, 10 mM EDTA and 1 mM dithiothreitol, PH 7.4-7.5 at 4 C. The homogenate was centrifuged at 4 C for 30 minutes and the supernatant was used for analysis of enzymes. Alkaline phosphatase enzyme was determined by the method of KING (1965) using Folin-ciocalteu reagent, while cholinesterase enzyme was assayed by the method of De La HUERGA, et al. (1952).

RESULTS

The mean concentrations of the elements Ca, Mg, Zn and Cu, and the activities of alkaline phosphatase and cholinesterase at zones A and B skin tissue homogenate were tabulated and graphed in correlation with the time of wound induction and are shown in table 1 & Fig. I & II respectively.

It is important to note that no alterations were detected in the mean concentrations of all the measured parameters between the zones A and B.

As is shown in Fig. (1), a slight decrease in the mean concentrations of Ca from the beginning of the investigation up to 30 minutes, then gradual notable elevation occur which reach maximum at 60 minutes and then decline.

Mg mean concentrations shows a slight decrease in the first 15 minutes then it gradualy increases until it reaches its maximal elevation at 120 minutes which then declines again.

As regards Zn mean concentrations, there is a decline in the first 15 minutes which becomes constant up to 30 minutes then it elevates markedly at 60 minutes and after that it decreases.

Cu mean concentrations exhibited a marked decrease up to 30 minutes, then elevation occurs gradually up to 60 minutes, then a gradual decline is notable.

As for the activity of alkaline phosphatase & cholinesterase in skin tissue homogenate (Fig. II), the results indicate that their activities decline from 0.5-15 minutes then a gradual elevation occurs which reaches its maximum at 120 minutes and lastly it declines gradually.

Fig. (III) shows the ratios of the elements mean concentrations versus time. Zn/Mg, Cu/Mg & Ca/Mg ratios were seemed to be the most valuable in estimating time of wound owing to their obvious alteration versus time.

Fig. (IV) shows the bar chart of the sum of the concentrations of the four elements/g tissue versus time. It can be seen that these elements reach their maximal elevations at 60 minutes.

Finally, table (II) shows the intercorrelations between the various parameters studied in zones (A) & (B) skin tissue homogenate in relation to time of wound induction. The 2 elements Zn & Mg were found to be correlated with alkaline phosphatase. On the other hand Mg & Ca were found to be correlated to each other besides their correlation with cholinesterase. Moreover, Zn & Cu were found to be correlated.

DISCUSSION

In this study, two biochemical procedures were used for determining the time of wounds; mean concentrations of elements (Ca, Mg, Zn and Cu) and the activities of alkaline phosphatase and cholinesterase enzymes.

No detectable changes in all the parameters studied, between the traumatic (A) and the peripheral (B) zones, could be observed. This may be due to the short distances (2 cm) between the two zones.

The elements which have been chosen in the present study play an important role in wound healing, so their concentrations in the wounded skin may have a useful concern to its timing.

Calcium is crucial to the dynamic organization of cell structure. It is required for the enzymatic activity of all collagenases (SELTZER, et al. 1976). Calcium acts via the intracellular calcium receptor calmodulin which has a direct cell cycle effect. Calmodulin is linked to DNA repair and DNA polymerase (RASMUSSEN and MEANS, 1989). The elevation of Ca that has been observed at the site of the injury could be due to its vital role in the coagulation systems and muscular contractility. Alterations in Ca concentrations are mediated via hormones and other related substances (NJAU, et al. 1991). These finally promote wound repair at the earliest stages of wound healing and intrench haemorrhage. Significant alterations of Ca have been found also in burns and other experimentally induced injuries (LOVEN, et al. 1984).

Elevation of Mg at the site of the injured tissues, indicates that it helps in wound healing. Furthermore, Mg plays a significant part in cell metabolism and enzyme regulation (e.g. alkaline phosphatase) at the injured sites. ALROWAIH, et al. (1987) and NJAU, et al. (1991) reported that Mg alterations occurred in both experimental animals and human after traumatic injuries and their subsequent repair.

The elevation of zinc at wound sites which was pronouncedly evident in correlation with time is in agreement with NJAU, et al. (1991). It has been also demonstrated that, after trauma, the amount of zinc in blood is decreased (VAN, et al. 1981). As stated before, zinc plays an important role in healing of wounds, as it increases rate of epithelization, rate of gain of wound strength and synthesis of collagen and other proteins (HUNT, 1980). A number of enzymes are zinc-dependent, notably alkaline-phosphatase, DNA-polymerase and reverse-transcriptase which are very important in the epithelial and fibroblast proliferation (PEACOCK and WINKLE, 1976 and CRAWFORD, 1987).

On the other hand, the elevation of copper at the site of injury could be explained by its role in the tissue repair as it is a catalyst of lysyl oxidase enzyme which initiates the cross-linking of collagen and elastin, a critical step in connective tissue repair (SIEGEL, 1979). It also limits immune activation and the associated stimulation of other cell types at the inflammatory site (HARRIS, et al. 1982).

The initial increase in enzyme activity represents among other things, an adaptive defence mechanism of the local connective tissue cells as an enzymatic response to injury (RAEKALLIO, 1966). The increase in enzyme activity in the peripheral zone may be called a positive vital reaction since there are no such changes in post-mortem wounds (RAEKALLIO, 1972). The activity of alkaline phosphatase and cholinesterase increases at 120 minutes after the injury which may be useful in the rough estimation of the age of vital wounds.

The true acetylcholinesterase is present in nerve tissues and red cells and is responsible for the hydrolysis of acetylcholine at synapses. It may have a role in restoring feeling and motor control in the second stage of wound healing in association with Ca and Mg that are essential for normal nerve function (VARLEY, et al. 1980). Alkaline phosphatase and cholinesterase are

participating in cells and tissue wound healing (NJAU, 1991) and could be used for wound timing.

The observed decreased values of the studied parameters in the wound area after their initial increase could be attributed to the inflammatory response resulting from trauma. It can lead to the physical interruption of blood vessels with immediate hemorrhage, extensive cell destruction at the site of injury permitting body fluids including blood to drain off (PEACOCK & WINKLE, 1976). The immediate response of small vessels in the area of injury is vasoconstriction. The vasoconstriction usually lasts only five to ten minutes at the most & is follwed by an active vasodilatation due to the attack of some proteases to the endothelium of skin microcirculation. Coincident with vasodilatation, leakage of fluid from venules occurs. This fluid has the same composition as plasma with its full complement of macromolecules (PEACOCK and WINKLE, 1976). Increased capillary permeability is the key to all subsequent events in inflammation within the wound area. Therefore, this depletion of the studied elements & enzymes in the early period after injury may be due to their leakage into the wound space. After that prostaglandins are synthesized by the injured cells & appear to be responsible for the late stages of the inflammatory reaction while simultaneously initiating early phases of injury repair (PEACOCK and WINKLE, 1976). The following major biochemical event is the rapid production of ground substances of skin tissue which consist of proteins, polysaccharides, glycoproteins, salts & water (HUNT, 1980).

In the present study, the two elements Zn & Mg were found to be significantly correlated with alkaline phosphatase & this is based on the fact that these elements are co-factors for its activity. Mg & Ca were found to be correlated to each other besides their correlation with cholinesterase & this explain their important role in wound healing. Moreover, Zn & Cu were found to be correlated; the two elements that are mainly involved in tissue repair.

In conclusion, the results showed that the }aximal increase in Ca, Zn & Cu mean concentrations & their ratios to Mg occurs at 60 minutes after wound injury, while the enzymes showed an increase at 60 min., then reach its maximum at 120 min. Moreover, all the parameters studied were found to be intercorrelated all over the period of the study & this evidenced by the maximal increase of the sum of the four elements concentrations at 60 min.

So, this represents a preliminary study relating changes in both elements and enzymatic activity levels and the time of wound induction in guinea pig's skin. Further studies are necessary for the provision of both human and other animals reference standards to be used as indices for timing of wound injury in medicolegal purposes.

REFERENCES

Abaton, S. (1976): burns in children, clinical symposia, Ciba–Geigy, Vol. 28 (4): p. 20.

Alrowaih, A.; Thorngren, K.G.; Abdulla, M.; Toerning, S. and Dashti, H. (1987): Alteration of trace elements in plasma of patients with traumatic bone injury. Acta Pharmacol. Toxicol., 59, Suppl., 7: 352–357.

Crawford, A.J. (1987): Excessive intracellular zinc accumulation in cardiac and skeletal muscles of dystrophic hamsters. Exp. Neurol., 92 (2): 265–276.

De La Huerga, J.; Yesinick, C. and Prpper, H. (1952): Quoted from Varley, H.; Gowenlock, A.H. and Bell, M. (eds) In: Practical clinical Biochemistry, (1980), 5th ed., William Heinemann Medical Books LTD London, p. 755.

Harris, E.D.; Disilvestro, R.A. Balthrop, J.E. (1982): Lysyloxidase a molecular target of copper In: Inflammatory diseases and copper. Edited by Sorenson, J.R.J. Humana Press, Clifton, New Jersey. 1st editon.

Hunt, T.K. (1980): Wound healing and wound infection. Appleton–century–crofts /New York, First Edition p. 135.

King, J. (1965): Practical Clinical Enzymology. D. Von. Nostrand Company LTD, London 1st edition p. 264.

Loven, L.; Nordstrom, H. and Lennquist, S. (1984): Changes in calcium and phosphate and their regulating hormones in patients with severe burns injuries. Scand. J. Plast. Reconsts. Surg., 18: 49–53.

Maeno, Y.; Takabe, F.; Inoui, H. and Iwasa, M. (1990): A study on the vital reaction in wounded skin: simultaneous determination of histamine and polyamines in injured rat skin by HPLC. Forensic Sci. Int., 46 (3): 255–268.

Margalioth, E.J.; Schenker, J.G. and Chevion, M. (1983): Copper & Zinc levels in normal and malignant tissues, Cancer, 52: 868–872.

Njau, S.N.; Epivationos, P.; Papadopoulou, H.T.; Fsaroulis, D. and Stratis, J.A. (1991): magnesium, Calcium and zinc fluctuations on skin induced injuries in correlation with time of induction. Forensic Sci. Int., 50: 67–78.

Peacock, E.E. and Winkle, W.V. (1976): Wound repair, 2nd ed., W.B. Saundirs Company, Philadelphia, London, Toronto, p. 184.

Raekallio, J. (1966): Enzyme histochemistry of vital and post-mortem skin wounds. J. Forens. Med., 13: 85–91.

Raekallio, J. (1972): Determination of age of wounds by histochemical and biochemical methods. forensic Sci. Int., 1: 3–16.

Rasmussen, C.D. and Means, A. (1989): Calmodulin, cell growth and gene expression. Trends Neurosci., 12: 433–437.

Seltzer, J.L.; Welgus, H.G.; Jeffrey, J.L. and Eisen, A.Z. (1976): The function of calcium in the action of mammalian collagenases. Arch. Biochem. Biophys., 173: 355–81.

Siegel, R.C. (1979): Lysyl oxidase, Int. Rev. Connect. Tiss Res. 8: 73–118.

Tendeschi, C.G.; Eckert, W.G. and Tendeschi, L.G. (1977): Mechanical traumas. In Forensic Medicine (a study in trauma and environmental hazards, W.B. Saunders Company, Philadelphia, London, Toronto, Vol. p. 24–26, 1st ed.

Van Rij, A.M.; Hall, M.T.; Bray, J.T. and Pories, W.J. (1981): Zinc as an integral component of the metabolic response to trauma. Surg. Gynecol. Obstet., 153 (5): 677–682.

Varley, H.; Gowenlock, A.H. and Bell, M. (1980): Practical Clinical Biochemistry, 5th ed., William Heinemann Medical Books LTD London, p. 872–881, 753.

Table (I): The mean concentrations ±SD of the elements & enzymes studied in zones A(traumatic) and B (peripheral) skin tissue homogenate in relation to time of wound induction.

Biochemical Investigations	Time (minutes)											
I - Elements concentrations	0.5		15		30		60		120		140	
	A	B	A	B	A	B	A	B	A	B	A	B
1) Ca (mg/g tissue)	2.91 ± 0.64	2.25 ± 0.41	2.54 ± 0.64	2.21 ± 0.23	2.25 ± 0.53	2.16 ± 0.24	3.10 ± 0.99	2.75 ± 0.47	2.07 ± 0.21	1.92 ± 0.32	1.78 ± 0.36	1.55 ± 0.24
2) Mg (mg/g tissue)	0.55 ± 0.08	0.61 ± 0.18	0.41 ± 0.04	0.45 ± 0.10	0.42 ± 0.06	0.43 ± 0.05	0.41 ± 0.07	0.48 ± 0.06	0.89 ± 0.14	0.88 ± 0.07	0.68 ± 0.10	0.60 ± 0.10
3) Zn (Ug/g tissue)	33.19 ± 5.42	35.25 ± 6.06	26.63 ± 5.14	25.13 ± 2.33	27.38 ± 5.36	26.64 ± 2.48	45.94 ± 8.58	41.06 ± 7.43	39.31 ± 3.48	38.06 ± 4.88	36.75 ± 2.60	36.75 ± 2.52
4) Cu (Ug/g tissue)	26.75 ± 4.70	19.57 ± 4.33	12.07 ± 3.22	8.52 ± 0.69	8.18 ± 1.11	8.87 ± 1.76	15.28 ± 1.15	17.53 ± 4.82	11.27 ± 1.71	9.55 ± 1.58	11.93 ± 1.31	10.91 ± 2.95
II- Enzymes activities												
1) Alkaline phosphatase. (U/mg protein)	2.06 ± 0.58	3.12 ± 2.76	1.26 ± 0.17	0.97 ± 0.39	1.44 ± 0.63	1.29 ± 0.23	1.77 ± 0.53	1.67 ± 0.66	3.72 ± 1.21	4.02 ± 1.98	2.99 ± 1.00	3.52 ± 1.50
2) Cholinesterase (U/mg protein)	0.14 ± 0.04	0.13 ± 0.03	0.13 ± 0.03	0.13 ± 0.03	0.14 ± 0.02	0.15 ± 0.03	0.17 ± 0.07	0.17 ± 0.03	0.18 ± 0.03	0.20 ± 0.03	0.15 ± 0.02	0.16 ± 0.09

Table (II): Intercorrelations between various parameters studied in zones A & B skin tissue homogenate in relation to time of wound induction.

				5 min.	15 min.	30 min.	60 min.	120 min.	240 min.
Alkaline phosphatase	vs.	Zinc	A	-	-	0.85***	0.57*	-0.79**	-0.59*
			B	-	0.90***	-	-	-	-
Alkaline phosphatase	vs.	Mg	A	0.69**	0.87***	-0.57*	-	-0.98***	-
			B	-	-	-	-0.58*	-0.75**	-
Cholinesterase	vs.	Mg	A	-	-	-	-	0.86***	-
			B	0.79**	-	-0.58*	-	0.72**	-
Cholinesterase	vs.	Ca	A	0.98***	0.90***	0.99***	-	-	-0.58*
			B	-0.56*	-	0.86***	-	-	-0.62*
Copper	vs.	Zinc	A	-	-	-	-0.98***	-0.86***	-
			B	-	0.75**	-0.90***	-	-0.75**	-0.82***
Calcium	vs.	Mg	A	-	-	-	0.96***	-	-
			B	-0.84***	-0.82***	-0.78**	-	-	-

- N.S. :non-significant
* < 0.05
** < 0.01
*** < 0.001

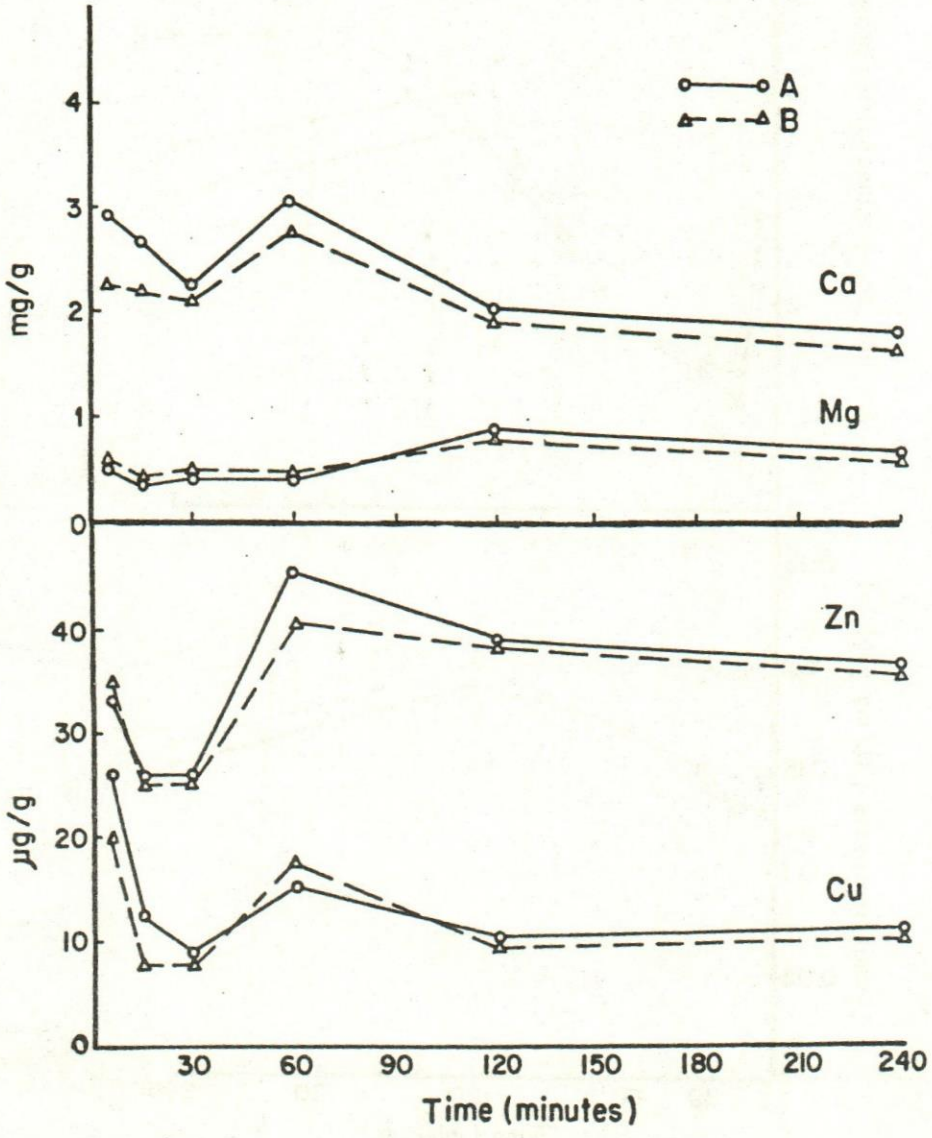

Fig.(I): The mean concentrations of Ca, Mg, Zn & Cu in zones A(Traumatic) and B(Peripheral) skin tissue homogenate in correlation with the time of wound induction.

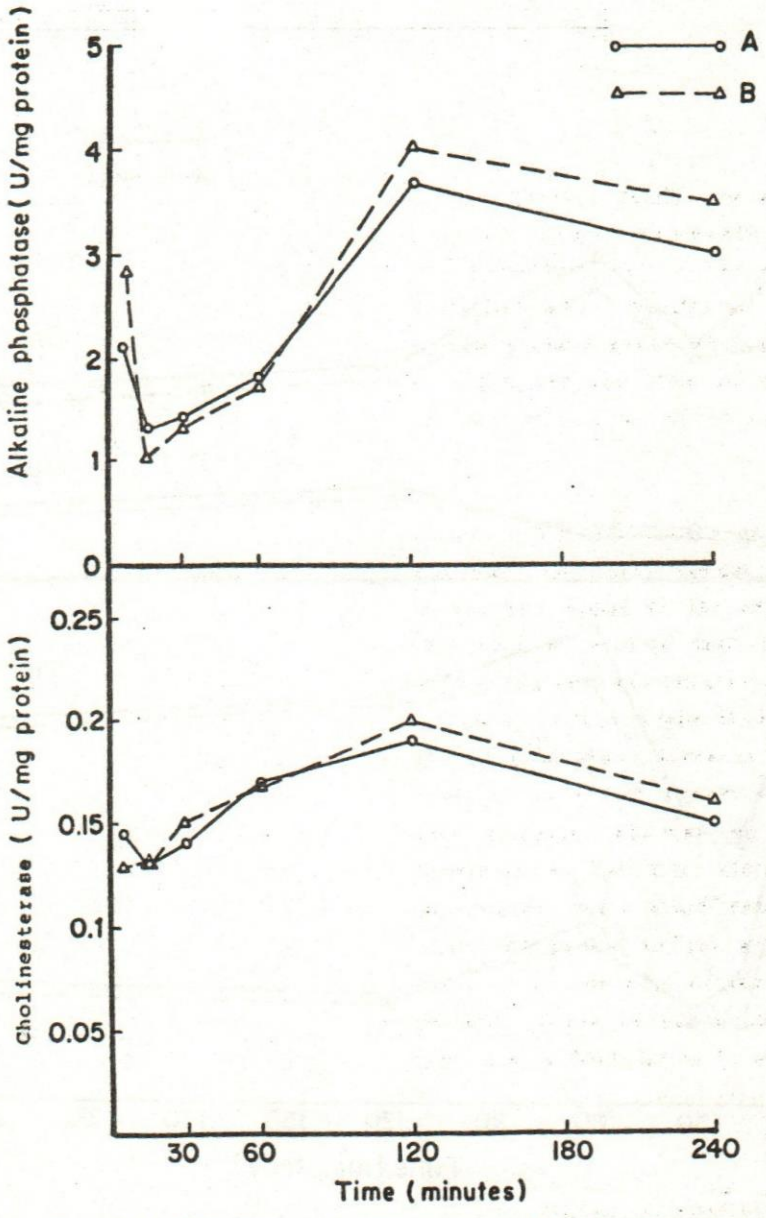

Fig. (II): The mean concentrations of the alkaline phosphatase & chalinesterase activities in zones A(traumatic) and B (peripheral) skin tissue homogenate in correlation with the time of wound induction.

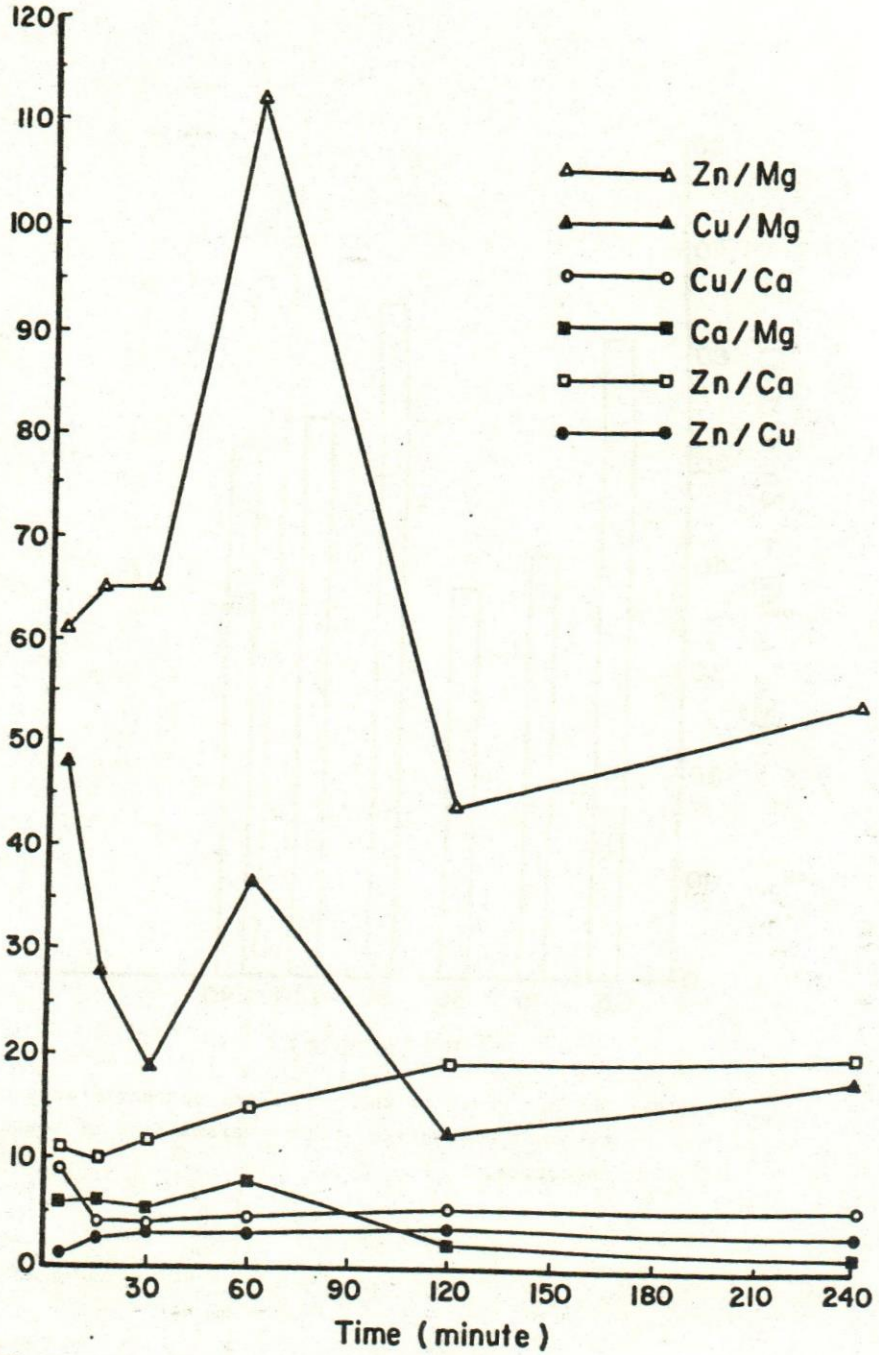

Fig.(III): The ratios of the elements' mean concentrations in correlation with the time of wound induction.

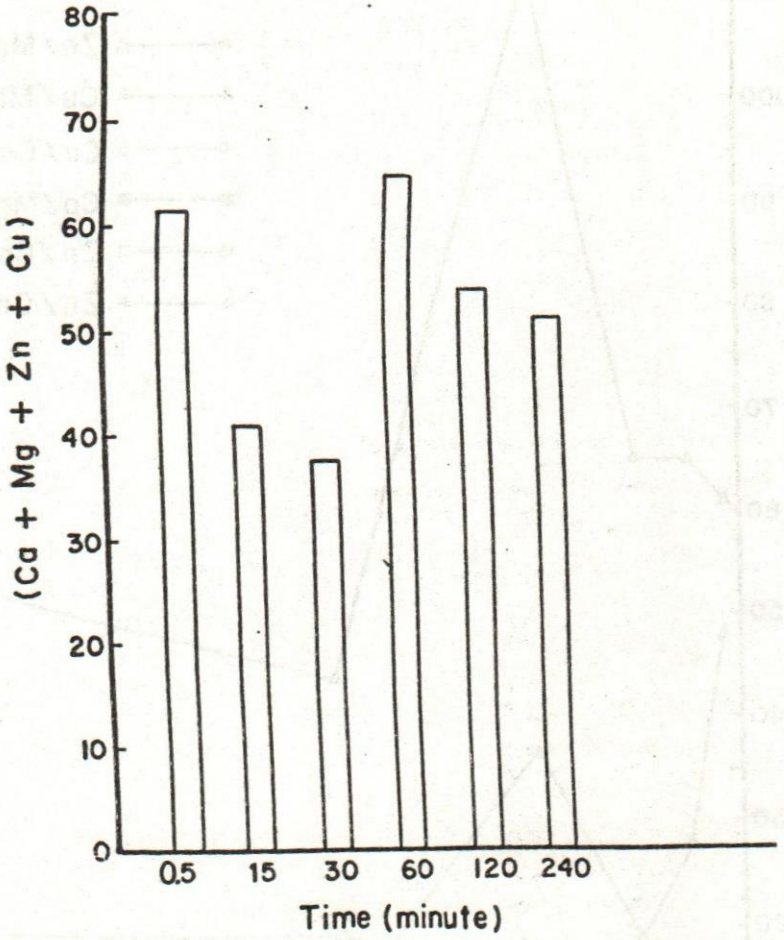

Fig.(IV): The bar chart of the sum of the concentrations of the four elements/g tissue versus time of wound induction.