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CHEMICAL ANALYSIS OF SERUM CHLORIDE, SODIUM AND POTASSIUM AS AN INDICATOR FOR THE POSTMORTEM TIMING IN ALBINO RATS

(With 1 Table and 4 Figures)

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التحليل الكيميائي لتركيز كلٍ من الكلوريد والصوديوم والبوتاسيوم في أمصال الفئران البيضاء كوسيلة لتقدير وقت حدوث النفوق

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يُعد تقدير وقت الوفاة من الأمور بالغة الأهمية خاصة في مجالات الطب الشرعي. كما أن إستحداث طرق وتقنيات جديدة لتقدير ذلك الوقت يُعد من الأمور المرغوب فيها والتي يجب معرفتها في جميع الأحوال و الأجناس. في هذه الدراسة تم إستخدام ٩٠فأراً أبيضاً قُسمت إلي ٩ مجموعات (بواقع ١٠فئران لكل مجموعة). تم حقن كل فأر ١سم من الهيبارين المخلط بالدكستروز و إحداث الوفاة عن طريق إستنشاق جرعة زائدة من الكلوروفورم. حُفظت الفئران بعد ذلك في درجة حرارة تتراوح بين ٣ــ٤ درجات مئوية قبل جمع الدم منها حيث تم جمع الدم من المجموعة أ بعد الوفاة مباشرة (وقت الصفر – ضابط التجربة) ومن المجموعات الأخرى (من ب إلى ذ) بعد ٦،١٢،٢٤،٣٦،٤٨،٦٠،٧٢ ثم ٩٦ ساعة بعد إحداث الوفاة وتم فصل المصل لقياس كلٍ من الكلوريد والصوديوم والبوتاسيوم. وقد أوضحت النتائج ما يلي: (١) وجود إرتباط سلبي بين كلٍ من الكلوريد ووقت إحداث الوفاة والوقت الذى مر بعده حيث إنخفض تركيز الكلوريد إنخفاضاً سريعاً خلال ٢٤ ساعة الأولى بعد الوفاة ثم إستمر في الإنخفاض بمعدل بطيء حتى ٩٦ ساعة وكانت نسبته وقت الوفاة (وقت الصفر) ٣٦٦و١٠٢±١٣٤ز٠ ملى مول حتى وصل إلى ٠٤٧و٦٥±٨٠٩و ملى مول عند ٩٦ ساعة بعد الوفاة.(٢) وجود إرتباط سلبى بين كلٍ من الصوديوم في المصل والوقت الذي مر على الوفاة فقد لوحظ ثبات تركيز الصوديوم في الست ساعات الأولى بعد الوفاة (إنخفاض غير معنوى) و كانت نسبته عند الوفاة٠٢٦و١٤٢±٢٤٤و ١ملىمول و بعد ٦ساعات بعد الوفاة وصلت إلى ٤٨٣و١٣٩±٩٦٧و٠ملىمول ثم أعقبه إنخفاض سريع حتى ٢٤ ساعة بعد الوفاة ثم إنخفاض بطئ حتى ٩٦ ساعة بعد الوفاة حيث وصل تركيزه إلى ٧٠٣و٦٨±٤٣٣و ١ملى مول.(٣) وجود إرتباط إيجابى بين كلٍ من البوتاسيوم والوقت الذى مر على الوفاة فإزداد التركيز في الـ ٢٤ ساعة الأولى بعد الوفاة (كان تركيز البوتاسيوم عند الوفاة ٤٤٥و٥±١٢٥و ٠ملى مول ثم إزداد إلى ٢٦٥و٤٧±٤١١و ١ ملى مول عند٢٤ ساعة بعد الوفاة) ثم أعقبه زيادة بطيئه حتى وصل إلى ٣٢٣و٧٤± ٣٠٧و ٢ملىمول عند ٩٦

ساعة بعد الوفاة. (٤) وجود إرتباط سلبى بين نسبة الصوديوم إلى البوتاسيوم والوقت الـذى مر على الوفاة حيث لوحظ إنخفاض سريع في ٢٤ ساعة الأولى بعد الوفـاة ثـم إنخفـاض بطيىء حتى ٩٦ ساعة بعد الوفاة (كانت النسبة ٠,٨٣و٢٦±٩٥٢و٩ عند وقت الصفـر ثـم ٩٢٤و٠±٦٢١و٠ عند ٩٦ ساعة بعد الوفاة). من هذه الدراسة نستنتج أن تركـيز الكلوريـد والصوديوم والبوتاسيوم وكذلك نسبة الصوديوم إلى البوتاسيوم في أمصال الفـئران يمكـن إستخدامهم كوسيلة لتقدير الوقت الذى مر على الوفاة (خاصة في فصـل الشـتاء أو فـي الظروف الباردة).

SUMMARY

Estimation of postmortem (PM) time is very important specially from the forensic medicine views as well as the development of a new accurate techniques for determining this time must be considered in these purposes. In this study, ninety male albino rats divided into 9 groups (10 rats per group) were used. Each rat was injected with 1 ml heparinized dextrose solution, then sacrificed by administration of an overdose of chloroform and the time of respiratory arrest (death) was recorded. Each cadaver was kept in a temperature of 3-4 °C prior to collection of the blood sample. The blood samples were collected from the rats of group A immediately after death (PMI= zero). While other groups of B to I the blood samples were taken after 6, 12, 24, 36, 48, 60, 72 and 96 hours PM. Each blood sample was centrifuged immediately at 14 000 rpm for 10 minutes. Estimation of chloride, sodium and potassium were adopted in collected serum at allover the various time. Our results revealed that: **(1)** A negative correlation between chloride and PMI. Serum chloride decreased rapidly during the first 24 hours after death, from an antemortem (zero time) mean value of 102.366 ± 0.634 mmol, then continued to decrease (at slow rates) to reach a mean value of 65.047 ± 0.809 mmol at 96 hours PM. **(2)** A negative correlation between sodium and PMI. Serum sodium level showed relatively some stability (very slow decrease) during the first 6 hours following death (mean values of 142.026 ± 1.244 mmol and 139.483 ± 0.967 mmol at PM intervals of zero time and 6 hours respectively), then decreased rapidly between 6 and 24 hours PM, followed by decline at slow rates to reach a mean value of 68.703 ± 1.433 mmol at 96 hours postmortem. **(3)** A positive correlation between potassium and PMI. Serum potassium concentration increased during the first 24 hours after death (from a mean value of 5.445 ± 0.125 mmol at zero time to a value of 47.265 ± 1.411 mmol at 24 hours postmortem), then continued to increase at progressively slow rates to reach a value of 74.323 ± 2.307

mmol at 96 hours PM. (4) A negative correlation between sodium/potassium concentration ratio and PMI. Serum sodium/potassium concentration ratio decreased very rapidly during the first 24 hours following death, from a mean value of 26.083 ± 9.952 at zero time, then this ratio continued to decrease in progressively slow manner, to reach a mean value of 0.924 ± 0.621 at 96 hours PM. From this study, I can conclude that the concentration of chloride, sodium, potassium and sodium/potassium concentration ratio in serum of rats can be used as an indicator for determining the time elapse after death (specially in winter season or in a cold conditions).

Key words: *Rat- chloride- sodium- potassium- serum. postmortem interval (PMI).*

INTRODUCTION

Estimation of postmortem time is important and the development of new accurate techniques for determining this time is highly desirable for use in forensic medicine. The evolution of methods based on body temperature (Knight, 1988), and recommendations for the practical application of the classical physical signs of rigor mortis, such as lividity and temperature, as well as supravital response of skeletal muscle and measurement of the central brain temperature were reported (Henssge *et al.*, 1984; Medea *et al.*, 1986). Furthermore, chemical methods such as the determination of potassium in vitreous humor had been applied (Wensing *et al.*, 1986; McLaughlin and McLaughlin, 1988; Coe, 1989; Ibrahim *et al.*, 1991; lange *et al.*, 1994; Sharkawy and Zaky, 2000).

A reliable chemical method for estimation of postmortem interval (PMI) have been directed towards revealing some constituents of a body fluid, the concentration of which ranges within narrow limits during life and varies in a time-related manner postmortem (Schleyer, 1963; Coe, 1977; Henry and Smith, 1980). Studies during the early postmortem period in rats, have revealed time-dependent changes in plasma total solute content (Querido and Knobel, 1986), in the product of erythrocyte intracellular water content and potassium concentration (Querido and Pillay, 1988), in plasma chloride concentration in rats (Querido, 1990 b), and in the plasma sodium/potassium concentration ratio (Querido, 1990 a).

Postmortem changes in plasma sodium and potassium concentrations have always been considered to occur too rapidly and with too great degree of individual variation, to be of any practical value

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in estimating PMI. Despite 40 years of available knowledge of PM changes in extracellular sodium and potassium concentrations (Jetter, 1959), Querido (1990 a) demonstrated that the relationship between the plasma sodium/potassium concentration ratio and PMI, during the 6 to 96 hours postmortem period, could be linearized by expression of the data in the form of a double logarithmic plot.

The aim of this study was (1) to investigate the biochemical changes occur in the serum after death through estimation of chloride, sodium, potassium and sodium/ potassium ratio, and (2) to relate any changes (either increase or decrease in these electrolytes) that occur in the serum in relation to the time since death in determining the time of death.

MATERIALS AND METHODS

Experimental animals:

Ninety male albino rats (150-200 g) divided into 9 groups (10 rats/group) were used. Each rat was injected with 1 ml heparinized dextrose solution, then sacrificed by administration of an overdose of chloroform and the time of respiratory arrest (death) was recorded. Each cadaver was kept in a temperature of 3-4 C prior to collection of the blood sample. The blood samples were collected from the rats of group A immediately after death (PMI= zero) and from other groups of B to I at 6, 12, 24, 36, 48, 60, 72 and 96 hours PM. Each blood sample was centrifuged immediately at 14 000 rpm for 10 minutes and the serum aspirated for chemical estimation of chloride, sodium and potassium concentration.

Estimation of serum chloride, sodium and potassium:

Serum chloride was measured by $AgN0_3$ titration method according to Jackson (1958). Sodium and potassium concentrations were estimated according to the method described by Williams and Twine (1960) using Carl-Zeiss Flame Photometer (M6D, Germany).

Statistical analysis:

Student's "t" test was used to calculate the significance between different postmortem intervals values and the value of zero time. Probability values 0.05 and 0.001 were considered statistically significant (Snedecor and Cochran, 1974).

RESULTS

Postmortem changes in serum chloride concentration:

The obtained results for serum chloride concentration are shown in Table 1 and Fig. 1. Serum chloride decreased rapidly during the first 24 hours after death, from an antemortem (zero time) mean value of 102.366 ± 0.634 mmol, then reach to 65.047 ± 0.809 mmol at 96 hours PM. The average rate of change (decrease) in serum chloride during the period from zero time to 96 hours after death (calculated from the mean values of serum chloride consecutive postmortem intervals) was 0.576 mmol/l/h.

Table 1: Serum chloride, sodium, potassium and sodium/potassium concentration ratio in albino rats in relation to PMI.

PMI (hours)	Cl (mmol/l)	Na (mmol/l)	K (mmol/l)	Na/K ratio
0	102.366 ± 0.634	142.026 ± 1.244	5.445 ± 0.125	26.083 ± 9.952
6	96.820 ± 0.735**	139.483 ± 0.967	20.325 ± 0.917**	6.862 ± 1.054
12	90.638 ± 1.088**	116.057 ± 2.856**	32.283 ± 1.113**	3.594 ± 2.566*
24	75.828 ± 0.966**	105.387 ± 3.179**	47.265 ± 1.411**	2.229 ± 2.253*
36	75.129 ± 1.019**	95.603 ± 2.158**	55.932 ± 3.107**	1.709 ± 0.694*
48	70.498 ± 0.694**	88.060 ± 3.315**	59.577 ± 2.635**	1.478 ± 1.258*
60	68.984 ± 0.787**	81.819 ± 2.450**	64.305 ± 2.095**	1.272 ± 1.169*
72	67.372 ± 0.614**	74.615 ± 2.517**	70.625 ± 3.669**	1.056 ± 0.686*
96	65.047 ± 0.809**	68.703 ± 1.433**	74.323 ± 2.307**	0.924 ± 0.621*
Correl. Coef.	-0.898	-0.945	0.923	-0.601
Slope	-2.110	-1.148	1.264	-2.404
Intercep.	206.446	155.623	-21.084	51.409

- The obtained results are mean ± S.E.M.
* Significantly different from the value of zero time at $p < 0.05$.
** Significantly different from the value of zero time at $p < 0.001$.

Postmortem changes in serum sodium and potassium concentrations:

Serum sodium and potassium concentrations are summarized in Table 1 and figure 2-3. Serum sodium level showed relatively some stability (very slow decrease) during the first 6 hours following death (mean values of 142.026 ± 1.244 mmol and 139.483 ± 0.967 mmol at postmortem intervals of zero time and 6 hours respectively), then decreased rapidly between 6 and 24 hours PM, followed by decline at slow rates to reach a mean value of 68.703 ± 1.433 mmol at 96 hours PM. serum potassium concentration increased during the first 24 hours after death (from a mean value of 5.445 ± 0.125 mmol at zero time to a value of 47.265 ± 1.411 mmol at 24 hours PM), then continued to

increase at progressively slow rates till reach 74.323 ± 2.307 mmol at 96 hours PM.

Serum sodium/ potassium concentration ratio:

Serum sodium/potassium concentration ratio (table 1 and Fig. 4) decreased very rapidly during the first 24 hours following death, from a mean value of 26.083 ± 9.952 at zero time, then this ratio continued to decrease in progressively slow manner, to reach a mean value of 0.924 ± 0.621 at 96 hours PM.

DISCUSSION

Most investigations of PM changes in chemical composition of body fluids have been directed towards elucidation of the antemortem biochemical status of the deceased and estimation of PMI (Schleyer, 1963; Sturner, 1972; Coe, 1977; Henry and Smith, 1980). Methods for estimating PMI. on the basis of the classical physical signs of death give only imprecise estimates and are affected by a number of variables (Henry and Smith, 1980) and therefore cannot be used to fix the time of death with any degree of certainty (Burton, 1974). Efforts to development of a reliable chemical means of determining PMI have been directed towards revealing some constituent (or combination of constituents) of a body fluid, the concentration of which: (1) ranges within narrow limits during life, and (2) Changes in a time-related manner PM. In addition, the concentration of the constituent at any given PMI should display minimal individual variation and should be independent of environmental factors. The existence of a constituent which displays all of these attributes remains to be demonstrated.

Postmortem changes in serum chloride concentration:

In the present study, the obtained results which revealed a decrease in plasma chloride concentration in postmortem intervals were in agreement and confirmed by the data recorded in other investigations (Jetter and Mclean, 1943; Jetter,1959; Coe, 1977; Querido, 1990a). Our results are also similar to that of these previous studies using plasma of human and animals, where the antemortem plasma chloride concentration in the present study was 363.515±2.254 mg/100 ml (102.336±0.634 mmol) which approximately similar to the average normal value of Albino rats (102 mmol) reported by Becker *et al.*(1979), and also within the normal range of human plasma chloride concentration (103 mmol) as mentioned by Tietz *et al.* (1986).

Jetter (1959) in a subsequent study of 20 human cadavers, found that plasma chloride concentration decreased to between 80 and 90 mmol at 24 h PM. In our study, the serum chloride concentration decreased from an antemortem value (zero time) of 363.515 mg/100 ml (102.366 mmol) to a value of 269.272 mg/100 ml (75.828 mmol) 24 h after death. Our obtained results run in the same manner of that obtained by Querido (1990b). He found that the antemortem mean plasma chloride concentration 101.0 ± 1.5 mmol decreased to 80.3 ± 3.0 mmol at 24 h post-mortem.

Schleyer (1963) reported a PM rate of change (decrease) in plasma chloride concentration ranged from 0.25 to 1.00 mmol/l/h (duration of PMI not specified), Coe (1974) estimated the rate of change (in a study of 155 hospitalized patients) during the first 15 h postmortem to be about 0.97 mmol/l/h. In the present study, serum chloride concentration decreased from an antemortem mean value of 102.366±0.634 mmol to 90.638 ± 1.088 mmol at 12 h PM, this is equivalent to a rate of change of 0.977 mmol/l/h. This value is almost identical to that obtained by Coe, 1974 (0.97 mmol/l/h) and Querido, 1990 a (0.94 mmol/l/h).

Postmortem changes of sodium and potassium concentrations:

The mean value of serum sodium (142.026±1.244 mmol) and potassium (5.445±0.125 mmol) immediately after death, zero time value (table 1 and Fig. 2-3) were of the same order of a manner as the normal values reported by Becker *et al.* (1979) for Albino rats despite the fact that the time zero value cannot be considered normal, since the blood samples were taken immediately after administration of an overdose of chloroform sufficient to inhibit respiration. The slight elevation in the mean value of serum potassium (5.445 mmol) may reflect the rapidity with which extracellular potassium concentration rises after death, particularly so, as Walia et al (1963) have recorded that heart blood values are not reliable indices of the terminal serum potassium levels if there has been even 5 minutes delay in the collection of the blood. The relative stability of serum sodium during the first 6 hours in our study (Fig. 2), is consistent with the early study of Jetter (1959) who reported that human serum sodium concentration remained constant during the first 12 h PM and with Querido (1990 a) who found that serum sodium level in Wister rats also remained constant during the first 6 hours PM.

The changes in serum sodium and potassium in our study during the period between zero to 96 hours PM are in accordance with the well-

establishes decrease in sodium level and increase in potassium level which occur in man (Coe, 1977; Henry and Smith, 1980) and in rat (Hodgkinson and Hambleton, 1969; Querido , 1990a). In addition, the time course of these changes appears similar to that in human. Coe (1974) reported that average rate of decrease in human serum sodium concentraion to be 0.9 mmol/l/h. Querido (1990 a) found that the rate of decrease of serum sodium in rat is about 0.8 mmol/l/h.. In our study, the rate of decrease in serum sodium concentration is about 0.763 mmol/l/h and an increase in serum potassium of about 0.717 mmol/l/h during the period between zero to 96 hours PM. The overall observations (table 1 and Fig. 2-3) confirm the generally-held view that postmortem changes in serum or plasma sodium and potassium levels occur too rapidly, and with too great degree of individual variation, to be of any practical value in estimating PMI.

The results of the present study indicate that absolute values and the rate of change in serum chloride, sodium and potassium concentrations during the period between zero to 96 hours PM in albino rats are virtually identical to those in dog, man and Wister rat. These findings obtained under laboratory conditions need further investigation under conditions encountered in routine forensic work.,

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Fig. 1. Serum chloride concentration in albino rats.

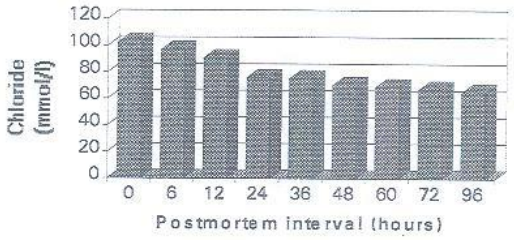

Fig. 2. Serum sodium concentration in albino rats.