Postmortem Biochemical Changes in Canine Cerebrospinal Fluid and Relation to Time after Death

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Summary
The effects of time on postmortem cerebrospinal fluid (CSF) samples taken from 10 adult dogs were studied. Dogs were maintained at room temperature 22°C for zero, 4, 12, 24, 36, and 48 hrs after death. Antimortem and postmortem cerebrospinal fluids was evaluated for potassium, sodium, chloride, zinc, glucose, lactate, total proteins, albumin, and urea. Results revealed significant and linear increase in the potassium concentration over the time in CSF where it was 0.34 mEq/hr during the first 12 hr PM, and then it became 0.25 mEq/hr during the next 24 hr. Also, the concentration of sodium was significantly increased as did lactate at different PM intervals, while, chloride and glucose concentrations declined significantly along the PM intervals. Zinc was not significantly changed but it slightly increased at 36 and 48 hrs PM. Total proteins, albumin and urea were significantly changed, only at late PM intervals. It was concluded that, a significant relationship exist between the concentrations of these constituents in canine CSF and with the time since death, and to determine the time since death, a combinations of chemical determinations gives a more reliable estimate than single measurements.

Introduction
Estimation of accurate time since death has always been an unclenched question of forensic pathologists all over the world. It becomes more intense when even the physical signs e.g. postmortem staining, cooling of body, rigor mortis etc. which gives rough estimation, are either in conflict or no longer gives any information. To overcome such situations, quotations of various body fluids had been attempted by various investigators (1 - 4). The most often investigated body fluids have been vitreous humor and cerebrospinal fluid in human and animals (5 - 13). These fluids showed significant changes in the
electrolyte concentrations in particular to potassium concentration in relation to time since death. Also, the most useful measurements on blood and cerebrospinal fluids (CSF) are amino nitrogen, nonprotein nitrogen, ammonia, creatine, and inorganic phosphate, which have a great value in the early PM interval (14). Potassium content of the vitreous humor and CSF has shown a linear rise with time in the interval 12 – 100 hours PM (14). The effect of time intervals of 3, 6, 12, 24, or 48 hours postmortem on CSF taken from adults dogs were studied by Schoning & Strafuss (5), the antimortem and postmortem CSF was evaluated for sodium, chloride, potassium, urea nitrogen, glucose, creatinine, calcium, phosphorus, and carbon dioxide. Results revealed that sodium and urea nitrogen values remain stable, carbon dioxide value dropped, calcium and creatinine levels increased slightly but continually after death. Moreover, chloride values decreased, while, potassium and phosphorus values increased markedly (6). Recently, Varela and Bossart (15), evaluated the biochemical analyses in vitreous humor collected from Indian manatees after death. Results revealed that, creatinine and urea nitrogen were significantly higher in severely autolyzed carcasses than in fresh carcasses, also, potassium concentrations were significantly higher in moderately autolyzed carcasses than in fresh ones but were highly variable in severely autolyzed carcasses. The aim of this work is to investigate the effect of time on certain biochemical parameters in cerebrospinal fluids taken from dogs at different time intervals after death.

Material and Methods

Animals:
Ten adult male dogs were used in this study. Antimortem cerebrospinal fluid samples were taken under general anesthesia and according to the procedure described by Klidle (16). Animals are then killed by rapid IV injection of over dose of thiopental sodium and their carcasses were kept at room temperature (22°C) for postmortem biochemical analysis. CSF samples were taken at intervals of zero, 4, 12, 36 and 48 hrs after death. All samples of CSF were stored –20°C till analysis.
Biochemical analysis of CSF:

Samples of cerebrospinal fluids are subjected for biochemical analysis, where, potassium, sodium and chloride, zinc, lactate, glucose, total proteins, albumin, and urea, were measured spectrophotometrically (17 – 23) using commercial kits purchased from BioMerieux and Randox Co.

Statistical analysis:

The data were analyzed by ANOVA as described by Snedecor and Cochran (24) and results are presented as mean ± S.D (P < 0.05) is considered statistically significant.

Results

Results presented in table (1) and figures (1 & 2) revealed that there are a significant changes in different element of the CSF at different intervals postmortem, where, potassium concentration increased gradually from 3.56 ± 0.39 antimortem to 11.22 ± 0.34 after 48 hrs postmortem. The rate of increase of potassium concentration over the time in CSF was 0.34 mEq/ hr, during the first 12 hr PM, then it became 0.25, 0.20, and 0.16 mEq/hr during the next 24 hr, 36 hr and 48 hrs postmortem, respectively. Also, sodium level was increased from 41.2 ± 5.26 antimortem to 133.4 ± 4.38 at 48 hr PM, while, chloride level was decreased along the pm intervals. Zinc was not significantly changed but it was slightly increased at 36 and 48 hrs PM. As concern glucose level in CSF, the results showed a significant decrease reaching 15.3 ± 1.96 mg/dl at 48 hr PM. In contrast, lactate was significantly increased from 9.3 ± 0.60 mg/dl antimortem to 65.8 ± 3.19 and 70.5 ± 4.19 mg/dl at 36 and 48 hr PM, respectively (table 1 & fig 2). Total proteins and albumin shows a significant decrease only at late (48 hr) PM interval. Finally, urea showed a significant increase only at 36 and 48 hrs PM (table 1 & fig 2).

Discussion

The determination of time elapsed after death by chemical examination of body fluids was restricted to compartments which are not as much exposed to autolysis and putrefaction as blood, the examination of vitreous humor was established by Coe (25), and
Madea et al., (9). Another possible compartment is the cerebrospinal fluids which can be used to estimate time since death and vitreous humor can not be used due to traumatic injuries or for other reasons. The results presented in table (1) and figures (1& 2) shown that, the concentration of some elements in CSF of dogs with in different intervals after death can be used as a reference indicators to estimate time after death with especial attention to potassium level which increased linearly after death, and such increase was rapid during the first 24 hrs of death, where, the rate of potassium increase was 0.34 and 0.25 mEq/ hr, during the first 12 and 24 hr PM respectively, then it decreased to , 0.20 , and 0.16 mEq/hr during the next 36 hr and 48 hrs postmortem, respectively. These results are in parallel with those obtained by Karkela (26) and Singha et al (4) who attributed the increase in potassium to its rapid release from the cells immediately after death. Similarly, sodium concentration in CSF of dogs was increased from 41.2 ± 5.26 mEq/l at antimortem to 129.8 ± 6.38 and 133.4 ± 4.38 mEq/l at 36 and 48 hrs PM, respectively (table 1 and figure 1). This result is inconsistent with those recorded by Schoning and Strafuss (5,6) who noticed non significant changes in sodium level in canine CSF at 3- 48 hrs after death. Chloride content of CSF of dogs showed gradual and slight decrease after death (table 1 and figure 1). As concerns glucose and lactate concentrations in CSF of dogs, results revealed a gradual and significant decrease of glucose after death whereas, lactate concentration showed the vice versa (table 1 and figure 2). These results are in agreement with those of Karkela (26) and Karlovsek (27) who, explained that, glucose in the blood and other body fluids in the corpse simply disappeared in the early post-mortem period, and the most important process contributing to this fact is glycolysis in which, the whole blood-sugar was metabolized to lactate (lactic acid is the final product of post-mortem glycolysis; one molecule of glucose gives two molecules of lactic acid). CSF total proteins, albumin and urea concentrations did not show significant changes till 36 hr after death where, total protein and albumins decreased while urea increased significantly 48 hr PM, these was attributed to the autolysis and putrefaction process that become evident after 24 hr PM (15). These results indicate that some constituents showed marked changes during early PM intervals (as potassium,
sodium, glucose, lactate) while others are markedly changed at late PM intervals (total proteins, albumin and urea). It can be concluded that, in canine cerebrospinal fluid, a significant relationship exist between the concentrations of some biochemical constituents -especially potassium, sodium, chloride, glucose and lactate- and with the time since death. Therefore, a combinations of chemical determinations for estimating PM time intervals gives a more reliable estimate than single measurements.

References
The Iowa State Univ. press Ames IA.
Table (1): Biochemical constituents of canine CSF at different time intervals after death.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Anti mortem</th>
<th>Time after death (PM)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>4 hrs</td>
</tr>
<tr>
<td>Potassium (mEq/l)</td>
<td>3.56 ± 0.39 de</td>
<td>5.54 ± 0.32 d</td>
</tr>
<tr>
<td>Sodium (mEq/l)</td>
<td>41.2 ± 5.26 e</td>
<td>64.2 ± 5.12 de</td>
</tr>
<tr>
<td>Chloride (mEq/l)</td>
<td>130.2 ± 6.38 a</td>
<td>120.2 ± 3.96 ab</td>
</tr>
<tr>
<td>Zinc (μg/dl)</td>
<td>91.4 ± 5.59</td>
<td>93.2 ± 2.38</td>
</tr>
<tr>
<td>Lactate (mg/dl)</td>
<td>9.3 ± 0.60 de</td>
<td>12.64 ± 0.61 d</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>70.6 ± 4.45 a</td>
<td>60.2 ± 3.35 ab</td>
</tr>
<tr>
<td>T.proteins (g/dl)</td>
<td>2.04 ± 0.11 a</td>
<td>2.04 ± 0.21 a</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>1.1 ± 0.16 a</td>
<td>1.16 ± 0.11 a</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>12 ± 1.58 c</td>
<td>13 ± 1.58 c</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± S.D.
Values with different letters are significantly differed (P ≤0.05).
Figure (1a): Potassium concentration in CSF of dogs at different PM intervals.

Figure (1b): Sodium concentration in CSF of dogs at different PM intervals.
Figure (1c): Chloride concentration in CSF of dogs at different PM intervals.

Figure (2a): Glucose concentration in CSF of dogs at different PM intervals.
**Figure (2b):** Lactate concentration in CSF of dogs at different PM intervals.

**Figure (2c):** Urea concentration in CSF of dogs at different PM intervals.
دراسة التغيرات البيوكيميائية لبعض مكونات السائل النخاعي الشوكي في الكلاب وعلاقتها بوقت حدوث النفوق
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أجريت هذه الدراسة لمعرفة تأثير الوقت على بعض مكونات السائل النخاعي المأخوذ من الكلاب قبل و بعد حدوث النفوق. حيث تم قتل مجموعة من الكلاب ووضعت في درجة حرارة الغريه (22 مئوية) لفترات 4 و 12 و 24 و 36 و 48 ساعه بعد النفوق. و لقد أظهرت التحليل البيوكيميائي للسائل النخاعي تغيرات في تركيزات بعض العناصر وهي البوتاسيوم - الصوديوم - الكالريوم - الزئبق - السكر - اللاكتيت - البروتينات - الزئل - و البولينات و ذلك على النحو التالي: زيادة تدريجية و ملحوظة بالنسبة للوقت في كمية عنصر البوتاسيوم حيث وصلت هذه الزيادة لأعلى معدلاتها 0.34 - 0.25 mEq/hr بعد 12 و 24 ساعه على التوالي من حدوث النفوق. أيضاً وصلت تركيزات كل من الصوديوم و اللاكتيت إلى مستويات عالية و ملحوظة عند مختلف الفترات بعد النفوق أما تركيزات الكالريوم و السكر فقد انخفضت بدرجة ملحوظة و تدريجية بعد النفوق و لم يظهر عنصر الزئبق تغيرات ملحوظة عند مختلف الفترات بعد النفوق. بالنسبة للبروتينات و الزئل فقد انخفضت فقط بعد فترات طويلة من النفوق و كان ذلك بعد 36 و 48 ساعه من النفوق. مماثلً بوضوح أن هناك علاقة قوية و ملحوظة بين تركيزات بعض العناصر (مثل البوتاسيوم - الصوديوم - السكر - اللاكتيت - البولينات) وبين وقت حدوث النفوق و أن قياس أكثر من عنصر قد يكون مفاضلاً من الإعتماد على قياس أحد العناصر لتحديد هذا الوقت.