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COMPARATI VE BIOCHEMICAL STUDIES BETWEEN AQUEOUS & VITREOUS FLUIDS AND SERUM OF DONKEYS

(With 6 Tables)

By

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مقارنة المحتوى الكيميائي لسوائل غرفتي العيـــن
مع المصل كوسيلة للتشخيص بعد الـــــوت

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إن بطء حدوث التغيرات الكيميائية مع ندرة التلوث في سوائل العين في غرفتها الأمامية والخلفية مع سرعة حدوثها في الدم والأنسجة بالإضافة إلى ثبات مستوى العديـــد من العناصر والمركبات بعد الموت ولفترة طويلة نسبيا يجعل من دراسة المحتوى الكيميائي الطبيعي لمحتويات سوائل العين والدم ذات دلالات هامة ليعطي مؤشراً ممتازاً في الكشـــف عن العديد من حالات التسمم والحالات المرضية المختلفة بالجسم خاصة بعد النفوق إضافـــة إلى قضايا الطب الشرعي والتي تتلازم معظمها مع حدوث تغيرات كيميائية بالجسم . وقـد تم إجراء هذا البحث على عشرين من ذكور الحمير المتواجدة ضمن حيوانات التجـــارب بقسم الجراحة بكلية الطب البيطري ـ جامعة أسيوط والتي تراوحت أعمارها بين أربـــع إلى ثمانية سنوات وتم أخذ عينات من دم وسائل الغرفتين الأمامية والخلفية للعيـــــن للتحليلات الكيميائية المختلفة لإيجاد العلاقة بين محتواها في الدم وفي سائل العيـــــن لتكون نواه للإستفادة بها كمؤشر لمعرفة سبب الموت . وقد تم قياس كل من الصوديـــوم والبوتاسيوم والكلوريد والكالسيوم والفسفور كمحتوى غير عضوي والبروتين الكلي واليوريا وحامض اليوريك والجلوكوز كمحتوى عضوي كما تم تقدير أنشطة بعض الخمائـــــر الهامة في الفوسفاتاز الحمضي والتراتزاميناز . وقد دلت النتائج أن هناك إرتبـــــاط معنوي شديد بين مستويات العناصر في الدم ومستوياتها في سائل غرفتي العين في داخـل الحيوان الواحد مما يؤكد ويؤيد ضرورة الإستفادة بالتحليلات الكيميائية لغرفتي العيـــن كوسيلة هامة جداً في تشخيص العديد من حالات السموم والأمراض وخاصة بعد النفوق مـع ضرورة الأخذ في الإعتبار تلك العناصر التي تتغير مع الموت .

SUMMARY

The present study was carried out to investigate the relationship between normal chemical constituent of donkey's serum and that of both aqueous and vitreous humour in the same animals. Analysis included sodium, potassium, chloride, calcium, phosphorus, uric acid,

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urea nitrogen, total proteins, glucose, ASAT, ALAT and acid phosphatase in the three biological fluids. Results indicated a highly significant correlation between levels of chemical constituent in serum, aqueous and vitreous humour.

INTRODUCTION

Metabolic disorder and poisonous cases are difficult or impossible for pathologist or toxicologist to be diagnosed due to advanced autolysis of absence of specific lesions. This is particularly a problem in cases of sudden or un observed death which frequently occurs. In these situation ante-mortem serum chemical values can be useful but are often unavailable (CANTOR, et al. 1989).

Moreover, postmortem blood undergoes rapid chemical changes or contamination. Aqueous or vitreous humour of the eye, on the other hand, has been found to retain some of its chemical values for a relatively long time after death, either in man or other species of animal under numerous type of diseases (COE, 1969 & 1972; PALMER, et al. 1985; LINCOLN and LANE, 1985 a).

In a recent study dealing with aqueous or vitreous humour of the eye, IBRAHIM, et al. (1991) and IBRAHIM & SHEHATA (1992) reported that both fluids contain various cations, proteins, urea nitrogen and some enzymes namely acid phosphatase, ALAL and ASAT. These constituents proved to be stable over 120 hrs. post-mortem with the exception of potassium and Acid phosphatase enzyme which showed relatively gradual elevation by time of death.

Lack of information on other constituents in donkeys encouraged the authours to present this work beginning firstly with a comparative evaluation of the chemical composition of Aq. & Vit.humour in correlation with their respective concentration in the blood of donkeys; these basic data may be of a further help in the field of forensic medicine & toxicology.

MATERIAL and METHODS

Twenty male donkeys between 4 to 8 years old, belonging to experimental animals of the dept. of surgery, Faculty of Veterinary Medicine, Assiut University were used in this study.

Blood samples from each examined animal were collected from jugular vein. The blood samples were left for clotting and then centrifuged at 3000 rpm for 5 minutes for obtaining serum.

Paracentesises of anterior and posterior chamber of the eye were performed for obtaining aqueous and vitreous humour respectively. The fluid was aspirated, using a 26-gauge (for aqueous humour) and 22-gauge (for vitreous humour), 2.5 cm needle and two-3 ml syringes connected to 3 way stopcock. The animals used in this experiment were free of clinical ocular abnormalities.

The paracentesis was performed under control of physical restraint and topical eye instillation of local analgesic (1% novasine).

For obtaining the vitreous humour, the needle was inserted through the sclera about 0.3 cm caudal to the limbus (corneo-scleral junction). The needle is directed ventrally and slightly caudally for about one cm to avoid the lens. Once the needle was in proper position, approximately one ml of vitreous humour was slowly aspirated with one syring.

For obtaining the aqueous humour, the needle was inserted cranial to the limbus. Once the tip of the needle centered over the pupil, most of the aquous humour was slowly aspirated with one syring. After aspiration of each humour, the 3-way stopcock was redirected and the exact volume of sterile normal saline solution was then injected to replace the aspirated fluid using the other syring.

Biochemical analysis of serum, aqueous and vitreous humour samples for potassium and sodium content were estimated by using flame photometer (corning 400), while chloride levels were determined, by corning chloride-meter 925. Uric acid and urea nitrogen were estimated after VARLEY (1975) and CHANEY and MARBACH (1962) respectively. Total proteins, calcium, inorganic phosphorus and glucose were determined according to the methods of WEICHSELBAUM (1946); GINDLER and KING (1972); GOLDENBERG (1966), respectively. Aspartic aminotransferse (ASAT) and alanine aminotransferase (ALAT) were determined using test kits supplied by Bio-Merieux / Bains / France, following the method of REITMAN and FRANKEL (1957). Acid phosphatase was estimated using test kits after KIND and KING (1954).

Multible correlation was made by the programing system in computer center of Assiut University.*

RESULTS

Results of inorganic constituents (potassium, sodium, chloride, phosphorous and calcium) of aqueous, vitreous humour and serum were recorded in table (1). Correlation coefficient of the three fluids were recorded in table (2).

Glucose, total proteins, uric acid and urea nitrogen as organic constituent concentration were recorded in table (3), and the correlation coefficient of the aqueous, vitreous and serum in the same animal were recorded in table (4).

Enzymatic activities of acid phosphatase, ALAT and ASAT of the three fluids were shown in table (5). The correlation coefficient recorded in table (6).

DISCUSSION

The results of the present investigation revealed that the content of different substance in aqueous, vitreous humour and serum were variable. These specific differences between the composition of aqueous, vitreous humour suggest a blood-retinal

*: According to PC. State, 1985, the University of Georgia, Athens, Georgia, U.S.A.

or a blood-vitreal barrier with selective properties similar to the blood-brain barrier (BLEEKER, et al. 1968 and GUNHA-VAZ, 1966).

The obtained results of inorganic constituents revealed that sodium, potassium and chloride levels in both aqueous and vitreous were higher than those present in serum. The direct passage of sodium toward the blood seems to be limited by a membrane of quite low permeability (REDDY and KINSEY, 1960).

The available literature lacks accurate knowledge about the mechanism of passage of these various substances between serum and both fluids under normal physiological conditions. It seem that there is a species differences concerning this matter. An example can be offered related to cat & rabbit where chloride is specially low in the aqueous humour and is associated in some way with a high bicarbonate concentration. In man and other primates the distribution ratio of chloride and bicarbonate is the reverse of that in the cat and rabbit (GLOOR, 1973). According to BITO and DAVSON (1964) an active carrier mechanism in the ciliary epithelium brings the potassium concentration in the posterior chamber to higher level than the plasma. On the other side calcium and phosphate contents of aqueous and vitreous humour were lower than their levels in serum. The low level of phosphate in the vitreous compared to that in the aqueous was previously recorded by ALDER (1975). Much less phosphate penetrates into the vitreous than into the anterior chamber As demonstrated by autoradiographic techniques the portal of entrance is the anterior vitreous bordering the ciliary body (CHRISTIANSSON and PALM, 1954). Regarding the aqueous and vitreous humour calcium and phosphorous, it is obvious that their concentration is rather lower than the level in blood (SOLIMAN and EL-AMROUSI, 1966).

The concentration of organic constituents (glucose, total proteins, uric acid and urea vitrogen) were found to lower in the aqueous and vitreous humour of donkey rather than in serum. Glucose diffuses into the vitreous across all its surrounding tissues. Its penetration into the vitreous is slower than into the aqueous (ADLER, 1975). The concentration of sugar was found to be lower in the aqueous humour of camels rather than in blood (SOLIMAN and EL-AMROUSI, 1966). There are two explanations for such a finding; either some of the sugar is held back in the blood stream and can not reach the anterior chamber (ADLER, 1953), or by its utilization and consumption by the surrounding tissues.

The low level of total proteins in both aqueous and vitreous humour recorded in our study related to serum is supported by the opinion of BALAZS (1960). The auther stated that blood vitreal barrier is quite effecient in dealing with soluble proteins, as the penetration rate of blood protein and haemoglobin is very low.

The obtained results of urea nitrogen were similar to that of ADLERS (1975) where urea content in aqueous and vitreous is lower than in the plasma.

The results of enzymatic activities (ACPH, ALAT and ASAT) showed lower levels in aqueous and vitreous humour rather than in serum. These records were previously reported by IBRAHIM, et al. (1991).

The statistical analysis of data (tables, 2, 4, 6) revealed a significant carrelation between serum and either aqueous and vitreous for all inorganic and organic contents and enzymatic activities except sodium.

The above examples clear that the penetration rate of the tested constituents varies between animals and more variations are expected under the infleunce of toxic substances or other causes (diseases) inducing death. This needs, in our opinion, further seperate detailed study on the action of currently famous toxic substances on the rate of pentration levels and distribution of constituents of both fluids. Degree of changes of these barriers must be taken in consideration.

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Table (1)

Concentration of Na, K, Ca, Cl & phosphate (mmol/kg H_2O) in aqueous, vitreous homours fluids and serum of donkey

Samples		Sodium	Potassium	Chloride	Calcium	Phosphate
Serum	Mean±S.E.	134.680±1.28	5.45±0.17	99.90±1.04	10.02± 0.21	2.16±0.11
	Min. - Max.	128.80 -140.0	4.90-6.70	94.00-106.00	8.99-10.80	1.68-2.75
Aqueous	Mean±S.E.	151.33 ±3.34	7.04±0.32	120.70±1.36	7.62±0.11	1.00±0.09
	Min. - Max.	126.00 -165.60	6.10-9.70	114.00-124.00	7.20-8.15	0.61-1.484
Vitreous	Mean±S.E.	143.94 ±1.33	6.42±0.26	112.60±1.03	7.32±0.12	0.63±0.07
	Min. - Max.	137.20 -151.20	5.80-8.50	107.00-116.00	6.60-7.85	0.21-0.93

Table (2)

Correlation coefficient/probability RHO=0 of Na, K, Ca, Cl & phosphate

Samples correlation between	Sodium	P <	Potassium	P <	Chloride	P <	Calcium	P <	Phosphate	P <
1 and 2	0.438	0.205	0.911	0.0002	0.912	0.0002	0.865	0.0012	0.964	10-4
1 and 3	0.118	0.745	0.926	0.0001	0.926	0.0001	0.869	0.001	0.923	10-4
2 and 3	0.598	0.068	0.933	0.0001	0.933	0.0001	0.878	0.001	0.960	10-4

1 : Serum
2 : Aqueous
3 : Vitreous

S.E. : Standard errors.
P : Significant at probability

Table (3)

Concentration of glucose, total proteins uric acid & urea nitrogen (mg/100 ml H_2O) of aqueous, vitreous humours fluide and serum of donkey

Samples		Glucose	Total protein	Uric acid	Urea nitrogen
Serum	Mean±S.E.	52.50± 1.31	110.20± 4.13	15.41± 1.14	17.89± 1.60
	Min. - Max.	45 - 57	86 - 130	10.00-20.20	8.50-22.80
Aqueous	Mean±S.E.	47.31± 1.26	9.75± 0.70	10.80± 0.72	12.32± 0.78
	Min. - Max.	40.50-53.80	6.00-14.00	6.90-14.23	7.66-15.40
Vitreous	Mean±S.E.	44.70± 1.13	7.67± 0.53	7.75± 0.83	14.80± 1.22
	Min. - Max.	38.00-49.00	4.60-11.00	3.63-12.36	10.22-22.04

Table (4)

Correlation coefficient/probability RHO=0 of glucose, total proteins uric acid & urea nitrogen

Samples correlation between	Glucose	P <	Total protein	P <	Uric acid	P <	Urea nitrogen	P <
1 and 2	0.958	10-4	0.936	10.4	0.696	0.025	0.909	0.0003
1 and 3	0.973	10-4	0.900	0.0004	0.631	0.051	0.692	0.027
2 and 3	0.970	10-4	0.938	10-4	0.766	0.009	0.715	0.020

1 : Serum
2 : Aqueous
3 : Vitreous

S.E. : Standard errors.
P : Significant at probability

Table (5)

Activity of acid phosphatase, ALAT & ASAT (U.L) of aqueous, vitreous humours fluid and serum of donkey

Samples		Acid phosphatase	ALAT	ASAT
Serum	Mean±S.E.	253.17± 6.13	11.00± 1.35	217.00± 8.72
	Min. - Max.	224.0 -287.5	6.00 - 18.00	180.0 -260.0
Aqueous	Mean±S.E.	93.60± 0.99	2.40± 0.19	22.80± 1.41
	Min. - Max.	88.5 -98.0	1.50- 3.00	18.0 -30.0
Vitreous	Mean±S.E.	69.60± 1.93	3.60± 0.38	61.10± 1.88
	Min. - Max.	60.0 -80.0	2.00- 6.00	52.00-68.00

Table (6)

Correlation coefficient/probability RHO=0 of acid phosphatase, ALAT & ASAT

Samples correlation between	ALAT	P <	ASAT	P<	Acid-phosphatase	P<
1 and 2	0.901	0.0004	0.928	0.0001	0.860	0.0014
1 and 3	0.957	10-4	0.904	0.0003	0.836	0.0026
2 and 3	0.881	0.0008	0.776	0.0083	0.847	0.0020

1 : Serum
2 : Aqueous
3 : Vitreous

S.E. : Standard errors.
P : Significant at probability