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AQUEOUS AND VITREOUS HUMOR ANALYSIS AS A DIAGNOSTIC AID FOR POSTMORTEM TIMING

(With 4 Tables & 2 Figs.)

By

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التحليل الكيميائي للسوائل المائية لغرفتي العين كوسيلة لتقدير وقت حدوث النفوق

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تظهر التغيرات الميتية بالعين عقب النفوق مباشرة ولفترة وجيزة إلا أن سوائل العين بما تتمع به من شفافية وصعوبة تلوثها تزيد من قيمتها بعد الموت . ولما كان تقدير وقت الموت في بقايا الجثث من الإمور الغاية في الصعوبة والدقة فقد حدى بنا ذلك لاجراء التحليلات الكيميائية لسوائل العين للاستفادة منها في تقدير الفترة التي انقضت على النفوق. وقد تم اجراء هذا البحث على خمسين من ذكور الحمير المتواجده بالمستشفى البيطرى جامعة أسيوط والتي قسمت الى عشر مجموعات ، تم أخذ عينات من حيواناتها بعد ٦، ١٢، ١٨، ٢٤، ٣٦، ٤٨، ٦٠، ٧٢، ٨٤، ٩٦ ساعة وقد سبق أخذ عينات من السائل المائي لكل من الغرفتين الأمامية والخلفية من نفس هذه الحيوانات قبل النفوق بمالايقل عن خمسة عشر يوما كضابط للتجربة . وقد أجريت التحاليل الكيميائية للسوائل المائية للعين لكل من البوتاسيوم والصوديوم والكلوريد والكالسيوم كمحتوى غير عضوى وكذلك البروتين الكلي واليوريا وحامض اليوريك كمواد عضوية كما تم تقدير أنشطة بعض الخمائر ممثلة في الفوسفاتاز الحمضي والتراثزامينار. وقد دلت نتائج البحث عن وجود علاقة ايجابية متزايدة ووثيقة بين وقت النفوق وتركيزات كل من البوتاسيوم والفوسفاتاز الحمضي والبروتين الكلي في الغرفة الأمامية وكذلك في البوتاسيوم والفوسفاتاز في الغرفة الخلفية. أما باقي النتائج فلم تعطى دلالات كافية لوجود هذه العلاقة. وقد اتضحت هذه العلاقة الخطيه بوضوح من التحليلات خاصة فى البوتاسيوم والفوسفاتاز الحمضي. ويتضح من هذا البحث الأهمية الكبرى لاستخدام السائل المائي لأى الغرفتين الأمامية والخلفية للعين لتقدير وقت النفوق عن طريق تقدير مستوى البوتاسيوم أو الفوسفاتاز الحمضي وان تزايد ت الأهمية في التقدير في الغرفة الأمامية عنها في الخلفية.

SUMMARY

The present study has been carried out on donkey's aqueous and vitreous humor to investigate the relationship between analytical changes and time of death.

Biochemical analysis of samples for sodium, potassium, calcium, chloride, uric acid, urea nitrogen, total protein, ALAT, ASAT and acid phosphatase were conducted.

INTRODUCTION

Aqueous and vitreous humor are stable, easily withdrawn fluids, and less susceptible than blood to postmortem chemical changes. It can be used to determine the time of death and as an aid to diagnosis of some diseases and poisonous cases (LANE and LINCOLN, 1985).

Aqueous humor resembles an ultrafiltrate of plasma. The chemical components of the aqueous humor are colloids, nonionized crystalloids and ionized crystalloids (DUKE-ELDER, 1968). The colloids are proteins, immunoglobulins, enzymes and lipids which are all present in much lower concentration than in the plasma as blood aqueous barrier. Sugar, urea and amino acids are the major nonionized crystalloids. The ionized crystalloids are anions and cations. The major cations in aqueous humor are sodium, potassium, calcium and magnesium with sodium making up 95% of the total cation concentration, but major anions in aqueous humor are chloride, bicarbonate, phosphate and lactate (COLE, 1974).

Vitreous humor (hyaloid body) occupies about 80% of the ocular globe volume and provides structural support and maintains intraocular pressure (TRIPATHI and TRIPATHI, 1984). It also acts as a "sink" for lens and retina so that metabolized substances are able to leake into the vitreous (BALYZS and DENLINGER, 1984). It has been stated that vitreous collected after death may be useful for determination the time of death by using potassium estimations (COE, 1989), but other tests may not be significantly important (BALASOORIYA, et al. 1984).

The chemical composition of aqueous and vitreous humor were previously recorded in horse, dog, cat and cow (BITO, 1965; DUKE-ELDER, 1968 and GRAYMORE, 1970).

The aim of the present study correlates between the biochemical changes in both aqueous and vitreous humor fluids and time after death.

MATERIAL and METHODS

Fifty donkeys from Faculty of Veterinary Medicine Hospital, Assiut University were used in this study. Samples of aqueous and vitreous humor were taken antemortem from all examined animals. The procedures were performed using thiopental sodium as a

tranquilizer in a dose of 5 mg/kg, i/v, premedicated with combelen in a dose of 0.2 mg/kg i/m for antemortem sampling. After death, the animals were divided into ten groups (5 animals each). Aqueous and vitreous humor samples were collected, 6, 12, 18, 24, 36, 48, 60, 72, 84 and 96 hours after death from the 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th and 10th group respectively. All eyes were normal in ophthalmic examination before sampling. All carcasses were kept in room temperature ranged between 5-20°C.

Aqueous and vitreous humor samples from both eyes were collected using a 62-gauge, 0.45 X 13 mm disposable needle, two 3-ml plastic syringe and a 3-way stopcock.

Biochemical analysis of aqueous and vitreous humor were established in the same manner for blood sera for potassium and sodium content estimation using flame photometer (Corning 400), while chloride levels were determined using chloride analyser model 925. Uric acid and urea nitrogen were detected after VARLEY (1975) and CHANEY and MARBACH (1962) respectively. Total protein and calcium were determined according to the methods of WEICHSELBAUM (1946); GINDLER and KING (1972) respectively. Aspartic aminotransferase (ASAT) and alanine aminotransferase (ALAT) were determined using test kits supplied from Biomerieux (Bains / France), folowing the methods of REITMAN and FRANKEL (1957). Acid phosphatase was estimated using test kits after KIND and KING (1954). The obtained data were statistically analysed according to KALTON (1967).

The simplest procedure for determining time of death is the use of the well known equation developed by STURNER (1963).

RESULTS

Results of inorganic constituents (potassium, sodium, chloride and calcium) of both aqueous and vitreous fluids of donkeys are recorded in table (1) and the effect of time of potassium level is shown in fig. (1).

Total protein, urea nitrogen and uric acid levels (organic constituents) are recorded in table (2).

Enzymatic activities of acid phosphatase, ALAT and ASAT of aqueous and vitreous humor are shown in table (3) and the effect of time on acid phosphatase activity is illustrated in fig. (2).

DISCUSSION

The results of the present study revealed that the normal levels of antemortem chemical composition of both vitreous and aqueous humor were more or less similar to

that reported in horse by BITO (1965); DUKE-ELDER (1968) and GRAYMORE (1970).

Our results dealing ten biochemical analysis parâmeters in this study, only acid phosphatase activity and potassium level showed a significant changes in both vitreous and aqueous humor in relation to time elapsed after death.

Obviously it appears that levels of aspartic aminotransferase (ASAT), alanine aminotransferase (ALAT), sodium, chloride and calcium in both aqueous and vitreous humor showed no significant variation between post and antemortem analyses. These findings are qualitatively similar to the results of DEVGUN and DUNBAR (1986) and MEDEA, et al. (1989). They reported that sodium, calcium, chloride and urea are stable in the postmortem interval up to 120 hours. Analysis of the previous mineral content may be useful in cataract eye disease, hyperparathyroidism and malignancy associated hypercalcaemia and uric acid in gout disease.

In traumatic or sudden deaths, the recorded rapid release of LDH and transaminases by hyalocytes may be due to the release of pteridine-like substances. The latter substances have a characteristic excitation band which is similar to the wavelength at which LDH, ASAT and ALAT are measured spectrophotometrically (MEDEA, et al. 1989).

A proportional significant elevation of acid phosphatase activity in correlation to time of death was recorded in this study. It must also be borne in mind that acid phosphatase is a lysosomal enzyme and its high level may confuse other lysosomal activity condition (WILKINSON, 1976). The release of the enzyme depend on membrane permeability which increases after death. Our results showed a significant elevation of potassium level in both aqueous and vitreous humor in correlation with time of death. This article is an historical review and critical evaluation of factors to be considered when using this test to determine the postmortem interval. JAFF (1962), found a consistent rise in the level of potassium commencing shortly after death and continuing for 125 hours. Therefore, it was no significant difference between refrigerated bodies and those kept at room temperature. ADELSON, et al. (1963) recorded that no significant difference was noticed in the potassium level of the two eyes. These workers found a straight line relationship between the vitreous potassium concentration and the postmortem interval. At the same line, our results are confirmed by that obtained by LEAHY and FARBER, (1967); LIE (1967) and COE (1973 & 1989).

Statistical analysis of data (table 4) revealed a significant level (0.0001) for the correlation between tha PMI and the cocentration of potassium, urea and acid phosphatase in aqueous humor fluid. On the other hand, only pctassium and acid phosphatase had the same correlation in vitreous fluid.

There was a linear relationship of the potassium values obtained by flame photometry and the PMI. It has a slope of approximately 0.085 and 0.0429 in aqueous and vitreous fluids respectively. The intercept was of approximately 5.885 and 6.704 and

correlation coefficient was of 0.995 and 0.928 for aqueous and vitreous fluid respectively.

Aqueous urea levels had a slope of aproximately 4.637, intercept of approximately 13.419 and correlation coefficient of 0.985.

The relationship between acid phosphatase levels and PMI was linear. There were a slope, intercept and correlation coefficient of (0.103 and 7.230); (72.683 and 35.112) and (0.735 and 0.975) for aqueous and vitreous humor respectively. It has been found a very good agreement between the actual and "expected" values in great majority of cases.

A number of both internal and external factors influencing the postmortem rise in aqueous and vitreous elements have been identified (COE, 1989). While all of them are of importance, certainly the environmental temperature is the most significant factor discovered to date and must be considered carefully whenever aqueous and vitreous studies are being used to determine the postmortem interval.

Finally, this work indicated the importance of aqueous and vitreous analysis for both potassium and acid phosphatase enzyme in determining time of death.

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Table (1)

Relationship of PMI and inorganic constituents (mmol/L.) in aqueous and vitreous humor of donkeys

Elements	Eye fluid	Time (hours)									
		6	12	18	24	36	48	60	72	84	96
Potasium	Aqueous P	6.25±0.140 *	7.16±0.310 **	7.37±0.560 **	7.85±0.120 ***	8.43±0.380 ***	9.76±0.230 ***	11.26±0.47 ***	12.2±0.62 ***	13.0±0.55 ***	14.0±0.670 ***
	Aqueous A	5.75±0.140	5.80±0.040	5.75±0.200	6.00±0.020	5.65±0.180	5.90±0.100	6.05±0.32	6.10±0.13	5.96±0.23	5.85±0.260
	Vitreous P	6.35±0.020 *	6.36±0.130 **	7.65±0.320 **	8.30±0.210 ***	9.00±0.290 ***	9.35±0.250 ***	9.46±0.440 ***	9.75±0.48 ***	9.92±0.31 ***	10.5±0.440 ***
	Vitreous A	5.90±0.150	5.85±0.180	5.95±0.020	6.05±0.130	5.80±0.200	6.00±0.200	6.10±0.260	5.96±0.20	6.00±0.20	5.95±0.300
Sodium	Aqueous P	161.2±4.90	164.2±2.91	153.0±13.0	164.3±3.23	157.7±4.50	142.2±6.20	147.7±7.80	154.9±10.5	158.2±6.40	151.7±2.61
	Aqueous A	158.7±5.20	154.2±7.31	150.7±2.40	156.4±5.20	158.2±1.40	150.0±3.20	152.4±3.20	152.6±1.70	149.8±3.40	150.5±3.50
	Vitreous P	144.2±9.01	144.6±2.70	142.8±6.93	162.4±2.14	161.4±4.06	155.4±4.93	158.6±3.28	151.2±1.07	153.6±3.44	155.0±2.50
	Vitreous A	156.8±5.55	152.6±3.40	154.6±4.20	156.7±3.21	155.5±5.00	158.8±5.20	153.5±4.00	154.6±1.50	152.4±2.77	158.5±3.00
Chloride	Aqueous P	114.0±2.82	132.5±13.56	125.7±3.95	154.0±16.0	150.5±8.94 *	160.0±14.6 *	160.0±14.6 *	126.5±3.20 *	158.0±7.06 **	152.5±10.0 *
	Aqueous A	112.5±0.96	116.7±1.230	114.4±6.00	120.2±6.80	118.6±6.40	120.2±3.00	116.0±2.00	120.0±10.0	119.7±5.00	121.0±6.80
	Vitreous P	100.0±0.50	107.5±3.10	110.7±4.20	105.0±2.80	128.5±3.10 **	132.5±5.40 **	133.0±12.8	122.0±7.20	132.5±8.95 *	116.6±7.21
	Vitreous A	105.0±3.22	110.6±2.60	109.6±3.40	108.6±3.45	111.0±2.50	110.7±2.40	115.5±3.40	113.1±1.13	111.0±2.40	114.4±2.00
Calcium	Aqueous P	6.66±0.690	6.26±0.600	7.020±0.54	8.71±0.330	7.68±0.490	6.22±0.450	6.04±0.17 *	6.20±0.46	6.38±0.320	7.05±0.62
	Aqueous A	7.76±0.430	7.60±0.590	7.820±0.74	7.96±0.620	7.65±0.500	7.58±0.700	7.32±0.40	7.40±0.62	7.65±0.620	7.70±0.52
	Vitreous P	7.20±0.420	7.87±0.700	6.96±0.430	7.12±0.290	5.91±0.040	6.33±0.620	8.5±0.65	5.21±0.53 **	7.37±0.050	7.01±0.33
	Vitreous A	7.65±0.210	7.55±0.600	7.65±0.520	7.62±0.400	7.00±0.500	7.20±0.400	7.7±0.50	7.13±0.24	7.62±0.420	7.50±0.48

* Significant at P 0.05
** Significant at P 0.01
*** Significant at P 0.001

P = Postmortem
A = Antemortem

Table (2)

Relationship of PMI and organic constituents (mg/100ml) of aqueous and vitreous humor of donkeys

Parameters	Eye fluid	Time (hours)									
		6	12	18	24	36	48	60	72	84	96
Total Protein	Aqueous P	9.31±1.71	8.66±0.96	9.22±0.97	4.77±0.09 ***	5.12±0.95 **	5.15±1.05 **	5.10±0.24 ***	5.00±0.90 **	4.82±0.65 **	4.85±0.72 **
	Aqueous A	9.75±0.70	9.02±0.33	9.85±0.15	9.33±0.33	9.45±0.44	9.99±0.50	9.70±0.62	9.22±0.42	8.99±0.90	9.04±0.80
	Vitreous P	6.96±0.31	7.66±0.40	4.75±0.40 ***	4.02±0.23 ***	3.88±0.40 ***	3.44±0.22 **	3.40±0.04 **	3.85±0.12 **	3.65±0.35 **	3.40±0.22 **
	Vitreous A	7.67±0.53	7.85±0.50	7.65±0.20	7.35±0.45	7.22±0.41	7.20±0.65	6.99±0.17	7.15±0.32	7.06±0.21	7.13±0.30
Urea nitrogen	Aqueous P	13.11±1.04	12.97±0.84	12.72±2.55	12.06±1.18	11.73±1.12	11.66±0.84	10.22±0.24 **	9.88±0.95 *	9.50±0.47 **	9.20±0.32 ***
	Aqueous A	12.29±0.89	12.80±0.40	12.02±0.30	12.86±0.80	13.04±0.62	13.50±1.10	13.28±0.60	12.90±0.65	13.26±0.60	12.88±0.60
	Vitreous P	16.75±2.11	14.62±1.23	14.32±1.05	14.49±2.02	14.64±2.32	12.04±3.01	13.99±1.06	14.86±1.22	13.44±1.17	12.32±0.70 *
	Vitreous A	14.82±1.22	15.59±1.59	14.98±0.69	14.66±0.70	15.02±0.85	14.00±1.60	15.66±1.66	15.00±0.77	16.04±1.82	15.38±1.44
Uric Acid	Aqueous P	8.36±1.57	10.18±0.29	12.57±0.35 ***	12.24±1.02 *	13.63±0.24 ***	14.15±0.48 ***	14.45±0.60 **	14.32±0.52 ***	14.82±0.56 ***	15.02±0.42 ***
	Aqueous A	9.92±0.82	9.78±0.32	8.55±0.42	9.04±1.35	8.86±0.78	9.16±0.86	9.45±0.98	8.88±0.44	7.85±0.37	9.16±1.01
	Vitreous P	9.87±0.99	10.22±1.04	11.25±0.82 *	11.00±0.92 *	14.40±0.44 ***	14.65±0.32 ***	14.25±0.58 ***	14.00±0.22 ***	13.72±0.36 ***	13.36±0.52 ***
	Vitreous A	7.97±1.70	7.88±0.48	8.02±0.78	7.32±0.60	8.14±0.66	7.75±0.65	8.00±0.50	7.45±0.32	7.92±0.42	7.65±0.32

* Significant at P 0.05
** Significant at P 0.01
*** Significant at P 0.001

P = Postmortem
A = Antemortem

Table (3)

Relation of PMI and some enzymatic activity (U/L) of aqueous and vitrous humor of donkeys

Enzymes	Eye fluid	Time (hours)									
		6	12	18	24	36	48	60	72	84	96
Acid phosphatase	Aqueous P	88.5±6.0	104±3.50 **	114.0±3.5 ***	132.0±5.50 ***	224.0±18.5 ***	316±27.0 ***	436±41.5 ***	666±2.00 ***	820±11.0 ***	980±25.0 ***
	Aqueous A	93.5±2.0	91±1.75	92.5±2.0	94.5±1.75	92.5± 3.0	91± 6.5	99± 3.5	91±2.75	95± 3.5	98± 2.0
	Vitreous P	68.0±3.3	71±2.0 *	120.0±3.5 ***	136.0±3.5 ***	168±11.0 ***	193.0±2.0 ***	400±50.0 ***	500±9.0 ***	570±3.00 ***	720±10.0 ***
	Vitreous A	64.5±1.5	64±1.5	67.5±4.0	69.5±2.5	66± 2.0	70.5±2.5	70± 3.0	71±3.5	69±1.75	69± 2.0
ALAT	Aqueous P	5.00±1.03	3.50±0.20	4.8±0.40	7.60±1.30 *	6.44±0.40 **	8.14±1.24 *	7.22±0.68 *	10.22±1.82 *	6.10±0.58 *	5.00±0.30
	Aqueous A	3.37±0.34	3.32±0.31	4.0±0.39	3.98±0.40	3.90±0.55	4.32±0.80	4.02±0.82	4.42±0.62	4.20±0.48	4.02±0.32
	Vitreous P	5.20±0.2	5.40±0.30	5.80±0.52	8.40±1.15 *	7.70±0.80 *	9.15±1.12 *	8.10±0.85 *	11.55±0.51 **	8.02±0.90 *	6.20±0.60
	Vitreous A	4.87±0.39	5.02±0.42	5.12±0.35	5.20±0.55	4.99±0.50	5.50±0.60	5.20±0.65	5.77±0.72	5.15±0.60	5.00±0.40
ASAT	Aqueous P	36.5±5.00	50.0±4.5 ***	58.4±6.2 **	70.2±13.2 *	61.0±8.50	71.0±10.2 *	61.0±7.02 *	85.0±14.7 *	40.4±3.9	39.6±3.5
	Aqueous A	24.0±2.09	23.0±2.3	32.0±3.5	31.0± 3.0	34.0±3.90	37.5± 3.6	36.0±3.50	39.0± 4.4	32.5±4.02	30.0±3.2
	Vitreous P	78.6±6.4	77.4±6.2	82.2±8.0	100.2±10.6 *	90.2±10.2	94.5±9.55	86.6±8.4	107.6±13.0	90.4±10.1	72.4±7.4
	Vitreous A	61.66±3.4	72.0±4.6	69.8±5.2	72.4± 5.1	68.0± 3.2	74.5±3.70	70.0±4.2	77.8± 3.4	69.5± 4.2	62.4±2.0

* Significant at P 0.05
** Significant at P 0.01
*** Significant at P 0.001

P = Postmortem
A = Antemortem

Table (4)
Statistical evaluation results of the chemical analysis in donkey's aqueous and vitreous humor

Parameters	Eye fluid	Intercept A	Slope B	Corr.Coeff.	t-Value	Sign.level
Potassium	Aqueous	5.885800	0.085050	0.995280	29.02297	< 0.0001
	Vitreous	6.704770	0.042970	0.928010	7.045360	0.0001
Sodium	Aqueous	160.136050	-0.101448	-0.449850	1.424650	0.1921
	Vitreous	148.895540	0.088520	0.391420	1.203110	0.2633
Chloride	Aqueous	130.323490	0.285010	0.530300	1.769180	0.1148
	Vitreous	107.153980	0.256050	0.641800	2.367100	0.0455
Calcium	Aqueous	7.147300	-7.133709	-0.268980	0.789900	0.4524
	Vitreous	7.155680	-4.554458	-0.152140	0.435380	0.6748
Total Protein	Aqueous	8.336500	-4.685319	-0.743260	3.142350	0.0138
	Vitreous	6.121540	-3.553813	-0.726410	2.989570	0.0173
Urea Nitrogen	Aqueous	13.41947	-4.637006	-0.985290	16.31014	< 0.0001
	Vitreous	14.700000	0.010000	0.509360	1.674130	0.1326
Uric Acid	Aqueous	10.266700	0.059370	0.854224	4.647620	0.0016
	Vitreous	10.685480	0.043560	0.735120	3.066990	0.0154
Acid Phosphatase	Aqueous	-72.683230	10.10380	0.975300	12.48887	< 0.0001
	Vitreous	-35.112730	7.230540	0.973370	12.01066	< 0.0001
ALAT	Aqueous	5.354150	0.022980	0.368810	1.122281	0.2943
	Vitreous	6.220220	0.029210	0.467780	1.496960	0.1728
ASAT	Aqueous	57.263680	0.000580	0.001160	0.003270	0.9975
	Vitreous	85.854810	0.047260	0.136920	0.390950	0.7060

Intercept A = Concentration at zero time
Slope B = Concentration difference in one hour

AQUEOUS & VITREOUS HUMOR ANALYSIS

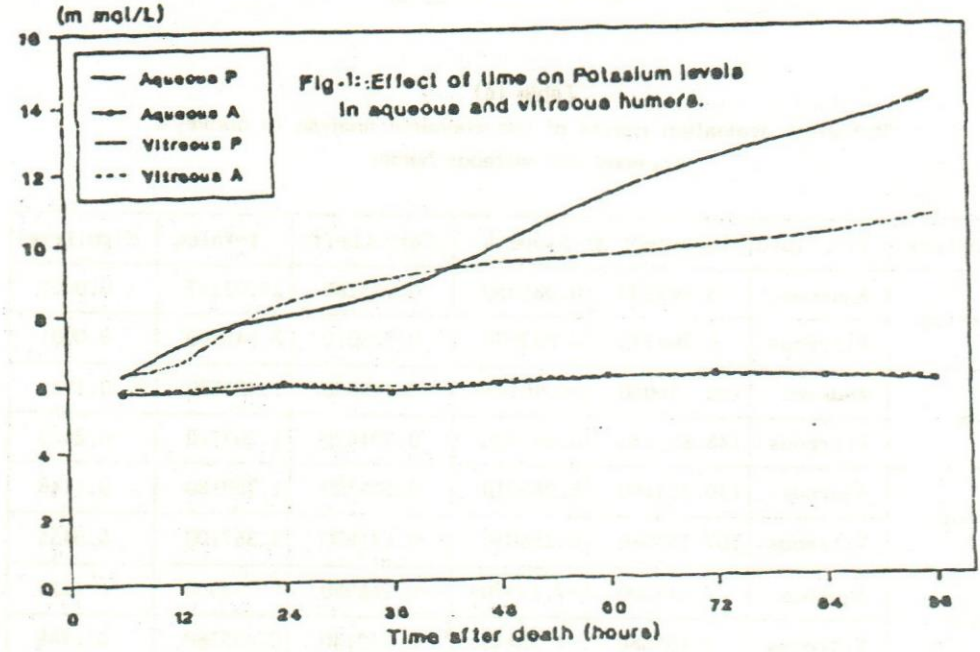

Fig.1::Effect of time on Potasium levels in aqueous and vitreous humers.

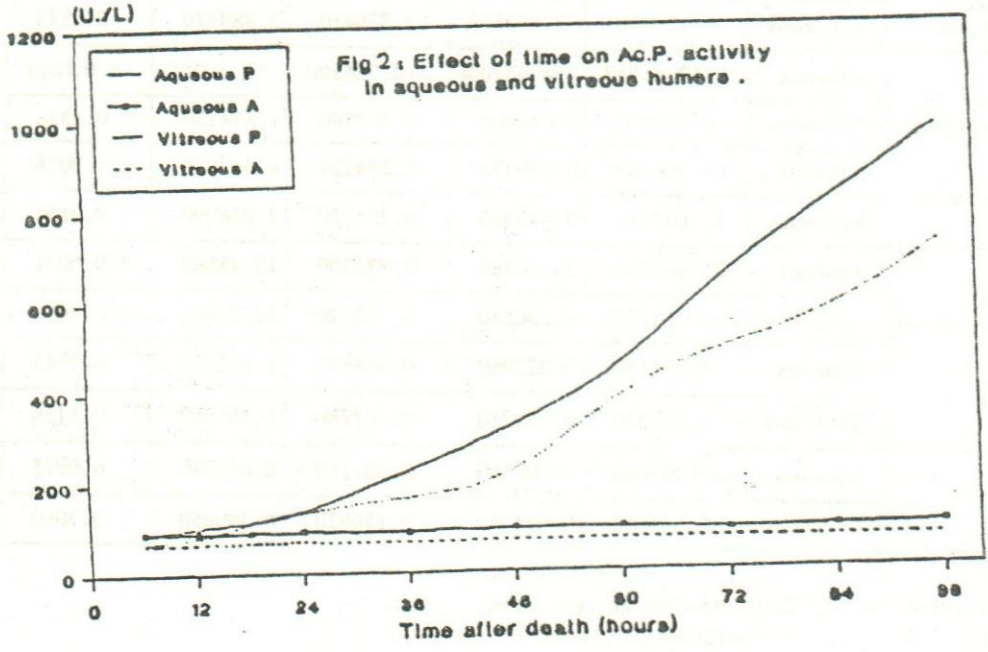

Fig 2 : Effect of time on Ac.P. activity in aqueous and vitreous humers .