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LABORATORY EVALUATION OF AQUEOUS HUMOUR IN HEALTHY SHEEP, GOAT, COW, BUFFALO AND DONKEY

(With Two Figs.)

By

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التحليل المعملي للسائل المائي للخزانة الأمامية للعين
في الاغنام والماعز والابقار والجاموس والحمير

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جمعت عينات من السائل الأمامي للخزانة الأمامية للعين من ٦ حيوانات من كل من الأغنام والماعز والابقار والجاموس والحمير السليمة اكلينيكياً. تم تحليل هذه العينات بالطرق المعملية الاكلينيكية المتاحة . وفحصت العينات من ناحية الخواص الطبيعية كاللون والرائحة ودرجة العكارة ودرجة التجلط والعدد الكلي للخلايا. كما حللت بيوكيميائيا للبروتين الكلي.

SUMMARY

Aqueous humour samples were collected from sheep, goats, cows, buffalos and donkeys (6 of each). The samples were evaluated using routinely available clinical laboratory methods. Physical examination of samples were performed including colour, odour, turbidity and coagulation. Biochemical analysis of samples for protein and total cell count were conducted.

INTRODUCTION

Aqueous humour is the transparent fluid that fills the anterior ocular segment. The constant flow of aqueous humour by the ciliary body, supplies the avascular cornea and lens with nutrients and remove their waste products. Aqueous humor resembles an ultra filtrate of plasma (GELATT, 1981).

The arrangement of the two layers of epithelial cells covering the ciliary body and processes provides the mechanism for active ion transport, including Na^+, Cl^-, and HCO_3. In addition, it provides the blood-aqueous barrier (DAMSON, 1963 and MOSES, 1970). Inflammation results in a breakdown of the barrier and a decrease in active production. The passive aqueous formation is increased, but the overall effect is a decreased aqueous formation. The eye becomes soft (hypotony), which is the hallmarke of anterior uveitis (HAVENER, 1970; MOSES, 1970 and WYMON, 1973).

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DUKE-ELDER (1968); GRAYMORE (1970); DAVSON (1974) and HAZEL, et al. (1985) studied the composition of aqueous humor in dogs, cats, horses and cows. GELATT (1981) stated that, the aqueous humor is normally transparent, with a protein level of 10 to 50 mg percent. With intraocular inflammation, aqueous protein level rapidly increase to 5 to 7 gm percent or nearly the same as plasma proteins. The equeous humor with elevated protein levels becomes turbid, thereby permitting demonstration of the Tyndall pheromenon, i.e. the visibility of floating particles (proteins) in the equeous humor (knowr clinically as "aqueous flare"). He added that, in acute iridocyclitis aqueous humor albumin is markedly elevated to near serum levels whearas in chronic inflammations aqueous proteins are mainly globulins.

Anterior chamber paracentesis is indicated in selected cases for diagnosis of anterior segment diseases, hypopyon and hyphema associated with glaucoma, malignant glaucoma prior to surgery, chronic nonresponsive iridocyclitis and intraocular neoplasia (GOLDBERG, 1967; MAIB, et al. 1967; HOWARD, 1968; HOGAN, et al. 1973; OLIN, 1977 and GELATT, 1974 & 1981). Because the quantity of aqueous sample is limited (0.2 to 0.5 ml for small animals and 1.0 to 2.0 ml for large animals), the types of analysis may be limited. Aqueous cytology may be the most informative, for best results. The usual low-cell populations need to be concentrated by millipore filteration, centrifugation and "hangingwell" methods. Protein analysis with the Folin-Lowry and paper chromatorgraphy may be informative (BLOGG and COLES, 1970 & 1971). Total protein was determined using the phenol method of DOUGHADUY, et al. (HAZEL, et al. 1985). HAZEL, et al. (1985), determined the total and differential cell counts, total protein and protein electrophoretic patterns in normal aqueous humor of dog, cat, horse and cow.

Because normal analysis of aqueous humor has not been reported in sheep, goats, cows (native breeds), buffalos and donkeys, investigation of normals is necessary for interpretation of some anterior occular segment disorders. The aim of the present study is to throw light upon the physical examination of aqueous humor and determination of total cell count and total protein in normal aqueous humor of native breeds including sheep, goats, cows, buffalos, and donkeys.

MATERIAL and METHODS

Anterior chamber paracentesis was performed in sheep, goats, cows, buffalos and donkeys, six animals of each of various age and sex. All animals proved to be clinically healthy by both clinical and laboratory methods of examination. The procedures were performed under the effect of tranquilizer using xylazine Hcl (0.5 mg/kg i.m. in sheep and goats and 0.05 mg/kg i.m. in cows and buffaloes) in combination with topical instillation of Novasen 1%. In donkeys, thiopental sodium in a dose of

5 mg/kg i.v. was used, premedicated with combelen in a dose of 0.2 mg/kg i.m. All eyes were normal on ophthalmic examination before sampling. Aqueous humour samples were collected using a 26-gauge, 0.45 x 13 mm disposable needle, two 3-ml plastic syringe and a 3-way stopcock. The eyelids were retracted by fingers. The globe was stabilized by thumb forceps grasping the bulbar conjunctiva. Samples were obtained by paracentesis of the anterior chamber through the limbus (Fig. 1&2). Once the tip of the needle centered over the pupil, most of the aqueous humor was slowly aspirated with one syringe, the 3-way stopcock was redirected and an equal volume of normal saline solution was injected, maintaining the normal intraocular pressure.

Physical examination of the samples were performed including, colour, odour, turbidity and coagulation. Within 30 minutes after sampling cell count was performed.

Total cell count:

Using special diluting fluid consisting of 10 ml of glacial acetic acid, 90 ml distilled water and 0.1 g crystal violet were used. The diluting fluid was drawn to mark 1 on white cell pipette and then compeleted to mark 11 with aqueous humor. The mixture was shaken and the first two to three drops were discarded, then using the ordinary haemocytometer in counting the sample. The results can be obtained by multiplying the number of counted cells in 0.6 to obtain number of cell/microliter.

The total protein concentration in aqueous humor is readily determined using trichloracetic acid-turbidimetric procedure (COLES, 1986). 0.5 ml of sample is combined with 1.5 ml of 5% T.C.A. and allowed to reach at room temperature for five minutes. The sample is agitated and turbidity measured against a bovine serum albumin standard at 420 nm.

RESULTS

Aqueous humor was clear, resembling distilled water, transparent and did not coagulate. Direct cell count and total protein values were summarized in table 1.

Table 1: Mean values and range of direct cell count and total protein in sheep, goat, cow, buffalo and donkey.

Species	Direct cell count (cell/µl)	Total protein (mg/dl)
Sheep	4.33 (2-7)	60.02 (33.4-70.5)
Goat	5.3 (3-8)	90.4 (77.7-99.9)
Cow	6.7 (3-15)	70.8 (56.9-78.3)
Buffalo	7.5 (3-12)	66.7 (56.9-77.8)
Donkey	6.8 (3-13)	48.6 (38.8-77.2)

DISCUSSION

In fact, paracentesis of the anterior chamber of the eye and aspiration of its contents, may result in an immediate alteration in the nature of the aqueous humor, due to the disruption of the blood-aqueous barrier. The penetration and aspiration have been used to produce experimental uveitis (BLOGG and COLES, 1971; STJERNSCHANTZ, et al. 1973 and BRIGHTMAN and HELPER, 1976). However, any increase in aqueous humor protein, enzymes and cells as a result of anterior chamber paracentesis was insignificant when compared with aqueous humor from patients with intraocular disease.

Direct cell counts, in the present investigation were very low and most cells were degenerated. HAZEL, et al. (1985), mentioned that, the intact cells in aqueous humor should probably be considered abnormal. The cells must be degenerated, due to the low protein content of aqueous humor. Mean values of total protein (mg/dl) in sheep, goat, cow, buffalo and donkey of the present study were nearly similar to those obtained by KRONFIELD (1941); BLOGG and COLES (1971) and HAZEL, et al. (1985) in dog, cat, cow and horse. Although, the number of animals in the present investigation is considered to be inadquate for obtaining a reference values, these findings may be of value, until a further study with more data can be performed.

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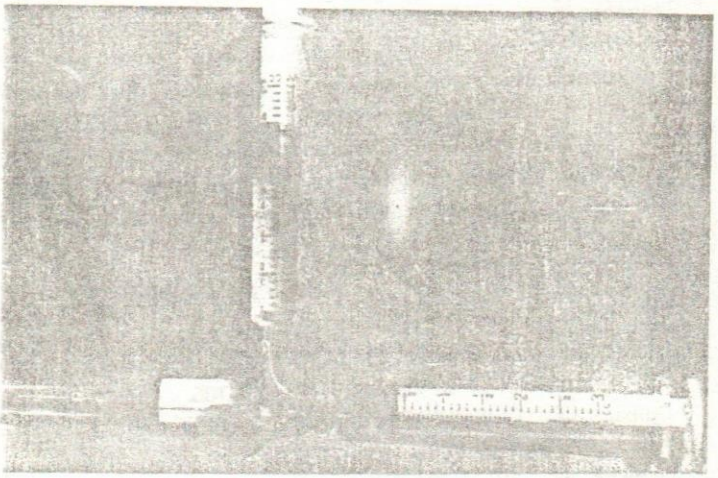

Fig. (1): Showing the 3-way stopcock, with two syringes used in paracentesis of the anterior chamber of the eye.

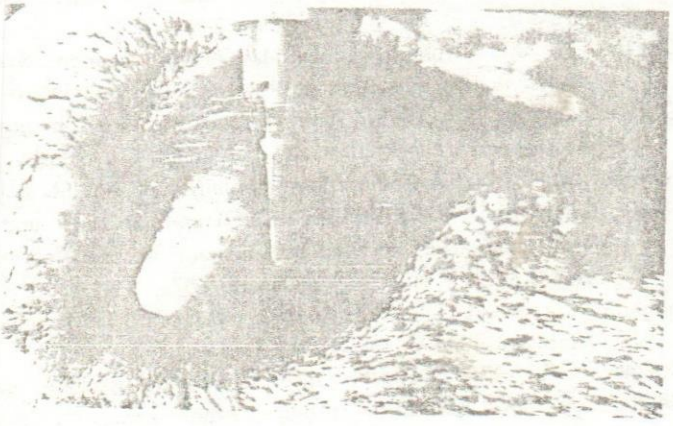

Fig. (2): Showing the needle in the anterior chamber of the eye.