A METHOD OF DETERMINING THE COLLOIDAL STATE OF THE VITREOUS BODY

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UDC 617.747-004.5-07

KEY WORDS: vitreous body, gel density, drying.

Destruction of the vitreous body (VB), accompanied by a change in its colloidal state, is often observed in myopia, glaucoma, diabetic retinopathy, and in cases of uveitis and hemophthalmia, especially traumatic [4, 5]. The most widely used method of quantitative assessment of the colloidal state of VB is viscosimetry. A horizontal hemoviscosimeter [2] or Ostwald's viscosimeter [1] have been used to determine the viscosity of VB in patients with hemophthalmia. In investigations into the effect of hypothermia on the viscosity of VB, Höppler's viscosimeter has been used [3]. However, these types of viscosimeters, like the technique of viscosimetry as a whole, can be used only to determine the physical state of homogeneous fluids and solutions with no internal skeleton structure. This method thus gives only an approximate estimate of the colloidal state of VB and is suitable only for determining viscosity of liquified VB. This narrows the range of usefulness of the method and reduces its value in experimental and diagnostic investigations.

VB in animals of different species and in man is a gel containing about 99% of water [7]. The main component retaining water in VB is considered to be hyaluronic acid [4, 5, 7], and the role of structural skeleton is performed by a fine network of collagen fibrils [6, 7]. These two components determine the elasticity of the VB gel and prevent free movement of the water in it, and this may affect the process of evaporation of water from VB in air. The rate of drying of VB can thus be used as an indicator of the state of its structural components.

The aim of this investigation was to develop a method of quantitative assessment of the colloidal state of VB. Parameters of measurements obtained during drying of VB had to be established, the dependence of these measurements on external conditions defined, and quantitative characteristics of the colloidal state of the solid and liquid parts of the normal VB and also of VB after destruction obtained.

EXPERIMENTAL METHOD

Bovine (two eyes), rabbits (five) and human (four) VB were used. Human VB from subjects aged 48-67 years were removed at autopsy not more than 14 h after death. The eyes were opened in the region of the flat part of the ciliary body and 50 µl of vitreous liquid was withdrawn into graduated glass tubes with an internal diameter of not less than 3 mm. Measured fragments of VB and the same volume of distilled water were spread on coverslips within a radius of 7.5 mm. The coverslips intended for water were smeared beforehand with a very small quantity of VB to improve wettability.

In the experiments of series I samples of liquid (central) and solid (peripheral) parts of human VB were applied to coverslips. The coverslips were then placed in separate Petri dishes on filter paper and the dishes were half covered with lids. Three samples of VB of different consistency were each dried at 15, 22, and 30°C. The samples of water were dried under the same conditions. The drying time of VB (T_{vb}) and water (T_w) was recorded with an accuracy of 1 min by noting disappearance of the moist spot on the coverslip.

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 98, No. 12, pp. 761-764, December, 1984. Original article submitted January 27, 1984.

 0007-4888/84/9812-1762$08.50

TABLE 1. Drying Index of Human VB (T_{vb}/T_w) for Drying at Different Temperature (M ± m)

Place of taking sample of VB	Temp., °C		
	15	22	30
Peripheral part of VB	1,76±0,02	1,78±0,02	1,77±0,02
Central part of VB	1,46±0,02	1,47±0,01	1,44±0,02

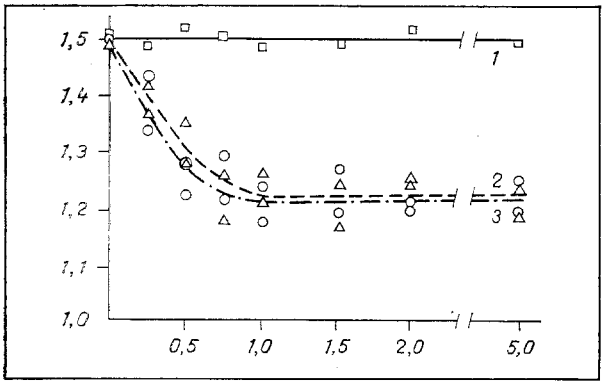

Fig. 1. Changes in drying index (T_{vb}/T_w) of rabbit VB treated with collagenase and hyaluronidase. Abscissa, duration of incubation of VB (in h). 1) (Squares) intact VB; 2) (triangles) VB + collagenase; 3) (circles) VB + hyaluronidase.

As a model of destruction of VB in pathology, its structural components were subjected to enzymic hydrolysis. Into each tube containing rabbit VB 50 μg of collagenase with an activity of 150 U/mg (from "Ferak," West Germany) or hyaluronidase (Mark A, from "Reakhim," USSR) was introduced. The tubes were then sealed at both ends with Parafilm and incubated in the same way as intact VB, for 5 h at 37°C. Samples of VB were dried every 15 min during incubation for 1 h, and then every 30 min.

In the experiments of series II, to obtain parameters characterizing the test object most completely, drying was carried out on the pans of a damped analytical balance. Coverslips with VB and water were placed on different pans of the balance. Deviation of the pointer of the balance, i.e., the difference in weight of VB and water $M_{vb} - M_w$), was recorded during the drying process every 5 min with an accuracy of 0.1 mg. Rabbit and bovine VB and also an artificial substitute for VB, namely Healonid (from "Pharmacia," Sweden), were investigated by this method. Healonid is a viscous 1% solution of sodium hyaluronate in phosphate buffer.

EXPERIMENTAL RESULTS

In the experiments with human VB the drying index (T_{vb}/T_w) of the solid (peripheral) part of VB was 1.77 and of the liquid (central) part 1.46 (Table 1). The drying method thus confirmed the spatial difference in gel density of the human VB [5, 6]. Values of T_{vb}/T_w were independent of the conditions of drying, so that the method can be used with sufficient accuracy and reproducibility of results over a range of temperatures exceeding the health norms for laboratories. The results of treatment of VB with collagenase and hyaluronidase showed that T_{vb}/T_w fell in the course of 1 h to 1.22 (Fig. 1), and subsequent incubation for 5 h caused no further change in the values of T_{vb}/T_w. Enzymic hydrolysis of the structural components of VB is known to give rise to two basic types of changes in the colloidal state of VB. Degradation of collagen leads to converstion of the gel of VB into a sol, and hydrolysis of hyaluronic acid leads to collapse of the collagen skeleton and to its separation from the liquid part of VB [4, 5]. Similar changes in the colloidal state of VB are observed in various kinds of ocular pathology and can be recorded by the method described above. By the use of a balance it was possible to obtain additional parameters of the drying process. It

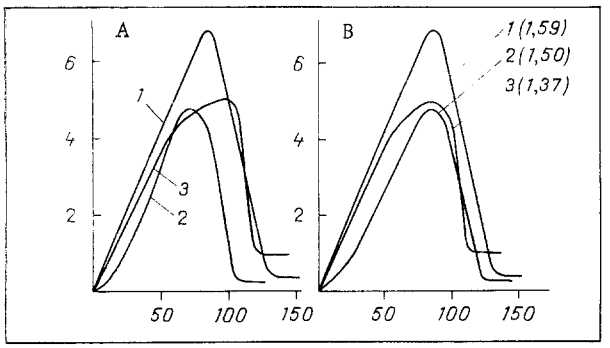

Fig. 2. Changes in difference of weight (in mg) of test gel and water during drying. Abscissa, drying time (in min). A) Curves showing difference in mass of bovine (1) and rabbit (2) VB, Healonid (3), and water at different drying temperatures; B) the same curves, reduced to identical external drying conditions (i.e., to the same value of T_w). Values of T_{vb}/T_w given in parentheses.

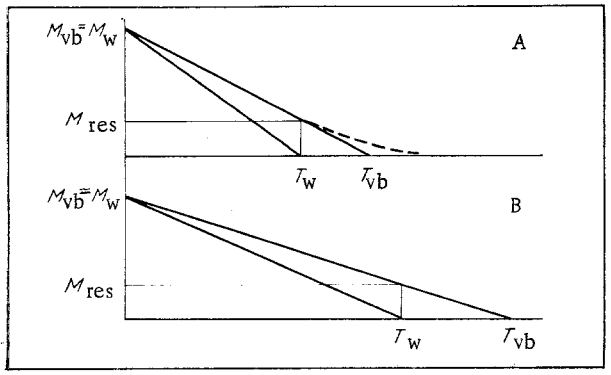

Fig. 3. Dependence of parameters of VB drying on rate of drying. Abscissa, drying time of VB (T_{vb}) and water (T_w); ordinate, weight of VB (M_{vb}) and water (M_w). A) Rapid drying of VB sample and water; B) slow drying of sample of same VB and water. Clearly constancy to T_{vb}/T_w during rapid and slow drying ensures constancy of M_{res}. With uneven gel density of VB in the sample, the value of $T_{vb} - T_w$ may rise appreciably (broken line).

will be clear from Fig. 2a that curves obtained during investigation of rabbit and bovine VB and also of Healonid were similar in character. The ascending parts of the curves show the difference in weight of the test object and water ($M_{vb} - M_w$) during drying. The descending parts of the curves give the rate of decrease of the weight of the test objects after complete evaporation of water. Projection of the ascending part of the curve on the abscissa shows the drying time of water (T_w), and of the whole curve until it flattens out on a plateau, the drying time of VB (T_{vb}). For greater clarity, the same curves, but reduced to identical external conditions of drying (i.e., to the same value of T_w) are shown in Fig. 2b. Analysis of the shape of the curves enables the following additional parameters to be distinghished: 1) residual mass (M_{res}), which, like T_{vb}/T_w, characterizes gel density. On the graph M_{res} is given by the height of the peak. For bovine and rabbit VB and for Healonid M_{res} was 6.8, 4.8, and 5 mg respectively; 2) the steepness of drying:

$$\left(\frac{M_{res}}{T_{vb}/T_w - 1}\right)$$

which characterizes inequality of density of the gel under investigation. The less the steepness of drying, the more uneven the distribution of the structural skeleton. Steepness

of drying of the test samples of bovine and rabbit VB and of Healonid was 11.3, 10.2, and 14.3 mg respectively; 3) dry residue (M_{dry}) shows the content of dry substance in the sample. On the graph M_{dry} is expressed as the level of the plateau, and for bovine and rabbit VB and for Healonid it was 0.4, 0.3, and 1 mg respectively. 4) The degree of dispersion of the skeleton (M_{res}/M_{dry}) shows the degree of distribution of dry substance in the sample giving solidity to the gel (M_{res}). For bovine and rabbit VB and Healonid this parameter has values of 17, 16, and 5 respectively.

Weighing thus gives a more complete and accurate picture of the test material. For example, the parameter M_{res} determines gel density more accurately than T_{vb}/T_w. The reason is that the value of M_{res} can be obtained without waiting for the material to dry completely, and in that way the uneven process of drying can be eliminated. The value of the parameter M_{res}, like that of T_{vb}/T_w, is independent of the rate of drying (Fig. 3). By drying on the pans of a balance, the terminal drying process can be allowed for in the form of the expression $\frac{M_{res}}{T_{vb}/T_w - 1}$. Since this parameter gives information on the heterogeneity of the structural skeleton in the test sample, it can be used as a test of biological compatibility of various materials with VB. This is particularly important during choice and evaluation of the quality of alloplastic materials intended for the manufacture of intraocular lenses. The suggested method enables the colloidal state of VB intended for transplantation to be estimated, and the quality of artificial hydrogels to be monitored. Aging is often accompanied by destruction of VB, so that there are good grounds for investigations of VB from the age aspect. The method also can be used to study different biological fluids, tears, and secretions, such as saliva in Sjögren's syndrome.

In conclusion, the suggested method is simple and requires only microdoses of gel, of whatever density, for its implementation. The use of derivatographs will enable the drying of the object to be completely automated.

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