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THE VITREOUS BODY AND GLAUCOMA*

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OF the variety of theories put forward to explain the raised intra-ocular pressure in primary chronic glaucoma, that suggested by one of us (Duke-Elder, 1930) to the effect that a swelling of the vitreous body is the prime agent in the process has found a fairly wide acceptance. This theory was put forward because it could account for such symptoms as the shallow anterior chamber† and the atrophy of the choroid and ciliary body in glaucoma, and appeared reasonable from a physico-chemical aspect in view of the gel nature of the vitreous body. The experimental evidence relating to this theory which has accrued since has been conflicting and often equivocal in its interpretation. Thus Baurmann (1924) and Löbeck (1929) failed to find any change in volume of the vitreous body on varying its reaction within narrow limits;

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* The blocks illustrating this article have been kindly loaned by the *Biochemical Journal*.

† Gonioscopic examination has demonstrated the possibility of a raised intra-ocular pressure with the drainage angle completely free. In these cases, if the vitreous was swelling, no rise in intra-ocular pressure could be expected from this cause unless some upset in the permeability of the membranes lining Schlemm's canal occurred at the same time.

Redslob and Reiss (1928) found considerable variations in the total volume of the intact eye in a modified dilatometer on varying the *Ph*, and Duke-Elder (1930) described variations in volume of the isolated vitreous body which were thought to demonstrate the possibility of a swelling of the vitreous under suitable conditions. The evidence to be brought forward in this paper allows of a different interpretation of these last results. Glaucoma, however, is not associated with any detectable abnormality in the *Ph* of the blood (see *e.g.*, Schmerl, 1928), so that experiments on the variation of this factor are only of interest to the extent that they show that the vitreous body is capable of modification of its volume in response to a changed environment. What can be made to occur in a test-tube need not necessarily occur in the living body.

A factor in the swelling of proteins often more potent than the *Ph* is the presence of specific ions, *e.g.*, I- or CNS-, or the so-called hydrotropic substances of Neuberg (1916). The recent work of Katz (1933) on the swelling of starch and gelatin has shown that substances such as the sodium and potassium salts of long-chain fatty acids, and, in general, dipolar substances with both hydrophilic and hydrophobic groups in their molecules, will increase the rate of swelling to an enormous extent. Since the increased swelling so produced is due to adsorption on to the micelles of the colloid, it is obvious that these substances may be effective in low concentrations, and hence it is not impossible that they are effective in the causation of glaucoma. The occurrence of abnormal quantities of substances of a soapy nature in the eye in advanced glaucoma and similar degenerative conditions of the eye lends support to this view.

In this paper attention will be focused entirely on the physico-chemical properties of the vitreous body in so far as they relate to the possibility of its swelling *in vivo*. In a later paper the adequacy of the theory from an ophthalmological point of view will be discussed in conjunction with a new theory based on different physico-chemical assumptions.

PART I. DIRECT SWELLING EXPERIMENTS ON THE VITREOUS BODY.

The apparatus of Schmidt (1931), used for the determination of the water-uptake of powders, was modified (Duke-Elder, Robertson, and Davson, 1935) to suit the peculiarities of the vitreous body (see Fig. 1). The tap, T_1 , allows connections to be made between the reservoir, R, and the capillary, C; between R and the chamber, P; between P and C, and between R, P and C. The vitreous body is placed in the chamber, P, which is separated from the fluid in the rest of the apparatus by a sintered glass disc, S, which has a mark on it showing the level of the tap, T_1 . Any uptake of water is measured by the movement of the meniscus in the

capillary. The procedure is to fill the reservoir with the given solution and make connection, with T_2 open, to P and C until fluid flows through T_2 when the latter is closed and connection is made between R and C alone until the latter is half filled. T_1 is then half-turned so that no connections are made, and the vitreous body of an ox is placed in P to just above the mark. When T_1 is turned to make connection between P and C the meniscus will move along the capillary to the right, due to the slight excess of hydrostatic pressure in P. To counteract this the whole apparatus is tilted slightly as the tap is turned so that the meniscus stops after it has moved a few inches. The tilting must not be

FIG. 1.

so steep that the meniscus starts to move backwards, otherwise water will enter P and the experiment must be started again. Solutions of 0.9 per cent. NaCl buffered with phosphate to pH 7.8 and 8.0 and solutions of 1 per cent. sodium oleate and laurate were used.

In all cases no water-uptake was observed in 24 hours. As will be appreciated, the apparatus described determines whether the gel takes up water, but not whether a volume increase occurs, although the one process is always associated with the other. Kuntzel (1929) has divided the swelling process of gels into two parts, one due to water-uptake and the other due to the mutual repulsion of charged micelles. In view of the possible independence of the two processes at certain stages of swelling it was thought that there was just a chance that the vitreous body might alter its volume by a re-orientation of the charged micelles, produced by the adsorption of a polar compound, without necessarily taking up water. To put this to test, the apparatus shown in Fig. 2 was devised.

FIG. 2.

The hollow hemisphere, A, fits into the dome, B, by a ground joint. B is connected to a capillary, C, and a reservoir, R. A is filled with vitreous and fitted into B. R is filled with a saturated solution of sodium laurate in amyl alcohol dried with sodium sulphate. The whole is allowed to equilibrate in a thermostat at 25° (constant to 0.002°). When thermal equilibrium has been established the tap T is turned and the solution is allowed to fill B and run along the capillary tube. The tap is turned off and the position of the meniscus fixed on the cross-wires of a telescope. Any increase in the volume of the vitreous due to the adsorption of sodium oleate from the amyl alcohol would be registered by a movement of the meniscus to the right. A control was run using pure dry amyl alcohol. Repeated experiments failed to show an increase of volume; in general a slight contraction in both control and test experiments was observed.

The absence of any water-uptake near Ph 8 in the first experiment is in agreement with the findings of Löbeck; the experiments with soaps show that under more extreme conditions than that of changed Ph the vitreous body does not increase in volume in 24 hours.

PART II. INDIRECT EXPERIMENTS.

The results of Part I can hardly be treated as absolutely demonstrating that the vitreous body is incapable of increasing in volume in primary chronic glaucoma, as the increase in intra-ocular pressure associated with this disease is usually slow, frequently

taking years to reach its maximum value. So far two possible theories of the mechanism of volume increase have occurred to us depending on different conceptions of the mode of origin of the vitreous body. First, if it is assumed that the vitreous is formed by a continuous process of swelling from an initial dry protein similar to the process of swelling of dry gelatin in water, then, considering it as at its swelling maximum at normal Ph , it seems possible that it could swell further if the Ph were altered in a direction favourable to increased hydration.

All previous experiments designed to show whether the vitreous body swells have tacitly assumed the truth of the latter proposition. However, although the colloid chemical literature contains many studies on the swelling of gels in the presence of foreign substances, no one, it seems, has studied the influence of these substances on a gel already at its swelling maximum. The procedure in all cases has been to cause a quantity of the dry material to swell at one Ph , and then to take another lot of dry material and cause it to swell at a new Ph , and so on (Duke-Elder, 1930 and others). On consideration, it is by no means evident that a swollen gel will respond to a change in its environment to the same extent or in the same way as the dry material, owing to the intervention of peptisation which is particularly to be expected when the micelles are already strongly hydrated.

If, on the other hand, the vitreous body is formed by a sol-to-gel change, another theory must be presented to show how it can swell.* In this case it is suggested that a minute fraction of the vitreous body is peptised owing to the presence of some lyophilic substance; if, then, the procedure is reversed whereby the comparatively protein-rich fluid is converted to its gel form, the presence of the adsorbed lyophilic substance enables the protein to orient more water than previously. The net result of this cyclical process is an increase in volume. If this were to proceed over a number of years the effect would be to produce the pressure symptoms characteristic of chronic glaucoma. The process may be illustrated in a crude way by placing a lump of 10 per cent. gelatin in a small volume of water. Addition of acid produces eventual peptisation; neutralization of the acid converts the sol back to a gel larger in volume than before. (The objection that the increased pressure will expel fluid after the peptisation of a portion of the vitreous body is overcome by postulating a uni-directional permeability of the surrounding membranes. This is not a further *ad hoc* assumption as some such state must be the case as will be shown in a later paper). Part II of this paper is

* Experiments of our own on gelatin clots, as yet unpublished, together with theoretical considerations lead to the conclusion that a dilute clot, as opposed to a swollen gel, will not increase in volume directly when its environment is changed.

divided into three sections according as the experiments throw light on the possibility of a gel at its swelling maximum swelling further on changing the *Ph* suitably (Section 1), on the structure

FIG. 3.

of the vitreous body and hence its mode of origin (Section 2), and on the possibility of peptising the residual protein (Section 3).

Section 1. Swelling of fibrin. In Fig. 3 the water-uptake per g. of dry fibrin, as determined in a Schmidt apparatus, is plotted

FIG. 4.

against *Ph*. From the curve it is evident that by allowing fibrin to swell to its maximum at *Ph* 6 and then changing the medium to one of *Ph* 2 the conditions necessary to show whether a gel at its swelling maximum can take up further water by changing the *Ph* favourably will be achieved.

To this end the Schmidt apparatus was modified as in Fig. 4 (Duke-Elder and Davson, 1935). A bulb B with a tap T_1 fits on the side-tube by a ground joint. With T_2 closed the bulb is filled with concentrated HCl and T_1 is closed. Fibrin is placed on the

porous plate, P, and, when the water-uptake, as measured by the movement of a meniscus in the capillary in communication with the chamber, C, has reached its maximum the tap T_1 is opened allowing acid to diffuse in, thus lowering the Ph of the fluid in contact with the swollen fibrin. In Fig. 5 the water-uptake is plotted against time. For convenience the initial part of the curve is not shown and the scale of ordinates above 2.8 ml. is a half of that below this value. Obviously the swollen fibrin adjusted itself to the changed Ph . If the acid had not diffused in, the water-

FIG. 5.

uptake would have reached a final value of 2.80 ml., whereas, owing to the changed acidity, it rose to a maximum of 3.18 ml.

Swelling of gelatin. Gelatin was allowed to swell at Ph 4.8, the isoelectric point, at which Ph swelling is minimal, and at Ph 1. Pieces of gelatin which had already swollen at Ph 4.8 were transferred to the solution of Ph 1 and their subsequent swelling followed. In Fig. 6, I and II are the swelling curves of gelatin in solutions at Ph 1.1 and 4.8 respectively. The upper branch of curve II represents the change in the rate of swelling on transferring the gelatin from a medium of Ph 4.8 to one of Ph 1.1. It is at once evident that the swollen gelatin has adjusted itself to the changed Ph .

In the light of these experiments the truth of the first assumption, made tacitly in all earlier experiments on the vitreous body, to the effect that a gel at its swelling maximum for certain conditions can be expected to increase in volume at all, is demonstrated. Hence if the vitreous body is formed by a continuous process of swelling without the intervention of a sol stage, the presence of soaps in

the surrounding tissues would favour an increased volume of the vitreous body.

Section 2. The structure of the vitreous body. Goedbloed (1934, 1) described ultra-microscopic changes in the vitreous on treatment with KCNS solutions which he interpreted as being due to an increased hydration of the micelles. If such were the case a change in the elastic properties of the vitreous body on treatment with this substance would be expected. To test this two techniques were used, first, the resistance of the treated vitreous body to the emulsification of Hg by ultra-sonic waves was compared with that of a control, and secondly, the technique of Freundlich and Seifriz

FIG. 6.

(1923) for the measurement of the elastic properties of gels, as modified by Robertson and Duke-Elder (1933) to suit the peculiarities of the vitreous body, was used.

Ultra-sonic waves. When water and Hg are submitted to ultra-sonic waves an emulsion of Hg in water is immediately formed, the whole mixture taking on a dark grey colour; if the water is replaced by a jelly the elastic properties of the latter hinder the emulsification of the Hg, so that after a given time there is a fairly well defined boundary between the clear jelly and that in which Hg particles have penetrated. The height of this boundary gives a rough idea of the elasticity of the jelly.

Ox vitreous was placed in 1.0 M, 0.5 M, and 0.14 M solutions of KCNS and in isotonic saline for 24 hours at 20°. They were

transferred to test tubes and 1 ml. of Hg added, and the tubes were held in succession over the vibrating column of oil above the quartz plate of the ultra-sonic apparatus. The heights of the emulsified Hg columns were compared.

There was no detectable difference in the heights of the columns of the vitreous treated with KCNS and of those treated with normal saline, so that it is concluded no very gross changes in the elastic properties of the vitreous body are caused by KCNS.

Nickel particle technique. Vitreous is placed in a rectangular glass cell 1 cm. edge and a nickel particle about 50 diameter is picked off a slide under the microscope and stabbed into the vitreous. The particle is focused and its extension due to an applied

FIG. 7.

magnetic field measured by a micrometer eye-piece. Portions of vitreous treated with KCNS, K_2SO_4 , and dilute HCl at Ph3 were studied together with normal untreated vitreous. The results are shown in Figs. 7 and 8. Curve I, Fig. 7 is the typical extension-time curve for normal untreated vitreous, characterized by a rapid initial elastic displacement followed by a viscous creep approaching a fairly constant velocity.

Before describing the remaining curves a few words must be said in regard to the limitations of the technique and the results expected should a hydration or dehydration of the colloid particles be achieved by the solutions mentioned. Firstly, a direct comparison of the absolute displacements of one given particle in different lots of vitreous in a given time and a fixed field strength is difficult, as a particle of the size necessary for its recovery from the first vitreous is very irregular and its extension will depend on its direction of motion. Furthermore, as the vitreous

is not stuck firmly to the cell walls there will be a certain amount of 'give' which will vary with the quantity of vitreous in the cell and the depth of the particle in it. On the other hand, the curve I, Fig. 7, is very typical and can be reproduced from vitreous to vitreous without difficulty. As far as the changes expected on treatment of the vitreous with the reagents mentioned are concerned, from the work of Leick (1904) and Kramer (1926) a decrease in elasticity is to be expected with K_2SO_4 and an increase with KCNS. This should be reflected in a diminution in the viscous creep, characterized by the upper part of the curve, in relation to the initial displacement, in the case of K_2SO_4 which normally acts as a dehydrating agent, and the reverse should hold

FIG. 8.

for KCNS. (Once again no work has been done on the effect of such solutions on gels already formed; workers in this field have added the substance to be examined to the sol which was then allowed to set, and the elastic modulus measured).

Curves II and III of Fig. 7 are those for vitreous treated with 0.5 M KCNS and K_2SO_4 respectively. They are representative of a number of curves determined similarly, but it should be mentioned that a few curves were obtained not conforming to these types; the reason for these irregularities, which were not observed in normal vitreous, will be brought out later. The displacements in the different curves are in no way comparable with one another as different particles were used. It is evident that 0.5 M KCNS has the reverse effect to that expected on the supposition that it increases hydration, the extension subsequent to the first 10 secs. being very small compared with that during this time. 0.5 M K_2SO_4 has no appreciable effect on the extension-time curve. The absence

of effect in this case is very surprising as a considerable contraction in volume was observed. The curves for isotonic solutions of these salts (not shown) were in general similar to those for normal vitreous. The curves obtained with vitreous treated with dilute HCl at Ph3 (Fig. 8) throw a little light on the problem. Curve I occurred by applying a small magnetic force; increasing the field strength with conditions, otherwise the same, gave Curve II which is quite normal. It is evident that the viscosity of the medium has increased under the influence of the acidity, so that a limiting force has to be exerted to allow the creep to proceed. A similar phenomenon was observed with K_2SO_4 but not so regularly.

Baurmann (1924) showed that between Ph3 and Ph2 a precipitate fall in the volume of the vitreous occurs; this change is accompanied by the precipitation of the mucoprotein in the gel (Goedbloed, 1934, 2). If it is assumed that the volume change is due to the collapse of the vitreous structure, as opposed to a semi-reversible dehydration, then at Ph3 the undermining of the vitreous structure would be expected to have already begun, and hence the changes in the elasticity could be put down to this cause. The decreased stability of the vitreous, which would tend to lower the elasticity, would at the same time increase the viscosity of the intermicellar fluid and the two effects would compensate each other to varying extents. If this is true, the irregularities in the shapes of the curves obtained by treating vitreous with KCNS, K_2SO_4 , and dilute HCl mentioned earlier are explained, as it is only to be expected that a body in a state of very unstable equilibrium will respond extravagantly to minute changes in its environment, thus prejudicing the reproductibility of results obtained on it.

In view of the results obtained on the extension-time curves, it was important to see whether the absolute values for the extension of a given particle varied greatly according as the vitreous was treated with K_2SO_4 , KCNS, or dilute HCl. The only way of achieving this end was to treat a large number of pieces of vitreous successively and analyse the results statistically.

TABLE I

Solution	$\bar{d}$	n	s	t	P
0.5 M KCNS -	+4.34	13	2.56	3.3	<0.01
0.5 M K_2SO_4 -	-1.10	12	4.21	0.9	0.3 - 0.4
$1 \cdot 10^{-3}$ M HCl -	-2.30	13	2.53	3.3	<0.01

TABLE II

Protein solution added	Change in stability	
	Setting time	Melting time
1 per cent. ovalbumen . . .	—	+
0.5 per cent. agar-agar . . .	—	+
2.0 per cent. agar-agar . . .	+ + +	+ + +
1.0 per cent. serum globulin . .	+	—
0.75 per cent. isinglass . . .	—	+ +
1.5 per cent. isinglass . . .	—	+ +
2.0 per cent. caseinogen . . .	— —	— —

In Table I d is the mean difference in extension for normal and treated vitreous; a positive value means that the treated vitreous gave a larger extension than its control, and hence was less elastic. n is the number of pairs of determinations, s is the standard deviation of the differences, t is the ratio of the mean difference to its standard error (Fisher, 1928), and P is the probability that such a value of t will occur by chance. When P is greater than 0.05 the difference is insignificant. It appears that in 0.5 M KCNS the extension is greater, in dilute HCl less than the control, whilst the difference with K₂SO₄ is insignificant. Here again the results are more in conformity with the supposition that the vitreous stability has been upset than that a reversible syneresis has occurred, or in the case of KCNS a reversible hydration.

As the large volume decrease of the vitreous body on treatment with dilute HCl appeared to be correlated with the precipitation of its muco-protein (Goedbloed, 1934, 2), it was decided to determine in what way the presence of a second protein influenced the stability of a protein clot. In Table II the changes in the stability of gelatin clots by the addition of a second protein as measured by changes in their setting and melting times are shown. Positive signs indicate degrees of increased stability. It is evident from the table that a second protein, or in the case of agar-agar a carbohydrate, may enter into the intimate structure of a gelatine gel. Hitherto, in the vitreous body, the residual protein has been considered as the determining factor in its degree of hydration. The results of this section lead to the view that the vitreous stability and hydration are bound up with the physical states not only

of the residual protein, but also of the muco-protein and perhaps of the albumen and globulin.

In so far as they show that the effects of acid, KCNS, and K_2SO_4 tend to lower the stability of the vitreous body, the results of this section also point to the vitreous body having been formed as a clot from an aqueous solution of proteins, since gels formed by a swelling process alone exhibit a certain degree of reversibility in their hydration and dehydration phenomena. When, furthermore, the similarity in the elastic and ultra-microscopic properties of the vitreous body and dilute plasma clots is considered (Robertson and Duke-Elder, 1933, 2) the balance of evidence points to the vitreous body having been formed as a clot. Hence the second of the two assumptions implied in earlier experiments on the vitreous body, to wit, that this body represents a stage in a continuous process of swelling from an initial xerogel, is shown to be unsound, so that further experiments of the nature of those of Löbeck, and those described in Part I are not likely to yield interesting results. If the theory of a swelling of the vitreous body is still to be maintained it must be on the basis of the alternative theory of a cyclical mechanism outlined earlier.

Section 3. Attempts at peptisation of the residual protein. The vitreous body was submitted to ultra-sonic vibrations, as a class of gels, the thixotropic gels, are converted to their sol form by this treatment. No liquefaction of the vitreous body was obtained in this manner. Solutions of KCNS of strengths varying from 0.5 M to a saturated solution, a variety of soaps, and thiourea, in the hot and the cold, were tried on the separated residual protein without any evidence of peptisation. Mörner's treatment (1894) by which the protein was heated with water at 105° under pressure gave a clear solution, which, however, failed to set on cooling as described by this author. Repeated attempts using this treatment failed to give a jelly, and it must be concluded that this vigorous treatment gave breakdown products.

The failure to peptise the vitreous proteins must not be interpreted as vitiating the second theory of vitreous swelling, as post-mortem changes in the ultra-microscopic picture of the vitreous body have been described (Duke-Elder, 1929)* which may be interpreted as being due to denaturation changes. Attention should be drawn here to the statement by Goedbloed (1934, 1) that KCNS, in concentrations of 2.5 M and greater, causes a complete solution of the vitreous body *in vitro*. Such is not the case; we have observed repeatedly that, whilst at 2 M concentration and greater there is a decrease in the volume of the vitreous body culminating at a concentration of 2.5 M in its shrinkage to a minute fraction of its

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* Baurmann (1932) denies the existence of these changes.

original volume, the effect is not one of dissolution but one of dehydration as the residual protein is to be seen invariably floating on the top of the solution. We may add that the context of Goedbloed's statement leaves no doubt that it was his intention to convey the idea that the residual protein had completely dissolved.

The same author (1934, 1, 2) denies that the vitreous body is a gel on the grounds that the phenomena associated with it are not reversible. We have some difficulty in taking this criticism seriously, but feel that a reply is necessary to prevent the introduction of confusion into a matter already obscure enough. Firstly we must point out that the exclusion of the vitreous body from the category of gels, on the count of the irreversibility of the phenomena associated with it, simultaneously rules out practically all substances up to now considered as belonging to this class. Secondly, the elasticity as exhibited in the nickel particle technique (Duke-Elder and Robertson, 1933) and the behaviour with ultrasonic vibrations and Hg, the vapour pressure isotherm (Duke-Elder, 1930), the anisotropy (*ibid.*), the formation of Liesegang rings (*ibid.*), and the ultra-microscopic appearances of the vitreous body represent a nexus of properties peculiar to gels. Goedbloed's attempt to discount the evidence of the elasticity of the vitreous body by ascribing this property to its 'framework' as distinguished from it itself is equivalent to denying mass to a piece of iron because the mass belongs to the constituent atoms in its lattice structure.

In conclusion, it must be admitted that the theory that a swelling of the vitreous body is a factor in chronic glaucoma is definitely weakened by the results described in this paper. The changes in volume with Ph recorded earlier (Baurmann, 1924; Duke-Elder, 1930; Goedbloed, 1934, 2) which were regarded as evidence for a possible hydration of the vitreous body under suitable conditions *in vivo* (Duke-Elder, 1930) find a new interpretation, in that they do not represent semi-reversible changes similar to those of swollen gelatin, but are to be attributed to varying states of instability produced by the changed Ph . The elucidation of the probable mode of origin of the vitreous body has shown the faultiness of certain implied assumptions as to the mechanism whereby the vitreous body could swell; although an alternative mechanism, reasonable from a physico-chemical point of view, has been put forward, it must be pointed out that the hypotheses involved are more numerous and less capable of experimental verification. If the actual peptisation of the vitreous proteins could be achieved, a basis for the experimental verification of this alternative theory would be obtained.

Summary

The chemical and physical properties and behaviour of the vitreous body have been studied with a view to testing the theory that a swelling of the vitreous body is the cause of the raised intra-ocular pressure in primary chronic glaucoma.

No increase in volume of hydration was obtained on treatment of the vitreous body with solutions of sodium oleate and laurate, or by changing the *Ph* to the alkaline side of normality.

The changes in the elastic properties of the vitreous body on treatment with K_2SO_4 , KCNS, and dilute HCl indicate that a decreased stability of the gel structure is produced by these reagents.

Attempts at peptisation of the vitreous proteins were unsuccessful.

The theoretical implications in the theory of vitreous swelling are discussed, and a mechanism whereby it may occur put forward.

Goedbloed's denial of the gel nature of the vitreous body is repudiated.

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