FURTHER STUDIES OF THE VISCOSITY OF ASPIRATED HUMAN VITREOUS FLUID: WITH SPECIAL REFERENCE TO ITS USE IN RETINAL DETACHMENT SURGERY

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IN SPITE OF THE ACCEPTANCE of vitreous implantation as a valuable supplementary technique in the surgical management of some cases of retinal detachment (since first reported by Shafer[1] in 1957) and the importance generally attributed to the viscosity of the implant,[2-5] there have been few studies of this quality of human vitreous fluid; and rarely is a surgeon aware of the viscosity of the fluid he is using.

Earlier studies from this Department[6] of 44 samples of aspirated vitreous fluid collected for use as implant material showed the feasibility of obtaining reproducible measurements of viscosity. Because of the wide range of values found, it was concluded that viscosity determinations should be made routinely before implantation, so that the less viscous samples might be discarded. It was noted that 0.75 cc. was sufficient for this measurement. If the vitreous from both eyes of a donor was pooled (providing a total amount of 3.0 to 4.0 cc.), enough became available for viscosimetry, culture, and implantation.

It was shown that the viscosity was the same after passage through a 27-gauge needle as after passage through an 18-gauge needle, and for this reason, the use of the wider gauge needle has no longer seemed justified when implanting vitreous.

The addition of alpha-chymotrypsin had no effect on viscosity. The addition of a small amount of hyaluronidase, however, resulted in a rapid reduction of viscosity to a level only slightly higher than that of water. It was therefore evident that the major part of the viscosity of aspirated vitreous was related to its hyaluronic acid content. The

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viscosity of reconstituted lyophilized vitreous was found to compare favorably with that of stored cadaver vitreous, supporting earlier observations by Paufique, *et al.*[7]

The investigations have been continued in order to obtain data possible only from the study of a larger amount of material. The present paper reports the results of viscosity studies of 189 more samples, bringing the total number to 233.

MATERIALS AND METHODS

The new material was from two sources.

1. *One hundred and seventy-seven samples obtained at post-mortem from 97 donors at the Institute of Pathology, McGill University.* These samples were obtained three to eighteen hours after death, under aseptic conditions, by aspiration through an 18-gauge needle introduced through conjunctiva and sclera 8 or 9 mm. behind the limbus, as previous described.[8] The vitreous fluid was withdrawn slowly, with the point of the needle in the center of the vitreous chamber. The amounts varied from 1.5 to 2.0 cc. The material from each eye was injected into a separate sterile rubber-stoppered vial through a second 18-gauge needle. All viscosities were measured within one hour of collection, and cultures were taken. The samples were then stored in a refrigerator at 4° C.

2. *Twelve samples of lyophilized (preserved) vitreous from eight donors.** These had been collected with a 15-gauge needle from eyes enucleated at autopsy. A 1.0 cc. antibiotic solution containing neomycin, polymyxin B, and gramicidin† had been added to each sample before freezing and lyophilization. The method of preservation has been described.[9] Each sample arrived as a dry white powder, indicating satisfactory preservation.

In the present studies, we were interested in determining the viscosities of *concentrated solutions* of lyophilized vitreous. Therefore, the least amount of water needed to dissolve the vitreous powder was slowly added and the viscosities then determined. Vigorous shaking of the vial was necessary to dissolve the powder and was done before each further addition of water. In all but two samples, solution was obtained by adding an amount equal to 50 per cent of the original volume.

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†Neosporin solution, Burroughs, Wellcome & Co. Inc., Tuckahoe, N.Y.

The technique of viscosimetry has been described.[6] An Ostwald type viscosimeter pipette with a 0.5-mm. diameter bore that would allow determinations of viscous solutions in quantities of 0.75 cc. was used. *Relative* viscosities were measured, with distilled water the reference fluid. Determinations were made at 37° C., using a constant temperature water bath with thermoregulator accurate to within 0.1° C. Flow time was recorded with a stopwatch with automatic starter, accurate to within 0.2 seconds. The average of five consecutive readings was taken. Differences in values for the five readings were generally extremely small. Some of the samples showed particulate matter; this usually settled to the bottom of the vial and could be avoided in collecting the fluid for viscosimetry. (The inclusion of insoluble particles in the viscosity pipette leads to false results which become readily apparent, however, because of variations in consecutive readings; such a sample would then be discarded.)

RESULTS

SAMPLES FROM DIFFERENT SUBJECTS

Figure 1 shows the frequency distribution of viscosities of 177 samples of aspirated human vitreous fluid from 97 donors tested within one hour of collection. There was a wide range in values, from a low of 1.06 to a high of 5.50. Average viscosity was 1.97; median was 1.86.

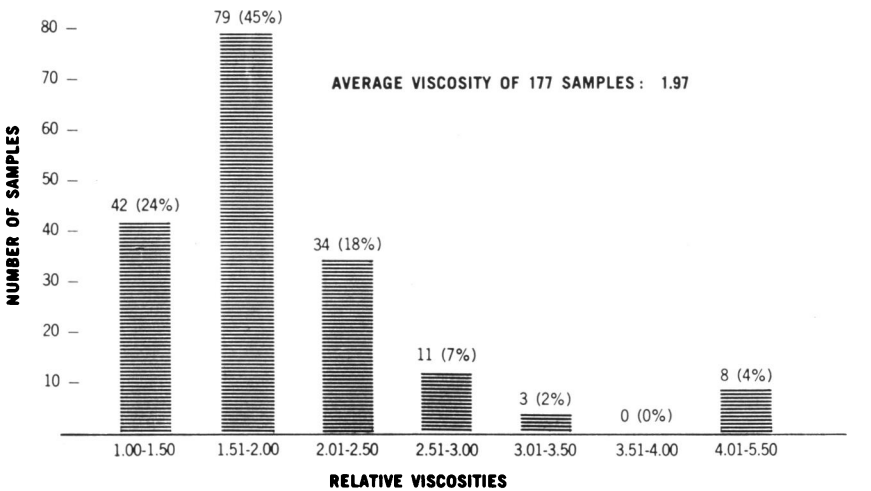

FIGURE 1. FREQUENCY DISTRIBUTION OF VISCOSITIES OF 177 SAMPLES OF ASPIRATED HUMAN VITREOUS FLUID FROM 97 DONORS TESTED WITHIN ONE HOUR OF COLLECTION.

Lowest figures were 1.061, 1.088, and 1.089, all others being greater than 1.15. Bacterial contamination may have caused the low viscosities in two of these samples (both from the same patient), since a heavy growth of *E. coli* was cultured repeatedly from each. The sample with lowest viscosity (1.06) was sterile, however.

Eight samples (from five individuals) had viscosities ranging from 4.2 to 5.5, much in excess of all the others. Study of the clinical and autopsy records of these patients failed to reveal anything unusual to explain these high values.

Twelve samples from six patients were cloudy. Viscosities of these, as well as of six icteric samples from three jaundiced patients, were not remarkable.

TABLE 1. RELATIVE VISCOSITY OF CONCENTRATED SOLUTIONS OF LYOPHILIZED VITREOUS

	Donor age (years)	*Before lyophilization*	*After solution*		
		Volume (cc.)	*Volume (cc.)*	*Per cent of original volume*	*Viscosity*
Single eyes	61	2.0	1.8	90	1.731
	88	1.5	0.75	50	2.800
Pairs of eyes	56	2.0	1.0	50	3.520
		1.5	0.85	57	3.875
	60	1.5	0.85	57	4.110
		1.5	0.80	53	4.120
	73	2.0	1.7	85	5.755
		2.0	1.2	60	6.269
	80	2.0	1.0	50	2.776
		2.0	1.0	50	2.900
	83	2.0	1.0	50	4.680
		2.0	1.0	50	4.686
AVERAGE		1.83	1.08	59	3.935

LYOPHILIZED VITREOUS. Greater viscosities were found for concentrated solutions of lyophilized vitreous (Table 1). Values ranged from 1.731 to 6.269, the average being 3.935. Although this was higher than the average for the fresh cadaver samples, it is of interest that ten samples in the latter group had viscosities between 3.00 and 5.50, that is, in a similar range.

EFFECT OF NEEDLE BORE. Viscosities of both fresh and reconstituted lyophilized samples were the same after passage through a 27-gauge needle as after passage through an 18-gauge needle, confirming an earlier finding.[6]

SAMPLES FROM RIGHT AND LEFT EYES OF THE SAME SUBJECTS

In contrast to the wide range of values for different individuals, the average difference in viscosity of the aspirate from right and left eyes of the same donor (80 pairs) was small, only 0.15 (Table 2). Forty-seven pairs of eyes (59 per cent of the total) showed no measurable difference; seventy pairs (or 88 per cent) showed differences of less than 0.25.

TABLE 2. DIFFERENCE IN VISCOSITY OF FLUID FROM RIGHT AND LEFT EYES OF THE SAME DONOR

Viscosity difference	*No. of donors*	*Per cent of donors*	
0.00	47	59%	88%
0.00–0.24	23	29%	
0.25–0.49	4	5%	12%
>0.50	6	7%	
TOTAL	80		

AVERAGE DIFFERENCE = 0.15

Only in six instances (7.5 per cent of the total) were the differences for the two eyes greater than 0.50. Two of these patients had unilateral eye disease; a record of eye examination was not available for the others. The greatest viscosity difference between right and left eyes was found in a patient noted at autopsy to have heterochromia iridis; unfortunately the basis of the heterochromia was not known. The hypochromic eye yielded vitreous fluid with a low relative viscosity of 1.19; the eye with the dark brown iris gave fluid with a viscosity of 4.24. In the second patient, there was unilateral corneal scarring of unknown etiology, sufficient to obscure a view of the vitreous and fundus; the other eye was normal. Viscosity of fluid from the affected eye was 2.84 and from the normal eye, 1.36.

Factors in method which might have been responsible for differences in viscosity in samples from different individuals and not in samples from the two eyes of the same individual were: (*a*) differences in time between death and collection; (*b*) differences in amount of fluid aspirated; (*c*) differences in site in the vitreous chamber from which the fluid was taken; and (*d*) differences in time between collection and measurement of viscosity.

In this study, the last two factors had been kept constant. Time between death and collection of the sample was known in each case. The majority of samples were collected within three to eighteen hours

of death. A few were obtained 18 to 26 hours after death. No correlation between viscosity and the length of these intervals could be noted.

EFFECT ON VISCOSITY OF VARYING THE AMOUNT WITHDRAWN. The amount of fluid aspirated varied from 1.5 to 2.0 cc.; no correlation between the amount withdrawn and viscosity could be noted. In order to determine the possible effect on viscosity of greater differences in the amounts withdrawn, 1.0 cc. was taken from the right eyes and 2.0 cc. from the left eyes of eleven patients. Table 3 shows that there were no significant differences in viscosities in the two groups.

TABLE 3. RELATION OF VISCOSITY TO AMOUNT OF VITREOUS FLUID WITHDRAWN

	Viscosity readings	
Patient no.	*1.0 cc. from O.D.*	*2.0 cc. from O.S.*
1	1.790	1.710
2	2.186	2.186
3	2.186	1.783
4	2.144	2.076
5	1.465	1.540
6	1.370	1.260
7	1.606	1.216
8	4.204	5.084
9	2.142	2.370
10	2.910	3.408
11	1.616	1.846
AVERAGE	2.147	2.225
DIFFERENCE BETWEEN AVERAGES = 0.078		

EFFECT OF AGE ON VISCOSITY

Median and average viscosities were about the same in all decades of life between ages 2 and 90 (Table 4), when the six extreme values noted in this age span were excluded in calculating the means.*

Ages of the patients in whom the extremely high values (over 4.00) occurred were 50, 57, 74, 81, and 93. Highest viscosities (5.50) were obtained from both eyes of the oldest patient in the series (age 93), but a value almost as high (5.43) was found for one eye of a patient age 50.

Values for the youngest patients were not remarkable. The youngest child, age 2, yielded fluid with a viscosity of 1.33. Four other patients in the first decade were four to seven years of age; viscosities of fluid from their eyes varied from 1.87 to 2.00.

*Since these extreme values satisfied Chauvinet's criterion, it was in order to omit them.[10]

TABLE 4. VISCOSITY OF ASPIRATED VITREOUS FLUID AT DIFFERENT AGES

Age (yrs.)	*No. of samples*	*Extreme values**	*Range of viscosity (extreme values excluded)**	*Mean viscosity (extreme values excluded)**	*Median viscosity*
2–10	10		1.33–2.00	1.81	1.86
11–20	4		1.36–1.67	1.51	1.52
21–30	4		1.35–2.19	1.80	1.85
31–40†	10†		1.47–2.66	1.87	1.76
41–50	26	5.43	1.26–2.40	1.84	1.83
51–60	41	4.30; 4.30	1.06–2.59	1.86	1.90
61–70	34		1.22–3.03	1.86	1.77
71–80	27	5.08; 4.02	1.16–3.41	1.80	1.83
81–90	17	4.24	1.19–2.56	1.86	1.85
93	2	5.50; 5.50			
ENTIRE GROUP	175†		1.06–3.41	1.84±0.45‡	1.86

*According to Chauvinet's criterion.
†Two contaminated samples with unusually low viscosities (1.08) from a patient aged 39 not included in table.
‡Mean ±S.D.

SYSTEMIC FACTORS AND VISCOSITY

A careful study of the autopsy reports, available on all donors, and of the clinical records available on 65 of the 97 donors, failed to show any correlation between the patient's general status and viscosity. Table 5 lists the data analyzed. The viscosity of fluid from diabetic patients, all of whom were under reasonable control, was not remarkable. Average viscosity of 18 samples from nine patients with uremia was 1.964. There was no correlation of viscosity with the state of hydration or nutrition of the patient. The ingestion of drugs, including diuretics, cardiac glycosides, and steroids had no apparent effect; none of the patients were receiving Diamox.

TABLE 5. DATA ANALYZED IN AUTOPSY REPORTS AND CLINICAL RECORDS OF DONORS FROM WHOM VITREOUS LIQUID WAS OBTAINED AT AUTOPSY

Eye history and examination
Primary and secondary clinical diagnoses
Whether death occurred suddenly in previously healthy person, or followed prolonged illness
Nutrition–hydration
Fever; duration and degree if present
Drugs administered (e.g., diuretics, antibiotics, steroids)
Adjuvants administered (e.g., glucose, saline, blood)
Blood chemistry (e.g., urea nitrogen, electrolytes, sugars, etc.)
Hemogram
Autopsy findings

OCULAR STATUS AND VISCOSITY

Unfortunately the refractive status of most of the patients was not known. The patient yielding the sample with lowest viscosity (1.06) had been examined by one of the authors, however, and was emmetropic, with normal vision. A few small vitreous floaters were noted in each eye. This 53-year-old female patient with manic-depressive psychosis died from unexpected complications of electroshock treatment, having been previously in good physical health.

Reports from ophthalmologists were found in only six of the 65 clinical records available; in none of these was the slit-lamp appearance of the vitreous recorded. Three patients were diabetics with early cataracts and mild diabetic retinopathy; viscosities of the samples from these eyes were not remarkable. The histories and physical examinations recorded in the charts did not indicate the presence of significant eye disease in any of the other patients.

EFFECT OF STORAGE TIME AND TEMPERATURE ON VISCOSITY

Earlier studies[6] suggested that aspirated vitreous fluid lost about 24 per cent of its viscosity after one week of storage, with little or no further loss thereafter. Samples stored as long as two to seven years retained a reasonable viscosity, which compared favorably with that of samples stored from two weeks to one year (see Table 8).

The present studies were carried out (*a*) to confirm the earlier findings with a larger series; (*b*) to determine if the loss in viscosity was related entirely to duration of storage, or whether other factors such as handling the samples or increasing their temperature to 37° C. for viscosimetry might also be responsible; (*c*) to study more precisely the decay curve of viscosity during the first week by doing daily determinations; and (*d*) to determine the effect of storage time on the viscosity of concentrated solutions of lyophilized vitreous.

The results are summarized in Tables 6 and 7.

1. Twenty-five samples (Table 6; Group A in Table 7) were each divided into two parts. One part was stored at 4° C., the other at 37° C. The samples were tested daily. Between tests they were kept in pipettes, the ends of which were sealed to prevent evaporation. Viscosity fell more rapidly at 37° C., but the total loss after one week was about the same for both groups (22.1 per cent and 25.3 per cent). In both groups, most of the loss occurred during the first four days, with the greatest drop in the first 24 hours. There was only a small further decrease between the fourth and sixth days and no additional loss between the sixth and seventh days.

TABLE 6. EFFECT OF TEMPERATURE AND DURATION OF STORAGE ON MEAN VISCOSITY OF 25 SAMPLES OF ASPIRATED HUMAN VITREOUS FLUID WHEN ONE-HALF OF EACH SAMPLE WAS STORED AT 4°C.* AND THE OTHER HALF AT 37°C.

Time after collection (days)	*Storage at 4°C.**		*Storage at 37°C.*	
	Mean viscosity	*Mean decrease in initial viscosity (per cent)*	*Mean viscosity*	*Mean decrease in initial viscosity (per cent)*
0†	1.90	—	1.90	—
1	1.74	8.4	1.60	15.8
2	1.68	11.6	1.58	16.8
3	1.61	15.3	1.53	19.5
4	1.52	20.0	1.50	21.0
5	1.50	21.0	1.46	23.2
6	1.48	22.1	1.42	25.3
7	1.48	22.1	1.42	25.3

*Temperature of the samples raised once daily to 37°C. for viscosimetry.
†First viscosity tested within one hour of collection.

TABLE 7. EFFECT OF TEMPERATURE OF STORAGE AND FREQUENCY OF VISCOSITY TESTING ON MEAN VISCOSITY OF ASPIRATED HUMAN VITREOUS FLUID DURING THE FIRST WEEK AFTER COLLECTION

	Group	*No. of samples*	*Frequency of determinations*	*Storage temperature (degrees C.)*	*Initial viscosity*	*Decline in viscosity after 6 days (per cent)*	
Vitreous obtained at post-mortem	A	25	Daily	37	1.90	25.3	
			Daily	4	1.90	22.1	
	B	12	Once—end of six days	4	2.38	13.3	13.0
	C	11	Once—end of six days	4		12.7	
			Daily	4	2.25	20.5	
Concentrated solutions of lyophilized vitreous	D	12	Twice—one hour and six days after solution	4	3.94	18	

2. Twelve other samples (Group B in Table 7) were each divided into two parts. One part was tested within one hour of collection; the other was stored at 4° C. and not tested until after six days. The decrease in viscosity after six days was less, only 13.3 per cent.

3. Another eleven samples (Group C in Table 7) were also each divided into two parts and treated in the same way except that the first part was tested daily in addition to the initial test within one hour of collection, to serve as a control. The second part was not tested

until after six days. The average decline in viscosity of the undisturbed samples was again smaller (12.7 per cent), whereas the viscosity of the portions that were tested daily declined 20.5 per cent.

4. Finally, twelve samples of concentrated lyophilized vitreous (Group D in Table 7) were tested one hour and again six days after solution and showed an average decrease in viscosity of 18 per cent.

5. The decreases noted in Tables 6 and 7 are the means for each group. In all the groups individual samples showed great variations in the amount of viscosity lost after six days, ranging from 3 to 33 per cent for samples tested daily, and from 3 to 26 per cent for the samples tested only at the end of the period.

DISCUSSION

Table 8 lists the mean viscosities of human vitreous liquid in three other studies[7,11,12] which involved smaller numbers of samples, including the previous study from this Department.[6] Allowing for variations on the basis of differences in the time interval between collection of the samples and measurement of their viscosities, there is a remarkable agreement in the mean values.

Collection of the vitreous fluid in the present study was intentionally by aspiration, and centrifugation was purposely omitted in order to duplicate the conditions under which the material was being prepared for use in surgery,[8] especially since it has been stated that "rough handling" reduces the viscosity of hyaluronic acid preparations,[13] and since it has also been suggested that passage through a small bore needle might affect it.[1] Because aspiration was carried out with the needle point in the center of the vitreous chamber, fluid from the

TABLE 8. MEAN VISCOSITIES OF HUMAN VITREOUS LIQUID

	No. of samples	*Time after collection*	*Viscosity*	
			Range	*Average*
Paufique, *et al.* (1959)[7]	6		1.4–3.0	1.90
Balazs (1960)[11]				1.97
Edwards and Locke (1961)[6]	7	1 hour	2.0–3.7	2.52
	7	24 hours		2.05
	6	1 week		1.65
	9	2–7 years	1.1–3.7	1.72
Berman and Michaelson (1964)[12]	37*			1.73
	32†			1.89
Locke and Morton (current study)	175	1 hour	1.1–5.5	1.97

*Ages 13–85, central portion of vitreous (includes 7 myopic eyes).
†Ages 13–85, cortical layer of vitreous (includes 7 myopic eyes).

most peripheral portions of the vitreous body was probably not included. The concentration of hyaluronic acid has been reported to be higher in the cortical layers than in the central core.[11] It is possible, therefore, that centrifuged fluid from the entire vitreous body might have given different values. However, mean values found by Balazs,[11] by Paufique, *et al.*,[7] and by Berman and Michaelson[12] for such fluid (Table 8) were similar to the mean value found by us. Berman and Michaelson found little difference in values for the cortical layer as compared to the central portion. Viscosity of fluid from seven myopic eyes was also not significantly different.

They[12] found no significant difference in the mean viscosity of four samples from patients 13 to 45 years of age compared to 26 samples from donors 50 to 85 years of age. On the other hand, the viscosities of three samples from donors less than two years of age were so low as to be barely measurable.

We found the mean viscosity to be the same in all decades between ages 2 and 90 (Table 4) when six extreme values (which satisfied Chauvinet's criterion[10]) were excluded from the calculations. The few extremely high values occurred in patients fifty years of age and over, including our oldest donor, age 93. Correlation of the slit-lamp appearance of the vitreous *in vivo* with its viscosity was not possible. It can be assumed, however, that there was a significantly higher incidence of vitreous degeneration, including liquefaction and shrinkage, in the older patients. Since the viscosity of samples from the oldest donors was not less than that from the younger ones, it can be concluded that the viscosity of vitreous fluid is not related to the integrity of the vitreous body, and that eyes with vitreous degeneration may yield fluid of good viscosity.

This is in accord with current concepts that the integrity of the vitreous body depends primarily upon the collagen, whereas the viscosity of its liquid component depends chiefly upon hyaluronic acid.[11,13] Our results indicate that these may vary independently. From their studies on beef vitreous humor, Brunish, Rowen, and Irvine[14] also concluded that viscosity was an inadequate criterion of vitreous integrity.

Gross clarity of aspirated vitreous also cannot be counted upon to signify the absence of degeneration in the vitreous body, since the incidence of cloudy samples in this series was only 6.9 per cent, much less than the usual incidence of vitreous degeneration in a group with this age distribution.

The large variation in values in samples from eyes of different

individuals indicates the desirability of routinely measuring the viscosity of vitreous prepared as implant material before surgery. Otherwise samples with values little greater than that of saline may be used. This could happen frequently. For example, Figure 1 shows that 24 per cent of the samples in this series had viscosities of 1.50 and less, when tested one hour after collection, and it was shown above that during the first six days after collection, undisturbed samples stored at 4° C. lost from 3 to 26 per cent of their initial viscosity, with an average loss of 13 per cent.

In striking contrast to the variations found in samples from different individuals was the similarity of results for samples from two eyes of the same individual. The results of Balazs' chemical studies[11] parallel these. He found considerable individual variation in the concentrations of hyaluronic acid, protein nitrogen, and sialic acid in vitreous bodies from 65 human eyes, but a remarkable agreement between the values for two eyes of the same individual. Berman and Michaelson,[12] as well as Paufique, *et al.*,[7] have also commented on the large variation in viscosity of samples from different individuals.

These findings raise the interesting question of which factors are responsible for high or low viscosity. Since age was not a significant factor, and eye disease was noted only exceptionally, systemic factors were studied. None could be implicated. Experimentally, in animals, such correlation has been shown.[15–18] For example, Christiansson[15] found that in alloxan diabetes in rabbits the amount of hyaluronic acid in the vitreous body was almost doubled, while the collagen content was significantly reduced; in our study, the viscosity of vitreous from diabetics showed no unusual tendencies. Systemic administration of steroids has been shown to increase the viscosity of vitreous fluid in rabbit eyes;[16–18] in our series, individuals receiving steroids did not have fluid of higher viscosity. Thyroidectomy also increased viscosity of the vitreous filtrate of rabbit eyes;[17,18] our thyroidectomized patients did not show unusual values. The viscosity of sodium hyaluronate has been shown to be influenced by electrolytes;[19] we could find no correlation between blood electrolyte levels and viscosity.

As far as the spontaneous loss of viscosity during storage is concerned, the more accurate index would seem to be derived from the samples left undisturbed at 4° C. for one week (13 per cent average loss with wide individual variations), since these were the conditions commonly used in preparing the material for surgery. It is interesting to note that the average loss was greater (20 per cent for one group, 22 per cent for another) when samples were measured daily at 37° C.,

even though the storage temperature between tests was 4° C. The daily temperature increase to 37° C. and agitation of the sample for viscosimetry would seem to be the possible factors involved. Brunish, *et al.*[14] in their work with beef vitreous humor found a measurable decrease in viscosity when determinations were made at 36° C., which did not occur when the measurements were performed at 0° C. Pirie and Van Heyningen[13] have said that rough handling reduces the viscosity of hyaluronic acid preparations.

Our earlier observation[6] that the viscosity of vitreous liquid remained the same after passage through a 27-gauge needle as after passage through an 18-gauge needle was again confirmed. Castroviejo[20] reported that the effectiveness of implanted vitreous in retinal detachment surgery did not seem to be altered by injecting it into the eye through a 30-gauge needle. Implantation through a 27-gauge needle was reported upon favorably by Pischel.[21] We also have had good results with this technique, which is simpler and less traumatic than when a larger needle is used. Regurgitation of vitreous does not usually occur, so that the volume of vitreous required is usually less. Great care has to be taken not to raise the intraocular pressure too high, which is all too easily done, since it may be more difficult to reduce the pressure to a safe level quickly except by paracentesis.

CONCLUSIONS

1. Since viscosity of vitreous implant material is generally considered of importance in surgery, and since there was a wide variation in viscosity from one sample to another, viscosities ought to be measured routinely so that samples with low viscosities can be discarded.

2. There will be some loss of viscosity during the first few days of storage. This has to be sacrificed in the interests of assuring sterility. There will be little additional loss after the first week, and many samples stored six months or longer will maintain a good level.[6] Since individual samples varied greatly in the amount of viscosity lost during the first few days of storage, it would seem wisest to wait until after the first week (when this value will have reached a stable level) before measuring viscosity, unless the sample has to be used before this time.

3. Pooling of all clear samples of cadaver or eye-bank vitreous, as is done in some centers, makes available to the surgeon a fluid of average viscosity. Our studies have shown that after one week, the relative viscosity of pooled vitreous will be between 1.50 and 1.70, having dropped from an initial level of 1.97. If all samples with viscosities below some

arbitrarily selected viscosity level are discarded and the remainder pooled, the viscosity of the resultant mixture can be made higher. Still higher viscosities can be obtained by not pooling the material, and by using individual samples with higher values. The highest viscosities of all can be obtained by using concentrated solutions of lyophilized vitreous.

4. Since samples from older individuals (who are known to have a much higher incidence of vitreous degeneration and liquefaction) were not less viscous than those from younger persons, it is concluded that the viscosity of aspirated vitreous fluid is not related to the integrity of the vitreous body as seen *in vivo* with the slit-lamp.

5. Aspirated vitreous fluid retained the same viscosity after implantation through a 27-gauge needle as after passage through a wider needle (e.g., 18-gauge needle). There no longer seems to be any justification for use of larger needles.

6. We were not able to ascertain in this study what factors determine whether aspirated vitreous fluid will have a high or a low viscosity. Studies on animal vitreous indicate a variety of systemic factors that may influence viscosity;[15–18] none could be incriminated here.

7. These studies do not permit any new conclusions about the value of vitreous implantation in retinal detachment surgery; nor do they allow us to conclude that samples with the highest viscosities are necessarily the best. For example, samples with unusually high viscosities, much in excess of all others, could conceivably have some abnormality responsible for the high viscosity which could make them less suitable for clinical use, in which case the best samples might be those with average or slightly above average viscosities. Our results do not allow us to speculate on these points.

REFERENCES

1. Shafer, D. M., The treatment of retinal detachment by vitreous implant, Tr. Am. Acad. Ophth., 61:194, 1957.
2. King, J. H., Jr., and S. B. Chavan, The preservation of eye tissues, Am. J. Ophth., 47:303, 1959.
3. Paufique, L., and J. Charleux, Lyophilized human vitreous as a therapeutic agent in retinal detachment of poor prognosis, Bull. Soc. opht. France, 74:345, 1961.
4. Shafer, D. M., In Importance of the Vitreous Body in Retina Surgery with Special Emphasis on Reoperations, ed. by C. L. Schepens. St. Louis, Mosby, 1960.
5. Widder, W., Hyaluronic acid as a vitreous implant in retinal detachments: Experimental principles, Graefes Arch. Ophth., 162:416, 1960.

6. Edwards, G. K., and J. C. Locke, Viscosity studies of aspirated human vitreous, with special reference to its use in retinal detachment surgery, Am. J. Ophth., 52:374, 1961.
7. Paufique, L., M. Fayet, and M. Revault, Comparative study of normal and lyophilized human vitreous, Ann. ocul., 192:241, 1959.
8. Edwards, G. K., and J. C. Locke, The collection, storage and selection of human vitreous for use in retinal detachment surgery, Am. J. Ophth., 50:108, 1960.
9. King, J. H., Jr., J. W. McTigue, and S. B. Chavan, Experiences with vitreous preserved with lyophilization, Tr. Am. Acad. Ophth., 64:287, 1960.
10. Documenta Geigy Scientific Tables, Fifth edition. Basle, J. R. Geigy, S.A., 1956.
11. Balazs, E. A., Physiology of the vitreous body, In Importance of the Vitreous Body in Retina Surgery with Special Emphasis on Reoperations, ed. by C. L. Schepens. St. Louis, Mosby, 1960.
12. Berman, E. R., and I. C. Michaelson, The chemical composition of the human vitreous body as related to age and myopia, Exper. Eye Res., 3:9, 1964.
13. Pirie, A., and R. Van Heyningen, Biochemistry of the Eye. Springfield, Thomas, 1956.
14. Brunish, R., J. W. Rowen, and S. R. Irvine, Proteins and hyaluronic acid of beef vitreous humor, Tr. Am. Ophth. Soc., 52:269, 1954.
15. Christiansson, J., The collagen content of the vitreous body in alloxan diabetes in rabbits, Acta ophth., 39:141–7, 1961.
16. Kaplan, D., and B. Fisher, Effect of methylprednisolone on mucopolysaccharides of rabbit vitreous humor and costal cartilage, Biochim. et biophys. acta, 83:102, 1964. Quoted by B. R. Straatsma, in Annual review: The lens and vitreous, A.M.A. Arch. Ophth., 73:577, 1965.
17. Larsen, G., The viscosity of the vitreous humor influenced by hormones, A.M.A. Arch. Ophth., 59:712, 1958.
18. Larsen, G., The hyaluronic acid in the rabbit vitreous body, A.M.A. Arch. Ophth., 60:815, 1958.
19. Morgan, S., Annual reports on the progress of chemistry, 42:236, 1946.
20. Castroviejo, R., Retinal detachment surgery: Scleral shortening by outfolding with titanium clips, Tr. Am. Acad. Ophth., 64:472, 1960.
21. Pischel, D., discussion of D. M. Shafer, The treatment of retinal detachment by vitreous implant, Tr. Am. Acad. Ophth., 61:194, 1957.

DISCUSSION

DR. JOSEPH A. C. WADSWORTH. Doctors Locke and Morton have completed a thorough and meticulous study of aspirated vitreous. The authors have made every effort to eliminate all variables in their determination. However, in spite of their precise study, there is a definite measurable variation in the viscosity of aspirated vitreous taken from cadaver eyes.

Strangely enough the older patients had the highest viscosity. In a personal study of fresh eyes it was found that a shrinkage and posterior detachment of the vitreous occurred with increased frequency in patients progressively older than 50 years. The posterior subvitreous space contained a clear fluid with an apparent viscosity somewhat greater than aqueous. However, the shrunken vitreous body had an apparent increase in viscosity over that of normal vitreous. It is entirely possible that the aspirated material

came from the shrunken vitreous from which a large part of the fluid element had been displaced into the posterior subvitreous space.

(Slide) This is a normal eye with the normal vitreous filling the entire vitreous cavity. (Slide) And this is an eye from a man over 50 years of age which shows the posterior detachment of the vitreous with a space here that is filled with a fluid substance. It is entirely possible that the aspiration took place from this area and giving a possibility of an increase in the viscosity of that portion.

By carefully opening fresh globes, the two elements could easily be collected and tested for their relative viscosity.

It is a valuable conclusion that the use of a 27-gauge needle for implantation does not affect the viscosity. The smaller needle facilitates the implantation of vitreous and decreases some of the technical difficulties and hazards of the procedure.

Fresh eyes opened where there has been a history of eye disease such as uveitis, intraocular surgery, or trauma very often show a marked liquefaction of the vitreous with a subsequent reduction in viscosity. In the case of heterochromia the blue eye is probably the diseased eye and one would expect a lower viscosity in such an eye as compared to the normal fellow eye.

If high viscosity is desired, there seems to be a greater preservation of viscosity in lyophilized vitreous than the fresh material. In addition, reconstitution can be carried out with a reduced volume to further increase the viscosity.

A great attempt was made by the authors to record the facts as they presented themselves. Their report is factual and unbiased.

DR. DONALD M. SHAFER. I regret that I did not have the opportunity of seeing Dr. Locke's paper before I came.

It is hard to weigh as to whether or not increased vitreous viscosity is desirable. Many people that do retinal surgery feel that the more viscous the vitreous is, the better. I certainly think that, on the table at the time of surgery, the more viscous the vitreous, the less likely it is to pass through a retinal hole and become subretinal. On the other hand, if this is carried to its logical conclusion, we should have it as viscous as possible, but could we not, at the same time, be setting the stage for true vitreous solidity which we so frequently associate with massive vitreous retraction. I wonder therefore if, surgically, there is not a happy medium. Unfortunately, we do not yet know the actual centistake value that is best.

I was very interested in Dr. Locke's report, and in Dr. Wadsworth's discussion. I think we will have to arrange to have all donors be 80 years of age or over.

I have been using an 18-gauge needle, though its use is more complex because of the sutures. This is because we drew the original samples, the original harvesting, with an 18-gauge needle. Surgically, I have found that when using a gauge 25 or larger, we have a leak back, so that a suture has

to be used when a 25-gauge or larger needle is used. When using a 27 or 30-gauge needle a scleral suture is usually not required.

There is one other point. Dr. Locke stated that he required .75 cc. to do the viscosity test. A good yield from a donor eye is frequently only one and a half cc. and that means using almost half your eye-bank deposit to find out the quality of the investment.

DR. LOCKE. I enjoyed Dr. Wadsworth's discussion. I found his comments pertinent, and I have no issue to take with any of them.

I also enjoyed Dr. Shafer's remarks. In the written version of the paper, we have made the point that we have no right to conclude that the most viscous samples are necessarily the best. In recommending that viscosity measurements be done routinely, we have suggested that vitreous from two eyes of the same donor be pooled. This provides from 3.0 to 4.0 cc. and makes enough available for culture and surgery after the amount needed for viscosimetry (0.75 cc.) has been removed.

In the same way that the dose and quality of every drug given should be precisely known, we are of the opinion that as much as possible should be known about the quality of vitreous to be implanted; there are probably other simple tests that could also be carried out to assist in this goal.