OX VITREOUS HUMOUR. 2.—HYALURONIC ACID RELATIONSHIPS*

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INTRODUCTION

This paper is a report of experiments bearing on the state of hyaluronic acid within the ox vitreous humour and the inter-relationships, both chemical and structural, between it and the soluble and insoluble proteins of the humour. Isolation of hyaluronic acid from ox vitreous humour by Meyer and Palmer (1934) naturally gave rise to the idea that this viscous polysaccharide is concerned with the maintenance of the vitreous body. Meyer and Palmer isolated it in a concentration of about 0.05 per cent. from ox vitreous humour filtrate and although Meyer, Smyth and Gallardo (1938) showed that the humours of other animals did not contain so much, yet it is obviously an important constituent of all that have been so far examined. In the ox vitreous humour there is more hyaluronic acid than soluble or insoluble protein. Table I gives values for the concentration of hyaluronic acid and the proteins of the vitreous humours of ox and man and demonstrates the great dilution in which they occur.

TABLE I.—COMPOSITION OF VITREOUS HUMOUR

Vitreous humour	Ox	Man	Ox	Man
	mg/100 ml.		mg/single humour	
Total nitrogen	22-24 ¹	23.5 ²	3.9-4.3	0.9
Soluble protein	39-43 ¹	20-40 ³	7-8	0.78-1.56
Residual protein	11-16 ³	—	2-3	—
Hyaluronic acid, calculated from hexosamine	41 ²	9.9 ²	7.5	0.38
.. .. .	—	11.5-19 ³	—	0.44-0.75

The volume of a single ox vitreous humour is taken as 18 ml.

The volume of a single human vitreous humour is taken as 3.9 ml. (Tab. Biol. 1947).

¹Krause (1934).

²Meyer, Smith and Gallardo (1938).

³Own results.

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The vitreous humour has been described in many different ways; histologists, physical chemists and ophthalmologists each have a different approach to the problem, which is basically to account for the stability of a structure which contains such low concentrations of its constituents. A study of the residual protein of the ox vitreous humour by Pirie, Schmidt and Waters (1948) led them to consider that the picture given by the histologists most nearly fitted their biochemical findings. According to this view, the vitreous humour is a tissue made up of a network of fibrils which are the prolongations of cells and this network is permeated by a viscous fluid, or jelly, which easily flows out of it. Pirie, Schmidt and Waters found that hydrolysis of the fibrillar protein by enzyme preparations containing collagenase caused liquefaction of the humour. This protein, however, occupies only a minute part of the total volume of the humour which is made up of a viscous solution or jelly of hyaluronic acid, soluble proteins derived from the blood and substances of small molecular weight.

Breakdown of this may also be expected to cause pathological changes in the humour. For example, it seems possible that disaggregation of the hyaluronic acid to a non-viscous form might also lead to disorganisation of the fibrillar network, perhaps with production of visible opacities, such as those seen in senile degenerations of the humour. It is well known that substances diffuse through the vitreous humour *in vivo* with extreme slowness. Haemorrhages remain localised for long periods and von Sallmann, Meyer and di Grandi (1944) found that penicillin injected intra-vitreally diffused through only one-third of the volume of the humour in 6 hours. This lack of spread may be due either to the way in which the fibrillar protein is laid down, or to the inhibiting effect on the diffusion of large molecules by the hyaluronic acid jelly. Probably both play some part and one might therefore expect that a change in the state of either would be reflected in changes, both in the state and in the behaviour of the vitreous humour. As a first step in the experimental consideration of these inter-relations, a study has been made of the state of hyaluronic acid in the normal ox vitreous humour and of the effect of hyaluronidase, the enzyme system that disaggregates and hydrolyses hyaluronic acid, on the behaviour of ox vitreous humour *in vitro*.

The state in which hyaluronic acid exists in the tissues and fluids of the body is not yet clear. Evidence derived from electrophoresis seems to show that it is not combined with protein at the pH of the body, but exists free, presumably as an ion. For example, Blix (1940) found that electrophoresis of filtered, dialysed and concentrated ox vitreous humour caused the hyaluronic acid

to migrate in the electric field faster than any of the proteins present. Meyer and Chaffee (1940) found that the hyaluronic acid of a viscous fluid obtained from a tumour behaved in the same way, yet many workers have been impressed by differences in physical properties between the native fluids of the body and hyaluronic acid solutions of comparable strength. For example, the viscosity of the tumour fluid studied by Meyer and Chaffee was far greater than the viscosity of solutions of hyaluronic acid of equal strength, perhaps showing that hyaluronic acid is combined with protein in the native fluid. On the other hand, it is possible that this difference in the viscosity of the native fluids and of solutions of hyaluronic acid isolated from them lies only in the different degree of aggregation of hyaluronic acid in the two situations.

Blix and Snellmann (1945) found that hyaluronic acid prepared from different sources had very different particle lengths, or degrees of molecular aggregation and that even hyaluronic acid from a single source might be polydisperse. For example, the material prepared from ox vitreous humour had particle lengths varying from 1,000 Å to 4,800 Å, judging from viscosity measurements. Hadidian and N. W. Pirie (1948) have recently isolated highly aggregated hyaluronic acid from umbilical cord with a relative viscosity greater than that of any previous preparations and they speak of hyaluronic acid as a family of substances differing slightly in architecture and greatly in particle size.

Consideration of the state of hyaluronic acid in the vitreous humour may, therefore, be directed to determine whether it is free or combined with protein and whether it is highly aggregated and, therefore, possibly immobile within the humour structure, or relatively disaggregated and freely diffusible. Meyer (1947) has suggested that hyaluronic acid is formed in the ciliary body and circulates in the vitreous humour, finally escaping into the aqueous humour, where it is disaggregated by hyaluronidase. Knowledge of the state and mobility of hyaluronic acid in the vitreous humour should help to evaluate these very interesting suggestions.

METHODS

State of aggregation of hyaluronic acid. Aggregated hyaluronic acid gives a fibrous precipitate with protein in dilute acid or acid acetone. Disaggregated hyaluronic acid gives either a flocculent precipitate or cloud under these conditions. This difference in precipitability has been used to estimate the disaggregating, or mucinase, effect of hyaluronidase and other systems by Robertson, Ropes and Bauer (1940). In the absence of protein, hyaluronic acid is not precipitated by dilute acid, but comes down with acetone. Aggregated hyaluronic acid gives a fibrous precipitate and disaggregated a flocculent one. I have used the change in type of precipitate with acid acetone as a qualitative test for the degree of aggregation of hyaluronic acid. The presence of 75 per cent. of the aggregated

form masks the presence of 25 per cent. of the disaggregated acid, but the test is sufficient to show any major disaggregation. The solution to be tested is acidified with dilute acetic acid and added to 2-3 vol. acetone. A fibrous clumped precipitate in a clear fluid is characteristic of aggregated hyaluronic acid, while a cloud or flocculent precipitate shows that the acid has been disaggregated.

Hexosamine was estimated by the method of Elson and Morgan (1933) and total nitrogen by micro-Kjeldahl followed by distillation and titration, using the apparatus described by Markham (1942). Total carbohydrate was estimated by the orcin colorimetric method described by N. W. Pirie (1936); this estimates the glucuronic acid but not the hexosamine part of the hyaluronic acid molecule.

MOBILITY OF HYALURONIC ACID THROUGH VITREOUS HUMOUR

Ox vitreous humour contains both proteins and hyaluronic acid in solution. Friedenwald and Stiehler (1934) found that all soluble organic material could be removed from a humour by prolonged washing in saline. It is, therefore, possible for hyaluronic acid to diffuse through and escape from the humour, but whether it does so combined with protein, or as an independent entity is not known. An attempt to answer this was made by analysing the material that stayed within the humour after varying times of washing. If protein and hyaluronic acid diffused out independently, then the composition of the material remaining should vary according to the time of washing.

Ox vitreous humours taken out of the eye $\frac{1}{2}$ —2 hours after death were suspended in water or saline for varying periods in the ice chest. The washing fluid was changed each day and finally the vitreous humour was filtered and the filtrate precipitated with acid acetone. The composition of this precipitate was compared with that from an ox vitreous humour filtered immediately after removal from the eye. In this way the rate of escape of protein and of hyaluronic acid could be roughly estimated. In some cases the composition of the precipitate from the washing fluid was also investigated. The first wash waters contained considerable salt and gave a flocculent precipitate with dilute acetic acid, indicating the presence of protein and hyaluronic acid which precipitate as a mucin compound in the dilute acid. The later wash waters contained no salt, no mucin and only traces of material precipitable by acetone. In contrast with this, the filtrates of fresh humours gave acetone precipitates with a carbohydrate content of 9—12 per cent., while filtrates of well-washed humours gave acetone precipitates with a carbohydrate content of 14—38 per cent. The acetone precipitate from the filtrate of a fresh humour after dialysis in a cellophane sac had a carbohydrate content of 14 per cent. This shows that the protein in the ox vitreous humour washes out more easily than the carbohydrate hyaluronic acid. This was further demonstrated by an experiment in which a single vitreous humour was suspended in water for

eight days, the water being changed each day. The wash waters were combined, concentrated and then added to acid acetone. The precipitate was centrifuged off, dried, weighed and its carbohydrate content was determined. On the eighth day the vitreous humour was filtered and the filtrate precipitated with acetone and the precipitate analysed in the usual way. The acetone precipitate from the collected wash waters weighed 17.8 mg. and contained 7.1 per cent. carbohydrate, while the acetone precipitate from the filtrate weighed 9.2 mg. and contained 20.4 per cent. carbohydrate. Table II gives the analysis of the acetone precipitates from the

TABLE II.—COMPOSITION OF ACETONE PRECIPITABLE MATERIAL FROM THE FILTRATE OF OX VITREOUS HUMOUR AFTER VARIOUS TIMES OF WASHING THE HUMOUR IN H₂O.

Washing time days	Wt. Precipitate from 1 humour mg.	Total N %	Total Carbohydrate (Glycuronic Acid — as glucose) %	Hexosamine %
0	19.8	—	10.1	—
0	20.7	—	9.2	—
0	—	6.7	12.6	11.3
0	—	6.5	—	7.6
3	15.9	—	14.6	—
9	6.5	—	14.6	—
14	>4.0	—	19.0	—
22	3.6	7.0	24.0	19.5
28	7.6	7.5	38.43	24.0
approx. 1 month	3.7	7.1	—	12.1
"	—	6.3	23.0	24.4
"	—	6.4	19.5	26.6
Dialysed fresh filtrate	15.9	7.9	14.6	16.6
Hyaluronic acid Meyer & Palmer (1936)	—	3.37	46.8	43.1

vitreous humours after various washing times and shows that there is a progressive increase in the carbohydrate content of the material remaining within the humour. The same progressive increase in the carbohydrate content of the relatively immobile material occurred when the vitreous humour was washed in 0.9 per cent. saline rather than in water.

The ash content of the acetone precipitate from fresh humours was 13—17.7 per cent. while that from a washed humour was 4.9 per cent. Calculation, on an ash free basis, of the protein and hyaluronic acid contents of these precipitates shows that the precipitate from a fresh humour has roughly 36 per cent. protein and 25 per cent. hyaluronic acid and the precipitate from a washed humour has 31 per cent. protein and 50 per cent. hyaluronic acid. This means that these experiments have not shown that protein and hyaluronic acid wash out quite independently of each other, but have demonstrated that they are relatively independent and that no compound similar to a mucin exists in the vitreous humour.

Two further points of interest arise from these experiments. The washing fluids from the vitreous humours always gave flocculent precipitates, either with acid alone or with acid acetone, whether the volume of the fluid was large or purposely kept small. This means that under the conditions of the experiment, aggregated hyaluronic acid does not diffuse out of the vitreous humour. All that diffuses is already disaggregated. The instability of aggregated hyaluronic acid is well known and Robertson, Ropes and Bauer (1940) showed that vitreous humour filtrate contained a disaggregating system, which they identified as ascorbic acid. It seems probable that gradual disaggregation of the hyaluronic acid goes on during washing. The material which stays within the washed humour and can be filtered out is always in the aggregated form giving a fibrous precipitate with acid acetone. This supports Meyer's idea of a circulation of hyaluronic acid in the eye with final escape through the aqueous humour of the disaggregated material.

A second point of interest is that the filtrate from a washed vitreous humour is not uniform in composition. After 7—10 days in repeated changes of distilled water, ox vitreous humour swells to 2—4 times its original size. Such swelling has already been described by Goedbloed (1935). I found that the filtrate from such swollen humours varied in composition, the first part, coming presumably from the outer part of the humour mass, being almost free from precipitable material and the last 5—10 ml. containing highly aggregated hyaluronic acid. This implies either that there is an internal structure of the vitreous humour, or that the jelly

of hyaluronic acid and protein is broken down gradually, the outer parts being washed away first. The very unstable character of the vitreous humour jelly makes this second explanation seem improbable and I think that this experimental finding is in favour of the view that there is an internal network which impedes to a certain extent the movement of large molecules.

INFLUENCE OF STRUCTURAL PROTEIN ON STATE OF HYALURONIC ACID

The action of hyaluronidase on the vitreous humour in the eye and excised from the eye and on the vitreous humour filtrate was studied in order to see whether the presence of the vitreous structure influenced in any way the action of the enzyme on the contained hyaluronic acid. The enzyme preparations were injected into the vitreous humour with a hypodermic syringe and the eyes, or the extracted humours or filtrates, were then incubated at 37° for varying times. Humours injected with saline were incubated to control the spontaneous disaggregation of hyaluronic acid. After incubation the vitreous humours were filtered directly into acid acetone in order to stop enzyme action and get immediate precipitation of mucin. The activity of the enzyme was judged by the type of precipitate obtained, a fibrous precipitate showing that the hyaluronic acid was still aggregated and a flocculent precipitate showing that the enzyme had disaggregated it. In some cases the insoluble residues from the humours were washed, dried and weighed in order to measure whether hyaluronidase had any effect on the residual protein.

Table III gives the results of these experiments and shows that in no case did hyaluronidase liquefy or have any grossly visible effect on the vitreous humour. Blix (1946) has already noted that hyaluronidase does not liquefy vitreous humour. Enzyme preparations that disaggregated hyaluronic acid instantly in the filtrate took a long time to disaggregate it when it was retained in the vitreous humour structure. The enzyme was slowed down even more when acting on the vitreous humour in the eye. This difference in the rate of action of hyaluronidase inside and outside the vitreous humour is probably due to the slow rate of diffusion of the enzyme through the vitreous body. If an injection of enzyme is made into the centre of an intact extracted humour contained in a small pot, the injected enzyme can be seen as a small bubble of different refractive index for several hours after injection. The vitreous humour structure prevents rapid diffusion of substances through it and in this way the structural protein influences the state of hyaluronic acid in the humour and tends to maintain it in the aggregated state.

TABLE III.—EFFECT OF HYALURONIDASE ON OX VITREOUS HUMOUR

Exp. No.	Vitreous Humour preparation	Enzyme prep : injected	Time at 37° hr.	Gross effect	Acetone ppt: from filtrate	Residue Wt : mg.
1	In ox eye	0.1 ml. <i>Cl. Welchii</i> preparation 500 v.r.u./ml.	1½	none	fibrous	—
	Freshly extracted	„	1½	none	flocculent	—
	Filtrate	„	none	none	flocculent	—
	In ox eye	none	1½	none	fibrous	—
	Freshly extracted	none	1½	none	fibrous	—
2	In ox eye	0.5 ml. of 1% Testis extract	15	none	fibrous	4.0
	Freshly extracted	„	15	none	flocculent	2.6
	In ox eye	none	15	none	fibrous	4.0
	Freshly extracted	none	15	none	fibrous	3.0
3	In ox eye	0.2 ml. Testis preparation 96 v.r.u./ml.	17	none	flocculent	3.0
	In ox eye	none	17	none	fibrous	3.9

One viscosity reducing unit (v.r.u.) of hyaluronidase is that concentration of enzyme that will reduce the viscosity of a standard substrate preparation to a level halfway between its original value and that of the solvent employed in 20 minutes, McClean (1942).

A further demonstration of this came from a study of another disaggregating system. Ascorbic acid, which is present in the normal vitreous humour in a concentration of 50 mg./100 ml. filtrate (Robertson, Ropes and Bauer, 1941), can disaggregate hyaluronic acid. This action is catalysed by copper, and addition of a small amount of copper to the filtrate of the ox vitreous humour caused immediate disaggregation of the hyaluronic acid. If, however, the same amount of copper was injected into the vitreous humour, no disaggregation occurred, even after 12–20 hours' incubation at 37°. This is probably because the copper combines with the structural protein and so becomes non-diffusible and inactive as a catalyst. Here again the presence of the structural protein has an influence on the state of hyaluronic acid.

INFLUENCE OF AGGREGATED HYALURONIC ACID ON DISSOLUTION OF
STRUCTURAL PROTEIN BY COLLAGENASE PREPARATIONS

Pirie, Schmidt and Waters (1948) showed that enzyme preparations from *Cl. Welchii* containing collagenase liquefied ox vitreous humour. They found that the liquefied humours always contained disaggregated hyaluronic acid. The enzyme preparations contained no active hyaluronidase, but were found to have 0.043 mg./ml. of Cu and this presumably acted as a catalyst for the disaggregation of hyaluronic acid by ascorbic acid in the liquefying humour. Table IV shows the disaggregating effect of the enzyme preparation and shows that disaggregation can be inhibited by addition of sodium diethyl dithiocarbamate which combines with copper and makes it catalytically inactive. Experiment 3 shows that the enzyme preparation does not disaggregate hyaluronic acid from umbilical cord unless ascorbic

TABLE IV.—DISAGGREGATION OF HYALURONIC ACID BY *C1Welchii*
COLLAGENASE PREPARATIONS

Expt. No.	Substrate	Saline added ml.	Enzyme added ml.	Time hr. 20°	Type of Acetone precipitate from filtrate
1	5 ml. ox vitreous humour filtrate	0.2	—	23	fibrous
	ditto	—	0.2	23	flocculent
2	2 ml. ox vitreous humour filtrate	0.1	—	5	fibrous
	ditto	—	0.1	5	flocculent
	ditto	—	0.1 + 0.05 ml. 0.5% sodium diethyl dithiocarbamate	5	fibrous
3	1.0 ml. 0.9% hyaluronic acid (umbilical cord)	—	0.05	3	fibrous
	1.0 ml. 0.9% hyaluronic acid (umbilical cord) + M/3000 ascorbic acid	—	0.05	3	flocculent
	1.0 ml. 0.9% hyaluronic acid (umbilical cord) + M/3000 ascorbic acid	—	—	3	fibrous

TABLE V.—EFFECT OF AGGREGATED HYALURONIC ACID ON COLLAGENASE ACTIVITY

The enzymes or other solutions were injected into the vitreous humours which were incubated overnight at 37° in the presence of toluene in small glass pots. 0.2 ml. 0.5 % sodium diethyl dithiocarbamate was used in experiments 1 and 2 and 0.3 ml. 1% in experiment 3. 0.3 ml. or 0.4 ml. collagenase containing 22 Q units of activity/ml. was used and 0.2 ml. testicular hyaluronidase containing 96 viscosity reducing units/ml.

Addition to vitreous humour	Gross effect	Type of acetone precipitate from filtrate	Weight of residue after filtration mg.
Saline	none	flocculent	3.6
Saline plus dithiocarbamate	none	fibrous	3.2
Collagenase ...	fluid	flocculent	none
Collagenase plus dithiocarbamate	mainly solid	fibrous	2.1
Saline	none	fibrous	3.8
Saline plus dithiocarbamate	none	fibrous	3.4
Collagenase ...	half fluid	flocculent	2.2
Collagenase plus dithiocarbamate	quarter fluid	fibrous	2.6
Saline plus dithiocarbamate	none	fibrous	1.5
Collagenase ...	part liquid	part fibrous	2.0 (very fragile)
Collagenase plus dithiocarbamate	solid	fibrous	1.8
Collagenase plus dithiocarbamate plus hyaluronidase	part liquid	flocculent	1.8 (very fragile)
Hyaluronidase ...	solid	flocculent	3.0
Hyaluronidase plus dithiocarbamate	solid	flocculent	3.0

acid is added. Both copper and ascorbic acid must be present for disaggregation to take place.

Mixture of diethyl dithiocarbamate with the collagenase preparation before injection into the vitreous humour slowed down

the liquefaction of the humour and the digestion of the insoluble protein. This effect is not due to inhibition of collagenase by the dithiocarbamate as corneal collagen is dissolved by collagenase in its presence. It seemed probable that dithiocarbamate prevented disaggregation of hyaluronic acid in the humour, and that aggregated hyaluronic acid prevented the spread of the collagenase through the vitreous humour mass, and so slowed down liquefaction.

I tried to verify this assumption by determining whether the inhibition of liquefaction by dithiocarbamate could be nullified by hyaluronidase. The experimental results were not entirely clear-cut, but showed that on the whole the addition of hyaluronidase to a mixture of collagenase and dithiocarbamate increased the liquefaction of the humour. In these experiments it was difficult to adjust the time of incubation so that no spontaneous disaggregation took place and yet the added hyaluronidase had sufficient time to disaggregate all the hyaluronic acid throughout the humour. The earlier series of experiments with hyaluronidase (Table III) had shown that the enzyme only acted slowly in the intact humour. Table V gives the results of three typical experiments, showing the inhibitory effect of dithiocarbamate on liquefaction and the effect of hyaluronidase on this. The last column records the weights of the insoluble residues from the humours after complete filtration. These residues were washed by suspension in water overnight whenever this was feasible, but in some cases they were so flimsy that this could not be done, and they were dried without washing, which means that the dry weight is probably too big.

DISCUSSION

The results of the experiments reported in this paper show that the soluble proteins wash out of the vitreous humour more easily than the hyaluronic acid, but that hyaluronic acid cannot be completely separated from protein in this way. The experiments do not therefore rule out the possibility that a compound of hyaluronic acid and protein occurs, but suggest that, if this is so, it is unlike the mucin type of compound obtainable by co-precipitation of hyaluronic acid and protein in dilute acid, where the hyaluronic acid forms a small part of the molecule.

Although hyaluronic acid and the insoluble collagen-like protein of the vitreous humour do not seem to have any chemical relation in the humour, yet they do influence each other. Aggregated hyaluronic acid has a very large particle size, and the hyaluronic acid-containing jelly of the vitreous humour may be considered as

an unstable structure that inter-acts with the structure of the insoluble protein. Liquefaction of the insoluble protein is slowed down by the presence of aggregated hyaluronic acid, and disaggregation of hyaluronic acid is slowed down by the presence of the insoluble protein. Each structure tends to preserve the other, and it seems possible that pathological changes in one might also lead to change in the other.

These experiments have been made on ox vitreous humour only. Meyer, Smyth and Gallardo (1938) have shown that the amount of hyaluronic acid is 3—4 times greater in the ox than it is in other animals. Von Sallmann (1948) has found that injection of hyaluronidase into the rabbit's eye causes partial liquefaction of the vitreous humour, and that the spread of injected haemoglobin in rabbit vitreous humour is accelerated by hyaluronidase. There are therefore big species-differences in the structure and behaviour of the vitreous humour, but it is likely that, even in species that have less hyaluronic acid in their vitreous humour than the ox, one must take into account the structural inter-actions between the collagen-like insoluble protein and the hyaluronic acid jelly when considering the biological behaviour of the vitreous body.

SUMMARY

1. Suspension of ox vitreous humour in water washes out protein and hyaluronic acid at different rates, and the analysis of the material remaining within the humour shows that there is no compound comparable to a mucin present in the humour.
2. Hyaluronidase acts more slowly within the vitreous humour than in the vitreous humour filtrate.
3. Collagenase preparations are inhibited by aggregated hyaluronic acid in the vitreous humour.
4. The conclusion is drawn that the presence of the insoluble structural protein of the humour may help to maintain aggregated hyaluronic acid, and that aggregated hyaluronic acid may help to maintain the structural protein.

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LEIOMYOMA OF THE IRIS*

Report of a Case

BY

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THE clinical and pathological diagnosis of tumours of the iris presents many difficulties. Because of their rarity it falls to the lot of few observers to have more than a passing acquaintance with them. Fear of their possible malignancy warps the judgment of the surgeon, and neither the clinician nor the pathologist approaches the problem with an unbiassed mind.

In 1943 Kahler, Wallace, Irvine and Irvine reported "the seventh case of leiomyoma of the iris." Following the lead of Verhoeff, several reported myomata of the iris and ciliary body were rejected because of insufficient histological proof. On the other hand, it is not unlikely that many cases have been wrongly labelled leuco-sarcoma (unpigmented melanoma) because their real nature was not apparent with the usual haematoxylin and eosin stain. Van Gieson's stain and Mallory's connective-tissue stain show that the tumour cells take the stain characteristic of

* This case was presented at a meeting of the clinical society, St. Vincent's Hospital, Melbourne, Victoria, Australia, on March 22, 1948. Received for publication, October 25, 1948

Some months ago I forwarded to you a report on a case of leiomyoma of the iris. Just recently, I have seen a report by E. C. Moulton, Senr. and E. C. Moulton, Jr. from Fort Smith, Arkansas, in the *American Journal of Ophthalmology* for February, 1948 (31, 214) in which they report a similar case. They mention the presence of cataract, and make a suggestion similar to mine, that the growth appears in the region of the foetal fissure. I was unaware of their article when I sent my report to you.