REFERENCES

1. Tsamparlakis, J. C.: Effects of transient induced elevation of the intraocular pressure on the visual field, Br. J. Ophthalmol. **48**: 237-249, 1964.
2. Gafner, F., and Goldmann, K.: Experimentelle Untersuchungen über den Zusammenhang von Augendrucksteigerung und Gesichtsfeldschädigung, Ophthalmologica **130**: 357-377, 1955.
3. Drance, S. M.: Studies in the susceptibility of the eye to raised intraocular pressure, Arch. Ophthalmol. **68**: 478-485, 1962.
4. Samojloff, A. J.: Weitere Untersuchungen über die Skotombildung, Zschr. Augenheilk. **58**: 282, 1925.
5. Bietti, G.: Di un nuovo "test" per la diagnosi precoce del glaucomo, Acta Sixteenth Congress Ophthalmology, London **2**: 895, 1950.
6. Lynn, J. R.: Modifications du champ visuel par l'augmentation experimentale aigue de la pression intra-oculaire et intra-orbitaire, *in*: ERC et Champ Visuel, Marseille, 1962, Maison Chibert pp. 317-318.
7. Jaeger, E. A., Weeks, S. D., and Duane, T. D.: Perimetric and visual acuity changes during ophthalmodynamometry, Arch. Ophthalmol. **71**: 484, 1964.
8. Bietti, G.: Effects of experimentally decreased or increased oxygen supply in some ophthalmic diseases, Arch. Ophthalmol. **49**: 491, 1953.
9. Vanderburg, D., and Drance, S. M.: Studies on the effects of artificially raised intraocular pressure: on retinal differential thresholds of the Bjerrum area, Am. J. Ophthalmol. **62**: 1048, 1966.
10. Phelps, C. D.: The pathogenesis of optic nerve damage in glaucoma: A review of the vascular hypothesis, *In*: Current Concepts in Ophthalmology, Blodi, F. C., editor. St. Louis, 1972, The C. V. Mosby Company.

Lymphocyte-induced vitreous membranes: a comparative study with leukocyte- and platelet-induced vitreous membranes.

META A. MEGUSAR, KWOK-WAI LAM, FELIPE I. TOLENTINO, AND HSIAO-SU LIU.

Vitreous membranes were induced in rabbit eyes by injecting an autogenous lymphocyte preparation. These membranes were compared with membranes induced by autogenous leukocyte and platelet preparations. Lymphocytes gave rise to faint, nonprogressive membranes, while leukocytes and platelets produced dense, long-standing membranes. It is suggested that lymphocytes may not be the cause of this weak response, as other cells in the preparation may be involved.

In 1966, Freilich, Lee, and Freeman[1] injected autogenous whole blood into rabbit eyes. Membranes appeared in all eyes receiving two blood injections spaced at an interval of six weeks, and in five of 24 eyes receiving only one injection. Vitreous membranes have also been produced by the injection of partially purified blood components. Lam, Ashrafzadeh, and Lee[3] produced dense vitreous membranes one week after the injection of leukocytes. Constable and co-workers[4] induced membranes with platelet-rich plasma. Red blood cells may also have a role in the formation of vitreous membrane.[2]

The present study reports the production of vitreous membranes with a lymphocyte preparation and compares them with membranes produced with leukocyte and platelet preparations.

Preparation of cells. Leukocytes, lymphocytes, and platelets were prepared from autogenous blood as described previously.[4, 5] There was less than 1 per cent red cell contamination and no platelet contamination in the leukocyte preparation. The lymphocyte preparation consisted of 90 per cent small lymphocytes. Cell concentrations, measured by a hemocytometer count of at least 400 cells per sample, were adjusted to correspond to normal blood: 1×10^6 cells per milliliter for lymphocytes and 1×10^9 cells per milliliter for platelets, suspended in saline.

Injection of cells. Adult pigmented and albino rabbits were anesthetized with intravenous sodium pentobarbital (30 mg. per kilogram). The fundus was examined with the indirect ophthalmoscope. Using aseptic techniques, 0.1 ml. of the cell preparation was injected with a tuberculin syringe with a 27-gauge needle into the vitreous cavity through the pars plana ciliaris 4 mm. behind the limbus. The injection was located in the central vitreous by guiding the needle tip with indirect ophthalmoscopy, which was also used to observe the fundus after the injection.

A total of 63 eyes were injected: 36 with lymphocytes, 13 with platelets, and 14 with leukocytes. Fewer leukocyte and platelet injections were performed, since they have been studied previously[3, 4] and were only intended for comparative purposes. Normal saline was injected into the vitreous cavity of ten eyes as a control.

Examination. Follow-up examination of the injected eyes was performed daily during the first week after injection and then at weekly intervals for eight months (263 days). Examination included biomicroscopy and indirect ophthalmoscopy. Visible vitreous membranes were photographed with a portable Kowa fundus camera.

Histopathology. All eyes were eventually enucleated at different time intervals and the membranes removed. The eyes were washed thoroughly with water to avoid contamination by blood. The globe was sectioned at the equator with a sharp razor blade and scissors. The exposed vitreous was searched for membranes with a

Table I. Density of lymphocyte-induced membranes (30 eyes)*

Interval (in days)	*None*	*Slight*	*Moderate*	*Severe*	*Total*
Immediate	15	15	0	0	30
1-2	1	24	5	0	30
3-10	0	13	11	0	24
11-20	0	6	10	0	16
21-40	0	4	8	0	12
41-60	0	1	5	0	6
61-80	0	4	2	0	6
81-100	2	1	2	0	5
101-140	2	2	1	0	5
141-180	2	2	1	0	5
181-263	2	0	0	0	2

Since a certain number of animals were killed for histopathologic study at each interval of observation, the total number of eyes decreased as the observation period lengthened.
*An additional six eyes were injected, but were withdrawn due to complications (see text).

Table II. Density of leukocyte-induced membranes (14 eyes)

Interval (in days)	*None*	*Slight*	*Moderate*	*Severe*	*Total*
Immediate	3	8	3	0	14
1-2	0	0	14	0	14
3-10	0	0	2	10	12
11-20	0	0	3	5	8
21-40	0	0	3	0	3
41-60	0	0	3	0	3
61-80	0	0	3	0	3
81-100	0	0	3	0	3
101-140	0	0	3	0	3
141-180	0	0	3	0	3

Since a certain number of animals were killed for histopathologic study at each interval of observation, the total number of eyes decreased as the observation period lengthened.

Zeiss operating microscope. Membranes were removed with fine forceps, mounted on glass slides, and stained with Wright's solution. Excess stain was washed away gently with water.

Results. All eyes injected eventually developed membranes. Six lymphocyte-injected eyes were withdrawn from the study, three because of traumatic cataract and three because of an infection.

The observation period was arbitrarily divided into eleven intervals. The membranes were graded according to their density (none, slight, moderate, and severe) as observed with the indirect ophthalmoscope and biomicroscope. Very mild vitreous flare was present in all eyes for the first few days after injection. Some eyes developed pigmented opacities at various time intervals in the central vitreous in the vicinity of the membranes. These opacities were more common in the lymphocyte- and platetlet-injected eyes than in leukocyte-injected eyes. As a rule, the vitreous gel around the membranes was clear. The controls showed none of these changes.

Ophthalmoscopic and biomicroscopic observations. LYMPHOCYTE-INDUCED MEMBRANES (TABLE I). One half of the eyes immediately developed a slightly dense membranous opacity around the injected lymphocytic preparation. After the second day, most eyes developed slight to moderate membranes, which were fine, mistlike, and whitish. Their density increased during the first week and then stabilized. After three months, these membranes grew very faint, and after six months they disappeared completely. In no case was their intensity comparable to membranes induced by leukocytes or platelets. Due to this fine appearance, photographs were often difficult to obtain. Similar difficulties were encountered in isolating and microscopically processing these membranes.

LEUKOCYTE-INDUCED MEMBRANES (TABLE II). The leukocyte preparation immediately produced slightly to moderately dense membranes in about three-fourths of the eyes. All eyes developed moderately dense membranes after the second day. The membranes were whitish and had processes projecting into the vitreous gel. After the first week, the density of the membranes in most eyes were graded as severe. It became moderate after one month and remained unchanged for the rest of the observation period.

PLATELET-INDUCED MEMBRANES (TABLE III). The injection of platelets immediately caused membrane formation in all eyes. The membranes were whitish and dense. By the end of the first week, the membranes had increased in density. Opaque finger-like processes were observed along the border of the membrane, giving it a

Table III. Density of platelet-induced membranes (13 eyes)

Interval (in days)	*None*	*Slight*	*Moderate*	*Severe*	*Total*
Immediate	0	12	1	0	13
1-2	0	1	12	0	13
3-10	0	1	2	7	10
11-20	0	0	2	7	9
21-40	0	0	0	6	6
41-60	0	0	0	4	4
61-80	0	0	2	1	3
81-100	0	0	1	1	2
101-140	0	0	1	1	2
141-180	0	0	1	1	2
181-182	0	0	1	1	2

Since a certain number of animals were killed for histopathologic study at each interval of observation, the total number of eyes decreased as the observation period lengthened.

sunburst appearance. The membrane density increased for four to six weeks and remained stable for the remainder of the observation period.

Histopathologic observations. LYMPHOCYTE-INDUCED MEMBRANES. Four days after injection, the predominant cells in the lymphocyte-induced membranes were lymphocytes, but macrophages and granulocytes were also observed. The number of cells was greatly reduced 45 days after injection.

LEUKOCYTE-INDUCED MEMBRANES. Leukocyte-induced membranes contained granulocytes, macrophages, and lymphocytes. Four days after injection, granulocytes were predominant. However, macrophages became predominant 12 days after injection.

PLATELET-INDUCED MEMBRANES. For the first three days, the platelet-induced membranes resembled an aggregation of platelets, but after one week, the platelet structure disappeared. Numerous granulocytes and macrophages appeared and persisted after 185 days.

Discussion. Recent attempts to specify the blood component responsible for the pathogenesis of vitreous membranes reveal the complexity of the problem. It is clear that different types of isolated blood cells can cause the formation of a vitreous membrane.

The lymphocyte preparation produced the weakest vitreous membranes. Lymphocyte-induced membranes were faint, did not become progressively denser, and did not fan out into strands. The leukocyte preparation induced a denser membrane which continued to progress for three weeks after injection, while the platelet preparation produced a very dense membrane which formed immediately.

Since the lymphocyte preparations were only 90 per cent pure (the best purification possible) and contained approximately 10 per cent other cells, the faint membrane formation observed might be explained in one of two ways: either lymphocytes do indeed precipitate a mild degree of membrane, or the formation observed is due to the contamination by the other cells. However, it is also possible that the lymphocytes interact in some way with the other cells present and both are responsible for membrane formation. The origin of the macrophages and granulocytes observed in the membrane remains unclear. These cells could be among the cells originally injected, or they could originate from the ocular vessels in response to the trauma of injection. The possibility that they are transformed from lymphocytes,[7] leukocytes, or hyalocytes also cannot be ruled out.

The relatively rapid formation and density of membranes caused by platelets is probably due to direct interaction with vitreous protein, similar to the mechanism of the collagen-platelet reaction.[8] We did, however, observe some macrophages and granulocytes in long-standing platelet-induced membranes. This appears to contradict the assumption that macrophages and granulocytes are transformed lymphocytes, since our platelet preparation contained no lymphocytes. Moreover, since the platelet-induced membrane was long-standing, it could have stimulated the migration of phagocytic cells into the vitreous gel from retinal vessels. The trauma of injection must also be considered as a possible mechanico-chemical causative factor, although no effects were observed in the saline-injected controls.

The findings of our animal experiments suggest that in clinical situations a vitreous membrane could arise from one or more factors. Future investigations identifying the cells and the chemical constituents of membranous materials in the human eye will contribute to an understanding of the pathogenesis of human vitreous membranes.

The authors wish to thank Grace Romano for technical assistance. Editorial assistance was provided by David F. Dobies and Aaron Seidman.

From the Department of Retina Research, Eye Research Institute of Retina Foundation, Boston, Mass. 02114. This work was supported by the

John A. Hartford Foundation, Inc. of New York, Public Health Service Research Grant EY-00227 from the National Eye Institute, National Institutes of Health, and by a Man Power Award from Research to Prevent Blindness, Inc. Submitted for publication Sept. 3, 1974. Reprint requests: Editorial Services Unit, Eye Research Institute of Retina Foundation, 20 Staniford St., Boston, Mass. 02114.

Key words: lymphocytes, leukocytes, platelets, vitreous membranes.

REFERENCES

1. Freilich, D. B., Lee, P. F., and Freeman, H. M.: Experimental retinal detachment, Arch. Ophthalmol. **76**: 432, 1966.
2. Regnault, F. R.: Vitreous hemorrhage: An experimental study. I. A macroscopic and isotopic study of the evolution of whole blood and hemoglobin, Arch. Ophthalmol. **83**: 458, 1970.
3. Lam, K. W., Ashrafzadeh, M. T., and Lee, C. B.: Vitreous membranes; induction in rabbits by intravitreous leukocyte injections, Arch. Ophthalmol. **88**: 665, 1972.
4. Constable, I. J., Oguri, M., Chesney, C. M., et al.: Platelet-induced vitreous membrane formation, INVEST. OPHTHALMOL. **12**: 680, 1973.
5. Li, C. Y., Yam, L. T., and Lam, K. W.: Studies of acid phosphatase isoenzymes in human leukocytes demonstration of isoenzyme cell specificity, J. Histochem. Cytochem. **18**: 901, 1970.
6. Yam, L. T., Castoldi, G. L., and Mitus, W. J.: Quantitative evaluation of phytohemagglutinin-stimulated lymphocyte cultures, J. Lab. Clin. Med. **70**: 699, 1967.
7. Elves, M. W.: The Lymphocytes. Chicago, 1972, Year Book Medical Publishers, Inc.
8. Hovig, T.: Aggregation of rabbit blood platelet produced in vitro by saline extract of tendons, Thromb. Diath. Haemorrh. **9**: 248, 1963.

Experimental serous and hemorrhagic uveal edema associated with retinal detachment surgery. THOMAS M. AABERG.

A study of the effects of hypotony, cryotherapy as the adhesive modality and vortex system obstruction in repair of experimental retinal detachment in the owl monkey is reported. The presence of serous uveal edema (detachment) correlated mainly with the degree of vortex obstruction. Hemorrhagic extravasation appeared to be a more exaggerated manifestation of such vascular stasis.

Serous or hemorrhagic accumulation of fluid in choroidal tissue and the suprachoroidea, most commonly known as choroidal edema in Europe or choroidal detachments in the United States, has been said to occur routinely in retinal detachment surgery. Some authors believe the expansion of the suprachoroidea occurs to some degree after all retinal detachment operations.[1] Serous choroidal edema appeared clinically on the first or second postoperative day in 23 per cent of the series reviewed by Hawkins and Schepens.[2] Actual hemorrhage into the choroid and suprachoroidea occurred most commonly at the time of drainage of subretinal fluid, occurring in one per cent of cases.

In an experimental study on squirrel monkeys, Hawkins and Schepens[2] showed the combination of scleral diathermy, vitreous aspiration, and destruction of two vortex veins produced serous choroidal detachment. Diathermy and vitreous aspiration alone also produced suprachoroidal edema but to a lesser degree. These animals did not have retinal detachments, however, and thus did not correspond to the human situation.

Hayreh and Baines[3] produced uveal venous stasis ischemia in rhesus monkeys by selective cauterization of vortex veins. Occlusion of three or all vortex veins caused eventual anterior segment ischemia and atrophy.

The present study was undertaken to study the effects in retinal detachment surgery of hypotony, cryotherapy as the adhesive modality, and obstruction to the vortex system to determine the importance of each factor when combined with scleral buckling techniques, using a model mimicking the human retinal detachment situation.

Methods. Rhegmatogenous retinal detachments were produced in owl monkeys by the technique of Machemer and Norton.[4] Four weeks after production of the total retinal detachment, retinal surgery was performed utilizing either a segmental meridional buckle, without obstruction to a vortex ampulla, an encircling buckle placed over an anterior tear with minimal vortex obstruction, a posterior encircling buckle compromising all vortex flow, or insufflation of the vitreous using air and no scleral buckle, the latter providing virtually no obstruction to vortex supply. Cryotherapy was constant in amount and extent, treating only the tear with lesions placed encircling the hole. In each of the surgical categories, the eye was left in a hypotonous state at the end of surgery to give the maximal chance for uveal edema. If, on histologic examination, uveal edema was found to be present, a similar series of animals in that category was investigated with care taken to avoid hypotony during or after the operation. Therefore, only surgical categories in which uveal edema resulted with hypotony had further investigation regarding uveal edema without hypotony. This was done in order to keep the experiment to a workable number of animals. No attempt was made to judge the effect of hypotony or cryotherapy alone since the ex-