0273-2289/96/6101-02—0129$07.50

Regulating Aspects of Biosoluble and Insoluble Film Release Systems Containing Protein Proteinase Inhibitor

NADEZHDA G. BALABUSHEVITCH,[1] NATALIA R. KILDEYEVA,[2] NATALIA A. MOROZ,[1] SVETLANA P. TRUSOVA,[2] ALEXANDER D. VIRNIK,[2] GENNADY L. KHROMOV,[3] AND NATALIA I. LARIONOVA*,[1]

[1]Department of Chemistry, M. V. Lomonosov Moscow State University, 119899, Moscow, Russia; [2]Moscow State Textile Academy, 117918, Moscow, Russia; and [3]Scientific Research Institute for Medical Engineering, 129301, Moscow, Russia

ABSTRACT

New types of potent aprotinin-containing medicinal polymer films are elaborated for topical applications. The higher the temperature of drying, the lower the steam absorption, the film swelling, and the velocity of aprotinin in vitro release. The presence of antimicrobials having the basic functional groups contributed to compacting the structure of the films and retention of aprotinin in them. The velocity of aprotinin release from the films is regulated by adding different polymers. Inclusion of polyvinylpirrolidone in the film resulted in acceleration and increase of aprotinin release. This was probably because of increasing the film swelling by a factor of 1.7. Additional retention of the inhibitor in films was achieved by inclusion of sodium alginate and cellulose powder capable of binding aprotinin. Soluble bioadhesive films derived from a copolymer of acrylamide, *N*-vinylpirrolidone, ethyl acrylate (M_r 30,000–600,000) and aprotinin were obtained and analyzed. Kinetics of aprotinin release from biosoluble films was studied under various conditions. The duration of aprotinin release was comparable with duration of gradual dissolution of the matrix. Bioavailability of aprotinin from soluble films was linear with time.

Index Entries: Aprotinin; protease; inhibitor; film; polymer; bioadhesive; release.

*Author to whom all correspondence and reprint requests should be addressed.

INTRODUCTION

Polyvalent proteinase inhibitor from bovine organs (aprotinin) effectively inhibits trypsin, chymotrypsin, plasmin, plasminogen activators, blood coagulation factors, tissue and serum kallikreins, and so forth (*1*). Because of its wide specificity, aprotinin has long been employed for the therapy of some diseases caused by unbalance of proteolytic systems (*2*). The main drawback of aprotinin is a short life-time (7–10 min depending on animal species and the dose) (*3,4*).

The immobilization of physiologically active substances on/in biocompatible carriers is known to be one of the approaches in the development of drug release systems (*5*). The goal of this study was to elaborate new types of potent aprotinin-containing preparations for topical applications. Modern dressings for wound healing must be atraumatic and easy to put into practice. They must accelerate regeneration of living tissues, suppress microorganism growth, provide a controlled drug release, and protect the wound from external action.

We have attempted to use multicomponent polymer compositions for creation of more advanced medicinal forms of aprotinin based on insoluble and bioadhesive soluble films.

MATERIALS AND METHODS

Materials

Trypsin was obtained from Merck; the content of active sites in the preparation was 62% as determined according to Chase and Shaw (*6*); *N*-benzoyl-L-arginine ethyl ester was a Sigma reagent; poly(vinyl alcohol) M_r 60,000 Dalton,- and polyvinylpirrolidone M_r 150,000 Dalton were obtained from Plastpolymer, Inc., Russia; powder cellulose was obtained from Baykalsky integrated pulp-and-paper mill, Russia; sodium alginate was purchased from Integrated Alga plant, Arkhangelsk, Russia; gentamicinsulfate was from Ferane, Inc. (Russia); chlorohexidin bigluconate was from Lekarstvo, Inc. (Russia); Sephadex G-50 sf was purchased from LKB-Pharmacia (Sweden); TSK-Gel Toyopearl HW-50 was obtained from Toyo Soda MFG (Japan); aprotinin and a highly purified pharmaceutical preparation—inhiprolum (Russia) from bovine pancreas (activity 5000 KIE/mg) were also used.

Preparation of Films Containing Aprotinin

The insoluble films were prepared from the polymer composition containing 10% poly(vinyl alcohol), 0.05% antimicrobial (gentamicinsulfate or chlorohexidin bigluconate), and 0.05% aprotinin. The biosoluble

films were prepared from the polymer composition containing copolymer of acrylamide, vinylpirrolidone, ethyl acrylate, and polyethylenglycol used as plasticizer and aprotinin. Copolymer:polyethylenglycole:aprotinin ratio was 170:1:1 (w/w). The mixture was thoroughly stirred. To remove bubbles of air, the compositions were centrifuged. The films were obtained by passing the compositions through a slit spinneret. The drying temperature of films was 20, 40, 60, and 80°C.

Measurement of Maximum Steam Absorption and Film Swelling

The maximum steam absorption by poly(vinyl alcohol) films was measured on a vacuum setup with the Mc Ben balance. The maximum film swelling was determined as a ratio (w/w) of swollen and dried film expressed in %.

Determination of the Activity of Aprotinin in Solution and in Films

To assay the activity of aprotinin in solution, a 10–100 μL aliquot was added to 790–700 μL 0.05*M* Tris-HCl buffer, pH 8.0, with 0.02*M* $CaCl_2$ containing 2 μg trypsin, and the mixture was incubated for 10 min. Residual trypsin activity was assayed according to Schwert and Takenaka (*7*) by adding 200 μL 1.5 m*M* solution of *N*-benzoyl-L-arginine ethyl ester in the same buffer. The concentration of active aprotinin was calculated from the equivalent concentration of active trypsin. To assay the initial inhibitory activity of poly(vinyl alcohol) films, 10 mg of film were swollen in 1 mL of 0.05 Tris-HCl buffer, pH 8.0, with 0.02*M* $CaCl_2$ for 1 h followed by addition of 50 μL trypsin solution (1 mg/mL). The mixture was shaken for 30 min. Then 100 μL of the mixture were added to 900 μL 0.3 m*M* solution of *N*-benzoyl-L-arginine ethyl ester in the same buffer. The concentration of active aprotinin was calculated from the equivalent concentration of active trypsin and expressed as a percentage of included active aprotinin. The residual inhibitory activity of poly(vinyl alcohol) films was determined as follows: 10 mg of film were soaked in 0.1 mL of water or 1 mL of the physiological solution for 24 h. The film was then dried with filter paper and placed in 1 mL 0.05*M* Tris-HCl buffer, pH 8.0, with 0.02*M* $CaCl_2$ containing (10–20 μg) trypsin. The following procedure was the same as above.

Study of In Vitro Release of Aprotinin

In vitro release of aprotinin was evaluated by soaking the films in water with or without shaking under various water:film (w/w) ratios (M) and following the assay of aprotinin activity in solution.

Table 1
Influence of Ingredients of the Polymer Composition and the Drying Temperature on the Maximum Steam Absorption, Maximum Film Swelling, and Inhibitory Activity of Aprotinin-Containing Poly(vinyl alcohol) Films

			Inhibitory activity of film	
			Initial	After contact with water, M=10, 24 h
Antimicrobials	Maximum steam absorption, %	Swelling max, %	% of included aprotinin	
Drying temperature 20°C				
	31	660	90	6
GMS[a]	29	620	89	6
CHB[a]		620	97	7
Drying temperature 40°C				
		420	90	14
GMS[a]	28	420	77	19
CHB[a]		400	97	20

[a]GMS—Gentamicinsulfate; CHB—Clorohexidin bigluconate.

RESULTS AND DISCUSSION

Insoluble Films Released Aprotinin

The results in Table 1 show the drying temperature to be the main factor influencing the structure and properties of poly(vinyl alcohol) films. The films become more compact; the higher the drying temperature, the lower the steam absorption and the film swelling.

The composition and preparation routine of multicomponent films should affect the activity of immobilized aprotinin and pharmacodynamics of wound dressing. The inclusion of aprotinin in poly(vinyl alcohol) films caused no appreciable loss of its inhibitory activity (Table 1). Figure 1 demonstrates that the velocity of in vitro release of aprotinin decreased threefold when the drying temperature increased from 20–60°C.

In addition to the drying temperature, the presence of antimicrobials having the basic functional groups contributed to compacting the structure of the films. It resulted in obtaining the materials that retained up to 20% of the activity after their contact with water at M = 10 for 1 d (Table 1). These conditions simulate the wound treatment with wound dressings (*8*).

The rate of aprotinin release from the films can be regulated by adding different polymers (water-soluble polyvinylpirrolidone, sodium alginate, and insoluble cellulose) to the composition.

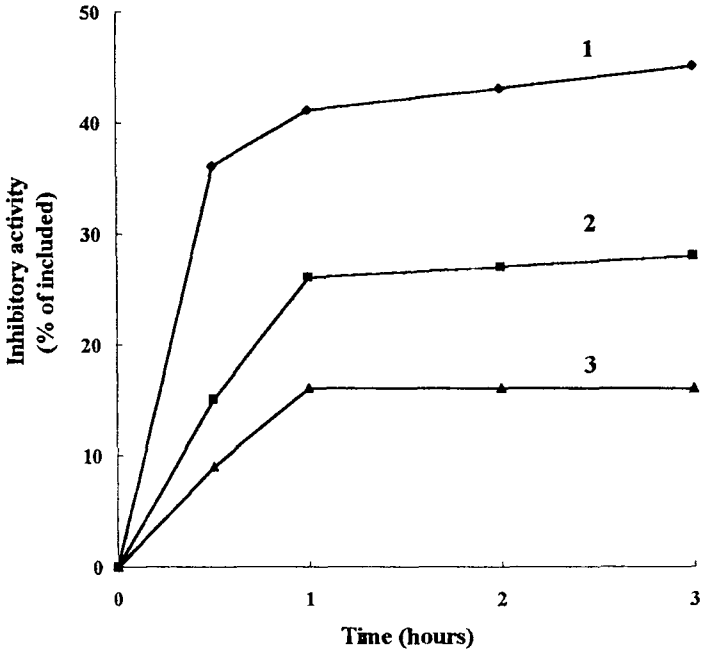

Fig. 1. In vitro release of aprotinin from poly(vinyl alcohol) films. The drying temperature: 20 (1), 40 (2], and 60°C (3). 100 mg of film were shaken in 30 mL of water (M = 300). The aliquots of solution were taken at selected time intervals. The activity of aprotinin in solution was determined as described in Materials and Methods.

Figure 2 shows that inclusion of 1% polyvinylpirrolidone, M_r 150,000 Dalton, to poly(vinyl alcohol) (w/w) accelerates and increases the aprotinin in vitro release from the films dried at 60°C. This occurs because of increasing the film swelling by a factor of 1.7 on addition of polyvinylpirrolidone.

The opposite effect was reached by inclusion of components capable of binding aprotinin. Sodium alginate, which is known to accelerate the wound healing, was chosen as such a polymer component. Appearance of high-molecular-mass active peak as well as the native aprotinin was proven by gel filtration of the polymer composition containing 0.1% sodium alginate (Fig. 3). The aprotinin release from the alginate-containing films was retarded fourfold compared to the film without additives (Fig. 2). The release of the inhibitor can be increased twofold by treating the films with 1*M* NaCl, which destroys polyelectrolyte complex between the carboxyl-containing polysaccharide and basic aprotinin.

In addition to soluble polymers, cellulose powder with a high adsorptive capacity was investigated as an additive influencing the aprotinin release from the poly(vinyl alcohol) films. Its effect on the aprotinin activity is shown in Fig. 4. Adding up to 1% cellulose results in a sixfold decrease in the aprotinin release. This may be because of the sorption of

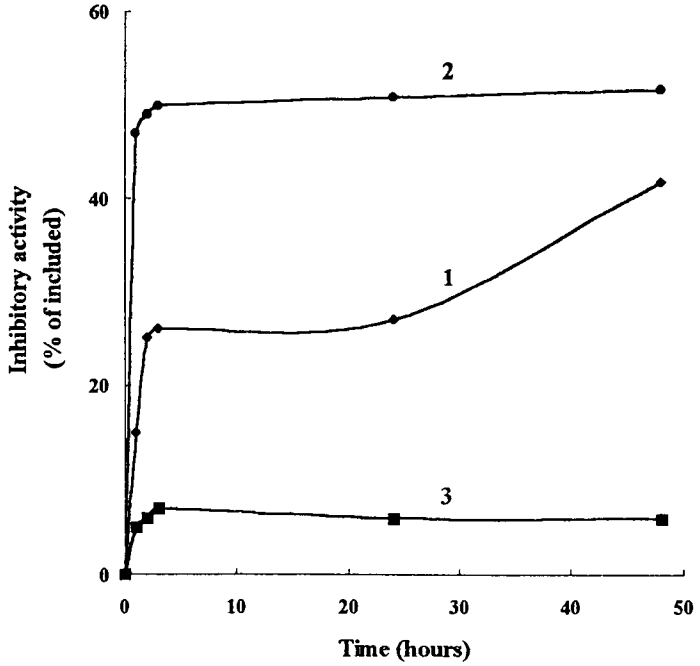

Fig. 2. Effect of soluble polymers in the composition on the aprotinin release from poly(vinyl alcohol) films dried at 60°C. (1) Without additives, (2) polyvinyl-pirrolidone, and (3) sodium alginate. Ratio of soluble polymer to poly(vinyl alcohol) in the forming composition was 1:100 (w/w).

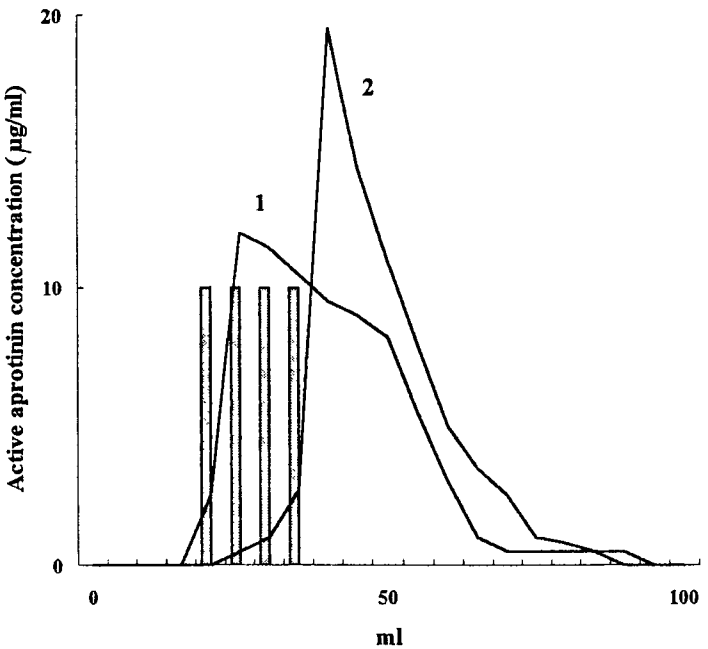

Fig. 3. Gel chromatography of the polymer composition (1), containing 10% poly(vinyl alcohol), 0.05% aprotinin, 0.1% sodium alginate, and native aprotinin (2) on a Sephadex G-50 column (1.8 × 22 cm). Eluent 0.9% NaCl. The hatched region illustrates the elution of sodium alginate determined with the phenol-sulfuric acid reagent according to Dubois et al. (*9*).

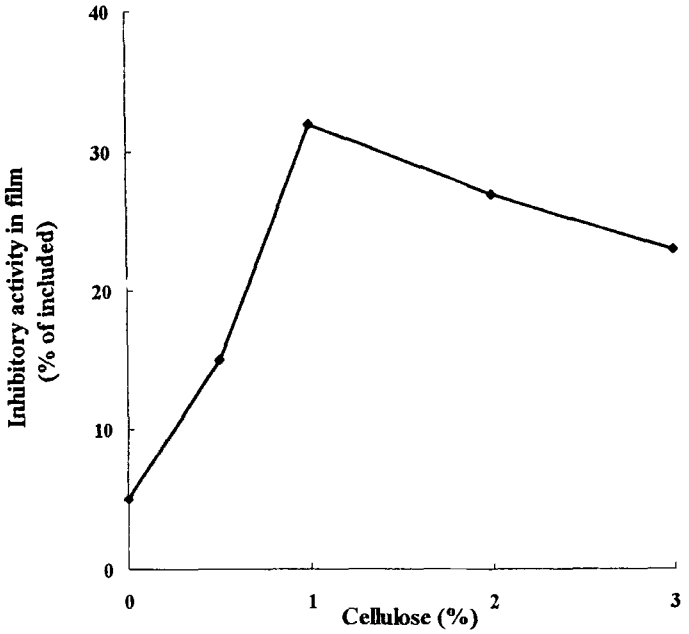

Fig. 4. Residual aprotinin activity in poly(vinyl alcohol)-cellulose films after treatment with the physiological solution as a function of the amount of cellulose in the composition. The drying temperature was 80°C. 10 mg film were soaked in 1 mL physiological solution for 24 h. For other details, *see* Materials and Methods.

the inhibitor on cellulose particles. Further increasing the amount of cellulose appears to loosen the film structure and lowers the aprotinin activity, which is retained in the films after soaking in the physiological solution for 24 h. Thus, inclusion of additional polymers in the composition proved to be an attractive and promising way to regulate the aprotinin release from insoluble films.

Biosoluble Films Released Aprotinin

The results of our previous study of soluble polymer matrixes of various compositions demonstrated that a copolymer of acrylamide, *N*-vinylpirrolidone and ethyl acrylate with a wide polydispersion (mol wt 30,000–600,000 Dalton) meets pharmaceutical requirements, and it is promising for preparation of biosoluble medicinal films (*10*). Such films ensure the programmed entry of a medicine into the organism by regulating the macromolecular structure and the chemical nature of a polymeric matrix (for instance, by changing its hydrophilic-hydrophobic balance). Numerous tests conducted in the ophthalmological clinics of Russia showed that application of biosoluble medicinal films leads to a qualitatively new medicinal effect. The treatment period and the number of procedures decreases 2–3 and 3–10 times, respectively. The expenditure of the medicinal preparations is reduced 5–10 times and the danger of crossinfection is almost eliminated (*10*).

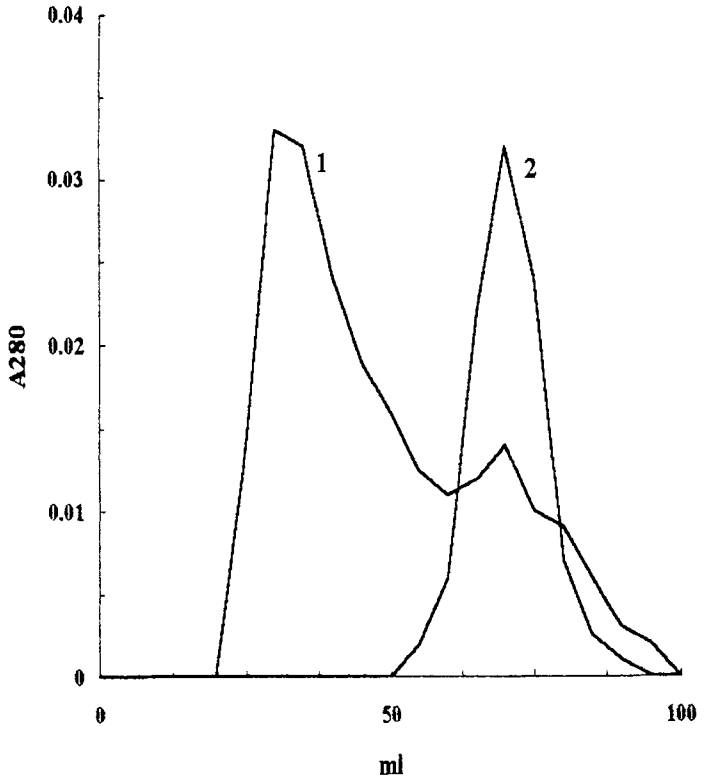

Fig. 5. Gel chromatography of the solution of biosoluble polymer film (1) containing aprotinin and native aprotinin (2) on a Toyopearl HW-50 column (2 × 20 cm). Eluent 0.9% NaCl.

The inhibitory activity of aprotinin immobilized in biosoluble medicinal films was retained by 80%. Figure 5 shows aprotinin is released from the films both as the native inhibitor and a complex with copolymer. Data illustrating the kinetics of release are presented in Fig. 6. Bioavailability of aprotinin from these films was linear with time when the film was in contact with the physiological solution at M = 60 (curve 2 in Fig. 6). These conditions are close to those used in ophthalmology, since one or two films (film weight 17 mg) are used daily, but an average tear volume produced for 24 h is 1–2 mL (*11*). Under these conditions, aprotinin was released in a matter of 5 h. An increase in the volume of solvent and intensity of shaking (curve 1 in Fig. 6) resulted in dissolution of the film and appearance of the entire aprotinin activity in solution in a matter of 1 h. For the comparison, poly(vinyl alcohol) film formed at the same temperature (40°C) released only 20% the aprotinin activity in a matter of 1 h.

The presence of copolymers of mol wt 30,000–40,000 Dalton secured a quick autoadhesion of the films on a mucous surface. It is a key parameter for intrabuccal drugs. Preliminary results of phase I of a human clinical trial of biosoluble films with aprotinin in patients with aphthous stoma-

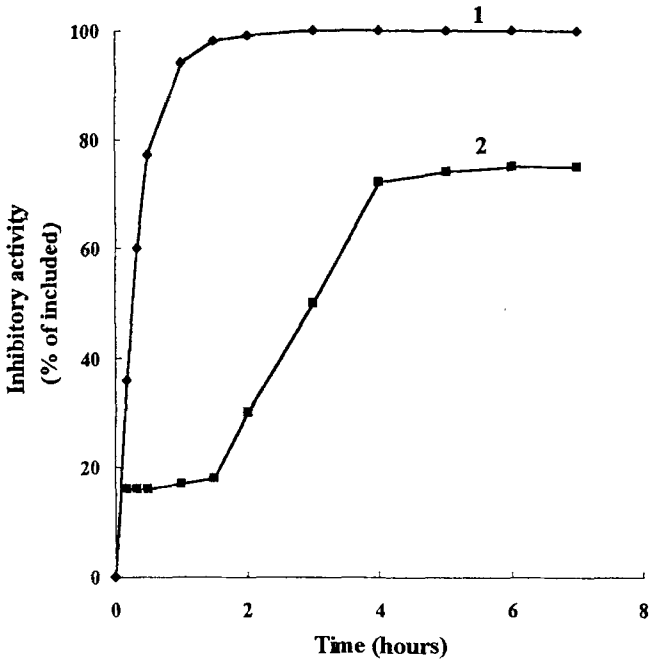

Fig. 6. Kinetics of aprotinin release from biosoluble films: (1) M = 300, with shaking; (2) M = 60, without shaking. The drying temperature was 40°C.

titis (n = 3) and the erosive-ulcerous form of the lichen planus (n = 12) demonstrated the improvement of patient status, as well as a twofold shortening of the epithelization time.

ACKNOWLEDGMENT

The support from the Russian State Program Enzyme Engineering and Universities of Russia is acknowledged.

REFERENCES

1. Fritz, H. and Wunderer, G. (1983), *Arzneim.-Forsch./Drug Res.* **33,** 479–494.
2. Haberland, G. L. and Lewis, D. H. (1973), *Neue Aspekte der Trasylol-Therapie.* Bd. G, Schattauer Verlag, Stuttgart.
3. Larionova, N. I., Sakharov, I. Yu., Kazanskaya, N. F., Zhuravlyov, A. G., Vladimirov, V. G., and Tolstich, P. I. (1980), in *Future Directions: Enzyme Engineering,* Plenum, New York, pp. 241–255.
4. Larionova, N. I., Balabushevitch, N. G., Gladysheva, I. P., Moroz, N. A., Kazanskaya, N. F., Polekhina, O. V., and Donetsky, I. A. (1994), *Vopr. Med. Khim.* **40,** 25–31 (in Russian).
5. Larionova, N. I., Kazanskaya, N. F., and Torchilin, V. P. (1984), in *Biotechnology* (in Russian), Bayev, A. A., ed., Nauka, Moscow, pp. 113–125.

6. Chase, T. and Shaw, E. (1967), *Biochem. Biophys. Res. Commun.* **29,** 508–514.
7. Schwert, G. W. and Takenaka, Y. A. (1955), *Biochim. Biophys. Acta* **16,** 570–576.
8. Timokhina, V. I., Lapo, V. G., and Lanina, S. Ya. (1982), in *Medical-Technical Problems of Human Individual Defence*, vol. 23, Katscheyev, V. S., ed., Institute of Biophysics Press, Moscow, pp. 97–102.
9. Doubois, M., Gilles, K. A., Hamilton, J. K., Roberts, P. A., and Smith, F. (1956), *Anal. Chem.* **28,** 350–356.
10. Khromov, G. L. (1992), *Med. Prog. Through Technol* **18,** 9–13.
11. Mishina, S., Gasset, A., and Klyce, S. (1966), *Invest. Ophthalmol. and Vis. Sci.* **5,** 264–276.