Raman Spectroscopic Study of the Effect of aldose Reductase inhibitor on experimental diabetic cataract

Hiroko Nozawa, Tokikage Yaginuma, Aritake Mizuno

Department of Ophthalmology, The Jikei University School of Medicine

ラマン分光法による実験的糖尿病性白内障に対するアルドースリダクターゼ阻害剤の効果の研究（図5，表1）

野沢　裕子*・柳沼　時影*・水野　有武*,**（東京慈恵会医科大学眼科学教室*／東京慈恵会医科大学医化学教室**）

要　約

ストレプトゾトシン誘発糖尿病ラットの水晶体内の相対的水濃度とチロシンダブレットの相対強度比を，レーザーラマン分光法を用い測定し，更にそれらに対するアルドースリダクターゼ阻害剤（M79175, エーザイ）の効果を検討した．コントロール群の水晶体核部分の相対的水濃度は加齢と共に減少したが，ストレプトゾトシン糖尿病群では核白内障の進行に伴い著明に増加した．ストレプトゾトシン処理後60日目の水晶体皮質部分の相対的水濃度は，コントロール群に比べ糖尿病群でははるかに高値を示したが，アルドースリダクターゼ阻害剤はこの変化を有意に抑制した．一方，核部分のチロシンダブレットの相対強度比は，混濁のある糖尿病群では高値を示したが，混濁のない糖尿病群，アルドースリダクターゼ阻害剤投与群は，コントロール群と差はなかった．（日眼　92：194—201，1988）

キーワード：レーザーラマン分光法，アルドースリダクターゼ阻害剤，実験的糖尿病，白内障，ラット水晶体

Abstract

Lens hydration during the development of streptozotocin (STZ)-induced diabetic cataract in rats was investigated using laser Raman spectroscopy. The effect of an aldose reductase inhibitor (ARI) (6-fluoro-2-methyl-spiro(chroman4, 4'-imidazolidine)-2', 5'-dione, M79175) on the diabetic lens was also studied.

The relative water content, represented by the intensity ratio of the water band (I_{3390}) and the CH band (I_{2935}), in the nucleus center of the untreated control rat lenses gradually decreased upon aging from 0.29 in 30 day-old rats to 0.18 in 150 day-old rats. However, that of the diabetic rat lenses markedly increased with the development of nuclear cataract. On the 60th day after STZ administration, the relative water content in the cortex near the equator increased from 0.48 in the controls to 1.12 in the diabetic lenses. On the same experimental day, the relative water content in the lenses of the diabetic rats treated with ARI remained within normal level. On the other hand, the intensity ratio of Raman bands of tyrosine doublet (I_{833}/I_{856}) from the nuclear portion of the lenses changed from 0.83 in the controls to 1.00 in the diabetic lenses according to the lens opacification. This change did not occur in the clear lenses of diabetic rats treated with ARI. (Acta Soc Ophthalmol Jpn 92 : 194—201, 1988)

別刷請求先：105　東京都港区西新橋3—19—18　東京慈恵会医科大学眼科学教室　野沢　裕子

（昭和62年7月20日受付）

Reprint requests to: Hiroko Nozawa, M.D.　Dept. of Ophthalmol., Jikei Univ. School of Med.
3-19-18 Nishi-shinbashi, Minato-ku, Tokyo 105, Japan

(Accepted July 20, 1987)

Key words: laser Raman spectroscopy, aldose reductase inhibitor, experimental diabetes, cataract, rat lens

I Introduction

The lens is a highly transparent organ which mainly consists of lens protein crystallins and water. Recently, laser Raman spectroscopy has been introduced into cataract research by several investigators. Ozaki and his coworkers reported that the change of the intensity ratio of tyrosine doublet (I_{833}/I_{856}) was observed in the diabetic cataractous rat lens[1]. They also proposed that the relative intensity of the Raman band at 3,390cm^{-1} assigned to an OH stretching mode of lens water was a useful marker for cataract indicating lens hydration from the result of the hereditary mouse cataract study[2]. It is of interest to see the variation in the relative intensity of the water band as a function of diabetic changes in the lens. In this study, the effect of an aldose reductase (EC 1.1.1.21) inhibitor (ARI) on the diabetic lens was observed by laser Raman spectroscopy monitoring the relative water content at several positions of the lens.

II Materials and methods

Male Sprague-Dawley rats (1—7 months of age, Charles River Japan, Kanagawa, Japan) were used in all experiments.

The experimental animals were divided into three groups, namely; an untreated control group, a streptozotocin (STZ)-induced diabetes group and a STZ-induced diabetes-plus-ARI group. Rats were injected intravenously with STZ (100mg/kg body weight) at the age of 4 weeks. The third group was fed a diet mixed with 5mg of ARI per kilogram of lab chow from immediately after the injection of STZ. The ARI which we used was 6-fluoro-2-methyl-spiro 〈chroman-4, 4′-imidazolidine〉-2′, 5′-dione (M79175, Eisai Company, Tokyo, Japan)(Figure 1). Blood glucose levels showed no significant difference on the 60th experimental day between the diabetic group and the diabetes-plus-ARI group, that is, 565±72mg/dl and 532±57mg/dl, respectively, whereas that in the control group was 123±15mg/dl.

The lenses were excised immediately after decapitation at various intervals after STZ administration. The lens was carefully transferred and placed in the cuvette cell (10×10×30mm) filled with Tris-buffered balanced salt solution containing 5.5mM glucose[3]. The osmolarity of this solution was 291mOsm. (Figure 2).

The Raman spectra were obtained using a JASCO NR1000 laser Raman spectrometer (JASCO CO., Tokyo, Japan). The lens was illuminated by the argon laser from the bottom of the cell and scattered light was collected at 90 degrees relative to the incident beam. The light source was the 514.5nm line of an argon ion laser (NEC GLC3200, Tokyo, Japan) and the laser power at the sample was usually 100mW. In the case of opaque lenses, 2-300mW laser power was used. In the case of opaque lenses. Peak frequencies were calibrated using the spectrum of indene and were believed to be accurate to ±1cm^{-1} for well-resolved bands.

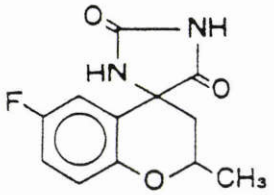

Fig. 1 The structure of M79175 (aldose reductase inhibitor).

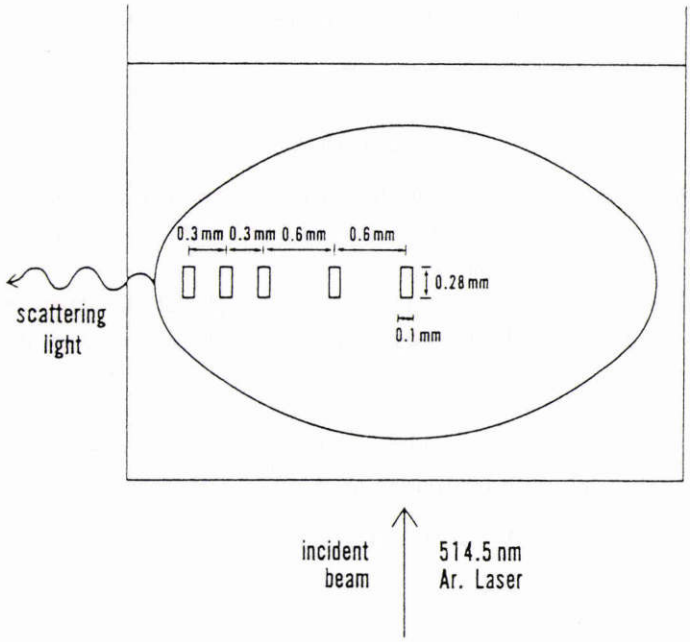

Fig. 2 The scheme of the laser illumination and Raman scattering detection.

Raman spectra were detected at five focused areas from the nucleus center to the lens equator, as shown in Figure 2. The laser diameter was 0.1mm and the scattered light was collected from the illuminated column, 0.28mm in height, restricted by the 4.0mm height entrance slit of the double monochromator. Raman spectra that were monitored repeatedly under the same conditions showed good reproducibility for each region (less than 1% error). We monitored the intensity ratio of the tyrosine doublet (I_{833}/I_{856}) in the 700—950cm^{-1} region and the OH band at 3,390cm^{-1} and the CH band of protein at 2,935cm^{-1} in the 2,800—3,800cm^{-1} region. The bands at 833 and 856cm^{-1} doublet indicate tyrosine bands from lens proteins. The band at 2,935cm^{-1} shows the CH stretching mode of lens proteins as a internal standare and the band at 3,390cm^{-1} indicates the OH stretching mode of lens water. Thus the ratio of the intensities (I_{3390}/I_{2935}) indicates the relative water content at the position monitored in the lens (Figure 3).

III Results

Figure 4 shows the relative content of lens water from the nucleus center plotted at various periods following induction of diabetes by STZ. In the control lenses, the relative content of lens water gradually decreased with age. The intensity ratio (I_{3390}/I_{2935}) changed from 0.29 in 30 day-old rats to 0.18 in 150 day-old rats. On the 60th day after STZ injection, most of the diabetic lenses had vacuoles at the equatorial portion spreading to the anterior center and apparently clear nuclei as examined by slit lamp microscopy. The relative water content in the apparently clear nuclei of diabetic lenses did not increase ($I_{3390}/I_{2935}=0.18$) until the 60th experimental day. Some of the 60th experimental day lenses (two of six) appeared to have hazy nuclear opacity, which showed a slight elevation of the relative water content ($I_{3390}/I_{2935}=0.35$) at the nuclear portion of the lenses. The relative water content increased rapidly with development of cataract. The relative content of water in the lenses was greatly increased when the nuclear cataract developed fully in the diabetic rats. On the other hand, the lenses in the STZ-induced diabetes plus ARI group were still transparent and the relative water content did not change until the 180th diabetic day. The ARI significantly inhibited the development of

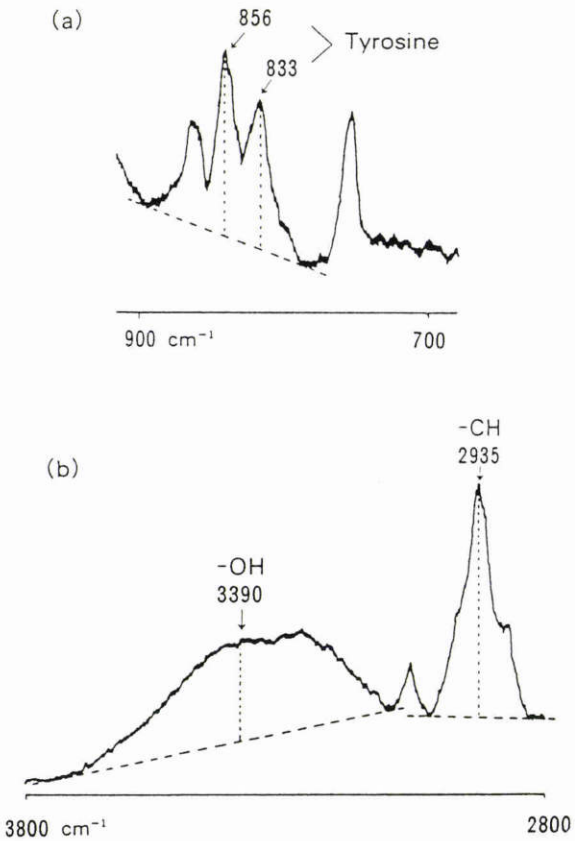

Fig. 3 The Raman spectra of the rat lens. (a): 700~900cm^{-1} region. The bands at 833 and 856cm^{-1} doublet indicate tyrosine bands from lens protein. (b): 2800~3800cm^{-1} region. The band at 2935cm^{-1} indicates the CH stretching mode of lens proteins and the band at 3390cm^{-1} indicates the OH stretching mode of lens water.

STZ-induced cataract.

Figure 5-a shows the relative content of water from the various positions of rat lenses in three groups on the 60th experimental day after STZ injection. No apparent opacity was observed in the lens of 4 of 6 diabetic rats, and the relative water content in the cortex was higher than that in the nucleus, that is, the nucleus center was mostly dehydrated. The relative content of water in the diabetic lenses greatly increased at all sites as compared with the controls but this increase was completely suppressed to a level equal to that in controls when ARI was added. The short-term effect of ARI on STZ-induced diabetic cataract was reported in a preliminary paper[4]. In this study, the short-term and the long-term effects of ARI on the diabetic cataract were investigated. Figure 5-b shows the relative content of water at the various sites of rat lenses of the control and the STZ-induced diabetes plus ARI groups at the 180th day after STZ injection. At this stage, all lenses of the diabetic group showed hypermature cataract and the lens water was not detectable by laser Raman spectroscopy because the laser beam could not penetrate the lens interior even in the cortex. On the contrary, the lenses of the STZ-induced diabetes plus ARI group maintained transparency, and the relative

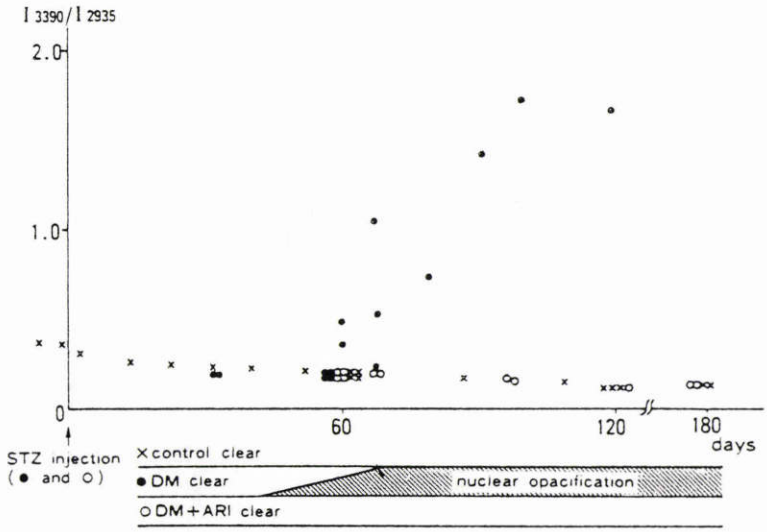

Fig. 4 The profile of the relative water content in the nucleus center of the lenses in three groups. DM=diabetes mellitus

content of water in these lenses was about the same as that in the control lenses. Therefore, ARI prevented this elevation of water content and cataract formation through the 180th diabetic day.

The microenvironmental change of tyrosine residues was also observed by the laser Raman spectroscopy. Table 1 shows the intensity ratio of the tyrosine doublet at the nuclear portion of the lenses in three groups at the 60th experimental day. The intensity ratio of the tyrosine doublet, 833 to 856cm^{-1}, changed from 0.83± 0.02 in the controls to 1.00±0.04 in the hazy lens of diabetic cataracts, but did not change in the diabetic clear lenses or in the lenses of the STZ-induced diabetes-plus-ARI group. The ARI prevented the environmental change of tyrosine residues in diabetic cataract.

IV Discussion

Cataract formation is one of the important complications of diabetes mellitus. It is recognized that aldose reductase plays on important role in the formation of sugar cataract in the early stages. The accumulation of sorbitol through the polyol pathway leads to lens hydration in the diabetic lens. The water content of galactosemic rat lens increased in parallel with the increase in the amount of galactitol, one of the polyols derived from galactose[5]. ARI has the potential to be a potent anticataract drug in diabetes. However, there are few suitable methods to objectively observe the efficacy of a given drug on cataract in vivo and in situ.

Laser Raman spectroscopy was introduced to obtain molecular-level information on lens changes in situ[6)7)]. This was previously applied to the study of ocular lens in situ by Yu and East[8)] and by Mizuno et al[9)].

In this study, we investigated the change of the relative content of lens water with aging and with the development of STZ-induced rat diabetic cataract in addition to the effect of ARI on the formation of cataract using laser Raman spectroscopy. The relative intensity of a water band in the control lens nucleus gradually decresed in accordance with the aging process (Figure 4). It suggests that the relative concentration of the lens water to the lens protein decreased with age because of the dehydration of older lens fibers in the nuclear portion. Using Raman spectroscopy Ozaki et al. reported that relative intensity of water band in mouse lenses clearly decreased with age[2)10)]. Cotlier revealed that a mammalian lens slowly increased in size, that new lens fibers developed throughout life and that older lens fibers in a nuclear portion became dehydrated[11)]. The

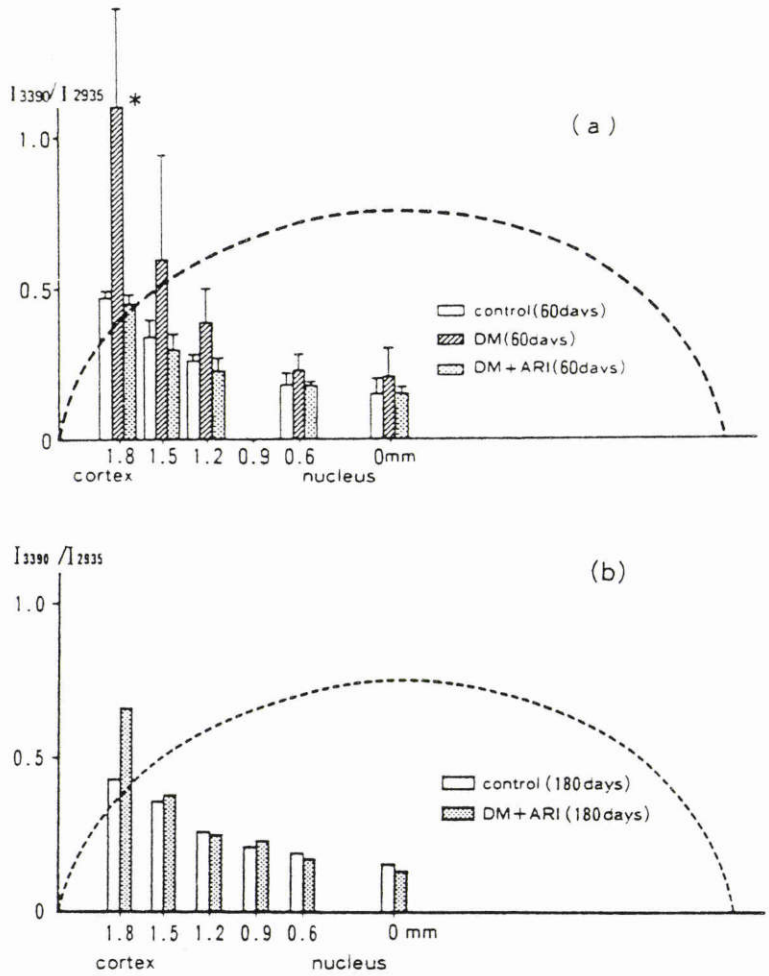

Fig. 5 (a) : The relative water content from the various positions of rat lenses at the 60th day after the streptozotocin injection in three groups (n=4~6). The bars indicate means±SD. * $p<0.01$ (b) : The relative water content from the various portions at the 180th day in the control (n=2) and the diabetes plus ARI group (n=2). DM=diabetes mellitus

Table 1 The intensity ratio of the tyrosine doublet (I_{833}/I_{856}) at the nuclear portion of the lenses of three groups at the 60th experimental day.

	Control	DM		DM+ARI
		opaque	clear	
I_{833}/I_{856}	0.83±0.02	1.00±0.04	0.85±0.01	0.84±0.03

relative intensity of the lens water band dramatically increased in the nucleus of diabetic cataractous lenses (Figure 4). This result was observed in an other type of cataract, hereditary cac-Nakano cataract[12]. There are many ARIs, for example, a compound of naphthoimide (AY-22, 284)[13], quercitrin (one of flavonoids)[14][15], and a derivative of spirohydantoin (CP-45, 634)[16]. The ARI we used, M79175 is one of the derivatives of

spirohydantoin. The activity of this enzyme inhibitor is very strong and IC_{50} is 9×10^{-8}M. From our results, the ARI we used suppressed cataract formation and completely inhibited the increase of the relative water content in the nucleus of STZ-induced diabetic lens through the 180th diabetic day (Figure 4). Ono et al. reported that sorbitol levels in lenses were markedly reduced when rats with STZ induced diabetes were treated orally with M79175. In addition, in galactosemic rats, orally administered M79175 effectively delayed the development of cataract formation[17].

The relative water content was significantly more elevated in the cortex than in the nucleus of the diabetic lenses at the 60th experimental day (Figure 5-a). The relative water content was greatly increased even in the apparently clear cortex on the 60th experimental day. In the nucleus, the relative water content was slightly elevated before this time and it further increased with the development of nuclear opacity (Figure 4, 5-a). The lens hydration started earlier in the cortex than in the nucleus. It may be that the accumulation of polyol in fiber cells leads to hydration in the cortex, creating vacuoles in precataratous stage. ARI (M79175) inhibited the lens hydration of diabetic rat significantly.

Table 1 shows the relative Raman intensity of tyrosine doublet of the lenses in three groups. This value changed from 0.83 in the control to 1.00 in the opaque lenses of the STZ-induced diabetic group and did not change in the diabetic clear lenses. The change in the intensity ratio of the tyrosine doublet has been observed in lens opacification, for example, diabetic cataract, cac-Nakano mouse cataract and cold cataract[1)12)18)], but not in aging[10)]. The intensity ratio of the tyrosine doublet correlates with the strength of the hydrogen bond of the phenolic hydroxyl group[19)]. It was suggested that the nature of hydrogen bonding of some tyrosyl residues in lens proteins changed with lens opacification and that probably some tyrosyl residues became hydrophobic and buried among aggregated proteins where the OH group of tyrosine is strongly bound to a native acceptor[1)]. The relative intensity ratio of the tyrosine doublet of the lenses in STZ-induced diabetes plus ARI group did not change through the 180th experimental day because of the transparency of lens.

ARI suppressed the hydration of the diabetic lens and lens opacification including the change of tyrosine doublet. Our results revealed that the Raman study was one of the most useful techniques to monitor the efficacy of ARI on diabetic lenses, because it provided objective data about lens hydration in situ.

Acknowledgements

We are grateful to Prof. Hiroshi Matsuzaki and Dr. Yoshio Kamada for their interest and helpful discussions during this investigation. We also thank Mr. Keiichi Nomura for his supplying ARI (M79175) and Mr. Teruki Ikeda for his technical advice for the Raman spectrometer JASCO NR1000.

Corresponding author: Hiroko Nozawa, M.D., Department of Ophthalmology, The Jikei University School of Medicine, 3-25-8 Nishi-Shinbashi, Minato-ku, Tokyo 105, JAPAN

References

1) **Ozaki Y, Mizuno A, Kamada Y,** et al: Laser Raman spectroscopic study of a diabetic cateractous lens. Chemistry Letters: 887—890, 1982.
2) **Ozaki Y, Mizuno A, Itoh K,** et al: Potential clinical application of laser Raman spectroscopy for cataract diagnosis. Innov. Tech. Biol. Med. 5: 269—286, 1984.
3) **Epstein DL, Kinoshita JH**: Effect of methylphenyldiazenecarboxylate (Azoester) on lens membrane function. Exp. Eye Res. 10: 228—236, 1970.
4) **Mizuno A, Nozawa H, Yaginuma T,** et al: Effect of aldose reductase inhibitor on experimental diabetic cataract monitored by laser Raman spectroscopy, Exp. Eye Res. in press: 1986.
5) **Kinoshita JH**: Biochemical Basis on Cataract Formation. Acta Soc. Ophthalmol. Jpn. 80: 1362—1371, 1976.
6) **Yu NT, East EJ**: Laser Raman spectroscopic studies of ocular lens and its isolated protein fractions. J. biol. Chem. 50: 2196—2202, 1975.
7) **Schachar RA, Solin SA**: The microscopic protein structure of the lens with a theory for cataract formation as determined by Raman spectroscopy of intact bovine lenses. Invest. Ophthalmol. 14: 380—396, 1975.

8) **Yu NT, Kuck JFR Jr., Askren CC**: Laser Raman spectroscopy of the lens in situ, measured in an anesthetized rabbit. Curr. Eye Res. 1: 615—618, 1982.
9) **Mizuno A, Ozaki Y, Kamada Y,** et al: Direct measurement of Raman spectra of intact lens in a whole eyeball. Curr. Eye Res. 1: 609—613, 1982.
10) **Ozaki Y, Mizuno A, Itoh K,** et al: Raman spectroscopic study of age-related structural changes in the lens proteins of an intact mouse lens. Biochemistry 22: 6254-6259, 1983.
11) **Cotlier E**: The lens, Moses RA: Adler's Physiology of the Eye. St. Louis, Missourri U.S.A., Mosby Company, 277—303, 1981.
12) **Itoh K, Ozaki Y, Mizuno A,** et al: Structural changes in the lens proteins of hereditary cataracts monitored by Raman spectroscopy. Biochemistry 22: 1773—1778, 1983.
13) **Dvornik D, Simard-Duquesne N, Kraml M,** et al: Polyol accumulation in galactosemic and diabetic rats: Control by an aldose reductase inhibitor. Science 182: 1146—1148, 1973.
14) **Varma SD, Kinoshita JH**: Inhibition of lens aldose reductase by flavonoids their possible role in the prevention of diabetic cataracts. Biochem. Pharmacol. 25: 2505—2513, 1976.
15) **Varma SD, Mizuno A, Kinoshita JH**: Diabetic cataracts and flavonoids. Science 195: 205—206, 1977.
16) **Peterson MJ, Sarges R, Aldinger CE,** et al: A novel aldose reductase inhibitor that inhibits polyol pathway activity in diabetic and galactosemic rats. Metabolism 28: 456—461, 1979.
17) **Ono H, Nozawa Y, Hayano S**: Effects of M79175, an aldose reductase inhibitor on experimental sugar cataracts. Acta Soc. Ophthalmol, Jpn. 86: 1343—1350, 1982.
18) **Mizuno A, Ozaki Y, Itoh K,** et al: Raman spectroscopic evidence for the microenvironmental change of some tyrosine residues of lens proteins in cold cataract. Biochem, Biophys. Res. Comm. 119: 989—994, 1984.
19) **Shiamwiza MN, Lord RC, Chen MC,** et al: Interpretation of the doublet at 850 and 830cm^{-1} in the Raman spectra of tyrosyl residues in proteins and certain model compounds. Biochemistry 14: 4870—4876, 1975.