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THE EFFECTS OF X-IRRADIATION
ON LENS REDUCING SYSTEMS

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KEY WORDS

rabbit lens, X-ray, pyridine nucleotides, sulfhydryl groups, hexose monophosphate shunt, glutathione reductase, glutathione peroxidase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase

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RUNNING TITLE

X-irradiation and Lens Reducing Systems

ABSTRACT

Studies have been made of the effects of X-ray on various lens reducing systems including the levels of NADPH and glutathione (GSH), the activity of the hexose monophosphate shunt (HMS) and the activities of certain enzymes including glutathione reductase, glutathione peroxidase and glucose-6-phosphate dehydrogenase (G-6-PD). It was found that during several weeks following X-irradiation but prior to cataract formation there was very little change in the number of reduced -SH groups per unit weight of lens protein but that, with the appearance of cataract, there was a sudden loss of protein -SH groups. In contrast, the concentration of GSH in the X-rayed lens decreased throughout the experimental period. Similarly, the concentration of NADPH in the X-rayed lens was found to decrease significantly relative to controls one week prior to cataract formation and the ratio of NADPH to NADP^+ in the lens shifted at this time period from a value greater than 1.0 in the control lens to less than 1.0 in the X-rayed lens. A corresponding decrease occurred in the activity of the HMS in X-rayed lenses as measured by culture in the presence of $1\text{-}^{14}\text{C}$ labelled glucose. G-6-PD was partially inactivated in the X-rayed lens. Of the eight enzymes studied, G-6-PD appeared to be the most sensitive to X-irradiation. The data indicate that X-irradiation results in a steady decrease in the effectiveness of lens reducing systems and that, when these systems reach a critically low point, sudden oxidation of protein -SH groups and formation of high molecular weight protein aggregates may be initiated.

INTRODUCTION

The formation of high molecular weight (HMW) proteins is believed to be responsible for scattering of light and the resultant opacification of a cataractous lens.^{1,2,3} The mechanism by which these protein aggregates are formed is not known but one possible contributing factor may be the oxidation of protein -SH groups. An increased concentration of disulfide bonds has been found to be present in the HMW protein of X-ray induced cataract, and, more importantly, there is an indication that intermolecular bonding is involved since treatment of the protein with disulfide bond reducing agents results in the release of lower molecular weight crystallins.⁴ Additional support for the concept that oxidation of sulfhydryls may play a role in protein aggregation comes from the observations of Spector and Roy⁵ who found an increase in the disulfide content in HMW proteins of human cataractous lenses.

The present study was designed to provide possible clues to the mechanism of oxidation of protein -SH groups and the formation of HMW protein in X-ray induced cataract in rabbits. A systematic investigation has been conducted on the effects of X-irradiation on various lens reducing systems which may normally act to maintain protein -SH groups in the reduced state. In this report, we present data on the levels of the reducing compounds NADPH and glutathione (GSH), the activity of the hexose monophosphate shunt (HMS) and the activities of several enzymes including glutathione reductase, glutathione peroxidase and glucose-6-phosphate dehydrogenase (G-6-PD) in normal and irradiated lenses. These parameters were examined at various times after X-ray until cataract formation, which occurred regularly 8 weeks later. The results suggest that a diminished supply of GSH and NADPH and decreased activities of G-6-PD and the HMS may promote the oxidation of protein sulfhydryl groups in this type of cataract.

METHODS

The albino rabbits used in this study were 5 weeks of age at the time of X-irradiation. The X-irradiation factors employed have been described previously.⁴ One eye was irradiated and the area surrounding the eye was protected with a lead shield. The non-irradiated eye served as the control. A single 2,000 rad dose was given to each rabbit.

Concentrations of protein -SH groups and GSH (non-protein -SH groups) were determined with 5-5'-dithio-bis-(2-nitrobenzoic acid)(DTNB).⁶ For this purpose, whole lenses were homogenized at 0-5°C in 0.02M sodium phosphate buffer, pH 7.4, containing 0.1M NaCl and 0.025M EDTA. Protein was assayed by the method of Lowry, et al.⁷ using bovine serum albumin as a standard.

The concentrations of pyridine nucleotides were determined with the use of a "cycling assay" system employing thiazolyl blue (MTT) as a terminal electron acceptor. This method was originally described by Nisselbaum and Green⁸ and was modified in the present study for use with lens tissue. A complete description of the modifications employed in the assay procedure is in preparation and will be shortly submitted for publication. Briefly, the cycling mixture consisted of MTT, phenazine ethosulfate (PES) and either ethanol and alcohol dehydrogenase for the determination of NAD⁺ and NADH or glucose-6-phosphate and glucose-6-phosphate dehydrogenase for the determination of NADP⁺ and NADPH. The rate of the reduction of thiazolyl blue to the corresponding formazan, which absorbs maximally at 570nm, is proportional to the concentration of coenzyme.

The procedure for extraction of pyridine nucleotides was a modification of that used by Sippel.⁹ Either NAD⁺ and NADP⁺ or NADH and NADPH were assayed in individual lenses. For extraction of NAD⁺ and NADP⁺ the lens was homogenized in 5 volumes of 0.23MKH₂PO₄ for 1 minute in a boiling water bath, chilled, neutralized with an equal volume of 0.2N KOH and centrifuged at 5°C for 30 minutes at 20,000g. The order of treatment of KH₂PO₄ and KOH was reversed for extraction

of NADH and NADPH. The recoveries of each coenzyme in the procedure were determined by adding a known amount of coenzyme just prior to the homogenization of a normal lens in acid or base. The recoveries were nearly 100 percent for NAD^+ and NADP^+ and approximately 85 percent for NADH and NADPH.

The activity of the HMS was estimated in control and experimental lenses by determining the amount of $^{14}\text{CO}_2$ liberated from lenses cultured in the presence of 1- ^{14}C labelled glucose.¹⁰ The lenses were cultured in capped tubes under sterile conditions for 8 hours at 37°C in 5 ml of KEI-4 medium¹¹ (6.94 mM in glucose) containing approximately 45,000 cpm/ml of radioactivity. The amino acid concentrations of the KEI-4 medium in the aforementioned paper are incorrectly given as four times the actual values; the values should read as mg per 4 liters of medium. The specific activity of the 1- ^{14}C labelled glucose (New England Nuclear Corp., Boston, Mass.) was 14 $\mu\text{Ci}/\text{mg}$. At the end of the culture period a vial containing 0.5 ml of hydroxide of hyamine was inserted into the tube, stoppered and 1 ml of 2N H_2SO_4 injected into the medium to release CO_2 . The released CO_2 was allowed to absorb into the hydroxide of hyamine overnight.¹² The vial and contents were then added to scintillation fluid and counted in a liquid scintillation counter. The lenses were removed from the culture tubes, dried overnight at 85°C and weighed. The amount of radioactivity released per lens was normalized to account for a slight day-to-day variation in the level of radioactivity present in the medium. A blank consisted of a vial of hydroxide of hyamine which was treated in the same manner as above except that a lens was not present in the culture medium.

The activities of glyceraldehyde phosphate dehydrogenase, lactate dehydrogenase (LDH), hexokinase, G-6-PD, phosphofructokinase, 6-phosphogluconate dehydrogenase and glutathione reductase were determined by measuring the change in absorbance at 340 nm of either NADH or NADPH as described by Bergmeyer.¹³ Glutathione peroxidase was assayed by the method of Lawrence et al.¹⁴

RESULTS

Under the conditions employed, 90% of the irradiated rabbits developed mature cataracts characterized by complete opacity and swelling 8 weeks ($\pm$ 3 days) after X-irradiation. One week prior to cataract development (7 weeks following X-irradiation), the X-rayed lenses were clear except for some haziness present in the posterior subcapsular region. These lenses, which weighed on the average 250mg, were 20 percent lighter than the controls (312 mg) (Table I). However, 4 weeks after X-irradiation there was little difference in weight between control and experimental lenses, both weighing about 260 mg.

During the weeks following irradiation there was very little change in the number of reduced -SH groups per unit weight of protein (Fig. 1). In contrast, there was a dramatic decline in the concentration of GSH during the same period so that one week prior to cataract formation the level of GSH in the experimental lens was only 25 percent of that in the control. It was only at the time of cataract formation (8 weeks after X-ray) that loss of protein sulfhydryl groups was observed; the decrease amounted to 35 percent relative to the control.

The sudden decrease in the number of reduced -SH groups per unit weight of protein and the previously reported finding of high disulfide content in the HMW protein of X-ray induced cataract⁴ prompted us to investigate changes in the reducing systems of the X-rayed lens which might be responsible for the observed phenomena. Table II summarizes the results of analyses for NADPH and other pyridine nucleotides in the lens 7 weeks post X-irradiation. The main feature of the data is that, one week prior to cataract, there was a significant reduction in the concentration of NADPH in the lens ($P < .001$), amounting to a 42 percent decrease on a per gram wet weight basis. The decrease in NADPH on a per lens basis was higher (52 percent, data not shown) due to the fact that the X-rayed lens was 20 percent lighter than the control (7 weeks post-irradiation). In addition to NADPH, the concentration of the other three coenzymes also decreased in X-irradiated lenses although to a lesser extent.

On a per gram wet weight basis, the decreases in NADH, NADP^+ and NAD^+ ranged from 12-24 percent; somewhat less than that observed for NADPH. Of the four coenzymes analyzed, only the decrease in NADH was not significant ($p < .05$).

Table II also indicates that a shift occurred in the ratio of NADPH to NADP^+ in the X-rayed lens 1 week prior to the appearance of cataract. In the control lens, at this time period, the ratio of NADPH to NADP^+ was 1.1, while in the X-rayed lens the value fell below 1 (0.8). Thus, in the precataractous lens, there was a significantly lower concentration of NADPH relative to NADP^+ . On the other hand, the ratio of the concentration of NAD^+ to that of NADH was almost 2 in both control and X-irradiated lenses.

In view of the observed decrease in the level of NADPH, it was of interest to study the effect of X-ray on the activity of the HMS which furnishes a continuous supply of NADPH in the lens. The $^{14}\text{CO}_2$ liberated from the oxidation of 1- ^{14}C labelled glucose was taken as a direct measure of HMS activity since it has been well documented that the activity of the tricarboxylic acid cycle in the rabbit lens is minimal and that there is about 40 times more CO_2 liberated from the oxidation of 1- ^{14}C labelled glucose than from 6- ^{14}C labelled glucose.¹⁰

Figure 2 shows the activity of the HMS in control and experimental lenses at various times after X-irradiation. It may be seen that on a per lens basis, there was a slight increase in the shunt activity in control lenses over the 7 week period. This increased activity was due, in part, to the growth of the lens, the dry weight increasing from 55mg at the beginning of the experiment to 111 mg at the end of the 7 weeks (Table I). However, in the case of X-irradiated lenses, there was a steady decline in the activity of the shunt; a 34 percent decrease relative to controls at the end of 4 weeks and a 45 percent decrease at the end of 7 weeks following X-irradiation.

In view of the differential growth of control and X-irradiated lenses, which was especially evident 7 weeks after X-irradiation (Table I), the results of HMS activity are also expressed on a per mg dry weight basis. When the data

are expressed in this manner (Fig. 2), a gradual decrease in shunt activity is noted in both control and X-irradiated lenses. However, the decrease in shunt activity was much greater in X-irradiated lenses; a 32 percent decrease relative to controls was observed at the end of 4 weeks and this level of inhibition remained through 7 weeks following X-irradiation.

It is seen from the data in Figure 3 that the activity of G-6-PD, the first enzyme present in the HMS, decreased gradually in the lens following X-irradiation. On a per lens basis, there was a 25 percent decrease in the enzyme activity at the end of 4 weeks while a 35 percent decrease was noted 7 weeks following X-irradiation. On the basis of unit wet weight, the decrease in the activity was 20 percent and 27 percent 4 and 7 weeks after X-ray.

In addition to the activity of G-6-PD, seven other enzymes were also assayed in lenses 7 weeks following irradiation or just prior to the formation of cataract. The activities of the various enzymes determined in whole lenses are shown in Table II. In contrast to a significant reduction in the activity of G-6-PD, the activity of 6-phosphogluconate dehydrogenase, the second dehydrogenase of the HMS, was not significantly affected. Similarly, the glycolytic enzyme LDH, as well as two enzymes of glutathione metabolism, glutathione reductase and glutathione peroxidase, were unaffected by X-irradiation. Some decrease in the activity of hexokinase, glyceraldehyde phosphate dehydrogenase and phosphofructokinase was, however, noted. The decrease in the activity of these enzymes ranged from 14 to 18 percent relative to controls compared with a 27 percent decrease in G-6-PD.

DISCUSSION

A major objective of this work was to investigate the possibility that a decline in the activity of reducing systems in the X-irradiated lens may initiate the oxidation of protein -SH groups observed in cataractous lenses. The data clearly show that the concentrations of GSH and NADPH, the ratio of NADPH

to NADP^+ and the activity of the HMS significantly decrease in the X-rayed lens prior to the appearance of mature cataract. In contrast, there is very little change in the level of protein -SH groups until the time of cataract formation when a sudden loss of protein -SH groups occurs. This relative stability of protein -SH groups in the lens following X-irradiation has also been observed by other investigators.¹⁵ It is possible that the -SH groups of lens crystallins remain reduced at the expense of lens reducing systems and when a critically low point is reached, the oxidation of protein -SH groups is promoted.

Merola and Kinoshita¹⁶ have also observed that the -SH groups of lens proteins are normally very stable; however, they found that the groups became very susceptible to oxidation after the proteins were denatured. A similar phenomenon may occur following X-irradiation of the lens; that is, at the point of cataract formation, an unfolding of lens proteins occurs and protein -SH groups, previously buried, become exposed and oxidized. The decline in the lens reducing systems observed in the present study following X-ray may hasten the unfolding process and subsequent oxidation.

The cycling assay procedure used in this study for measurement of pyridine nucleotides was found to be highly suitable for lenses and more sensitive than previously employed fluorometric methods. The only published data for the concentrations of pyridine nucleotides in the rabbit lens are those of Bullard¹⁷ who used a fluorescent assay technique with an extraction method identical to that employed in the present study. In general, the present procedure yielded higher values for NAD^+ and NADH and lower values for NADP^+ and NADPH. A major difference was that the ratio of NADPH to NADP^+ (1.1) determined in the present study for normal rabbit lenses was considerably higher than the value of 0.4 reported in the earlier investigation.

The observed decreases in the concentration of NADPH and of the ratio of NADPH to NADP^+ in the X-rayed lens can, for the most part, be attributed to the inactivation of the HMS. However, in attempting to explain the decrease in HMS

activity, a number of factors have to be considered. The observed inactivation of G-6-PD (27 percent on a unit weight basis) may have not been sufficient to account for the decrease in HMS activity. It has been reported that, even though the activity of G-6-PD is relatively low in rabbit lens¹⁸, the HMS can be significantly stimulated in vitro.¹⁹ Thus, G-6-PD is probably present in excess in rabbit lens and the inactivation of the HMS by X-irradiation may involve other factors such as the decreased activity of hexokinase, a lower level of NADP^+ or, possibly, a reduction in glucose uptake.

Our data indicate that X-irradiation results in decreased activity of four of the eight enzymes assayed: G-6-PD, hexokinase, glyceraldehyde phosphate dehydrogenase and phosphofructokinase. These results confirm earlier findings of X-ray induced inhibition of G-6-PD,²⁰ hexokinase²¹ and glyceraldehyde phosphate dehydrogenase.²² In the present study, G-6-PD was inhibited to the greatest extent suggesting that this enzyme is more sensitive to X-irradiation. The effect may vary within species, however, since, in short term studies of rat lenses, G-6-PD appeared to be unaffected by X-irradiation.²³

Van Heyningen et al.²² have reported that sulfhydryl dependent enzymes in the lens are affected by X-irradiation earlier than those enzymes which do not require free -SH groups for activity. Consistent with this idea, we have observed that the activities of the enzymes G-6-PD, hexokinase and glyceraldehyde phosphate dehydrogenase, which are known to be sulfhydryl dependent,^{24,25,26} decreased following X-irradiation. LDH, although reported to be a sulfhydryl dependent enzyme, was unaffected by X-ray in the present study as well as in that of Pirie et al.¹⁵ This is possibly due to the fact that the critical -SH groups of lens crystallins may also be protected and less susceptible to oxidation by X-irradiation.

Our investigations show that the activity of the enzyme glutathione reductase

is unaffected by X-irradiation prior to cataract formation. Pirie and co-workers¹⁵ also studied the effect of X-ray on this enzyme and reported a decrease in activity. However, in examining their data, it appears that inactivation of the enzyme was seen primarily in lenses which showed evidence of swelling. We also noted inactivation of glutathione reductase at this cataractous stage (data not presented); however, we could not observe any change in the activity of the enzyme prior to the development of mature cataract. The latter finding is in contrast to the observations of Swanson²⁰ who noted a decrease in glutathione reductase activity soon after X-irradiation of the lens. However, in the aforementioned study, the assays were performed on capsule-epithelium preparations. Since our assays were performed on whole lenses, the data are difficult to compare. The possibility that inactivation of the enzyme may be confined to lens capsule-epithelium is currently under investigation.

In any event, even though the activities of glutathione reductase and glutathione peroxidase in the lens may be unaffected by X-irradiation, it is reasonable to conclude that the functions of these enzymes may be impaired since the concentrations of their cofactors, NADPH (glutathione reductase) and GSH (glutathione peroxidase) are significantly lower than normal. Thus, glutathione reductase would be less effective in reducing oxidized glutathione as well as the mixed disulfides of protein and glutathione.²⁸ Similarly, glutathione peroxidase would be inhibited in its function of reducing hydrogen peroxide and organic hydroperoxides. The decreased effectiveness of these two enzymes could result in oxidative damage to lens epithelial proteins and lens crystallins.

TABLE I

WEIGHTS OF CONTROL AND X-IRRADIATED RABBIT LENSES

Time post X-irradiation	Dry weight (mg)		Wet Weight (mg)	
	Control	X-irradiated	Control	X-irradiated
1 hour	55 $\pm$ 3 (4)	55 $\pm$ 3 (4)	---	---
2 weeks	75 $\pm$ 4 (3)	75 $\pm$ 5 (3)	---	---
4 weeks	83 $\pm$ 2 (8)	81 $\pm$ 1 (8)	262 $\pm$ 15 (14)	256 $\pm$ 12 (14)
5.5 weeks	100 $\pm$ 8 (4)	87 $\pm$ 5 (4)	---	---
7 weeks	111 $\pm$ 6 (3)	89 $\pm$ 5 (3)	312 $\pm$ 12 (12)	250 $\pm$ 21 (12)

The results are expressed as the means $\pm$ S.D. The data for dry weights were obtained from lenses used in measurement of hexose monophosphate shunt activity (Fig. 2).

TABLE II

CONCENTRATION OF PYRIDINE NUCLEOTIDES IN CONTROL
AND EXPERIMENTAL RABBIT LENSES ONE WEEK PRIOR TO
THE FORMATION OF X-RAY INDUCED CATARACT

	Control	X-Irradiated
	nmoles/g wet wt.	nmoles/g wet wt.
NADP ⁺	15.6 ± 1.1 (6)	12.5 ± 1.2 (6)
NADPH	17.7 ± 1.2 (8)	10.3 ± 1.2 (8)
NADPH/NADP ⁺	1.1	0.8
NAD ⁺	988 ± 79 (6)	748 ± 181 (6)
NADH	487 ± 71 (8)	428 ± 91 (*)
NAD ⁺ /NADH	2	1.8

The results are expressed as the means ± the standard deviation. The number of experiments is indicated in parentheses. The pyridine nucleotide levels on a per lens basis can be calculated from the wet weights of lenses, at 7 weeks, listed in Table I.

TABLE III

THE ACTIVITIES OF VARIOUS ENZYMES IN CONTROL AND EXPERIMENTAL LENSES
ONE WEEK PRIOR TO X-RAY INDUCED CATARACT

ENZYME	CONTROL Units/g wet wt.		X-IRRADIATED Units/g wet wt.	
Lactate dehydrogenase (7)	51	± 6.2	51	± 6.0
Glyceraldehyde phosphate dehydrogenase (7)	40	± 3.3	34**	± 3.6
Phosphofructokinase (5)	19	± 0.5	16**	± 0.9
Glutathione peroxidase (3)	1.2	± 0.04	1.2	± 0.08
6-phosphogluconate dehydrogenase (6)	0.28	± 0.02	0.26	± 0.02
Glucose-6-phosphate dehydrogenase (8)	0.24	± 0.01	0.17*	± 0.02
Hexokinase (6)	0.18	± 0.01	0.15**	± 0.02
Glutathione reductase (5)	0.04	± 0.01	0.04	± 0.01

Assays were performed on whole lenses; one enzyme unit corresponds to the conversion of 1 μ mole of substrate per minute at 30°C under optimal conditions.

The number of experiments is shown in parentheses. Values marked with asterisks differ significantly from control (*p < 10⁻³)(**p < 10⁻²)

LEGENDS

Figure 1

The effect of X-irradiation on the concentrations of protein -SH groups and GSH in the lens. Lenses were analyzed at various times following a single X-ray dose of 2,000 rads to the rabbit eye. Values for protein -SH groups are shown by circles and values for GSH are shown by triangles. Open symbols represent experimental values; solid symbols represent control values for non-irradiated contralateral lenses. The values at 8 weeks post X-ray are for mature cataractous lenses. Each value is the mean of at least three experiments and the bars represent standard deviations.

Figure 2

The effect of X-irradiation on the activity of the hexose monophosphate shunt in the lens. Lenses were analyzed at various times following a single X-ray dose of 2,000 rads to the rabbit eye. The activity of the shunt was compared in control (open symbol) and experimental (solid symbol) lenses by measuring $^{14}\text{CO}_2$ liberated from 1- ^{14}C labelled glucose. Controls consisted of non-irradiated contralateral lenses. Values of CO_2 liberated per lens are shown by circles and those per mg dry weight are shown by triangles. Each value is the mean of 3 to 8 experiments and the bars represent standard deviations.

Figure 3

The effect of X-irradiation on the activity of glucose-6-phosphate dehydrogenase in the lens. Lenses were analyzed 4 and 7 weeks following a single X-ray dose of 2,000 rads to the rabbit eye. Open symbols represent experimental values; solid symbols represent control values for non-irradiated contralateral lenses. Values for activity per lens are shown by circles and those per gram wet weight are shown by triangles. Each value is the mean of 8 experiments and the bars represent standard deviations.

REFERENCES

- (1) Benedek, G. B.: Theory of transparency of the eye, *Appl. Optics* 10:459, 1971.
- (2) Spector, A., Freund, T., Li, L.-K. and Augusteyn, R. C.: Age dependent changes in the structure of alpha-crystallin, *Invest. Ophthalmol.* 10:677, 1971.
- (3) Jedziniak, J. A., Kinoshita, J. H., Yates, E. M., Hocker, L. O. and Benedek, G. B.: On the presence and mechanism of formation of heavy molecular weight aggregates in human normal and cataractous lenses, *Exp. Eye Res.* 15:185, 1973.
- (4) Giblin, F. J., Chakrapani, B. and Reddy, V. N.: High molecular weight protein aggregates in X-ray induced cataract, *Exp. Eye Res.* 26:507, 1978.
- (5) Spector, A. and Roy, D.: Disulfide-linked high molecular weight protein associated with human cataract, *Proc. Natl. Acad. Sci. USA* 75:3244, 1978.
- (6) Sedlak, J. and Lindsay, R. H.: Estimation of total, protein-bound, and non-protein sulfhydryl groups in tissue with Ellman's reagent, *Analyt. Biochem.* 25:192, 1968.
- (7) Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J.: Protein measurement with the folin phenol reagent, *J. Biol. Chem.* 193:265, 1951.
- (8) Nisselbaum, J. S. and Green, S.: A simple ultramicro method for determination of pyridine nucleotides in tissues, *Analyt. Biochem.* 27:212, 1969.
- (9) Sippel, T. O.: Pyridine nucleotides in normal and diabetic rat lenses, *Exp. Eye Res.* 1:368, 1962.
- (10) Kinoshita, J. H. and Wachtl, C.: A study of the C^{14} -glucose metabolism of the rabbit lens, *J. Biol. Chem.* 233:5, 1958.
- (11) Kinsey, V. E. and Reddy, D. V. N.: Studies on the crystalline lens. XI. The relative role of the epithelium and capsule in transport, *Invest. Ophthalmol.* 4:104, 1965.
- (12) Reddy, V. N., Chakrapani, B., Rathbun, W. B. and Hough, M. M.: Evidence for lens oxoprolinase, an enzyme of the gamma-glutamyl cycle, *Invest. Ophthalmol.* 14:228, 1975.
- (13) Bergmeyer, H. U.: *Methods of Enzymatic Analysis*, 2nd English edition, Volumes 1 and 2, New York, 1974, Academic Press.
- (14) Lawrence, R. A., Sunde, R. A., Schwartz, G. L. and Hoekstra, W. G.: Glutathione peroxidase activity in rat lens and other tissues in relation to dietary selenium intake, *Exp. Eye Res.* 18:563, 1974.
- (15) Pirie, A., van Heyningen, R. and Boag, J. W.: Changes in lens during the formation of X-ray cataract in rabbits, *Biochem. J.* 54:682, 1953.
- (16) Merola, L. O. and Kinoshita, J. H.: The reactivity of the sulfhydryl groups in normal bovine lens, *Amer. J. Ophthalmol.* 44:326, 1957.
- (17) Bullard, B.: Pyridine nucleotides in the lens of different species, *Exp. Eye Res.* 4:108, 1965.

references cont.

- (18) Bullard, B. and Pirie, A.: Glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in lens and blood of different species, *Exp. Eye Res.* 3:118, 1964.
- (19) Kinoshita, J. H., Futterman, S., Satoh, K. and Merola, L. O.: Factors affecting the formation of sugar alcohols in ocular lens, *Biochim. Biophys. Acta* 74:340, 1963.
- (20) Swanson, A. A.: The effect of radiation on lenticular epithelial enzymes in young rabbits, *Exp. Eye Res.* 4:231, 1965.
- (21) Nordmann, J. and Mandel, P.: Enzyme studies in radiation cataract, *Amer. J. Ophthalmol.* 40:871, 1955.
- (22) van Heyningen, R., Pirie, A. and Boag, J. W.: Changes in lens during the formation of X-ray cataract in rabbits, *Biochem. J.* 56:372, 1954.
- (23) Lerman, S., Devi, A. and Banerjee, R.: The effect of ionizing radiation on protein and carbohydrate metabolism in the rat lens, *Invest. Ophthalmol.* 1:95, 1962.
- (24) Glock, G. E. and McLean, P.: Further studies on the properties and assay of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase of rat liver, *Biochem. J.* 55:400, 1953.
- (25) Sols, A. and Crane, R. K.: The inhibition of brain hexokinase by adenosine-diphosphate and sulfhydryl reagents, *J. Biol. Chem.* 206:925, 1954.
- (26) Harris, I., Meriwether, B. P. and Park, J. H.: Chemical nature of the catalytic sites in glyceraldehyde-3-phosphate dehydrogenase, *Nature* 198:154, 1963.
- (27) Webb, J. L.: *Enzyme and Metabolic Inhibitors. Volume 1*, New York, 1963, Academic Press, pp. 546-547.
- (28) Srivastava, S. K. and Beutler, E.: Cleavage of lens protein-GSH mixed disulfide by glutathione reductase, *Exp. Eye Res.* 17:33, 1973.

FIGURE 1

FIGURE 2

FIGURE 3