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Glutathione and its Function in the Lens—An Overview

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This paper presents an overview of the current state of our knowledge concerning the metabolism and function of glutathione (GSH) in the lens, with particular reference to the contributions of Dr Jin H. Kinoshita to this field. Glutathione in the lens is synthesized from its constituent amino acids and degraded by mechanisms involving transpeptidation and hydrolysis. The turnover of GSH in the lens is due to its catabolism rather than transport of GSSG as is the case in red blood cells and some other tissues. Three aspects of the functional role of GSH in cataract formation are considered. First, GSH may be important in maintaining protein thiols in the reduced state, thus preventing the formation of high molecular weight protein aggregates which are the basis for light scattering and lens opacification. A second function may be to protect membrane -SH groups that are important in cation transport and permeability. A third functional role is to detoxify hydrogen peroxide and other organoperoxides. The glutathione redox cycle is intimately involved in the detoxification of H_2O_2 which is normally present in the aqueous humor.

Key words: glutathione; lens; cataracts; hexose monophosphate shunt; cation transport; protein thiols; oxidation; glutathione redox cycle; H_2O_2; oxidative damage; glutathione reductase; glutathione peroxidase.

1. Introduction

One of Dr Jin Kinoshita's many important contributions to our understanding of the biochemistry of the lens has been his insight into the metabolism and function of glutathione in this tissue. His first publication in this area appeared in 1955. This was of particular significance since it was the first to report the relationship of carbohydrate and glutathione metabolism and how glutathione in the lens could be maintained largely in the reduced form (GSH). These studies demonstrated that the enzyme glutathione reductase was able to catalyze the interaction of NADPH and oxidized glutathione (GSSG) and that in lens homogenates the 'dehydrogenases' in the hexose monophosphate shunt could be coupled with GSH reductase (Kinoshita, 1955). Thus the hexose monophosphate shunt, which he had shown to be an important pathway for glucose metabolism in the lens, might also play a key role in maintaining GSH in the reduced state. He suggested that in the lens, in place of the cytochrome system, the transfer of electrons from NADPH to oxygen may be accomplished through GSH-ascorbic acid oxidation. According to the proposed scheme, GSH may react non-enzymically with dehydroascorbic acid to form ascorbic acid. The oxidation of ascorbic acid was thought to be by oxygen (Kinoshita, 1955). In the absence of an enzyme for the conversion of ascorbic acid to dehydroascorbic acid it was considered possible that the latter compound was furnished by the aqueous humor [Fig. 1(A)]. In this classic paper, he made other important observations. He noted that 'GSSG stimulates the oxidation by 88%, presumably by increasing the rate of oxidation of TPNH'. Pentose shunt was also found to be stimulated by dehydroascorbic acid although to a lesser degree (Table I). The participation of ascorbic acid in the oxidation–reduction of glutathione was later clarified by the work of Pirie (1965) who suggested that the respiratory link between ascorbic acid and glutathione may be hydrogen peroxide rather than oxygen [Fig. 1(B)].

In a subsequent study, Kinoshita examined the factors that may contribute to the maintenance of high concentrations of GSH in the lens (Kinoshita and Masurat, 1957). These studies revealed that the lens has a low activity of the enzyme which could split the γ-glutamyl bond of the tripeptide. Furthermore, it was noted that diffusion of GSH out of the lens was limited, suggesting the possibility that a major fraction may be bound via hydrogen bonding since in the presence of concentrated urea or guanidine solutions GSH could be rapidly extracted from an intact lens. Since it was known that lens synthesized glutathione, the question arose about its normal turnover.

In 1964, in one of the most thought-provoking reviews on the subject, Kinoshita considered the functional role of GSH in the lens. He drew attention to many of the similarities between the metabolism of red blood cells and the ocular lens and speculated that the function of GSH in the lens fibers in cataract formation may have a parallel with Heinz body formation in red cells due to deficiency of GSH. It was suggested that in the cataractous lens the aggregation of proteins may also be the result of loss of GSH.

Some years later, Kinoshita and his associates proposed yet another functional role for GSH in that it may be involved in the protection of membrane sulfhydryl groups which are critical for cation transport. These studies demonstrated that lowering of GSH in the lens had an adverse effect on both active transport and permeability of lens membranes to cations (Epstein and Kinoshita 1970a, b). Only recently several lines of experimental evidence in support of

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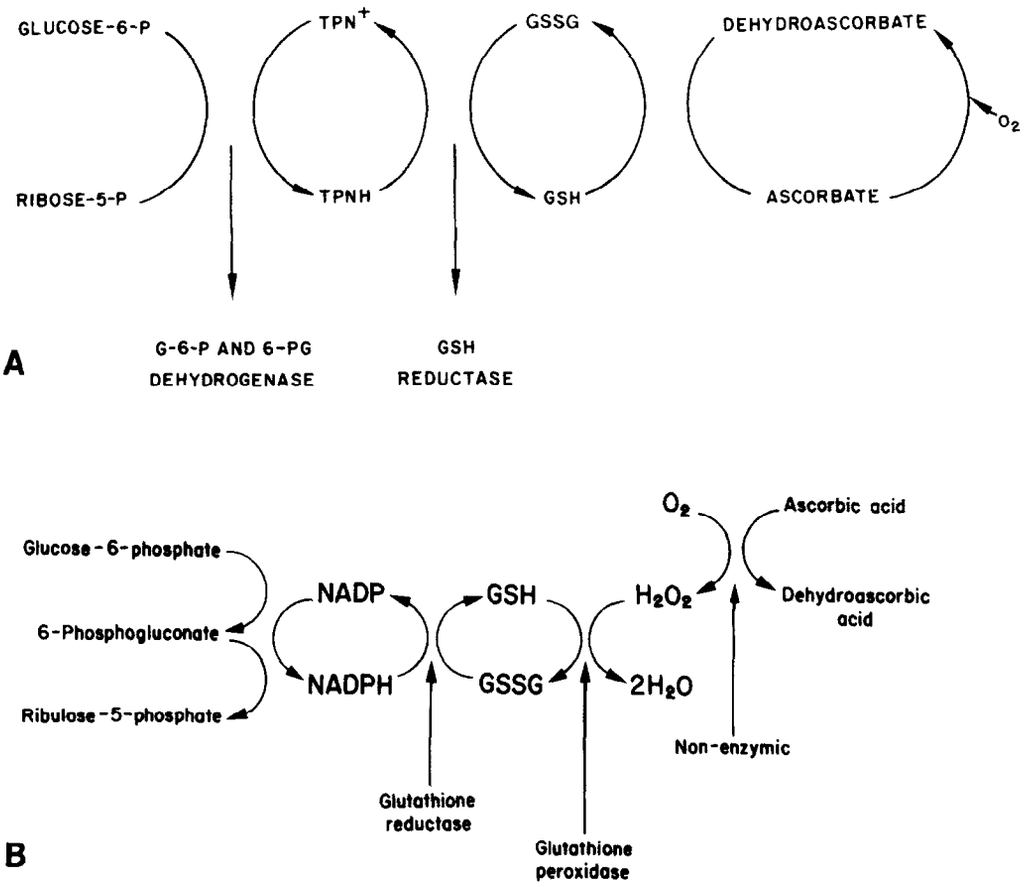

FIG. 1. Scheme for reactions coupling the oxidation-reduction of glutathione and ascorbic acid with the hexose monophosphate shunt [A, adapted from Kinoshita (1955); B, from Reddy (1971)].

these hypotheses have come from a number of laboratories (Reddy and Giblin, 1984).

Thus, many of Dr Kinoshita's investigations in this area have provided a fundamental conceptual framework for further investigations and continue to engage the interests of many ophthalmic biochemists. The purpose of my presentation is to provide an overview of the metabolism and function of GSH in the lens.

2. Metabolism of GSH

It has long been known that the lens contains a high concentration of GSH, a tripeptide composed of the amino acids glycine, cysteine and glutamic acid. Depending on the species its concentration in the lens has been found to vary over a wide range from 2 to 17 μmol g^{-1} (Reddy, 1971). It is generally agreed that the bulk of glutathione is present in the reduced state and is unevenly distributed. Its concentration is higher in the cortex than in the nucleus, the highest being in the epithelium (Reddy, Giblin and Matsuda, 1980). Lens epithelium, a single layer of cells interposed between aqueous humor and the anterior portion of the lens, is metabolically the most active part of the tissue. It is no wonder that the concentration of pyridine nucleotide in epithelium parallels that of GSH. While many substances are transported into the lens by the epithelium (Reddy, 1979), it is well accepted that GSH is synthesized within the lens. The first evidence for in vivo synthesis of GSH in the lens was based on the incorporation of labeled glycine (Kinsey and Merriam, 1950; McMillan, Ryerson and Mortensen, 1959; Reddy, Klethi and Kinsey, 1966) and glutamic acid into GSH of an intact lens. Subsequent studies with tissue homogenates and purified enzymes have firmly established that the synthesis of GSH follows sequential steps involving the enzymes, γ-glutamylcysteine synthetase and glutathione synthetase respectively (Cliffe and Waley, 1958; Rathbun, 1980).

While synthetic reactions were well established, the

TABLE I

Effects of various agents on the oxidation of [1-^{14}C]glucose by lens

Compound added	Recovered in $^{14}CO_2$ (cpm)	Percent of control
0	367±14	
0·01 M NaCN	240±22	65
0·01 M Na azide	300±12	82
0·01 M Oxidized glutathione	690±95	188
0·01 M Dehydroascorbic acid	410±18	114

Kinoshita (1955).

TABLE II

Studies in support of the reactions for the synthesis and catabolism of GSH in the lens as outlined in Fig. 2

Reaction mechanisms	Reference
Incorporation of constituent amino acids into GSH	Kinsey and Merriam (1950), Reddy et al. (1966), McMillan et al. (1959).
Synthesis of GSH to constituent amino acids	Cliffe and Waley (1958), Rathbun (1980)
Breakdown of GSH to constituent amino acids	Reddy et al. (1973).
γ-Glutamyl transpeptidase	Rathbun and Wicker (1973), Reddy and Unaker (1973), Ross et al. (1973).
Cyclotransferase	Cliffe and Waley (1961).
Oxoprolinase	Reddy et al. (1975).

fate of GSH once synthesized was much in doubt. As mentioned earlier, Kinoshita and Masurat (1957) were the first to show that lens homogenates, which effectively split most peptides, were relatively inactive against the γ-glutamyl bond of glutathione. These findings were later confirmed by Cliffe and Waley (1961), who showed that lens extracts could not bring about the breakdown of GSH.

The possibility that GSH may be lost from the lens by leakage was also ruled out by the studies of Kinoshita and Masurat (1957) and Reddy (1971). In rabbit lenses cultured in physiological medium less than 3% of GSH was lost from the lens in a 24-hr culture period. Thus, for many years the mechanism for the turnover of lens GSH remained a mystery. Based on the loss of GSSG under oxidative stress from the lens, Srivastava and Beutler (1969) suggested that the turnover of GSH in the lens may be similar to that in the red cell where GSSG is believed to be transported from the cell against a concentration gradient. In the lens, however, the turnover of GSH may not be due to 'transport' of GSSG but appears to be due to its catabolism, as shown in my laboratory (Reddy, Varma and Chakrapani, 1973). Evidence for catabolism of lens GSH was based on studies in which degradation of labeled GSH was demonstrated. When ^{35}S-labeled GSH was introduced into an intact lens it was 'totally' degraded giving rise to labeled cysteine. Furthermore, the rate of degradation of GSH was time-dependent with a rate constant of 0·014% hr^{-1}. While the loss of some GSH from the lens through diffusion, possibly as GSSG, cannot be entirely excluded, these studies provided conclusive evidence that the rate of degradation of GSH (0·014% hr^{-1}) was in good agreement with its rate of synthesis derived from the incorporation of either ^{14}C-labeled glycine or glutamic acid (0·018% hr^{-1}) into GSH of an intact rabbit lens cultured in vitro.

In extending these studies on the catabolic fate of GSH in the lens, it has been shown that all of the enzymes of the γ-glutamyl cycle are present in the lens. In most tissues, breakdown of GSH involves transpeptidation and hydrolysis. According to this scheme GSH is catalyzed by γ-glutamyl transpeptidase, a membrane-bound enzyme. This enzyme catalyzes the transfer of the γ-glutamyl moiety of GSH to an acceptor amino acid to yield γ-glutamyl amino acid and cysteinyl glycine. The γ-glutamyl amino acids give rise to free amino acids and oxoproline by the enzyme cyclotransferase while cysteinyl glycine is hydrolyzed by a peptidase into free glycine and cysteine. The generation of free glutamic acid from oxoproline by the enzyme oxoprolinase thus provides the necessary free amino acids for the resynthesis of GSH. The presence of all of these enzymes in the lens

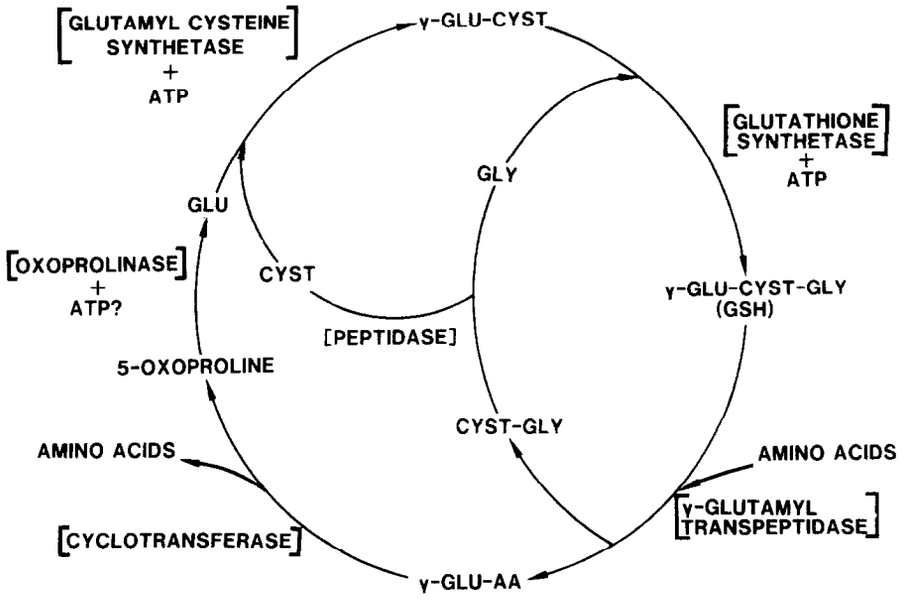

FIG. 2. Reactions involved in the synthesis and degradation of glutathione in the lens.

has been well documented in the studies referred to in Table II. Thus, it may be concluded that the metabolic scheme for lens GSH is comparable to that in many other tissues and that the lens continually synthesizes and catabolizes GSH for its normal turnover as shown in Fig. 2. It is of interest that three moles of ATP, derived mainly from glycolysis, appear to be needed for the normal metabolism of lens GSH, two for its synthesis and one for its degradation. In contrast to the observations in other tissues, ATP requirement for lens oxoprolinase has not been demonstrated (Reddy et al., 1975).

3. Function of GSH

(a) Role in the Maintenance of Protein Thiols in Reduced State

A common feature of most types of cataracts is a dramatic decrease in the level of GSH. The concentration of this tripeptide also decreases with age. Therefore, it has been considered plausible that GSH may play an important role in maintaining lens transparency. Although it has long been postulated that one of the important functions of GSH in the lens may be to protect protein thiol groups and thus prevent cross-linking of soluble crystallins and formation of insoluble proteins (Dische and Zil, 1951; Kinoshita and Merola, 1959; Kinoshita, 1964), experimental evidence for such a hypothesis has been lacking. The presence of high molecular weight (HMW) proteins in aging and cataractous human lenses was reported by Jedziniak et al. (1973) and Spector, Staufter and Sigelman (1973). In X-ray induced cataract, where changes in the level of GSH have been well documented, a significant increase in the content of HMW proteins (Liem-The et al., 1975; Giblin, Chakrapani and Reddy, 1978) has also been demonstrated. Analysis of the -SH groups present in isolated HMW protein, determined in the presence of 8 M urea, showed that 50% of the -SH groups were present in the oxidized state. Treatment of HMW aggregates with disulfide-bond reducing agents resulted in a partial deaggregation of the protein with release of low molecular weight crystallins identified as α-crystallin and low molecular weight β-crystallins. These results clearly suggested that covalent disulfide bond formation was involved in protein aggregation in X-ray cataracts. Additional support for the concept that oxidation of sulfhydryl groups may play a role in the formation of protein aggregates was provided by the studies of Spector and Roy (1978) who found an increase in the disulfide content in HMW proteins isolated from human cataractous lenses.

While a decrease of GSH in the lens is one of the earliest effects of radiation cataract, the loss of protein thiols occurs almost abruptly and is coincident with the development of mature cataract (Giblin, Chakrapani and Reddy, 1979). It appears that protein thiols become oxidized only when the level of GSH in the lens drops below some critical level. In addition to a decrease in GSH, the activity of hexose monophosphate shunt, the level of NADPH and the ratio of NADPH to $NADP^+$ are also significantly lower just prior to the formation of mature X-ray cataract (Giblin et al., 1979). Therefore, these factors may also contribute to the oxidation of protein thiols and lens opacification.

It is of interest that there is a significantly greater oxidation of protein thiols in the nucleus of irradiated lens (Garadi, Giblin and Reddy, 1987) and aging human lens (Harding, 1970) than in the cortex. To the extent that GSH concentration in the nucleus is low, it is possible that higher protein disulfide is the result of low levels of GSH. Similar to the observations in X-ray cataracts, a progressive oxidation of protein -SH has been shown to take place in the human nuclear cataract (Truscott and Augusteyn, 1977) and in advanced stages more than 90% of the -SH was found to be oxidized (Anderson and Spector, 1978). In addition to the oxidation of crystallin -SH groups, disulfide linkage of polypeptides to membrane proteins in human senile cataracts have also been reported (Spector et al., 1979; Takemoto and Hansen, 1982; Kodama and Takemoto, 1988). In rabbit lenses subjected to hyperbaric oxygen there was a decrease in GSH with a concomitant increase in disulfide cross-linking of crystallins in the urea-insoluble fraction (Padgaonkar, Giblin and Reddy, 1989). Also, the lens nucleus was more susceptible to formation of protein disulfide than the cortex.

Inherent in the concept that the role of GSH is to prevent aggregation or cross linking of protein thiols is the formation of mixed disulfides as was first proposed by Kinoshita and Merola (1959). Since then, a number of investigators have attempted the measurement of mixed disulfides of glutathione and protein in the lens (Harding, 1970; Reddy and Han, 1976; Srivastava and Beutler, 1973; Lou, McKellar and Oliver, 1986). Increased amounts of mixed disulfides have been found in cataractous lens, although there appears to be an equilibrium between reduced and oxidized forms of glutathione. Glutathione reductase and NADPH can not only regenerate GSH from GSSG but can cleave mixed disulfides of glutathione (Srivastava and Beutler, 1973), although, in one report, free GSH was shown to exchange with protein-GSH mixed disulfide (Mostafapour and Reddy, 1982–3). In any case, the formation and cleavage of mixed disulfides may serve as a mechanism for the protection of protein thiols of lens crystallins. In a few attempts in which the mechanism of disulfide formation has been investigated it was noted that GSSG rather than GSH interacts with protein thiols to form mixed disulfides (Srivastava and Beutler 1973; Mostafapour and Reddy 1982–3).

(b) Protection of Critical Thiol Groups Involved in Cation Transport and Permeability

Another function of GSH in the lens may be to protect membrane -SH groups that are important for cation transport. Epstein and Kinoshita (1970a, b) first reported that critical thiol groups are involved in the regulation of cation transport in the lens and that depletion of GSH in the lens resulted in the oxidation of -SH groups of cell surface membranes leading to an increase in permeability to cations and inhibition of ^{86}Rb uptake. The relationship of GSH to cation transport in the lens was studied by lowering lens GSH with azoester and diamide. Giblin, Chakrapani and Reddy (1976) investigated the relationship of GSH and transport function in the lens by oxidizing a significant portion of GSH in the epithelium which is the site of major transport function. They examined the rates of influx and efflux of ^{86}Rb following oxidation of 60–90% of GSH in the epithelium with tertiary butyl hydroperoxide (TBHP) while total lens GSH was oxidized by only 25%. From an evaluation of the transfer coefficients for active transport and permeability, it was concluded that 70% of active transport was inhibited while the permeability was doubled. Furthermore, the changes in epithelial function were elicited only when the amount of GSH in the epithelium was decreased by more than 60%.

The mechanisms by which intracellular GSH influences the -SH groups involved in membrane permeability, which are presumably in the surface membranes, are unknown. However, the effect on active transport resulting from the depletion of lens GSH appears to be due to the inactivation of Na^+,K^+-ATPase, a sulfhydryl-containing enzyme (Giblin et al., 1976; Epstein and Kinoshita, 1970a, b). Giblin's studies indicated that when GSH was oxidized with TBHP, Na^+,K^+-ATPase was inactivated irreversibly, probably through a conformational change in the enzyme. These investigators suggested that formation of disulfide bonds within the enzyme may have occurred during the initial oxidation of epithelial GSH.

The lens has a remarkable ability to regenerate reduced glutathione through a series of coupled reactions involving carbohydrate and GSH metabolism (Kinoshita, 1955; Reddy, 1979). Therefore, in studies designed to investigate lens membrane function and GSH, where glutathione levels are lowered through oxidation, varying amounts of GSH are regenerated during subsequent experimental periods so that the exact relationship between GSH level and cation transport cannot be ascertained. A useful reagent to lower cellular GSH is 1-chloro-2-4-dinitrobenzene (CDNB) which is known to conjugate GSH stoichiometrically by a reaction catalyzed by GSH-S-transferase (Keen, Habig and Jakoby, 1976). The irreversible binding of GSH to CDNB makes it possible to maintain GSH at any desired level in assessing the relationship of GSH to lens membrane function. In a recent study (Reddy et al., 1988) it was shown that depletion of GSH in the lens leads to a reduction in the uptake of ^{86}Rb and an increase in its efflux similar to the findings in which oxidizing agents were used to lower lens GSH. When GSH in the epithelium was depleted by 70–90% of the normal level, the efflux rate of ^{86}Rb increased nearly 2·5-fold, but the Na^+,K^+-ATPase activity was unaffected. These studies showed that the endogenous level of lens GSH itself was not the principal factor in maintaining Na^+,K^+-ATPase activity. However, GSH-deficient lenses were more susceptible to oxidative stress as evidenced by a 55% inactivation of membrane ATPase when CDNB-treated lenses were exposed to 0·05 mM H_2O_2 (Fig. 3). These findings are also consistent with the observations that GSH-depleted rat lenses became cloudy upon incubation with peroxide or superoxide anions (Ansari and Srivastava, 1982–3).

The regulation of normal cell volume, involving the transport of electrolytes, is an important factor in the maintenance of lens transparency (Kinoshita, 1965). Many types of cataracts appear to involve changes in electrolytes. In the X-ray cataract model a correlation exists between the loss of GSH, inactivation of Na^+,K^+-ATPase and increased permeability of lens membranes to cations (Reddy and Giblin, 1984). The effect of GSH on cation transport in the lens appears to have an indirect effect on protein synthesis. Studies in several cataract models have suggested that alterations in the concentrations of cations in the lens may influence the

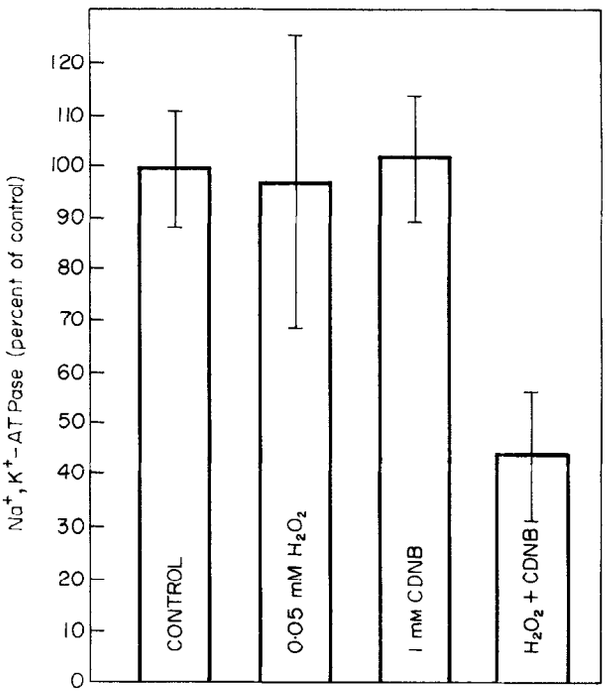

FIG. 3. Effects of H_2O_2 and CDNB on the activity of Na^+,K^+-ATPase in rabbit lenses expressed as percent of control. All lenses were cultured for 30 min in the presence or absence of 1 mM CDNB and Na^+,K^+-ATPase was measured after a 4-hr culture period in normal Tyrode's medium in the presence or absence of H_2O_2. Values are expressed as percent of control (from Reddy et al., 1988).

rate of protein synthesis (Shinohara and Piatigorsky, 1977; Piatigorsky, 1980; Piatigorsky, Kador and Kinoshita, 1980). Garadi et al. (1984) showed that in X-ray cataracts there was a significant decrease in the incorporation of [^{35}S]methionine into crystallins, cytoskeletal and membrane proteins of fiber cells. These changes in protein synthesis were correlated with changes in Na^+ and K^+ levels.

(c) *Protection Against Oxidative Damage*

Among the various postulated functions of GSH in the lens, the one which has received considerable attention during the last decade is its role in protection against oxidative damage. The generation of free radicals and/or hydrogen peroxide in various tissues has been considered a prime factor in the deleterious effects of certain drugs or their metabolites. GSH may act either directly as a protective agent by scavenging the damaging free radicals or by protecting other reactive thiols of enzymes or membrane proteins, as I have already mentioned. H_2O_2 has been shown to be present in normal aqueous humor of various species. Pirie (1965) proposed that peroxide is formed in the aqueous humor during the oxidation of ascorbic acid which is normally present in high concentration in these fluids [Fig. 1(B)]. A correlation between the level of ascorbic acid and H_2O_2 has been shown recently (Giblin et al., 1984). The oxidation reaction is thought to be catalyzed by light and riboflavin (Pirie, 1965). The peroxide so formed diffuses into the lens where it is detoxified enzymically by means of coupled reactions involving glutathione peroxidase, glutathione reductase and the hexose monophosphate shunt. The epithelium appears to be primarily responsible for the detoxification of H_2O_2 (Reddy and Giblin, 1984; Giblin et al., 1987). Consistent with this role, the epithelium has a high concentration of GSH and NADPH along with substantial activity of the related enzyme.

If peroxide was not detoxified by the lens, oxidative damage to lens membranes and susceptible protein thiol groups would result (Fukui, Epstein and Kinoshita, 1973; Fukui, 1976; Garner, Garner and Spector, 1983; Giblin et al., 1987; Ikebe, Susan, Giblin, Reddan and Reddy, 1989). Since high concentrations of H_2O_2 have been found in aqueous humor in association with some human cataracts, it has been suggested that in aging human lenses H_2O_2 may be a factor in the development of nuclear cataracts (Spector and Garner, 1981). The central role of GSH and the glutathione redox cycle in the detoxification of H_2O_2 in the lens has been extensively studied in our laboratory (Reddy and Giblin, 1984). These studies showed that exposure of the lens to H_2O_2 results in significant stimulation of hexose monophosphate shunt and that there was a direct correlation between the amount of GSSG formed and the level of shunt activity. Above the physiological level of peroxide, the activity of the shunt decreased

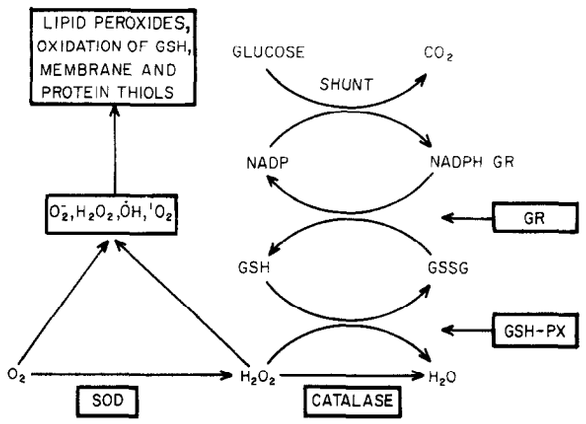

FIG. 4. Schematic representation of possible cellular oxidation-reduction reactions in the lens. GR, glutathione reductase; GSH-Px, glutathione peroxidase; SOD, superoxide dismutase; OH, hydroxyl radical; $^1O_2$, singlet oxygen (from Reddy et al., 1980).

and normal levels of GSH could no longer be maintained, thus leading to oxidative damage to the lens.

The relationship of GSH metabolism to the detoxification of H_2O_2 in the lens was also studied by blocking one of the key enzymes of glutathione redox cycle. Inhibition of glutathione reductase with 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), a relatively specific inhibitor, resulted in lower shunt stimulation, accumulation of GSSG, inhibition of Na^+,K^+-ATPase activity, and reduced ^{86}Rb uptake (Reddy and Giblin, 1984; Giblin et al., 1987).

In conclusion, although I have emphasized the role of GSH metabolism in the detoxification of H_2O_2, there are other mechanisms (Bhuyan and Bhuyan, 1977a, b) for protection against oxidative damage by free radicals and other oxidizing species (Fig. 4). Free radicals, such as superoxide, OH radical and singlet oxygen, could originate from oxygen as well as the products of ionizing radiation, UV and visible light. Some of these could be directly removed by reaction with GSH. Superoxide dismutase may render the highly reactive O_2^- radical to the less reactive H_2O_2 (Varma, Kumar and Richards, 1979). Catalase, also present in the lens, may react with H_2O_2 to form H_2O and molecular O_2. However, at the physiological concentration of H_2O_2 present in the eye, glutathione peroxidase, rather than catalase appears to be more important in the detoxification of H_2O_2 as discussed elsewhere in this symposium (Giblin et al., 1990). Furthermore, while glutathione peroxidase shares the substrate H_2O_2 with catalase, it alone can react effectively with lipid and other organic hydroperoxides. Thus glutathione peroxidase may also have a role in the repair mechanisms by removing organic peroxides.

Acknowledgments

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