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# Changes in Lens During the Formation of X-ray Cataract in Rabbits. 2

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There is progressive and finally almost complete loss of glutathione and of protein sulphydryl groups during the formation of lens opacity. This has long been known and has led to many investigations to determine at what stage the change in the sulphydryl groups takes place. Bellows & Rosner (1937) showed that the glutathione decreased before any opacity developed in the lenses of rats fed with galactose, and Bellows & Schoch (1950) observed the same in rabbits after injection of alloxan. Kinsey & Merriam (1950) measured glutathione synthesis by culturing lenses *in vitro* in the presence of [$C^{14}$]glycine, and in a small series they found that such lenses, from rabbits that had been fed on naphthalene 2–5 days previously, synthesized less glutathione than lenses from normal rabbits. Twelve days after the last naphthalene dose, the rate of glutathione synthesis had returned to normal. Kinsey & Merriam therefore considered that during the development of cataract the synthesis of glutathione became impaired, but that it might recover.

The earliest change which we have found in the rabbit lens during development of a cataract induced by X-rays is a diminution of glutathione (GSH) level which begins before any clinical change can be detected (Pirie, van Heyningen & Boag, 1953). (This paper is to be considered as Part I of the series.) This decrease is accompanied by a decrease in the activity of glutathione reductase, but the activity of four other enzymes was not affected, even in the opaque lens. None of these four enzymes (lactic dehydrogenase, malic enzyme, cytochrome *c* reductase and *iso*citric dehydrogenase) is thought to contain functional sulphydryl (SH) groups.

Since protein sulphydryl is also diminished during the formation of cataract, though at a later stage than glutathione (Pirie *et al.* 1953), we have investigated the effect of X-irradiation of the eye of a rabbit, with consequent cataract formation, on three enzymes and one coenzyme, all known to depend on SH groups for their activity. The enzymes we have investigated are glyceraldehyde-phosphate dehydrogenase, which contains GSH as a prosthetic group (Krimsky & Racker, 1952), glyoxalase, whose substrate is a compound of glutathione and methylglyoxal (Racker, 1951), and an acetaldehyde oxidase, which is probably an SH enzyme. The activity of aldolase, a non-sulphydryl enzyme, was also determined.

It also seemed logical to try to assess the change in coenzyme A (CoA) after irradiation of the lens, since its sulphydryl group is essential for activity (Lynen, Reichert & Rueff, 1951). As the method of extraction of CoA from the lens by boiling is the same as that used to extract coenzymes containing nicotinic acid, we took the opportunity, whenever material was available, of estimating nicotinic acid as well as CoA. While it is possible that changes in the concentration of nicotinic acid would reflect changes in that of the active coenzymes diphosphopyridine nucleotide (DPN) and triphosphopyridine nucleotide (TPN), we do not know what proportion of the nicotinic acid in lens is present in the form of these

coenzymes. Robinson, Levitas, Rosen & Perlzweig (1947) showed that, in rat liver, kidney and muscle, all the nicotinic acid was, in fact, in the form of DPN and TPN. A change in one or more of these coenzymes might be of particular importance, as they mediate so many steps in metabolism.

## EXPERIMENTAL

### *Materials*

*Lens suspension and extract.* These were prepared as before (Pirie *et al.* 1953).

*Diphosphopyridine nucleotide (DPN).* A crude preparation of nucleotides containing 30% DPN and 12% TPN was used (Lepage & Mueller, 1949).

### *Methods*

Cataract was produced exactly as previously described, by irradiation of the right eye of a young rabbit, of known age, with X-rays (1400 r), leaving the left eye and lens barely affected. Change in enzymic activity and coenzyme content is expressed per lens rather than per g., as the weight of the irradiated lens changes, and we are interested in the change in the whole lens.

*Reduced glutathione.* GSH in the lens suspension was estimated by the method of Grunert & Phillips (1951). The total amount was measured in the right and the left lenses wherever possible, to supplement the slit-lamp and ophthalmoscopic assessment of cataract development. The GSH begins to diminish before any visible change is apparent, and continues to fall as the degree of opacity increases (Pirie *et al.* 1953). The concn. in the left lens (263–440 mg./100 g. wet wt.) was within the normal range. The percentage decrease in GSH after irradiation is given in Tables 1 and 3 in order that a comparison may be made between GSH loss and diminution of enzymes and coenzymes.

*Glyceraldehyde phosphate dehydrogenase (GPD).* This was estimated in Warburg manometers by measuring the rate of liberation of $CO_2$ from $NaHCO_3$ due to production of phosphoglyceric acid in the following reaction sequence:

Hexose diphosphate $\rightarrow$ glyceraldehyde phosphate (GP) + dihydroxyacetonephosphate (*aldolase*).

Dihydroxyacetonephosphate $\rightarrow$ GP (*triosephosphate isomerase*).

$2GP + 2DPN^+ \rightarrow 2$ phosphoglyceric acid $+ 2DPNH$ (*GPD*) (in presence of arsenate).

2 Pyruvate $+ 2DPNH \rightarrow 2$ lactate $+ 2DPN^+$ (*lactic dehydrogenase*).

Crystalline aldolase (prepared by the method of Cori, Slein & Cori (1948) and once recrystallized) and lactic dehydrogenase (kindly given by Dr D. M. Needham, F.R.S.) were added in excess so that the GPD in lens should be the limiting enzyme. No acid was formed in the absence of added lens. Arsenate was added, to enable the oxidation of GP to go to completion, and fluoride to inhibit phosphatases. The activity of the enzyme was always measured at two different concentrations, usually 0·3 and 0·6 ml. of 1:10 lens suspension in 3·0 ml. total vol., and the rate of acid production was proportional to the amount of lens. The enzymes were mixed with glutathione in the manometer flasks before adding the substrate, to ensure that any diminution of activity in the irradiated lens could not be due to the lowered concentration of GSH therein.

The contents of the manometer flasks were as follows: suitable dilutions of lens suspension, aldolase and lactic dehydrogenase, sodium arsenate (18 $\mu$moles), DPN (approx. 0·15 $\mu$mole), sodium pyrophosphate (3 $\mu$moles), glutathione (6·5 $\mu$moles), $NaHCO_3$ (111 $\mu$moles), KF (150 $\mu$moles); total volume 2·6 ml. The side arm contained magnesium hexose diphosphate (12 $\mu$moles) and sodium pyruvate (10 $\mu$moles) in 0·4 ml. The gas phase was 95% $N_2$ + 5% $CO_2$ and the temperature 37°. The pH of the reaction mixture was calculated to be 7·8.

The activity of the enzyme is expressed as $\mu$l. $CO_2$ evolved/min. by the whole lens. The evolution was measured during 15–20 min.

*Glyoxalase.* This was measured in Warburg manometers by the method of Crook & Law (1952). Portions of 0·1 and 0·2 ml. of a 1:10 lens suspension were used and the rate of reaction was proportional to the amount of lens used. The activity of the enzyme is expressed as $\mu$l. $CO_2$ evolved/min. by the whole lens. The evolution was measured from 5 to 15 min. after starting the reaction.

*Aldolase.* This was measured at 37° by a modification of the method of Taylor, Green & Cori (1948). The activity of the enzyme is expressed as the $\mu$g. triose P formed/5 min. by the whole lens. The duration of the experiment was 15 min.

*Acetaldehyde oxidase.* This enzyme oxidizes acetaldehyde to acetic acid and requires DPN. The reduction of DPN was followed in a Beckman spectrophotometer in a 1 cm. cell at 340 m$\mu$. The system consisted of lens extract (0·2–0·5 ml.), acetaldehyde (10 $\mu$moles), DPN (0·14 $\mu$mole) and sodium pyrophosphate, pH 9 (60 $\mu$moles); total volume 3·0 ml. The activity of the enzyme is expressed as 100 $\times$ the increase in the optical density ($\Delta \log I_0/I$) at 340 m$\mu$./10 min. for the whole lens, readings being taken for 10 min. from 15 sec. after the addition of DPN, during which time the action was linear.

*Acetic acid* was estimated by the method of Bartley (1953).

*Coenzyme A.* The method of Kaplan & Lipmann (1948) was used, the sulphanilamide being estimated by the method of Bratton & Marshall (1939) and the CoA expressed as 'Lipmann units'. The lens was dissected from the eye, weighed and immediately dropped, whole, into a tared tube containing 1·5 ml. of 0·2 M sodium acetate buffer, pH 5·7, standing in a boiling-water bath. After 3 min., the tube was removed, cooled and re-weighed. Any loss in weight due to evaporation was made up by addition of water, and the lens, which had usually partly disintegrated during the boiling, was rubbed up in the fluid with a glass rod. The suspension was centrifuged at 8000 *g*, and the CoA estimated in the supernatant fluid. The dilution of the lens in the sodium acetate was approximately 1:4 and 0·2–0·3 ml. were used for each test. Several dilutions of a standard CoA extract from liver were tested simultaneously each time the lens was tested. Owing to the small concentration of CoA in the lens extract, the mixtures were incubated at 37° for 3 hr., rather than for the 2 hr. recommended by Kaplan & Lipmann (1948).

*Estimation of nicotinic acid.* The lens was boiled as described for extraction of CoA and the supernatant fluid used. Nicotinic acid was determined by the method of Kodicek (1940), the final colour being read at 430 m$\mu$. using the Beckman spectrophotometer.

## RESULTS

### *Clinical course of formation of lens opacity*

This has been described in the previous paper. The left lens was affected only very slightly, or not at all, and for simplicity we shall refer to it as normal (Pirie *et al.* 1953).

### *Changes in enzymes and coenzymes after irradiation*

The results for the enzymes studied are given in Table 1. The rabbits are listed in decreasing order of severity of their cataracts, judged by slit-lamp and ophthalmoscopic appearance the day before death; the numbers are consecutive with those in our earlier paper. The order of cataract severity was decided by one of us before knowing the order of the changes of lens constituents. The lenses were opaque in nos. 21–24 inclusive. Nos. 25 and 26 showed major, but not complete, opacities and nos. 35, 36 and 37 showed only a trace of opacity in the lens.

*Glyceraldehyde phosphate dehydrogenase and glyoxalase.* The GPD activity of normal (left) lenses ranged from 63 to 183 $\mu$l. $CO_2$/min./lens and glyoxalase activity from 115 to 393 $\mu$l. $CO_2$/min./lens. The two enzymes were affected to roughly the same extent in any particular, irradiated lens. On two occasions (rabbits nos. 29 and 33), their activity was considerably diminished before gross changes in the

Table 1. *Comparison between right (irradiated) and left lenses. Changes in lens weight, concentration of reduced glutathione, and activity of glyceraldehyde phosphate dehydrogenase, glyoxalase, aldolase, and acetaldehyde oxidase*

(Rabbits are listed in order of decreasing severity of lens opacity. Methods of estimation of enzymic activity given in the Methods section.)

| Rabbit no. | | Lens weight (mg.) | Time after irradiation (weeks) | Decrease in irradiated lens (%) | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | GSH | Glyceraldehyde phosphate dehydrogenase | Glyoxalase | Aldolase | Acetaldehyde oxidase |
| 21* | R | 577 | 42 | — | 75 | 88 | — | 67 |
| | L | 499 | | | | | | |
| 22* | R | 631 | 43 | — | 100 | 100 | — | — |
| | L | 529 | | | | | | |
| 23* | R | 626 | 45 | 95 | — | — | 70 | — |
| | L | 548 | | | | | | |
| 24* | R | 611 | 26 | 82 | 28 | 22 | — | 61 |
| | L | 474 | | | | | | |
| 25 | R | 494 | 17 | 87 | — | — | — | 42 |
| | L | 450 | | | | | | |
| 26 | R | 448 | 25 | 70 | 15 | 16 | — | 45 |
| | L | 487 | | | | | | |
| 27 | R | 389 | 32 | 61 | — | 41 | 29 | — |
| | L | 468 | | | | | | |
| 28 | R | 447 | 32 | 48 | — | 23 | 0 | — |
| | L | 497 | | | | | | |
| 29 | R | 463 | 22 | — | 52 | 38 | — | 18 |
| | L | 473 | | | | | | |
| 30 | R | 457 | 22 | 37 | 0 | 6 | — | 12 |
| | L | 473 | | | | | | |
| 31 | R | 475 | 21 | 38 | 9 | 6 | 0 | 40 |
| | L | 495 | | | | | | |
| 32 | R | 470 | 20 | 49 | — | 9 | 0 | — |
| | L | 479 | | | | | | |
| 33 | R | 450 | 16 | 49 | 40 | 46 | 0 | — |
| | L | 470 | | | | | | |
| 34 | R | 398 | 18 | 40 | — | — | — | 23 |
| | L | 424 | | | | | | |
| 35 | R | 441 | 17 | 22 | 0 | 11 | — | 30 |
| | L | 488 | | | | | | |
| 36 | R | 420 | 15 | 32 | — | — | — | 24 |
| | L | 433 | | | | | | |
| 37 | R | 436 | 17 | 9 | — | — | — | 10 |
| | L | 444 | | | | | | |

* Lens opaque.

lens had occurred, but in rabbits nos. 24 and 26, where the cataract was well advanced, there was only slight loss of activity. Fig. 1 compares the activities of GPD in right and left lens of rabbit no. 29, and Fig. 2 compares the activity of glyoxalase in right and left lens of rabbit no. 33.

*Aldolase.* This enzyme was considerably affected only in the opaque lens (Table 1).

*Acetaldehyde oxidase.* This enzyme, which has not previously been described in the lens, is present in the lenses of both cattle and rabbits. It is similar to that described by Racker (1949) in ox liver. No attempt has been made to purify the enzyme, but some of its properties have been studied in lens extracts. Activity is assessed by measuring the increase in absorption at 340 m$\mu$. in the presence of DPN. Under the conditions that we used, the enzyme reduces DPN not TPN; the pH optimum is near 9·0 and it has been established, using the method of Bartley (1953), that the product of the reaction is acetic acid.

We found that the enzyme was almost inactive in any buffer other than pyrophosphate, and at first considered that this showed that coenzyme A took part in the reaction (cf. Lipmann, Jones, Black & Flynn, 1952). However, no evidence for this could be obtained. Dialysis of the lens extract for 48 hr. against running tap water did not alter enzymic activity, although it would probably have removed the small amount of CoA present. Lynen *et al.* (1951) showed that incubation with 0·08M iodoacetate for 2 hr. at room temperature inactivated CoA through combination of the iodoacetate with the SH group of the coenzyme. Such incubation before the testing of the DPN preparations used (one our own and one commercial), both of which were found to contain CoA, did not inhibit enzymic activity.

Pyrophosphate would seem to exert its activating effect through its chelating properties. The enzyme was almost inactive in phosphate, borate, glycine, veronal, and tris(hydroxymethyl)aminomethane buffers but was active in pyrophosphate, or in other buffers plus ethylenediaminetetraacetate. In the presence of small amounts of pyrophosphate, addition of $Cu^{2+}$ inactivated the enzyme, but the addition of ethylenediaminetetraacetate prevented inactivation. These results are shown in Table 2. The pH optimum in ethylenediaminetetraacetate was near 9·0, as in pyrophosphate. The most active enzymic preparations were obtained by grinding and extracting the lens in the presence of ethylenediaminetetraacetate. We wish to thank Dr S. M. Altmann for bringing this effect of pyrophosphate on enzymes to our notice. Altmann & Crook (1953) found that chelating agents, among them pyrophosphate, markedly stimulated succinoxidase under certain conditions.

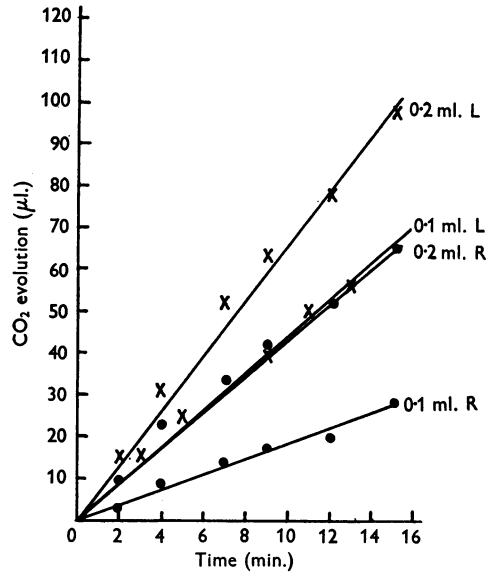


Fig. 1. Activity of glyceraldehyde phosphate dehydrogenase in right (irradiated) and left (normal) lens of rabbit no. 29.

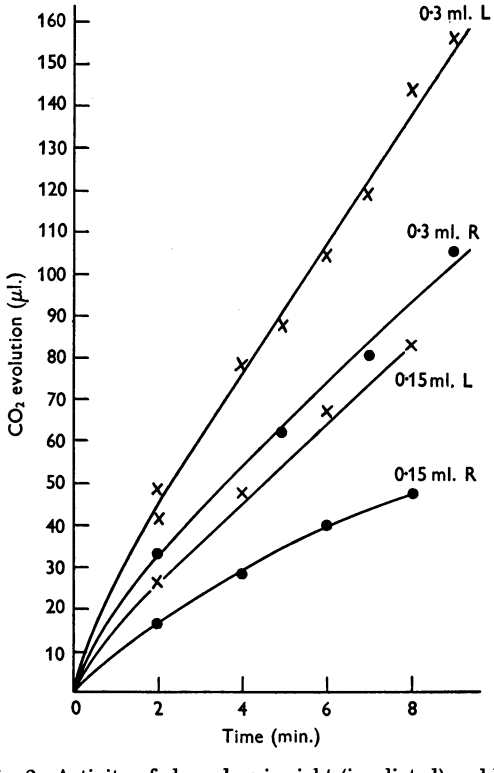


Fig. 2. Activity of glyoxalase in right (irradiated) and left (normal) lens of rabbit no. 33.

Table 2. *Activation of acetaldehyde oxidase by chelating agents and inactivation by copper*

(Activity was determined by measuring increase of absorption at 340 mμ. during 5 min. ($\Delta E$). Each cell contained 0·4 ml. 1:4 cattle-lens extract, dialysed 48 hr.; acetaldehyde (10 μmoles), DPN (0·14 μmole), and other components as indicated, in a total vol. of 3·0 ml. All tests were at pH 9·0, the lens extract being adjusted before test.)

| Addition | $\Delta E$ Expt. 1 | $\Delta E$ Expt. 2 |
|---|---|---|
| — | 0·012 | 0·001 |
| 60 μmoles $Na_4P_2O_7$ | 0·084 | 0·118 |
| 16 μmoles ethylenediaminetetraacetate | 0·104 | 0·134 |
| 60 μmoles $Na_4P_2O_7$ + 16 μmoles ethylenediaminetetraacetate | 0·104 | — |
| 15 μmoles $Na_4P_2O_7$ | 0·077 | — |
| 15 μmoles $Na_4P_2O_7$ + 3 μmoles $CuSO_4$ | 0·014 | — |
| 15 μmoles $Na_4P_2O_7$ + 3 μmoles $CuSO_4$ + 16 μmoles ethylenediaminetetraacetate | 0·109 | — |

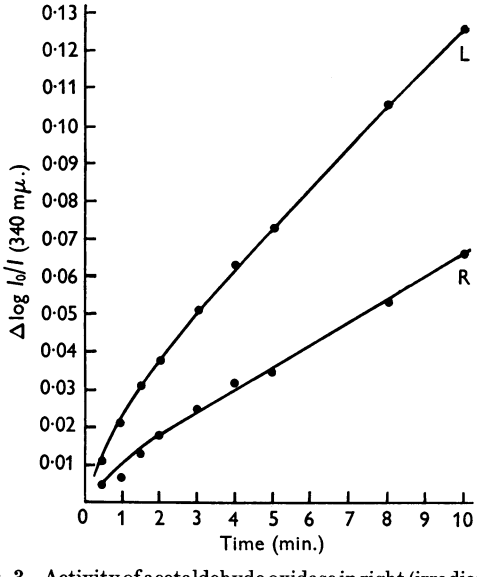


Fig. 3. Activity of acetaldehyde oxidase in right (irradiated) and left (normal) lens of rabbit no. 25.

Treatment of dialysed lens extract with iodoacetate for short periods at room temperature had no effect on activity, but after 2 hr. in the presence of 0·08 M iodoacetate activity dropped by about half. Immediate inactivation of the enzyme occurred if dialysed lens extract were treated with 3 mM iodoacetamide or with even lower concentrations (0·2–1·2 mM) of *o*-iodosobenzoate. Such inactivation took place in the presence of pyrophosphate or ethylenediaminetetraacetate. We take this as evidence that the activity of the enzyme depends on the presence of SH groups in its molecule.

Fig. 3 compares the activity of acetaldehyde oxidase in the right and left lenses of rabbit no. 25, the right lens showing a fall to about half the activity of the left. Table 1 shows that the activity of acetaldehyde oxidase is more regularly depressed at an early stage after irradiation than is that of glyceraldehydephosphate dehydrogenase, glyoxalase, or aldolase. The decrease in activity was the same whether pyrophosphate buffer, or phosphate buffer in the presence of ethylenediaminetetraacetate, was used. The decrease is, however, variable and is never complete. Rabbit no. 35, which showed a 30 % decrease in acetaldehyde oxidase activity in the right lens, was killed 4 months after irradiation and showed only traces of opacity in the lens, yet rabbit no. 30, killed 6 months after irradiation, had multiple peripheral opacities and yet the acetaldehyde oxidase activity was only diminished by 12 %. In rabbit no. 34, comparison was made between loss of aldehyde oxidase and loss of protein sulphydryl. It was found that protein SH had diminished by 11 % and the acetaldehyde oxidase by 23 %.

*Coenzyme A after irradiation.* The normal rabbit lens contains about 10 units CoA/g., about one-tenth of the concentration in liver (Table 3). The two lenses from a single normal animal differ by as much as 20 %, but this may be experimental error, which is large when such small amounts of coenzyme A are estimated. We found that the opaque lens contained little or no CoA, but that in the early stages of opacity there was little change. For example, rabbit no. 44, killed 23 weeks after irradiation, showed definite posterior opacities when examined with the ophthalmoscope, but the CoA content was a little higher in the irradiated than in the normal lens. The glutathione content of the irradiated lens had fallen by 39 %. It seems, therefore, that loss of CoA is not a primary change attendant on formation of opacity. The glutathione content of lenses used to estimate CoA and nicotinic acid was necessarily estimated in the boiled extract (see Methods). The values found were on the whole slightly higher than the values found in extracts prepared in the usual way.

*Nicotinic acid after irradiation.* Change in nicotinic acid followed much the same course as change in CoA (Table 3). No change was found when glutathione had already fallen, but by the time the lens was opaque there was a 77 % loss of nicotinic acid. Kodicek (1940) gave a value of 50 μg./g. of nicotinic acid in ox lens and found 100 μg./g. in a human cataract. He concluded that the nicotinic acid level was unaffected in cataract. Our results show that it is impossible to generalize, and that nicotinic acid does gradually fall in the rabbit lens during the formation of opacity after X-ray injury. Such a fall cannot, however, be considered as a cause of the opacity, as it takes place later than the fall in glutathione, and indeed after the opacities are clinically visible.

Table 3. *Change in coenzyme A and in nicotinic acid content in lens after irradiation*

(Rabbits listed in order of decreasing severity of lens opacity. Methods of estimation are described in Methods section.)

| Rabbit no. | | Lens weight (mg.) | Time after irradiation (weeks) | GSH decrease (%) | Coenzyme A | | Nicotinic acid | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Units per lens | Difference (%) | μg. per lens | Difference (%) |
| 38* | R | 518 | 44 | — | Nil | — | — | — |
| | L | 519 | | | — | | — | |
| 39* | R | 639 | 46 | — | Nil | −100 | — | — |
| | L | 548 | | | 5·1 | | — | |
| 40* | R | 479 | 39 | — | 2·0 | −64 | 25 | −77 |
| | L | 510 | | | 5·6 | | 113 | |
| 41* | R | 467 | 28 | — | 1·2 | −86 | — | — |
| | L | 518 | | | 9·0 | | — | |
| 42 | R | 550 | 22 | 86 | 2·1 | −53 | 29 | −42 |
| | L | 512 | | | 4·5 | | 49 | |
| 43 | R | 451 | 26 | 63 | 1·1 | −70 | 23 | −46 |
| | L | 516 | | | 3·7 | | 43 | |
| 44 | R | 460 | 23 | 39 | 5·1 | +8 | 28 | −8 |
| | L | 482 | | | 4·7 | | 30 | |
| 45 | R | 441 | 19 | 10 | 4·1 | +13 | 33 | −11 |
| | L | 441 | | | 3·6 | | 37 | |
| Normal | R | 569 | — | — | 3·7 | +9 | 30 | −12 |
| | L | 560 | | | 3·4 | | 35 | |
| Normal | R | 572 | — | — | 5·9 | +22 | — | — |
| | L | 563 | | | 4·8 | | — | |
| Normal | R | 443 | — | — | 4·5 | — | 37 | −10 |
| | L | 446 | | | 4·6 | | 41 | |

* Lens opaque.

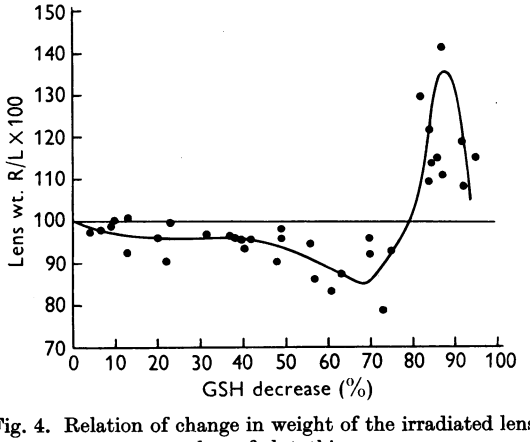


Fig. 4. Relation of change in weight of the irradiated lens to loss of glutathione.

*Lens weight*

In Fig. 4, the weight of the right lens is plotted against the loss in its GSH content, both values being expressed as percentage of that in the left lens. The figure includes points calculated from the data in Tables 1 and 3, others from data given in a previous paper (Pirie *et al.* 1953), and two further values from rabbits used in experiments outside the scope of this paper. Our previous suggestion, that in the early stages of formation of opacity the affected lens weighs less than the normal lens, is confirmed. There appears to be a rather sharp border line between this condition and a state in which the lens is intumescent and considerably heavier than the normal one. The change takes place when the GSH content has decreased by about 80 %. There is a suggestion that, as the cataract develops and the loss of GSH becomes even greater, the relative weight of the irradiated lens falls, but more observations are needed to confirm this.

## DISCUSSION

The results reported here, together with those in our earlier paper (Pirie *et al.* 1953), support the suggestion of Bellows & Rosner (1937) and of Bellows & Schoch (1950) that loss of glutathione occurs early in cataract development. We have now studied altogether eight different enzymes in lens after irradiation, and the results, in general, show that, of these particular enzymes, those activities which depend on sulphydryl groups are affected before those which are not so dependent. We found that cytochrome *c* reductase, lactic dehydrogenase, *iso*citric dehydrogenase, and malic enzyme, are normally active in the opaque lens (Pirie *et al.* 1953), and aldolase only loses activity in the very late

stages. These enzymes are not dependent on sulphydryl groups. On the other hand, glyceraldehyde phosphate dehydrogenase, glyoxalase, and acetaldehyde oxidase, which progressively lose activity during the formation of opacity, need sulphydryl groups. Change in the enzymic activity occurs later than change in glutathione concentration, which suggests that the concentration of enzyme sulphydryl groups is maintained to some extent at the expense of glutathione.

The tests of activity of those enzymes needing glutathione, either as a coenzyme or as a stabilizer, have been made in the presence of an added excess of glutathione. Fall in enzymic activity is therefore not simply due to a fall in the glutathione content, although it may be a consequence of this. If glutathione acts as a stabilizer of protein SH, then the enzymes may become irreversibly inactivated when the glutathione in the lens falls below a certain level. It is possible that this is the reason why enzymes dependent on sulphydryl groups appear to be more susceptible to damage.

Glutathione reductase appears to be an exception, in that loss of activity can be detected very early in cataract formation, and it is doubtful whether it is an enzyme activated by sulphydryl groups (van Heyningen & Pirie, 1953). It is possible that the loss of glutathione in the irradiated lens is due both to fall in the activity of glutathione reductase and to a decrease in the rate of its synthesis, as described by Kinsey & Merriam (1950) in naphthalene-induced cataract.

With the small amount of tissue available, it is difficult to estimate the coenzyme content with accuracy, but it appears as if coenzyme A, and also nicotinic acid, diminish progressively in the lens as the opacity develops, but no striking change occurs at an early stage.

Apart from changes in certain enzymes and coenzymes, our results show that a general change must take place before clinical opacities are advanced. The irradiated lens may be 5–10 % lighter than the lens in the normal fellow-eye when only very slight changes can be seen with the slit-lamp or ophthalmoscope. Salit (1939) and Bloch & Salit (1946) have shown that, in development of nuclear sclerosis or of cataract in the human lens, there is a loss of lens protein. Salit, Swan & Paul (1942) showed that in rats fed with galactose, the wet weight of the lens fell progressively during the development of cataract and, at the same time, the dry weight fell even more sharply, until a completely opaque lens had only 31 % of the dry weight of a normal lens. Merriam & Kinsey (1950) calculated from studies with radioactive glycine that the turnover of protein in the lens of the rabbit is 2·5–5 %/day. It is possible that the loss in weight of the lens after irradiation may be explicable as a failure of protein synthesis.

We must record that all the changes we have described occur some time after irradiation. They provide no direct evidence on the initial physical and chemical changes in the lens caused by the radiation, but they are of interest in the general study of cataract production.

## SUMMARY

1. Glyceraldehyde phosphate dehydrogenase, glyoxalase, acetaldehyde oxidase, and aldolase, are present in the rabbit lens.

2. The activities of glyceraldehyde phosphate dehydrogenase, glyoxalase, and acetaldehyde oxidase, fall during development of opacity in the lens after X-irradiation.

3. The activity of aldolase is unchanged except in the opaque lens, when it is diminished.

4. The content of coenzyme A and of the nicotinic acid falls gradually during the development of opacity.

5. The weight of the irradiated lens is less than that of the fellow-eye during the early stage of opacity formation.

6. These results are discussed in relation to the known changes in glutathione and in protein sulphydryl groups in cataract.

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# Metabolism of Thiamine Phosphates in Washed Suspensions of Kidney Particles

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Whilst it is certain that the oxidation of pyruvate and α-oxoglutarate in animal tissues requires, among other cofactors, cocarboxylase (Lohmann & Schuster, 1937; Barron, Goldinger, Lipton & Lyman, 1941; Stumpf, Zarudnaya & Green, 1947), the precise role of this coenzyme is still obscure. As the oxidative decarboxylation of α-ketonic acids is accompanied by the synthesis of pyrophosphate bonds of ATP, it seemed possible that cocarboxylase might act as a phosphate carrier (Kiessling & Lindhal, 1952). This hypothesis became capable of experimental testing with the availability of isotopic phosphorus and of methods capable of separating small quantities of organic phosphates by paper chromatography (Hanes & Isherwood, 1949; Eggleston & Hems, 1952; Bartley, 1953*b*; Krebs & Hems, 1953). Experiments are reported in this paper in which the rate of incorporation of $^{32}PO_4$ into thiamine phosphates was measured and compared with the rate of oxidation of α-ketonic acids.

## EXPERIMENTAL

*Special chemicals.* Thiamine triphosphate, thiamine diphosphate, oxythiamine diphosphate and thiamine were commercial samples (Roche Products Ltd.) of more than 95% purity. Thiamine monophosphate was prepared by the method of Karrer & Viscontini (1946) and oxythiamine by the method of Rydon (1951). Adenosine triphosphate (ATP) preparations contained about 80% ATP and 10% adenosine diphosphate (ADP). α-Oxoglutarate was prepared by the method of Friedman & Kosower (1946) and was not less than 98% pure, as estimated by the method of Krebs (1950). Pure sodium pyruvate was prepared according to Robertson (1942). All other reagents were of analytical grade.

*Chemical estimations.* Keto-acids were estimated by the 'specific' extraction method of Friedemann & Haugen (1943), succinate manometrically according to Krebs (1937), and free thiamine directly in the trichloroacetic acid (TCA) fitrates by the method of Melnick & Field (1938), the optical density of the red colour extracted into xylene being measured spectrophotometrically at 520 mμ. Measurements of radioactivity were made on a sample of the diluted, wet-ashed material or on the *iso*butanol extract obtained by the phosphate estimation. An M/6 liquid counter (20th Century Electronics) was used.

*Preparation and incubation of the particulate suspensions.* Particulate suspensions of sheep-kidney cortex, consisting mainly of mitochondria suspended in 0·9% (w/v) KCl, were prepared as described by Bartley (1953*a*). In all cases the '$R_3$ residue' was used. Samples (2 ml.) of the tissue suspension (60–100 mg. dry wt.) were added to 2 ml. of medium in the main compartment of conical Warburg vessels which included ATP, $MgCl_2$ (both at 0·001 M final concn.) and phosphate buffer, pH 7·4 (final concn. usually 0·005 M). The centre wells contained NaOH and filter paper, with $O_2$ in the gas space. The $O_2$ uptake was measured at 40° and the incubation was stopped at varying times by transfer of the vessel to ice-water and addition of 0·5 ml. 30% (w/v) TCA to each vessel. The precipitated protein was removed by centrifugation and the TCA extract was stored at −18° until it could be analysed.

*Paper-chromatographic separation, identification and estimation of thiamine phosphates.* The method of Viscontini, Bonetti, Ebnöther & Karrer (1951) proved unsuitable, as it failed to separate thiamine mono- and di-phosphates, and a new method was therefore elaborated. Whatman no. 1 filter paper washed with ethylenediaminetetraacetic acid (Eggleston & Hems, 1952) was used throughout. Of several solvent systems tested, namely benzyl alcohol/formic acid, cellosolve/water, dioxan, *tert.*-pentyl alcohol/formic acid, *iso*propyl ether/formic acid and *p*-toluenesulphonic acid/*tert.*-pentanol/water (Hanes & Isherwood, 1949), only the last was satisfactory. Solutions were delivered from an Agla syringe (Burroughs Wellcome Ltd.) either as spots or as bands along a line 8 cm. from the end of a rectangular filter paper, 18 × 45 cm. After drying in a current of cold air, the papers were irrigated with the solvent by descending