SUMMARY

1. The phosphorylation quotient

$$\left(\frac{\text{equivalents of organic phosphate formed}}{\text{atoms of oxygen consumed}} \right)$$

associated with the oxidation of pyruvate by suspensions of washed subcellular particles of kidney cortex was calculated from measurements of the rate of incorporation of ^{32}P into adenosinetriphosphate.

2. When pyruvate, in the presence of bicarbonate, was completely oxidized (as indicated by an O_2 /pyruvate ratio of 2.5) the average phosphorylation quotient was 2.23 (range 2.08–2.6).

3. When pyruvate was incompletely oxidized phosphorylation quotients up to 3.92 were obtained. From the regression line obtained by plotting phosphorylation quotients against the O_2 /pyruvate ratio, a phosphorylation quotient of 4 was obtained for the oxidation of pyruvate to acetate.

4. Either bicarbonate, oxaloacetate or a precursor of oxaloacetate must be present if pyruvate is to be oxidized beyond acetate. The average phosphorylation quotient in the presence of fumarate (1.7) or oxaloacetate (2.13) was lower than that obtained with bicarbonate. Unfractionated suspensions completely oxidizing pyruvate gave a phosphorylation quotient of 1.3. Reasons for phosphorylation quotients below the expected value of 3 are considered.

5. In presence of 0.001M-fumarate or oxaloacetate, pyruvate (0.0025M) was oxidized beyond the stage of acetate (O_2 /pyruvate ratio greater than 0.5) but was not completely oxidized.

6. At pH 7.8, a phosphorus-containing compound other than adenosinetriphosphate or orthophosphate, probably pyrophosphate, was formed.

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

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Experimental work on cataract is directed towards finding some change which precedes or occurs early in opacity formation. Work has been hampered by lack of suitable experimental material, and though there is considerable information about the end results of senile cataract (Bellows, 1944), little is known of the changes occurring in animal lenses during the production of experimental cataract.

The chemistry of the two lenses of a single normal animal seems to be very similar (Bellows, 1944), just as their weights are practically identical. It is therefore an advantage to be able to produce cataract in one lens only, leaving the second as a control. We have chosen to irradiate the right eye of rabbits and thus produce a cataract in this eye while barely affecting the other. Irradiation

cataract due to X-rays has been known for a long time and has been described in detail in rabbits by Poppe (1942), von Sallmann (1951), Cogan & Donaldson (1951) and others. Standardization of age of rabbit and technique of irradiation and dosage leads to a predictable course of opacity formation. In our experiments the lens is usually opaque and intumescent about 7 months after irradiation, and the metabolism of the right lens can be directly compared with that of the left lens. Such a comparison may be made at any stage after irradiation.

We have compared the activities of five enzymes in such lenses: (i) glutathione (GSSG) reductase, so far the only enzyme in lens known to use GSSG as substrate; (ii) and (iii) malic enzyme and isocitric dehydrogenase, both of which can be coupled in lens with the GSSG-reductase through triphosphopyridine nucleotide (TPN) (van Heyningen & Pirie, 1953); (iv) lactic dehydrogenase; and (v) cytochrome *c* reductase. Cytochrome *c* has been shown to be present only in the epithelium of the lens (Ely, 1952) and the function of this enzyme throughout the substance of the lens is not known.

Decrease of SH groups in senile cataract is well established, and diminution of the ascorbic acid in the aqueous humour has also been reported (see Bellows, 1944). We have therefore studied the effect of irradiation on the reduced glutathione (GSH) and protein SH (PSH) of the lens and on the ascorbic acid of the aqueous humour, vitreous humour and, where possible, the lens.

EXPERIMENTAL

Animals. Dutch rabbits 6 ± 2 months old were used. Nearly all were 6 months old.

Clinical examination. The eyes were examined with the slit-lamp and ophthalmoscope before irradiation and at various times afterwards in order to determine the course of cataract formation.

Irradiation. The irradiation was carried out with a Philips Metalix 200 kV. X-ray therapy set, operated at 190 kV., 6 ma. with a filter of 0.5 mm. Cu + 1 mm. Al. A lead tube 2.5 cm. int. diam. and 18 cm. long defined a vertical X-ray beam. The rabbit to be treated lay on its left side with the right eye centrally placed in the end of the lead tube. The distance from the X-ray target to the end of the lead tube was 28 cm. The dose rate at the level of the rabbit's lens was measured before each set of irradiations with four small condenser ionization chambers (BD2 chambers made from bakelite graphite and calibrated at the National Physical Laboratory) placed side by side in a Perspex block. On different occasions the dose rate varied from 67 r./min. to 72 r./min., but the exposure time was chosen to give a total dose of 1400 r. to the right lens in every case. Although the measurement of dose rate and of time were both accurate to 1%, variations in position of the rabbits might lead to errors of a few per cent in the dose to the lens, but certainly not as much as 5%. The dose received by the left lens during this treatment was measured by irradiating in the usual way a rabbit carcass from which the left eye had been removed

and replaced by a condenser chamber enclosed in a thin waterproof sheath. It was found that the left eye, if on the axis of the beam, received a dose of 548–596 r. In setting up the rabbits, however, it was the position of the right eye which was considered important and the left eye was not always central in the X-ray beam. On many occasions, therefore, the left eye probably received much less than this. The position of the X-ray beam both at entry and at exit became visible on the rabbit as a patch of grey hair, and in many cases this showed that the left lens must have been outside the beam of X-rays.

Removal of eyes and preparation of eye tissues and fluids for examination. The rabbit was killed with intravenous Nembutal. The eyes were at once excised and blotted to remove blood. A capillary glass pipette was then pushed through the cornea, and the aqueous humour, which ran into the pipette, was blown out into a weighed tube containing 0.2 ml. 5% (w/v) metaphosphoric acid which was then reweighed. The weight of the aqueous humour was usually 0.1–0.2 g. The cornea and the iris were then cut off and the lens removed in its capsule by cutting round the zonule. Small tags of pigmented ciliary processes could be removed by blotting gently with filter paper. The vitreous body was next expressed from the back part of the eye, weighed and dropped into 2 ml. 5% (w/v) metaphosphoric acid. The weight of the vitreous body was between 1.0 and 2.0 g. The metaphosphoric acid solutions of the aqueous humour and vitreous body were centrifuged and the supernatants used for the estimation of ascorbic acid.

Lens suspension. The lens was weighed, chopped, and ground with a little sand and three times its weight of distilled water. The sand quickly settled and samples of the suspension were taken for estimation of GSH and some of the enzymes; the rest was dialysed 24–48 hr. against running tap water.

Lens extracts. The lens extracts were opalescent and were prepared by centrifuging the suspension for 20 min. at 7000 g.

Diphosphopyridine nucleotide (DPN) and TPN. A mixture of the crude nucleotides was prepared by the method of LePage & Mueller (1949).

o-Iodosobenzoic acid. This was prepared by the method of Askenasy & Meyer (1893).

Methods

Ascorbic acid. This was estimated by titration with 2,6-dichlorophenolindophenol made up so that 1.0 ml. $\equiv$ 0.1 mg. ascorbic acid. Aqueous humour (0.05 ml.) was titrated with dye contained in an Agla micrometer syringe, using mechanical stirring of the drop. Vitreous humour was titrated from a 2.0 ml. burette using 0.2 ml. dye.

Reduced glutathione. 0.2 ml. undialysed lens suspension was precipitated with 1.5 ml. 2.5% (w/v) metaphosphoric acid and GSH was estimated in the extract by the method of Grunert & Phillips (1951).

Protein SH. This was estimated in undenatured lens protein by an adaptation of the method of Hellerman, Chinard & Deitz (1943). 1.0 ml. *o*-iodosobenzoate (approx. 0.005 N, standardized against 0.005 N- $\text{Na}_2\text{S}_2\text{O}_3$) was added to a suitable volume of dialysed lens extract or suspension (0.1–0.3 ml.) in 2.0 ml. 0.1 M-potassium phosphate buffer, pH 7. After exactly 2 min. a freshly prepared solution of 0.5 g. KI in 5 ml. 0.1 N-HCl was added, and the iodine liberated from the excess *o*-iodosobenzoate titrated against

0.005N- $\text{Na}_2\text{S}_2\text{O}_3$. During the 2 min. incubation we found that the colour given by the nitroprusside reaction decreased from a bright puce to a very pale rose, but was not quite abolished. Further incubation decreased the colour only very slowly and at the same time the amount of *o*-iodosobenzoate reacting with the lens slowly increased. However, as groups in proteins other than SH may react with *o*-iodosobenzoate, the incubation time was restricted to the 2 min. suggested by Hellerman *et al.* (1943).

Malic enzyme. This was estimated by following the reduction of TPN as previously described (van Heyningen & Pirie, 1953).

isoCitric dehydrogenase. Reduction of TPN was followed in a Beckman spectrophotometer according to the method of Lotspeich & Peters (1951). 0.3 ml. was used and readings were taken at 1 min. intervals for 10 min.

Lactic dehydrogenase. Reduction of DPN was followed spectrophotometrically according to the method of Askonas (1951). 0.01 or 0.02 ml. undialysed lens extract was used and readings were taken at 0.5 min. intervals for 2.5 min.

Activity of the malic enzyme, and of isocitric and lactic dehydrogenases is expressed as increase in optical density ($\Delta \log I_0/I$) at 340 $\text{m}\mu$ /min. for the whole lens.

Cytochrome-c reductase. Reduction of cytochrome *c* was measured at 550 $\text{m}\mu$. TPN was reduced in the Beckman cell using isocitric dehydrogenase in a dialysed extract of acetone heart powder (Lotspeich & Peters, 1951) which itself had no cytochrome reductase activity. The system consisted of D-isocitrate (1.4 μ moles), MnCl_2 (2.0 μ moles), TPN (approx. 0.28 μ mole), heart extract (0.1 ml.), cytochrome *c* (prepared by the method of Keilin & Hartree, 1937) (0.2 ml.) and 0.029M-veronal-acetate buffer (Michaelis, 1931), pH 7 (2.0 ml.); total vol. 2.95 ml. Reduction of TPN was followed at 340 $\text{m}\mu$. until nearly complete (5 min.); 0.05 ml. undialysed lens extract was then added and the increase of absorption at 550 $\text{m}\mu$. read at 1 min. intervals for 5 min. The activity is expressed as increase in optical density at 550 $\text{m}\mu$ /min. for the whole lens.

Glutathione reductase. The activity of this enzyme was measured by coupling it with the decarboxylation of isocitrate (Ochoa, Mehler & Kornberg, 1948), according to the following equations:

where TPN^+ represents oxidized TPN and TPNH represents reduced TPN. Although lens will decarboxylate isocitrate, a dialysed extract of acetone heart powder (which we found contained no GSSG-reductase) was added to ensure that the reductase was always the limiting enzyme. Dialysed lens extract or suspension (0.5 ml.) was incubated at 37° with heart extract (0.1 ml.), GSSG (8.1 μ moles), isocitrate (9.4 μ moles), TPN (approx. 0.28 μ mole), MnCl_2 (0.5 μ moles) and 0.029M-veronal-acetate buffer pH 7.25 (0.8 ml.); total vol. 1.8 ml. A blank with all the reagents except lens was always included. One drop of CHCl_3 was added to each tube and 0.4 ml. samples were pipetted into 2.0 ml. freshly prepared cold 10% (w/v) trichloroacetic acid at intervals, usually 0, 1.5, 3 and 4.5 hr. α -Ketoglutarate was estimated in the extract by the method of Friedemann & Haugen (1943) and the activity of the enzyme was expressed as mg. α -ketoglutaric acid produced by the whole lens/hr. Under these conditions it was not possible to estimate the increase in GSH, owing to the uncatalysed interaction between GSH, GSSG and the SH and S.S groups in protein.

RESULTS

Clinical course of formation of lens opacity

This was very similar in all the rabbits. Within 24 hr. of irradiation both eyes showed cells in the anterior chamber with a slight flare and debris on the lens capsule. This quickly subsided and was followed in about a week by a cellular invasion of the anterior vitreous body, which might last for a week or a fortnight. About 3 weeks to 1 month after irradiation many rabbits showed a massive pigment slide on the cornea caused by wandering of the pigmented epithelium of the limbus on to the cornea. The fur came off the lids in 4–7 weeks and was later replaced by sparse grey fur. First signs of lens opacity occurred in about 4 months when small greyish opacities could be seen with the slit-lamp at the back of the lens. Water clefts at the equator and changes in the anterior cortex were evident in about 5 months and the opacity was often sufficiently dense to prevent view of the fundus 6 months after irradiation. We found, like Cogan & Donaldson (1951), that rate of opacity formation depended very much on the age of the rabbit when irradiated. The left lens is estimated to receive a maximum of about 40% of the radiation received by the right lens and the eye showed the cellular reactions in the anterior chamber and vitreous body. In a few rabbits there was a small granular opacity at the centre back surface of the left lens but at the time the rabbit was killed this involved only a minute part of the total lens.

The results from nineteen rabbits are arranged in Table 1, in which the rabbits are listed in decreasing order of the severity of their cataract, judged by slit-lamp examination on the day before death. The order of cataract severity was decided by one of us before knowing the order of the changes of lens constituents. The lens was opaque in nos. 1–8 inclusive. Nos. 9–13 showed major but not complete opacities. The last seven (nos. 14–20) were killed at intervals before the development of severe cataract, in order to find out at what stage metabolic changes could first be observed. Nos. 14–17, inclusive, had relatively slight opacities confined to the front and back of the lens. No. 18, killed 3 months after irradiation, showed no changes in the lens on slit-lamp examination. The lenses of nos. 19 and 20, killed 20 hr. after irradiation, showed no abnormality, although the usual initial inflammatory reactions were present in both eyes. In these young rabbits the two right lenses were combined and the two left lenses were combined, as a single lens was too small for all the determinations.

In no animal did the left lens, on removal, show any changes visible to the naked eye, although in some of the rabbits with severely affected right

lenses the slit-lamp appearance of the left lens had been slightly abnormal. Any abnormality in the left lens was certainly tiny and for simplicity we shall refer to the left lenses as normal.

It seems that the lens in the earlier stages of cataract weighed rather less than the normal lens (Table 1, rabbits nos. 9-17 inclusive). On the other hand, the severely cataractous lens was usually considerably heavier than the normal one, apparently because of an increase in water content (rabbits nos. 1-8 inclusive). To take into account this variation between the weights of the two lenses, comparisons are made throughout between whole lenses

and not on a wet weight basis. This tends to minimize losses in the heavy cataract lenses but to maximize losses at the early stage.

Effect of irradiation on GSH and protein SH in lens

The GSH content of right and left lenses was compared on fifteen occasions (Table 1). In the eight rabbits in which the cataract was complete there was a decrease of GSH in the right lens of 84 % or more. In the rabbits in which only a small part of the lens was affected, the decreases ranged from 57 % in no. 14 to 20 % in no. 17. In general, the

Table 1. *Comparison between right (irradiated) and left lenses. Changes in reduced glutathione, protein SH and glutathione reductase*

(The rabbits are listed in order of decreasing severity of lens opacity. GSSG-reductase activity is given in mg. α -ketoglutaric acid formed by the whole lens/hr. Detailed methods are given in the Methods section.)

		Lens wt. (mg.)	Time after irradn. (months)	SH per lens (mg.)					GSSG-reductase		
No.				GSH	Loss (%)	PSH susp.	Loss (%)	PSH extract	Loss (%)	Extract	Loss (%)
1	R	546	9	0.029	85	0.37	53	0.14	75	—	
	L	481		0.199		0.79		0.55		—	
2	R	726	5.5	—		—		—		0.042	48
	L	500		—		—		—		0.081	
3	R	595	5	0.037	84	—		—		—	
	L	490		0.232		—		—		—	
4	R	547	7	0.027	84	0.59	44	0.35	55	0.058	54
	L	502		0.166		1.06		0.77		0.125	
5	R	592	8	0.013	92	—		—		—	
	L	499		0.162		—		—		—	
6	R	495	6	—		0.68	39	0.50	45	—	
	L	486		—		1.12		0.91		—	
7	R	725	5.5	0.021	87	—		—		0.073	41
	L	514		0.165		—		—		0.123	
8	R	663	6	0*	>84	—		—		0.029	63
	L	473		0.131		—		—		0.079	
9	R	499	7	0.057	70	—		0.81	17	0.055	49
	L	527		0.188		—		0.98		0.109	
11	R	500	7	—		—		—		0.011	88
	L	518		—		—		—		0.089	
12	R	459	4.5	0.044	75	—		—		0.038	54
	L	498		0.175		—		—		0.082	
13	R	389	5	0.053	73	0.56	19	0.46	31	0.083	49
	L	496		0.195		0.69		0.67		0.162	
14	R	439	7.5	0.071	57	0.65	13	0.58	18	0.106	34
	L	509		0.167		0.75		0.71		0.161	
15	R	406	5	0.075	56	—		0.63	2	0.089	17
	L	430		0.172		—		0.64		0.107	
16	R	430	7.5	0.146	23	—		—		0.112	11
	L	499		0.189		—		—		0.126	
17	R	472	4.5	0.130	20	0.74	0	0.66	3	0.054	23
	L	491		0.162		0.74		0.68		0.070	
18	R	470	3	0.167	13	0.69	1	0.54	4	—	
	L	468		0.193		0.70		0.56		—	
19	R	400	20 hr.	0.158	4	0.56	0	0.40	2	0.034	0
20	L	412	20 hr.	0.165		0.56		0.41		0.034	

* Less than 0.021.

more severe the opacity, as judged by slit-lamp appearance, the greater the reduction in GSH content of the lens. In no. 18, which had no opacity, the GSH had already fallen by 13%; in nos. 19 and 20 there was no significant change. Thus the GSH content, while unaffected on the day following irradiation, began to decrease before any visible change had occurred in the lens and continued to fall as the degree of opacity increased. The GSH of the left lenses were all within normal limits (Bellows, 1944).

The PSH content of lens extract was compared in ten rabbits. In general, the decrease in PSH in the affected lens was greater the more severe the cataract, but in all cases the decrease was less than that of the GSH. Furthermore, there was no change in PSH in rabbits nos. 15, 17 and 18, whereas the GSH was significantly diminished. Thus it appears that GSH is affected before PSH and to a greater extent.

The PSH content of the suspension was reduced to a rather smaller extent than that of the extract (Table 1). As there appears to be little change in the SH content of the insoluble protein (i.e. difference between values for suspension and extract) (Table 1), the decrease in SH must be mostly in the soluble protein.

Effect of irradiation on enzymes in lens

Of the five enzymes which have been compared (Tables 1 and 2) only one, glutathione reductase, was affected by irradiation.

Glutathione-reductase. This is the only enzyme in lens known to use GSSG as its substrate and so it is interesting to find, in each of twelve comparisons, that enzyme activity was less in extracts of the right, cataractous, lens than it was in extracts of the corresponding left lens (Table 1). (The results are expressed as mg. α -ketoglutaric acid formed by the whole lens/hr. by coupling the reductase, through TPN, with the decarboxylation of isocitrate (see Methods section).) There was no difference in activity between the right and left lenses of the rabbits killed on the day after irradiation (nos. 19 and 20), and in comparisons between extracts of lenses from five normal rabbits the activity of the right and left lenses did not differ by more than 5%. The decrease in GSSG-reductase activity could be detected as soon as there was any sign of the opacity in the lens. In rabbits nos. 15–17 there was some decrease in both GSH and GSSG reductase, while the PSH was unchanged.

Malic enzyme. In the lenses of seven normal rabbits the difference in activity between the right and left lens of the same animal was never more than 13%. The activities ranged between 1.2 and 1.8. Table 2 shows that there was no consistent difference between the right and left lenses of four

experimental animals, and the activities were of the same order as in the normal animals.

Table 2. *Comparison between right (irradiated) and left lenses. Activity of malic enzyme, isocitric and lactic dehydrogenases and cytochrome-reductase*

(The rabbits are listed in order of decreasing severity of lens opacity. The methods of test of the individual enzymes are given in the Methods section.)

No.		$\Delta \log I_0/I \times 10^2/\text{min.}$ for the whole lens			
		Malic enzyme	isoCitric dehydro- genase	Lactic dehydro- genase	Cyto- chrome reductase
1	R	—	—	—	35
	L	—	—	—	39
2	R	1.2	2.8	—	—
	L	0.8	2.5	—	—
3	R	—	—	130	42
	L	—	—	131	23
7	R	—	—	220	43
	L	—	—	260	56
8	R	—	—	318	—
	L	—	—	318	—
10	R	1.7	1.3	—	—
	L	1.8	1.1	—	—
11	R	1.3	2.8	—	—
	L	1.2	2.1	—	—
12	R	1.5	2.3	—	—
	L	1.8	3.0	—	—

isoCitric dehydrogenase. The activity of this enzyme was compared between the right and left lenses of three normal rabbits. They differed by 3, 12 and 33% respectively, and the activities ranged from 1.8 to 2.6. A comparison of the right and left lenses of four experimental animals showed that the isocitric-dehydrogenase activities did not differ significantly and were of the same order as in the normal animals.

Lactic dehydrogenase and cytochrome reductase. These two enzymes are both much more active in lens extracts than are the malic enzyme or isocitric dehydrogenase. Each enzyme was estimated in three irradiated animals and there was no consistent difference in activity between the right and left lenses.

Effect of irradiation on ascorbic acid in the eye

The concentration of ascorbic acid in all parts of the normal rabbit eye is many times greater than that of the blood. In any particular animal the level varies with age and with intake of ascorbic acid in food but it is the same in the two eyes of one animal. Goldmann & Buschke (1935–6) found that the ascorbic acid of the aqueous humour in the two eyes of one rabbit might differ by ± 4.1 mg./100 ml., and consider that variations larger than this are abnormal.

Table 3. *Ascorbic acid content of aqueous humour and vitreous body in right (irradiated) and left eyes*

(The rabbits are listed in order of decreasing severity of lens opacity. Nos. 1-8 had opaque lenses when killed. Ascorbic acid was estimated by titration with 2:6-dichlorophenolindophenol (see Methods section).)

No.	Ascorbic acid (mg./100 g.)					
	Aqueous humour			Vitreous body		
	Right	Left	Difference	Right	Left	Difference
1	29	34	-5	20	18	+2
2	—	40	—	15	14	+1
3	24	32	-8	13	13	0
4	49	52	-3	30	17	+13
5	29	33	-4	19	14	+5
6	25	26	-1	18	14	+4
7	12	30	-18	14	13	+1
8	40	46	-6	37	25	+12
9	30	40	-10	16	14	+2
10	12	29	-17	18	12	+6
11	49	64	-15	25	19	+6
12	15	22	-7	12	12	0
13	15	20	-5	8	8	0
14	29	29	0	22	20	+2
15	34	37	-3	17	14	+3
16	32	28	+4	14	13	+1
18	35	40	-5	17	19	-2
19	49	44	+5	19	16	+3
20	44	35	+9	18	16	+2

We have estimated ascorbic acid in the aqueous humour and vitreous humour and occasionally in the lens. Table 3 shows that of sixteen irradiated rabbits with varying degrees of lens opacity, nine have lower ascorbic acid in the aqueous humour from the irradiated right eye and six, on the basis of Goldmann & Buschke's results, show no change. The two rabbits killed 20 hr. after irradiation show no change. Of those showing a reduction in ascorbic acid four had opaque lenses and five were killed before the opacity was fully developed. Of those showing no change three had complete and three incomplete opacities. It therefore seems that though there is a tendency for the ascorbic acid of the aqueous humour to fall after irradiation the fall is not correlated with opacity formation in the lens.

The ascorbic acid of the vitreous body is lower than that of the aqueous humour. After irradiation there is no decrease and in some cases there seems even to be an increase in ascorbic acid content. It may be that the level of ascorbic acid, like that of glucose (Adler, 1930), is dependent on retinal metabolism. Damage to the retina by irradiation might diminish its use of ascorbic acid which would then tend to accumulate in the vitreous body.

We have been able to estimate ascorbic acid in only a few lenses, but have found that there is little change even when the opacity is far advanced. For example, in rabbit no. 1 the right lens contained 27 mg. ascorbic acid/100 g. and the left lens 23 mg./100 g.

DISCUSSION

The course of development of cataract in these rabbits is the same as that described by Cogan & Donaldson (1951) who concluded that '... the lens is a relatively susceptible organ to irradiation with energies used in therapy (200 kV.-1000 kV.) and that the threshold for the production of cataract is less than that for any other gross abnormality of the eye or periocular tissue...'. The earliest changes found in the lens after irradiation occur in the dividing cells of the epithelium. von Sallmann (1952) found complete inhibition of mitosis 2 hr. after a dose of 1500 r. to a rabbit. This inhibition persisted for 3-4 days and was followed a few days later by excess mitoses. Nuclear fragmentation and extrusion of Feulgen-positive materials could be seen 2 hr. after irradiation.

von Sallmann (1951), using a staining reaction, found no change in deoxyribonucleic acid (DNA) of the epithelium up to 3 days after irradiation, but 2-3 weeks later the DNA was lower in the irradiated lens. von Sallmann & Locke (1951) found that lenses with complete irradiation cataract took up more radioactive sodium and iodine and less radioactive potassium and phosphorus than normal, but early cataract changes did not alter phosphorus uptake. In fact, no early biochemical change has been found after irradiation, yet it would seem possible that irradiation causes some change in all lens cells, not only in the epithelium, to lead to complete opacity and disorganization.

The earliest change we have found is diminution of GSH which begins before any abnormality is visible by slit-lamp examination of the lens. Bellows & Rosner (1937) showed a decrease of GSH in the lenses of rats fed galactose before any opacity developed, and Bellows & Schoch (1950) found that GSH diminished in rabbit lenses 72 hr. after alloxan treatment, if by then the rabbit had developed a high blood sugar; the first abnormality in the lens *in vivo* was noticed at the first weekly examination with the slit-lamp. Waters (1950), who also examined the lenses of rabbits made diabetic with alloxan, found that while the GSH remained within normal limits the protein SH was significantly lowered immediately after injection and did not regain its normal value even after 2 years. Dische & Zil (1951) found reduction in both PSH and GSH in human senile cataract.

In our experiments the first signs of clinical opacity were accompanied by a loss in GSSG-reductase activity and a further decrease in the GSH content. On the basis of this correlation between GSH and enzyme activity it is tempting to consider that glutathione reductase plays a part in the changes in SH occurring in cataract formation, especially as it is the only enzyme we have found to be affected. It is remarkable to find that in the opaque lens the activity of the other enzymes tested so far, cytochrome reductase, lactic dehydrogenase, isocitric dehydrogenase and malic decarboxylase is normal.

The fact that the loss in PSH began at a later stage in the development of opacity than that of

GSH, and was always less pronounced, suggests that the level of PSH is maintained to some extent at the expense of GSH. Some of the protein SH will consist of SH groups in enzymes. Experiments are being done to find out whether enzymes known to contain functional SH groups are affected in the development of radiation cataract.

SUMMARY

1. Decrease of reduced glutathione occurred in the lens after X-radiation before any clinical change could be detected. Loss of reduced glutathione increased as the opacity progressed.

2. Protein SH groups decreased at a later stage of cataract formation than reduced glutathione, and to a smaller extent.

3. Decrease of reduced glutathione in lens was accompanied by a decrease of glutathione-reductase activity. The activity of four other enzymes was unaffected even in completely opaque lenses.

4. There was a slight fall of ascorbic acid in the aqueous humour after irradiation, but it was not correlated with the formation of opacity in the lens. There was no decrease of ascorbic acid in the lens or vitreous body.

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