EXPERIMENTAL STUDIES ON EARLY LENS CHANGES AFTER X-RAY IRRADIATION

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THE EFFECT of x-rays on the crystalline lens has been studied in the past by many investigators but there is as yet no more than controversial evidence as to the pathogenesis of roentgen ray cataract and the primary site of the changes which lead to formation of the opacity. The problem was taken up again because of the paucity of the experimental series reported in the old literature and because of the opportunity to utilize relatively new methods of examination. In the present approach, biological lines were followed rather than those the radiologists would choose, that is, histological, histochemical, and cytochemical changes and alterations of the permeability in the irradiated lens were recorded as well as some influences of the radiations on environmental factors, as, for instance, on the permeability of the blood aqueous barrier. The part of the work reported here comprises the data obtained with one type of radiation only, that is, with a single dose of 2000 r of penetrating x-rays. The radiation factors were: KV 220 volts, MA 20, Filter ½ mm. Cu., 1 mm. Al., TSD 30 cm., Field 20 mm., round, HVL 1 mm. Cu., r/min. in air 145. The early effects observed in these experiments of acute exposure to penetrating x-rays cannot be compared directly with the late results reported by other workers, especially when they used softer x-rays, another

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dosage, or divided doses. For this reason only occasional reference will be made to previous investigations (la,lb,2,3,4), important as they may be.

Technique. The x-ray beam was directed from above with the eyes exposed by keeping the upper lid updrawn with Scotch tape so that the upper limbus was in the center of the field and the upper lid margin was within the field. In a small series (6 rabbits) the incidence of the beam was perpendicular to the cornea. One eye of each animal (chinchilla rabbit) was irradiated; the other, shielded, was outside the beam. Routine observations with focal illumination, with the biomicroscope, the slit lamp and the opthalmoscope were carried out at intervals ranging from 24 hours to 2 weeks. The longest observation was 9 months.

Results. The early effects on the lens of rabbits will be described first as they present themselves ophthalmoscopically and biomicroscopically or by inspection of the isolated lens under the dissecting microscope. The irradiation with the technic used gave rise to clinically recognizable changes within 4 to 10 weeks in 44 out of 46 irradiated eyes which were kept under observation for a suitable period. At this time there first appeared in the periphery close to the equator a fine and slightly irregular dark ring which could be seen ophthalmoscopically under maximal dilatation of the pupil. A few vacuoles scattered in the posterior cortex at some distance from the equator were often visible at this stage (Fig. 1a). These became usually more numerous in the following days or weeks and formed clusters. The latter then developed into a ring of a garland pattern located in the posterior cortex between the most peripheral zone and the central portion (Fig. 1b). They were composed of a great number of small vacuoles. The appearance of the posterior horizontal suture remained either unaltered or showed side branches in either an upward or downward direction, but this occurred also in the untreated eyes. From the garland or ring opacity, tongue-shaped extensions spread later toward the posterior pole. At this time a mosslike or net-shaped superficial opacity appeared underneath the anterior capsule starting from the periphery of the pupillary area (Fig. 1c). The cataractous process thereupon progressed for several weeks, but the opacity of the cortex remained translucent for periods of varying length. More

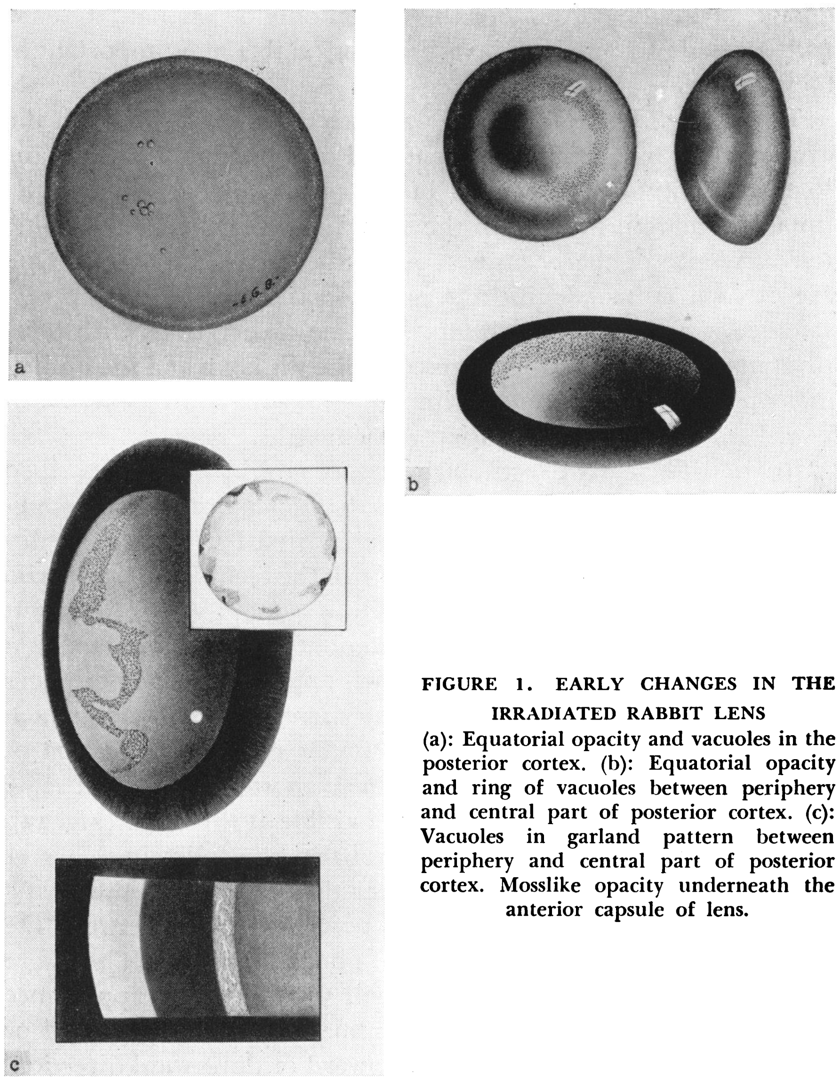


FIGURE 1. EARLY CHANGES IN THE IRRADIATED RABBIT LENS

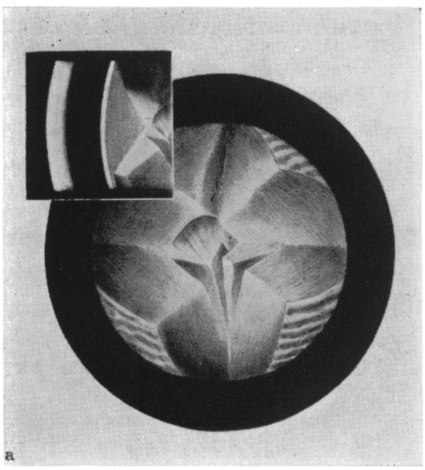
(a): Equatorial opacity and vacuoles in the posterior cortex. (b): Equatorial opacity and ring of vacuoles between periphery and central part of posterior cortex. (c): Vacuoles in garland pattern between periphery and central part of posterior cortex. Mosslike opacity underneath the anterior capsule of lens.

deeply opaque fragments of the nucleus could be distinguished before the stage of full cataract was reached, 3 to 4 months after irradiation (Fig. 2a).

As described by previous workers, especially by Poppe (4), histological changes preceded those detectable by clinical means or with the dissecting microscope by several weeks.

Technique for the histological examinations. The lenses were carefully freed from the attachments in the equatorially opened globe and simultaneously fixed by injecting Carnoy[1] solution around them. A large calotte of the posterior cortex containing the entire horizontal suture was then removed so that the peripheral part of the posterior cortex, that is, the equatorial area, was not injured. Through this opening the nucleus was delivered by carefully separating it with a fine spatula from the surrounding cortex. The preparations of the experimental and control eyes consisting of the intact anterior portion of the cortex and including the equator of the lens were then treated according to Stowell's technic (5) recommended for studies of nucleic acids in paraffin

[1] Glacial acetic acid, 1 part; alcohol 95%, 3 parts.



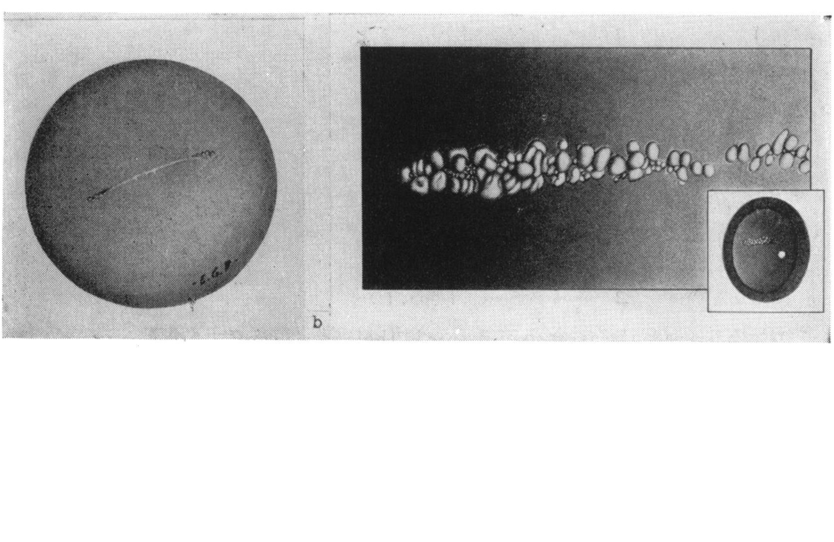


FIGURE 2. LATER CHANGES IN THE IRRADIATED RABBIT LENS
(a): Advanced x-ray cataract. (b): Vacuoles at the posterior suture of the rabbit lens 4 to 5 weeks after irradiation.

sections. The preparations of the treated and normal lenses were imbedded in the same block, cut in meridional directions, and mounted and stained together on the same slide. The posterior calottes of experimental and control lenses were also treated together and sectioned perpendicular to the horizontal suture. Ten irradiated and 10 control eyes were examined.

Results. A displacement of some cell nuclei in the lens bow backward but occasionally forward was the first deviation from the norm. This was noticed as early as one week after irradiation (Fig. 3). At the two-week interval some disorder of cell nuclei in the preequatorial zone was suggested by their less regular and less dense spacing and their slightly abnormal shapes, but the evaluation of these earliest changes was subject to trial and error. About one or two weeks later the palisade arrangement of the epithelium in this zone vanished, the cells became flatter, and the row of nuclei showed occasional interruptions (Fig. 4). The nuclei demonstrated a variety of degenerative changes, such as chromatin fragmentation, pyknosis or karyolysis. Cell counts per unit area in this zone gave much lower readings than in the control lenses (Table 1).

Based on the reports of the blocking effect of x-rays on the formation of nucleic acids, and on the turnover of desoxyribonucleic acid in normal organs of adult animals, microscopic demonstration

CELL COUNTS IN PRE-EQUATORIAL EPITHELIUM OF IRRADIATED AND CONTROL LENSES

Time after Irradiation in Weeks	*Number of Rabbit Lenses*	*Counts**	
		Irradiated Eyes	*Control Eyes*
1	4	O.D. 72.66	O.S. 99
		O.D. 60.16	O.S. 76
2	4	O.D. 69.3	O.S. 91
		O.D. 57.33	O.S. 92
3	1	O.D. 64.2	(No control. 89.7 av. of all controls)
4	6	O.D. 47.3	O.S. 91
		O.D. 41.0	O.S. 86.66
		O.D. 56.0	O.S. 81.66
8	2	38.12	103.66

*Each figure is the average of counts in three sections of each lens.

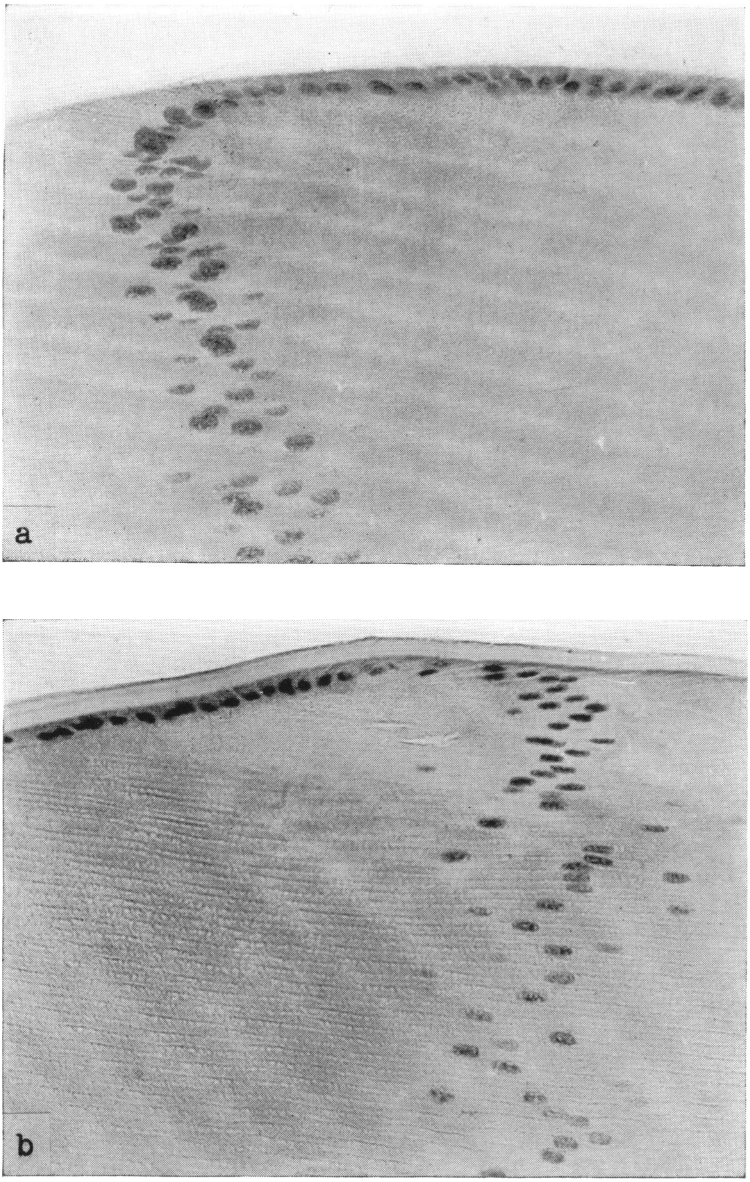


FIGURE 3. DEVIATION FROM THE NORM
Lens bow of control eye (a) and of irradiated lens (b) with displacement of nuclei one week after treatment. Hematoxylin and eosin. Magnification 225 x.

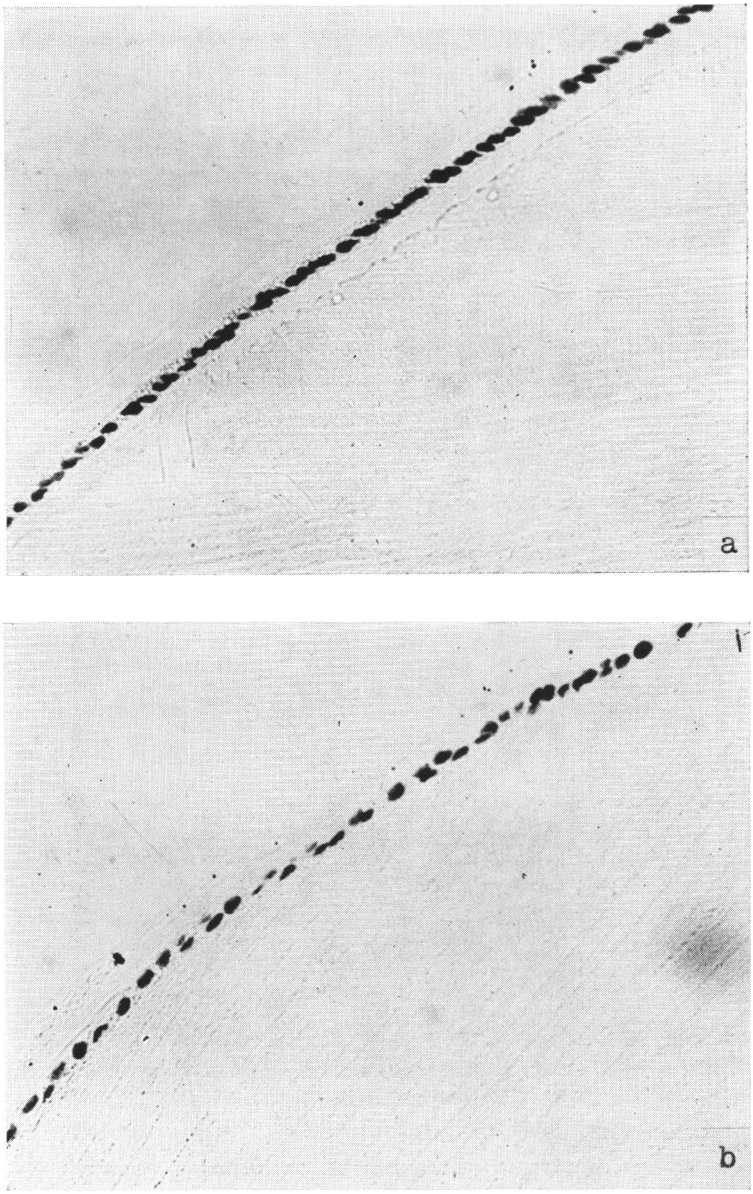


FIGURE 4. CHANGES IN THE EPITHELIUM
Preequatorial zone of lens epithelium in control eye (a) and irradiated eye (b), with spatial disarrangement and morphological changes of nuclei, 4 weeks after irradiation. Feulgen stain. Magnification 225 x.

of the nucleic acids was attempted on the sections of the anterior segments of the lenses.

Technique of special staining procedures for nucleic acids. Stowell's modification of the Feulgen technic (5) was supplemented by a staining procedure which indicates with alleged specificity the degree of polymerization of desoxyribonucleic acid as described in the work of Brachet (6), Kurnick (7), and Pollister and Leuchtenberger (8). Staining with methyl green and pyronin alone or together was carried out with dyes purified by recrystallization as recommended by the last-mentioned authors. Ten pairs of sections for each of the 4 staining procedures and for hematoxylin-eosin staining were studied in the present series of experiments.

Results. The Feulgen technic and the methyl green pyronin stain as an indicator for the degree of polymerization of this acid suggested a decrease in content of the acid and in its polymerization in the nuclei of the preequatorial zone 2 to 3 weeks after irradiation. No difference between the normal and irradiated eyes was evident at earlier intervals. As a rule, morphological changes in the nuclei accompained the variations in the staining characteristics. Quantitative data of intensities of staining are not available as yet.

It is not known whether or not these cytochemical changes in some of the nuclei of the lens epithelium express the blocking of the formation of desoxyribonucleic acid by x-rays or the inhibition of its turnover rate as described by Euler and Hevesy (9), Ahlström and coworkers (10), and Stowell (11) in normal organs of adult animals a few hours after irradiation. Nor can it be concluded from these experiments that the demonstrable changes in the degree of staining of this most important chemical constituent of the nuclei were the first alterations in the sequence of events.

The elongated form of the young nucleated fibers provide suitable conditions for comparison of lesions in the nuclei with those in the cytoplasmic part. Initial changes of the latter cannot be distinguished from artefacts with any degree of certainty. However, in 6 eyes of the series, changes on the ends of the fibers—that is, at great distance from the nuclei—became very conspicuous 4 weeks after irradiation. They consisted of large cystic spaces partly filled with fine granular matter (Fig. 5). Some of these cavities obviously arose from intrafibrillary vacuoles, others may have developed from interfibrillary clefts. These systems of cysts formed complete rings about 1 mm. in front and behind the equator. Brilliantly reflecting vacuoles were observed in these eyes with focal illumination located either at the lateral ends of the posterior horizontal suture or along its entire length (Fig. 2b). These vacuoles disappeared in a few weeks without residue in the eyes, which were not removed for histologic examination.

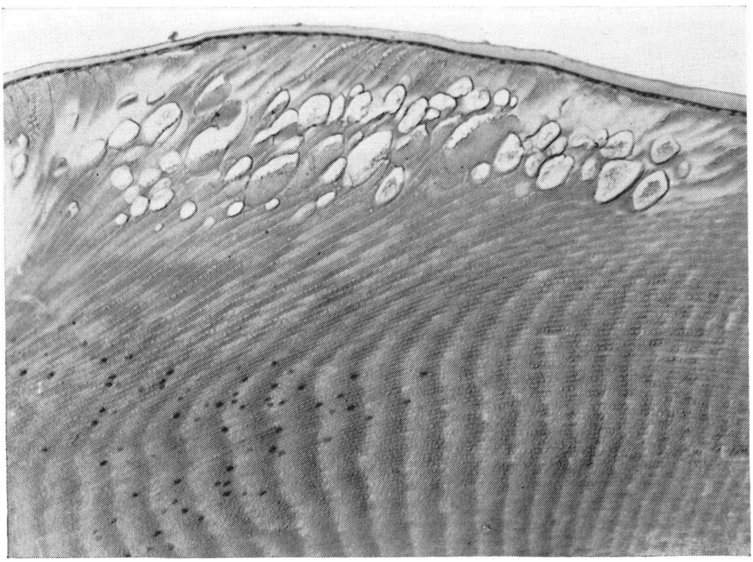


FIGURE 5. CYST FORMATION AT SUBCAPSULAR ENDS OF FIBERS IN FRONT OF EQUATOR OF LENS, 4 WEEKS AFTER IRRADIATION
Hematoxylin and eosin. Magnification 65 x.

In regard to the histologic picture the severity of the radiation damage at the subcapsular ends of the fibers contrasted with the moderate degree of morphological and staining changes in the nuclei of the preequatorial zone and of the bow. It led one to believe that in these six instances the irradiation had affected the cytoplasm directly, although degenerations of the nuclei may have preceded the lesions in the fibers. Except for these observations the histologic studies conveyed the impression that the degenerative changes in the nuclei of the germinative zone, their reduction in number, and the decrease of cytochemically demonstrable desoxyribonucleic acid in some of the nuclei were well advanced before the fibers showed swelling, cystic formation and loss of direction of growth.

One of the many theories of the biologic effects of x-ray considers the interference of membrane permeability in vital systems by the radiation. Changes in the permeability of the nuclei- and cell- membranes of the lens and of the lens capsule therefore enters into the discussion of factors responsible for the formation of cataract. Radioactive indicators provided a useful tool for studying permeability particularly when autoradiographic technics were employed. These technics were described in a previous paper (12). In the present series (15 experiments), radioactive sodium, potassium, sulfur, phosphorus, and iodine were selected. Differences in the distribution of these tracers between control and experimental lenses were usually noticeable and often striking after extensive cataractous changes had been diagnosed clinically. Differences from the norm could not be detected in the autographs of irradiated lenses before vacuoles had accumulated in the cortical region.[2] The most remarkable dissimilarity in the autographs of cataractous and normal lenses were seen when radiophosphorus was used by the systemic route under standard conditions (Fig. 6a). The decrease of easily hydrolyzable phosphorus in the cortex of cataractous lenses could explain the lack of darkening of the film emulsion which took place in this area of control eyes. Radiosodium had diffused into the cataractous lens 4 hours after intraperitoneal injection, whereas the autographs did not show any darkening in the area corresponding to the lens in control eyes or when the cataractous process was beginning. The high water content of the cataractous lens may have caused this behavior, since sodium is distributed in the extracellular fluid. Radiosulfur did not produce a photographic effect in the area of the lens independent of the presence or absence of cataractous changes. The results in the experiments with radiopotassium were ambiguous. Radioiodine which proved not to be bound to the lens proteins was present in considerable amounts in the cataractous lens as shown in the autoradiographs. Its content

[2] The densitometric measurements of the films will be reported in another paper.

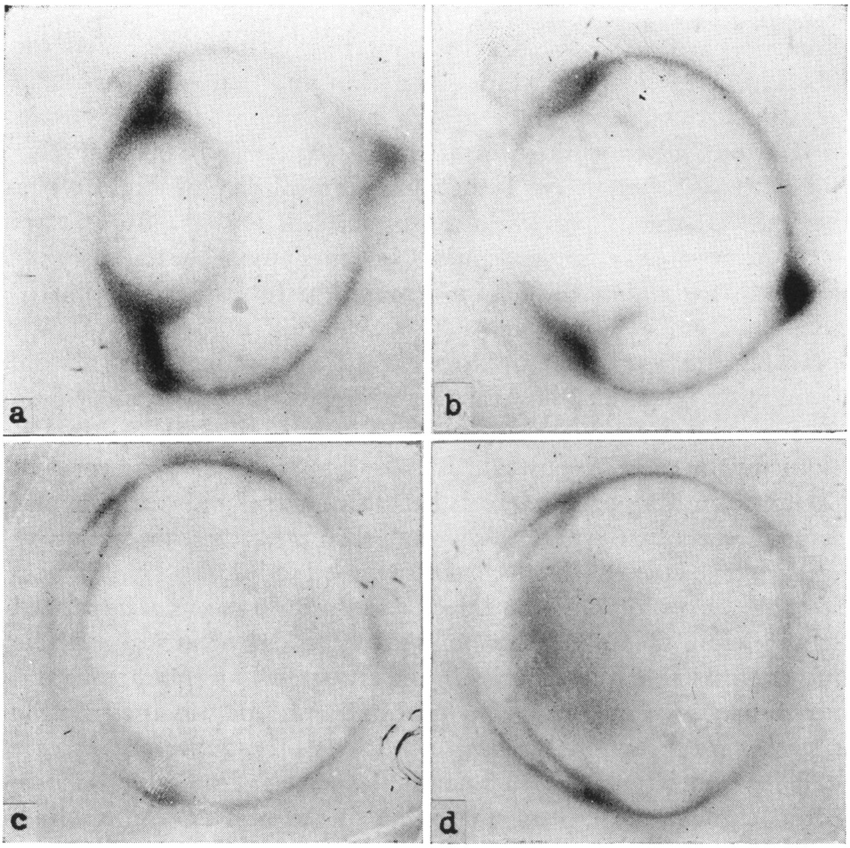


FIGURE 6. RADIOGRAPHS OF FROZEN SLICES OF RABBIT EYES
(a): Normal eye. (b): Cataractous eye, after intraperitoneal injection of P 32. (c): Normal eye. (d): Cataractous eye, after intraperitoneal injection of I 131.

in the lens was also increased in the early stages of visible radiation damage. This observation would point to an enhanced permeability of the lens for radioiodine in the initial phase of cataract formation. In summary the experiments suggested that increased permeability of the capsule or cortex of the lens did not precede visible radiation injuries of the lens but accompained them if the cataractous process advanced.

The radiobiological term "indirect effect of radiations" is usually confined to those effects produced as a result of injury to the blood supply. In a wider sense it may be used on the eye for damage to the blood aqueous barrier. It was shown in a previous study (13) that exposure of the rabbit's eye to penetrating radiations of more than 250 r is followed by an increase in permeability of the barrier for fluorescein, but this disturbance is of short duration (less than one week) when the dose does not exceed 1000 r. When 2500 r were used the disturbance lasted 7 to 8 weeks. Thus these experiments indicate that a radiation injury of the blood aqueous barrier is not connected with the development of radiation cataract, particularly if one considers that cataract in rabbits can be produced by a dose of 1000 r.

SUMMARY

1. Cataractous changes became demonstrable with the ophthalmoscope in 44 out of 46 eyes 4 to 10 weeks after irradiation with 2000 r of penetrating x-rays. The opacities started at the equator of the lens, progressed in the posterior cortex and then in the anterior cortex in characteristic patterns.

2. Acute exposure of rabbits' eyes to a dose of 2000 r's of penetrating rays was followed by spatial disarrangement and initial morphological changes of the nuclei in the bow and in the preequatorial zone of the lens epithelium, as early as 1 week after irradiation. In the second and third week degenerative changes of the nuclei in this area became more pronounced, with a considerable decrease in the number of the nuclei and with gaps in the epithelial row of the preequatorial zone.

3. Specific stains for nucleic acids indicated that a few weeks after irradiation many of the nuclei in this area contained less desoxyribonucleic acid of high polymerization than comparable nuclei in the control lenses, but it was not clear whether the cytochemical differences preceded the signs of nuclear degeneration.

4. Swelling and disorganization of lens fibers were usually observed after changes in the cell nuclei had advanced; but in 6 of 46 irradiated eyes of the series, extensive cystic formations at the subcapsular ends of the lens fibers 4 weeks after irradiation suggested a direct radiation effect on the cytoplasm.

5. The permeability of the lens to radioactive sodium and iodine appeared increased in autoradiograms of lenses with beginning cataract. No divergence from the norm was observed in irradiated

lenses prior to ophthalmoscopically visible radiation damage.

6. Experiments with fluorescein did not lend support to the theory that an indirect radiation effect on the blood aqueous barrier played a significant part in the development of radiation cataract produced by acute exposure to 2000 r's.

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DISCUSSION

DR. P. J. LEINFELDER. I have enjoyed Dr. von Sallmann's presentation very much and have been impressed with the demonstrations he presented. He has confined his experimental work to the effects that are produced by a single, and, may I say, overwhelming or lethal dose of x-ray to the lens epithelium. This has resulted in cataractous changes that I believe cause complete opacification of the lens in approximately 100 days. The first observed morphological changes occurred after a period of approximately six weeks. These were progressive, and eventually the first formed vacuoles changed to a posterior subcapsular and anterior subcapsular type opacity and was followed by intumescence and complete opacification of the lens. I would like to point out that there is a time interval between the irradiation and the complete opacification of the lens. Dr. von Sallmann has indicated, however, that histologic changes could be noted in the nuclei as early as one week, changes that he attributed to chemical alteration in desoxyribonucleic acid of the nucleus. He then proceeds to study the effects, or possible effect, of irradiation on the permeability of the lens capsule and of the lens cell itself, as well as to attempt to demonstrate a change in the blood aqueous barrier. Some of us might say, "So what?"

The physiologist uses all forms of tools to study the results of changes that may occur to produce pathology, and x-ray is one of the means that can be used, and at the present time we are intimately interested in irradiation effects. Unfortunately, however, radiation energy is not unitary; we cannot expect the same specific effect from radiation that we will obtain from, let us say, dinitrophenol. With x-rays, even if the energy range is limited, when the x-rays strike the tissue, new radiation wave-lengths are set up and alteration in the character of the energy occurs. With any given dosage of x-ray we may get effects that are the result of denaturation of the protein such as coagulation, changes in surface tension, changes in permeability, and we also may obtain physicochemical changes, such as ionization, alterations in the desoxyribonucleic acid, and profound effects on the respiratory mechanism, the enzymes within the cell. Dr. von Sallmann at the present time has not taken up the question of the respiratory enzymes. That is a field that needs investigation and is being investigated by other workers. He has attempted to show the physical changes in permeability of the lens capsule, and there is an alteration. Whether that alteration in the lens capsule is the immediate effect of x-ray or is a consequence of something else that has occurred in the cell, he admits that he cannot tell. However, we must appreciate that this work is leading to an understanding of the mechanism, the physiology of the lens, and the changes that result in the formation of cataract, whether it be cataract induced by x-ray, by metabolic conditions, by specific poisons, or as a result of senility. I think x-ray cataract should also point out to us that senile cataract may not be the result of something that is occurring at this moment in the individual who has the senile cataract, but that his cataract may be the result of injuries that have taken place repeatedly throughout his life, or within the previous year or two. Sometimes, for instance, the diabetic develops his cataract a year after he has been put on perfect control, because the injury caused by impaired glucose metabolism that occurred during the period prior to diagnosis has been sufficient to result in complete opacification of the lens after a period of months.

I am very grateful to Dr. von Sallmann for this study. Some of these changes are in accord with those that we previously had reported. One cannot, as he says, compare results obtained with the irradiation that he used with those that may occur with smaller doses, whether used singly or repeatedly.

DR. F. H. VERHOEFF. I was very much interested in the fact that Dr. von Sallmann found an increase in the permeability of the blood-aqueous barrier after irradiation, and it seems to me this could be of importance in connection with the question of glaucoma. Radiation has been used in the treatment of glaucoma. I have never used it for this purpose myself, but others have claimed it is beneficial, so I should like to ask Dr. von Sallmann if he thinks that at first this increased permeability would increase the intraocular pressure, and then subsequently the permeability, and therefore the pressure, might be decreased.

DR. LUDWIG VON SALLMANN. I think it was very gracious of Dr. Leinfelder to comment favorably on this skeleton of a study which I was allowed to present here. He added much more on the radiobiological effects and the mechanism of these effects than I could cover in the time, but I would like to say we are interested in the effects on enzyme systems, and work is going on in this direction in cooperation with Dr. Dische. I do not wish to convey the idea that the changes in desoxyribonucleic acid are really the primary lesion, or the trigger mechanism, for whatever follows. We could not prove this at all. However, it was shown by Euler and Hevesy, and Ahlström abroad, and by Stowell here, that the turnover of the desoxyribonucleic acid is suppressed soon after x-ray irradiation, in other organs of the normal animal body.

I am sorry that I cannot answer Dr. Verhoeff's question because I did not measure the intraocular tension in these rabbits which showed increase in permeability of the blood-aqueous barrier for one week; the increase in permeability went hand in hand with hyperemia of the conjunctiva and signs of primary reactions on the lids.