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Early Ultrastructural Changes After Low-Dose X-Irradiation in the Retina of the Rat

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Summary

In this study Lister rats were given doses of X-rays ranging from 200–2,000 Rads to the retina of one eye, sacrificed at various time intervals between one hour and one month later and the irradiated eye processed for electron microscopy. The rod photoreceptor cells were by far the most radiosensitive cells in the retina, their outer segments showing distinctive membrane damage at one hour after 200 Rads of X-rays. Photoreceptor cell death was not seen at doses less than 1,000 Rads in the time period of the experiment. The retinal pigment epithelial (RPE) cells showed damage in the form of mitochondrial swelling but only in doses over 500 Rads. Retinal pigment epithelial cell loss did not occur under 2,000 Rads. The inner retinal neurones, glial elements and the retinal vasculature did not show any ill effects in the time period of this study.

Several recent clinico-pathological studies[1,2,3] have suggested that the inner retina is differentially vulnerable to the effects of ionising radiation as compared to the outer retina. As these interpretations are at variance with the extensive experimental evidence reported by Noell *et al.*[4,5] and Cibis *et al.*,[6] a study was undertaken to identify the earliest ultrastructural effects of ionising radiation (X-rays) on the retina and to titrate the level of radiation at which acute ultrastructural damage is manifested.

The morphological effects of ionising radiation on the retina have been documented light microscopically[4,5,6,7] but the early ultrastructural changes, particularly with low doses of radiation are not known.

This study describes the early ultrastructural changes occurring in the rat retina at times up to one month after low doses of x-irradiation.

Materials and Methods

Male hooded Lister rats (OLAC) weighing 250–350 g were anaesthetised with Hypnorm (dose 0.3 ml/kg body weight) and the right eye of each animal irradiated using a 90 KV X-ray machine (Therapax) with a dose delivery rate of 239.08±16.17 Rad/min at the surface and a 77.5% depth dose at 5 mm from the surface. Groups of three animals were given single doses of 200, 500, 1,000, 1,500 and 2,000 Rad to the retina, and a group from each dose sacrificed at 1 hour, 6 hours, 12 hours, 48 hours, 96 hours, 1, 2 and 4 weeks post irradiation. At sacrifice the animals were deeply

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anaesthetised with pentobarbitone and the irradiated eyes enucleated. The anterior segments were removed before overnight immersion fixation in 2.5% glutaradehyde in 0.1 M cacodylate buffer containing 10 mM magnesium chloride. Representative tissue blocks of retina, choroid and the overlying sclera were osmicated and embedded in Spurrs resin. Before sectioning for electron microscopy the blocks were orientated using 1 μm sections stained with toluidine blue. The left eyes of the test animals were regarded as unsuitable for control material as it was impossible to completely shield this eye from stray radiation. Two separate groups of untreated animals were used as controls. One group was sacrificed at the beginning of the study and one group at the end.

Results

As the inner retinal neurones, glial elements and the retinal vasculature showed no pathological change in the time and dose range covered by this study, the following description will be confined to ultrastructural changes occurring in the outer retina.

The earliest ultrastructural change observed in irradiated retina was the appearance of small (80–130 nm) membranous whorls within the rod cell outer segment (ROS) membrane stacks. Whorls were not seen in the control retinas but were present in 200 Rad rats at one hour post irradiation (Fig. 1). 500 Rad rats showed a much larger number of outer segment whorls when compared to 200 Rad animals at the same stage. In addition to whorls, doses of 1,000 Rad and over produced gross separation of the ROS membrane discs (Fig. 2) and swollen mitochondria in the retinal pigment epithelial (RPE) cells (Fig. 3). In Table I it can be seen that ROS disc separation and swollen RPE mitochondria occurred at lower doses than 1,000 Rad but took longer to become manifest. 2,000 Rad animals at one hour post irradiation were the first group to show severe

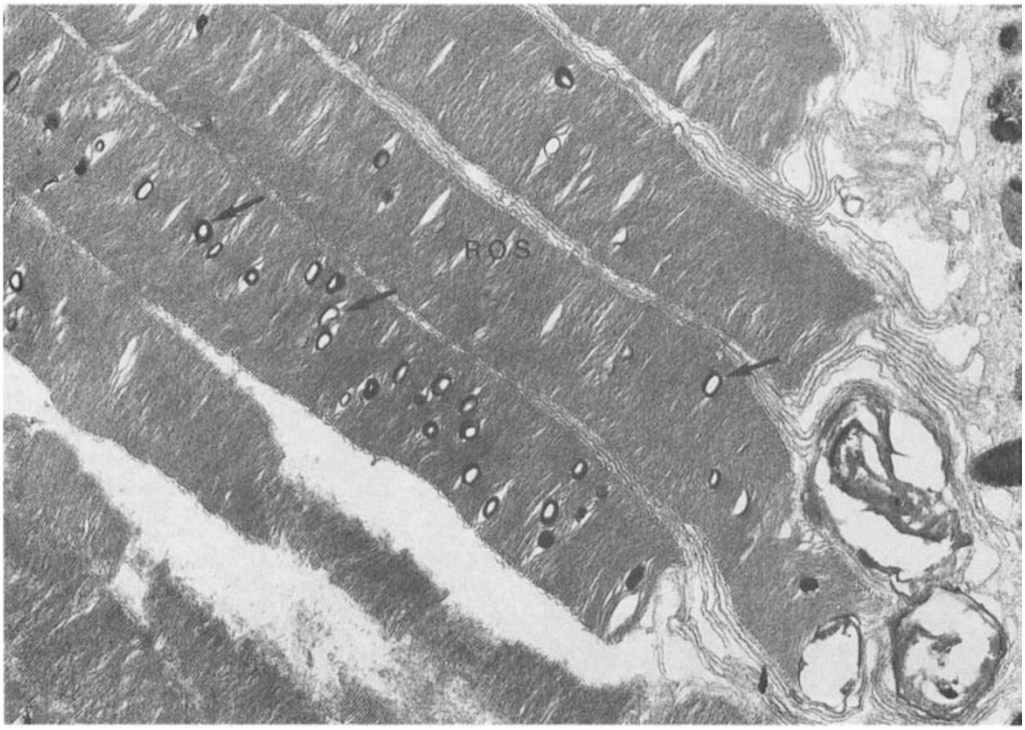

Fig. 1 *Rat retina 1 hour after 200 Rad. Rod outer segments (ROS) show many membranous whorls (arrows).* ×*18,000*

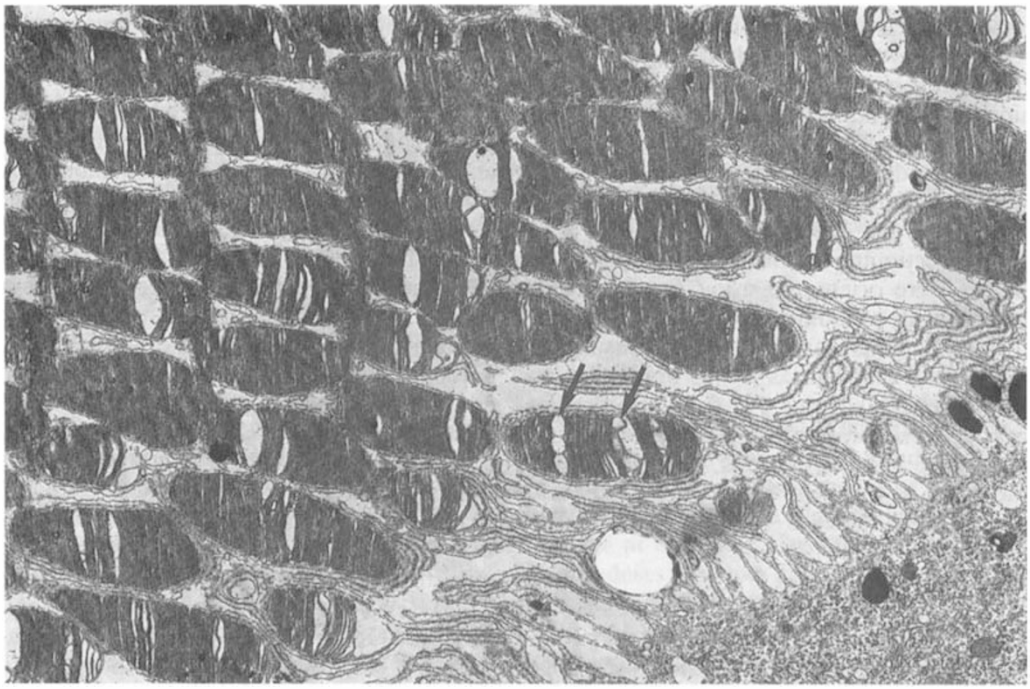

Fig. 2 *Rat retina 24 hours after 1,500 Rad. ROS show gross separation and vesiculation (arrows) of discs. ×10,400*

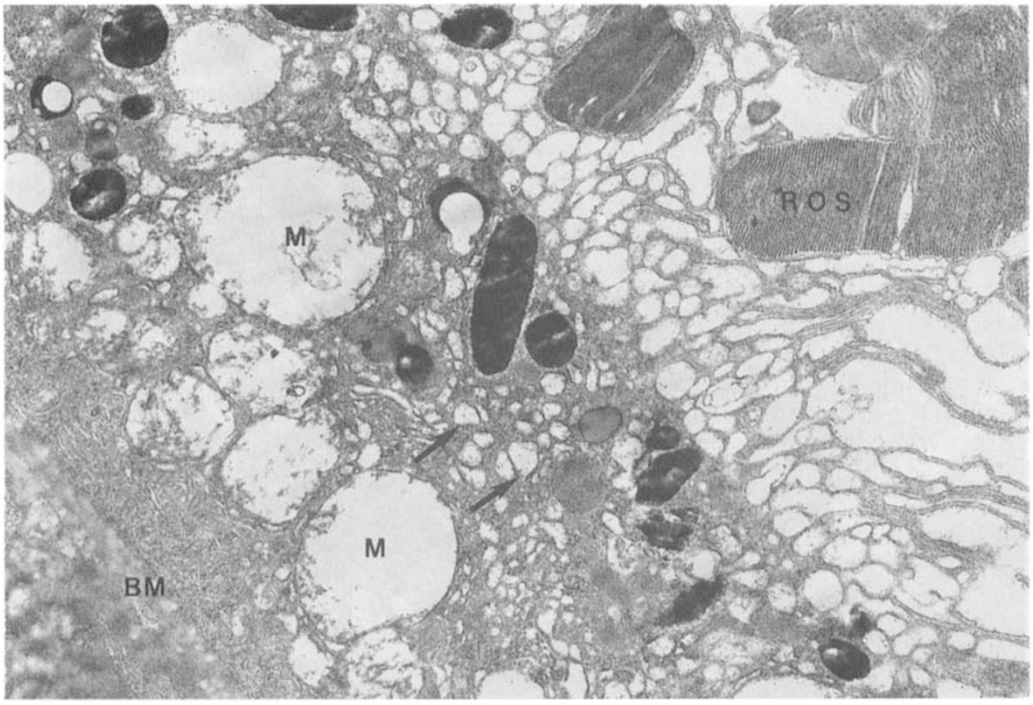

Fig 3. *Rat retina 6 hours after 2,000 Rad. The mitochondria (M) of RPE cells show gross swelling. The cisternae of smooth endoplasmic reticulum also appears swollen (arrows). Rod outer segment (ROS). Bruch's membrane (BM). ×18,000*

Table I *Summary of ultrastructural changes in the retina*

	200R	*500R*	*1,000R*		*1,500R*		*2,000R*	
1 hour	W	W	W DS SM		W DS SM		W DS SM RNPK	
6 hours	W	W SM	W DS SM		W DS SM		W DS SM RNPK	RISD
12 hours	W	W SM	W DS SM		W DS SM RNPK	RISD	W DS SM RNPK	RISD
24 hours	W	W SM	W DS SM		W DS SM RNPK	RISD	W DS SM RNPK	RISD
48 hours	W	W SM	W DS SM		W DS SM RNPK	RISD	W DS SM RNPK	RISD
96 hours	W	W SM	W DS SM		W DS SM RNPK	RISD	W DS SM RNPK	RISD
1 week	W	W SM	W DS SM RNPK		W DS SM RNPK	RISD	W DS SM RNPK	RISD
2 weeks	W	W DS SM	W DS SM RNPK	RISD	W DS SM RNPK	RISD	SM RNPK	L-RPE ORG
1 month		DS	DS RNPK	RISD	W DS SM RNPK	RISD	 RNPK	L-RPE ORG

Key:
W = Rod outer segment whorls present.
DS = Rod outer segment disc separation present.
SM = Swollen oedematous mitochondria present in RPE cells.
L-RPE = Rod cell nuclear pyknosis present.
RISD = Rod inner segment damage present.
L-RPE = Loss of RPE cells.
ORG = Outer retinal gliosis.

nuclear damage as evidenced by the presence of a small number of pyknotic nuclei in the outer nuclear layer. The number of pyknotic rod cell nuclei in the outer nuclear layer subsequently increased to reach a maximum in 2,000 Rad animals between 24 and 48 hours post irradiation (Fig. 4a). 1,500 Rad and 1,000 Rad were the only other doses in which pyknotic photoreceptor nuclei were encountered and then only at times longer than 12 hours (Fig. 4b) and one week respectively.

Damage to the photoreceptor inner seg-

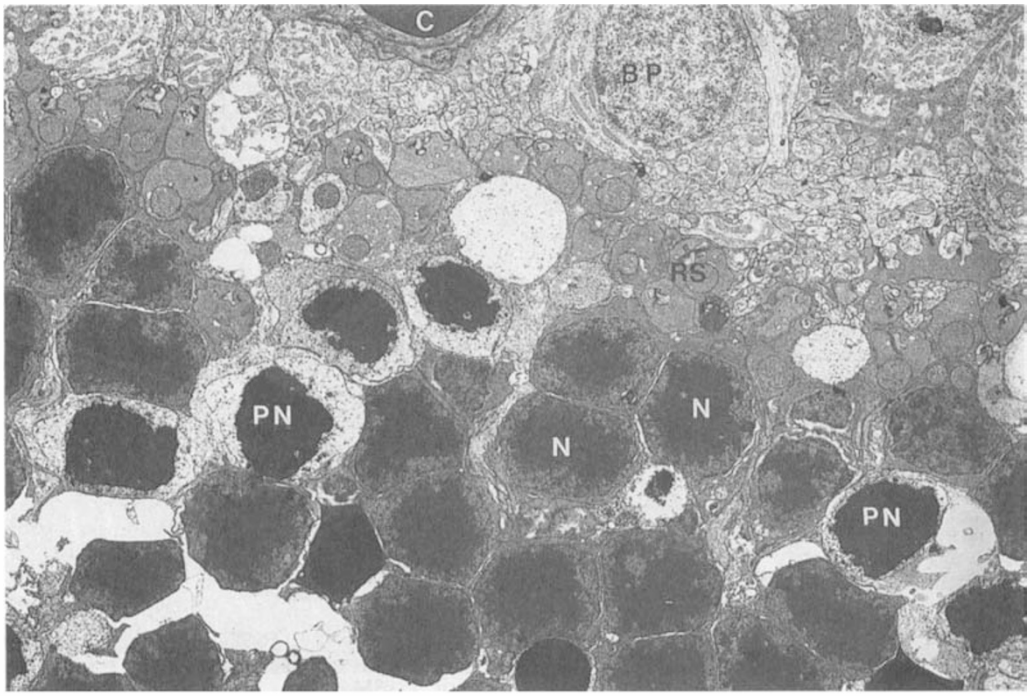

Fig. 4a *Rat retina 12 hours after 2,000 Rad. The outer nuclear layer contains many pyknotic rod cell nuclei (PN), although some rod nuclei (N) still appear normal. The majority of the rod spherules (RS) in the outer plexiform layer appear normal as do adjacent bipolar cells (BP) and a retinal capillary (C). ×4,400*

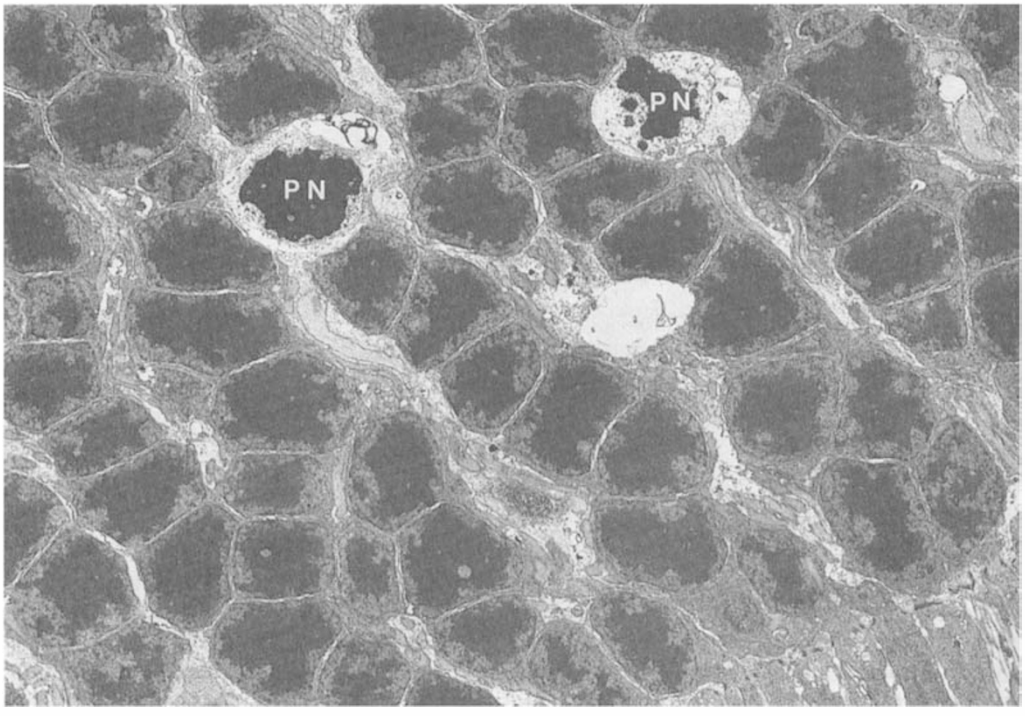

Fig. 4b *Rat retina 12 hours after 1,500 Rad. The outer nuclear layer shows two pyknotic nuclei (PN). ×4,400*

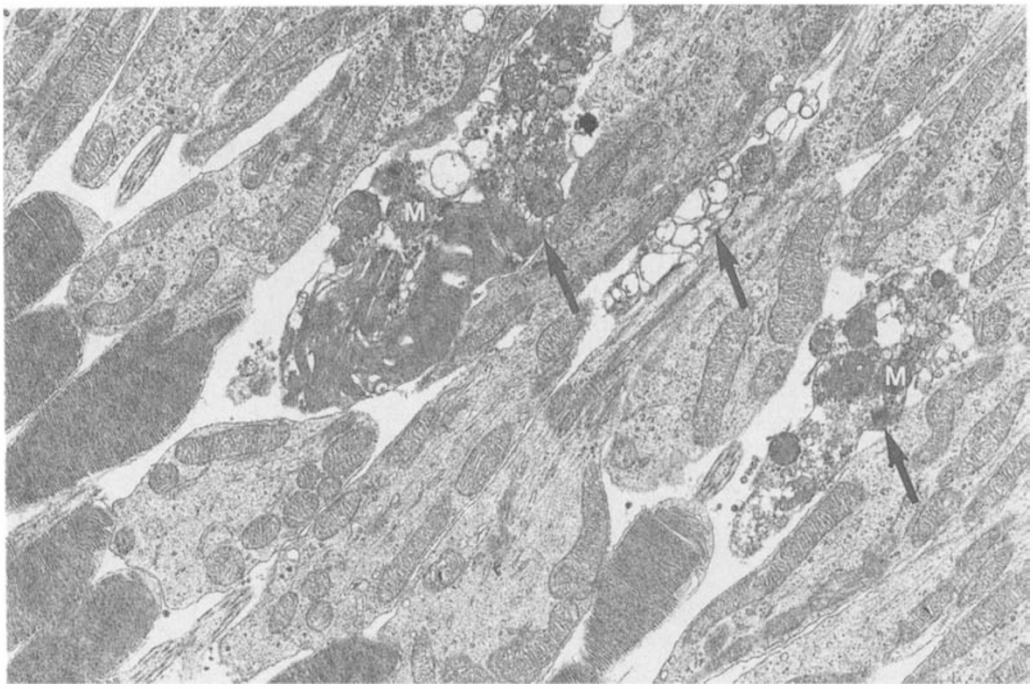

Fig. 5 *Rat retina 24 hours after 1,500 Rad. Degenerate rod inner segments (arrows) lie between apparently normal cells. The mitochondria (M) of the damaged inner segments are rounded and dense. ×13,200*

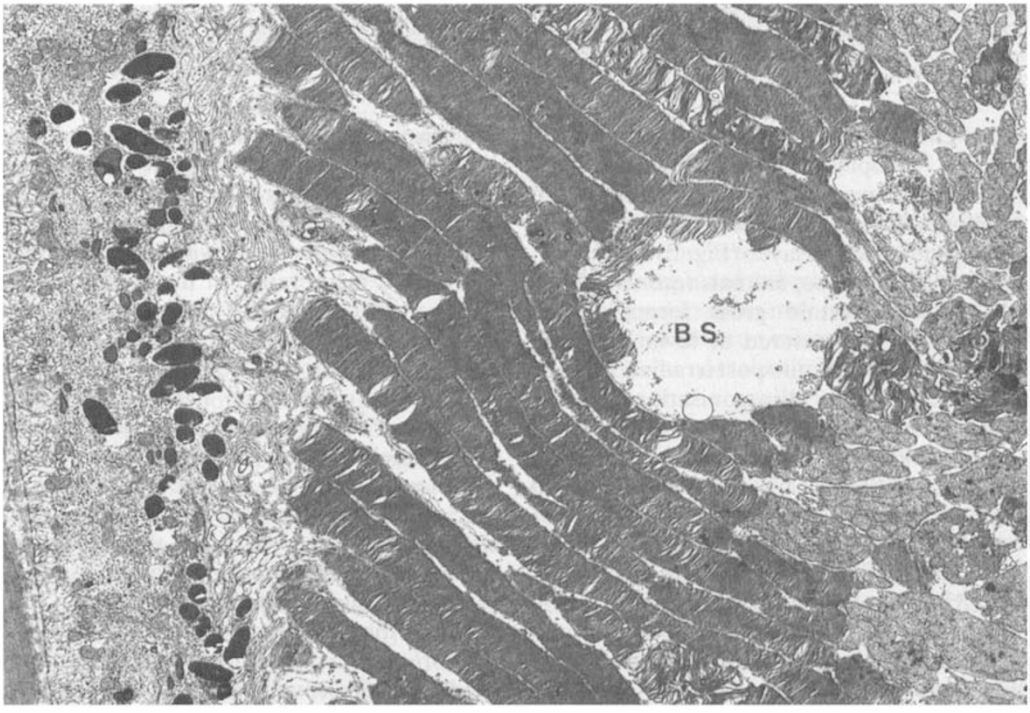

Fig. 6 *Rat retina 12 hours after 2,000 Rad. The grossly damaged rod outer segments show occasional balloon-like swellings (BS) at the base of stacks. ×5,900*

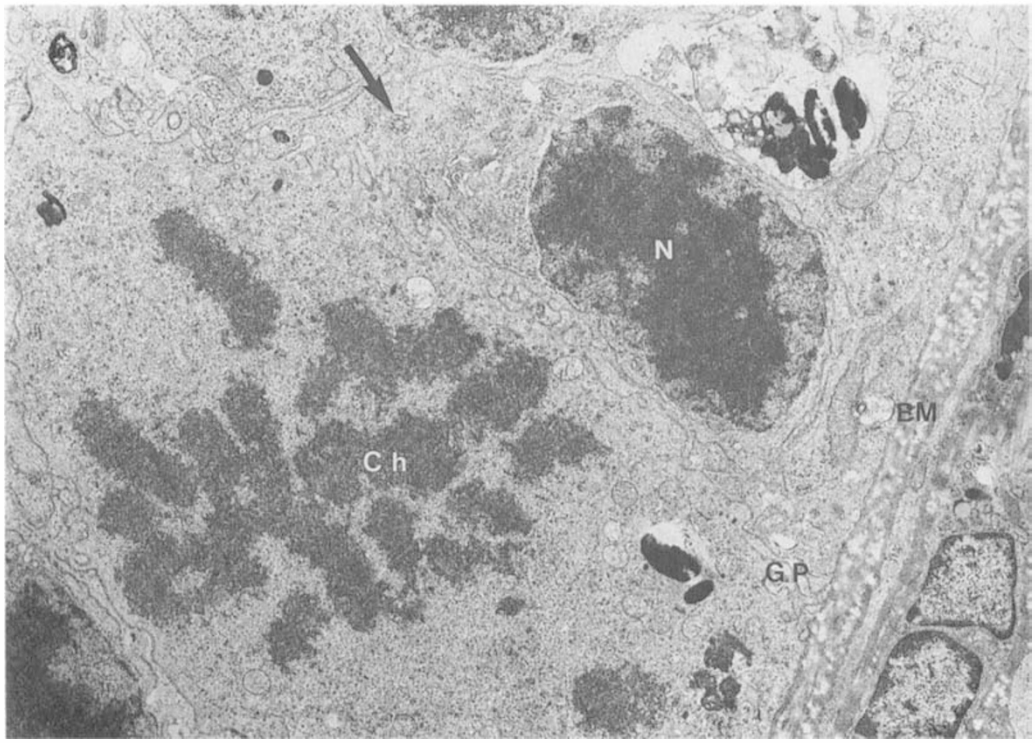

Fig. 7 *Rat retina 2 weeks after 2,000 Rad. The processes of hypertrophic glial cells (GP) have filled the outer retina to make direct contact with Bruch's membrane (BM). One such glial cell is engaged in mitosis as evidenced by the presence of chromosomal bodies (Ch). Entrapped within the glial processes is a residual rod cell nucleus (N) and perikaryon. The rod cell shows a remnant of its cilium (arrow). ×7,800*

ments characterised by a shrunken electron opaque cytoplasm and dark rounded mitochondria was only a feature in doses and at times, where pyknotic nuclei were present in the outer nuclear layer (Fig. 5).

In the acute phase, animals receiving 2,000 Rad showed certain gross forms of cell damage not encountered at lower doses of irradiation. At 12 hours post irradiation many ROS showed gross disorganisation and balloon-like swellings at the bases of the membrane stacks (Fig. 6). These changes were often accompanied by the inner segment damage described above.

Although the retinal pigment epithelial cells showed mitochondrial damage characterised by a swollen washed-out appearance in the low dose ranges, only in 2,000 Rad animals, two weeks post irradiation was there obvious evidence of cell death. At two weeks post irradiation many areas of RPE cell loss could be identified in 2,000 Rad animals. In such instances the retinal glial cells had proliferated as evidenced by mitotic figures (Fig. 7), encompassed the few surviving rod cell perikarya, and filled the subretinal space coming into close apposition with denuded areas of Bruch's membrane. In these regions the glial cell cytoplasm had often enclosed pigment granules from degenerate RPE cells. RPE cells persisting under these conditions had usually 'rounded up' having lost contact with Bruch's membrane (Fig. 8). In such cells the organelles were poorly preserved and the nuclei were pyknotic. The poor condition of the cell organelles clearly distinguished degenerate RPE cells from the occasional mononuclear phagocytes which had ingested pigment granules and other cell debris.

When the dose of radiation was small the photoreceptor cells showed ultrastructural evidence of recovery soon after the initial insult. In 200 Rad rats there was a reduction in the number of ROS membrane whorls one

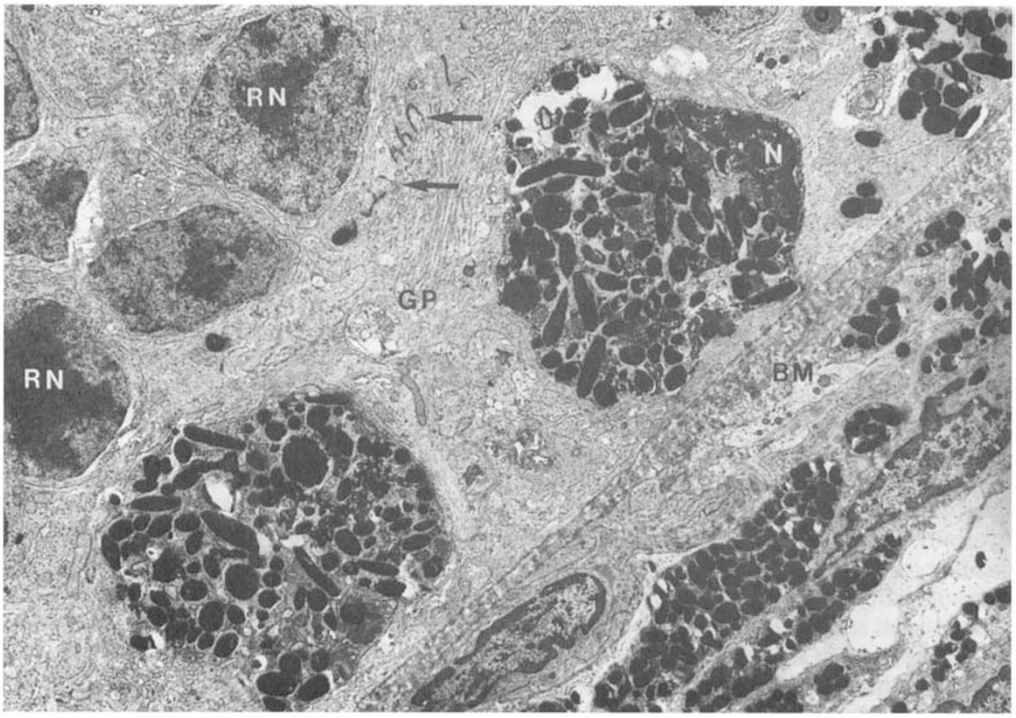

Fig. 8 *Rat retina 2 weeks after 2,000 Rad. The outer retina shows complete loss of the normal architecture, although Bruch's membrane (BM) and remnants of the external limiting membrane (arrows) persist. A few residual rods cell nuclei (RN) survive among the processes of hypertrophic glia (GP). Degenerating RPE cells have rounded up and lost contact with Bruch's membrane. The nucleus of one of these cells (N) is pyknotic.* ×*5,900*

week after irradiation. In these animals the residual ROS whorls were localised at the RPE end of the membrane stacks and in RPE phagosomes containing ingested outer segment material.

Discussion

The membranous whorls observed in the photoreceptor outer segments were the first definitive sign of radiation damage to the fine structure of the retina. The whorls were thought to represent specific damage to the highly labile outer segment membranes which show extreme sensitivity to the destructive effects of various forms of radiation due to the high proportion of unsaturated fatty acids and protein in their constitution. It was notable in 200 Rad rats, where the radiation injury to the visual cells appeared to be confined to the outer segments, that the outer segment whorls moved towards the RPE as the normal process of disc shedding and renewal continued[8] and were not encountered at times longer than that required for outer segment renewal in the rat (9 days).[9]

A second parameter of early radiation damage in the retina was the presence of swollen or ruptured mitochondria in the RPE. As this phenomenon may occur in any tissue prepared for electron microscopy due to poor fixation it was not taken into account in specimens where the quality of fixation was in doubt. Our conviction that the mitochondrial changes described in the present study reflect real radiation damage is supported by the following observations. First, the frequency of swollen mitochondria in the RPE appeared dose dependent. Second, for doses in which frank RPE cell death did not occur (<1,500 Rad), following the acute phase (96 hours) the frequency of damaged mitochondria decreased until by one month post irradiation they were no more common than in control RPE.

The damage described in the rod inner segments was not thought to represent a further degree of sublethal cell injury. As such damage only occurred in concert with pyknotic nuclei in the outer nuclear layer, the inner segment changes were regarded as the cytoplasmic expression of the cell death heralded by nuclear pyknosis.

This study has shown that the photoreceptor cells of the retina are highly vulnerable to the effects of even relatively small doses of ionising radiation and are by several orders of magnitude the most radiosensitive cells in the retina. The inner retinal neurones, glial and vascular cells showed no ill effects in the dose range and times covered. These data provide ultrastructural confirmation of the extensive experimental studies of Noell[4,5] and Cibis *et al.*,[6] Baily and Noell[10] and Cibis and Brown[11] who demonstrated conclusively the extreme radiosensitivity of the rod cells. However, several recent studies[1,2,12,13] have reported that the inner retina is more radiosensitive than the outer retina. Confusion has arisen because these studies were limited to the long term effects of relatively high doses of radiation and the bulk of the described pathological changes were those of an ischaemic retinopathy which occurred secondary to radiation injury of the retinal vasculature. Furthermore, the cone cells in primates are highly resistant to radiation[6] and their survival would have maintained the structure of the outer retina. Some of the recent clinico-pathological studies do in fact illustrate cell loss in the outer nuclear layer but do not comment on it.[3,12] To compound these problems many investigators are still working with badly preserved thick paraffin sections incorrectly orientated to make any meaningful comment on the outer retina.

Conclusions

It is obvious that the clinical entity known as radiation retinopathy needs to be redefined in light of the findings of this and other experimental studies. Radiation retinopathy as we see it is a compound condition representing the cumulative effects of early direct radiation damage to the outer retina upon which is superimposed an ischaemic retinopathy resulting from a late manifestation of vascular cell injury.

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