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Age- and Light-Dependent Changes in the Rat Eye*

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Summary. In a total of 400 untreated rats which had achieved ages of up to three years, the age-related morphological changes in the eye, and more particularly in the retina, resulting from three years of artificial lighting were studied. In addition to this, from ten 1067-day old and five 90-day old rats, a total of 9427 measurements of nuclear size were made and the number of nuclei per unit area at the 1st and 2nd retinal neurones were determined.

The most marked morphological changes in the senile rat eye are those shown in the retina: namely a rarefication of the nuclei of the outer nuclear layer of 35.7% ± 6.1 and of the inner nuclear layer of 26.9% ± 4.4 with a simultaneous highly significant ($p < 0.005$) age-dependent increase in area of the nuclei of the inner nuclear layer. In one third of the aging animals the retinal capillaris were characterized both by an increase in calibre and in PAS staining, sharper contouring, and thickend walls. There were few signs of age in the choroid. The anterior sections of the bulbus showed a thickening of Descemet's membrane in the cornea by approximately a factor of three in all aging animals, and a sclerosis of the ciliary body and the iris root.

The retinal damage resulting from up to three years of exposure to light intensities of 30, 62 and 223 lux was to a large extent uniform. It was characterized by a general atrophy of the 1st neurone, a partial degeneration of the 2nd neurone and destruction of the retinal structure as well as vascularization of the in part multi-layered pigment epithelium. The influence of the light intensity on the number of damaged retinae was highly significant ($p < 0.01$).

Knowledge of such findings in the rat would appear to be an important factor in long term toxicity studies for differentiating between changes in the eye dependent on age and environment, and those occasioned by the compound being tested.

Introduction

Basically it is most important to know which of the changes which occur in animals during the testing of pharmaceuticals over long periods of time are related to age, which are related to environment and finally which are a result of the compound being studied. In many of the animals used for such studies this problem has not been sufficiently clarified. On the on hand we were therefore interested in the changes in the rat eye which are related to age, and on the other in every light-dependent pathological change.

For this study we used animals in which we were initially seeking to establish the spontaneous tumour rate. These animals were a strain of rat which we routinely use in the toxicological testing of pharmaceuticals. To this end we allowed an un-

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treated group of 400 rats to live up to three years. This group allowed us on either a single organ or an organ system basis both to become familiar with and to judge the morphological changes which occur with age. At the same time we were able to approach more closely the question of precisely what retinal damage is produced as a result of artifical lighting.

In an earlier study (Stötzer, Weiße, Knappen and Seitz, 1970) which ran over a period of three months, we were able to show that in the rat there is a highly significant relationship between light intensity and retinal degeneration. The animals in this study which were exposed to a light intensity of 60 lux or more were seen to have retinal damage, principally in the form of a gradually differentiated degeneration of the rods and outer nuclear layer (1st neurone). In extreme cases, in addition to these observations there was a degenerative change in the outer plexiform layer and the outer cell layers of the inner nuclear layer (2nd neurone). These results provide an insight into the subject of light-dependent retinal damage. However, because the study was of such short duration we were unable to follow such damage, even in a single animal, through to its logical conclusion: namely retinal degeneration.

This study was therefore designed so that results would be obtained in respect of both the changes dependent on age and those dependent on light.

Materials and Method

400 6-week-old SPF albino rats (200 male and 200 female) of the Chbb/THOM(SPF) species were used in this study. The animals were kept under SPF conditions in pairs in Macrolone® type III cages, at a temperature of 22° C ± 1, and a relative humidity of 55% ± 3. Sniff-bedding was provided and the whole system was closed. The standard diet was Altromine-R® and drinking water was available ad libitum. All animals were in *one* windowless room with 12 hour light/dark periods without twighlight intervening. The animals were divided by sex with the 200 males and 200 females on opposite sides of the room and the cages were stacked in six rows. The source of light was 6 Osram strip-lights (Universal White), each of 40 Watts. These were mounted on the ceiling in two rows of three strip-lights each and at a distance of 175 cms from each other. A differential light intensity was achieved as the cages were stacked in six rows. The distance of the upmost row of cages (row 6) to the light source was 150 cms while that of the lowest row (row 1) was 260 cms. The light-intensity in each cage was repeatedly determined using a standard light meter. In this way the following average values were determined:

Row 1 =12 Lux; Row 2 = 15 Lux; Row 3 = 19 Lux;
Row 4 = 30 Lux; Row 5 = 62 Lux; Row 6 = 223 Lux.

The animals in rows 1 to 3 were used to evaluate the age-dependent changes while those in rows 4 to 6 served for the study of the light-dependent changes. During the whole period of the study each animal remained in the same cage and no cage position was altered. This implies that the animals were kept under constant light conditions up to a maximum of three years.

The animals were sacrificed at 945 respectively 1067 days and after removal of the lens were prepared separately for histology. From the 248 animals which died intercurrently, only the bulbi of those showing no autolysis were used. Staining: H. and E., PAS reaction (Schüller), trichrome staining (Masson/Goldner), luxol fast blue (Klüver/Barrera), toluidine blue, congo red (Bennhold), alcian blue (Lisson), reticulin fibre stain (Gomori). All staining was carried out according to Romeis, 1968. In addition, 10 of the 1067-day old animals were examined with a Zeiss particle size analyser and determinations were made of the nuclear size in the inner and outer nuclear layer. Five ninety-day old animals were used as controls. In the same animals the number of nuclei per unit area in the inner and outer nuclear layers was determined using a grid and a magnification of 1300. In this determination we took into account the mean values and standard deviation for the single groups, as well as for the comparison calculation using the propagation of errors method (Fenner, 1931). The frequency evaluation was carried out using the 2×2 table up to a total of 60 hyper-geometric distribution (Documenta Geigy, statistical tables). For over 60 and for $m \times n$ tables the χ^2 test using a continuity correction was used (Yates, 1934).

Results

1. Age-Dependent Changes

72 animals (41 female and 31 male) were used in the histological evaluation of age-dependent change. Of these 21 died intercurrently at an average age of 870 days, 40 animals were sectioned at 945 days and 11 at 1067 days. By and large the age-dependent changes were more pronounced in the females than in the males of the same age.

The most obvious structural changes in the senile rat eye were shown histologically at the 1st and 2nd retinal neurone (Fig. 1). The inner retinal layer, including the inner plexiform layer was to a large extent, both in its disposition and structure comparable with that of the young animal. The young animals however demonstrated an inner nuclear layer of 3 to 5 nuclear rows while the older animals showed only 3 to 4 nuclear rows in the peripapillary area and in the retinal periphery only 2 to 3 nuclear rows. Over the same total area, the reduction in the nuclear rows in the inner nuclear layer, when compared with the young animal was $26.9\% \pm 4.4$. A similar relationship was evidenced in the outer nuclear layer which was 8 to 10 nuclear rows in the young animal. In the old animal the peripapillary area was reduced to 6 to 8 layers and the mid-retinal periphery was reduced to 4 to 6 nuclear layers. In the outer retinal periphery however the outer nuclear layer and outer plexiform layer were so reduced in the older animals that they were no longer demonstrable (Fig. 2). This rarefication resulted in the remaining cells of the inner nuclear layer bordering directly on the lamina limitans externa retinae which itself was in good condition. The numerical atrophy described in the outer nuclear layer started at the papilla and ended at the ora serrata. This reduction in the nuclei of the outer nuclear layer in old rats represents a rarefication of $35.7\% \pm 6.1$ when compared with the young animals.

There was also a difference in nuclear size in the inner and outer nuclear layers. While in a comparison of the nuclei in the nuclear layer in both young and old animals a variation in nuclear surface of between 10.0 μ^2 and 11.6 μ^2 was seen in 4199 determinations and while this variation was not statistically significant, in the nuclei of the inner nuclear layer a highly significant ($p < 0.0005$) age-dependent increase in area was seen. A total of 5228 nuclear determinations were made and a nuclear surface of between 24.6 μ^2 and 28.5 μ^2 was demonstrated in the 90-day old animals, while the values for the old animals lay between 28.5 μ^2 and 32.6 μ^2. It should be stressed that these areas expressed in μ^2 represent only comparative values as no nuclear volumes were calculated.

The changes found in one third of the animals in the vascular system of the retina were not comparable in their intensity to the aging process at the 1st and 2nd neurone. While the arterioles and venules showed no marked changes in the old animals, the retinal capillaries, the superficial and deep capillary networks were marked by increased calibre, a sharper contouring and a thickening of the capillary wall. The capillary walls demonstrated less cell—and in places there was even complete acellularity—and this was particularly well shown up with PAS staining (Fig. 3).

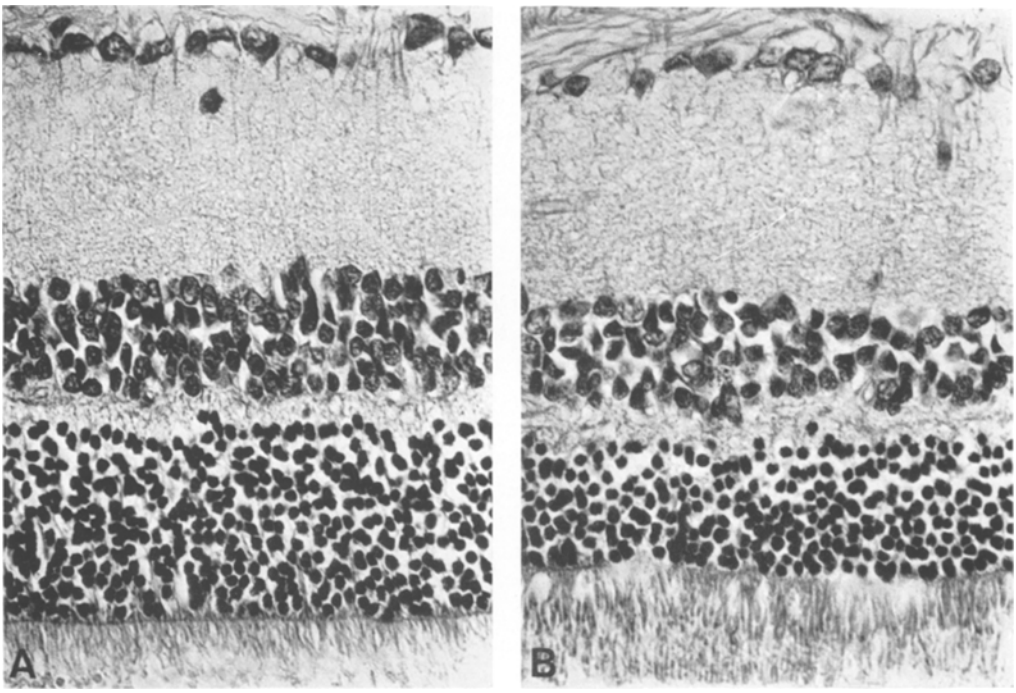

Fig. 1. Normal rat retina showing mid-fundus (A) in a 90-day old animal and (B) in 1067-day old animal. Masson/Goldner × 400

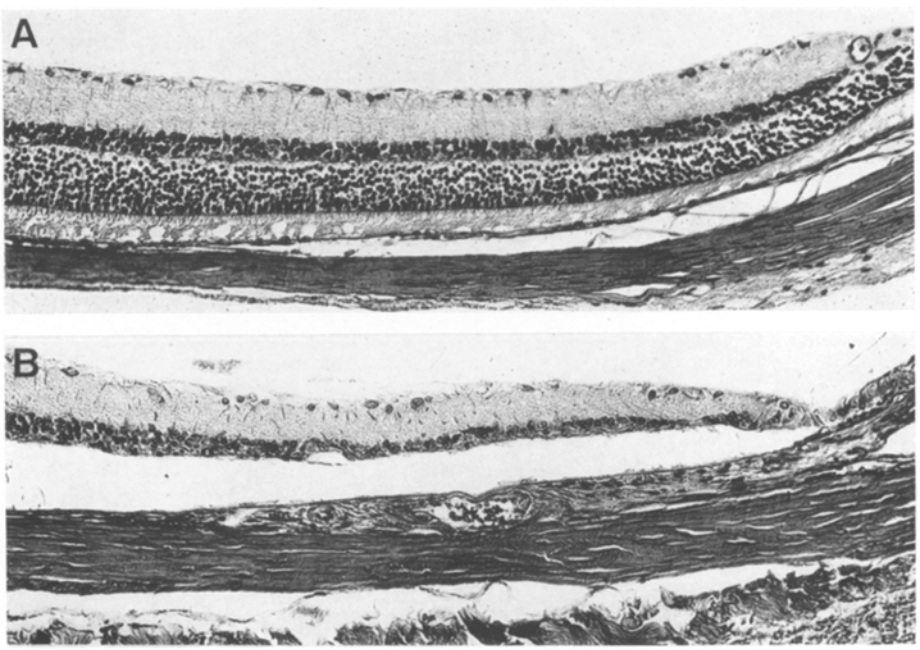

Fig. 2. Rat retinal periphery in a 90-day old animal (A) and a 1067-day old animal (B) showing generalized atrophy of the 1st neurone in the older animal. Masson/Goldner × 160

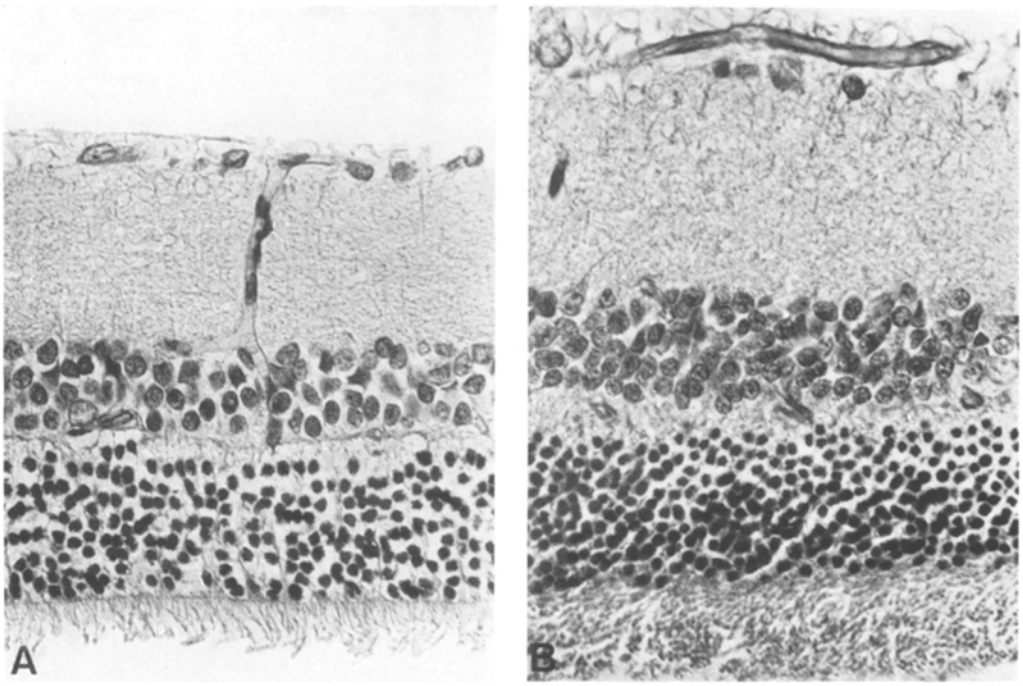

Fig. 3. Retinal capillaries in the rat, in a 90-day old animal (A) and a 1067-day old animal (B) with reduction in cells and thickened wall in the older animal. PAS reaction × 640

In contrast to those in the retina, the age-dependent changes in the choroid were relatively few. Only in three cases did cell proliferation occasion a marked narrowing or occlusion of the vessel and such observations were made in single arterioles only. In two cases subendothelial cells were singled out by light microscopy as having inflated nuclei and single intracytoplasmic vacuoles. In the third animal, besides a total displacement of the lumen, sclerosis of the vessel wall was observed together with a reactive increase of the fibrocytes and histocytes in the adventitia.

In the anterior portion of the bulbi, age-dependent changes were seen principally in the cornea and the anterior uvea. In the old animals the most marked change was the thickening of Descemet's membrane. In young animals this membrane measured 2 to 3 μ at the centre of the cornea and 1 to 2 μ at the periphery. In contrast to this comparable values in the old rats were increased threefold, giving figures of up to 10 μ in the centre and 4 to 6 μ in the region of the chamber angle (Fig. 4). The changes in the anterior uvea were characterized by the reductions in the number of stroma cells with simultaneous increase and thickening of the collagen fibre elements. This sclerosis was particularly marked in the areas of the ciliary body and the iris root.

2. Light-Dependent Retinal Changes

In order to be able to give an opinion on the retinal damage after almost three years of artifical lighting, the findings in 93 rats (50 female, 43 male) from the

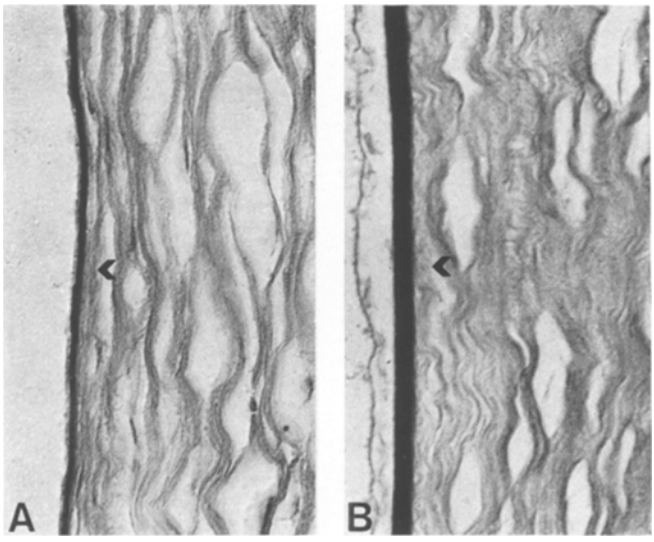

Fig. 4. Descemet's membrane (→) in rat cornea, in a 90-day old animal (A) and a 1067–day old animal (B). PAS reaction × 400

Table 1. Distribution of animals showing light-dependent changes in the upper three rows of cages

Row	Light intensity (Lux)	Male		Female		Total animals showing light-dependent changes
		animals examined	animals with light-dependent changes	animals examined	animals with light-dependent changes	
6	223	24	19 (79.2%)	23	22 (95.7%)	41
5	62	11	3 (27.3%)	19	9 (47.4%)	12
4	30	8	3 (37.5%)	8	4 (50.0%)	7
		43		50		60

upper three rows of cages were evaluated. 18 animals averaged 790 days, 66 animals 945 days and 9 animals 1067 days under these conditions of light intensity. As can be seen from Table 1, 60 of these 93 rats showed light dependent changes of the retina.

Although these animals were kept for varying periods and at varying light intensities, the resulting morphology of the light-dependent retinal damage, which was always oberved to the same extent in both eyes, was to a large extent uniform in all cases both in respect of its extent and its degree.

The rods, the outer nuclear layer and the outer plexiform layer were no longer demonstrable in the total extent of the retina (Fig. 5). The remaining cells in this rarified and often only 2 to 3 layer, inner nuclear layer bordered directly

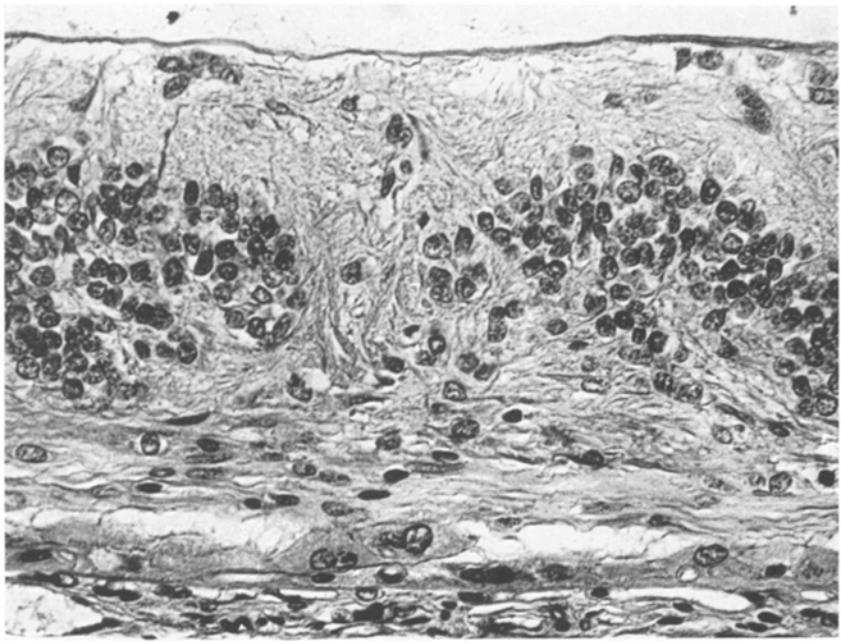

Fig. 5. Rat retina with total degeneration of the 1st neurone and advanced loss of layered structuring. The animal was kept for 790 days at a light intensity of 30 lux. H. and E. $\times 400$

into the lamina limitans externa retinae. As a result of the better retained layered structuring in the peripheral retinal sections, this latter is particularly well demonstrated, especially as it lies directly in the pigment epithelium. Apart from individual nerve cell degeneration, the sections of the ganglion cell layer, the nerve fibre layer, and the inner plexiform layer in the region of the ora serrata showed no marked changes. In the peripapillary area and in the mid-retinal periphery were numerous capillaries, running from the superficial network transversely through the retina and branching into the pigment epithelium (Fig. 6). This pigment epithelium, which originally comprised a single layer, was penetrated along its entirety by a dense capillary network and locally was in 2 to 3 layers. In addition to this, at the entry points of these capillary branches to the pigment epithelium a considerable increase in the size of the cells of the pigment epithelium could be observed: at times even increased by a factor of 2. Individual enlarged cells were observed to have made their way to lie along the vessels in the retina. At numerous points in this vascularized pigment epithelium, marked coiling of the vessels was seen. The walls of the vessels in these sections which contain many cells are remarkable and remeniscient of new vessel formation. The fact that in the areas in which the capillaries from the retinal surface pass to the pigment epithelium, the retinal structure is to a large extent destroyed, seems to us worth noting (Fig. 7). In these destroyed sections cystoid cavities of varying sizes were sometimes found. In many animals drop-shaped intra-retinal hyaline deposits were noted. These deposits were particularly to be found in the region immediately

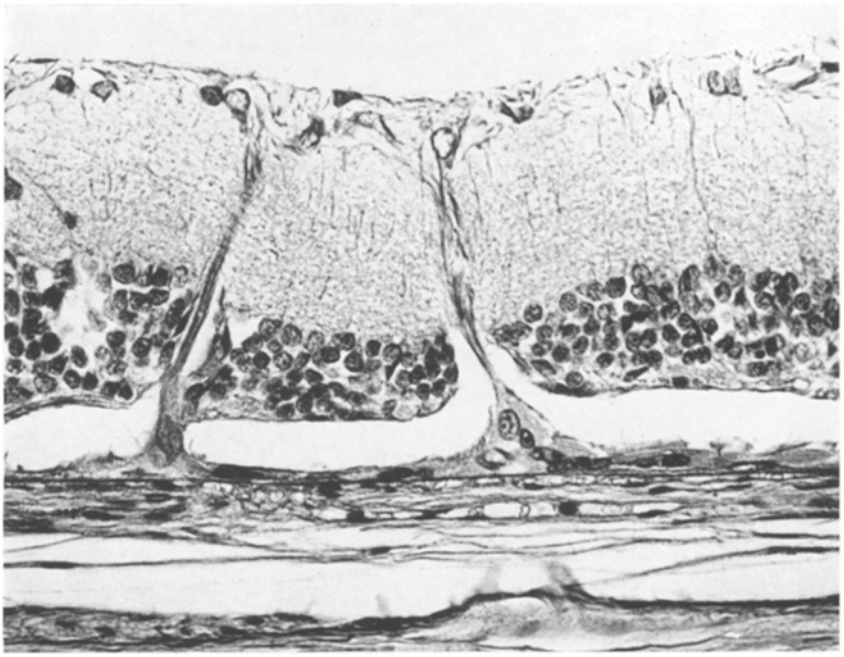

Fig. 6. Rat retina showing two capillary branches running from the superficial capillary network to the pigment epithelium and branching there. This animal was kept for 1067 days at a light intensity of 60 lux. Masson/Goldner × 350

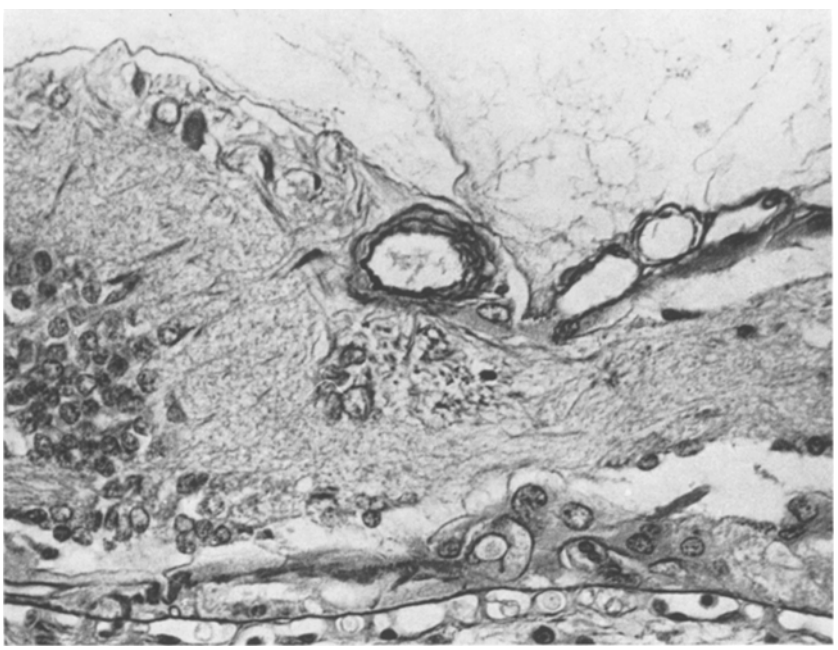

Fig. 7. Rat retina with total structural loss, clustering of the nuclei of the inner nuclear layer and irregular surface. The vascularized pigment epithelium shows in places 2 to 3 layers. This animal was kept for 1067 days at a light intensity of 60 lux. Masson/Goldner × 400

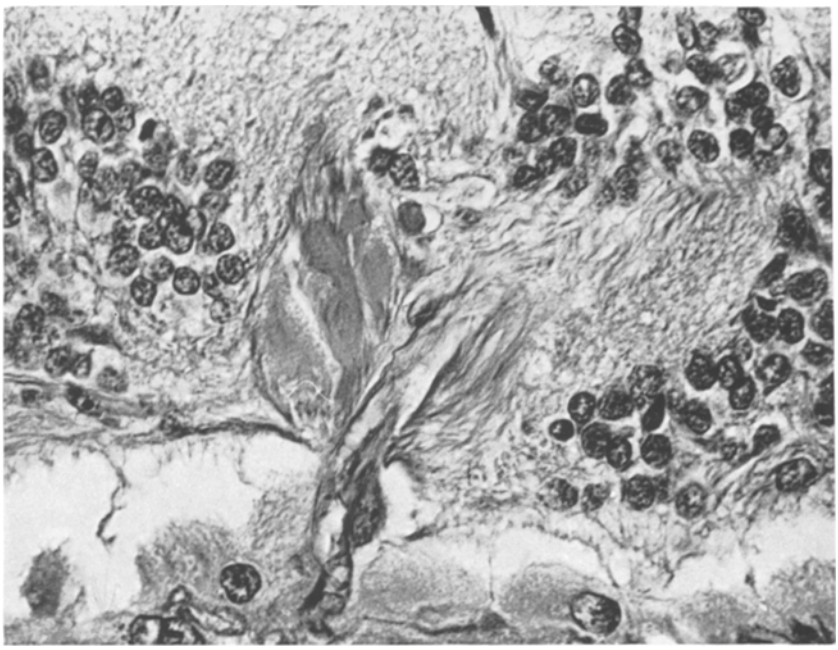

Fig. 8. Rat retina showing hyaline deposits in the region of a capillary leading to the pigment epithelium. Amyloids and muco-polysaccharides could not be demonstrated. This animal was kept at a light intensity of 60 lux for 1067 days. H. and E. × 640

bordering the capillaries, or at the points of capillary branching (Fig. 8). Bruch's membrane, which was practically always easily visible under the light microscope, represented an intact boundary between the retina or rather the pigment epithelium and the choroid. The choriocapillaris and the choroidea were also unchanged.

The distribution of the 60 animals showing light-dependent damage in the upper three cage rows clearly demonstrates (Table 1) that there is a highly significant ($p < 0.01$) relationship in both sexes between the light intensity and the number of damaged retinae. The number of animals affected at 223 lux (6th row) is significantly higher than at 62 lux (5th row), while the difference between 62 lux and 30 lux (4th row) cannot be demonstrated statistically. The expected difference between the sexes in these three rows was also nor able to be statistically demonstrated. In 33 animals the described light-dependent damage was not seen. In these animals the retinae showed comparable age-dependent changes to the animals in the lower three cage-rows.

Discussion

In order to avoid misinterpretation of the cause of retinal damage in toxicological studies it is important to have knowledge of the possible causes which can lead to real or supposed pathological findings. In this study the effect of age and long term artificial lighting were examined as being the possible causes of structural changes in the rat eye and in particular the retina. In order to ensure

that other factors did not influence the results, we endeavoured to ensure that the requisite environmental conditions prevailed.

A hereditary component can be excluded as a possible additional cause of the retinal changes observed in the old animals in the light study. Bourne, Campbell and Tansley (1938), Dowling and Sidman (1962), Dowling (1964), Tilgner-Peter (1966) and Saunders (1967) demonstrated that hereditary retinal degeneration has already commenced in the rat by the 3rd week and that the postnatal reduction in the photoreceptor cells progressed so fast that total absence of the first neurone is achieved by about day 60. In an earlier study (Stötzer, Weisse, Knappen and Seitz, 1970) our strain was examined histologically for just such degeneration and regular microscopic controls in routine experiments have all resulted in no indication of such a factor.

A chronic *vitamin A deficiency* can be ruled out, be it either as the sole cause of or as a contributory factor to the retinal damage recorded in this study. Chemical analyses of the diet given, namely Altromine-R® showed after autoclaving an average vitamin A content of 8800 I.U./kg of feed[1]. If this figure is compared with the recommendation of Coates *et al.* (1969) that rats should have 5000 I.U./kg of feed, it can be seen that the animals were given sufficient vitamin A.

As a further important factor interfering in the physiological aging of the rat retina, the *lighting conditions* were taken into account as we had already (1970) been able to demonstrate that in the rat a light intensity of less than 30 lux did not lead to any light-dependent changes. Therefore those animals used in this study for the evaluation of the age-dependent factors lived up to three years under conditions of light intensity from between 12 and 19 lux. From this one can therefore say with some certainty, even when considering the other two factors, that the demonstrated morphological criteria for the "aging eye" are solely the result of senescence.

To our knowledge only Leopold and Calkins (1951) have to date published work on age-dependent changes in the rat eye. We are able to confirm the collagenization of the stroma of the ciliary body demonstrated by these authors. Nevertheless the typical corneal change in old rats was, in our opinion, the increase in thickness of Descemet's membrane. According to Lauber (1936) and Vrabec (1961) this is a result of the secretory function of the particularly active metabolism of the endothelium. In man (Rother, 1965) the thickness of Descemet's membrane is approximately proportional to the square root of age: so that theoretically the approximate age can be fixed by measuring the thickness of this membrane. Similarly, we believe that it is possible to differentiate with certainty between young and old rats on the basis of the thickness of Descemet's membrane.

Equally noteworthy are the retinal changes corresponding to age. Whereas Leopold and Calkins (1951) found no morphological variations in their aging rats, we noted a *rarefication in the number of cells in the 1st and 2nd neurones of* about one third, as well as *changes in the capillary networks*, as being characteristic, age-related changes of the retina. The fact that both of these findings were particularly marked in the retinal periphery, accords well with studies in man (Sachsenweger, 1971).

1 These analyses were carried out at the "Institut für Ernährungsphysiologie" in the veterinary faculty of Munich University (Chair: Prof. Dr. Dr. J. Tiews †).

Unexpectedly high at 35.7% and 26.9% were the figures arrived at by cell count for the degree of rarefication of the neuroepithelium respectively the ganglion cells of the 2nd neurone. This in spite of the fact that the choriocapillaris feeds the neuroepithelium by diffusion and that age-dependent changes in the vessels in the choroid were only demonstrated in single instances. This rarefication of the neuroepithelium in the retinal periphery is also typical in aging man where according to Hogan and Zimmermann (1964) it is accompanied by a simultaneous proliferation of the glial cells. However in the rats under study we observed no such increase in glial tissue.

The morphometric increase in surface area of the nuclei of the inner nuclear layer found in the old animals can only be presented as a finding. An interpretation must be left until specific studies have been carried out.

The senile capillary changes in man demonstrated by Kuwabara, Carroll and Cogan (1961) both histologically and also in trypsin digested flat preparations, are typified by a reduction in the cells of the capillary wall and by irregularities in the calibre of these vessels as well as by an affinity for PAS stain. Inasmuch as it was possible to given an opinion on the retinal capillaries in old rats by using histological sections, with the exception of the vessel irregularities the same characteristics apply. Friedmann, Smith and Kuwabara (1963) and Friedmann and Smith (1965) showed that these aging characteristics in the capillaries are not limited to the retina but are also seen in other sections of the bulbus.

The influence of artificial lighting on the rat retina has been the subject of considerable study in recent years (Noell *et al.*, 1966; Gorn and Kuwabara, 1967; Kuwabara and Gorn, 1968; Hansson, 1970; Green, 1971).

Basically the difference between these studies, which include our previous study (1970), and the one here described, lies in the fact that we did not study the effects of short term for the most part high intesity light, but the effect of intensities up to a maximum of 223 lux over a period of years. The study has shown that in the rat retina, which is morphologically that of a nocturnal animal, even relatively low light intensities (30 to 60 lux) result over a long period of time in the same retinal damage as that evinced by high intensities of shorter duration. The degree of degeneration and destruction demonstrated after up to three years of exposure to light is to be seen as the final stage of light-induced damage in the retina of the albino rat. In this process the more morphologically discrete age-dependent damage was completely hidden. The time of onset of such a degenerative process under the given lighting conditions is however not able to be stated from this study. It seems to us important to note that the given lighting conditions are quite usual in the majority of studies carried out using rats.

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