made from each block, stained with toluidine blue and used for selection of areas for ultrastructural observation. Thin sections were stained with uranyl acetate and lead citrate prior to examination in a JEOL JEM 100C transmission electron microscope.

Results. At 3 days, axon bundles in both the lamina propria and submucosa of all experimental rats were composed of a mixture of normal and swollen degenerative axons. These swollen axons were electron translucent, contained few neurotubules and small scattered strands of axoplasm. Phospholipid membrane debris was common, usually in the form of myelin figures. A mixture of normal and degenerating axons within the same Schwann cell sheath was common. Fibroblasts were frequently seen near degenerating axon bundles.

By 3 to 6 weeks, degenerating axons were found in decreasing numbers. Healthy axons could be divided into 2 types. These were either small and densely packed with neurotubules or large with sparse neurotubules (fig. 1). Areas of apparent synapse were seen between adjacent nerve fibers on rare occasions. Collagen fibers were abundant around the nerve bundles.

At each 3-week interval postoperatively, from the ninth to the fifteenth, an increasing number of normal appearing axon bundles could be seen in the mucosa and submucosa. Small fibers with numerous neurotubules and large fibers with relatively few neurotubules were seen in the same Schwann cell sheaths. Synaptic contact was noted between adjacent fibers within bundles and also between individual nerve fibers and parietal cells. The synaptic vesicles were electron translucent and similar in morphology to cholinergic neurotransmitter vesicles (fig. 2).

The nerve fibers in the mucosa of the sham operated rats were normal in appearance. While some variation in diameter was common, the degree of this variability was less than that seen in the experimental groups. No degenerating axons were seen in any sham rats.

Discussion. The changes noted in the axons of the gastric mucosa at 3 days were typical of nerve degeneration following injury. Degeneration could be found up to 6 weeks after surgery, decreasing with time. As degeneration proceeded, small axons with densely packed neurotubules appeared in both the PCV and PCV + antrectomy experimental rats. The numbers of these fibers showed a definite increase up to the 9–12 week intervals. These were interpreted as regenerating axons. At 12 to 15 weeks the diameter of the smaller axons was greater than in earlier weeks. Schwann cells of these vagotomized rats contained a larger number of axons than those of the controls and the size and shape of the fibers was more heterogeneous. Increased fibroblast activity and collagen deposition was noted in the early period of healing[5]. The rapid appearance of the small axons (2–3 weeks) raises some question as to their source. Division of the vagal nerve fibers is accompanied by retraction of the cut end of the nerves. While regeneration along the vasculature is a possibility, it seems unlikely that the regenerating axons could advance that rapidly in an organized way from outside the walls of the stomach. The findings in the PCV rats and the PCV + antrectomy rats were identical which would indicate that these fibers do not arise from the nerves located in the antrum. It would appear that recruitment of axons from some other nearby source such as the fundus may be involved in this rapid regeneration.

1 Kennedy, T., Johnston, G.W., MacRae, K.D., and Spencer, F.F.A., Br. med. J. *2* (1975) 301.
2 Wastell, C., Colin, J., Wilson, T., Walker, E., Gleeson, J., and Zeegen, R., Br. med. J. *2* (1977) 851.
3 Koffman, C.G., Elder, J.V., and Gillespie, I.E., Br. J. Surg. *66* (1979) 145.
4 Amdrup, E., Andersen, D., and Hostrup, H., Wld J. Surg. *2* (1978) 85.
5 Joffe, S.N., Crocket, A., and Doyle, D., Am. J. Surg. *143* (1982) 80.

0014-4754/84/080850-03$1.50 + 0.20/0

Light dependent accumulation of macrophages at the photoreceptor-pigment epithelial interface in the retina of albino mice

S. Sanyal, A. De Ruiter and Ch. Dees[1]

Department of Anatomy, Erasmus University, Postbox 1738, NL-3000 DR Rotterdam (The Netherlands), 10 October 1983

Summary. In the subretinal space of albino mice, macrophages appear from the time of eye opening and increase in number for 6 months; thereafter they decline with age. Dark rearing retards the accumulation of these cells, and exposure to constant light results in a rapid increase. Observations suggest that macrophages appear as a response to visual cell decay in albino mice and supplement the phagocytic activity of the pigment epithelium.

In the developing retina of mice, macrophages appear during vascular growth and remain located within the vascularized part of the inner retina, up to the level of the outer plexiform layer[2,3]. As a result of visual cell death due to hereditary degeneration[2,4,5], similar cells appear in the outer retina including the photoreceptor-pigment epithelial interface. In the albino rat Ling[6,7] has observed that macrophages are present in the inner retina but are noticeably absent from the receptor layer and has commented[7] that the pigment epithelial cells normally carry out the phagocytic activity. However, in the course of pathogenesis in dystrophic[8] and light damaged[9,10] retinas in the rat macrophages have been observed in the outer retina. Braekevelt[11] has shown that phagocytic cells occur at the photoreceptor-pigment epithelial interface of teleostean fishes under normal conditions. Recently, we observed similar cells in the retina of albino mice. Light and electron microscopic observations and enzyme histochemical studies were undertaken and a survey was made for the presence of such cells in mice and rats from different strains. In addition, groups of mice were reared in darkness or exposed to constant light and their eyes were examined for the presence of similar cells. In this report, we describe the macrophage nature of the cells and their changes with age and light conditions.

Material and methods. Eyes from albino and pigmented mice and rats from many inbred strains were examined for the presence of phagocytic cells. For other studies albino mice of the Balb/cLiA strain were used. For electron microscopy eyes were fixed in aldehyde mixture and post-fixed in 1% osmium tetraoxide. Small pieces were dissected out and embedded in epoxy resin as described earlier[12]. For the localization of peroxidase

activity, the eyes were sliced in a tissue chopper after aldehyde fixation. The sections were stained by the benzidine method[13,14] and post-fixed and embedded in the usual way. For the staining of other enzymes eyes were fixed in 4% paraformaldehyde and 15-µm-thick cryostat sections, attached on slides were used. The activity of N-acetyl-β-D-glucosaminidase was localized according to Hayashi[15] as reported earlier[2]. Acid phosphatase was localized by the Gomori procedure with β-glycerophosphate as substrate and by the azo dye method with α-naphthyl acid phosphate as substrate[16]. Nonspecific esterase activity was stained with α-naphthyl acetate as already described[17]. For inosine diphosphatase the method of Novikoff and Goldfischer[18] was used with inosine diphosphate as substrate at pH 7.2.

For the light deprivation experiment, animals were born and maintained in total darkness. A dim red lamp was used during cage-cleaning etc. For exposure to constant light, groups of mice in transparent cages were placed between fluorescent tubes. The intensity was 2400 ± 200 lux inside the cage.

For the estimation of the cells, materials stained for β-glucosaminidase were most suitable. The cells, marked by enzyme activity and located between the outer limiting membrane and the pigment epithelium were included. The total number of cells in 5 medial sections, passing through the optic nerve, were counted under the microscope. The average from 5 sections was used for each eye and 3–5 individuals were used for each group.

Results and discussion. Eyes from albino mice of inbred strains, A, AKR, Balb/c, GR/A, and Swiss, and from pigmented mice of the inbred strains, C57BL, DBA, and C3H^{rd+} (a substrain of C3H in which the *rd* gene has been substituted by the normal allele, and which hence has a normal retina) were screened for the presence of β-glucosaminidase positive cells. Presence of stained cells could be noted between the outer limiting membrane and the pigment epithelium in the albino mice (fig. 1) of all strains examined. However, similar cells were not observed in any of the pigmented mice. It should be mentioned that 2 strains of albino rats, Amsterdam, and Wag/Rij and 1 strain of tan hooded rat, R×U, were also examined but similar cells were not found.

Figure 3 shows the frequency of the β-glucosaminidase-positive cells in the retinas of albino Balb/c mice at different ages. At 14 days, when the eyes had just opened, these cells were absent or rarely observed. The cells increased in number and reached maximum frequency at the age of 2–3 months and remained more or less unchanged up to 6 months. Thereafter a slow decline in the frequency of these cells was seen in the retinas of older mice.

In albino Balb/c mice, reared in darkness, the number of β-glucosaminidase positive cells in the photoreceptor-pigment epithelial interface remained very low. At 6 months very few cells

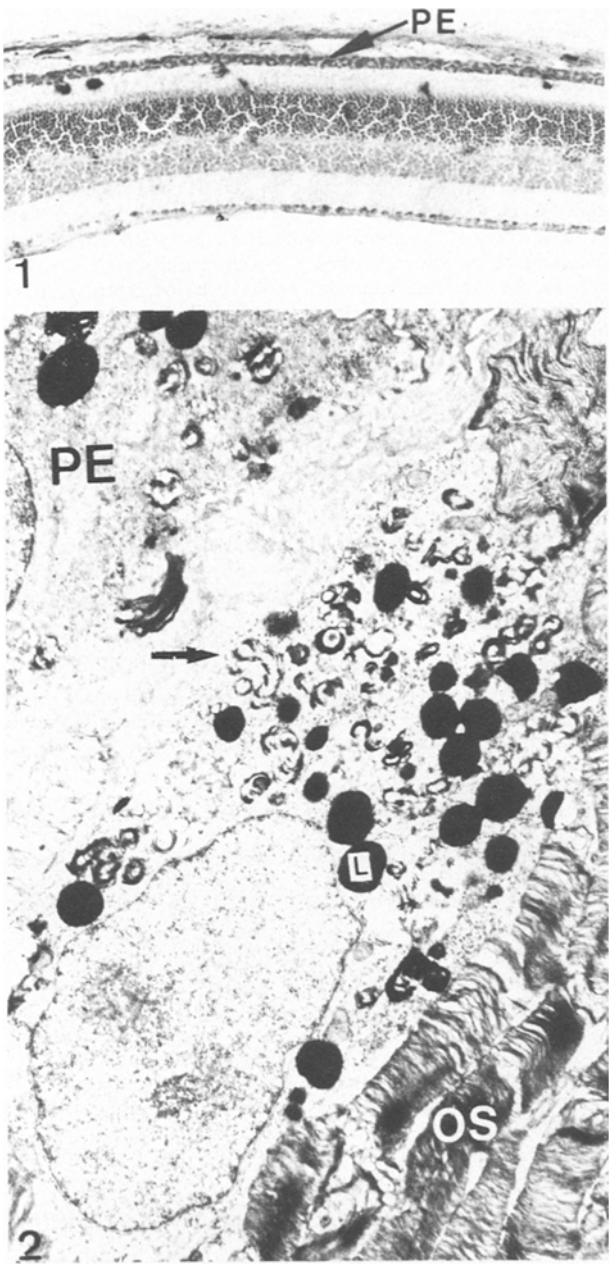

Figure 1. Cryostat section of retina from a 3-month-old albino mouse, maintained in cyclic light, stained for the enzyme β-glucosaminidase. Positively stained cells (macrophages) are seen scattered in the receptor layer below the pigment epithelium (PE). ×200.

Figure 2. Electron micrograph of a macrophage, between pigment epithelium (PE) and outer segments (OS) of an albino mouse which has been exposed to constant light for 48 h. Note phagosomes including receptor outer segment debris (arrow) and lipid droplets (L). ×7000.

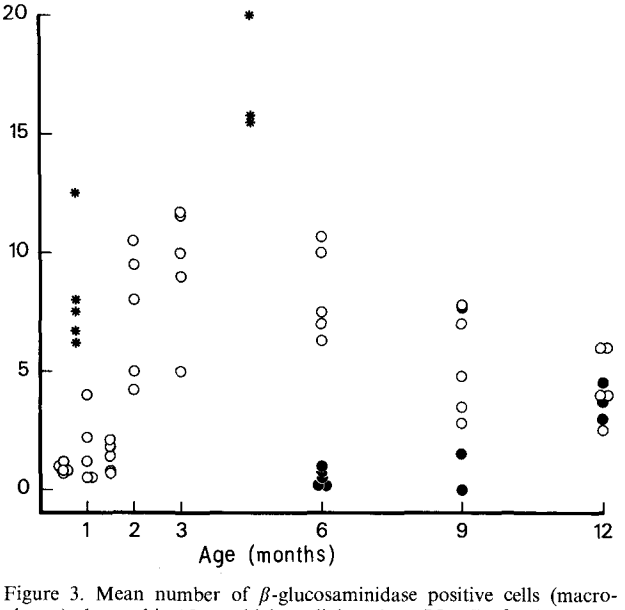

Figure 3. Mean number of β-glucosaminidase positive cells (macrophages) observed in 15-µm-thick medial sections (N = 5) of retinas from albino mice reared in cyclic light (open circles) and in total darkness from birth (closed circles). Asterisks represent individuals exposed to constant light for 48 h prior to fixation.

could be observed and their frequency was the same as at 2 weeks. At 9 months 2 of the 3 mice examined also showed few or no β-glucosaminidase positive cells. In 1 individual these cells appeared with the same frequency as in the control group. In the retina of 12-month-old mice, more β-glucosaminidase positive cells were seen and their frequency was comparable to that in the control group.

Albino Balb/c mice of 2 different age groups, at 14 days and at 4–5 months, were exposed to constant light for 48 h and the eyes were examined for the frequency of β-glucosaminidase positive cells. In both groups, a higher frequency of these cells, was recorded than in those maintained in cyclic light (fig. 3). It should be mentioned that constant light also caused disruption and loss of visual cells. Longer exposure caused further deterioration of the retinal morphology and made it difficult to count the cells.

Figure 2 shows the electronmicrograph of an amoeboid cell located between the outer segments and pigment epithelium of an albino mouse which has been exposed to constant light for 48 h. Large phagosomal inclusions of varying sizes are present in the cytoplasm. Some of these inclusions contain debris of receptor outer segments. These are similar to the inclusions within the pigment epithelial cells. Similar phagosomal inclusions have also been observed in such cells from mice of the same age, maintained in cyclic light (fig. 4), but these are less frequent and smaller in size. In materials stained for peroxidase, enzyme activity was observed in large peroxisomes within the macrophage cytoplasm whereas the retinal cells were completely unstained. In the pigment epithelium some activity was present in microperoxisomes. At the light microscopic level, the cells showed strong reactions for non-specific esterase and β-glucosaminidase. The pigment epithelial cells reacted positively for these enzymes but the staining was less intense. The cells showed a faint activity for acid phosphatase in contrast to the intense activity seen in the pigment epithelium. The cells were also stained for inosine diphosphatase, an enzyme present in the vascular tissue of the retina but not in the pigment epithelium.

Presence of β-glucosaminidase and nonspecific esterase in these cells, in which they resemble the macrophages in the degenerating retina[2,8]; of IDPase activity as in vascular tissues and cells of vascular origin[19] and of peroxidase activity as in murine macrophages[14,20] indicates that the cells are vascular derived macrophages. Presence of phagolysosomal inclusions, containing receptor outer segment debris, suggests that the macrophages are engaged in active phagocytosis. Since phagocytic function of the pigment epithelial cells in the renewal and shedding of the outer segments is well established[21], it would appear that the macrophages in the photoreceptor-pigment epithelial interface in the albino mice supplement the phagocytic activity of the pigment epithelial cells. Accumulation of the macrophages from the time of eye opening, increased frequency after exposure to constant light, and retarded accumulation in dark-reared albino mice suggest that the process is light dependent. In contrast to the pigmented and dark-reared mice, light-induced changes in the visual cells and their consequent decay in albino mice appear to be of a higher order and sufficient to stimulate macrophage reaction. However, absence of similar cells in the albino rat[7] and their presence in pigmented teleostean fishes[11] point to a species difference in factors which make visual cells more vulnerable in some species than in others.

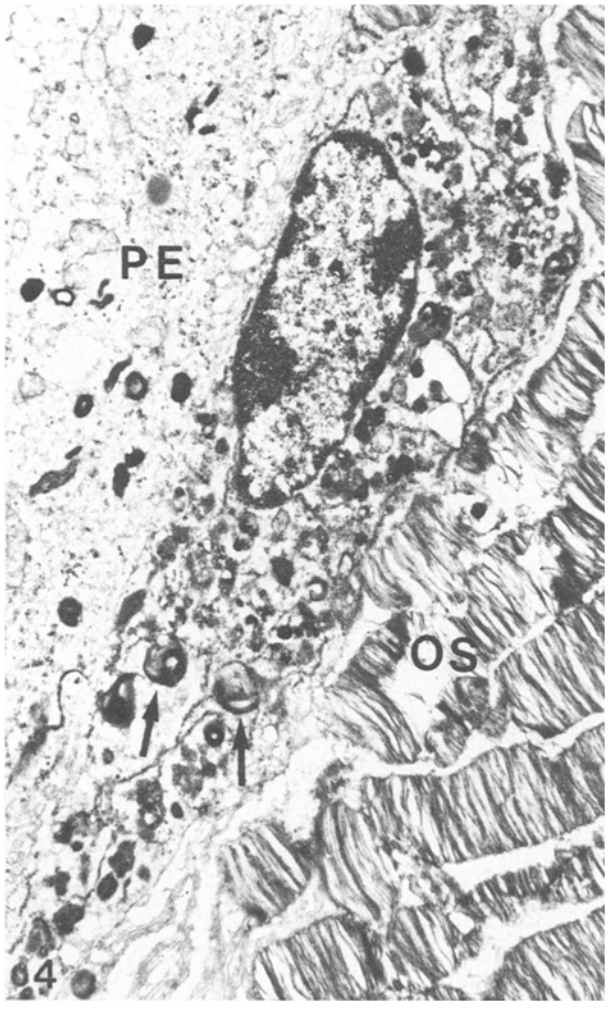

Figure 4. Electron micrograph of a macrophage, between the pigment epithelium (PE) and outer segments (OS) of an albino mouse maintained in cyclic light. Small phagosomes containing outer segment debris are present (arrow) ×5000.

1 We thank R.K. Hawkins for efficient management of the animal colony, H.G. Jansen for cooperation in the use of electron microscope and Ms Paula van Alphen for help in preparation of the illustrations.

2 Sanyal, S., Histochemie *29* (1972) 28.
3 Hume, D.A., Perry, V.H., and Gordon, S., J. Cell Biol. *97* (1983) 253.
4 Sanyal, S., De Ruiter, A., and Hawkins, R.K., J. comp. Neurol. *194* (1980) 193.
5 LaVail, M.M., Blanks, J.C., and Mullen, R.J., J. comp. Neurol. *212* (1982) 217.
6 Ling, E.A., Archvm histol. jap. *44* (1981) 167.
7 Ling, E.A., Archvm histol. jap. *45* (1982) 37.
8 Essner, E. and Gorrin, G., Invest. Ophthal. vis. Sci. *18* (1979) 11.
9 O'Steen, W.K., and Karcioglu, Z.A., Am. J. Anat. *139* (1974) 503.
10 Gloor, B.P., Albrecht v. Graefes Arch. Ophthal. *190* (1974) 183.
11 Braekevelt, C.R., Vision Res. *20* (1980) 495.
12 Sanyal, S., and Jansen, H.G., Neurosci. Lett. *21* (1981) 23.
13 Graham, R.C., and Karnovsky, M.J., J. Histochem. Cytochem. *14* (1966) 291.
14 Ogawa, T., Koerten, H.G., and Daems, W.Th., Cell Tiss. Res. *188* (1978) 361.
15 Hayashi, M., J. Histochem. Cytochem. *13* (1965) 355.
16 Barka, T., and Anderson, P.J., J. Histochem. Cytochem. *10* (1962) 741.
17 Bhattacharjee, J. and Sanyal, S., Histochemistry *46* (1975) 53.
18 Novikoff, A.B., and Goldfischer, S., Proc. natl Acad. Sci. USA *47* (1961) 802.
19 Sanyal, S., and De Ruiter, A., in preparation.
20 Daems, W.Th., and Koerten, H.K., Cell Tiss. Res. *190* (1978) 47.
21 Young, R.W., J. Cell Biol. *33* (1967) 61.

0014-4754/84/080852-03$1.50 + 0.20/0