KINETICS OF ROD OUTER SEGMENT RENEWAL IN THE DEVELOPING MOUSE RETINA

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ABSTRACT

The kinetics of rod outer segment renewal in the developing retina have been investigated in C57BL/6J mice. Litters of mice were injected with [^{3}H]amino acids at various ages and killed at progressively later time intervals. Plastic 1.5 μm sections of retina were studied by light microscope autoradiography. The rate of outer segment disk synthesis, as judged by labeled disk displacement away from the site of synthesis, is slightly greater than the adult level at 11–13 days of age; it rises to more than 1.6 times the adult rate between days 13 and 17, after which it falls to the adult level at 21–25 days. The rate of disk disposal, as measured by labeled disk movement toward the site of disposal, is less than 15% of the adult level at 11–13 days of age; it rises sharply to almost 70% of the adult level by days 13–15 and then more gradually approaches the adult rate. The net difference in rates of synthesis and disposal accounts for the rapid elongation of rod outer segments in the mouse between days 11 and 17 and the subsequent, more gradual elongation to the adult equilibrium length reached between days 19 and 25. The changing rate of outer segment disk synthesis characterizes the late stages of cytodifferentiation of the rod photoreceptor cells.

INTRODUCTION

Vertebrate rod photoreceptor cells continually renew their photoreceptive outer segments (Young, 1967; Young and Droz, 1968; Young, 1971 *b*). Protein is synthesized in the rod inner segments and migrates into the bases of the rod outer segments where it is thought to be incorporated into the invaginating outer plasma membrane of the outer segments to form membranous outer segment disks (Nilsson, 1964; Young, 1968, 1969). This labeled protein is primarily opsin, the protein moiety of the rod visual pigment (Bargoot et al., 1969; Hall et al., 1968, 1969; Matsubara et al., 1968) which may constitute as much as 80% of the outer segment protein (Hall et al., 1969; Robinson et al., 1972; Heitzmann, 1972). As new outer segment disks are formed, they displace the previously synthesized disks progressively outward. Some proteins in the displaced disks may be turned over locally, but opsin apparently is not (Bok and Young, 1972). When the disks reach the apex of the rod outer segment they are either intermittently shed in groups of 10–30 disks surrounded by their outer plasma membrane and then ingested by the pigment epithelial cells (Young, 1971 *a*), or the pigment epithelial cells play the initial active role in breaking off the apical groups of disks (Spitznas and Hogan, 1970). Within the pigment epithelial cell cytoplasm, the ingested groups of outer segment disks become surrounded by pigment cell membranes to form inclusions called phagosomes (Young and Bok, 1969; Spitznas and Hogan, 1970; Young, 1971 *a*). The phagosomes subsequently are degraded within the pigment epithelial cells (Ishikawa and Yamada, 1970).

In the normal adult animal the system is in

equilibrium. Rod outer segments are maintained at a constant length, so the rates of outer segment formation and disposal must be equal. The disk disposal mechanism consists of at least two components, phagocytosis and degradation by the pigment epithelial cells. There could also be membrane lysis independent of the pigment epithelial cells. The equilibrium among these processes can be altered experimentally, as evidenced by accumulation of phagosomes well above the normal low concentration in pigment epithelial cell cytoplasm when the eye is exposed to excessive light (Kuwabara, 1970).

When rod outer segments are elongating in the developing retina, the rate of synthesis of outer segment disks must be greater than the rate of disposal. This implies either differences in the time of onset of the synthetic and disposal components of the renewal mechanism, changes in their rates as development proceeds, or both. This study was carried out to explore the kinetics of the two components during generation of rod outer segments in normal animals.

MATERIALS AND METHODS

Mice were selected for this investigation because their photoreceptors are almost entirely rods and their outer segments are generated postnatally, for the most part between days 10 and 20 (Olney, 1968; LaVail and Reif-Lehrer, 1971; Caley et al., 1972). Genetic uniformity among experimental animals was obtained by the use of inbred C57BL/6J mice, a readily available strain known to be homozygous for the wild-type allele at the retinal degeneration locus (Sidman and Green, 1965). The mice were fed 18RF mouse pellets (26% protein, 7% fat; Charles River Breeding Laboratories, Inc., Wilmington, Mass.) ad lib. and were maintained in a 12 h light-12 h dark environment at a room illumination of approximately 50 footcandles. All experimental manipulations were carried out in ordinary laboratory illumination near the middle of the light phase of the cycle.

In single adult eyes the autoradiographic method allows rod outer segment renewal to be calculated from measurement of disk synthesis alone since the rates of disk synthesis and disposal are equal (Young, 1971 *b*). In the developing retina it was necessary to measure synthesis and disposal independently. The rate of disk synthesis was studied in the conventional fashion by measuring the displacement with time of the radioactive disks away from their site of synthesis at the base of the outer segment. The rate of disk disposal was determined by measuring the movement with time of radioactively labeled disks towards their site of disposal at the apex of the outer segment.

Single litters of mice at 10, 11, 12, 14, 16, 18, 20, and 53 days of age were injected with a mixture of equal volumes of L-[4,5-^{3}H]leucine and L-[3-^{3}H]-phenylalanine (sp act 38.6 and 15.7 Ci/mmol, respectively; both 1.00 mCi/1.00 ml; New England Nuclear, Boston, Mass.). Each animal received a single intraperitoneal injection of 25 μCi/g body weight, a dose much less than other workers (e.g., Young and Bok, 1969; Bok and Hall, 1971; Young, 1971 *b*) have successfully used with no apparent radiation damage.

The animals in each litter were killed at 2-day intervals ranging from 1 to 11 days after injection (Table I) by vascular perfusion for 10 min with a mixture of 1% formaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, 40°C. The animals were then decapitated, the corneas were slit, and the heads were immersed in fixative and stored overnight at 4°C. The following day the eyes were dissected out and bisected in the dorsoventral meridian passing through the optic nerve head. The four eye hemispheres were then rinsed in the same phosphate buffer with 5% sucrose added, postfixed in 2% osmium tetroxide in the same buffer for 1 h at room temperature, rinsed in saline, rapidly dehydrated in ethanol, rinsed in propylene oxide, and embedded in an Epon-Araldite mixture.

The embedded blocks were oriented in such a way that sections through an eye hemisphere would contain a full length of retina including the optic nerve head and both regions of ora serrata. In this way the rod outer segments were aligned in the plane of section, or very nearly so. Sections were cut at 1.5 μm on a Porter-Blum MT2 ultramicrotome (Ivan Sorvall, Inc., Newtown, Conn.), mounted on glass slides, and subsequently dipped in Kodak NTB-2 bulk emulsion (Eastman Kodak Co., Rochester, N. Y.) diluted 1:1 with water. The slides were exposed for 18 wk, processed by conventional methods used in our laboratory (Sidman, 1970), and then stained through the emulsion with toluidine blue.

Camera lucida drawings of the autoradiographs were made with a Zeiss microscope outfitted with a Wild drawing tube (Wild Heerbrugg Instruments, Inc., Farmingdale, N. Y.). Beginning at a distance of 250–350 μm from the optic nerve head, lines indicating the base of the outer segments, the approximate middle of the band front of silver grains over the outer segments, and the apex of the outer segments were traced for a distance of 200–250 μm in a direction away from the optic nerve head. The drawings were made on lined paper oriented so that the lines were parallel to the outer segments. Measurements from the drawing of each specimen were made of (1) outer segment length, (2) the distance from the base of the outer segment to the band of silver grains, denoted as *b* (Figs. 6–9) and equivalent to the *labeled disk location* defined by Young (1971 *b*), and (3) the distance from the heavily labeled disks

to the apex of the outer segment, denoted as *a* (Figs. 6–9). Ten measurements were made at 15–18 μm intervals on each side of the optic nerve head, and the means of the 20 values were calculated for each specimen. Mean outer segment lengths were obtained by averaging the 40–220 values at each age (Table I).

Rod outer segment renewal time in the adult animals was calculated according to the method of Young (1971 *b*): outer segment renewal time =

$$\frac{\text{outer segment length}}{\text{labeled disk location}} \times \text{days after injection.}$$

TABLE I

Rod Outer Segment Lengths (μm) and Displacement (μm) of Heavily Labeled Disks After Injection of [^3H]Amino Acids

Age at Injection	Interval after injection					
	1d	3d	5d	7d	9d	11d
P10						
OS length*	4.28	8.29	14.38	17.74	18.52	17.20
Corrected OS length	3.79(40)	7.97(60)	12.03(60)	16.69(80)	18.56(100)	18.56(100)
Labeled disk location (b)*	1.36	6.25	12.85‡	—‡	—‡	—
Corrected (b)	1.20	6.01	10.75‡	—‡	—‡	—
P11						
OS length*	8.13	13.07	14.73	17.10	18.20	
Corrected OS length	7.56(40)	11.13(40)	14.65(40)	17.25(40)	18.08(40)	
Labeled disk location (b)*	1.33	8.70	12.98‡	—‡	—	
Corrected (b)	1.24	7.41	12.91‡	—‡	—	
P12						
OS length*	8.48	13.32	15.41	18.25	17.67	19.44
Corrected OS length	7.97(60)	12.03(60)	16.69(80)	18.56(100)	18.56(100)	20.44(100)
Labeled disk location (b)*	1.04	8.94	13.73‡	17.54‡	—‡	—
Corrected (b)	0.98	8.07	14.87‡	17.84‡	—‡	—
P14						
OS length*	11.01	15.68	15.59	17.47	17.06	
Corrected OS length	12.90(60)	16.69(80)	18.56(100)	18.56(100)	20.44(100)	
Labeled disk location (b)*	0.97	7.95	13.61	15.60‡	—	
Corrected (b)	1.14	8.46	16.20	16.57‡	—	
P16						
OS length*	20.05	18.98	20.85	19.37	20.24	22.84
Corrected OS length	16.69(80)	18.56(100)	18.56(100)	20.44(100)	22.09(60)	22.19(220)
Labeled disk location (b)*	1.85	7.61	12.98	16.57	—‡	—‡
Corrected (b)	1.54	7.44	11.55	17.48	—‡	—‡
P18						
OS length*	21.04	19.68	23.01	23.06	21.68	23.96
Corrected OS length	18.56(100)	18.56(100)	20.44(100)	22.09(60)	22.19(220)	22.19(220)
Labeled disk location (b)*	1.70	6.78	12.49	16.50	19.17	—‡
Corrected (b)	1.50	6.39	10.09	15.80	19.62	—‡

TABLE I (*continued*)

Age at Injection	Interval after injection					
	1d	3d	5d	7d	9d	11d
P20						
OS length*		23.23	22.94	23.67	24.91	21.99
Corrected OS length		20.44 (100)	22.09 (60)	22.19 (220)	22.19 (220)	22.19 (220)
Labeled disk location (b)*		6.49	11.86	17.37	22.45	—‡
Corrected (b)	§	5.71	11.42	16.28	20.00	—‡
P53						
OS length*	22.74	23.45	23.38	26.58	22.02	19.34
Corrected OS length	22.19 (220)	22.19 (220)	22.19 (220)	22.19 (220)	22.19 (220)	22.19 (220)
Labeled disk location (b)*	1.55	6.27	10.64	18.10	19.73‡	—‡
Corrected (b)	1.51	5.93	10.10	15.11	19.88‡	—‡

* Based on 20 measurements.

‡ Distinct band of silver grains could not clearly be distinguished.

§ Since a 1 day postinjection animal was not available in this series, the corrected b_{1d} value of 1.50 μm obtained in the 18- and 53-day series was used in calculating the synthesis rates (Fig. 10).

—, no silver grains present; b, distance from base of outer segment to heavily labeled disks; OS, outer segment; P, postnatal day.

The number of measurements upon which the corrected outer segment length at each age was based is indicated in parentheses.

Rod outer segment synthesis in the developing retina was determined by the change in length of *b* from one postinjection interval to another. For example, the labeled disk location at 3 days postinjection (b_{3d}) minus the location at 1 day postinjection (b_{1d}) yields the displacement over a 2 day period (Fig. 10, upper illustration). Since this method requires the comparison of two specimens of different ages, and because there is some variation in outer segment lengths even in animals of the same age, the value of *b* for each specimen was normalized by multiplying it by the ratio of mean outer segment length for that age to the mean outer segment length for that specimen (Table I).

Disposal of rod outer segments in the mouse retina was obtained by determining the change of *a* from one postinjection interval to another. Thus, a_{1d} minus a_{3d} yields the movement of labeled disks *toward the apex of the outer segment* over a 2 day period (Fig. 10, lower illustration). The values of *a* which were used in the calculation were corrected as described for the values of *b* (Table I).

Ultrathin sections were cut from blocks of retinas of selected ages (Table II, column 1) in the above series of mice and of 8- and 10-day old specimens. Electron micrograph montages were prepared of the pigment epithelium from the posterior region of the eye 250–350 μm from the optic disk and from the peripheral region of the eye, 200–350 μm from the ora serrata. The number of phagosomes and dense granules of all forms associated with degradative activity as described by Ishikawa and Yamada (1970) were counted. The area of the pigment epithelial cell cytoplasm (excluding basal and apical processes and the nucleus) was estimated by weighing cut-out portions of the micrographs; the results were expressed as inclusions per 100 μm^2 pigment epithelial cell cytoplasm (Table II).

RESULTS

Rod Outer Segment Length

Rod outer segment length was measured at the posterior region of the eye in mice from 11 to 64 days of age (Fig. 1). At 11 days of age (Fig. 2) the outer segments are variable in length and are usually not vertically aligned; the thickness of the outer segment layer as a whole is about 4 μm which is only 18% of the average adult outer segment length of 22.2 μm. By day 13 the outer segments are more uniform in length and more accurately aligned with one another, but many vertically oriented membranous saccules are present between the parallel rod outer segments, as

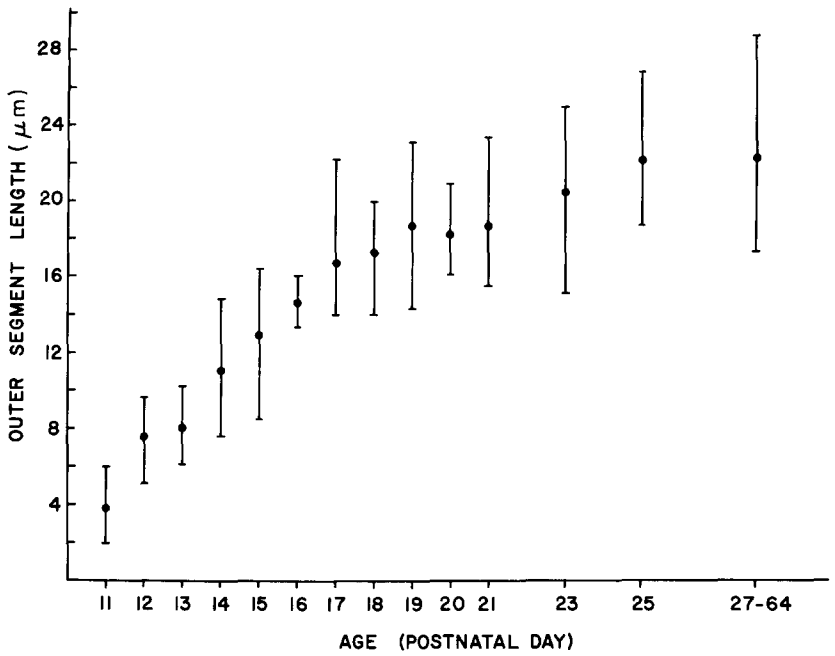


FIGURE 1 Outer segment lengths in retinas of C57BL/6J mice. The brackets indicate the total range of values at each age. It should be noted that standard errors of the mean were small (the largest at postnatal day 14 was ± 0.34 μm, and the average was ± 0.22 μm). They have been omitted from the graph.

noted in the developing rat retina by Dowling and Gibbons (1962). By day 15 (Fig. 3) the outer segments have undergone slightly more than 50% of their net growth (Fig. 1).

The average length of rod outer segments at 17 days of age is about 75% of the average adult length, but the range of lengths at this age and at all subsequent ages overlaps the adult range (Fig. 1). Thus, in many instances, 17-day old (Fig. 4) and slightly older retinas cannot be distinguished from adult retinas (Fig. 5).

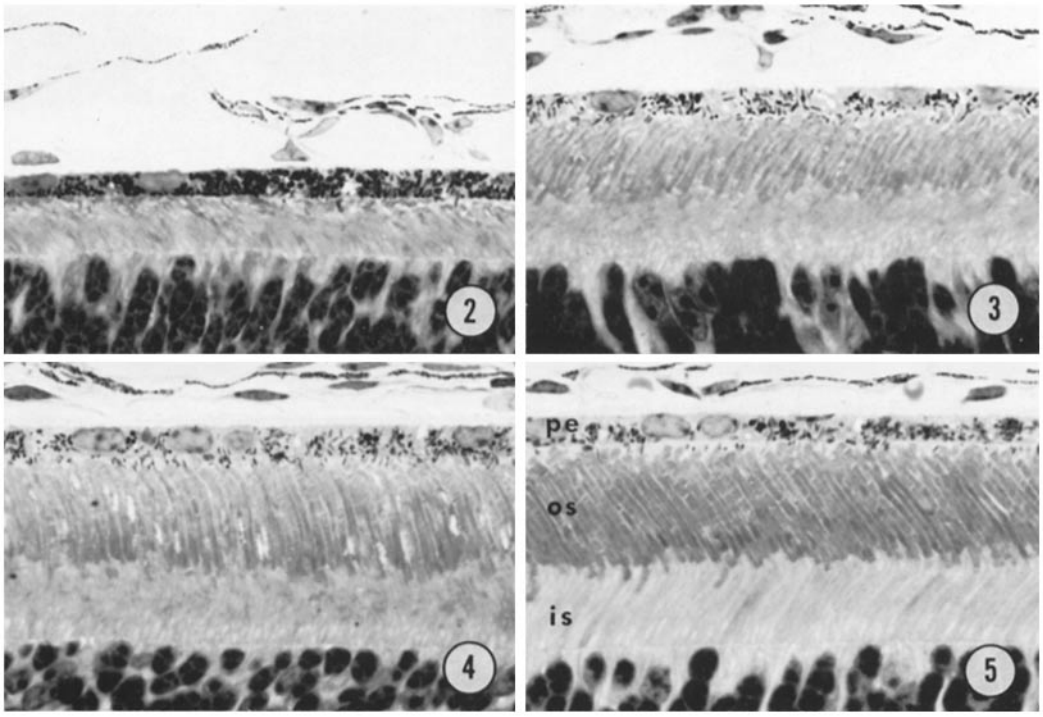
The rate of elongation of outer segments between days 11 and 17 is rapid and almost linear; however, the subsequent elongation is much more gradual (Fig. 1 and Table I). The adult length of mouse outer segments appears to be achieved between 19 and 25 days of age. Since by 25 days the average length was virtually identical to that of 54–64 days, the 27- to 31-day values were pooled with those of older animals to obtain the average adult length.

Rod Outer Segment Renewal

The total rod outer segment renewal time in the adult mouse is 10.4 ± 1.0 days. This is based on the average of six specimens injected at 20 or 53 days of age and fixed 3, 5, and 7 days later. This renewal time agrees with that found for mice by Young (1967).

The number of rod outer segment disks produced and shed daily by each adult photoreceptor cell can be calculated. Division of average adult outer segment length (22.2 μm) by total renewal time (10.4 days) gives a daily renewal rate of 9.6%, or 2.13 μm of rod length. Adult mouse outer segments were found to contain 35.1 ± 1.8 disks/μm. Thus, an average of about 75 disks are renewed daily in each adult mouse rod cell.

The rate of synthesis of outer segment disks in the developing mouse retina is plotted in the upper curve of Fig. 10. In the 11–13 day period, the rate of synthesis is already slightly greater than the adult rate. The rate rises sharply so that during the 13–17 day period it is more than 1.6 times the adult rate. Outer segment disks in the developing retina at all ages are uniformly stacked at a concentration of 35.8 ± 2.4 disks/μm, indistinguishable from the adult value. Thus at the peak rate of growth during the 13–17 day period, about 120 disks are synthesized per day. The rate then declines to the adult level of synthesis at about day 21–25. The data in the upper curve of Fig. 10



FIGURES 2–5 Light micrographs of posterior regions of retinas from C57BL/6J mice. Epon-Araldite 1.5 μm. Toluidine blue. × 800.

FIGURE 2 Postnatal day 11. Rod outer segments are quite disorganized and the thickness of the layer is only about 7% of the adult outer segment length.

FIGURE 3 Postnatal day 15. Rod outer segments are slightly greater than 50% of the adult length.

FIGURE 4 Postnatal day 17. Rod outer segments average about 75% of the adult length. In this field they are almost as long as adult outer segments.

FIGURE 5 Postnatal day 31. *is*, rod inner segment; *os*, rod outer segment; *pe*, pigment epithelial cell.

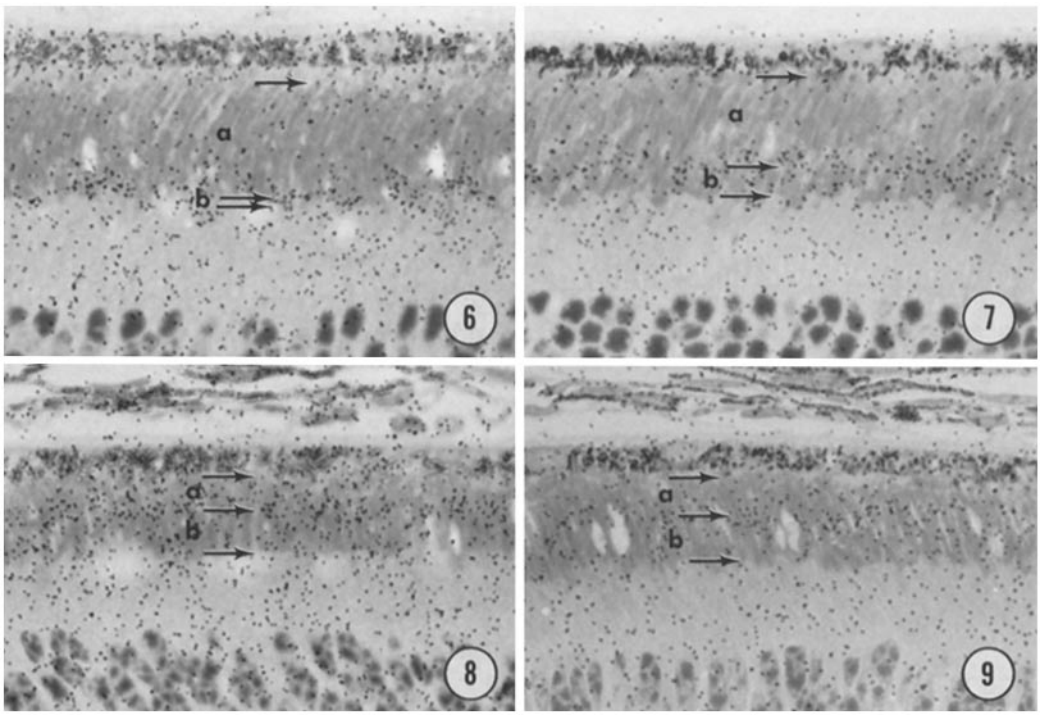
were normalized as described in Materials and Methods. The uncorrected data mimic the synthesis curve and show an even greater rate of synthesis at the 12–15 day period.

Autoradiographs of the 1- and 3-day postinjection intervals from the 53-day injection series are illustrated in Figs. 6 and 7. Examples of 3-day postinjection specimens from the 11- and 12-day injection series (Figs. 8 and 9) can be compared with one from the 53-day injection series (Fig. 7).

The plot of the rate of disposal of rod outer segments in the developing mouse retina is presented in Fig. 10, lower curve. The rate of disposal sharply increases from less than 15% of the daily adult rate at 11–13 days to almost 70% of the adult rate at 13–15 days of age. The rate then more gradually approaches the adult rate and reaches it between 19 and 21 days of age. The uncorrected data show a similar sharp rise at early ages.

The disposal rates in most instances show somewhat larger standard errors than do the values of synthesis rates (Fig. 10). This may reflect the fact that rod disks are continually synthesized but only intermittently shed (Young and Bok, 1969; Young, 1971 *a*).

Outer segment renewal was not measured systematically in the periphery of the developing retina near the ora serrata, since (1) the outer segments are much shorter there, (2) the outer segments develop later there, presumably because most of the peripheral photoreceptors are generated several days later than those at the posterior pole of the eye (Sidman, 1961), and (3) the rapidly



FIGURES 6–9 Light microscope autoradiographs of posterior regions of retinas from C57BL/6J mice injected with [^{3}H]amino acids on various days of age and killed at subsequent time intervals. On each micrograph the upper arrow represents the apex of the outer segments and the lower one the base of the outer segments. The middle arrow indicates approximately the middle of the heavy band of silver grains. *a*, the distance from the heavily labeled disks to the apex of the outer segments; *b*, the distance from the base of the outer segments to the heavily labeled disks; these distances are measured along a line parallel to the outer segments. Epon-Araldite 1.5 μm. Toluidine blue. × 800.

FIGURE 6 Injected on day 53, killed 1 day postinjection. A band of silver grains lies just past the junction of the inner and outer segment.

FIGURE 7 Injected on day 53, killed 3 days postinjection. The band of silver grains has been displaced to just less than one-third the length of the outer segment.

FIGURE 8 Injected on day 11, killed 3 days postinjection.

FIGURE 9 Injected on day 12, killed 3 days postinjection.

expanding size of the eye and surface area of the retina between days 10 and 30 does not give a set point of reference such as that provided by the optic nerve head in the posterior region of the eye. Cursory examination revealed that when rod outer segments were generated in the peripheral retina (several days later than in the posterior retina), the outward displacement of labeled outer segment disks was usually equal to or slightly less than the displacement in the posterior retina. However, the differences were very slight and difficult to quantify.

In adult mouse retinas, the synthesis rate of peripheral outer segments was the same as in the posterior retina. With shorter outer segments, the peripheral outer segments had a shorter renewal time, which is in agreement with Young's (1971 *b*) observations on peripheral rods in the rhesus monkey.

Phagocytic Activity of the Pigment Epithelial Cells

In order to study further the disposal component of the outer segment renewal mechanism

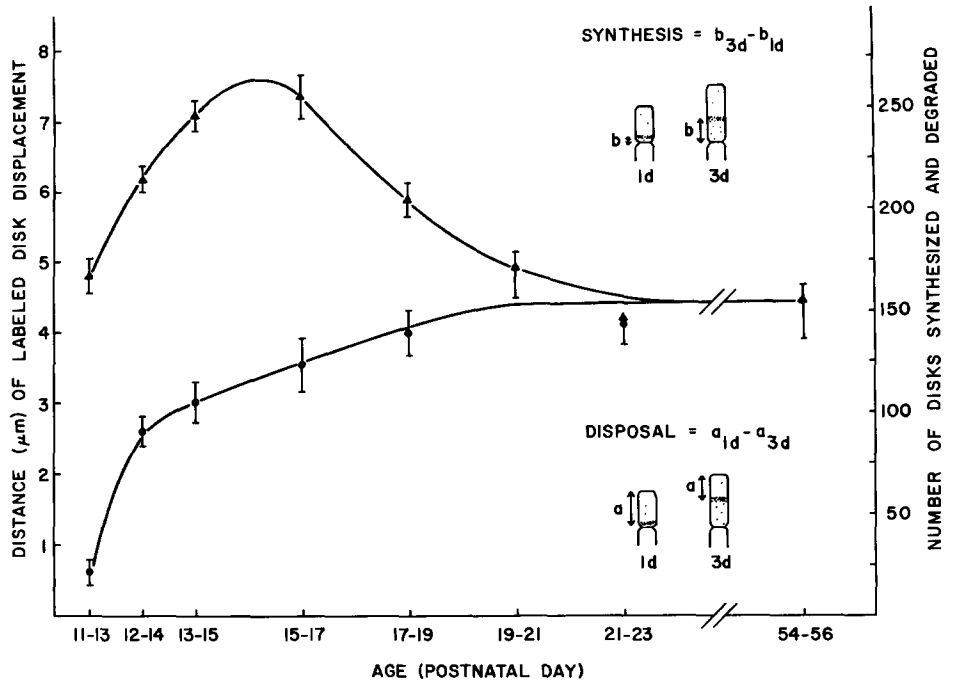


FIGURE 10 Rates of rod outer segment disk synthesis (upper curve) and disposal (lower curve) in developing C57BL/6J mouse retinas. Each value represents the distance of movement of labeled outer segment disks during an interval of 2 days. *a*, distance from the heavy band of silver grains to the apex of the outer segments; *b*, distance from the base of the outer segments to the heavy band of silver grains. The bars represent the standard error of the differences of the means at each interval. The synthesis and disposal values for each of the last three 2-day intervals are virtually coincident. The bars above these points are the standard errors of the synthesis values, and those below are of the disposal values. No bar for the synthesis value is given for the 21–23 day interval because b_{1d} of that interval is estimated (see footnote in Table I). The disposal value indicated for the 21–23 day age was actually a_{3d} minus a_{5d}, since no 20 day a_{1d} value was available (Table I).

in the developing mouse retina, the pigment epithelium was studied by electron microscopy. The number of cytoplasmic organelles and inclusions presumably associated with degradative activity was tabulated at selected ages (Table II). Dense granules of many sizes and forms have been shown to be acid phosphatase positive and are thought to be lysosomes (Ishikawa and Yamada, 1970); all were included in the dense granule count.

Although the results (Table II) showed considerable variation in both phagosome and dense granule count, two points were clearly demonstrated in the posterior region of the eye. First, some inclusions are present (suggestive of degradative activity) as early as the 8th and 10th postnatal days, when rod outer segments have not yet begun to elongate rapidly. Second, the number of phagosomes per unit area shows a sharp rise at day 11 and is maintained at 3–7 times the 10 day value at all subsequent ages. The number of dense granules per unit area shows a similar but less dramatic rise at day 11. The sharp rises in the frequency of phagosomes and dense granules are consistent with the general shape of the disposal rate curve in the autoradiographic analysis (Fig. 10, lower curve), but they occur 1 day earlier than the autoradiographic data would have suggested. These quantitative data confirm the finding of Ishikawa and Yamada (1970) that the number of organelles and inclusions associated with degradative activity in the pigment epithelial cells increases as outer segments elongate; however, the increases correlate more closely with the rise in rate of disk disposal than with the rate of outer segment elongation.

TABLE II

Concentration of Phagosomes and Dense Granules‡ in Mouse Pigment Epithelial Cells*

Age	Area measured	Phagosomes	Dense granules	Total inclusions
	μm^2			
Posterior retina				
P8	396	0.2	9.8	10.1
P10	606	0.3	9.6	9.9
P11	388	1.8	14.4	16.2
P12	523	1.2	12.6	13.8
P13	542	0.9	15.9	16.8
P15	565	2.3	7.4	9.7
P17	853	2.1	11.6	13.7
P19	622	1.1	17.7	18.5
P21	574	1.7	13.1	14.8
P25	474	1.7	11.8	13.5
P62	327	2.1	15.3	17.4
P64	658	1.7	20.4	22.0
Peripheral retina				
P11	784	0.5	3.6	4.1
P13	719	0.6	8.2	8.8
P14	898	0.9	7.8	8.7
P15	682	1.9	6.3	8.2
P17	625	2.7	7.0	9.8
P19	708	1.8	5.5	7.3

* Number per 100 μm^2 of pigment epithelial cell cytoplasm.

‡ All forms described by Ishikawa and Yamada (1970).

P, postnatal day.

Phagosome and dense granule counts in the peripheral retina near the ora serrata show changes similar to those in the back of the eye, but the increases in number in the periphery occur several days later (Table II). It should be emphasized that in the peripheral retina at day 11, many rods have generated no outer segments, but by day 13 the outer segments clearly separate the inner segments from the pigment epithelial cells. Furthermore, the lengths of outer segments in the periphery are more variable than at the back of the eye throughout the developmental period.

DISCUSSION

Elongation of rod outer segments in the C57BL/6J mouse proceeds at a rapid and almost linear rate from day 11 to 17. Although in some specimens outer segments have reached the adult length by day 17, most attain their average adult length of 22.2 μm at 19–25 days of age. The increase in outer segment length parallels the almost linear rise in rhodopsin content from day 8 to 23 when the adult level is reached in DBA mice (Caravaggio and Bonting, 1963).

Outer segment elongation in the developing mouse retina reflects the excess in rate of synthesis compared to rate of disposal of outer segment disks. The maximal growth period from days 11 to 17 after birth is characterized by a rapid acceleration in the rate of disk synthesis, which presumably reflects an increased rate of rhodopsin synthesis, from just above the adult level to greater than 1.6 times the adult level. In the same period the rate of disk disposal rises from 15% of the adult level at days 11–13 to almost 70% of the adult level at days 13–15, followed by a more gradual rise (Fig. 10). From 17 to approximately 19–25 days of age, the rate of synthesis falls to the adult level, and the rate of disposal rises to the adult level, probably reaching it somewhat earlier than the rate of synthesis.

There are wide ranges of outer segment lengths at each age in the mouse retina (Fig. 1). A deliberate attempt was made to obtain autoradiographic data at as constant a distance as possible from the optic nerve head, but variation nevertheless was considerable. In the 22 groups of ten measurements each (two groups from a single animal) which were used to obtain the average adult outer segment length, the maximum range within any one group was ± 5.71 μm; the average was ± 3.64 μm. Furthermore, outer segment length in any single specimen varies across the retina. The length not only decreases as one proceeds from the back of the eye to the ora serrata, but also the outer segments are extremely short immediately surrounding the optic nerve head. Given this intraretinal variability, it is not surprising that there might also be substantial variation in outer segment lengths among different animals of the same age. It should be noted that comparable ranges in outer segment length exist in the rat (Dowling and Sidman, 1962).

Variation in outer segment length was not due to mechanical compression or elongation or other artifacts of the preparative procedure. The procedure used in this study causes minimal distortion of the eye. In more than 90% of the specimens the retina remained completely attached to the pigment epithelium, and no signs of compression were recognized.

Three features of the data represented in the

synthesis and disposal curves in Fig. 10 indicate the validity of the method of analyzing the separate components of the rod outer segment renewal system. First, at each 2-day time interval the difference in outer segment synthesis and outer segment disposal gives a net increment of outer segment elongation which closely matches the measured increment (Fig. 1). Secondly, the two curves meet at essentially the same age that adult outer segment length is reached. Thirdly, the equilibrium rate of synthesis and disposal of just less than 2.2 μm/day agrees well with the value of 2.13 μm/day calculated by Young's method (1971 *b*).

The fact that the adult rod outer segment reaches and then maintains a state of equilibrium suggests that synthesis and disposal mechanisms may be interrelated. The equilibrium holds over a range of experimental conditions such as elevation of the ambient temperature in adult frogs and sustained, increased intensity of ambient illumination in both adult frogs and adult rats (Young, 1967).[1] However, the rates of synthesis and disposal are clearly separable under certain conditions. The rates differ during elongation of outer segments in the normally developing mouse retina. The equilibrium length is established only when the rate curves of synthesis and disposal meet (Fig. 10). Similarly, synthesis and disposal must also proceed at different rates in the adult during recovery after limited destruction of outer segments by visible light (Kuwabara, 1970; Tso, 1973), vitamin A deficiency (Dowling and Gibbons, 1961), or experimental retinal detachment (Kroll and Machemar, 1969). Further studies are necessary to determine what sort of control mechanism(s) exists to regulate the quite different events of outer segment synthesis and disposal.

The importance of distinguishing protein synthesis from protein degradation is becoming recognized in other cell systems, for example in cases where cell size is externally regulated by alteration of degradation rate (e.g., Goldberg, 1973). The rod outer segment is a favorable system for such studies because separation of the site of synthesis and the site of degradation (Young, 1967; Young and Droz, 1968; Young and Bok, 1969) simplifies independent measurement of the two processes.

[1] In Young's diagrams, Figs. 21 and 22 in Young, 1967, the outer segment lengths are drawn as unchanged by the different environmental factors.

The synthesis of specific proteins is being used increasingly as a criterion of cellular differentiation (Kafatos, 1972). Phases of development can be defined on the basis of changing rates of specific protein synthesis (Rutter et al., 1968; Kafatos, 1972). In a "predifferentiated" phase, specific protein synthesis is below threshold for detection. During phase I of the "differentiated" period (Kafatos, 1972), or "protodifferentiated" phase (Rutter et al., 1968) the rate of protein synthesis increases to a low but detectable level. The rate of synthesis rises rapidly during phase II and then is "modulated," often downwards, during phase III. The differentiation of mouse rod outer segments, and presumably their major protein, opsin, follows this general pattern. Photoreceptor cells in the posterior regions of the eye are generated from late fetal life through the first several postnatal days (Sidman, 1961). Rhodopsin or opsin has not been detected in this period. On about day 5, the rod cells begin synthesizing outer segment membranes (Olney, 1968). Rhodopsin is present at least by day 7, but its net accumulation during the next few days (phase I) is low (Caravaggio and Bonting, 1963). By day 11, the rod has entered phase II, the period of rapid acceleration of specialized synthesis. Adjustment of the rate of specific protein synthesis downward to the adult equilibrium level in phase III begins on about day 17 (Fig. 10).

Rates of protein degradation have not been reported in other cell systems during the phases of differentiation. In the case of the photoreceptor cell, some outer segment membranes must be degraded during phase I, from postnatal days 5–10, because signs of phagocytic activity within the pigment epithelial cells are evident at least by day 8 (Table II). Further, if there were no disposal one might expect the outer segment layer to be thicker than it is at days 10 and 11 (Fig. 2; see also Fig. 1 in LaVail and Reif-Lehrer, 1971). The disposal rate curve does not show the sharp inflections characteristic of the synthesis curve in the transitions between phases.

It should be possible to extend the analysis of the control of photoreceptor cell differentiation by study of mutant mice and rats which have abnormalities in rod cell differentiation, pigment epithelial cell differentiation, or both. The retinal degeneration, *rd*, mutation in the mouse results in a rapid degeneration of rod cells just after they enter phase II of outer segment differentiation, i.e., just after the rate of rhodopsin syn-

thesis begins its rapid acceleration (Caravaggio and Bonting, 1963). The mouse has the added advantage that congenic stocks are available which provide genetic controls for analysis.[2] The *rd* mutation in the RCS rat produces an apparently more complex phenotype in which both components of the outer segment renewal mechanism are abnormal. The pigment epithelial cells display no phagocytic activity (Herron et al., 1969; Bok and Hall, 1971; LaVail et al., 1972), and outer segment synthesis is slowed from the normal rate (Herron et al., 1969). This slowing occurs before day 17 (LaVail et al., 1972), a time when a very high rate of synthesis would be expected if the kinetics of outer segment elongation in the rat are like those of the mouse.[3] It is not yet clear whether the defects in the outer segment synthesis and disposal mechanisms in the RCS rat are independent of one another.

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[3] The rate of rod outer segment elongation and increased rhodopsin content are essentially identical in the mouse and rat (Bonting et al., 1961; Dowling and Sidman, 1962; Caravaggio and Bonting, 1963).

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