Proc. Natl. Acad. Sci. USA
Vol. 77, No. 8, pp. 4998–5002, August 1980
Neurobiology

Retinal rhythms in chicks: Circadian variation in melatonin and serotonin *N*-acetyltransferase activity

(oscillation/retina/birds/arylamine acetyltransferase)

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Communicated by Colin S. Pittendrigh, April 18, 1980

ABSTRACT **There is a large-amplitude circadian rhythm of indoleamine metabolism in the retina–pigment epithelium of the chicken. *N*-Acetyltransferase activity (arylamine acetyltransferase; acetyl-CoA:arylamine *N*-acetyltransferase, EC 2.3.1.5) and melatonin content are 15-fold higher at night than during the day in a cycle of 12 hr of light and 12 hr of dark; in constant darkness there is a 4-fold increase during the subjective night. Light at midnight inactivates *N*-acetyltransferase and lowers melatonin. *N*-Acetyltransferase activity is found predominantly in the retina. The circadian rhythm of this enzyme activity persists in pinealectomized chicks. Thus the pineal is not responsible for retinal indoleamine rhythms. Retinal and pineal levels of *N*-acetyltransferase activity behave similarly under several conditions. In the chicken, the eye is a major site of rhythmic indoleamine metabolic activity.**

Many features of photoreceptor cell physiology fluctuate with daily rhythmicity. Evidence suggesting that a general feature of retinal metabolism in all vertebrate classes is its modulation by daily rhythms has accumulated rapidly (1–14). This is not surprising in cells that are exquisitely specialized for reception of light, which varies with an environmentally imposed periodicity. What is more interesting is that this rhythmicity continues even in the absence of the light–dark cycle that normally synchronizes it. In constant conditions there is endogenously rhythmic regulation of daily disc shedding of photoreceptor outer segments (1–3).

Some aspects of photoreceptor metabolism that exhibit daily rhythms can be directly affected by melatonin (*N*-acetyl-5-methoxytryptamine) (15–17). The retina contains the enzymes necessary to make melatonin (18–21). Quay (18) has found hydroxyindole *O*-methyltransferase in the retina of a variety of vertebrates, including chickens. Recently Binkley *et al.* (21) have found a day–night change in *N*-acetyltransferase (arylamine acetyltransferase; acetyl-CoA:arylamine *N*-acetyltransferase, EC 2.3.1.5) activity in the eyes of chickens, sparrows, and rats. Serotonin is also found in the chick retina (22–24). Thus it seems possible that the retina makes melatonin. We have examined the possibility that the melatonin content of the retina, and the activity of retinal *N*-acetyltransferase, a controlling enzyme in melatonin synthesis, have circadian rhythms similar to their well-studied oscillations in the pineal gland.

MATERIALS AND METHODS

Animals. Chicks (White Leghorn pullets, Babcock B-300 strain, *Gallus domesticus*) were purchased locally on the day of hatching and kept in warmed brooders with food (Purina Chick Starteena) and water available ad lib, on a light–dark cycle (LD 12:12, lights on 0000–1200 central standard time) until age 2–3 weeks. The light source was Sylvania Cool White fluorescent bulbs, which produced an intensity at the level of the brooders during the light period of 700–800 lux, measured with a Gossen Luna Pro photometer (model B650920). When light was given in the middle of the dark period, its intensity was 1400–1500 lux. For the experiment shown in Fig. 1 *B* and *D*, a group of animals age 23 days was transferred into constant darkness (DD) at the time of the normal L-to-D transition. Beginning after 44 hr of constant darkness, animals were killed by decapitation at 2-hr intervals. In another experiment (Fig. 1 *A* and *C*), 14-day-old animals that had continued in LD 12:12 were killed at similar intervals. During dark periods, animals were killed in complete darkness.

Dissection and Tissue Preparation. Eyes were dissected out under dim red light (Kodak Wratten filter no. 24, approximately 20 lux), quick-frozen on dry ice, and stored at −80°C until assay. At the time of assay, frozen eyes were hemisected, vitreous humor and pecten were removed from the posterior hemisphere, and the retina–pigment epithelium was removed and sonicated. *N*-Acetyltransferase activity was immediately measured by a modification (25) of the method described by Deguchi and Axelrod (26). The remaining sonicate was quick-frozen and stored until melatonin radioimmunoassay and protein assay were performed.

***N*-Acetyltransferase Activity Assay.** One retina–pigment epithelium was sonicated in 200 μl of 0.64 mM acetyl-coenzyme A (Sigma) in 0.5 M KPO_4 buffer, pH 6.5. A 50-μl sample was added to a 20-μl reaction mixture containing 4.167 nmol of [^{14}C]acetyl-coenzyme A (0.2 μCi, ICN; 1 Ci = 3.7×10^{10} becquerels) and 0.2 μmol of tryptamine to give a final concentration of 0.36 M KPO_4 buffer, 0.517 mM acetyl-coenzyme A, and 2.86 mM tryptamine. The reaction was carried out for 10 min in a 37°C water bath. It was stopped with 1 ml of toluene/isoamyl alcohol (97:3, vol/vol) and stirred in a Vortex mixer for 15 sec. After centrifugation at 2000 × *g* for 15 min., a 0.5-ml aliquot of the organic phase was transferred to a scintillation vial and evaporated overnight. Ten milliliters of ACS (aqueous counting scintillant, Amersham/Searle) was added and the radioactivity was measured. Two-dimensional thin-layer chromatography and autoradiography showed that the product comigrated with a pineal product and with authentic *N*-acetyltryptamine. Retinal *N*-acetyltransferase activity shows similar biochemical characteristics to the pineal enzyme, and differs from the brain and liver enzymes (unpublished data).

Melatonin Radioimmunoassay. Melatonin was measured by a modification of the radioimmunoassay method of Rollag and Niswender (27). The retina–pigment epithelium sonicate

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Abbreviations: LD, light–dark cycle; DD, constant darkness.

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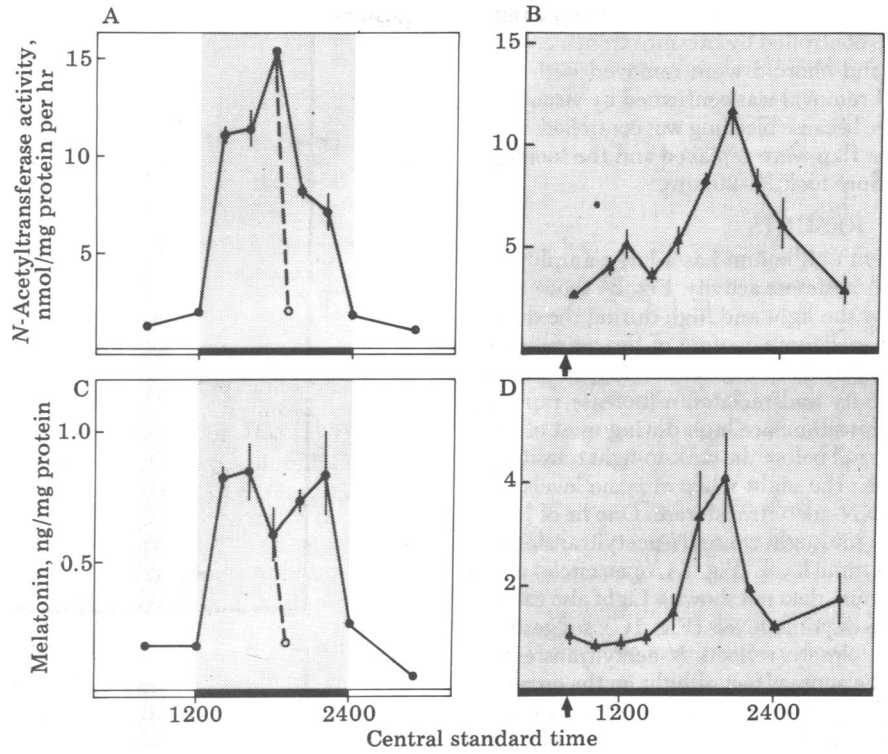

FIG. 1. Circadian changes in indoleamine metabolism in the retina–pigment epithelium of the chick. Shaded areas and black bars represent darkness; ●, chicks killed every 2 hr; ○, chicks treated with 1 hr light (1300–1400 lux) at mid-dark before sacrifice. (*A*) *N*-Acetyltransferase activity in LD 12:12; (*B*) activity in DD; (*C*) melatonin content in LD 12:12; (*D*) content in DD. Arrow is placed 44 hr after animals were transferred to DD. *N*-Acetyltransferase activity and melatonin were measured from the same eye in LD 12:12 (only one eye from each bird was used); in DD, *N*-acetyltransferase activity was measured from one eye, melatonin from the other eye of each chick. $n = 5$ chicks in all groups. The standard errors of the means are indicated when they exceed the size of the symbol.

was thawed, then centrifuged at 12,000 × *g* for 1 min in a Beckman Microfuge. Samples (100 μl) of the supernatant were diluted 1:4 with glass-distilled H_2O and extracted with 2 ml of glass-distilled chloroform (Anspec, Ann Arbor, MI) and washed with 500 μl of 0.1 M sodium bicarbonate buffer, pH 10.25. A 1-ml sample of the chloroform extract was evaporated to dryness under N_2, redissolved in 0.5 ml of phosphate-buffered saline/gelatin (0.01 M sodium phosphate buffer/0.9% NaCl/0.1% gelatin, pH 7.0) and then assayed for melatonin by using an equilibrium assay procedure. In an ice bath, 100 μl of ^{125}I-labeled melatonin analog in phosphate-buffered saline/gelatin and 200 μl of antisera (1:64,000 dilution of rabbit 1055) in phosphate-buffered saline containing 0.05 M EDTA and 0.005% normal rabbit gamma globulin was added to each assay tube. After incubation at 4°C for 48 hr, 3 ml of ice-cold 95% (vol/vol) ethanol was added to each reaction mixture to precipitate the antigen–antibody complex. The tubes were centrifuged at 2000 × *g* for 30 min at 4°C, the supernatant was poured off, and radioactivity in the precipitate was measured in a Nuclear-Chicago gamma counter. Computer programs of Rodbard (28) were used to calculate melatonin content of the retina.

Validation. The radioimmunoassay was validated for the quantification of melatonin in chicken retina–pigment epithelium. Inhibition curves were obtained with various quantities of melatonin and chloroform extracts of retina–pigment epithelium homogenates taken from chicks during the day and night. Inhibition curves were linearized by using a logit transformation and the slopes were determined for a nonweighted least squares fit. Parallel lines with slopes of −1.88, −1.80, and −1.97 were obtained for melatonin, day, and night retina–pigment epithelium, respectively. Melatonin (5, 10, 25, 50, 100, 250, or 500 pg) added to retina–pigment epithelium sonicate before extraction was quantitatively recovered ($y = 0.82x + 213$, in which y is melatonin recovered and x is melatonin added; correlation coefficient $r = 0.68$).

Protein Assay. Protein content was determined for each sample with the Coomassie blue method of Bradford (ref. 29; Bio-Rad). A 3-μl sample of supernatant from each centrifuged sonicate was assayed for protein. This was used to standardize each sample's *N*-acetyltransferase activity and melatonin content to its protein content, controlling for small losses of retina–pigment epithelium during dissection. The range of protein in all samples in the experiment in Fig. 1 *A* and *C* was 2.18–6.62 mg per retina–pigment epithelium. The mean ± SEM was 5.28 ± 0.1.

Retina–Pigment Epithelium Separation. In the experiment shown in Fig. 2, the retina was separated from the pigment epithelium by a modification of the method of Woodruff *et al.* (30). The eye was cut with a shallow circumferential incision; then the anterior half was pulled away, with the vitreous body and the retina attached. The pecten and optic nerve were cut, freeing the retina completely from the attachment to the back half of the eye. It was then cut from the front half of the eye and quick frozen on solid CO_2. The pigment epithelium remained attached to the posterior eyecup and was removed and frozen.

Pinealectomy. Pinealectomy was performed by a modification of the method of Binkley *et al.* (31). Chicks were anesthetized with Nembutal (40 mg/kg), and the pineal was removed surgically under a dissecting microscope. A flap of bone was drilled and pulled back from the pineal region. Then the dura mater on either side of the pineal was cut. Finally the anterior sinus was cut and held closed with a mouse-tooth iridectomy microforceps, forming a V-shaped flap that was pulled back, exposing the pineal gland attached to the inner surface

of the membrane and the choroid plexus. The forceps was removed and bleeding was controlled by pressure from a cotton swab while the pineal and choroid were removed with the forceps; completeness of removal was confirmed by visual inspection. This was possible because bleeding was controlled. The meningeal flap and bone flap were replaced and the incision was sutured. The procedure took 15–20 min.

RESULTS

The chick retina–pigment epithelium has a large-amplitude daily rhythm of *N*-acetyltransferase activity. Fig. 1*A* shows that the activity is low during the light and high during the dark. Radioimmunoassayable melatonin content of the retina–pigment epithelium has a similar daily fluctuation (Fig. 1*C*). Both *N*-acetyltransferase activity and melatonin increase rapidly after the light-to-dark transition, are high during most of the dark period, and begin to fall before the dark-to-light transition. Bright light, given during the night when enzyme levels are high, quickly inactivates *N*-acetyltransferase. One hr of light (1400–1500 lux) given at midnight causes *N*-acetyltransferase activity to decrease to daytime levels (Fig. 1*A*, open circle) with a halving time of 10–12 min (data not shown). Light also causes melatonin to decrease to daytime levels (Fig. 1*C*), suggesting that melatonin content closely reflects *N*-acetyltransferase activity in the retina. The acute effect of light on the enzyme accounts at least partially for the low levels of *N*-acetyltransferase activity and melatonin during the light portion of a LD cycle (Fig. 1 *A* and *C*). The high nighttime levels of *N*-acetyltransferase activity and melatonin could be the result of release from light inhibition. Thus light inhibition alone could explain *N*-acetyltransferase cyclicity in an LD cycle. We assayed the levels of retinal *N*-acetyltransferase from chicks held in DD to explore the possibility that the light cycle was directly driving the rhythm. Chicks were kept for 2 days in DD, then enzyme levels of groups of five chicks were measured every 2 hr. The rhythms of enzyme activity and melatonin persist in constant darkness (Fig. 1 *B* and *D*), showing that there is an underlying circadian modulation of the serotonin-to-melatonin pathway that does not depend on cyclic light. There are qualitative differences between the LD and DD results. *N*-Acetyltransferase and melatonin during the subjective day (the projected day if the LD cycle had continued) are not as low as in the light portion of an LD cycle, the nighttime rise is more gradual, and the shape of the curve is different. *N*-Acetyltransferase activity levels are similar in LD and DD but, interestingly, retinal melatonin content in DD is higher than in LD (cf. ordinate values in Fig. 1 *C* and *D*). Increased melatonin content in constant darkness could result either from increased melatonin synthesis or uptake, or from decreased melatonin release or breakdown. Clearly, under some conditions melatonin levels in the retina do not directly reflect *N*-acetyltransferase activity.

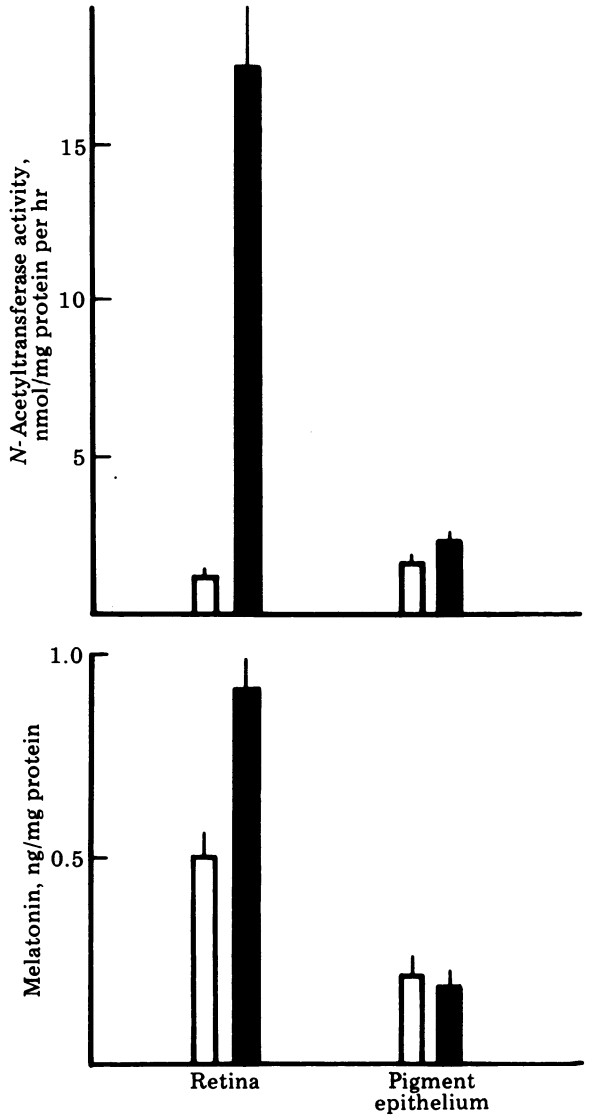

FIG. 2. Localization of *N*-acetyltransferase activity and melatonin in the retina–pigment epithelium of chicks. Solid bars, mid-dark; open bars, mid-light. The retina was separated from the pigment epithelium by a modification of the method of Woodruff *et al.* (30). At mid-dark, eyes were dissected out under dim red light (approximately 4 lux). Mean ± SEM; $n = 6$ eyes in all groups.

We attempted to localize *N*-acetyltransferase activity and melatonin within the retina–pigment epithelium by assaying each tissue component separately. Rhythmic enzyme activity was found predominantly in the retina, where there was a

Table 1. Comparison of retinal and pineal *N*-acetyltransferase activities

Treatment	Retina		Pineal	
	Total activity, nmol/tissue per hr	Specific activity, nmol/mg protein per hr	Total activity, nmol/tissue per hr	Specific activity, nmol/mg protein per hr
LD 12:12				
Mid-light	4.4 ± 0.24	0.95 ± 0.11	1.5 ± 0.18	4.7 ± 0.74
Mid-dark	63.4 ± 2.3	15.6 ± 2.5	61.4 ± 4.5	184.2 ± 17.1
1 hr light at mid-dark				
Control (no light)	48.1 ± 1.9		52.5 ± 3.1	
Light (1400–1500 lux)	8.1 ± 0.52		11.1 ± 2.1	

Retinal and pineal values are from the same animals (mean ± SEM per retina or pineal; $n = 5$ in all groups).

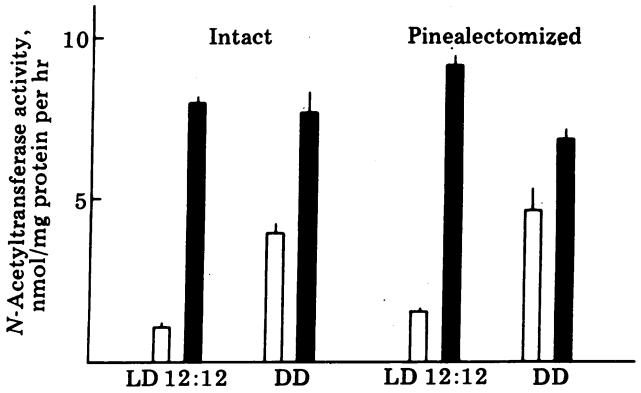

FIG. 3. Effect of pinealectomy on day–night differences in *N*-acetyltransferase activity. In the LD experiment: open bars, mid-light; solid bars, mid-dark. In the DD experiment: open bars, projected mid-light; solid bars, projected mid-dark. Chicks had been in DD for 42 hr when the mid-light point was taken and 54 hr when the mid-dark point was taken. $n = 5$ chicks in all groups.

15-fold increase at night. There was a small amount of nonrhythmic *N*-acetyltransferase activity in the pigment epithelium (Fig. 2). Similarly, the retina contained more melatonin than did the pigment epithelium, and only retinal melatonin content showed a day–night change.

There are great similarities between retinal and pineal indoleamine metabolism in chicks: levels of *N*-acetyltransferase activity; amplitude of the day–night differences; phase of the rhythms, with high levels at night and low levels during the day; inactivation of *N*-acetyltransferase by light during the dark portion of the LD cycle (Table 1). We tested the possibility that the pineal is directly involved in the control of retinal *N*-acetyltransferase rhythmicity. One-week-old chicks were pinealectomized, and after 10 days in a LD 12:12 cycle, *N*-acetyltransferase activity in the retina–pigment epithelium was examined at mid-light and mid-dark. Another group of pinealectomized chicks was transferred into DD, and after 42 hr chicks were killed at their subjective mid-light and 12 hr later at their subjective mid-dark. Fig. 3 shows that the rhythm in retinal *N*-acetyltransferase activity persists after pinealectomy in both an LD cycle and in DD.

DISCUSSION

We have shown that *N*-acetyltransferase and melatonin are found in the retina and that enzyme activity and melatonin content are correlated under several conditions: they have similar phases in LD and DD; when retinal enzyme is inactivated by light, retinal melatonin levels are also decreased. Serotonin and hydroxyindole *O*-methyltransferase, the other enzyme in the pathway from serotonin to melatonin, are found in the retina of most vertebrates (18–20, 22–24). The isolated retinae of rats (32) and trout (33) are able to make melatonin from serotonin. The retinal rhythm of *N*-acetyltransferase activity persists in pinealectomized chicks. On the basis of these facts we suggest that melatonin is rhythmically synthesized in the chick retina by retinal *N*-acetyltransferase, not synthesized elsewhere and taken up from the circulation.

The function of the retinal melatonin rhythm is not yet known. Retinal melatonin could be released into the general circulation to affect distant targets, or transported via the optic nerve to the brain. It could also act within the eye to drive or modulate the circadian rhythms of photoreceptor metabolism, or to modulate neural retinal function.

In sparrows, pinealectomy abolishes circadian rhythms of locomotor activity (34, 35), and transplantation experiments have demonstrated that the pineal is a dominant circadian pacemaker (36). Pinealectomy in chickens (37) and quail (38), however, does not appear to abolish circadian rhythms of locomotor activity (37). Because the retinal *N*-acetyltransferase rhythm persists after pinealectomy in chickens (Fig. 3) and because melatonin may be involved in the control of locomotor activity rhythms (39, 40), it is possible that a blood melatonin rhythm resulting from retinal secretion is involved in the persistence of the circadian locomotor rhythm after pinealectomy. If retinal melatonin is released into the general circulation, and can be shown to affect a nonretinal target, the retina might usefully be considered an endocrine organ.

In all vertebrate classes, rod and cone outer segments shed packets of disks, with a daily rhythm (1–8). In chickens, rods have a burst of shedding during the first few hours of light in a LD 12:12 cycle and cones shed during the first few hours of dark (6). Synthesis of new membranes in the rod outer segment also occurs with a daily rhythm in *Xenopus* (3). Furthermore, both photomechanical movements of rods and cones (6, 9, 10) and pigment migration in the retinal pigment epithelium (11, 12) occur with a daily rhythm in some vertebrate classes.

Several recent studies suggest that rhythms of photoreceptor metabolism are controlled within the eye (41–45). Pinealectomy does not abolish the daily rhythm of disc shedding in albino rats or frogs (43–45). Experiments in which light input is applied differentially to the two eyes (by patching one eye) indicate that control of rhythmic disc shedding resides at least partially within the eye. Rats kept in constant light lost the rhythm of disc shedding in the open eye; the rhythm continued in the patched eye (42). Retinal melatonin may well be the primary agent through which control within the eye is exerted, because it has a large-amplitude circadian rhythm, and because exogenous melatonin, given by injection to amphibians, causes movements of cones similar to those caused by light (15). It also produces pigment aggregation in the retinal pigment epithelium when administered intraocularly or systemically in fish (16) and mammals (17). Any or all of these rhythms of photoreceptor metabolism might be regulated by retinal melatonin.

Because the enzymes necessary for melatonin synthesis are present in the retina of all vertebrate classes (18), circadian rhythmicity in melatonin content of the eye may be widespread. If so, the vertebrate eye may be found to have a variety of unsuspected endocrine and neuroendocrine functions.

We thank J. S. Takahashi and S. Binkley for comments on the manuscript and M. Rollag for the melatonin antibody. The research was supported by National Institutes of Health Grant HD-07727.

1. LaVail, M. M. (1976) *Exp. Eye Res.* **23**, 227–280.
2. LaVail, M. M. (1976) *Science* **194**, 1071–1074.
3. Besharse, J. C., Hollyfield, J. G. & Rayborn, M. E. (1977) *J. Cell Biol.* **75**, 507–527.
4. Young, R. W. (1978) *J. Cell Biol.* **76**, 593–604.
5. Young, R. W. (1977) *J. Ultrastruc. Res.* **61**, 172–185.
6. Young, R. W. (1978) *Invest. Ophthalmol. Visual Sci.* **17**, 105–116.
7. Bunt, A. H. (1978) *Invest. Ophthalmol. Visual Sci.* **17**, 90–104.
8. Basinger, S., Hoffman, R. & Matthes, M. (1976) *Science* **194**, 1074–1076.
9. Burnside, M. B. (1976) *Exp. Eye Res.* **23**, 257–275.
10. Ali, M. A. (1971) *Vision Res.* **11**, 1225–1288.
11. Pang, S. F., Yew, D. T. & Tsui, H. W. (1978) *Neurosci. Lett.* **10**, 221–224.
12. Walls, G. C. (1967) *The Vertebrate Eye and Its Adaptive Radiations* (Hafner, New York).
13. Young, R. W. (1978) *Vision Res.* **18**, 573–578.

14. Hollyfield, J. G. & Basinger, S. F. (1978) *Invest. Ophthalmol. Visual Sci.* **17**, 87–89.
15. Quay, W. B. & McLeod, R. W. (1968) *Anat. Rec.* **160**, 491.
16. Cheze, G. & Ali, M. A. (1976) *Can. J. Zool.* **54**, 475–481.
17. Pang, S. F. & Yew, D. T. (1979) *Experientia* **35**, 231–233.
18. Quay, W. B. (1965) *Life Sci.* **4**, 983–991.
19. Wainwright, S. D. (1979) *J. Neurochem.* **32**, 1099–1101.
20. Cardinali, D. P. & Rosner, J. M. (1971) *Endocrinology* **89**, 301–303.
21. Binkley, S., Hryshchyshyn, M. & Reilly, K. (1979) *Nature (London)* **281**, 479–481.
22. Suzuki, O., Noguchi, E., Miyake, S. & Yagi, K. (1977) *Experientia* **33**, 927–928.
23. Florén, I. & Hansson, H. (1979) *Invest. Ophthalmol. Visual Sci.*, in press.
24. Hauschild, C. & Laties, A. (1974) *Invest. Ophthalmol.* **12**, 537–540.
25. Hamm, H. & Menaker, M. (1979) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **38**, 1043.
26. Deguchi, T. & Axelrod, J. (1972) *Anal. Biochem.* **50**, 174–179.
27. Rollag, M. D. & Niswender, G. D. (1976) *Endocrinology* **98**, 482–489.
28. Rodbard, D. & Hutt, D. M. (1974) in *Symposium on RIA and Related Procedures in Medicine*, International Atomic Energy Agency, Vienna (UNIPUB, New York), Vol. 1, pp. 165–189.
29. Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–253.
30. Woodruff, M. L., Bownds, D., Green, S. H., Morrisey, J. L. & Shedlovsky, A. (1977) *J. Gen. Physiol.* **69**, 667–679.
31. Binkley, S., Kluth, E. & Menaker, M. (1971) *Science* **174**, 311–314.
32. Cardinali, D. P. & Rosner, J. M. (1971) *J. Neurochem.* **18**, 1769–1770.
33. Gern, W. A., Owens, D. W. & Ralph, C. L. (1979) *Science* **204**, 183–184.
34. Takahashi, J. S. & Menaker, M. (1980) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **38**, 2583–2588.
35. Gaston, S. & Menaker, M. (1968) *Science* **160**, 1125–1127.
36. Zimmerman, N. H. & Menaker, M. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 999–1003.
37. MacBride, S. A. (1973) Dissertation (Univ. of Pittsburgh, PA).
38. Simpson, S. M. (1980) Dissertation (Univ. of Wales, Wales).
39. Gwinner, E. & Benzinger, I. (1978) *J. Comp. Physiol.* **127**, 209–213.
40. Turek, F., McMillan, J. P. & Menaker, M. (1976) *Science* **194**, 1441–1443.
41. Easter, S. S., Jr. & Macy, A. (1978) *Vision Res.* **18**, 937–942.
42. Terstein, P. S., O'Brien, P. J. & Goldman, A. I. (1978) *Invest. Ophthalmol. Visual Sci.*, Suppl. 17, 134.
43. LaVail, M. M. & Ward, P. A. (1978) *Invest. Ophthalmol. Visual Sci.* **17**, 1189–1193.
44. Tamai, M., Tierstein, P., Goldman, A., O'Brien, P. & Chader, G. (1978) *Invest. Ophthalmol. Visual Sci.* **17**, 558–562.
45. Currie, J. S., Hollyfield, J. G. & Rayborn, M. E. (1978) *Vision Res.* **18**, 995–1003.