Photochemistry and Photobiology Vol. 45, No. 5, pp. 637–641, 1987
Printed in Great Britain.

0031-8655/87 $03.00+0.00

PENETRATION OF LIGHT INTO THE UTERUS OF PREGNANT MAMMALS

STEVEN L. JACQUES[1], DAVID R. WEAVER[2] and STEVEN M. REPPERT*[2]
[1]Wellman Laboratory, Department of Dermatology and [2]Laboratory of Developmental Chronobiology, Children's Service, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114, USA

(*Received* 17 *September* 1986; *accepted* 10 *November* 1986)

Abstract—The fraction of incident light which reaches the uterine lumen in pregnant rats and guinea-pigs was studied with a surgically implanted optical fiber. Penetration of visible spectrum light (400–650-nm wavelengths) into the uterus was examined. The effects of clipping the hair, retracting the skin, and retracting the abdominal wall on the penetration of light was also assessed to determine the relative amount of light transmitted through each layer between the external environment and the uterus. The presence of significant transmission at biologically relevant wavelengths raises the possibility that environmental lighting may reach the fetus and influence development.

INTRODUCTION

Light has generally been considered to be the environmental characteristic least likely to reach the fetus *in utero* (Bradley and Mistretta, 1975). A number of studies from this and other laboratories indicate that the environmental light–dark cycle during the prenatal period does influence pre- and postnatal physiology and behavior, however [see Reppert *et al.*, 1985 for review]. In those cases where the mechanism for a prenatal influence of environmental lighting has been determined, it has been found that the fetus perceives lighting indirectly, following transduction of relevant aspects of the environment by the dam. For example, fetal perception of the phase of the light–dark cycle in rats is the result of maternal–fetal communication and is not due to direct fetal perception of light (Reppert and Schwartz, 1983). Similarly, perception of daylength by fetal Djungarian hamsters is dependent upon the maternal pineal gland and its hormone, melatonin (Weaver and Reppert, 1986). While these studies have not demonstrated any direct influence of light on the fetus, the state of neural and sensory development may be insufficient in these altricial species for direct responsiveness to light during the prenatal period. In more precocious species, however, the development of responsiveness to light may occur prenatally (Sedlacek, 1971; Persson and Stenberg, 1972).

To assess whether environmental lighting could potentially have direct effects on fetal physiology, we determined the amount of light which actually reaches the fetus in pregnant rats and guinea-pigs using a surgically implanted optical fiber bundle. Penetration of visible spectrum light to the uterus through all tissue layers and the effects of clipping the hair, retracting the skin, and retracting the abdominal wall were examined.

*To whom correspondence should be addressed.

MATERIALS AND METHODS

Animals. Five timed-pregnant CD strain (albino) rats were obtained from Charles River Laboratories (Boston, MA). The experiments were conducted on gestational day 20 (of 21–22 days gestation), when each dam weighed *ca.* 400 g. The dams were anesthetized with pentobarbital (50 mg/kg, i.p.) for surgical implantation of the optical fiber bundle and remained anesthetized throughout the period of light measurements; following completion of the measurements the dams were sacrificed by an overdose of pentobarbital.

Three timed-pregnant Hartley strain (albino) guinea-pigs (Camm Research Laboratories, Wayne, NJ) were used. Each dam weighed 1200–1400 g and was carrying six fetuses (25–45 g) at the time of the experiment on gestational day 51 (of 65–70 days gestation). An anesthetic preparation consisting of ketamine (55 mg/kg), xylazine (2 mg/kg) and atropine (0.08 mg/kg) was administered intraperitoneally and as with the rats, the guinea-pigs were sacrificed before recovering from anesthesia.

Spectrophotometric measurements. Spectrophotometric measurements were made between 400 and 650 nm *via* an optical fiber bundle (4 mm dia.). A vertical plastic supporting sheath around the shaft of the fiber bundle fixed the position of the bundle relative to the light source; the length of the sheath was varied to compensate for the size of the animals (*ca.* 4 cm for rats and 8 cm for guinea-pigs). A diffuse light source was constructed by placing 14 42-cm cool white fluorescent tubes (GE type F15T12-CW) 15 cm above and to either side of the sensor. Light entering the fiber bundle was transmitted to a spinning diffraction grating spectrophotometer (Rofin, model 6001, 5-nm bandwidth, scan time 0.1 s), detected by a photomultiplier tube (Hamamatsu), and the data were stored on a Hewlett–Packard computer in 5-nm bandwidths from 400 to 650 nm.

A calibration scan (100% of source) was recorded with the fiber bundle in position without an animal, which provided an unobstructed measurement of the light source. (The acceptance angle of the optical fiber bundle as it viewed a diffuse light source was not significantly different whether it was in air or submerged in water.) The response of the system reflected both the spectral distribution of the light source and the sensitivity of the detector. During each subsequent measurement of light transmission through animal tissues, 100 scans were recorded and averaged over a 20-s period, and the results were normalized by the 100% calibration scan to yield per cent transmission. Three such determinations consisting

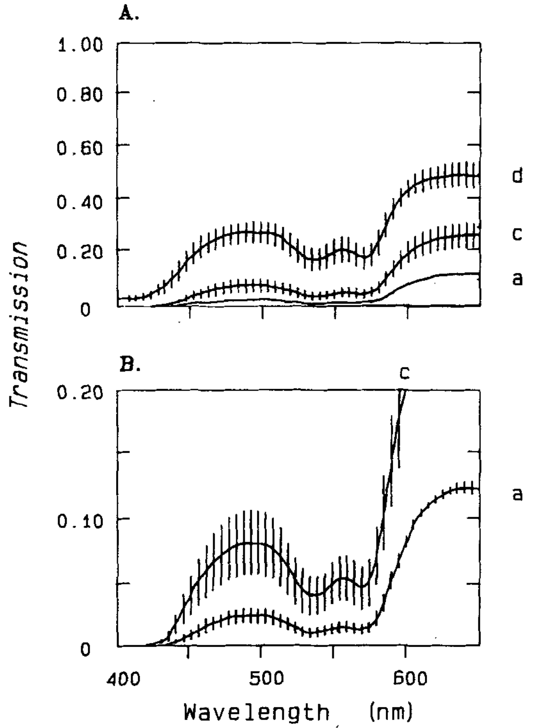

Figure 1. Transmission of light into the uterus of the rat. The mean transmission level by wavelength for 5 rats are indicated; the vertical lines represent SEM. The lower graph represents the same data on an expanded scale. Spectral scans for the following conditions are shown: (a) through full thickness, (c) after retraction of the skin, and (d) after retraction of the abdominal wall.

of 100 scans each were made for each placement of the fiber bundle in each animal, and the mean values of transmission were calculated for each fiber placement. The mean and standard error (SEM) are reported for 5 rats (n=5), and for two sides in each of 3 guinea-pigs (n=6).

Procedure for surgery and transmission measurements. For measurement of light transmission into the rat uterus, the fiber bundle was acutely implanted in the uterus of the anesthetized dam. The plastic supporting sheath around the fiber bundle was used to maintain the sensor in a constant position relative to the light source when placed in the uterus. The gravid uterus was exposed by a midventral incision and the fiber bundle was inserted through a small incision in one uterine horn. The dam was then placed on her side so that the uterine horn containing the fiber bundle was up, toward the light source. Transmission of light from the source through the full thickness of maternal tissues was first examined. Additional measurements were performed following retraction of the dam's skin in the region of the sensor and following retraction of the abdominal wall.

For guinea-pigs, measurements of light transmission into the uterus were performed across both ventral and dorsal surfaces (examined individually). For each pregnant guinea-pig, an incision was placed *ca.* 4 cm to the right of the midline on the ventral surface, and the fiber bundle was inserted through a small incision in the right uterine horn. The dam was then rolled onto her ventral surface with the probe in place, so that transmission through the dorsal surface could be assessed. Following all measurements for the right side, the fiber bundle was removed and the abdominal and skin incisions were sutured. An incision was then made on the left side on the dam's dorsal surface, the fiber bundle was implanted in the left uterine horn, and transmission through the dam's ventral surface was measured. For each position in each dam, measurements were made through the full thickness of maternal tissues. Subsequent measurements were made after shaving the hair in the region of the fiber bundle's tip and (as in the rat) following retraction of the skin and the abdominal wall.

To illustrate the scattering of white light during propagation across the maternal tissues, the optical fiber bundle was positioned near a fetal eye within the rat uterus and held by sutures. A white light source (tungsten/halogen lamp, Sylvania type ENZ 50 W–30 V) was transmitted into the uterus *via* the optical fiber bundle, and a photograph was taken of the scattered light that emerged at the abdominal surface.

RESULTS

The penetration of light into the rat uterus is shown in Fig. 1. The transmission across the full thickness of maternal tissues (hair, skin, abdominal wall and uterus) was relatively low for wavelengths below 600 nm, but increased to 12% at 650 nm (curve a). Retraction of the skin approximately doubled the amount of light entering the uterus (curve c). Retraction of the abdominal wall further increased transmission to 20–30% below 600 nm and to 50% at 650 nm (curve d).

The transmission pattern across the full thickness of maternal tissues in the guinea-pig (Fig. 2, curve a) was very similar in shape and magnitude to that seen in the rat. No differences in transmission were observed between the dorsal and ventral surfaces, so the results have been pooled. Clipping the hair in the area overlying the fiber bundle approximately doubled transmission into the uterus (curve b), suggesting that reflection of light by the white coat of albino animals may represent a significant barrier to penetration of light into the abdomen. Retraction of the skin (curve c), and retraction of the abdominal wall (curve d) also increased transmission, reaching 30–40% at wavelengths below 600 nm and 60% at 650 nm. These values for guinea-pigs are about 10 percentage points higher than the corresponding values for the rat.

A window of transmission was observed at about 500 nm, and the data at that wavelength are summarized in Fig. 3. The measured transmission was the product of the individual tissue transmissions. Each tissue layer contributed approximately the same amount of optical attenuation. It was surprising that the abdominal wall, which was 3.5 mm thick, and the uterus wall, which was only 0.6 mm thick, had equal transmissions. In the guinea-pig, the hair contributed nearly as much to optical attenuation as did the skin.

The wavelength dependence of scattering is demonstrated in Fig. 4, which shows the pattern of scattered light that emerged from a rat with a fiber bundle light source surgically inserted in the uterus. The center of the spot was white, which indicated

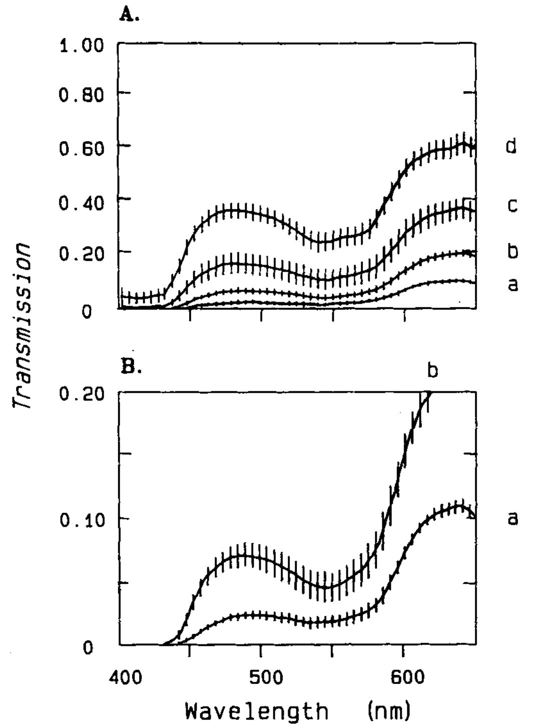

Figure 2. Transmission of light into the uterus of the guinea-pig. The mean transmission for 6 placements (2 positions in each of 3 animals) with SEM indicated for each wavelength. The lower graph represents the same data on an expanded scale. Spectral scans for the following conditions are shown: (a) through full thickness, (b) with the hair shaved, (c) after retraction of the skin, and (d) after retraction of the abdominal wall.

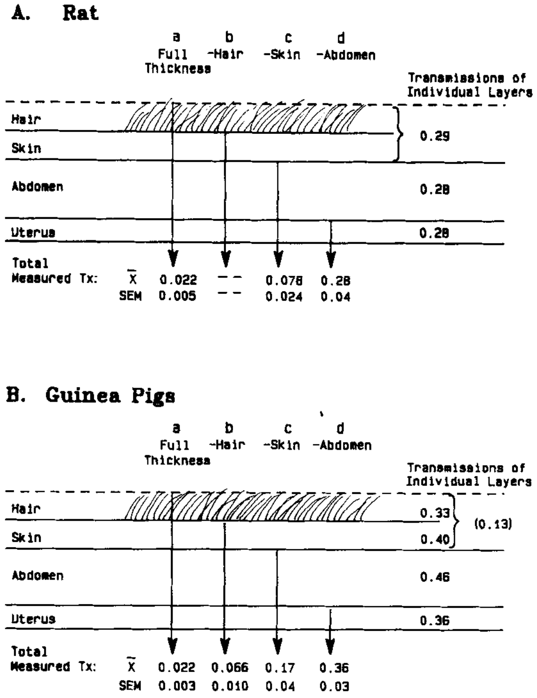

Figure 3. Transmission through each of the maternal tissue layers between the fetus and the external environment. Transmission (T_x) is expressed as the fraction of incident light which passed through the hair (T_{hair}), skin (T_{skin}), muscle and facia of the abdominal wall ($T_{abdomen}$) and the uterine wall (T_{uterus}) when that tissue is considered individually. For the guinea-pig, four measurements were made and four tissue layers were involved:

$$T_{hair}T_{skin}T_{abdomen}T_{uterus} = T_{full\ thickness} \quad (a)$$

$$T_{skin}T_{abdomen}T_{uterus} = T_{clipped\ hair} \quad (b)$$

$$T_{adomen}T_{uterus} = T_{retracted\ skin} \quad (c)$$

$$T_{uterus} = T_{retracted\ abdomen} \quad (d)$$

The solution of Eqs. a–d specified the transmission for each individual tissue layer. For the rat, the "clipped hair" measurement (Eq. b) was not made, therefore only the product $T_{hair}T_{skin}$ could be specified. (A) Rat. (B) Guinea-pig.

contributions from the entire visible spectrum were present. At the periphery of the spot, the light appeared yellow, then red. A great deal of scattering was necessary to cause this light to wander so far from the central axis, and the effective path length traveled by the light that emerged at the periphery was much greater than the on-axis path. The increase in path length for light emerging at the periphery caused blue and green light to be significantly attenuated, whereas the longer wavelengths of yellow and red were less attenuated by tissue absorbance.

DISCUSSION

Our results indicate that a small but significant portion of incident light is transmitted into the uteri of guinea-pigs and rats during pregnancy. The wavelength *vs* transmission relationship observed is consistent with the higher scattering and absorption of light at shorter wavelengths described for bloodless tissues, combined with absorption peaks due to oxy- and deoxyhemoglobin of the blood around 420 and at 540–580 nm (Anderson and Parrish, 1981; Wilson *et al.*, 1985).

Interestingly, there is a window of increased transmission centered around 500 nm. Light in the spectral range of 500 ± 50 nm is most biologically relevant to mammals, as these wavelengths coincide with the peak wavelengths for absorption by the visual pigment, rhodopsin (Brown and Wald, 1964). Similarly, wavelengths around 500 nm are maximally effective in influencing the circadian timing system of mammals, as indicated by efficacy in producing phase shifts in circadian rhythms, suppressing pineal melatonin content or synthesis, or maintaining testicular function (Cardinali *et al.*, 1972; Takahashi *et al.*, 1984; Brainard *et al.*, 1984, 1986).

In this biologically relevant wavelength range, *ca.* 2% of incident light is transmitted into the uterus.

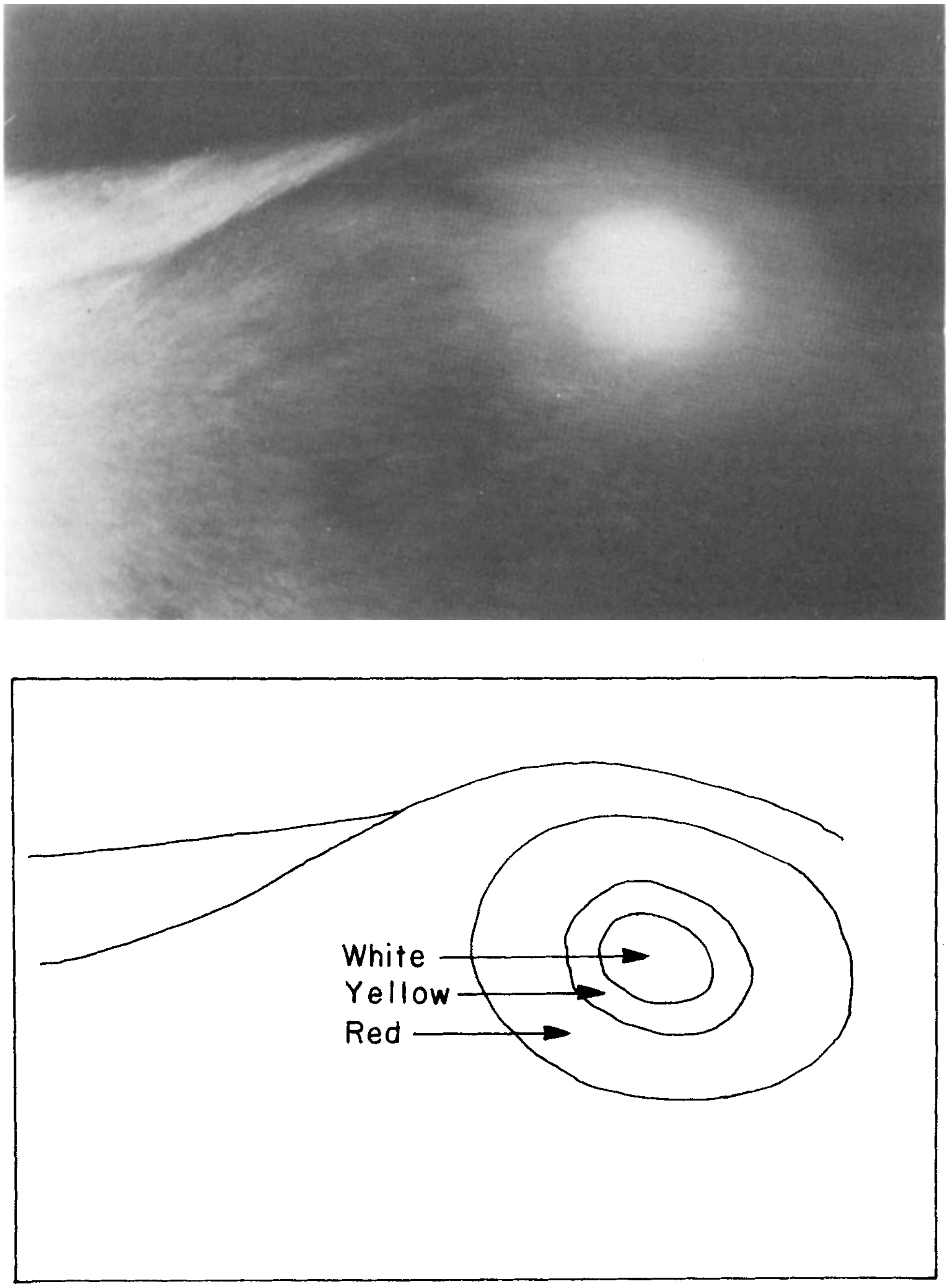

Figure 4. Pattern of scattered white light from an optical fiber bundle surgically implanted within the uterus of a pregnant rat. Directly over the light source, light of all wavelengths was transmitted through the maternal tissue, and the center of the spot appeared white. Towards the periphery, where the path length of light was longer, the higher attenuation of blue and green produced rings of yellow and red light. (upper) Photograph of light emerging from rat; (lower) scheme of color pattern.

Since irradiance levels in the blue-green range as low as 0.2 μW/cm^2 clearly influence physiological processes related to the circadian timing system in adult rats and hamsters (Brainard *et al.*, 1983, 1986), a dam exposed to only 10 μW/cm^2 of light would have enough light transmitted into the uterus to influence the fetus, if the fetus is as responsive to light as are adults. The developing mammal may be even more sensitive to light than adults, as exposure to light may reduce the future sensitivity to light (Lynch *et al.*, 1981).

In addition to the circadian system, light may influence fetal physiology through the primary visual system. The threshold light intensities for vision are several orders of magnitude lower than the threshold for affecting the circadian timing system (Reuter, 1972; Groos and Mason, 1980). Direct fetal perception of light may facilitate development of synaptic connection in the primary visual system, as postnatal critical periods for development of neural responsiveness to visual stimuli occur in some species (*cf.* Hubel and Weisel, 1970).

Several factors will influence transmission of environmental lighting into the uterus. In this study, we placed the fiber bundle into the uterus and then positioned it gently against the inside of the abdominal wall. This reduced the path length for light relative to that which would occur if the uterus were not distended in this manner. While our method may increase transmission somewhat, we feel it is probably similar to that which would occur during late gestation, when the volume of the conceptus presses the fetuses against the abdominal wall. The physiological effect of intrauterine light exposure would also be influenced by the orientation of the fetus relative to the external body wall (e.g. whether it is the fetal head or fetal rump which is maximally exposed to light). In addition, the blood volume of the uterus (hence hemoglobin and oxyhemoglobin absorbance), maternal coat color and thickness, and position of the source relative to the fetuses would influence transmission. This latter point is important for quadrupeds, as the fetus will normally be closer to the dam's ventral surface, while environmental lighting will most likely impinge upon the dam's dorsal surface. In diurnal primates, bipedal posture may allow for even greater likelihood of exposure of the fetus to environmental lighting.

The results of this study provide a basis for estimating the amount of light that will penetrate into the uterus under laboratory conditions. Furthermore, the data are consistent with the idea that environmental lighting can reach the fetus at intensities sufficient to influence fetal physiology

Acknowledgements—We would like to thank Mark J. Banister and Daniel Henshaw for expert technical assistance in these studies. This work was supported in part by NIH grants AM25395-08 and HD14427, a grant from the Arthur O. and Gullan M. Wellman Foundation, and March of Dimes Basic Research Grant 1-945. SMR is an Established Investigator of the American Heart Association.

REFERENCES

Anderson, R. R. and J. A. Parrish (1981) The optics of human skin. *J. Invest. Derm.* **77**, 13–19.

Bradley, R. M. and C. M. Mistretta (1975) Fetal sensory receptors. *Physiol. Rev.* **55**, 352–382.

Brainard, G. C., B. A. Richardson, T. S. King, S. A. Matthews and R. J. Reiter (1983) The suppression of pineal melatonin content and N-acetyltransferase activity by different light irradiances in the Syrian hamster: a dose–response relationship. *Endocrinology* **113**, 293–296.

Brainard, G. C., B. A. Richardson, T. S. King and R. J. Reiter (1984) The influence of different light spectra on the suppression of pineal melatonin content in the Syrian hamster. *Brain Res.* **294**, 333–339.

Brainard, G. C., M. K. Vaughan and R. J. Reiter (1986) Effect of light irradiance and wavelength on the Syrian hamster reproductive system. *Endocrinology* **119**, 648–654.

Brown, P. K. and G. Wald (1964) Visual pigments in single rods and cones of the human retina. *Science* **144**, 45–52.

Cardinali, D. P., F. Larin and R. J. Wurtman (1972) Control of the rat pineal gland by light spectra. *Proc. Nat. Acad. Sci. USA* **69**, 2003–2005.

Groos, G. A. and R. Mason (1980) The visual properties of rat and cat suprachiasmatic neurons. *J. Comp. Physiol.* **135**, 349–354.

Hubel, D. H. and T. N. Weisel (1970) The period of susceptibility to the physiological effects of unilateral eye closure in kittens. *J. Physiol. Lond.* **206**, 419–436.

Lynch, H. J., R. W. Rivest, P. W. Ronsheim and R. J. Wurtman (1981) Light intensity and the control of melatonin secretion in rats. *Neuroendocrinology* **33**, 181–185.

Persson, H. E. and D. Stenberg (1972) Early prenatal development of cortical surface responses to visual stimuli in sheep. *Exp. Neurol.* **37**, 199–208.

Reppert, S. M., M. J. Duncan and B. D. Goldman (1985) Photic influences on the developing mammal. In: Ciba Foundation Symposium 117, *Photoperiodism, Melatonin and the Pineal.* Pitman: London, pp. 116–128.

Reppert, S. M. and W. J. Schwartz (1983) Maternal coordination of a fetal biological clock *in utero. Science* **220**, 969–971.

Reuter, J. H. (1972) A comparison of flash evoked ERGs and ERGs evoked with sinusoidally modulated light stimuli in a number of rodents. *Pfleugers Arch.* **331**, 95–102.

Sedlacek, J. (1971) Cortical responses to visual stimulation in the developing guinea pig during prenatal and postnatal period. *Physiol. Bohemeslov.* **20**, 213–220.

Takahashi, J. S., P. J. DeCoursey, L. Bauman and M. Menaker (1984) Spectral sensitivity of a novel photoreceptor system mediating entrainment of mammalian circadian rhythms. *Nature* **308**, 186–188.

Weaver, D. R. and S. M. Reppert (1986) Maternal melatonin communicates daylength to the fetus in Djungarian hamsters. *Endocrinology* **119**, 2861–2863.

Wilson, B. C., W. P. Jeeves and D. M. Lower (1985) *In vivo* and post mortem measurements of the attenuation spectra of light in mammalian tissues. *Photochem. Photobiol.* **42**, 153–162.