Histol Histopath (1992) 7: 1-6

Histology and Histopathology

Effects of white light on the pineal gland of the chick embryo

V. Aige-Gil and N. Murillo-Ferrol

Department of Animal Anatomy, Embryology and Genetics, Faculty of Veterinary, University of Zaragoza, Zaragoza, Spain

Summary. Chick embryos were directly exposed to a source of white light during incubation and sacrificed before hatching. The light caused a number of teratological effects such as high mortality, delay in development, celosomy, hepatomegaly, auricular dilation and micrognatia. The pineal gland of the illuminated embryos showed an increase in number and size of the intracytoplasmic lipid droplets of the follicular pinealocytes. These findings suggest that the pineal gland of the chick embryo is sensitive to light.

Key words: Pineal gland, Light, Lipid droplets, Morphometry, Chick embryo

Introduction

The pineal gland arises in the diencephalic roof and lies between the habenular and posterior commissures (Calvo and Boya, 1979). In the adult, the pineal gland extends from the intercommissural region to the roof of the skull and is covered by the meninges. Its characteristic indol is melatonin and its levels increase in serum during the night (Binkley et al., 1973, 1975; Pang et al., 1974, 1977; Pelham, 1975; Ralph, 1976).

In the adult, the pineal activity is regulated by the environmental enlighting (Binkley et al., 1977a,b; Deguchi, 1979a,b; Kasal and Pérez-Polo, 1980). According to Morita (1966), Ralph and Dawson (1968) and Okshe et al. (1969) the avian pineal organ is not directly light sensitive. But, in cultured chick pineals exposed to different light-dark periods, N-acetyltransferase (NAT) activity has been demonstrated (Binkley et al., 1977a,b, 1978a,b,c; Wainwright and Wainwright, 1978; Deguchi, 1979a,b; Kasal and Pérez-Polo, 1979, 1980). Although a direct photoreception has not been proven in vivo, there is enough evidence to suggest that the pineal gland may be sensitive to light (Cuello et al., 1972; Hisano et al., 1972; Binkley et al., 1975).

In ovo hydroxyindole-O-methyltransferase (HIOMT) activity has been detected whether in 10-day chick embryos (Wainwright, 1974a,b) or in pineal culture (Mezei and Wainwright, 1979). However, no studies about pineal light activity in the embryo have been carried out. In the present report the pineal sensitivity to light *in ovo* and the effect of light on the embryo development are studied.

Offprint requests to: Dr. D. Vicente Aige-Gil, Departamento de Anatomía, Embriología y Genética Animal, Facultad de Veterinaria, C/ Miguel Servet nº 177, 50013 Zaragoza, Spain

Materials and methods

Eggs of Rhode Island Red domestic fowl, supplied by a local farm, were used in this experiment. They were incubated at 38.5° C and 75% relative humidity for 48 hours, at which time they had reached stage 12-13 in the Hamburger and Hamilton (1951) development series. Then, after removing 2 ml of albumen, the embryos were exposed by cutting a 2 cm^2 window in the equatorial region of the shell and sealed with transparent cellophane.

Two bulbs of cold white light (PHILIPS prismatic SL. 9 and SL. 13) placed 10 cms above the eggs gave a light intensity of 100 watt.

From a total of 175 eggs exposed, 95 eggs were incubated in light. The remaining 80 eggs were taken as controls.

Twice a day the embryos were checked and the dead ones removed and dissected under the microscope and studied for malformations. Some of the embryos which showed abnormalities were diaphanized following the Simons and Van Horn (1971) procedure.

At 18 days of incubation, the embryos were sacrificed and the pineal glands fixed in 2.5% glutaraldehyde in Sorensen's buffer (0.1 M phosphate, pH 7.4) for 30 min at 4ºC. After postfixation in 2% osmium tetroxide in the same buffer for 30 min at 4ºC,

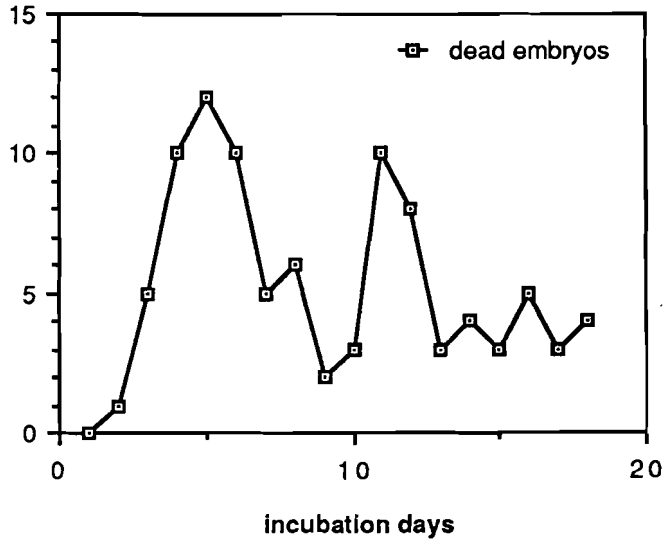


Fig. 1. Effect of light on the mortality of the expossed embryos.

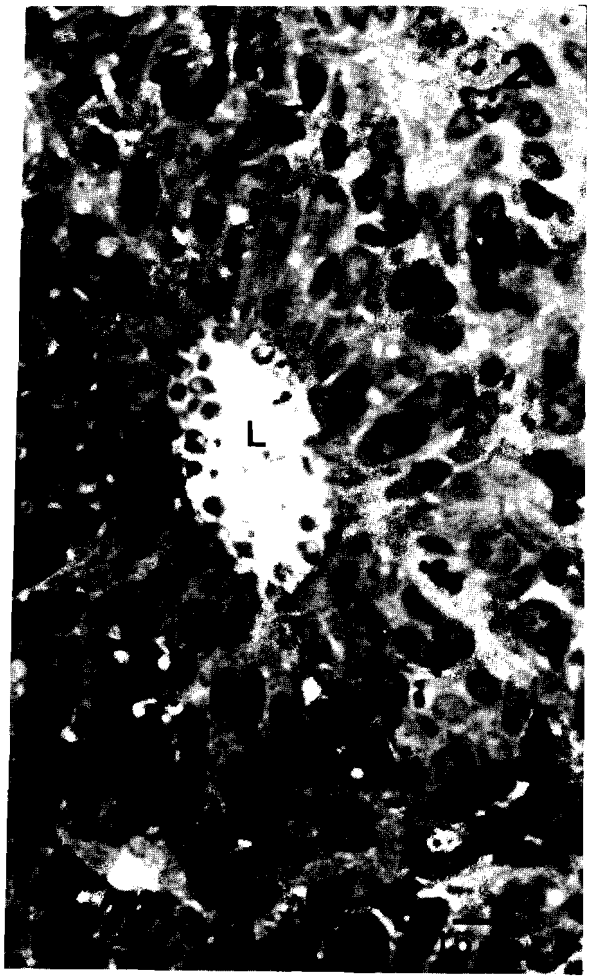


Fig. 2. Follicular structure of a control pineal gland. (L) Follicular lumen. (P) Follicular epithelium.

the samples were dehydrated, stained in uranyl acetate and embedded in Epon. Transversal semithin sections, 2 μm thick, were stained in basic toluidine blue and studied under the light microscope. Ultrathin sections were counterstained in lead citrate and examined with a JEOL JEM-T8 electron microscope.

Morphometric analysis

The number of intracytoplasmic lipid droplets in the pineal follicles was counted and the diameter was measured in 10 fields of 7500 μm^2 selected at random from each pineal gland. These data were analyzed in a MESIMAG (Nachet) morphometer.

Results

After 18 days of incubation, the embryo mortality increased from 19% in controls to 83% when the embryos were incubated at light (Table 1), showing two maximum peaks of mortality: one between 4 and 6 days and another between 11 and 12 days (Fig. 1).

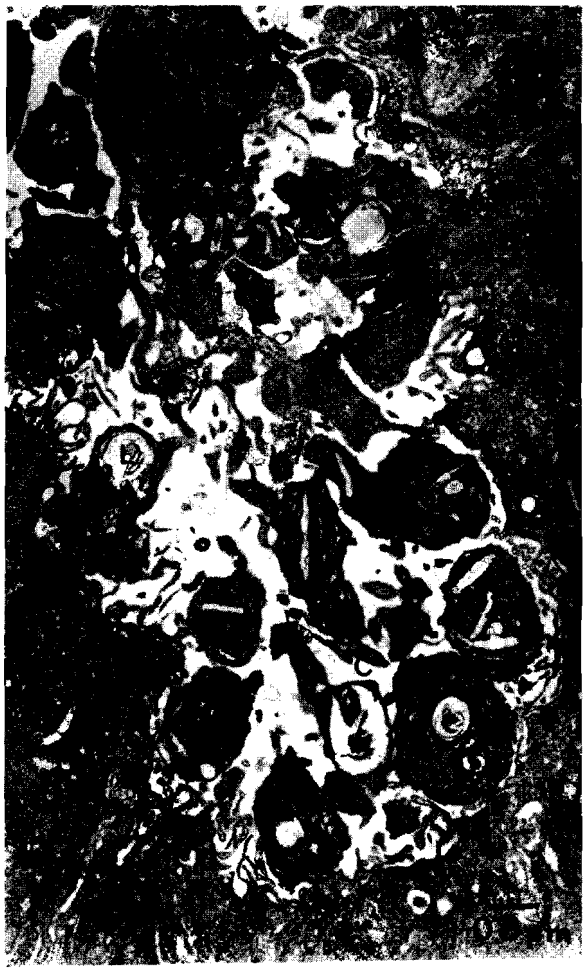
The anomalies found in the light-exposed embryos and in controls were of two types, as set out in Table 1. In the first type the malformations appeared to be caused simply by exposing the embryo through the window, whereas in the second type they were induced by the light.

Among the malformations associated with opening and resealing the egg is a high percentage of spina bifida, affecting either totally or partially the spinal column, macrognatia, mesencephalic anomalies, microphthalmia, anophthalmia and beak anomalies.

Exposure of the embryos to light produced the abnormalities shown in Table 1 and included: high mortality; delay in development; celosomy; auricular dilation, hepatomegaly and micrognatia.

In the light-exposed embryos and in controls, the epithelium forming the pineal follicles could be divided into two layers: one around the central lumen with nuclei located towards the basal zone and a second peripheric one surrounded by the basal lamina (Fig. 2). Boya and Zamorano (1975) call these two layers follicular and parafollicular respectively.

Two main cellular types were found in the follicular layer: one with a large nuclei and lax chromatin with one or two nucleoli; and the other with the nuclei located more deeply than in the previous type and a dense chromatin. The first type showed cellular prolongations towards the basal lamina containing few vesicles and dense bodies. The supranuclear cytoplasm was rich in ribosomes, rough endoplasmic reticulum, and mitochondria, and showed a well-developed Golgi system. In the apical portion, this cellular type projected a dilation or «drumstick» to the central lumen of the follicle (Fig. 3). This projection contained ribosomes, mitochondria and a pair of diplosomes that formed the basal portion of a ciliar projection. The neck of the drumstick was rich in microtubules and at this level there were mechanisms



of union with the neighbouring cells (Fig. 3).

In the second type, the cytoplasm was also projected towards the basal lamina. The supranuclear cytoplasm contained fewer organelles than that of the previous cellular type and a more dense hyaloplasm. The luminar portion showed many projections of small size as in the ependymal cells (Fig. 3).

In both cellular types lipid droplets were present (Fig. 4a). In the first type, they were located in the supranuclear cytoplasm and showed a laminar aspect. In the second type, the lipid droplets were also found in a supranuclear position, but they were never located so close to the central lumen as in the first type. In both cellular types the size and number of lipid droplets had increased (Table 2) and, in some cases, they occupied the whole drumstick (Fig. 4b). The histograms (Table 2) show that the lipid droplet diameter increased from an average of 1 μm in controls to 2.5 μm in those incubated with light.

The parafollicular layer of the follicular epithelium was formed by cells similar to those found in the follicular layer, but the cell shape was rounder. The

Fig. 3. Follicular lumen of a pineal gland of an 18-day embryo exposed and incubated in light. (L) Lipid droplet. (C) Ciliar projection. (D) Cellular dilation or «drumstick». (d) Cytoplasmic projections of small size. (M) Mitochondria.

Figs. (4 a,b). Pineal follicles of a pineal gland of an 18-day embryo exposed and incubated in light. The size and number of lipid droplets (arrows) are increased filling, in some cases, the cellular dilations or «drumstick» projected to the lumen.

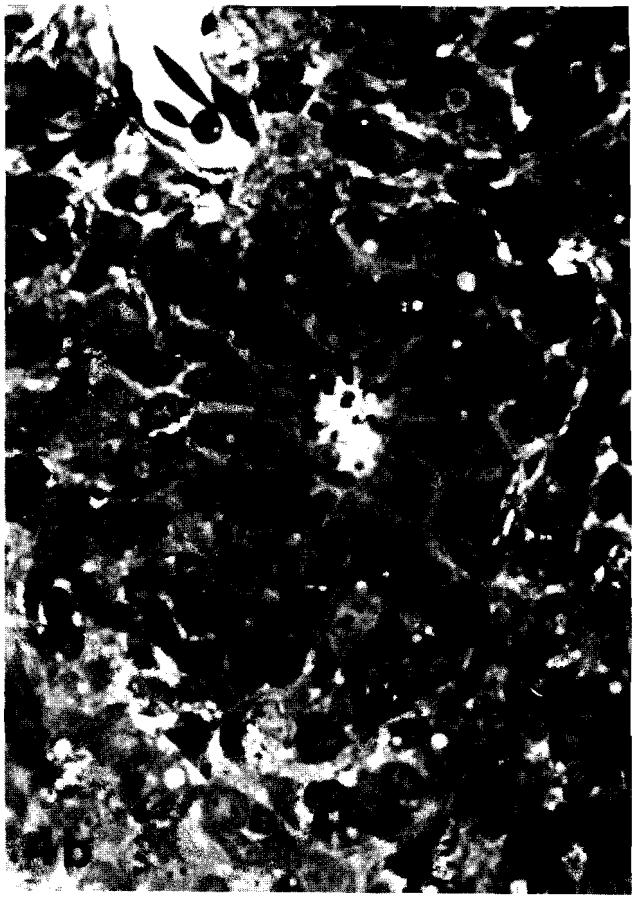


Table 1. Percentages of embryos showing various abnormalities after exposure or exposure and incubation in light.

Group 1 induced by exposure		
	Incubated in light	Controls
Spina bifida	3	1
Macrognatia	3	0
Mesencephalic anomaly	4	0
Microphthalmia	7	1
Unilateral anophthalmia	3	0
Beak anomaly	6	0
Group 2 mainly induced by light		
Mortality	95	20
Delay in development	83	19
Celosomy	28	1
Auricular dilation	26	0
Hepatomegaly	28	0
Micrognatia	11	0

number and size of the lipid droplets had also increased as happened in the follicular layer, showing the same laminar and non laminar aspect.

Nerve fibres in the pineal gland of 18-day embryos were not found in either control or light-exposed embryos.

Discussion

The influence of the pineal gland in the control of the daily rhythms is of considerable importance in birds pointing out the possibility of being the site of a biological clock (Binkley et al., 1977a,b, 1978a,b). In pinealectomized sparrows, the grafting of a pineal gland can restore the lost rhythms (Zimerman and Menaker, 1975). In mammals, on the other hand, the suprachiasmatic region seems to act as a timekeeper (Ralph et al., 1990).

Intracytoplasmic lipid droplets have already been reported by Collin (1969), Boya and Zamorano (1975) and Calvo and Boya (1979) in the chick pinealocytes both before and after hatching. But in the present work, it has been found that the number and size of these lipid droplets increases when the embryos are exposed and incubated in continuous light (Table 2). Similar results have been found in adults by Thillard (1968), who observed an increase in the amount of sudanophilic material in cells forming follicles in constant light and no change in constant darkness in adults. McFarland et al. (1969) found that the pineal cells decreased in volume, had fewer cytoplasmic lipid granules and showed a complete loss of the interlobular sudanophilic material in light-deprived chickens. By contrast, Milcou and Postelnicou (1964) reported a decrease in size of the intrinsic pineal cells and a diminution of the number of mitochondria induced by exposure to continuous illumination.

Simply exposing early embryos through a window appears to have significant teratogenic effects (Ancel, 1956) that decrease according to the time of incubation. So, Petite et al. (1990) found that just opening the shell of eggs before incubation resulted in 91% mortality, whereas the mortality dropped to 64% when the shell was opened at stage 4 and 24% at stages 8-10 (Aige-Gil and Simkiss, 1991).

There is a difference between the teratogenic effects found in the embryos incubated in light and the embryos incubated in darkness, showing that the light induces high mortality, delay in development, celosomy, auricular dilation, hepatomegaly and micrognatia.

In 28% of the embryos incubated in continuous illumination celosomy was detected. The diaphanization of the affected embryos showed either a failure in the closure of the sternal plates or a misplaced sternum with the ribs moved craneally. Moreover, the fact that all the celosomic embryos showed hepatomegaly suggests the possibility that celosomy could have been secondary to hepatomegaly.

According to Harrison (1951) and Murillo-Ferrol (1985) there is a natural predisposition in the chick embryo to the left microphthalmia. In the present paper, 1% of the control embryos were affected whereas the percentage went up to 7% in the embryos incubated with light. The cause of this anomaly is unknown but, as with the rest of the abnormalities, it may be light-induced.

Garwood et al. (1973) and Walter and Voitle (1973) found an increase in hatchability and body weight when the chicken eggs were incubated in light. However, Tamimie (1967) reported delay in development, low body weight at hatching, anomalies in limbs, eyes and jaw. In the present paper, delay in development was also found among other abnormalities reported previously (Table 1). However, in this experiment, the embryos were exposed and incubated under constrant white illumination using the same intensity (100 watt) as employed by these earlier authors.

In conclusion, the present study demonstrates that light induces teratological effects when the chick embryos are exposed through a window in the egg shell and that the pineal gland of the chick embryo is sensible to light *in ovo*, showing an increase in number and size of the cytoplasmic lipid droplets in the cells forming the pineal follicles. This fact needs further studies in order to prove whether this increase is a consequence of an advanced differentiation and development of the pineal gland.

Acknowledgements. The authors wish to thank Dr. L. Domínguez for critically reviewing the manuscript and to Mercedes Jaime for her help in the revision of the English text.

References

Aige-Gil V. and Simkiss K. (1991). Sterilizing embryos for transgenic chimeras. Br. Poul. Sci. 32, 427-438.

Ancel P. (1956). Recherche sur les effets tératogénes de l'overture de l'oeuf de Poule au cours des trente-quatre

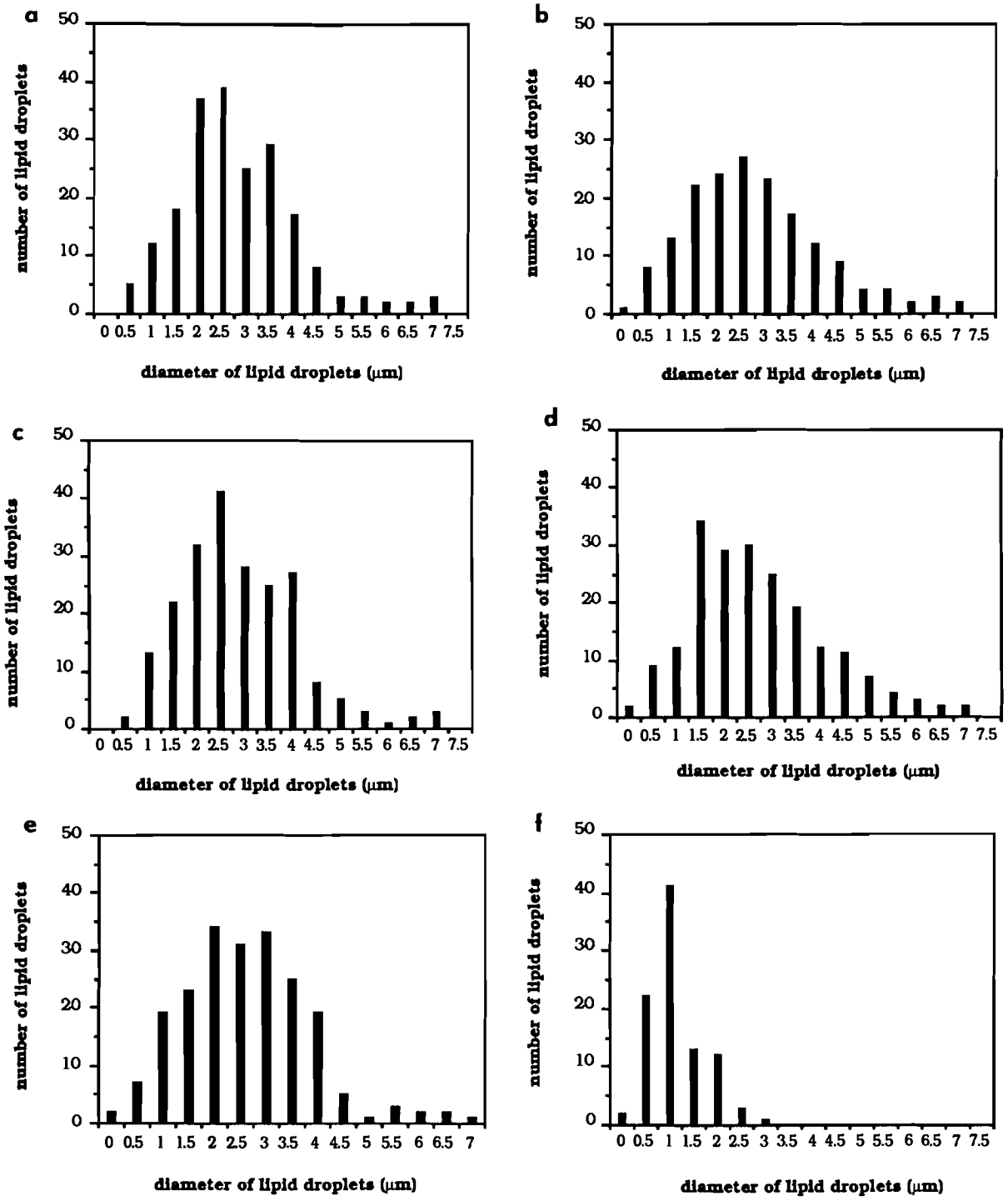


Table 2. Number and diameter (µm) of lipid droplets found in 10 fields of 7500 µm^2 selected at random in five pineal glands from 18-day embryos incubated with light (a, b, c, d, e) and in the control (f).

premières heures de l'incubation. Arch. Anat. Microsc. Morphol. Exp. 45, 203-217.

Binkley S., MacBridge S., Klein D. and Ralph C. (1973). Pineal enzymes: Regulation of avian melatonin synthesis. Science 181, 273-275.

Binkley S., MacBridge S., Klein D. and Ralph C. (1975). Regulation of pineal rhythms in chickens: Refractory period and nonvisual light perception. Endocrinology 96, 848-853.

Binkley S., Stephens J.L., Riebman J.B. and Reilly K.B. (1977a). Regulation of pineal rhythms in chickens: photoperiod and dark-time sensitivity. Gen. Comp. Endocrinol. 32, 411-416.

Binkley S., Riebman J. and Reilly K. (1977b). Timekeeping by the pineal gland. Science 197, 1181-1183.

Binkley S., Riebman J. and Reilly K. (1978a). Regulation of pineal rhythms in chickens: inhibition of dark-time N-acetyltransferase activity. Comp. Biochem. Physiol. (c) 59, 165-171.

Binkley S., Riebman J. and Reilly K. (1978b). The pineal gland: vitro a biological clock in. Science 202, 1198-1201.

Binkley S., Riebman J. and Reilly K. (1978c). Pineal N-acetyltransferase activity: a rhythm in vitro. Science 202, 1198-1201.

Boya J. and Zamorano L. (1975). Ultrastructural study of the pineal gland of the chicken (*Gallus gallus).* Acta Anat. 92, 202-226.

Calvo J. and Boya J. (1979). Ultrastructural study of the embryonic development of the pineal gland of the chicken *Gallus gallus.* Acta Anat. 103, 39-73.

Collin J.P. (1969). Contribution a l'étude de l'organe pinéal. De l'épiphyse sensorielle a la glande pinèale. Modalités de transformation et implications fonctionelles. Annales de la station biologique de Besse-en-Chandesse. Supplément. N° 1.

Cuello A.C., Hisano N. and Tramezzani J.H. (1972). The pineal gland and the photosexual reflex in female ducks. Gen. Comp. Endocrinol 18, 162-168.

Deguchi T. (1979a). Circadian rhythm of serotonin N-acetyltransferase activity in organ culture of chicken pineal gland. Science 203, 1245-1247.

Deguchi T. (1979b). A circadian oscillator in cultured cells of chicken pineal gland. Nature 282, 94-96.

Garwood V.A., Thorton E.J. and Lowe P.C. (1973). The effect of continuous illumination of incubating chicken eggs on embryonic development. Poult. Sci. 52, 337-340.

Hamburger V. and Hamilton H.L. (1951). A series of normal stages in the development of the chick embryo. J. Morphol. 88, 49-92.

Harrison J.R. (1951). In vitro analysis of differentiation of retina pigment in the development of the chick embryo. J. Exp. Zool. 111, 209-241.

Hisano N., Cardinali D.P., Rosner J.M., Nagle C.A. and Tramezzani J.H. (1972). Pineal role in the duck extraretinal photoreception. Endocrinology 91, 1318-1322.

Kasal C.A. and Pérez-Polo J.R. (1980). In vitro evidence of photoreception in the chick pineal gland and its interaction with the circadian clock controlling N-acetyltransferase (NAT). J. Neurosci. Res. 5, 579-585.

Kasal C.A., Menaker M., Pérez-Polo J.R. (1979). Circadian clock in culture: N-acetyltransferase activity of chick pineal glands oscillates in vitro. Science 203, 656-658.

McFarland L.Z., Wilson W.O. and Winget C.M. (1969). Response of the chicken pineal gland, blood and reproductive organs to darkness. Poult. Sci. 48, 903-907.

Mezei C. and Wainwright S.D. (1979). Hormone-induced increase of hydroxyindole-O-methyltransferase activity in the embryonic chick pineal gland in organ culture. Life Sci. 24, 1111-1118.

Milcou S.M. and Postelnicou D. (1964). L'influence de l'illumination prolongée sur la structure de l'épiphyse, chez de canard. Rev. Roum. Endocrinol. 1, 175-177.

Morita Y. (1966). Absence of electrical activity of the pigeon's pineal organ in response to the light. Experientia 22, 402.

Murillo-Ferrol N.L. (1985). Obtención de microftalmias tras la interrupción de los vasos vitelinos derechos. Estudio experimental en el embrión del pollo. Trabajos de Investigación. Facultad de Medicina de Madrid. pp 331-342.

Okshe A., Morita Y. and Vaupel-von Harnack M. (1969). Zur feinstruktur und funktion des pinealorgans der taube (*Columba livia*). Z. Zellforsch 102, 1-30.

Pang S.F., Ralph C.L., Reilly D.P. (1974). Melatonin in the chicken brain: its origin, diurnal variation and regional distribution. Gen. comp. Endocrinol 22, 499-506.

Pang S.F., Brown G.M., Grota L.J., Chambers J.W. and Rodman R.L. (1977). Determination of N-acetylserotonin and melatonin activities in the pineal gland, retina, Harderian gland, brain and serum of rats and chickens. Neuroendocrinology 23, 1-13.

Pelham R.A. (1975). A serum melatonin-rhythm in chickens and its abolition by pinealectomy. Endocrinology 96, 543-546.

Petite J.N., Clark M.E., Liu G., Gibbins A. and Etches M.V. (1990). Production of somatic and germ line chimeras in the chicken by transfer of early blastodermal cells. Development 108, 185-195.

Ralph C.L. (1976). Correlations of melatonin content in pineal gland, blood and brain of some birds and mammals. Am. Zoologist 16, 35-43.

Ralph C.L. and Dawson D.C. (1968). Failure of the pineal body of two species of birds *(Coturnix coturnix japonica* and *Passer domesticus)* to show electrical responses to illumination. Experientia 24, 147-148.

Ralph M.R., Foster R.G., Davis F.C. and Menaker M. (1990). Transplanted suprachiasmatic nucleus determines circadian period. Science 247, 975-978.

Simons E.V. and Van Horn I.R. (1971). A new procedure for whole mount alcian blue staining of the cartilaginous skeleton of chicken embryos, adapted to the procedure in potassium hydroxide. Acta Morphol Neerl. Scand. 8, 281-292.

Tamimie H.S. (1967). Effect of continuous and intermittent light exposure on the embryonic development of chicken eggs. Poult. Sci. 46, 1327.

Thillard M.J. (1968). Mise en évidence histochimique des lipides dans l'épiphyse du poulet. C.R. Soc. Biol. (Paris). 162, 1074-1080.

Wainwright S.D. (1974a). Course of the increase in hydroxindole-o-methyltransferase activity in the pineal gland of the chick embryo and young chick. J. Neurochem. 22, 193.

Wainwright S.D. (1974b). Development of hydroxytryptophan decarboxilase activity in the pineal gland, cerebral hemispheres and cerebelum of the chick embryo and young chick. Can. J. Biochem. 52, 149.

Wainwright S.D. and Wainwright L.K. (1978). Regulation of the diurnal cycle activity of serotonin acetyltransferase in the chick pineal gland. Can. J. Biochem. 56, 685-690.

Walter J.H. and Voitle R.A. (1973). Effects of photoperiod during incubation on embryonic during on embryonic and post-embryonic development of quail and chickens. Br. Poult. Sci. 14, 533-540.

Zimerman N.K. and Menaker M. (1975). Neural connections of sparrow pineal role in circadian control of activity. Science 190, 477-479.

Accepted June 1, 1991