Morphologic Changes in the Pineal Parenchyma Cells of Rats Exposed to Continuous Light or Darkness

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ABSTRACT. Young female rats were maintained for 8 weeks in continuous light or darkness, after which their pineal organs were removed and studied with light microscopy. The pineals from animals kept in light appeared to be functionally inhibited in that the parenchymal cells were smaller in size, contained fewer prominent nucleoli and had much less cytoplasmic basophila than those of the pineals from animals kept in darkness. Taken together with previously reported physiologic data, these findings suggest that the pineal is an important functional part of the neuroendocrine mechanism regulating the gonads with regard to varying conditions of illumination.

CONSIDERABLE evidence exists which suggests an antigonadotrophic function for the mammalian pineal organ (1). Furthermore, Wurtman *et al.* (2), in confirming and extending the work of others, have shown that pinealectomy in the immature female rat produces premature vaginal opening and hypertrophy of the ovary, adrenal and pituitary, whereas chronic administration of an aqueous pineal extract counteracts these effects of pinealectomy. In addition, we have recently presented evidence indicating that the pineal mediates some of the effects of varying illumination on the rat ovary (3). In that study, it was suggested that continuous light inhibits pineal activity, thereby diminishing its inhibitory influence on the pituitary-ovarian axis; continuous darkness, on the other hand, allows greater pineal activity and thus leads to smaller target organs. The hypothesis was based on two lines of evidence: 1. Pinealectomy or exposure to continuous light produced similar but not additive increases of ovarian weight, whereas administration of the pineal extract prevented the ovarian hypertrophy of animals kept in constant light. 2. Constant light exposure produces a decreased pineal weight, whereas constant dark leads to an increased pineal weight. The latter finding has been reported by Fiske *et al.* (4) and by Quay (5), who also noted a decrease in pineal lipid during continuous illumination.

Little information is available, however, on the cellular morphology of pineals of rats maintained under conditions of varying illumination. Therefore, it seemed necessary to obtain direct cytologic confirmation that the changed pineal weight reflected a true functional change in the pineal parenchyma cells and not merely a hyperplasia or increased blood volume. This report describes the results of a cytologic study of the pineal organs of some of the same animals used to obtain the physiologic data presented in our previous paper (3).

Materials and Methods

One group of 15 female rats of the Charles River strain was maintained for 56 days in almost continuous darkness, while 2 groups of animals were maintained for the same period in continuous light. Of these latter 2 groups, one group of 17 animals received

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daily saline injections, whereas the other group, comprising 15 animals, received daily injections of an aqueous bovine pineal extract. The pineals were placed in modified Bouin's solution immediately after weighing and were fixed for several days. Subsequently, the glands were sectioned at 5μ and stained as follows: hematoxylin and eosin and the Halmi paraldehyde-fuchsin trichrome technique (6) for general cellular morphology and for detection of possible cytoplasmic inclusions; methylene blue-eosin at a pH of 5.3 for the nucleic acid components.

Approximate cell size was determined by counting the number of nuclei per standard field in an ocular grid at a magnification of 1,080 diameters. Ten to 20 such fields were counted for each pineal from random sections in different areas, the counts were averaged for each gland and the reciprocal was taken as a rough index of cell size for each gland.

Results

No differences were noted in the appearance of the blood vessels or connective tissue capsule of the pineals from animals in the various groups. The parenchymal cells in any given gland were largely randomly arranged and fairly uniform in appearance, although small circular nests of cells rather like follicles were found occasionally in all glands. No regional differentiation of the gland such as into cortex or medulla could be seen. Rare, scattered, very small, dense cells could be found in all glands, but their number was so small as to be insignificant. Only one instance of a cell in mitosis was found in the entire study, and it could not be identified as a parenchymal cell. Consequently, the mitotic rate can be considered to be negligible for all the pineals.

The parenchymal cells of pineals from animals kept in constant dark showed several striking cytologic differences

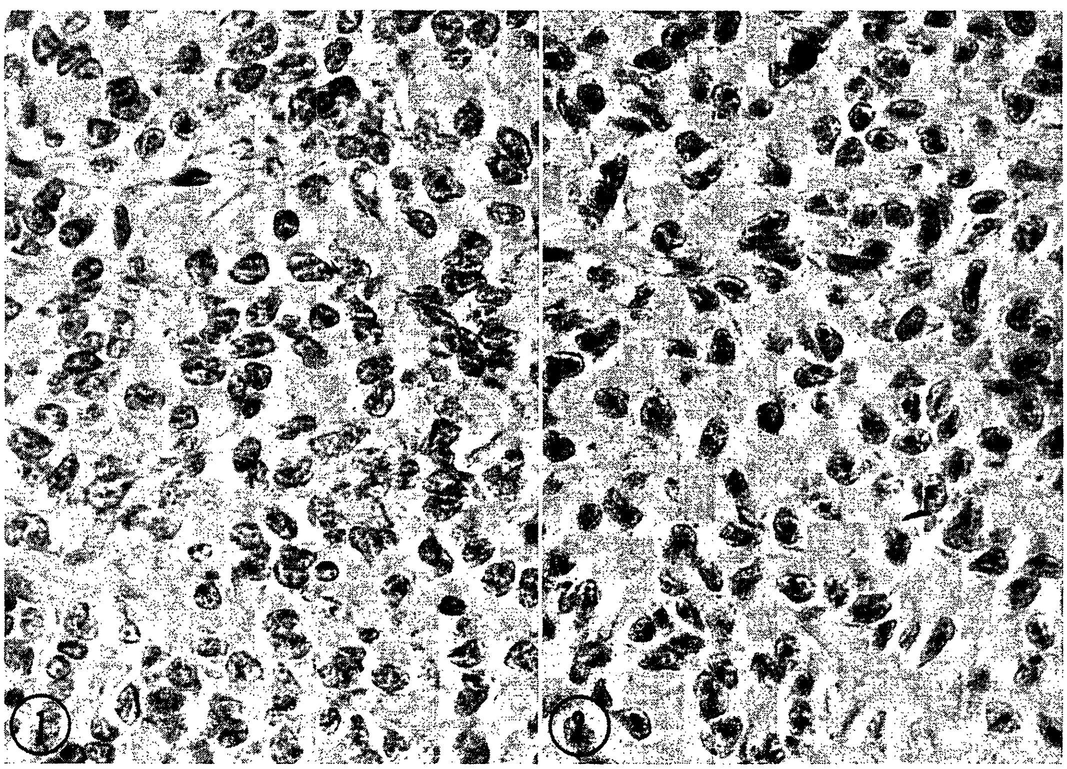

FIG. 1 and 2. Pineal parenchymal cells from an animal kept in constant light (FIG. 1), and from an animal kept in constant dark (FIG. 2). Note the greater cell volume of the cells in FIG. 2, as indicated by the spacing of the nuclei, and the appearance of prominent nucleoli in nearly all the nuclei. (Hematoxylin and eosin ×300)

TABLE 1. Effect of illumination on pineal cell size in rats

Experimental treatment	No. glands counted	Nuclei/field ± =95% Conf. limit	% decreased in light 100 (1 − dark) / light
Dark	13	39.2 ±2.2	
Light+Saline	10	55.8 ±2.5*	30
Light+Extract	11	49.8 ±2.8*	21

* $P < 0.001$ for different population from group 1; $P > 0.05$ that groups 2 and 3 are different populations.

from those of both groups maintained in the light. It was immediately apparent that the cells from the animals kept in the dark were much larger (Fig. 1 and 2). The relative volume of cells in each of the three groups is shown in Table 1. The cells of the group of animals kept in the dark constitute a population distinct from that of the cells of both groups kept in the light, which statistically form a single population.

The cells of animals kept in the dark differed also in the fact that their cytoplasm showed very large amounts of basophilia after the methylene-blue eosin staining, whereas cells from the two light groups had little or no such basophilia (Fig. 3 and 4). In the former cells, the basophilia takes the form of a diffuse light blue background with many dense blue granular bodies, which are located in clusters or spread in lines along the boundary of the cell (Fig. 4). Occasional clear perinuclear regions, which presumably are Golgi areas, appear in cells from animals kept in the dark. These areas are rare in animals from the light-exposed groups. In H and E preparations, the cytoplasm of cells from dark animals is moderately eosinophilic and is granular or vacuolar in appearance. By contrast, the cytoplasm of cells from animals kept in the light is more eosinophilic, more homogeneous and less granular.

Finally, the cells from the two populations differ in the shape and appearance of the nuclei. In animals kept in the dark, the nuclei vary from oval to piriform to triangular, with highly irregular and very much indented nuclear membranes. Cells from the animals exposed to light have more consistently oval nuclei with somewhat smoother walls. Nearly all the nuclei from the dark-exposed animals show very prominent nucleoli (Fig. 2, 4), which are often stained bichromatically, showing a deeply basophilic central area and an irregular-shaped eosinophilic outer area as well. Nuclei from the animals kept in light show few prominent nucleoli (Fig. 1, 3), and these are seldom bichromatic. In both cases the nuclei show scattered eosinophilic droplets, and these appear somewhat more numerous in the nuclei from animals in the dark, but this cannot be clearly determined without tedious and technically difficult counting. It also appears that the average size of the nuclei from animals kept in the dark may be slightly larger but they are so irregular in shape that valid comparative measurements cannot be made.

Discussion

The observations reported above seem sufficient to demonstrate that the parenchymal cells of the pineal organ are functionally altered by the conditions of illumination to which the immature and maturing female rat is exposed. They are entirely consistent with the physiologic data we reported earlier (3), which indicate that exposure to constant light produces an inhibition of pineal growth and an antiovarian function, whereas exposure to constant dark is associated with a large pineal and small ovaries.

Quay and Levine (7) have shown that mitotic activity in the rat pineal decreases almost to zero about two weeks after birth. This fact, coupled with our finding of a complete lack of mitotic activity, indicates that the change in size of the pineal under the conditions of this experiment cannot be accounted for on the basis of increased or decreased rates of cellular division, but is related directly to the changed size of the individual cells. In addition, the changes in cell volume, as measured in this study, correlate extremely well with the pineal weight changes detected on autopsy. It should be noted that cells of the two groups of animals kept in the light are approximately 21 and 30% smaller than those of the animals kept in the dark. The data in the previous paper (3) showed that the pineals of the animals kept in the light were approximately 15 and 20% lighter in weight. Since the weight of the connective tissue and blood may be considered to be approximately the same for the pineals of all the groups, it seems reasonable to conclude that the change in pineal weight in animals under different conditions of illumination is due very largely, if not exclusively, to the change in volume of the parenchymal cells.

However, the cytologic changes other than in size are perhaps the most interesting and suggestive data obtained in this study. The changes in the nuclei and nucleoli are in keeping with the findings of other authors: Holmgren *et al.* (8) showed that injections of bovine pineal extract produced decreased numbers of

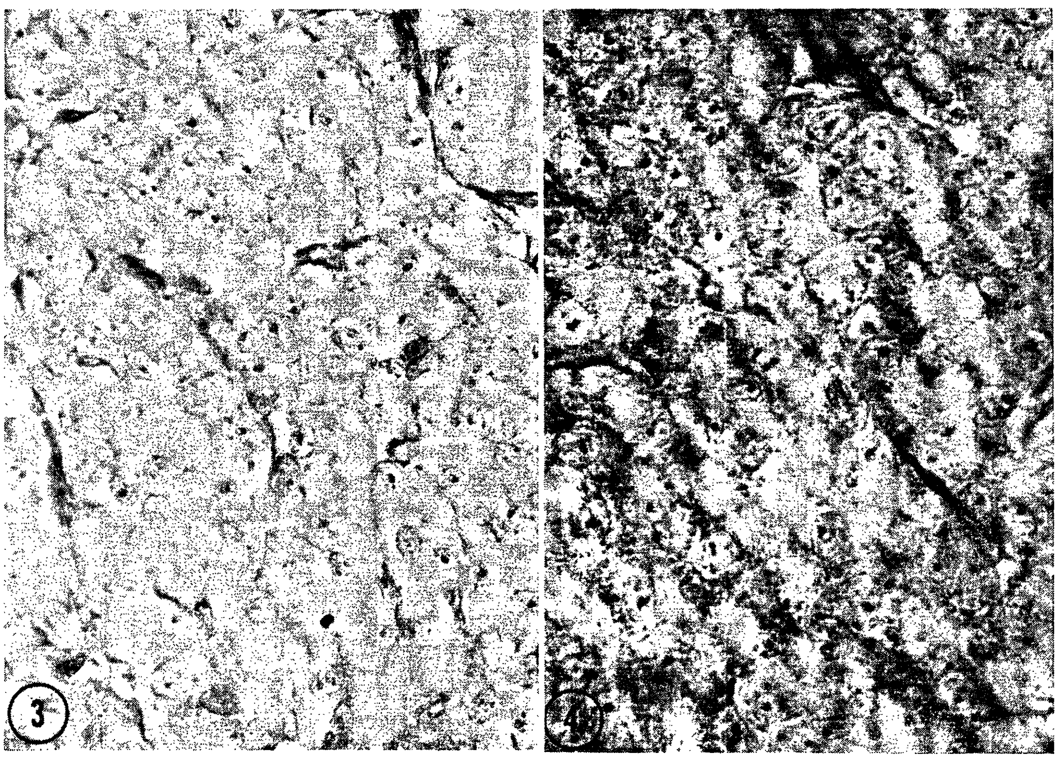

FIG. 3 and 4. Methylene-blue eosin preparations of pineal parenchymal cells from an animal kept in constant light (FIG. 3) and from an animal kept in constant dark (FIG. 4). Note the intense, granular cytoplasmic basophilia and prominent nucleoli of the cells in Fig. 4 as contrasted with those in Fig. 3. (×300) nucleoli and intranuclear granules in pineal cells of the rat; Quay (9) found that the pineal parenchyma cells of adult white-footed mice maintained in constant darkness contained significantly more phloxinophilic intranuclear granules than those of animals exposed to light. It should be noted that the combination of fixative and pH of staining with methylene blue used here is such that it is highly probable that the cytoplasmic basophilia present is RNA. This is in accordance with the finding of large amounts of cytoplasmic RNA by Wislocki and Dempsey (10) in pineal cells of the young rhesus monkey. It is generally accepted that the amount of cytoplasmic basophilia and the number and size of nucleoli provide a rough index of the rate of synthesis of nucleoprotein and protein. Thus, these results make it appear likely that the quantity of illumination to which the animals were exposed directly influenced nucleoprotein and protein synthesis in the pineal and, presumably, its endocrine activity.

Unfortunately, no "normal controls," animals exposed to normal nocturnal-diurnal variation in light, were studied in the present case. However, Fiske *et al.* (4) observed that the average pineal weight of maturing rats kept in constant darkness was approximately equal to that of animals exposed to natural light periods, and Quay (5) found little difference in the weight and lipid content of the pineals of rats exposed to 14 hours or 15 minutes of light per day. In addition, the pineal cells of normal rats of the same strain and only two weeks younger than the ones used in this study closely resemble those of the animals here kept in the dark (Roth: unpublished results). These facts suggest, but do not prove, that constant darkness has a permissive rather than an obligatory stimulatory effect on the pineal organ. The effect of pineal extract on the pineal glands of animals exposed to natural light conditions (8), and the absence of any additional effect of pineal extract in the pineal glands of animals kept in constant light, together suggest that light by itself can maximally inhibit the pineal and that too much light is the sole significant factor.

In conclusion, it can be stated that the combination of these cytologic findings with the physiologic results reported previously constitutes a compelling argument that, in the rat, the pineal is an important functional part of the neuroendocrine regulatory system, at least with regard to maturation of the gonads. If this conclusion can be generalized by experiments on other species, it would suggest some new lines of investigation of the well-documented effects of light on the maturation and seasonal activity of the reproductive system in vertebrates.

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Addendum. Fiske *et al.* (*Endocrinology* **71:** 130, 1962) reported that reduction in weight of the pineal organs of rats kept in continuous light is a direct effect *via* the central nervous system and not dependent on secretions of the pituitary, gonads, thyroid or adrenal.

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