Table 1. Effects of administration of melatonin or of deprivation of water on serotonin content of various regions of rat pituitary gland. Rats received melatonin (1 mg/kg per day intraperitoneally) or its diluent for 5 days. Other animals were deprived of access to water for 5 days. Data are given as micrograms of serotonin per milligram of protein ± standard error of the mean.

Region	Melatonin	Control	Water deprivation	Control
Pars distalis	0.14 ± 0.024	0.11 ± 0.015	0.08 ± 0.011*	0.14 ± 0.020
Pars intermedia	0.36 ± 0.026*	0.25 ± 0.038	0.23 ± 0.052	0.21 ± 0.040
Pars nervosa	0.16 ± 0.023	0.15 ± 0.015	0.08 ± 0.019*	0.16 ± 0.025

* $P < 0.05$. Differs from control.

gions of the pituitary. In contrast, deprivation of water modifies serotonin concentrations in the pars distalis and pars nervosa, but it has no effect on the amine in the pars intermedia.

Male Sprague-Dawley rats, weighing 500 to 600 g, received daily injections of melatonin (1 mg/kg per day intraperitoneally), dissolved in 10 percent ethanol, for 5 days; control animals received only the diluent. Animals were housed three per cage under standard laboratory conditions (that is, lights on from 6:00 a.m. to 6:00 p.m.; light provided by cool white fluorescent bulbs yielding approximately 50 mphot at head height) and given free access to rat chow and water. For dehydration experiments, uninjected animals were given access to food, but not to water, for 5 days. All injections were administered between 10:00 a.m. and noon, and all rats were decapitated between noon and 1:00 p.m. The pituitary was rapidly removed and placed on a chilled glass surface, and the pars distalis, neural lobe, and pars intermedia were separated under a dissecting microscope. Pools of three tissue samples were homogenized in 0.01*N* HCl and frozen until they could be assayed fluorometrically for serotonin (*6*). Portions of each homogenate were also assayed for total protein (*7*). Data from two experiments, each with treatment groups of 9 to 12 animals, were pooled and analyzed by Student's *t*-test.

Serotonin was present in relatively high concentrations in all three regions of the rat pituitary, but especially in the pars intermedia (Table 1). Melatonin administration caused a 44 percent increase in serotonin concentration (micrograms per milligram of protein) within the pars intermedia (Table 1). Since this tissue also contained more protein in melatonin-treated rats (0.22 versus 0.15 mg), its net increase in serotonin content was probably almost twofold. The pineal indole had no effect on the serotonin concentrations or protein contents of the pars distalis or neural lobe.

The effects of deprivation of water for 5 days on pituitary serotonin contrasted sharply with those of melatonin. Pituitaries of dehydrated rats showed striking decreases in the serotonin concentrations of the pars distalis and neural lobe (Table 1), and significant declines in their protein contents as well. However, dehydration had no effect on the pars intermedia.

These data suggest that: (i) The serotonin-containing structures in the various regions of the rat pituitary respond to stimuli (melatonin, dehydration) which are known to affect pituitary secretion. (ii) The responses of these structures appear to be specific, that is, melatonin, which rapidly reduces MSH concentrations in the pars intermedia (*3*), alters serotonin concentrations in this region but not in the remainder of the pituitary. (iii) The response of the serotonin-containing structures in the pars intermedia to melatonin may participate in its effects on MSH secretion. Several drugs which affect serotonin metabolism elsewhere in the body are also known to influence secretion of MSH (*8*). (iv) One general mechanism by which melatonin produces its physiological effects may be to modify the disposition of serotonin, its close structural analog, within target tissues. Hence, melatonin influences serotonin concentrations in the brain (*4*) and pars intermedia (Table 1), and blocks the contractile effects of serotonin on several types of smooth muscle in vitro (*9*). The exact significance of the elevation of concentration of serotonin in the pars intermedia after treatment with melatonin should become clearer once the site of serotonin storage is known.

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3 April 1970

Birefringent Filamentous Organelle in BHK-21 Cells and Its Possible Role in Cell Spreading and Motility

Abstract. *A birefringent structure consisting of a mass of filaments, 100 to 125 angstroms in diameter, appears at certain times during the spreading of a BHK-21 cell in culture. It is involved in the formation of the birefringent streak found in fully spread cells. The structure may be in part responsible for various aspects of cell motility.*

When living BHK-21 fibroblasts are observed with the polarization microscope, birefringent streaks are observed within the major cell processes of spread cells (*1*) maintained in culture. These streaks run longitudinally along the long axis of the major cell processes (*1*) and have been correlated by electron microscopy with similarly oriented subcellular fibrils that have been classified as microfilaments, microtubules, and filaments (*1*). As birefringence has been correlated with various aspects of cell motility (see, for example, *2*), it was thought that a study of the formation of the birefringent streaks during cell spreading might lead to some new information on the ultrastructural basis of the spreading phenomenon, about which virtually nothing is known (*3*).

The BHK-21/C13 fibroblast-like cells

were allowed to spread on glass cover slips for light microscopy and on Millipore filters for electron microscopy. The cells were grown in Eagle medium containing 10 percent calf serum and 10 percent tryptose phosphate broth. Details of the techniques used for growing cells and for making preparations for microscopy have been described in detail in previous publications (*1*, *4*).

Freshly trypsinized cells were obtained from growing populations of BHK-21 cells and were observed with polarization optics at various time intervals as they spread on glass cover slips from a spherical shape to a flattened fibroblast-like configuration (*4*). Birefringence was not evident immediately within these cells, but, as they began to flatten slightly, birefringence appeared adjacent to the nucleus in most of the cells. The birefringence in this region was detected with a $\lambda/30$ Brace-Koehler type compensator. In most instances, this juxtanuclear birefringent region was spherical and appeared as alternating bright and dark bands (Figs. 1 and 2). The central portion of this region appeared to be isotropic and usually contained granules (Fig. 2). The birefringence within this region is positive with respect to its circumferential axis [see Bennett (*5*)], which is indicative of fibrous material being wound around the center of the sphere. Retardation measurements were made according to the method of Bear and Schmitt (*5*, p. 653), and yield values of 1 to 2 nm, indicating a rather low level of oriented material. With phase contrast or differential interference microscopy, this region appeared as a relatively clear (that is, free from most large cytoplasmic particles) sphere adjacent to the nucleus (Figs. 3 and 4). As the cells begin to extend major cell processes (*1*), what we shall refer to as the "birefringent sphere" disappears and birefringent streaks are formed (Fig. 5). In several cells, birefringent streaks are seen to arise from the birefringent sphere (Fig. 6).

The BHK-21 cells cultured on cover slips maintained at 37°C for 24 hours were also observed with polarization optics. The majority of the cells in these cultures have elongated major cell processes within which are found the typical birefringent streaks (Fig. 5). However, a few cells are always found with distinct spheres adjacent to the nucleus (Figs. 1 and 2), but most of these cells are not fully spread and do not contain distinct birefringent streaks. Cells with streaks as well as birefringent spheres (see Fig. 6) can occasionally be found.

Cells placed on cover slips and maintained at room temperature for 24 hours do not spread out into the typical fibroblast-like configuration (*4*). The cells do, however, spread out slightly, and, when observed with the phase contrast microscope, a large spherical clear region can be seen adjacent to the nucleus in every cell (see Figs. 3 and 4). When observed with polarization optics, these regions appear as the typical birefringent sphere (see Figs. 1 and 2). When the cells are returned to 37°C and observed at different time intervals up to 6 to 8 hours, the majority of them become fully spread and, coincident with spreading, the birefringent spheres gradually elongate into the birefringent streaks.

When thin sections of partially spread cells obtained 1 to 2 hours after plating at 37°C are observed with the

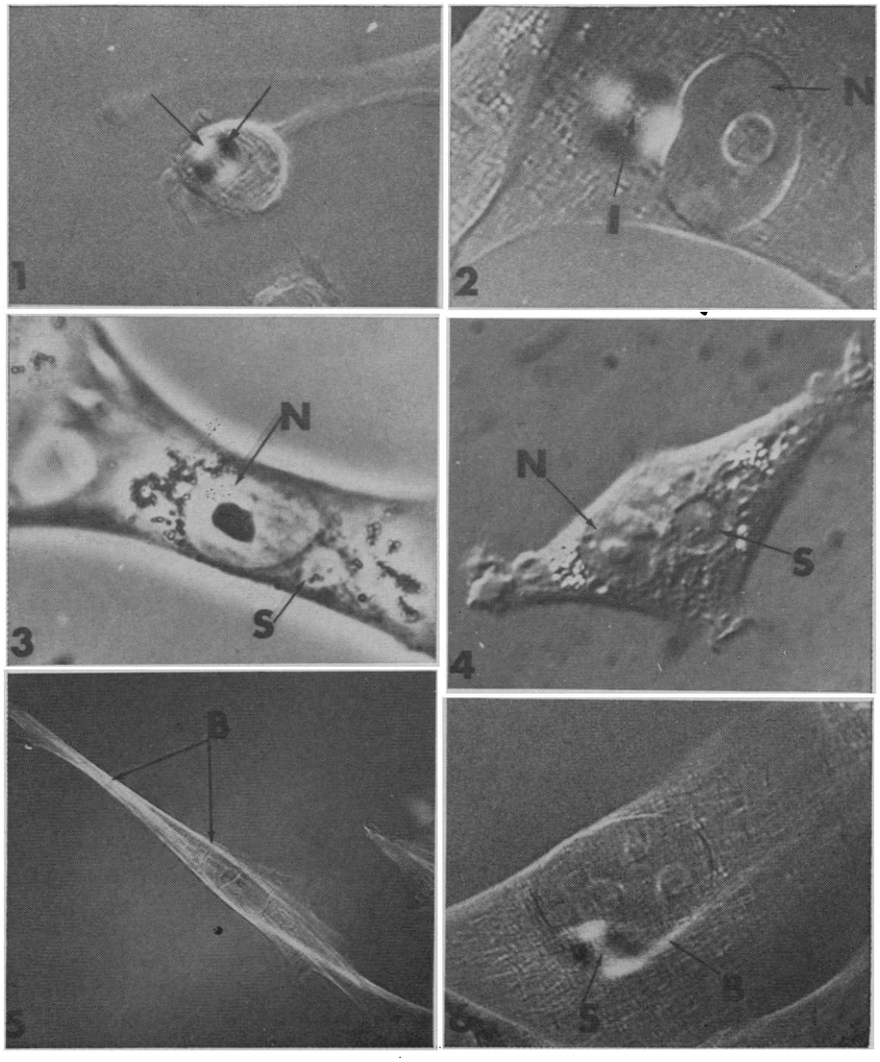

Fig. 1. A BHK-21 cell observed with polarization optics 1½ hours after being placed on a glass cover slip at 37°C. Note birefringent sphere appearing as dark and bright bands (arrows) (× 540). Fig. 2. A BHK-21 cell observed with polarization optics 3 to 4 hours after being placed on a glass cover slip at 37°C. Note isotropic region (*I*) in center of birefringent sphere and nucleus (*N*) (× 520). Fig. 3. A BHK-21 cell observed with phase contrast optics approximately 3 hours after being placed on a cover slip maintained at 37°C. Note clear birefringent sphere region (*S*) adjacent to nucleus (*N*) (× 430). Fig. 4. A BHK-21 cell placed on a cover slip and maintained at room temperature for 24 hours in an atmosphere of 95 percent air and 5 percent CO_2. Note birefringent sphere (*S*) adjacent to nucleus (*N*). Differential interference optics (× 600). Fig. 5. A fully spread BHK-21 cell observed with polarized light optics. Birefringent streaks (*B*) appear as bright fibers running along the long axis of the major cell processes (× 260). Fig. 6. A spreading BHK-21 cell demonstrating a birefringent streak (*B*) arising from a juxtanuclear birefringent sphere (*S*). Polarized light optics (× 560).

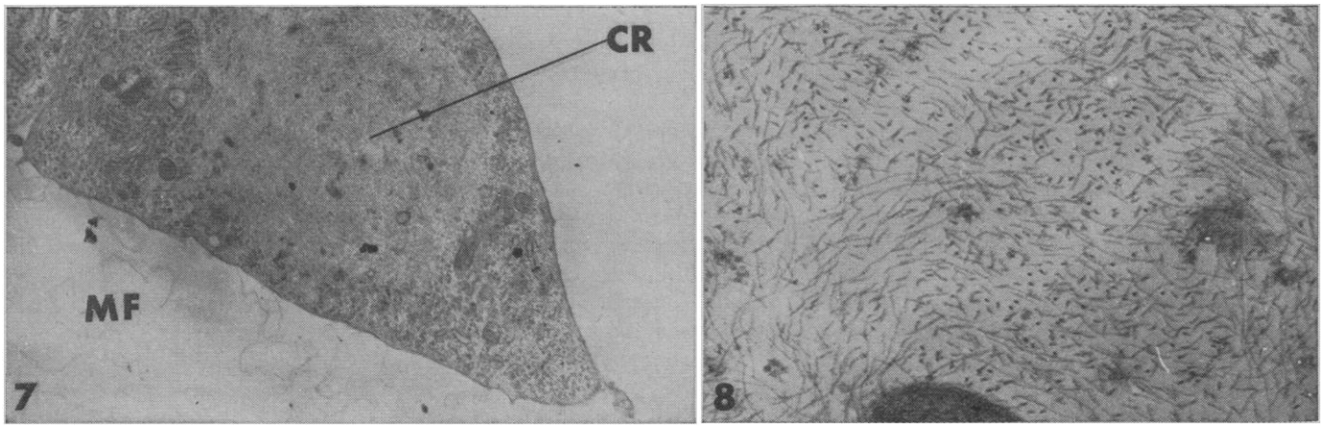

Fig. 7. An electron micrograph (low-power magnification) of a BHK-21 cell placed on a Millipore filter (*MF*) for 90 minutes at 37°C. The large circular clear region (*CR*) excludes most of the larger cell organelles (× 9,750). Fig. 8. An electron micrograph (higher magnification) of a portion of the clear region shown in Fig. 7. This demonstrates the high concentration of filaments, 100 to 125 Å in diameter, in the clear region (× 41,000).

electron microscope, a large circular region adjacent to the nucleus, and excluding most large cytoplasmic organelles, can be seen in many of the cells (Fig. 7). This region contains filaments 100 to 125 Å in diameter (Fig. 8), which in most cases seem to be arranged circumferentially. Linear arrays of these filaments can also be found leading into a ball of circumferentially oriented filaments. Microtubules and microfilaments are never observed within the circular region; however, they can be seen elsewhere within the cells.

The birefringent sphere is considered to be a cell organelle because (i) it is found in the same juxtanuclear location in almost all partially spread cells observed during the course of this study, (ii) it has uniform structural organizaiton, (iii) it may be related to the physiological phenomenon of spreading and major cell process formation in BHK-21 cells, (iv) it can be found in normally growing populations of cells, and finally, (v) under certain experimental conditions (that is, cells maintained at room temperature), it is present in all cells in a population. The birefringent sphere seems consistently to arise adjacent to the nucleus. In the electron microscope a ball of essentially circumferentially oriented filaments is also observed adjacent to the nucleus in partially spread cells. This is the type of ultrastructure we would expect to find making up this type of birefringent sphere (*5*).

During cell spreading, filaments are pictured as being "reeled out" from the ball of filaments (birefringent sphere) as a major cell process is formed. On the other hand, filaments oriented longitudinally along the long axis of a fully formed major cell process may be "reeled in" to form a juxtanuclear ball of filaments during the withdrawal of a major cell process. The latter phenomenon would occur in normally growing populations during the rounding up of a cell for division or the respreading of daughter cells following division.

In a previous study, the filaments, 100 to 125 Å in diameter, were shown to be tubular (*1*) and are thus very similar in structure to the "small microtubules" found in other cultured cells (*6*) and to the neurofilaments found in nerve axons and dendrites (*7*).

Similar juxtanuclear zones have been observed in chick embryonic cells with the phase contrast microscope. These zones have been termed "slate colored bodies" (*8*). We must disagree with the statement that "slate colored bodies" appear only in embryonic muscle cells (*8*), as similar bodies appear as the clear birefringent sphere region in the nonmuscular BHK-21 cells observed in this study.

In summary, our evidence indicates that the birefringent sphere is apparently involved in birefringent streak formation (*1*). We propose that it may be at least in part responsible for the formation of the major cell processes of BHK-21 and other similar cell types, which occurs during the spreading of a cell on a glass substrate. As extension and contraction of processes similar to the major cell processes of BHK-21 cells are thought to be involved in translatory movements of cells (*9*), the birefringent sphere and streak may also be directly involved in this type of cell movement. In addition, the correlation of dynamic birefringent structures seen in living cells with structures seen in electron microscope preparations offers a unique opportunity for "bridging the gap" between the light and electron microscope. Similar correlations have been made in the case of the mitotic apparatus (*10*), the shelled amoeba *Difflugia* (*11*), and the fibers of cultured epithelial cells observed with the phase contrast microscope (*12*).

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30 April 1970