purine generally demonstrated little or no tubercu'in skin reactivity on day 21 after injection of the mycobacteria (Table 2). In some of the treated animals skin test reactivity did not develop for as long as 6 weeks after the mycobacteria injection. However, lymphocytes obtained on day 21 during this period of depressed skin test reactivity showed a similar pattern of proliferative responses to tuberculin when compared to cells of animals not receiving 6-mercaptopurine (Table 2).

Some of the animals treated with 6-mercaptopurine developed moderate lymphopenia and marked leukopenia during the study. However, there was no good correlation (correlation coefficient, 0.16) between the degree of depressed skin reactivity and the extent of lymphopenia (Table 2). In addition, the cessation of 6-mercaptopurine treatment was followed by return of lymphocyte counts to normal levels prior to the appearance of tuberculin skin reactivity.

These studies suggest that administration of 6-mercaptopurine did not lead to a qualitative alteration in the proliferative response of guinea pig blood lymphocytes to tuberculin despite concomitant suppression of the development of tuberculin skin reactivity. If the tuberculin-induced lymphocyte proliferation indicates the response of cells which have become "immunologically committed" to this antigen (*7*), several theories can be postulated for the depressed skin reactivity in face of in vitro evidence of immune capability. First, it should be emphasized that the lymphocyte responses compared here occurred in aliquots of 1.0×10^6 lymphocytes each, from either 6-mercaptopurine–treated or untreated animals. Since in vivo lymphopenia of varying degrees was common in the group treated with 6-mercaptopurine, it is conceivable that this substance may suppress the formation in vivo of all lymphoid cells including the subpopulation of cells which would become immunologically committed to tuberculin. Therefore there would be fewer such lymphocytes to recruit into an immune response. Additionally, 6-mercaptopurine therapy might somehow alter in vivo conditions for antigen-responsive lymphocyte activity in a way not reflected by the in vitro assay.

Evidence from other studies suggests possible alternative explanations for the suppressive effect of 6-mercaptopurine on delayed skin test reactivity. The large majority of mononuclear inflammatory cells appearing at the delayed hypersensitivity skin test site appear to be of bone marrow origin and are not specifically sensitized to the antigen in question (*8*). In several studies (*9*), 6-mercaptopurine has been found to exert a profound nonspecific anti-inflammatory effect associated with suppression of the appearance of these mononuclear cells of bone marrow origin in the inflammatory reaction. Therefore, it is possible that the animal with depressed tuberculin skin test reactivity due to 6-mercaptopurine may have sufficient lymphocytes which have become immunologically committed to tuberculin but does not have sufficient numbers of the relatively short-lived cells (*10*) which "nonspecifically" may migrate to and/or be retained at the skin test site in response to some stimulus from a small number of sensitized cells there.

Other experimental observations suggest that a significant portion of the long-lived lymphocyte population persists in the face of 6-mercatopurine administration to become immunologically committed to tuberculin in delayed hypersensitivity response. Delayed skin test reactivity is suppressed only transiently after discontinuation of in vivo administration of 6-mercaptopurine (*1*). In addition, 6-mercaptopurine given for a period prior to but not after the sensitization injection does not lead to suppressed skin test reactivity (*2*).

Since administration of 6-mercaptopurine appears to lead to no qualitative alteration in the response of the sensitized lymphocyte to tuberculin (by at least one criterion), further studies utilizing cell transfer and other in vivo and in vitro techniques may clarify the mechanisms involved.

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Pituitary Serotonin Content: Effects of Melatonin or Deprivation of Water

Abstract. *Relatively high concentrations of serotonin are found in the three regions of the rat pituitary gland. Administration of melatonin causes a selective increase in the serotonin concentration of the pars intermedia; deprivation of water for 5 days causes a selective decrease in the serotonin concentrations of the neural lobe and pars distalis.*

Relatively high concentrations of serotonin have been identified in the pituitary glands of several mammalian species (*1, 2*), especially in the pars intermedia and pars nervosa (*2*). It is not known whether the amine in these regions is localized within pituitary cells, in the terminals of neurons whose cell bodies lie outside the pituitary, or in both. The possible functions of pituitary serotonin also await elucidation. Several observations have led us to examine the effects of administered melatonin on pituitary serotonin concentrations.

These are (i) the decline in the content of melanocyte-stimulating hormone (MSH) in rat pituitaries which occurs soon after animals receive injections of melatonin (*3*), (ii) the changes in brain serotonin contents which follow administration of melatonin (*4*), and (iii) the tendency of circulating ^{3}H-melatonin to be taken up and retained within pituitary tissue (*5*). We have found that melatonin administration causes a significant increase in the serotonin concentration of the pars intermedia without influencing concentrations of serotonin in the other re-

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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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