

PART V. Possible Consequences

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13 The Emerging Role of the Pineal Gland and Melatonin in Oncogenesis

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One hallmark of the pineal gland and melatonin function is the pineal gland's ability to inhibit reproductive organ growth in prepubertal animals as well as induce a marked regression in reproductive organ size in adults. This is typically accomplished either by placing animals in short photoperiods or by injecting long-photoperiod-exposed animals with melatonin

in the late afternoon on a daily basis for several weeks (Reiter, 1980). Therefore, it is not surprising that investigators have begun to apply these same principles to examine the question of whether the pineal gland, and more specifically its primary hormone melatonin, influences the growth of malignant neoplasms (Lapin, 1976; Tapp, 1982; Blask, 1984). It is interesting to note that the initial work on pineal effects on oncogenesis (Georgiou, 1929) actually predates the seminal work of Reiter and colleagues in the 1960s and 1970s, which demonstrated that the pineal gland (under short-day conditions) and injections of melatonin inhibit the growth of (or cause the regression of) the reproductive organs in the rat and the Syrian hamster.

The basic and clinical aspects of the pineal gland and cancer have been exhaustively reviewed elsewhere by the author (Blask, 1984; Blask and Hill, 1988); therefore, in this chapter the focus is on more recent work that strongly implicates an inhibitory role for the pineal gland and melatonin in the processes of oncogenesis. Of all neoplasms studied to date, the experimental results obtained on breast cancer and melanoma are perhaps the most compelling with respect to pineal and melatonin influences. Therefore, these two tumor types, both of which are endocrine responsive, are featured as model systems currently holding the most promise for elucidating an important role for the pineal gland and melatonin in oncogenesis.

MELANOMA

In Vivo Studies

Reports by Das Gupta and co-workers (Das Gupta and Terz, 1967; Stanberry et al., 1983) indicated that the removal of the pineal gland from Syrian hamsters, before their inoculation with the transplantable and hormone-responsive melanotic melanoma No. 1 (MM1), resulted in a rather dramatic increase in the growth and metastases of the tumor in a 5-week period of observation during which the animals were maintained on a long photoperiod. Using a somewhat different system, Aubert and colleagues (1970) also found that pinealectomy promoted the growth of melanomas induced in long-photoperiod-exposed Syrian hamsters by the chemical carcinogen 7,12-dimethyl-benzanthracene (DMBA). However, when performed on hamsters (with MM1 melanomas) maintained in short photoperiods, pinealectomy further exacerbates the tumor growth-retarding effects of short days (Stanberry et al., 1983). This seemingly paradoxical finding was explained on the basis that the photoperiod in which hamsters are kept governs the melanoma growth response to pinealectomy.

Das Gupta and associates also reported that daily melatonin injections had no effect on melanoma growth in hamsters unless they were pinealectomized. Under these circumstances, melatonin administration inhibited

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the pinealectomy. Domeiri and Das Gupta (1984) in a recent investigation found that late-afternoon implants stimulated tumor growth, maintained in a long photoperiod. Melatonin implants of the same type of photoperiod administration are effective in the regression of melanoma to the extent of promoting or turning on the neuroendocrine system to ascertain the role of melatonin.

In addition to the work of Reiter, melatonin was reported to inhibit the growth of B16 melanoma (Blask and Kudo, 1985). In a study by Walkley and Kudo (1985), melatonin inhibited the growth of gonadal and/or adrenal melanocyte-stimulating hormone directly (Narita, 1985).

In Vitro Studies

A study by Walkley and Kudo (1985) showed that the effects of melatonin on a cloned melanoma cell line, which has previously developed in vitro, were studied. The molecule was suggested that melatonin actually stimulates the growth of Bartsch et al. (1981) caused about 60% inhibition (ing) human melanoma cells. These concentrations led to 20% stimulation of late-passaged cells. 90% inhibition of concentrations in vitro.

In another study (1981) demonstrated that concentrations of melatonin derived from bioassay of melanoma. For e

the pinealectomy-induced promotion of tumor growth and metastases (El-Domeiri and Das Gupta, 1973, 1976; Ghosh et al., 1973). However, a more recent investigation by this same group revealed that daily, morning, or late-afternoon injections of melatonin or subcutaneous silastic melatonin implants stimulated melanoma growth in pineal-intact hamsters maintained in a long photoperiod. Conversely, in short-day-exposed hamsters, melatonin implants had a tumor-inhibiting effect, suggesting that both the type of photoperiodic regimen employed and the method of melatonin administration are important factors affecting the responsiveness of this type of melanoma to melatonin (Stanberry et al., 1983). Whether the tumor-promoting or tumor-inhibiting effects of melatonin result from an influence on the neuroendocrine axis or directly on the tumor tissue itself is difficult to ascertain from these results.

In addition to melatonin injections or implants, oral administration of melatonin was recently found to be highly effective in slowing the growth of B16 melanoma cells transplanted into BALB/c athymic mice (Narita and Kudo, 1985). Narita and co-workers postulated that oral melatonin inhibited the growth of these hormone-responsive melanomas by inhibiting gonadal and/or adrenal function, by decreasing the pituitary secretion of melanocyte-stimulating hormone (MSH), or by blocking cell proliferation directly (Narita, 1988).

In Vitro Studies

A study by Walker et al. (1978) demonstrated that millimolar concentrations of melatonin caused an approximately 25% inhibition of the growth of a cloned melanoma cell line (B7) derived from a tumor that had spontaneously developed in a male golden hamster. However, a biphasic effect of this molecule was suggested by the fact that micromolar concentrations of melatonin actually stimulated cell proliferation by about 37%. In a similar study, Bartsch et al. (1986) found that micromolar concentrations of melatonin caused about 60% inhibition of the growth of early-passaged (slower growing) human melanoma cells, whereas in late-passaged cells (faster growing) these concentrations were ineffective. Millimolar melatonin concentrations led to 20% stimulation of cell proliferation in the early-passaged cells; in late-passaged cells, these extremely high melatonin concentrations induced 90% inhibition of cell growth by the end of a 7-day culture period; lower concentrations in the physiological range were without effect.

In another study using human melanoma cells, Meyskens and Salmon (1981) demonstrated varied responses of these cells to a wide range of concentrations of melatonin; the cells were clonogenic melanoma cells derived from biopsy material taken from metastatic nodules of malignant melanoma. For example, in slightly more than half the biopsy specimens

evaluated in the clonogenic assay, total cloning efficiency decreased as the melatonin concentration increased from 10^{-15} M to 10^{-5} M, with no accompanying change in either colony type or mean colony diameter. However, in one particular patient biopsy, a physiological concentration of melatonin (10^{-9} M) caused a fourfold increase in cloning efficiency. In a clone from another patient, 10^{-11} M melatonin induced a dramatic ninefold increase in the number of dark, small-cell colonies over the total colonies present in the control plates, which consisted predominantly of the light, large-cell variant; however, the number of colonies decreased as the concentration of melatonin increased. The remaining 27% of biopsy specimens exhibited no response to melatonin in the clonogenic assay.

BREAST CANCER

In Vivo Studies

Experimental breast cancer, typically induced in female rats with the chemical carcinogen 9,10-dimethylbenz(a)anthracene (DMBA), represents the most extensively studied malignant neoplasm with respect to the effects of pinealectomy, photoperiod, and/or melatonin administration (Blask, 1984). The development and growth of DMBA-induced breast cancers are primarily responsive to the direct mitogenic effects of prolactin (PRL) secreted from the pituitary gland and secondarily to estrogen (E) through its ability to stimulate PRL release (Welsch, 1985).

Effects of Pinealectomy

The initial studies on the effects of pinealectomy on DMBA-induced mammary cancer growth were essentially negative. For example, Lapin (1976) found that neonatal pinealectomy before DMBA administration had no effect on mammary oncogenesis as compared with control animals unless the rats were also treated with reserpine (a catecholamine-depleting drug) for 40 consecutive days after an initial intragastric feeding of DMBA. Three years later, Aubert and colleagues (1980) also reported that pinealectomy in adult female rats before DMBA administration had no effect on subsequent tumor development. The following year, Tamarkin et al. (1981) were the first to report that pinealectomy before intragastric administration of DMBA promoted tumor growth when a low dose of the carcinogen was given; at a high dose, however, no effect of pinealectomy was observed. The mechanism by which pinealectomy promoted tumor development at low doses of DMBA could not be ascertained from this investigation. Subsequently, Kothari et al. (1984) found that pinealectomy before DMBA administration had no effect on mammary oncogenesis in short-photoperiod-exposed rats (10:14 light:dark cycle) when compared with short-photoperiod-exposed, intact animals. A surprising result by Chang et al.

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(1985) was that the denervation of the pineal gland by bilateral superior cervical ganglionectomy before DMBA administration, which creates a functional pinealectomy, actually inhibited tumor growth and development.

Taking these discrepancies into account, we reexamined the effects of pinealectomy before tumor initiation in the *N*-methylnitrosourea (NMU) model of breast cancer (Pelletier et al., 1987; Blask et al., 1988). The advantages of using this model system in lieu of the DMBA model is that NMU-induced mammary cancers are more akin to human breast cancers in terms of their growth characteristics. Furthermore, NMU tumors, like human breast cancers, respond to the direct mitogenic effects of E in addition to PRL (Welsch, 1985). In our study, pinealectomy before tumor initiation with NMU led to a modest enhancement of mammary cancer development and growth (Fig. 1). This perhaps occurred via a pinealectomy-induced increase in PRL levels and tumor E receptors (Pelletier et al., 1987; Blask et al., 1988; Blask and Grosso, unpublished results). In most of the studies cited, the hypothesis has been promulgated that pinealectomy promotes breast cancer growth by eliminating the daily, endogenous nocturnal melatonin signal (Fig. 1) (Blask et al., 1988).

Effects of Photoperiodic Manipulations

Another approach by which the effects of the pineal gland on neoplastic growth have been assessed involves the manipulation of the photoperiod in such a way that pineal activity is either diminished or enhanced. A number of investigators, with the notable exception of Aubert and associates (1980), have shown that prolonged exposure of rats with DMBA mammary tumors to constant light stimulates breast cancer development and growth as compared with animals maintained on an alternating, daily light:dark cycle (Hamilton, 1969; Kothari et al., 1982, 1984; Shah et al., 1984). However, the problem in interpreting the data of Kothari's group (Kothari et al., 1982, 1984; Shah et al., 1984) is that oncogenesis in constant light-exposed rats was compared with that in rats kept on short photoperiod (10:14 light:dark); no long-photoperiod control group (14:10 light:dark) was included. Thus, it is difficult to assess whether tumor growth was actually greater under constant light or merely less in a short photoperiod; the inclusion of a long-photoperiod control group could have resolved this issue.

Constant light exposure, because of suppression of melatonin production, has been regarded as being tantamount to a "physiological pinealectomy" (Reiter, 1980). Not only does constant light eliminate the melatonin signal but it also stimulates the secretion of PRL (Vatican et al., 1980), which may in turn be part of the mechanism by which tumorigenesis is enhanced, particularly since DMBA breast cancers are PRL dependent (Welsch, 1985).

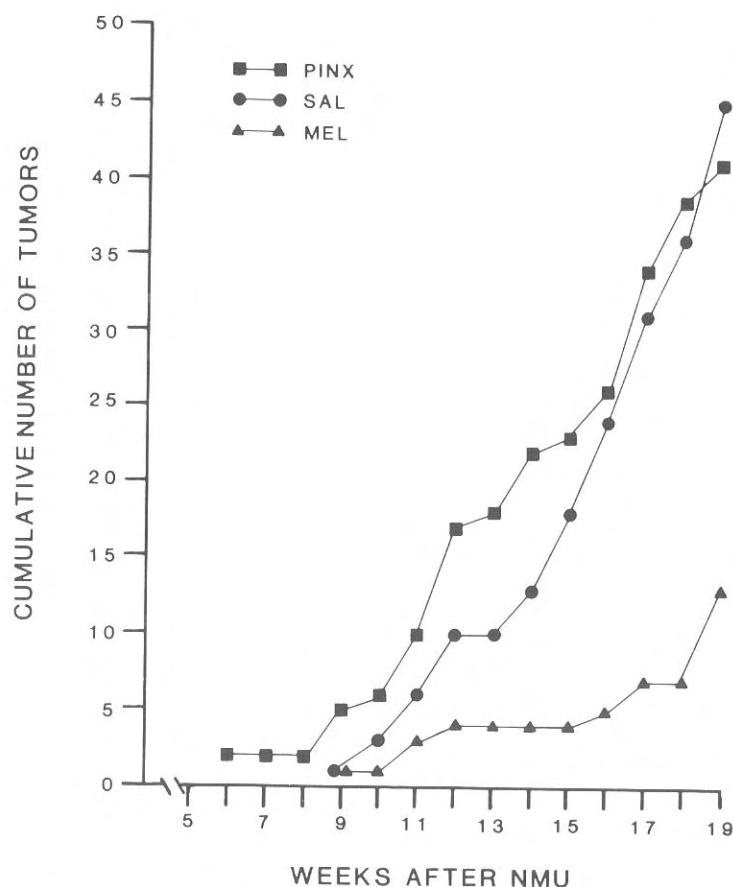


Fig. 1. Effect of pinealectomy (PINX), daily afternoon melatonin injections (MEL) (500 μ g/day), or saline (SAL) injections on *N*-methylnitrosourea- (NMU-) induced mammary carcinogenesis in female rats. Treatments were initiated before tumor induction with NMU and continued for 19 weeks.

As most strains of albino rat are less photoperiodically sensitive than some other species such as the hamster, light deprivation does not inhibit the neuroendocrine-reproductive axis unless it is combined with a potentiating factor such as olfactory bulbectomy or underfeeding (Reiter, 1980). In fact, both these potentiating factors make the rat more sensitive to the antigonadotrophic effects of daily, afternoon melatonin injection (Blask and Nodelman, 1979; Reiter, 1980; Blask et al., 1981), and ostensibly to the endogenous melatonin signal emitted from the pineal gland as well. Therefore, light deprivation (blinding) in combination with olfactory bulbectomy leads to a decrease in tumor incidence and growth (Chang et al., 1985;

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Sanchez Barcelo et al., 1988) as well as an increase in tumor regression (Blask, 1984; Chang et al., 1985). Not only does pinealectomy prevent the oncostatic effects of blinding/olfactory bulbectomy, but tumor growth is even further promoted beyond the level observed in the intact controls (Blask, 1984; Sanchez Barcelo et al., 1988). Interestingly, the combination of blinding with underfeeding is even a more potent inhibitor of breast cancer growth in rats with DMBA-induced tumors in that no tumors develop during a 20-week observation period. In this model system, pinealectomy totally cancels the antitumorigenic effect of blinding/underfeeding (Blask, 1984; Sanchez Barcelo et al., 1988). Interestingly, blinding alone decreases tumor incidence with no change in tumor number. Pinealectomy not only prevents this effect but causes a substantial increase in tumor number over that seen in both the controls and blinded animals (Blask et al., 1988).

Although the exact mechanism(s) by which light deprivation in combination with either olfactory bulbectomy or underfeeding inhibits DMBA tumor growth are unknown, these may involve a pineal gland and perhaps melatonin-induced neuroendocrine suppression of PRL (Leadem and Blask, 1981; Blask, 1984) and E secretion (Vaughan, 1984; Sanchez Barcelo et al., 1988) as well as a decrease in E-receptor number in the tumors themselves (Sanchez Barcelo et al., 1988).

Effects of Melatonin

A study by Hamilton (1969) was the first to document the effects of melatonin on DMBA-induced breast carcinogenesis. Although melatonin had no effect on the percentage of rats with palpable mammary tumors, 80% of tumors in the melatonin group were adenocarcinomas as compared with only 30% in the vehicle-treated controls. These results indicated that melatonin injections begun before tumor initiation actually enhanced mammary oncogenesis. Interestingly, virtually every subsequent study on the influence of melatonin on breast cancer growth has been at odds with the Hamilton study. Regardless of whether animals are maintained in diurnal or constant lighting, daily afternoon injections of melatonin begun on the day of or before tumor initiation with DMBA and continued thereafter decrease the incidence of rats with palpable mammary tumors as compared with vehicle-injected controls (Aubert et al. 1980; Tamarkin et al., 1981; Shah et al., 1984). While Aubert et al. (1980) reported that melatonin injections were still effective in pinealectomized rats, two other groups (Tamarkin et al., 1981; Shah et al., 1984) failed to observe an oncostatic effect of melatonin in animals in which the pineal gland had been removed, suggesting that the presence of the endogenous melatonin signal may be required for the antitumorigenic effect of exogenous melatonin to be expressed. However, if this is indeed the case, it is puzzling that melatonin apparently retains its effectiveness in animals maintained in constant

lighting (Shah et al., 1984), a photoperiodic scheme that functionally pinealectomizes the animal and extinguishes the melatonin signal (Reiter, 1980). As alluded to earlier in this chapter, melatonin is also effective in inhibiting melanoma growth in pinealectomized hamsters.

As was the case for transplanted B16 mouse melanoma cells (Narita and Kudo, 1985), oral melatonin, supplied in the drinking water before DMBA administration and continued throughout the period of carcinogenesis, effectively suppressed tumor incidence in rats maintained in a short photoperiod or in constant light (Kothari, 1987). Orally administered melatonin (400–500 µg/day) is also effective in suppressing tumorigenesis in rats with NMU-induced tumors (Blask, unpublished results).

It has been proposed by Kothari's group (Shah et al., 1984; Kothari, 1988) that melatonin administration before carcinogen treatment decreases the susceptibility of the mammary epithelium to neoplastic transformation via neuroendocrine mechanisms, leading to a decrease in the secretion of PRL (particularly the mitogenic 16-kdalton fragment of PRL) and E and/or by compromising the ability of the parenchymal cells to metabolize DMBA to its putative reactive intermediates. We have never observed a clear-cut melatonin-induced suppression in either circulating PRL or E (Blask et al., 1986; Pelletier et al., 1987; Blask, unpublished results), and thus we have some reservations about the neuroendocrine hypothesis (Blask, 1984; Blask and Leadem, 1987). Furthermore, if the increased susceptibility hypothesis is true, then restricting melatonin treatment to the tumor initiation period alone (before and during the period of carcinogen administration) should be sufficient to inhibit mammary oncogenesis. We have tested this hypothesis in both the DMBA and NMU breast cancer models, and have found that daily afternoon injections of melatonin, restricted to the tumor initiation phase only, are completely ineffective in suppressing tumor development and growth (Blask, unpublished results). However, if melatonin injections encompass both the initiation and promotional phases of oncogenesis, a potent oncostatic effect of melatonin is observed (Fig. 1) (Pelletier et al., 1987; Blask et al., 1988).

To test the postulate that melatonin inhibits oncogenic processes associated primarily with tumor promotion, we began treating female rats with daily afternoon melatonin injections 3 weeks after the administration of DMBA but before the appearance of overt, palpable tumors in animals that were either fed ad libitum or subjected to the potentiating factor of moderate underfeeding; the injections were continued throughout the remainder of the experimental period (Blask et al., 1986). Although melatonin treatment restricted to the promotional phase suppressed tumorigenesis in both fed and underfed rats, it was clearly much more effective in the underfed animals, suggesting an increased sensitivity of underfed animals to melatonin (Blask et al., 1981). Preliminary results in rats with NMU-induced breast cancers also indicate that melatonin injections

restricted to the tumor initiation phase only, are completely ineffective in suppressing tumor development and growth (Blask, unpublished results). This hypothesis is supported by the demonstration of regression of mammary tumors in rats after pinealectomy (Moreover, pinealectomy of transplanted tumors (Blask, 1973; Karmali, 1973).

In Vitro Studies

To further explore this hypothesis, we studied the effect of melatonin on breast cancer cells in culture. We have found that melatonin has a physiological effect on the growth of these cells (Vaughan, 1986). After 7 days of treatment, melatonin has a reversible, stimulatory effect on the growth of melatonin-concentrated cells (indoleamine). This effect was studied by microscopy of cells that correlate with the degree of exposure to melatonin (microvilli) (Fig. 1). Disruption of the endoplasmic reticulum, and the characteristic changes observed (1986a; Hill et al., 1986).

In addition to the effects of melatonin on precursors, melatonin also affects serotonin, *N*-acetylserotonin, and 6-hydroxytryptophan. Melatonin and 6-hydroxytryptophan are inhibiting cell growth in the in vitro and in vivo. Melatonin's activity is specifically related to melatonin's metabolism (Young et al., 1986). Its activity is caused by the effects of melatonin on the products. Moreover, melatonin (Danforth et al., 1986) that 6-hydroxytryptophan

restricted to the promotional phase inhibit mammary oncogenesis (Blask, unpublished observations). Further support for the antipromotion hypothesis is also provided by the study of Aubert et al. (1980), who demonstrated that melatonin injections were effective in causing the regression of established, palpable DMBA-induced mammary tumors. Moreover, previous reports that melatonin injections inhibit the growth of transplantable breast cancers in both mice and rats (Anisimov et al., 1973; Karmali et al., 1978) lend further credence to this postulate.

In Vitro Studies

To further examine the antipromotion hypothesis using a more direct approach, we studied the effects of melatonin on the ability of the human breast cancer cell line MCF-7 to proliferate in culture. For example, we have found that concentrations of melatonin corresponding to the physiological range (10^{-9} M to 10^{-11} M) present in the blood of humans (Vaughan, 1984) cause from 60% to 80% inhibition of cell proliferation after 7 days of continuous incubation; higher or lower concentrations of melatonin had no effect on cell growth. Melatonin's inhibitory effect is reversible, since the logarithmic growth of MCF-7 cells was restored after melatonin-containing medium was replaced with fresh medium lacking this indoleamine. Additionally, both scanning and transmission electron microscopy of these cells reveals several striking morphological changes that correlate with melatonin's inhibition of cell growth. After only 4 days of exposure to melatonin, MCF-7 cells exhibit reduced numbers of surface microvilli (Fig. 2), nuclear swelling, cytoplasmic and ribosomal shedding, disruption of mitochondrial cristae, vesiculation of the smooth endoplasmic reticulum, and increased numbers of autophagic vacuoles (Fig. 3), all characteristics of sublethal but reversible cellular injury (Blask and Hill, 1986a; Hill and Blask, 1988).

In addition to melatonin, we have tested the ability of a number of its precursors, metabolites, or analogues to inhibit MCF-7 cell growth. Neither serotonin, *N*-acetylserotonin, 5-methoxytryptophol, 5-methoxytryptamine, nor 6-hydroxymelatonin have any effect on cell proliferation; however, the melatonin analogue, 6-chloromelatonin, is as effective as melatonin itself in inhibiting cell growth (Blask and Hill, 1986a,b). Therefore, it appears that the in vitro antiproliferative effect of melatonin on MCF-7 cells is a relatively specific characteristic of this molecule. Furthermore, the failure of melatonin's major metabolites, 6-hydroxymelatonin and *N*-acetylserotonin (Young et al., 1985), to inhibit cell proliferation suggests that the oncostatic activity is caused by melatonin itself rather than one of its metabolic products. Moreover, MCF-7 cells apparently do not metabolize melatonin in vitro (Danforth et al., 1983). By contrast, Leone and colleagues (1988) reported that 6-hydroxymelatonin was markedly more potent than melatonin in

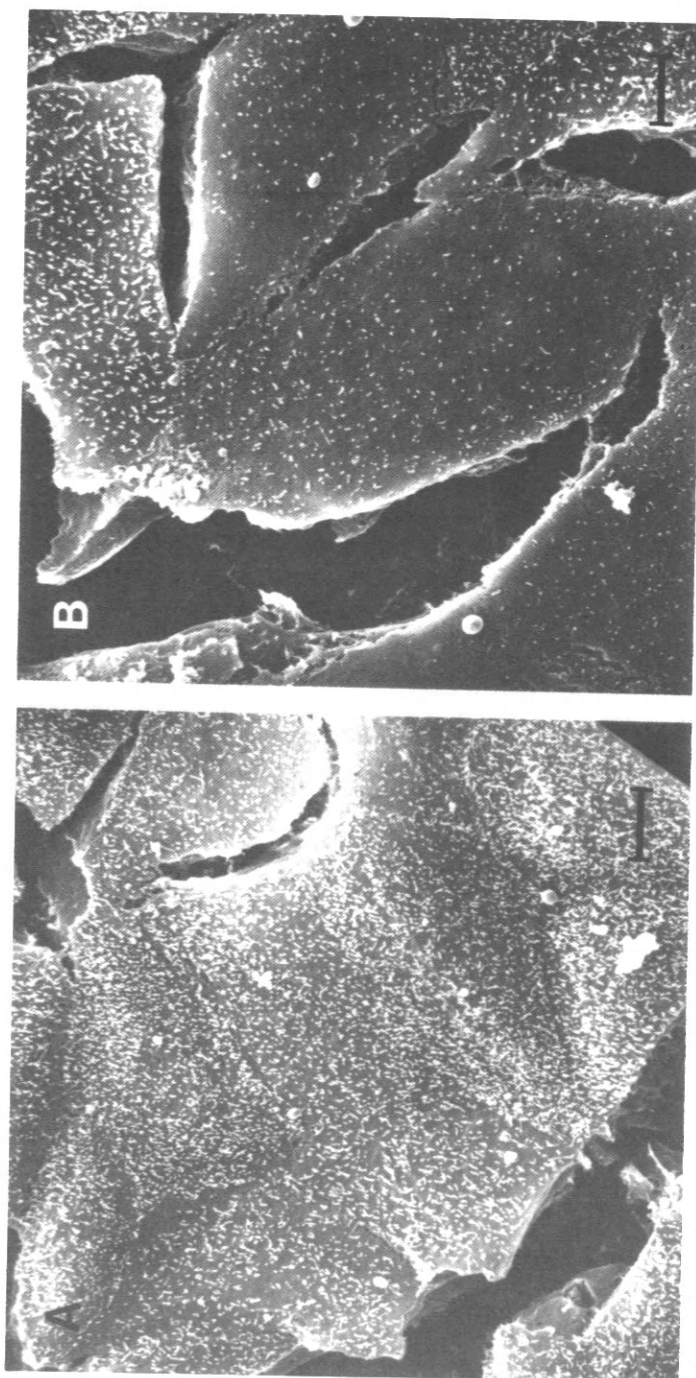


Fig. 2. Scanning electron micrograph of surface morphology of (A) control MCF-7 human breast cancer cells cultured for 4 days and (B) MCF-7 cells exposed to 10^{-9} M melatonin for 4 days. Bar = 1 μ m. [Reprinted with permission from Hill, S. M., Blask, D. E., 1988. Effects of the pineal hormone melatonin on the proliferation and morphological characteristics of human breast cancer cells (MCF-7) in culture. *Cancer Res.* 48:6121-6126.]



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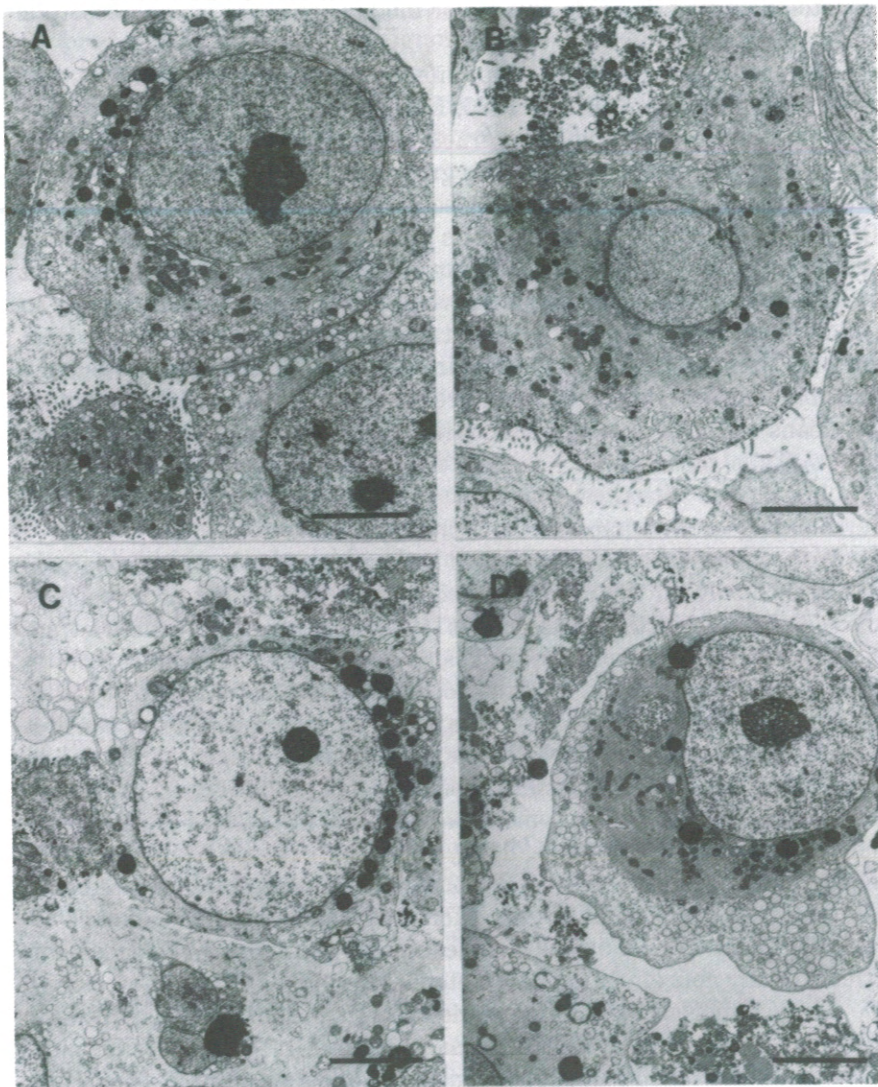


Fig. 3. Transmission electron micrograph of control (A, B) MCF-7 cells or cells treated with 10^{-9} M melatonin (C, D) in culture for 4 days. Bar=5 μ m.

[Reprinted with permission from Hill, S. M., Blask, D. E. 1988. Effects of the pineal hormone melatonin on the proliferation and morphological characteristics of human breast cancer cells (MCF-7) in culture. *Cancer Res.* 48:6121-6126.]

inhibiting the proliferation of a human ovarian carcinoma cell line than melatonin, suggesting that this major metabolite is the active oncostatic form of melatonin for this cell line.

The fact that melatonin's antiproliferative effect is a serum-dependent phenomenon (Hill and Blask, 1986; Blask and Hill, 1986a) initially suggested to us that it might be interacting with some component(s) in the serum such as a hormone(s) and/or a growth factor(s). Because MCF-7 cells are responsive to the mitogenic effects of both E and PRL (Shafie and Brooks, 1977; Osborne, 1985; Biswas and Vonderhaar, 1987) and contain receptors for both hormones, it occurred to us that these may represent at least two constituents of serum which may play an important role in melatonin's ability to directly inhibit breast cancer cell growth. Indeed, not only does melatonin inhibit E-stimulated MCF-7 cell growth, but the loss of its inhibitory effect in serum-free medium is restored by nearly 40% by means of the addition of PRL (Hill and Blask, 1986; Blask and Hill, 1986a). Thus, melatonin's oncostatic effect in vitro may involve an important interaction with these mitogenic hormones in the microenvironment surrounding breast cancer cells.

We became particularly interested in the interaction between melatonin and E, not only on the basis of our own growth data but as a result of the report of Danforth and associates (1983) that melatonin causes a transient increase in the number of nuclear and cytoplasmic E receptors in MCF-7 cells. We decided to test the postulate that melatonin's ability to inhibit breast cancer growth may relate, in part, to the degree of responsiveness of breast cancer cells to E; that is, melatonin's oncostatic effect should decrease as the E responsiveness of breast cancer cells decreases. We confirmed this hypothesis by demonstrating that, in the less E-responsive human breast cancer cell line T47D, melatonin's antiproliferative effect in physiological concentrations was considerably reduced as compared with the more responsive MCF-7 cells. Moreover, the E-unresponsive and E-receptor-negative cell line BT20 was completely immune to the oncostatic effects of any concentration of melatonin (Hill, 1986; Blask et al., 1988).

Another approach to examining the melatonin-E interaction hypothesis involved studying the interaction of melatonin with the nonsteroidal anti-estrogen tamoxifen in both serum-containing and a serum-free, chemically defined medium. Tamoxifen, which binds to the E receptor with high affinity, is currently the antiestrogen of choice in the treatment of E-receptor-positive breast cancer (Osborne, 1985). Melatonin at 10^{-9} M not only was more effective than 10^{-9} M tamoxifen (the therapeutic level) in inhibiting the growth of MCF-7 cells in serum-containing medium, but its antiproliferative effect was completely blocked by tamoxifen when the two compounds were coincubated. Interestingly, in serum-free medium, melatonin completely blocks the ability of tamoxifen to inhibit E-stimulated cell growth (Hill, 1986; Blask et al., 1988). Additionally, we found (Hill, 1986;

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and Hill, 1986; Blask and Hill, 1986a) that melatonin's antiproliferative effect is a serum-dependent phenomenon. Because MCF-7 cells are responsive to the mitogenic effects of both E and PRL (Shafie and Brooks, 1977; Osborne, 1985; Biswas and Vonderhaar, 1987) and contain receptors for both hormones, it occurred to us that these may represent at least two constituents of serum which may play an important role in melatonin's ability to directly inhibit breast cancer cell growth. Indeed, not only does melatonin inhibit E-stimulated MCF-7 cell growth, but the loss of its inhibitory effect in serum-free medium is restored by nearly 40% by means of the addition of PRL (Hill and Blask, 1986; Blask and Hill, 1986a). Thus, melatonin's oncostatic effect in vitro may involve an important interaction with these mitogenic hormones in the microenvironment surrounding breast cancer cells.

CONCLUSIONS

It would be premature to conclude that the failure of pinealectomy to inhibit breast cancer growth is due to the removal of melatonin from the pineal gland. The pineal gland is a source of this indoleamine, but it is not the only source of this indoleamine. Other sources of this indoleamine (e.g., tryptophan, 5-HT, and melatonin) that also have antiproliferative effects may be involved.

The failure of pinealectomy to inhibit breast cancer growth when its antiproliferative effect is blocked suggests that its antiproliferative effect is mediated through a non-pineal source. Furthermore, the failure of melatonin to inhibit breast cancer growth suggests a direct antiproliferative effect of melatonin with the fact that melatonin is a mitogenic hormone. The failure of melatonin to inhibit breast cancer growth when its antiproliferative effect is blocked suggests that its antiproliferative effect is mediated through a non-pineal source.

Although the failure of pinealectomy to inhibit breast cancer growth has been emphasized, the pineal gland may have an important function (Aronow, 1986). It is important if not more important to study much more work on the pineal gland as the organismal functions of melatonin. Nevertheless, the failure of pinealectomy to inhibit growth of two clinical breast cancer cell lines and breast cancer growth suggests consideration of melatonin in the diagnosis, the treatment, and the prevention of breast cancer.

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Blask and Hill, 1988) that melatonin inhibits the release of an E-inducible cathepsin-like 52-kdalton glycoprotein, which is an autocrine growth factor produced by MCF-7 cells (Vignon and Rochefort, 1985). Although indirect, these results further support the melatonin-E interaction hypothesis and suggest that part of the mechanism(s) of melatonin's antipromotion effect may be an interaction directly at the level of the E receptor.

CONCLUSIONS

It would be premature to tacitly assume that the tumor-promoting effects of pinealectomy in experimental animals result from the elimination of melatonin from the circulation, particularly because there are alternate sources of this indole. Perhaps more importantly, pinealectomy is also likely to quench other nonmelatonin compounds (i.e., low molecular weight peptides) that also have potent oncostatic properties (Ebels et al., 1988).

The failure of melatonin to inhibit carcinogen-induced breast cancer growth when its administration is restricted to the initiation phase strongly suggests that its influence on oncogenic mechanisms during the promotional phase is more crucial for its antineoplastic properties to be manifest. Furthermore, the evidence for a neuroendocrine mechanism by which melatonin inhibits breast cancer growth is not as compelling as that for a direct antiproliferative effect on the cancer cells themselves. Coupled with the fact that melatonin can inhibit the action of tumor-promoting mitogenic hormones such as E, this evidence lends even more credence to the antipromotion hypothesis proposed here.

Although the interaction of melatonin with the endocrine system has been emphasized in this discussion of oncogenesis, its relationship to immune function (Angeli et al., 1988) may ultimately turn out to be as important if not more important with respect to its oncostatic role. Obviously, much more work is required to understand the cellular-molecular, as well as the organismal, mechanisms by which melatonin inhibits oncogenic processes. Nevertheless, the ability of melatonin to inhibit *in vivo* and *in vitro* growth of two clinically important malignant neoplasms, namely melanoma and breast cancer, is perhaps the best argument yet for the serious consideration of melatonin as a potentially useful biological agent in the diagnosis, the treatment, and possibly the prevention of certain forms of cancer.

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