quantum yield (*9*) for the reaction and the number of photons available to and absorbed by the starting isomer. The number of photons absorbed is, in turn, determined by the overlap of the source's spectral irradiance with the absorption cross section of the starting isomer. Hence, in this reaction, those isomers showing good absorption in spectral regions of high intensity will be preferentially converted to other isomers. Solar spectral irradiance (Fig. 1B) shows an increase of about 3.5 orders of magnitude, from 290 to 320 nm. The UV absorption spectra for 7-DHC, $preD_3$, $lumisterol_3$, and tachysterol (Fig. 1C) demonstrate that 7-DHC and $lumisterol_3$ (in both protic and aprotic solvents) show negligible absorption above 315 nm, whereas both $preD_3$ and $tachysterol_3$ absorb radiation to at least 325 and 335 nm, respectively. Thus, the extinction coefficients for $preD_3$ and $tachysterol_3$ at 320 nm (for example) are relatively high (480 and 1700, respectively) compared with 0.1 or less for lumisterol and 7-DHC. The fact that the solar irradiance at 320 nm is 3.5 orders of magnitude greater than at 290 nm makes these absorption characteristics of $preD_3$ and $tachysterol_3$ significant. Even though the quantum yield for $tachysterol_3$ to $preD_3$ is low (Fig. 1), tachysterol, which has the highest extinction coefficient above 315 nm, is the most photoreactive isomer when exposed to sunlight, and the reaction is therefore driven from $tachysterol_3$ to $preD_3$ to $lumisterol_3$, which accumulates because it is the least photoreactive isomer. To test this hypothesis, we exposed tachysterol and $preD_3$ dissolved in an organic solvent to radiation between 315 and 340 nm and observed an accumulation of $lumisterol_3$.

During the past century, scientists have begun to appreciate several biologic effects of sunlight on the human body (*10*). Stratospheric ozone, a major component in the atmospheric path of sunlight, determines the 290-nm short-wavelength cutoff that is characteristic for the terrestrial solar spectrum (*11*) (Fig. 1B). In addition, the spectral characteristics of natural sunlight that penetrates to the earth's surface vary with altitude, latitude, time of the day, and season of the year. Little is known about whether the spectral properties of sunlight promote unique biologic actions in humans. Our observation that the spectral power distribution of sunlight has a dramatic effect on the cutaneous photosynthesis of $preD_3$ and its photoisomers, however, suggests that the spectral character of the radiation in the surrounding natural and artificial environments may be important for regulating subtle radiation-induced physiological and biochemical responses in humans. Our observations may also be helpful in the design of radiation sources that could enhance the production in vivo of $preD_3$ in human skin and the commercial production in vitro of previtamin D and previtamin D metabolites.

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Decreased Nocturnal Plasma Melatonin Peak in Patients with Estrogen Receptor Positive Breast Cancer

Abstract. *Plasma melatonin concentrations were determined over a period of 24 hours in 20 women with clinical stage I or II breast cancer. In ten of the patients, whose tumors were estrogen receptor positive, the nocturnal increase in plasma melatonin was much lower than that observed in eight control subjects. Women with the lowest peak concentration of melatonin had tumors with the highest concentrations of estrogen receptors. A significant correlation was found between the peak plasma melatonin concentration and the tumor estrogen receptor concentration in 19 of the patients. These data suggest that low nocturnal melatonin concentrations may indicate the presence of estrogen receptor positive breast cancer and could conceivably have etiologic significance.*

We recently suggested that there might be a relation between the development of breast cancer and impaired pineal function in women (*1*). There is evidence that the pineal product melatonin can decrease the number of breast tumors in dimethylbenz[*a*]anthracene (DMBA)-treated rats (*2*), and alter estrogen receptor (ER) concentration in vivo in ovariectomized rats and in vitro in human breast cancer cells (MCF-7) (*3, 4*). We undertook to examine pineal function by monitoring plasma melatonin concentrations over 24-hour periods in patients with breast cancer.

Biosynthesis of melatonin occurs in the pineal gland in a diurnal pattern that is reflected in the circulation by low concentrations during the day and high concentrations at night (*5*). This daily plasma profile has been essentially conserved over a wide range of vertebrate phylogeny (*6*), and in seasonally breeding species the daily melatonin rhythm may be the hormonal signal transducing environmental light information to the reproductive system (*7*). However, in nonseasonal breeders the physiologic role of this molecule remains unclear.

In nonseasonally breeding rats, daily afternoon melatonin injections significantly reduced the incidence of breast tumors in animals treated with a high dose (15 mg) of DMBA. Conversely, pinealectomy enhanced the incidence of tumors in rats given a low dose (7 mg) of DMBA. Assessment of circulating prolactin in DMBA-treated rats revealed significantly reduced levels in melatonin-treated rats, suggesting that melatonin may inhibit the induction of these prolactin-dependent tumors via prolactin suppression. Alternatively, melatonin may inhibit mammary tumor induction by its action on estrogen receptors. Oophorectomized animals are resistant to tumor induction by DMBA, but tumor induction can be restored by estrogen administration (*8*). These data suggest that daily treatment with melatonin or the presence of a normally functioning pineal gland can affect mammary tumorigenesis, perhaps by regulating the hormonal milieu of breast tissue or its intrinsic responsiveness to regulatory hormones.

Twenty women (aged 30 to 67 years) with clinical stage I or II breast cancer participated in a 24-hour blood sampling

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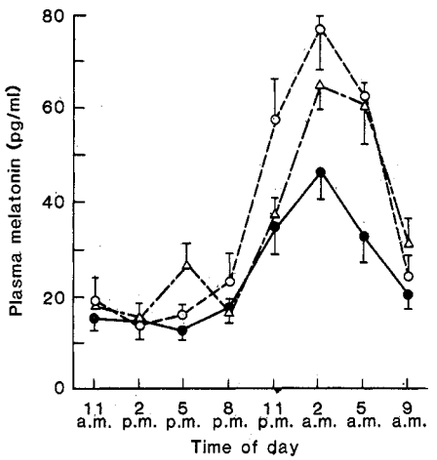

Fig. 1. Twenty-four-hour profiles of plasma melatonin concentrations in women with ER+ (●) (N = 10) or ER− (○) (N = 10) breast cancer, and in eight normal women (△). Data are presented as means ± standard error for each time of day.

protocol 1 day prior to tumor removal or 5 days after surgery. These patients were not taking any medication during the study, and samples were obtained every 3 hours through an indwelling intravenous needle and collected in heparinized tubes. The plasma was assayed for melatonin by a radioimmunoassay previously validated for use with human plasma (*9*). After surgery, a section of the malignant breast tissue was analyzed for ER concentration (*10*). In addition, blood samples of eight age-matched normal female volunteers were taken every 3 hours for 24 hours. All subjects conformed to normal ward routine.

A daily plasma melatonin rhythm was observed in all normal subjects (Fig. 1). These subjects had low melatonin concentrations (< 20 pg per milliliter of plasma) during the day, and reached peak concentrations of 60 pg/ml at 2 a.m. This agrees with previously published data (*11*). The daily melatonin rhythms of some of the breast cancer patients differed from those of the normal subjects. These patients were divided into two groups: those with tumors in which the ER concentrations were more than 10 fmole per milligram of protein (ER+), and those with tumors in which the ER concentrations were less than 10 fmole per milligram of protein (ER−). These differences correspond to clinically distinct subpopulations of patients with breast cancers (*12*). Inspection of individual plasma melatonin profiles revealed that in a substantial number (four out of ten) of ER+ patients there was little or no nocturnal increase in plasma melatonin; neither the ER− patients nor the normal subjects lacked this increase.

The 24-hour profiles for the normal subjects and the ER+ and ER− breast cancer patients are summarized as means ± standard error for each time of day (Fig. 1). A nocturnal increase in plasma melatonin, peaking at 2 a.m., occurred in all groups. However, ER+ patients had lower nocturnal plasma melatonin than ER− patients and normal subjects (46 ± 5 pg/ml compared to 78 ± 9 pg/ml and 65 ± 5 pg/ml, respectively).

The peak and nadir plasma melatonin levels of each of 19 of the 20 breast cancer patients were examined for a correlation with the ER concentration found in that patient's tumor (Table 1). We found a significant inverse relation between ER concentration and peak melatonin level (r_s = −.72, P < .001, Fig. 2). We found no relation between the ER concentration and nadir level of plasma melatonin. In addition, we found no significant relation between plasma melatonin levels and age, weight, parity, or menopausal status. Further analysis of the ER+, ER−, and normal groups showed that four of the ten ER+ women, five of the ten ER− women, and four of the eight normal women were postmenopausal. Of those patients in the ER+ group that had no nocturnal increase in melatonin, two were premenopausal. Thus, the relation between peak plasma melatonin and tumor ER concentration indicates that women with lower peak concentrations of plasma melatonin have higher tumor ER concentrations.

A relation between ER concentration and plasma melatonin was recently suggested by studies in the rat, where melatonin administration decreased brain ER concentration 40 minutes later (*3*). Similarly, we observed in vitro that melatonin can affect ER concentrations in MCF-7 cells. However, in our study, melatonin caused a transient increase in ER in MCF-7 cells (*4*). The difference in response to melatonin treatment may reflect the two different biological systems or, alternatively, may be due to methodological differences. Nonetheless, melatonin appears to alter estrogen receptors, and perhaps the daily melatonin rhythm plays a role in the daily regulation of estrogen receptors, as suggested previously (*3*).

Table 1. Tumor ER concentration and peak and nadir plasma melatonin concentration in 19 patients.

Estrogen receptor (fmole/mg protein)	Plasma melatonin (pg/ml)	
	Peak	Nadir
< 0.8	62.8	27.4
< 0.8	81.2	8.6
< 0.8	84.2	9.9
< 0.8	127.3	4.6
0.8	121.1	9.2
0.9	59.4	8.9
1.8	96.4	8.6
4.5	65.7	3.6
12.0	74.0	8.7
16.9	118.5	10.2
18.5	58.7	5.9
27.6	70.4	15.8
30.0	26.7	15.8
31.0	50.0	21.0
38.0	51.5	7.5
43.2	55.4	5.6
48.6	65.6	13.4
50.0	29.7	8.6
124.9	15.3	8.5

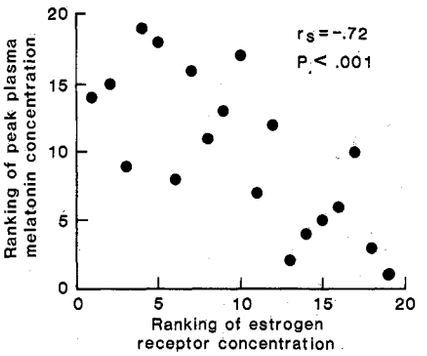

Fig. 2. Correlation between tumor ER concentration and peak plasma melatonin concentration in 19 patients. Data are presented as the rank order of ER concentration versus the rank order of peak plasma melatonin level for 19 breast cancer patients. The Spearman correlation coefficient is −.72 and the probability that this correlation came from a random population is < .001.

We were concerned that the period of study of most of the breast cancer patients occurred just prior to surgery, potentially introducing a stress factor into this study. The plasma samples collected from all subjects were analyzed for cortisol concentration and the data from all subjects were similar, with no significant differences between any groups at any time of day. Plasma cortisol levels were low throughout the day (5 ± 3 μg/dl), with a peak occurring between 5 and 7 a.m. (15 ± 5 μg/dl). Qualitatively and quantitatively, our data are consistent with previously published data for the 24-hour plasma cortisol profile in humans (*13*), suggesting stress-related hormonal changes were probably not a factor in this study.

We do not know if the decline in the nocturnal peak of plasma melatonin represents a long-term pineal failure, or whether a change occurs at the time of breast cancer development. Malignant tissue was already present in the breast cancer patients we studied. To determine whether an alteration in the plasma

melatonin profile precedes the presence of malignant tissue it will be necessary to conduct longitudinal and familial studies.

A number of studies have suggested that ER concentrations in breast tumors indicate the degree of hormonal dependence of an individual's breast cancer (*14*). The present data suggest that the lower the peak concentration of plasma melatonin, the greater the probability that the primary tumor may be hormonally dependent. Thus, the absence of nocturnal peak melatonin may serve as a biochemical marker for increased risk of developing ER+ breast cancer.

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Insulin Stimulation of Nucleoside Triphosphatase Activity in Isolated Nuclear Envelopes

Abstract. *The activity of nucleoside triphosphatase, an enzyme that regulates nuclear messenger RNA transport, was measured in highly purified nuclear envelopes isolated from rat liver. Addition of picomolar concentrations of insulin to freshly prepared nuclear envelopes directly increased the enzyme activity. The major effect of insulin on this enzyme was to increase the maximum velocity of its activity; no significant effects were seen on the affinity constant. These studies raise the possibility, therefore, that the nuclear envelope is a site where insulin regulates nuclear functions.*

The nuclear envelope is a double-membrane structure that separates the nucleoplasm from the cytoplasm. Although the exact organization and function of the nuclear envelope are unknown, several studies (*1*) have demonstrated that the nuclear envelope contains a distinctive enzyme, nucleoside triphosphatase (E.C. 3.6.1.15). This Mg^{2+}-dependent enzyme has several features generally not shared by other triphosphatases, including a broad substrate specificity (that is, the triphosphates of adenosine, uridine, cytidine, and guanosine are all effective), stimulation by RNA (*2*), and regulation by adenosine 3′,5′-monophosphate (cyclic AMP) (*3*).

Several studies in liver and other tissues indicate that a major function of this nucleoside triphosphatase is to provide the energy necessary for the transport of messenger RNA (mRNA) out of the nucleus. Either adenosine triphosphate (ATP) or another nucleoside triphosphate is necessary for this transport process to occur, and mRNA transport increases linearly with increasing nucleoside triphosphate concentrations (*4*). Also, nucleoside triphosphatase activity and RNA transport have a similar activation energy and a similar Michaelis constant (K_m) for ATP (*3*). Furthermore, several compounds regulate both processes in a similar manner; for example, cyclic AMP increases mRNA transport and nuclear envelope nucleoside triphosphatase activity (*3*, *5*), whereas compounds such as sodium fluoride, colchicine, and proflavin inhibit both functions (*3*, *6*).

There are a number of reports that insulin increases mRNA levels in liver and other tissues (*7*). Schumm and Webb (*8*) observed that the direct addition of insulin to isolated rat liver nuclei

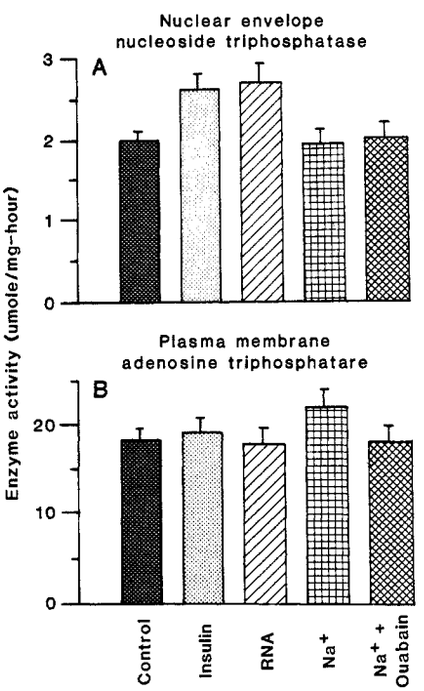

Fig. 1. Effects of insulin and other agents on the activity of (A) nuclear membrane nucleoside triphosphatase and (B) plasma membrane adenosine triphosphatase. Female Sprague-Dawley rats, 140 to 160 g, were deprived of food for 24 hours and injected through the tail vein with streptozotocin (Sigma) at 75 mg/kg. When the rats were studied 7 to 14 days later, the blood glucose concentrations were greater than 500 mg/dl. Purified liver nuclei were isolated (*20*), and nuclear envelopes were prepared from these nuclei as in (*21*), except that glycerol was omitted during the gradient centrifugation step. Purified plasma membranes were isolated (*22*), and nucleoside triphosphatase and adenosine triphosphatase activities were measured (*3*). In brief, either freshly prepared nuclear envelopes (0.25 to 0.5 mg of protein per milliliter) or plasma membranes (0.05 to 0.2 mg of protein per milliliter) were placed into buffer at 37°C containing 25 m*M* KCl, 5 m*M* $MgCl_2$, 50 m*M* tris-HCl (*p*H 7.5), and bovine serum albumin (0.1 g/dl). To this was added 10 p*M* insulin (Elanco), RNA (Millipore) at 200 μg/ml, 100 m*M* Na^+, or 100 μ*M* ouabain plus 100 m*M* Na^+. After a 10-minute incubation, the reaction was started with adenosine 5′-[γ-^{32}P]triphosphate (Amersham) added to a final concentration of 1 m*M*. The reaction was then stopped after 10 minutes with the addition of perchloric acid at 4°C; the intact ATP was adsorbed to charcoal; and the hydrolyzed ^{32}P was measured by liquid scintillation counting (*3*). Samples without membrane protein were included in each assay, and their values were subtracted from each result. Each value is the mean ± S.E. of three separate experiments.

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