8 ELF Electromagnetic-Field Effects on the Pineal Gland

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In this chapter, we review data, primarily from chronobiological and neuroendocrine studies, that suggest that ELF electric and magnetic fields can affect pineal function. We then propose ways in which alterations or deficits in the normal function of the pineal gland might be implicated in the etiology of certain cancers. To consider these data in context, we begin with a brief historical introduction regarding the various physiological roles ascribed to the pineal organ.

BACKGROUND

Anatomical description of the pineal gland probably predates that of any other endocrine organ. From the writings of Galen in the second century B. C., it is clear that he recognized the pineal. In the seventeenth century, Descartes postulated, possibly because of its central location in the brain, that the pineal organ was the "seat of the soul." He also put forward the idea that psychic phenomena and thoughts have their origin and come to fruition in the pineal.

In 1879, Koelliker felt justified in stating, on the basis of microscopic examination of both calcified and uncalcified glands, that the pineal was "undoubtedly an insignificant organ." (It was later noted, however, that in the rat the blood-flow-to-tissue-weight ratio of the pineal was exceeded only by that of the kidney.) In 1898 Heubner noted that pineal tumors in man were sometimes associated with precocious puberty. Wurtman et al. (1968a) later argued that precocious puberty followed destruction of pineal tissue, whereas tumors resulting in hyperfunction of the gland were associated with delayed puberty.

Early in the twentieth century, pineal extracts attracted interest because they could reversibly blanch the skin of amphibia (McCord and Allen, 1917). In 1958 Lerner isolated an active compound from approximately 250,000 bovine pineal glands, and showed it to be the most potent frog-skin-lightening agent known, active at concentrations of 10^{-13} g/ml (Lerner et al., 1958, 1959). This compound was called melatonin and, in an early application of organic mass spectrometry, was shown to be *N*-acetyl-5-methoxytryptamine.

In 1965, Wurtman and Axelrod set forth a hypothesis that has continued to be the basis for successful experiments in pineal function and physiology; namely, that the pineal organ, like the adrenal medulla, functions as a "neuroendocrine transducer," converting neuronal input to hormonal output (Wurtman and Axelrod, 1965).

PINEAL GLAND PHYSIOLOGY

Biosynthesis of Melatonin

Metabolic pathways for the biosynthesis of melatonin from serotonin are shown in Fig. 1. All enzymes involved in this pathway are found in the

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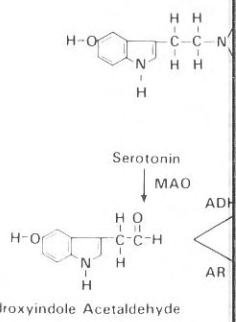

Fig. 1. Biosynthetic pathw
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mammalian pineal gland. The enzyme that adds the methyl group to the 5-hydroxy group of the indole nucleus (Axelrod and Weissbach, 1960), namely hydroxyindole-*O*-methyltransferase (HIOMT), was thought to be virtually exclusive to the pineal (Axelrod et al., 1961). More recently, however, there have been reports of its occurrence in other structures. For example, it has been detected in the retina (Cardinali and Rosner, 1971), which is not surprising considering the evolution of the pineal, and in the Harderian gland (Vlahakes and Wurtman, 1972), an endocrine organ associated with the nictitating membrane in rodents. Harderian glands are not present in humans; the principal site of HIOMT activity in the human is the pineal, and the bulk of circulating melatonin is derived from that organ.

Calcification of the pineal gland increases with age, but enzyme studies of pineals from humans up to 70 years of age show that they retain their capacity to synthesize melatonin (Wurtman et al., 1964). Histological studies from puberty to old age have shown that the proportion of the gland occupied by parenchymal cells is not decreased (Tapp and Huxley, 1972). More recent studies indicate that there are age-related changes in melatonin synthesis by the pineal (Waldhauser and Steger, 1986). Melatonin levels appear to decrease around the time of puberty, and become progressively less with age. The reduction in melatonin synthesis at puberty presents a consistent picture of pineal function with regard to sexual maturation, considering the antigonadotropic action of melatonin.

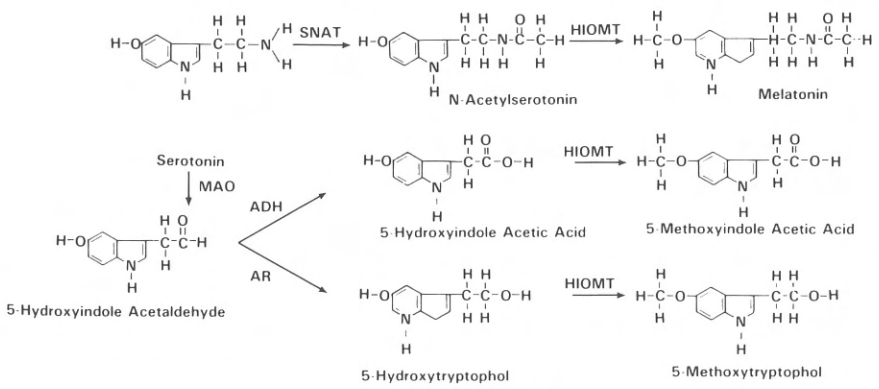

Fig. 1. Biosynthetic pathways for melatonin and other serotonin metabolites. Abbreviations: AR, aryl reductase; AH, aryl hydrogenase; MAO, monoamine oxidase; SNAT, serotonin *N*-acetyltransferase.

Other Pineal-Specific Indoles

As noted, the enzyme HIOMT, which is necessary for the synthesis of melatonin, is localized mainly in the pineal gland. This enzyme can participate in the synthesis of other 5-methoxy indoles: 5-methoxytryptamine (*O*-methyl serotonin), and 5-methoxytryptophol, for example, are physiologically active compounds that exhibit circadian rhythms in the pineal (McIsaac et al., 1965; Koslow, 1976; Wilson et al., 1978). Figure 1 depicts a fairly complete scheme for the biosynthesis of serotonin metabolites.

There is strong evidence to suggest that 5-methoxytryptophol is an antigonadotropic compound, its primary action being directed against follicle-stimulating hormone (FSH) rather than luteinizing hormone (LH) (Fraschini and Martini, 1970). In human plasma, 5-methoxytryptamine is quickly converted to its major urinary metabolite, 5-methoxyindole acetic acid (Wilson and Snedden, 1979). Alterations in 5-methoxytryptamine content have been measured in the cerebrospinal fluid (CSF) and in the brain tissue of depressive suicides (Shaw et al., 1967). McIsaac (1961) has put forward the hypothesis that certain 5-methoxy indoles may be implicated in behavioral changes. For example, he suggests that the 5-methoxylation of the indole nucleus is an important factor in the enhanced psychomimetic activity of the various hallucinogenic drugs, such as the ergot alkaloids, that are structurally related to serotonin.

Melatonin Measurement

Before the work of Wilson et al. (1977) and Lewy and Markey (1978), there was no reliable, sensitive assay for melatonin. Indeed, its existence in the human circulation was not unambiguously demonstrated until 1976 (Smith et al., 1976), although it had been previously inferred from results of bioassays (Pelham et al., 1973). Consequently, possible physiological functions of melatonin had been investigated only by indirect means, such as analysis of pineal enzyme activity or observations after pinealectomy and the exogenous administration of the hormone. Early mass spectrometric assays have largely been abandoned in favor of specific radioimmunoassays that have become commercially available (Arendt, 1978; Arendt et al., 1985). Pineal function can now be assessed by measurement of melatonin or its metabolites in the blood, urine, and CSF as well as in the pineal itself.

A particularly important addition to the analytical tools available for study of pineal function has been the development of a radioimmunoassay for 6-hydroxy melatonin sulfate (6-OHMS), a stable melatonin metabolite excreted in the urine. This assay allows noninvasive determination of pineal function. Levels of this metabolite have been compared with those of plasma melatonin (Bojkowski and Arendt, 1988) and exhibit the expected circadian rhythm. Morning void urines, which represent accumulation of the metabolite in the kidneys overnight, contain nominally 5- to 10-fold higher concentrations than do urines collected during the daytime.

Chronobiology

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Chronobiology

Some of the earliest observations suggested that melatonin production had a circadian variation, high at night and low during the day (Axelrod et al., 1965). It was further shown that this rhythm directly correlated with the light:dark conditions of the environment: constant darkness leading to continuously elevated melatonin production (as evidenced by increased HIOMT activity) and the reverse in constant light (Wurtman et al., 1963; Klein and Weller, 1971). This probably relates to the curious evolution of the pineal. In fish, amphibia, and birds it contains two parts, one of which is a sensory end-organ with discrete photoreceptor cells responding directly to light and having neuronal connections to the central nervous system (CNS). Because it lies near the surface of the skull, the pineal gland has been logically termed the "third eye." In mammals the pineal has evolved significantly, losing the photoreceptor cells but retaining specific endocrine cells of the pineal, the pinealocytes. This evolutionary change does not prevent the gland from responding to light, since it receives indirect sensory input from the retina (Moore et al., 1968).

Thus, serotonin and its catabolic metabolites, including *N*-acetyl serotonin, melatonin, 5-methoxytryptamine, and 5-methoxytryptophol, exhibit strong circadian rhythms in most reptiles, birds, and mammals that have been studied. A number of ultradian rhythms superimposed on pineal gland function have also been reported. These include an approximately monthly rhythm in women that is synchronized to the menstrual period (Arendt et al., 1985; Hariharasubramanian et al., 1985), and an annual rhythm in plasma melatonin levels that may be especially pronounced at higher latitudes (Dubbels and Khoory, 1986). In studies on the annual melatonin rhythms in man, Bojkowski and Arendt (1988) have shown that the total 24-hr excretion of 6-0HMS does not vary significantly with the time of year. The timing, or acrophase, of the nighttime peak does vary significantly however (by approximately 2 hr) at 52°N latitude (Fig. 2). It is also important to note that exposure to light of sufficient intensity during the dark cycle leads to an immediate drop in circulating melatonin content in several species, including man (Lewy et al., 1982).

Neuroendocrine Function

The pineal organ is described as a "neuroendocrine transducer" (Wurtman and Axelrod, 1965) because it serves as a locus for the conversion of a neural input concerning the environment to a hormonal output, primarily melatonin. Melatonin acts as a neuroendocrine mediator for the biological clock located in the suprachiasmatic nucleus, and produces the primary hormonal signal for synchronizing endocrine rhythms within the body (Cardinali, 1974). In mammals, it is clear that one important target of melatonin is the CNS (Anton-Tay, 1974). Innervation of the pineal is

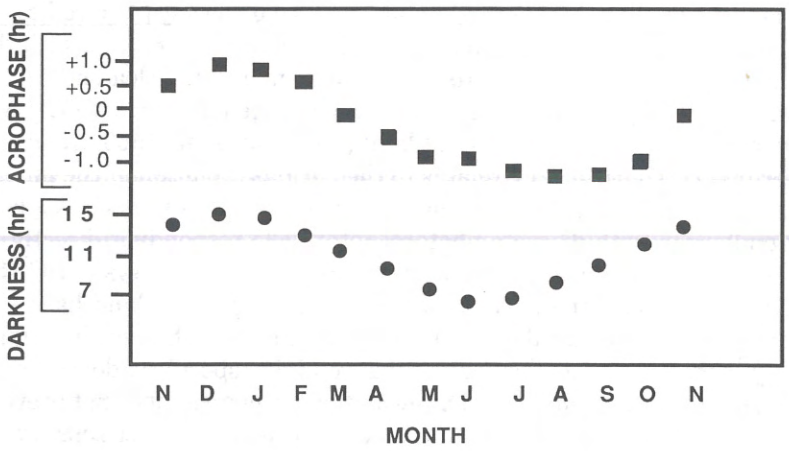


Fig. 2. Change in acrophase for humans in relation to the duration of the daily dark period. (Adapted from Bojkowski and Arendt, 1988.)

mainly from fibers of the superior cervical ganglion (Moore et al., 1968). However, fibers originating in the hypothalamus and in the optic system regions of the brain also innervate the gland (Moller and Mikkleson, 1988). The neuroendocrine function of the pineal as related to stress and reproduction is discussed in Chapter 5 by Reiter (this volume).

It is now recognized that pineal function in the endocrine system is inhibitory for most other endocrine glands (Wurtman and Cardinali, 1974; Nir, 1978). Pinealectomy in rats results in endocrine changes that include increases in circulating levels of gonadal steroids as well as FSH and prolactin (Kamberi et al., 1971). Pineal ablation also causes measurable functional changes in the hypothalamus, pituitary, and gonads, as well as in the thyroid and parathyroid glands (Nir, 1978). Although some of these effects may be secondary, there is evidence for direct interaction of the pineal gland with the pituitary (Bindoni and Raffaele, 1968) and the gonads (Reiter, 1981).

Research into the effects of melatonin on the brain and behavior has shown that exogenous melatonin readily enters the brain; uptake from CSF is even greater than from blood (Wurtman et al., 1964; Anton-Tay and Wurtman, 1969). Melatonin has the effect of inhibiting protein synthesis in brain slices (Orsi et al., 1973), and pinealectomy in neonatal rats is reported to significantly decrease myelin formation and brain development (Relkin et al., 1973; Relkin and Schreck, 1975). Zisapel et al. (1988) have

identified melatonin receptors in the hypothalamus and optic system regions of the brain. These receptor populations exhibit a clear circadian rhythm that appears to be largely light:dark driven.

Bowers et al. (1984) demonstrated the pineal gland's sensitivity to neuronal input during experiments wherein the intercarotid nerve, which together with the superior cervical ganglion innervates the pineal gland, was either sectioned, crushed, or frozen. Each procedure was effective in inhibiting pineal synthesis of *N*-acetyltransferase (NAT) and melatonin. On recovery from the freezing treatment, the intercarotid nerve was able to conduct nerve impulses, and an artificial 5-Hz signal was effective in eliciting pineal NAT response. However, spontaneous pineal rhythms were not observed at any time after treatment. Similarly, Kneisley et al. (1978) observed disruptions in the rhythms of excreted melatonin in human patients with cervical spinal cord transection. Cervical ganglion lesions also resulted in changes in electroencephalograph patterns (Adey et al., 1968). These investigators also noted sleep disruptions in quadriplegic patients.

Melatonin can induce sleep in both man (Anton-Tay, 1974; Cramer et al., 1974) and a variety of animals (Barchus et al., 1967; Hishikawa et al., 1969). Specific effects of melatonin on rapid eye movement (REM) sleep have been reported in man (Mouret et al., 1974). Rapidly fluctuating photoperiods, a presumed pineal stimulant, also affect REM (Rechtschaffen et al., 1969; Chamblin and Drew, 1971). Studies employing computer analysis of continuous EEG recordings found no significant difference between melatonin-induced sleep in man and natural sleep.

Only recently has there been a recognition of the variety in neurotransmitter receptor sites present in the pineal organ. Along with the well-established β-adrenergic receptors that are primarily responsible for stimulation of melatonin synthesis, the mammalian pineal contains α-adrenergic receptors and receptors for gamma amino butyric acid (GABA), as well as serotonergic, dopaminergic, glutamatergic, and muscarinic cholinergic receptors (Ebadi and Govitrapong, 1986).

Depression and Affective Illness

Pineal gland dysfunction has been implicated as a possible factor in affective illness. This is owing partially to the alterations in normal biological rhythms, including the sleep/wake cycle, that are often symptomatic of affective disorders. Some 30 years ago, Altschule (1957) and Eldred et al. (1961) showed that bovine pineal extracts were effective in lessening the severity of schizophrenic episodes in institutionalized patients. The importance of their findings seemed to diminish with the development and introduction of antipsychotic drugs such as haloperidol, although they were essentially confirmed by the work of Bigelow (1974).

In the 1970s some workers suggested ways in which the pineal may play a role in the etiology of schizophrenia. Their ideas were based largely

on the fact that slight changes in the metabolism of melatonin could give rise to potential hallucinogens (McIsaac, 1961; Greiner, 1970). Interest in the pineal as a possible mediator of schizophrenia has diminished in the 1980s; however, the pineal is now thought to be involved in the etiology of depressive illness, although its exact role remains unclear.

Lewy et al. (1982) and others have shown that extending the daily photoperiod by exposure to bright light can be effective in reducing symptoms in patients suffering from seasonal affective disorder syndrome (SADS). SADS patients become depressed during the winter months, but do not show such symptoms during the summer. In a study of 28 patients suffering from major depression, Brown et al. (1987) reported that decreased nocturnal melatonin levels in plasma correlated with depressed mood and reality disturbance, as determined by the Hamilton depression rating. Other studies, including those of Mendlewicz et al. (1980), Branchey et al. (1982), Beck-Friis et al. (1985), and Claustrat et al. (1984), have associated disruptions in melatonin circadian rhythmicity with "major depression." Specific mechanisms by which pineal dysfunction affects mood have not been worked out. On a systemic level, both reduction and phase-shifting of the circadian peak in melatonin concentration have been suggested as factors, although current views favor changes in the acrophase (timing of the melatonin peak) as the most relevant to these mood changes. [For a review of possible ELF field effects on mood and depression, see Wilson (1988).]

Papke et al. (1986) have generated evidence that melatonin is a neuromodulator. Neurons from rat superior cervical ganglia were cocultured with pinealocytes and exhibited action potentials that were longer and of higher amplitude than potentials from neurons grown in the presence of nonneuronal cells. In these same experiments, melatonin caused an increase in action potential duration that was not associated with increases in amplitude. Increasing levels of circulating steroids resulting from reduced melatonin secretion could be a factor in ELF-induced mood changes. Su et al. (1988) have identified specific gamma receptors in the brain and showed that certain gonadal steroids, including estradiol, inhibit gamma-receptor binding. The authors speculated that these sites may be involved in mediation of steroid-induced mood changes. Alterations in mood and affect may follow directly from the loss of melatonin as a neuromodulator or from changes in circulating steroid levels resulting from the loss of melatonin as a gonadal inhibitor.

An interesting consequence of the ELF field-induced reduction in NAT activity, in experiments carried out in our laboratory, was the compensatory increase in the levels of 5-methoxytryptophol (5-MTOL), which is an apparent effect of increased substrate availability in the face of reduced NAT capacity to metabolize serotonin. We hypothesized in this situation that

serotonin was being shunted down the monoamine oxidase (MAO) pathway (see Fig. 1). Murphy et al. (1986) conducted experiments to measure the effect of MAO inhibitor treatment on melatonin levels in depressive patients. They found a pineal response complementary to that observed in the case of ELF exposure; when the MAO pathway was shut down, more serotonin was shunted down the NAT pathway, resulting in an approximate threefold increase in melatonin levels. This increase in melatonin was believed to contribute to the antidepressive effects of the MOA inhibitor treatment.

In reviewing the physiology of melatonin thus far, a range of disparate activities have been surveyed. These can be integrated if one considers that the primary targets of melatonin are certain centers of the brain, where melatonin modifies the serotonin-containing neurons (as in the hypothalamus), to affect pituitary secretion, or acts in the CNS, to affect behavior.

PINEAL FUNCTION AND CANCER

Three fundamental assumptions are important to the discussion presented in this section:

1. Maintenance of environmentally mediated rhythms including intradian, circadian, and ultradian biological rhythms, particularly in endocrine hormone and neurotransmitter synthesis and release, is vital to adaptation and homeostasis in animals (see Moore-Ede et al., 1985).
2. Control and feedback mechanisms exist among the nervous, endocrine, and immune systems and allow neural mediation of certain endocrine and immune system functions (see Cotman et al., 1987). Mechanisms of this kind involving pineal gland function have been demonstrated in some of the work reviewed here, and they may be especially important with respect to ELF electric- and magnetic-field effects.
3. ELF electromagnetic fields interact with the nervous systems in a variety of animals, including humans (see O'Conner and Lovely, 1988). Of particular importance is evidence, obtained in several laboratory experiments, that pineal circadian rhythms in serotonin, melatonin, and other tryptophan metabolites are altered or obliterated by appropriate exposure to ELF electric fields (Wilson et al., 1981, 1986). More recent work with healthy human subjects suggests that ELF electric- or magnetic-field exposure may alter pineal function in certain individuals (Wilson et al., 1988).

Pineal function might be linked to the etiology of cancer in at least three fundamental ways:

First, melatonin itself is oncostatic and appears to be a humoral factor that inhibits the proliferation of certain cancer cells.

Second, melatonin enhances certain facets of the immune response, again possibly helping to protect against the development of cancers.

Third, melatonin functions as an inhibitor of the hypothalamic–pituitary–gonadal axis. As such, it may reduce the availability of hormones that are required for the growth of certain hormone-dependent breast, ovarian, and prostate cancers.

Oncostatic Properties of Melatonin

Oncostatic properties of pineal extracts were first demonstrated in 1940 by Nakatani et al. (1940), who observed that an unknown component from bovine pineal gland could inhibit growth of tumor cells in culture. Several subsequent studies confirmed that pinealectomy led to increased growth and proliferation of several cancer cell lines (Rodin, 1963; Das Gupta and Terz, 1967). By the early 1970s, it was clear that melatonin was a pineal factor that could reduce the growth and metastasis of several cancers (Bostelmann et al., 1971; El-Domeiri and Das Gupta, 1973).

Proper pineal function appears protective against cancer (Blask et al., 1988). Blask and Hill (1986) demonstrated that physiological concentrations of melatonin were effective in inhibiting the growth of breast tumor cell lines in vitro, while subphysiological and superphysiological concentrations had no significant effect. [See Regelson and Pierpaoli (1987) for a review of melatonin as an endogenous antitumor factor.]

Melatonin and Immune Function

Melatonin appears to have a stimulatory effect on immune function in the whole animal. Maestroni et al. (1986), for example, showed that pharmacological inhibition of pineal function in mice results in depressed immune response to sheep red blood cells. Appropriately timed injection of melatonin restored normal immune function as determined by this assay. Evening injections of melatonin antagonized the suppression of antibody production resulting from adminstration of the stress hormone corticosterone in mice (Pierpaoli and Maestroni, 1987). These workers have suggested that the endogenous opioid system may mediate the immunoregulatory effects of melatonin. This view was supported by experiments in which the opioid antagonist naltrexone completely abolished the immunoenhancing effects of melatonin.

Melatonin as an Endocrine Inhibitor

A third effect of pineal hypofunction is the loss of gonadal inhibition that is normally exerted by melatonin. Thus, Cohen et al. (1978) proposed that

reduced pineal melatonin secretion may be a factor in breast cancer etiology. Women with breast cancer showed reduced urinary melatonin levels (Bartsch et al., 1981). Tamarkin et al. (1982) and Danforth et al. (1982) extended these findings, noting altered melatonin secretion in patients with estrogen receptor- (ER-) positive breast cancer.

Androgenic prostate cancer is analogous to ER-positive breast cancers in that appropriate steroidal hormones are required for continued growth of the tumor. Buzzell et al. (1988) showed inhibition of growth in rat prostatic adenocarcinoma with melatonin. These findings have been confirmed by Philo and Berkowitz (1988) working with the same Dunning tumor cell line. Bartsch et al. (1988) reported that men with cancer of the prostate had lower nocturnal melatonin levels than men who did not have the disease. A pilot study by Leone and colleagues (Leone and Skene, 1989) has confirmed findings of altered melatonin secretion in prostate cancer as compared to controls with benign hypertrophy of the prostate. These investigators also reported alterations in 6-OHMS secretion associated with ovarian cancer in women.

Functional pinealectomy, as defined by Shah et al. (1984), can be achieved by exposing young virgin rats to constant light, thus inhibiting the release of melatonin. This approach has been used in studies on tumor formation and promotion in mammary epithelial cells. Tamarkin et al. (1981) and Shah et al. (1984) have shown a direct inhibitory effect of melatonin on the incidence and latency of dimethylbenzanthracene- (DMBA-) induced breast cancer in rats.

Noting these findings, Stevens (1987) suggested that reductions in melatonin concentration arising from ELF field exposure in rats may result in increased circulating estrogen levels. This may stimulate mammary tissue proliferation and thus increase breast cancer risk. To test this hypothesis, experiments analogous in design to those of Shah et al. (1984) were conducted in our laboratory; ELF electric fields were used instead of light to reduce pineal melatonin output. This work has shown that DMBA-induced rats exposed to 60-Hz electric fields since birth had more tumors per tumor-bearing animal than sham-exposed controls (Leung et al., 1988a). In the course of these studies, Leung found that mean pineal melatonin tended to be lower in ELF electric-field-exposed animals compared to controls. This is consistent with the findings of Tamarkin and others, who identified reduced melatonin and increased prolactin as risk factors in DMBA-induced mammary cancer in rats.

ELF ELECTRIC- AND MAGNETIC-FIELD EFFECTS IN HUMANS AND ANIMALS

Self-reported human symptoms related to ELF exposure are most often associated with subjective-state changes, including headaches, and often involve feelings of fatigue and general malaise (Asanova and Rakov, 1966;

Gavalas-Medici and Magadaleno, 1976; Shandala et al., 1984). In other studies on populations exposed to 50- to 60-Hz fields, increased excitability and irritability were reported. In many cases, a state of hyperactivity that interfered with normal physiological and mental function was observed and attributed to ELF field exposure (Sheppard and Eisenbud, 1977).

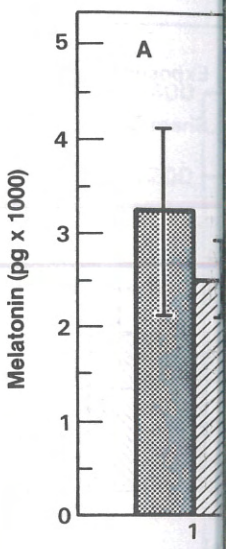
In experiments with 60-Hz electric fields, Graham et al. (1984) reported minor changes in evoked response measures subsequent to field exposure. Magnetic (ELF) field exposure can affect perceived flicker fusion frequency and low-light visual acuity in humans (Krause et al., 1985). Wever (1968) reported that 50-Hz field exposure arrested the lengthening of the human circadian cycle that normally occurs in the absence of time cues.

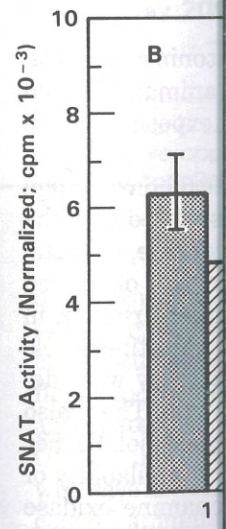
It is clear from studies on laboratory animals that the nervous system is affected by ELF exposure. Hjeresen et al. (1980) reported that electric-field exposure increased avoidance activity, and Jaffe et al. (1980) showed that exposed rats had faster recovery from slow-twitch muscle fatigue than did controls. Ehret et al. (1980) reported that exposed mice entered a state of torpor at electric-field strengths at or above about 75 kV/m. Ehret and Groh (see Chapter 4, this volume) have argued that an important effect of ELF exposure is loss of normal timing in biological circadian rhythms. Such "circadian dyschronism" appears to be a primary consequence of ELF exposure.

Both electric and magnetic fields can alter pineal gland function. Welker et al. (1983) showed that artificial magnetic fields could inhibit NAT activity in the rat pineal. Subsequently, a number of reports have confirmed that low-strength magnetic fields can alter pineal function both in vitro (Semm, 1983) and in vivo (see review by Olcese et al., 1988). It should be noted, however, that most of the work to date on magnetic-field effects has been carried out using low-intensity static fields. Laboratory data on the effects of higher strength AC magnetic fields are still being gathered and assessed, although early data from female rats indicate no alteration.

We have shown that chronic (21-day) exposure to ELF electric fields between approximately 2 and 40 kV/m can abolish the normal nocturnal peak in pineal melatonin in male rats first exposed to these fields at approximately 55 days of age (Wilson et al., 1981, 1983) (Fig. 3). This response appeared to have a threshold between 0.2 and 2 kV/m. At field strengths above approximately 2 kV/m there was no apparent dose response, suggesting the electric fields gave rise to an "all-or-none" effect. In the animals first exposed at 55 days of age, normal melatonin rhythms were reestablished within 3 days after cessation of ELF field exposure at 39 kV/m (Wilson et al., 1986) (Fig. 3C).

Rats exposed from conception until 23 days after birth at field strengths of either 10, 65, or 130 kV/m showed a decrease in the amplitude of the nocturnal melatonin peak, as well as an approximate 1.4-hr delay in the occurrence of peak melatonin concentration, compared to controls (Reiter





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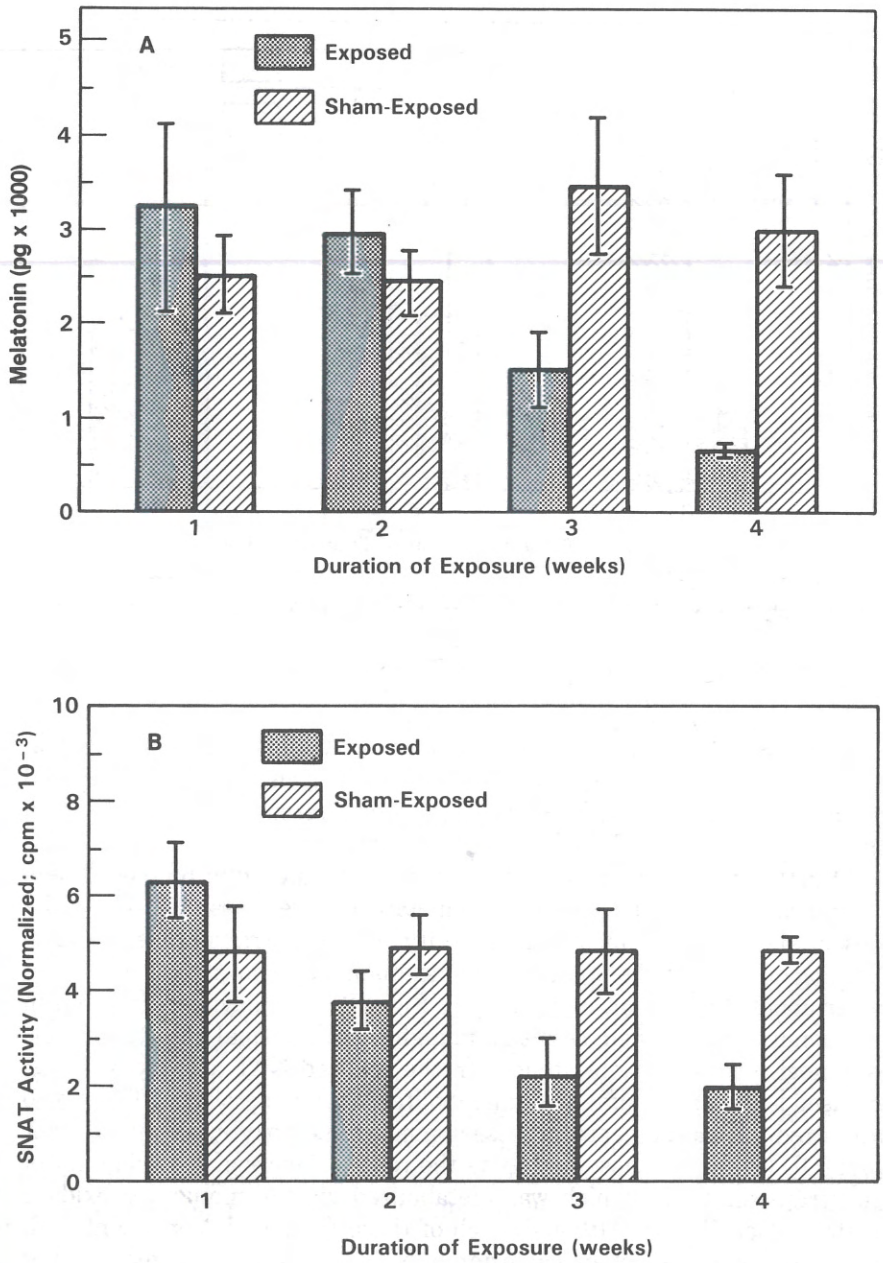

Fig. 3. (**A**) Mean nighttime pineal melatonin levels for rats exposed to 39-kV/m electric fields. Exposed animals were significantly different from controls in third week. (**B**) *N*-Acetyl serotonin activity in animals shown in A. (Figure continued on next page.)

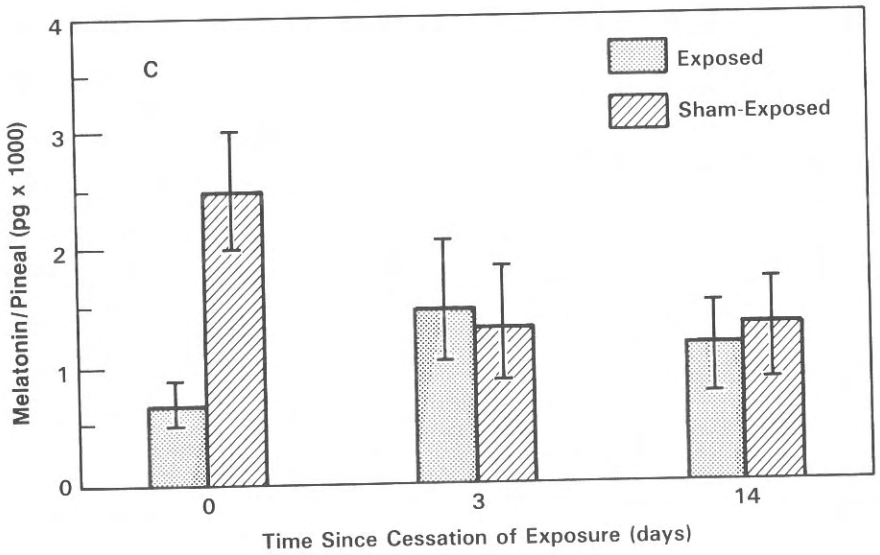

Fig. 3. (continued) (C) Recovery of melatonin rhythms in animals at cessation of exposure.

et al., 1988). Figure 4 shows a comparison of pineal melatonin concentration at several time points during the circadian cycle for animals exposed in utero through weaning at 23 days of age versus unexposed control animals.

Further work was done with animals first exposed at 55 days of age to determine what changes in pineal metabolism were associated with the observed reduction in melatonin. Activity of *N*-acetyltransferase, the rate-limiting enzyme for the production of melatonin, was shown to be depressed after about 3 weeks of exposure (see Fig. 3B). The day:night rhythm in serotonin was abolished, resulting in higher-than-normal nighttime levels of pineal serotonin. Consistent with the reduced NAT activity were decreased levels of its immediate metabolite, *N*-acetyl serotonin. There also appeared to be a compensatory rise in pineal 5-methoxytryptophol. Higher 5-MTOL levels were assumed to be the result of increased availability of substrate serotonin, which was metabolized via the monamine oxidase pathway (see Fig. 1). Although each of these findings is consistent with the induction of a biochemical lesion in NAT synthesis, the effect has not been studied thoroughly enough to give an indication of what changes in neuronal function may be involved.

Several factors appear to influence rat sensitivity to electric fields. There appear to be marked strain differences, for example. Males appear

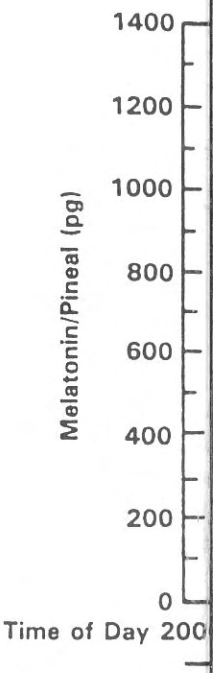

Fig. 4. Effect of ELF
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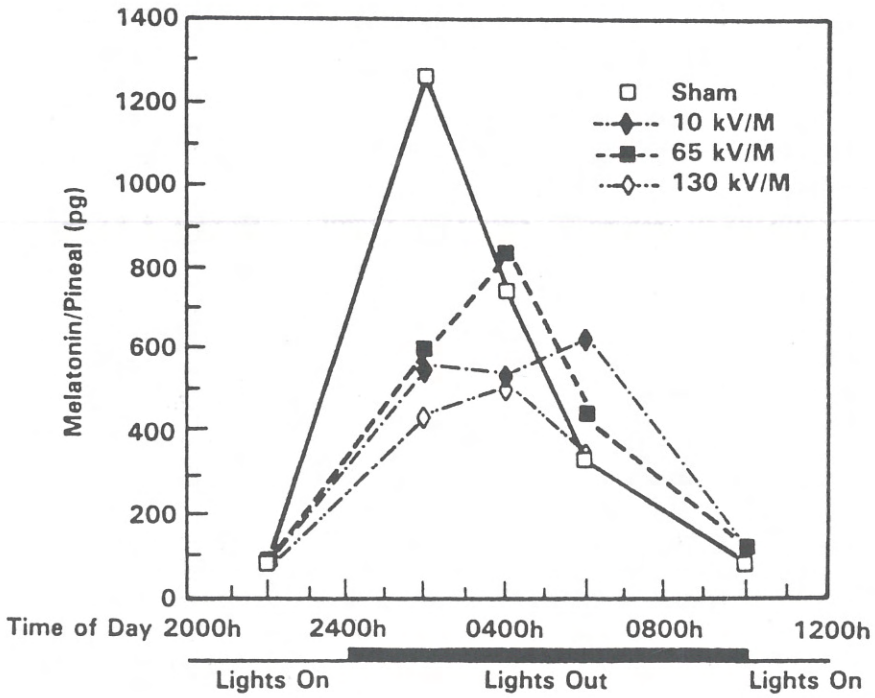


Fig. 4. Effect of ELF electric-field exposure at 10, 65, and 130 kV/m on rats exposed in utero. Note apparent shift in acrophase of exposed animals relative to controls. (Adapted from Reiter et al., 1988.)

more sensitive to magnetic fields than do females, and nonpigmented strains appear more senstive than do pigmented strains. There are a number of studies on rats that have shown no difference in the status of ELF field-exposed animals compared to controls using paradigms that may be anticipated, on the basis of the foregoing discussion, to show an effect. Of most interest among these are the studies of Morris and Phillips (1982), who have consistently shown no difference between ELF field-exposed and control animals in cellular or whole-animal immune response to several antigens.

Evidence for ELF Field Effects on Human Pineal Function

We have presented evidence from various experiments that suggest a possible contributory role for the pineal in the etiology of several disorders,

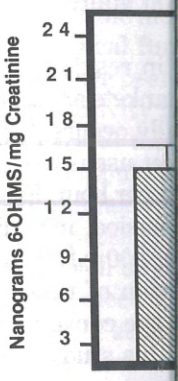
including cancer. It has also been demonstrated, in this laboratory and others, that chronic exposure to ELF fields can affect pineal gland function. The work of Wever (1968) on the effects of 10-Hz electric fields on human circadian rhythms constitutes indirect evidence for an interaction between the pineal gland and ELF fields. Although it is not our intention to argue that ELF field exposure contributes to increased cancer risk, we have conducted experiments to determine if a direct effect on pineal gland function in humans could be demonstrated as a result of exposure to electric and magnetic fields normally encountered in a residential environment.

Domestic electric blanket use occurs at night when the pineal is most active, and represents a highly periodic exposure to ELF fields. Use of these blankets, however, does not require alteration in the normal lifestyle or daily routine of the subjects. Electric and magnetic fields associated with the blankets are readily measurable, especially in comparison to other domestic sources of ELF fields. We therefore designed and executed a study to determine if domestic ELF electric- and magnetic-field exposure, as represented by the use of standard or modified electric blankets, could affect human pineal gland function. Preliminary findings from this study were reported by Wilson et al. (1988) and are only summarized here.

Our primary measure for determination of effect on pineal gland function was to monitor, by radioimmune assay, the nighttime excretion of urinary 6-OHMS. Two types of blankets were used in the study: conventional (snap safety switch), and continuous polymer wire (CPW). The latter had a shorter duty (on-off) cycle and exhibited an approximately 50% higher magnetic field, compared to the former.

Because of the wide variation in 6-0HMS excretion among individuals (Bojkowski and Arendt, 1988), the study was designed so that the volunteers served as their own controls. When the data were considered by individuals, seven subjects from the CPW groups exhibited statistically significant differences in their mean 6-OHMS excretion between at least two of the last three exposure periods. One individual, for example, showed a response pattern typical of that for the overall CPW user population; that is, no change in 6-OHMS excretion between preexposure and exposure period 2 (week 1–5), followed by a significant decrease in exposure period 3 (week 6–10), and a rebound to higher values at the cessation of exposure (Fig. 5). Figure 6 plots raw data of the nighttime melatonin excretion values for study subject 2045. Note the rapid increase in 6-OHMS excretion immediately after cessation of exposure. No statistically significant changes in 6-OHMS excretion occurred in the conventional blanket users among any of the exposure periods on either an individual or a group basis.

Those specific electric- or magnetic-field characteristics of CPW blankets that were responsible for the apparent changes in 6-OHMS excretion have not been determined. There was little difference in the magnitudes of the electric fields generated by the conventional AC as compared



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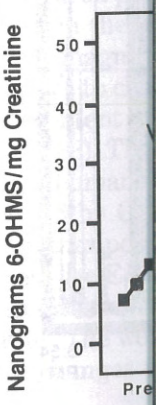

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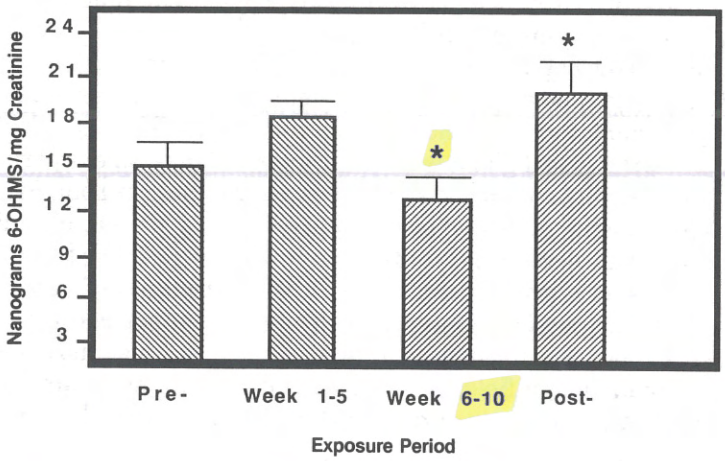

Fig. 5. Overnight excretion of 6-hydroxy melatonin sulfate in female volunteer (#2055) before, during, and after use of a continuous polymer wire (CPW) electric blanket. Values with asterisk (*) are significantly different from those for previous exposure period.

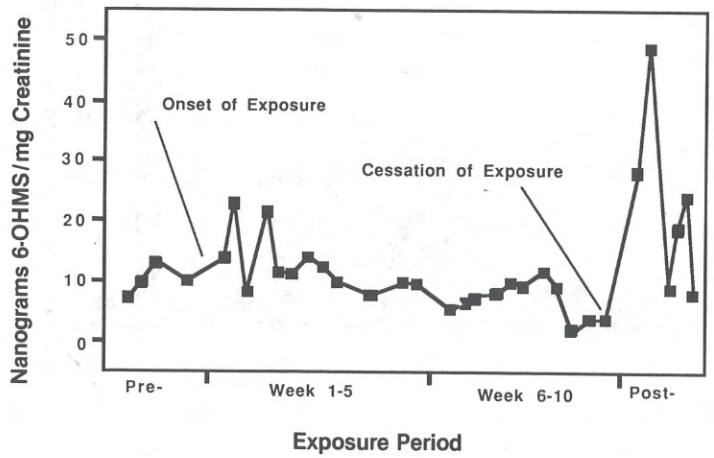

Fig. 6. Overnight excretion of 6-hydroxy melatonin sulfate for female volunteer #2045. Note substantial increase in urinary 6-OHMS after cessation of exposure.

to the CPW AC blankets. Magnetic-field strength, however, was as much as 50% higher in the CPW blankets. Graham et al. (1988) observed an enhancement of electric-field effect on humans when the field was rapidly switched on and off; CPW blankets switched on and off at approximately twice the rate of the conventional units.

Electric blanket use may constitute a special case in residential exposure. Both electric and magnetic fields under electric blankets are among the highest encountered in the home. Exposure generally occurs during the night when the pineal gland is active. Electric blanket use is periodic and may represent 50 to 60 changes in field exposure state per hour. Figure 7 is a graph of magnetic-field exposure during a 24-hr period, including time spent under an electric blanket from approximately 10:00 P.M. to 7:00 A.M., and serves as a graphic example of the contribution of these appliances to daily magnetic-field exposure. The fact that the conventional blanket users showed no response to their blankets in this study might indicate that reduction of fields or changes in duty cycle associated with these blankets may reduce or eliminate their effect on pineal function.

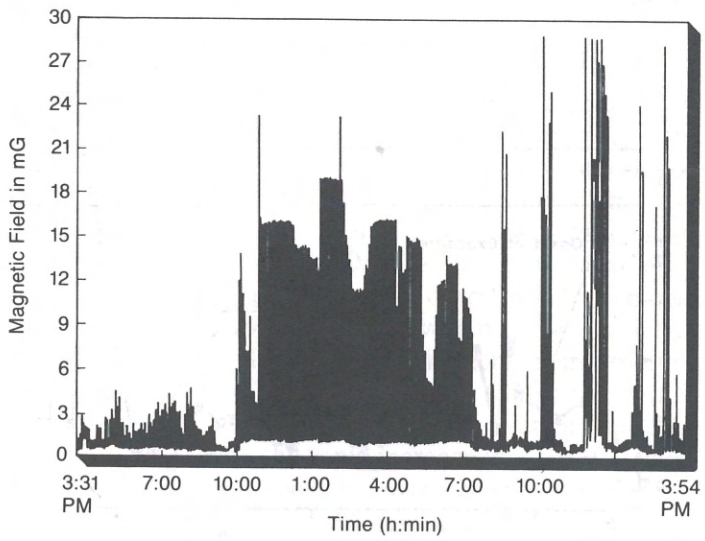

Fig. 7. Magnetic-field exposure for an 8-year-old child shows a large increase in field level from electric blanket use at night. "Spikes" during daytime are mostly from exposures received traveling to and from school.
[Reprinted with permission from Sussman, S. 1987. EMDEX (electric and magnetic field exposure) system. *Tech. Brief RP799-16.* Palo Alto, California: Electric Power Research Institute.]

POSSIBLE MECHANISMS

It now appears very likely that ELF electric and magnetic fields can alter pineal gland function by decreasing melatonin synthesis and release. On the level of the intact organism, there are a number of possible mechanisms for the pineal function response to ELF electromagnetic fields. Several of these have been discussed previously (Wilson et al., 1981). Although NAT enzyme activity is an apparent site of action for the fields, it is likely that more careful measurements will show that the requisite (β-adrenergic) neuronal input for stimulation of NAT activity is altered or missing in exposed animals.

It is possible that the alteration in pineal gland function observed in rats, as well as in certain humans, is a manifestation of stress. Animals placed in electric fields of approximately 39 kV/m or more show clear indications of stress resulting from such exposure. Leung et al. (1988b), for example, have shown that the occurrence and severity of a porphyrin-containing exudate from the Harderian gland of rats correlates with the level of field exposure. Occurrence of the exudate, termed chromodacryorrhea, is an indicator of stress in several rodents, including rats and hamsters. Leung has also reported that ELF exposure at 39 kV/m reduces or eliminates the change in circulating prolactin levels that normally occurs in response to the stress associated with restraining the animal. Thus, the ability of the ELF field-exposed animal to respond appropriately to an additional stressor is impaired.

Considering the location of the pineal gland, both in rats and in humans, it seems unlikely that ELF electromagnetic fields act directly to produce the functional changes that have been observed. However, it should be noted that cultured pinealocytes (i.e., outside the skull) do appear to respond to changes in the local magnetic field (Welker et al., 1983). In pigeons and rodents, magnetic fields are detected by receptors in or near the eyes. Responses to changes in magnetic field can be detected by changes in melatonin content in both the retina and pineal glands of these animals (Olcese et al., 1988). Thus, the possibility of subconscious retinal sensing of ELF fields in humans cannot be ruled out until experiments analogous to those described by Olcese et al. (1988) are performed in humans.

Another possible mechanism is one that we term ganglionic coupling, wherein the ELF field interacts with the nervous system directly to alter aspects of the natural electrical information that is transmitted between neurons. The work of Bowers et al. (1984), cited earlier, suggests that very subtle changes in neural signaling may affect pineal function.

Possible Consequences of ELF-Induced Alterations in Pineal Gland Function

In a report examining possible ELF field effects from the Sanguine/Seafarer project (see Chapter 1, this volume), the author stated that ELF

exposure appeared to act as an agent of stress on the organism and seemed to lower the organism's resistance. This summary of ELF effect from some two decades ago would still seem to be appropriate in describing those findings of the most recent studies which show effects.

In the discussion that follows, we speculate on the ways in which observed pineal effects may be manifested as stress. ELF electric and magnetic fields may be thought of as agents or stimuli from the environment that call for or elicit some adaptive response on the part of the organism. There are almost certainly thresholds for the response, which may be in terms of field strength, flux density, orientation, waveform, rate of change, polarization, or other characteristics. Which, if any, of these characteristics call for an adaptive response may be dependent on the neurological, immune, and endocrine state of the exposed organism or individual. The general response pattern of the CPW blanket volunteers described earlier, and particularly that of the subject shown in Fig. 6, can be used to illustrate how this adaptation process may proceed.

At initial presentation of the field, the subject showed a slight increase in melatonin metabolite excretion. We view this as an initial response on the part of the pineal to a detectable change in the environment that may be termed a stressor. This initial increase was then extinguished as exposure to the stimulation or stress represented by nightly exposure to the field continued. For several individuals in the study, but not for the population in general, 6-OHMS excretion was lower after 4 to 5 weeks of exposure than during preexposure baseline. It appears that the longer term exposure antagonized melatonin production. If allowed to continue, such suppression of melatonin may put the exposured individual at a disadvantage in responding to further stimuli requiring adaption. When the exposure ceased, 6-OHMS levels increased significantly, indicating the removal of the antagonistic "agent." Whether or not this adaptive response constitutes any significant challenge in homeostasis for humans is unclear. It can be imagined, however, that the presence of the field may act as a stimulus or additional stressor agent for individuals with compromised neurological or immune function. Its effect may be manifested in insufficient immune system performance or by changes in endocrine status or mood and affect. These changes may only occur or be observable in certain individuals. Any untoward effects resulting from ELF exposure are likely to result from long-term rather than acute exposure.

CONCLUSIONS

It remains to be determined whether ELF field effects on pineal gland function represent a health risk to humans. The pineal gland and melatonin affect a variety of physiological functions, and it may, therefore, be

coincidental that the three main areas of concern that have emerged from epidemiological studies (emotional depression, miscarriages, and cancer) appear to be associated with changes in physiological function that can be directly affected by melatonin. Indeed, removal of the pineal gland in human adults, although a rare occurrence, has not been reported to result in significant adverse affects to the patient. Nonetheless, it should be considered that pineal rhythms are generally strong and reproducible on an individual basis. They are conserved in most of the animals studied, even in the face of severe tryptophan restriction. It is therefore unlikely that they are of no importance to the organism.

Because of melatonin's activity relative to the etiology of cancer, the pineal organ has been fairly termed an "oncostatic gland." Evidence from several sources suggests that ELF electric-field exposure may affect pineal function. Perturbations in the melatonin rhythm have been reported in a number of disease states. These include colonic cancers, prostate adenocarcinomas, and ovarian cancers as well as ER-positive breast cancers. Hence, data such as those indicating increased susceptibility to chemically induced mammary tumors demonstrated in animals with compromised pineal function, and the increased number of tumors per tumor-bearing animal after exposure to ELF electric fields, suggest that further work in the area is needed.

It is now important to determine the relative importance of the electric and magnetic components of the fields in altering pineal function. The effects of changes in variables such as exposure duration, magnitude, waveforms, and relative phase of the fields should be studied. Initial data suggest that these effects are manifest in some individuals and not in others. Further, the physiological significance of these effects is not well understood. Research is also required to determine what factors, including possible genetic and environmental influences, may predispose certain individuals to exhibit an endocrine response to ELF field exposure.

Galen is credited with the observation, in the second century B. C., that melancholy women seemed at greater risk of cancer than women with sanguine dispositions. Galen also left us with the first recorded description of the pineal gland. We do not know if Galen associated pineal function with the occurrence of cancer. Nevertheless, two millenia after he recorded them, his observations would appear to be relevant to a central question regarding the possible health effects of ELF field exposure.

ACKNOWLEDGMENTS

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