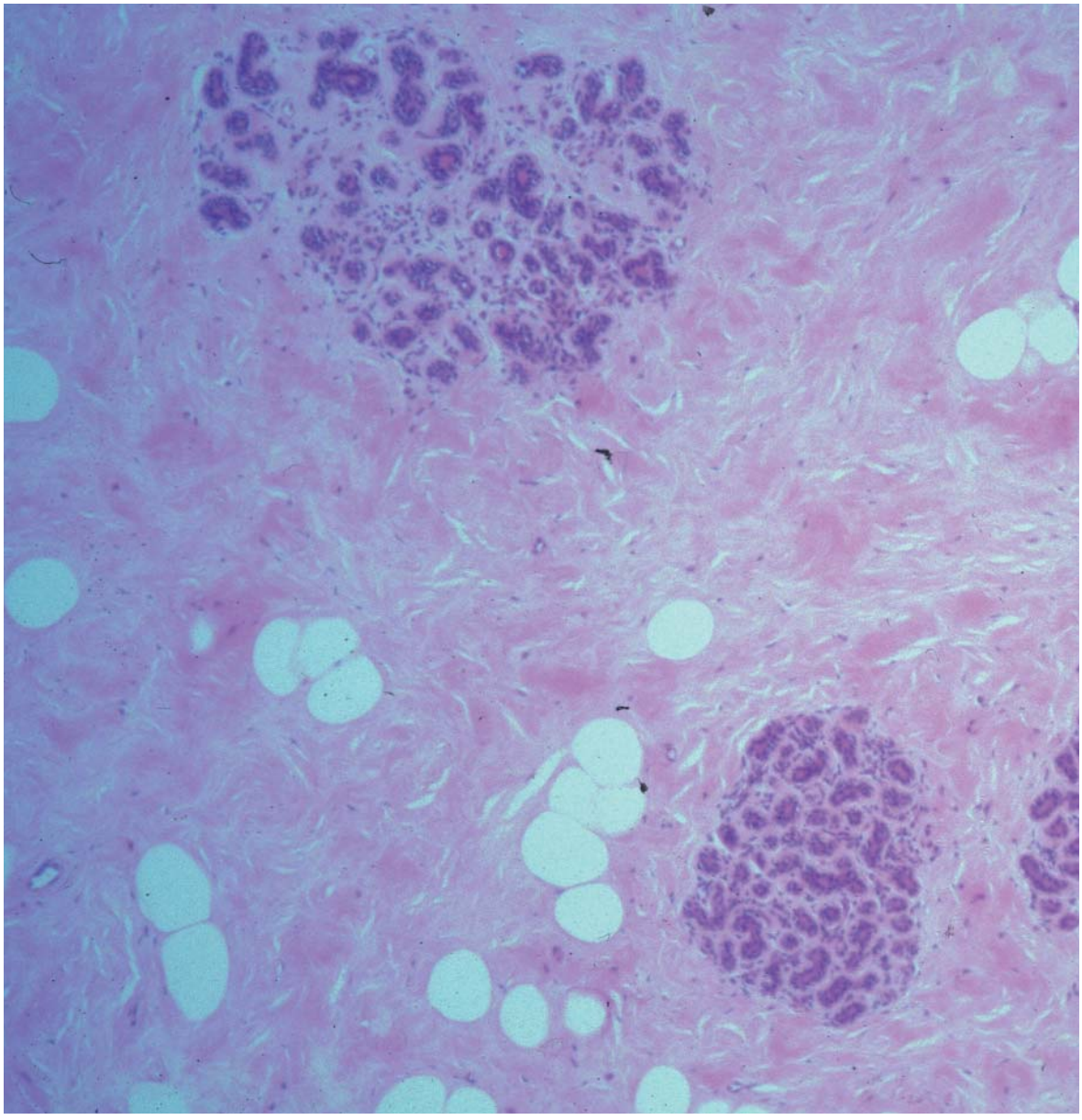
Health Protection Agency

Power Frequency Electromagnetic Fields, Melatonin and the Risk of Breast Cancer

Report of an independent Advisory Group on Non-ionising Radiation



Cover illustration: photomicrograph of the breast tissue of a premenopausal woman showing groups of normal resting lobular alveolar units within fibrous tissue and fat.

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Documents of the Health Protection Agency
Series B: Radiation, Chemical and Environmental Hazards
February 2006

Contents

Foreword

Until 1 April 2005, the National Radiological Protection Board (NRPB) had a statutory responsibility for advising UK government departments on health effects and standards of protection for exposures to ionising and non-ionising radiations. This responsibility now lies with the Radiation Protection Division (RPD) of the Health Protection Agency (HPA).

In 1990, to provide support for the development of advice on non-ionising radiations, the Director of the NRPB set up an Advisory Group on Non-ionising Radiation with terms of reference:

> 'to review work on the biological effects of non-ionising radiation relevant to human health and to advise on research priorities'

The Advisory Group was reconstituted in 1999 as an independent body and now reports directly to the Board of the HPA. Its current membership is given on page 3 of this report. For details of its current work programme, see the website www.hpa.org.uk.

The Advisory Group has, to date, issued a number of reports concerned with exposures to electromagnetic fields. It has considered their possible association with an increased risk of cancer, including childhood leukaemia. It has also reported on health effects related to the use of visual display units, on neurodegenerative disease and on corona ions and increased particle deposition near power lines. The Advisory Group has also considered the potential health effects of radiofrequency fields. Details of publications by the Advisory Group are given in an appendix.

In this report the Advisory Group considers the available scientific evidence from studies with humans, animals and cells relating to power frequency electromagnetic fields, melatonin and the risk of breast cancer.

Power Frequency Electromagnetic Fields, Melatonin and the Risk of Breast Cancer

Report of an independent Advisory Group on Non-ionising Radiation

Chairman Professor A J Swerdlow

This report from the independent Advisory Group on Non-ionising Radiation reflects understanding and evaluation of the current scientific evidence as presented and referenced in this document.

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Acknowledgements

We would like to thank Professor C Blakemore (University of Oxford) and Professor M Dowsett (Institute of Cancer Research, London) for contributing towards the early work of the Subgroup.

Summary

Exposure to power frequency electromagnetic fields (EMFs) is ubiquitous in modern life. The hypothesis that chronic exposure to EMFs may increase the risk of breast cancer, via a reduction in secretion of the hormone melatonin from the pineal gland, was first made almost 20 years ago, and has led to a great deal of research. To review this hypothesis, this report addresses evidence on three issues, namely, whether:

(a) EMFs affect the production or action of melatonin,
(b) melatonin affects the risk of breast cancer,
(c) EMFs affect the risk of breast cancer.

Investigations using cells, animals and humans have not given consistent or convincing evidence that EMF exposure affects melatonin production or action. However, there are deficiencies in the existing research, which leave open the possibility of an effect.

There is stronger evidence that melatonin can inhibit the growth of cancer cells in laboratory culture and in animals. Data on the possible relation of melatonin levels to risk of subsequent breast cancer in humans are limited and inconclusive. Studies investigating the effect of light exposure (which affects melatonin) on breast cancer risk in humans have given some evidence for an association, but left it unclear whether, if there is an association, it is causal in nature.

There is no consistent evidence, from research using cells, animals and humans, that EMF exposure is a cause of breast cancer, nor has any mechanism for such an association been demonstrated.

The report concludes with recommendations for further research.

Overall, the evidence that melatonin, and the timing and extent of light exposure, may affect breast cancer risk is intriguing but not conclusive. In aggregate, the evidence to date does not support the hypothesis that exposure to power frequency EMFs affects melatonin levels or the risk of breast cancer.

1 Introduction

Exposure to power frequency electromagnetic fields (EMFs) is ubiquitous in modern life. Sources of exposure include the national grid system, the local electricity supply network, and mains wiring in homes, offices and other buildings. In addition, EMFs are produced by all machines, appliances and devices powered by electricity: electric fields are related to voltage differences, whereas magnetic fields are associated with the flow of electric current.

In recent years there has been concern about possible health effects of exposure to EMFs arising from the electricity supply system (50 Hz in the UK) and in particular about a possible increased risk of childhood leukaemia. This has been a public issue in many countries and been the subject of a number of national and international reviews (eg Ahlbom et al, 2000; IARC, 2001).

The suggestion has also been made that exposure to EMFs may increase the risk of breast cancer. This was first made almost 20 years ago, and has led to a great deal of research: melatonin, a hormone produced by the pineal gland in the brain, has been suggested as playing a pivotal role in this.

The purpose of this report is to consider the available scientific evidence from studies with humans, animals and cells relating to power frequency EMFs, melatonin and the risk of breast cancer. Specifically, it has addressed three questions, namely, whether:

(a) EMFs can affect the production or action of melatonin,
(b) melatonin can affect the risk of breast cancer,
(c) EMFs can affect the risk of breast cancer.

1.1 Background to the report

Melatonin is a hormone produced by the pineal gland in a distinct daily rhythm governed by daylength. Levels of melatonin in the blood are very low during the day and are elevated at night in all animals, including humans (Arendt, 1995). Melatonin has been shown to influence the control of daily activities such as the sleep/wake cycle and seasonal rhythms such as those of reproduction in animals that respond to daylength. It has been found to diminish the effect of some chemical carcinogens on mammary tissue in animal experiments and to reduce the rate of growth of human breast cancer cells in culture.

Cohen and colleagues first proposed the hypothesis that diminished function of the pineal gland may promote the development of human breast cancer (Cohen et al, 1978). In addition, Stevens (1987) suggested that chronic exposure to electric fields (or to visible light at night) may reduce melatonin secretion by the pineal gland and so increase the risk of breast cancer.

Taken together, these ideas form the basis of the so-called melatonin hypothesis, which attempts to link electrical power use with increased risk of breast cancer via melatonin. This hypothesis has aroused wide interest and attention and has stimulated considerable experimental and epidemiological research.

Given the diversity of effects caused by melatonin and the complexity of its actions, long-term changes in this hormone might have wide ranging consequences for health. It has been proposed that these might include altered incidence of solid tumours or leukaemias, as well as effects on ageing and neurodegenerative diseases. While these endpoints are of potential interest, most melatonin-related EMF research has been focused on breast cancer.

As part of a previous comprehensive evaluation of the potential of power frequency EMFs to cause cancer, the Advisory Group on Non-ionising Radiation (AGNIR) concluded that melatonin rhythms were not substantially affected by exposure to magnetic fields (AGNIR, 2001), although the preliminary data from one investigation suggested some effects may occur, possibility in a sensitive subgroup of the study population. Since the publication of that report, further studies have been published relevant to an understanding of possible effects of EMFs on melatonin. As a consequence, the Board of the NRPB asked the Advisory Group to examine the evidence related to effects of EMFs on the production of melatonin and to advise on whether this could be implicated in the development of breast cancer.

1.2 Structure of the report

The report has been written in eight chapters. Following this introduction which provides an overall background and perspective to the report, the next two chapters give further background information important to the interpretation of the epidemiological and experimental evidence.

Chapter 2 describes the main physical characteristics of EMFs and common sources of exposure. The likely size of the fields in various residential and occupational settings is considered, as are fluctuations in exposure that occur during the day and night. Exposure to either an electric or magnetic field will induce electric fields and currents in biological tissues that depend on the magnitude of the external field.

Chapter 3 reviews the basic physiology of the pineal gland and its role in the production and secretion of melatonin into the blood. The roles of melatonin in reproductive and other seasonal functions that depend on the response of organisms to the length of the day are discussed as well as those such as sleep activity that have a near 24 hour period (circadian rhythm). The characteristics of melatonin production in humans and the effects of melatonin treatment are also described.

In addition, there is compelling evidence that hormones substantially influence the development of breast cancer. This evidence is reviewed in Chapter 3 together with information on the possibility that melatonin may mediate or interact with the mechanism of sex hormone action. A range of other possible mechanisms have been postulated whereby melatonin could influence cell growth and differentiation, through changes in the communication between cells, and in the immune system. These possibilities are also reviewed, as are melatonin receptors and their pharmacology.

The next three chapters consider the experimental and epidemiological evidence for each of the three main themes of the report. The experimental studies are further divided into investigations using cultures of cells (*in vitro* studies), studies using animals (*in vivo* studies), and studies using human volunteers.

Chapter 4 considers the effect of EMFs on melatonin production and action. If exposure to EMFs is to influence the development of cancer through an effect on melatonin secretion, then an understanding of how such fields may influence melatonin is essential.

The *in vitro* evidence divides into two types of study: those investigating effects on the production of melatonin by cultures of pineal cells, and those investigating effects on the action of melatonin on cells. Most *in vivo* studies have used rats and mice, although some studies have used Djungarian hamsters. Very few data have been obtained using non-human primates. Laboratory-based studies in humans investigating the effects of exposure to magnetic fields are described. Few of these studies, however, have used appropriate control procedures or monitored conditions sufficiently to enable the potential of magnetic fields to be properly assessed. Only a few epidemiological studies on EMF exposures and melatonin have been published. These are mainly concerned with melatonin levels in relation to residential exposures in women, and occupational exposures in both women and men.

Chapter 5 explores the relationship between melatonin and risk of breast cancer. As indicated above, the potential relationship between melatonin and the development of cancer is a subject that has aroused much interest after early work suggesting that pineal secretions decreased the rates of cell proliferation and transformation. *In vitro* studies of melatonin and breast cancer that have focused on this aspect are reviewed: more than 60 *in vitro* studies have been published, with the vast majority using the same breast cancer cell line (MCF-7) that was first described in 1973.

The potential of melatonin to modulate the incidence and growth of mammary tumours in various animal models of breast cancer is also reviewed. Most of these studies have assessed the effects of melatonin treatment on the growth of chemically induced mammary tumours; however, a few studies investigated effects on transplantable tumours, while others used normal and transgenic mouse strains that express a high spontaneous incidence of mammary tumours. Further studies are described that investigated the effects caused by inducing changes in pineal function either by removing the pineal gland (pinealectomy) or by altering the apparent day length.

Studies assessing melatonin secretion in breast cancer patients and in individuals without cancer are also reviewed in Chapter 5. These studies are difficult to interpret because the presence of breast cancer may have affected melatonin levels, and also because many of the studies lack control of potentially confounding factors that may affect melatonin secretion. Two cohort studies have given data on risk of subsequent breast cancer in women for whom information was available on urinary melatonin metabolite levels; these are reviewed. Epidemiological studies of several groups thought to have an unusual extent or timing of light exposure, as for instance shift workers or blind women, are also reviewed for evidence that might be relevant to the melatonin hypothesis. None of these latter studies, however, has furnished any data directly on melatonin levels.

Chapter 6 considers whether EMFs can affect the risk of breast cancer. Previously, the Advisory Group (AGNIR, 2001) concluded that there was no convincing evidence that exposure to EMFs at levels likely to be encountered directly damaged DNA (ie was genotoxic) or that EMFs could bring about the transformation of cells in culture. EMFs were therefore considered unlikely to initiate cancer. Since the completion of that report, some *in vitro* studies specifically relevant to breast cancer have been published; these newer studies are reviewed.

Although limitations exist in using carcinogen-induced rodent models of breast cancer, they are a widely used assay, and a number of animal studies are described which have used such models to study the effects of magnetic fields on mammary tumour development.

The final part of Chapter 6 considers the relation of EMF exposure to risk of female breast cancer. This includes the studies of breast cancer risks in women in relation to residential proximity to electricity transmission power lines, and to use of electric blankets.

The principal conclusions of the Advisory Group are given in Chapter 7, and the report finishes with recommendations for further research in Chapter 8. A glossary of less familiar scientific terms is also included.

1.3 References

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2 Sources, Measurements and Dosimetry

The aim of this chapter is to describe the main physical characteristics of power frequency electromagnetic fields (EMFs), the common sources of exposure, and to consider aspects of exposure that might be relevant to the melatonin hypothesis. Exposures at work and in the home are considered at the end of the chapter.

2.1 Characterisation of power frequency EMFs

Power frequency EMFs consist of electric and magnetic fields. The electric field describes the force created by electric charges, and the magnetic field describes the force caused by moving charges in the form of electric current. In the UK the fields alternate at a frequency of 50 Hz, the frequency of the electricity supply. The unit of electric field strength is normally volts per metre (V m^{-1}) or kilovolts per metre (kV m^{-1}). Magnetic field strength is usually measured in units of ampere per metre (A m^{-1}). A related quantity is magnetic flux density, measured in tesla (T), millitesla (mT), microtesla (μT) or nanotesla (nT). The other unit frequently seen in the literature is milligauss (10 mG = 1 μT).

EMFs are vector quantities, with a direction and magnitude. The fields can be linearly or elliptically polarised, depending on the arrangement of source current. Figure 2.1 shows the polarisation ellipse, which describes the rotation of the magnetic flux density vector (**B**) generated by sources of electric current. A single source current produces a linearly polarised field vector that oscillates in a discrete direction in space. Several current sources with different phases produce an elliptically polarised vector, which rotates in space. The fields in homes tend to have limited elliptical polarisation (Swanson, 1999). Those from power lines, however, have varying degrees of ellipticity depending on the source current arrangement.

In general, the electricity supply produces undistorted sinusoidal waveforms. However, in some situations the EMFs may contain additional frequency components called harmonics, which are multiples of the fundamental frequency.

2.2 Sources of power frequency EMFs

The sources of power frequency EMFs are divided broadly into those produced by natural processes and those generated by human activity. Naturally occurring EMFs arise from electrical processes associated with the Earth and the atmosphere. In most environments the dominant source of exposure is that associated with the generation, transmission and use of electricity. People are exposed directly through the use of electrical appliances or equipment, or incidentally through working close to heating systems and power supplies.

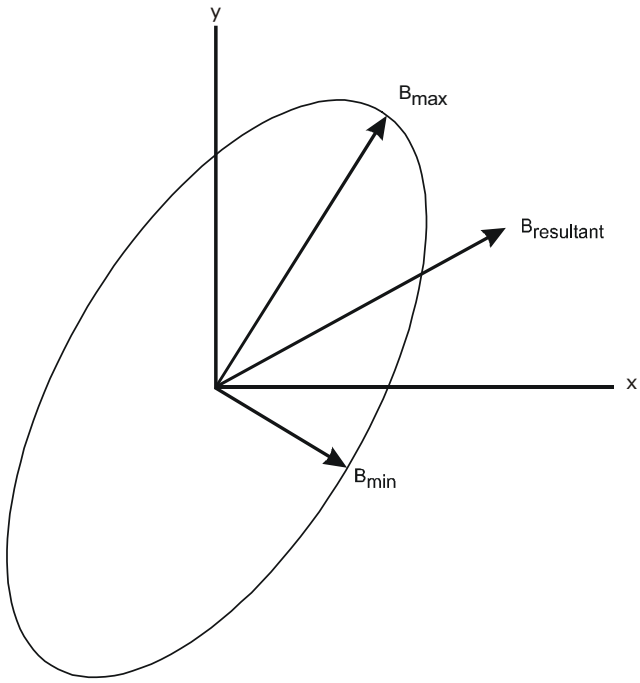

FIGURE 2.1 The polarisation ellipse describes the rotation of the magnetic flux density vector (B) that is produced by different sources of electric current. In this figure the ellipse lies in the x–y plane. More generally, the field will have orthogonal components

2.2.1 Natural sources

2.2.1.1 Electric fields

Processes in the atmosphere and magnetosphere produce a wide range of signals with frequencies reaching up to several megahertz (1 MHz = 10^6 Hz). Extremely low frequency variations arise mainly from the effects of solar activity in the ionosphere and atmospheric effects such as lightning discharges that cause the resonant oscillations in the Earth–ionosphere cavity known as Schumann resonances. The natural electric field strength at the power frequency of 50 or 60 Hz is about 10^{-4} V m^{-1} (EC, 1996).

2.2.1.2 Magnetic fields

The Earth's magnetic field changes continually at periods ranging from a few milliseconds up to 10^{12} years. The Schumann resonances produce magnetic fields around 10^{-5} μT at frequencies of 6–60 Hz. The measurement of signals with frequencies below 100 Hz is extremely difficult because of the interference from man-made signals. At 50/60 Hz the natural magnetic field is typically around 10^{-6} μT (Polk, 1974).

2.2.2 Man-made sources

The intensities of man-made EMFs at power frequencies usually far exceed those produced naturally. The principal sources are high voltage transmission lines, and electrical appliances and equipment used in industry and the home. Although the demand for electrical devices in modern society has led to increasing use of electricity, exposures have not necessarily increased in the same way, and in some situations new technologies have resulted in lower exposures.

2.2.2.1 Electric fields

High voltage (HV) power lines produce some of the largest electric fields encountered in the environment. In the UK, HV transmission lines operate principally at voltages of 400 kV and 275 kV and the distribution lines work at voltages from 132 kV down to 0.4 kV.

The strength of the electric field produced by a power line depends primarily on the operating voltage, the conductor size and geometry, the number of circuits, the phase arrangement, and the distance from the line. Maximum field strengths up to several kilovolts per metre are encountered at ground level below the highest voltage lines. For example, a 400 kV dual circuit transmission line can produce 11 kV m^{-1} near to ground level at the lowest point of the conductors; however, in practice, due to conservative design margins, the levels are usually no more than a few kilovolts per metre. The fields decay with increasing distance, depending on how the phase conductors are arranged. Electric field strengths of a few hundred volts per metre are encountered a few tens of metres from an HV transmission line, decreasing to a few tens of volts per metre at 100 m. In general, the fields become weaker as the operating voltage decreases; field strengths near the ground beneath distribution lines range typically from a few tens to a few hundreds of volts per metre.

The strongest electric fields inside homes are usually encountered close to the surfaces of domestic appliances, light fixtures and supply wiring. Electric field strengths up to several kilovolts per metre have been measured at the surface of certain appliances, although maximum field strengths of no more than several hundred volts per metre are more typical. The high fields near to appliances are usually very localised and field strengths weaken typically to a few tens of volts per metre at distances of a few tens of centimetres. Appliances with electric motors produce a broad spectrum of signals, with typical emission maxima near to 10–20 kHz and field strengths around 20 V m^{-1} (EC, 1996).

Electric fields are readily perturbed by conducting objects, including building materials and people, and the levels encountered in homes are very variable. The electric field strength inside homes away from appliances and electrical wiring is typically in the range 0–20 V m^{-1} (Swanson, 1999). In a pilot study carried out as part of the UK Childhood Cancer Study (UKCCS), arithmetic mean values of 13 V m^{-1} (standard deviation, SD 14.2) and 10.5 V m^{-1} (SD 13.1) were recorded for the bed and centre of the family living room, respectively (UKCCS Investigators, 2002). Inside buildings near to transmission lines, the electric fields are heavily attenuated, usually by a factor of between 10 and 1000 depending on the structure of the building. In these situations, the highest fields associated with the line, usually encountered close to open windows, are typically of the order of a few hundred volts per metre.

Electricity substations are another source of electric fields, although those encountered at the boundaries of substations are usually very weak due to effective screening. These are less than a few volts per metre

and certainly no more than a few hundred volts per metre near the largest installations. Further away from substations, connecting overhead power lines become the main source of electric field exposure. Electric fields from buried cables are negligible due to the shielding provided by the cable sheath and ground material (Maslanyj, 1996).

2.2.2.2 Magnetic fields

Common sources of exposure

There are many different man-made sources of magnetic fields in the environment. Figure 2.2 shows the pattern of magnetic field in a typical home in the UK (Swanson, 1999). The background levels generally arise from power lines and net currents in distribution circuits and conducting services (Merchant et al, 1994; Swanson, 1999). In some circumstances, currents flowing in the wiring inside the home can be important (Maslanyj et al, 2005).

Some of the largest magnetic fields encountered in homes are produced by appliances, and the fields they produce have been reviewed extensively elsewhere (Gauger, 1985; Preece et al, 1997; Maslanyj and Allen, 1998; AGNIR, 2001; ICNIRP, 2003). The strength of the field from an appliance is determined by the magnitude of the current used, the size and shape of conducting parts, the number of turns of wires in coils, and whether any shielding has been included in the design. Magnetic fields up to a few thousand microtesla can occur close to the body when using small appliances such as electric shavers. More

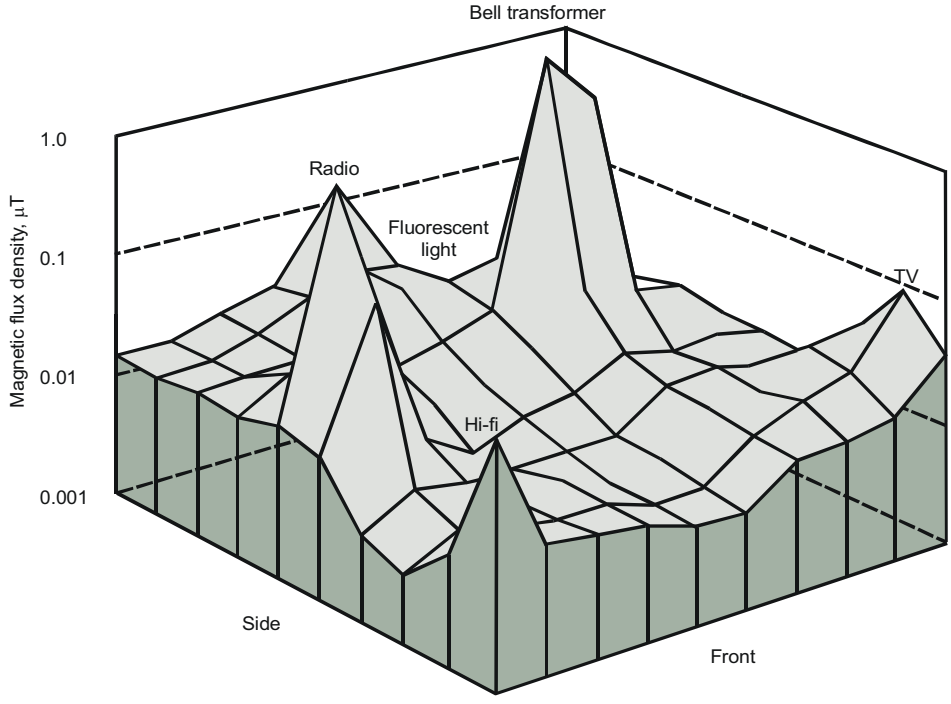

FIGURE 2.2 Power frequency magnetic field measured on a 1 m grid at 1 m above ground floor level in a typical home in the UK. After Swanson, 1999

typically, maximum fields are rarely more than a few hundred microtesla and the levels usually fall with distance, becoming negligible within 1–2 m or so. Large electrical appliances often produce smaller but more extensive fields because of the position and structure of the field source, usually the transformer, inside the equipment. Preece et al (1997) reported mean magnetic fields of 0.26 and 0.07 μT at 50 cm and 1 m, respectively, in front of TV sets in the UK.

HV power lines are potentially important sources of magnetic fields in homes located near to them. The main determinants of the strength of the field are the load current(s), the relative phasing of circuits, the spacing of conductors, and the distance from the line. Maximum fields up to a few tens of microtesla can occur close to the ground beneath the largest HV lines operating at maximum rating in the UK. However, because in practice they normally operate below rated conditions, the levels actually encountered rarely exceed more than a few microtesla. The fields weaken with distance to give typical values of a few tenths of a microtesla at distances of several tens of metres from the lines. In general, the maximum field decreases as the operating voltage decreases, because currents and conductor separations become progressively smaller.

HV underground cables can result in larger accessible magnetic fields than overhead power lines because they can be approached more closely at ground level. A buried 400 kV cable operating at 2 kA can produce fields in excess of 100 μT at 1 m above ground level directly over the cable. However, because the phase conductors are spaced more closely than for the equivalent overhead line, the field falls more rapidly, approaching background levels within a few tens of metres, and the region of elevated field associated with the cable is more restricted. The HV cables that operate at lower voltages tend to have phase conductors that are enclosed in a single metallic casing, which produce much weaker fields due to more efficient field cancellation – usually no more than a few tenths of a microtesla close to the ground.

Electricity substations are a common source of magnetic fields. Maximum fields of a few microtesla have been encountered at the boundary of the local area substations that serve residential areas in the UK, and the levels fall rapidly to become indistinguishable from normal domestic background, usually within a few metres. The maximum fields tend to occur opposite the feed pillar, transformer and switching units of the substation, and continue along the connecting power cables and lines (Maslanyj, 1996).

Sources of exposure in the workplace

Sources of exposure to power frequency EMFs in the workplace have been reviewed extensively elsewhere (Allen et al, 1994; AGNIR, 2001; Cooper, 2002; ICNIRP, 2003).

The main occupational environment where strong electric fields are encountered is the power industry, and particularly in electricity generation and transmission. Electric field strengths in the range of a few volts per metre to several kilovolts per metre have been reported close to electricity generating equipment at power stations in the UK (Cooper, 2002); higher levels may be encountered at specific locations in substations.

In general, data on electric fields in the workplace are sparse because many industrial processes rely on large currents rather than high voltages; thus most of the interest has been in magnetic fields. The industries most commonly associated with high magnetic fields are the electrical utilities, electrically powered transport systems, and those where the fields generated are integral to the processes used in the electrochemical, welding and induction heating industries (ICNIRP, 2003).

The main sources of magnetic fields in power stations are the HV power line conductors, where fields in excess of 1000 μT have been reported. Bus bars and switchgear produce a few tens to a few hundred microtesla, and generators and alternators at power stations produce fields between a few tens and a few hundred microtesla (Cooper, 2002).

Mainline electric trains in the UK have an overhead 25 kV, 50 Hz supply, and on-board rectification can give rise to alternating components in the static or 'quasi-static' field in the traction parts of the train. Magnetic fields up to 50 μT have been measured on a train, most likely related to the layout of the auxiliary power supplies (Allen et al, 1994).

The rectification processes used in the electrochemical industry can produce harmonics up to several hundred hertz at multiples of the power frequency. Fields up to several hundred microtesla have been measured close to rectifier banks and ambient levels of several microtesla have been measured in the areas around cells.

In the UK, AC welding equipment usually operates at 50 Hz. Magnetic fields of more than 1000 μT have been measured at the surface of a welding cable and in excess of 100 μT close to the power supply, and harmonics are often present (Allen et al, 1994).

Induction heaters operating at frequencies of a few tens of hertz are used for volume heating of metals. Power frequency fields of several hundred microtesla have been reported close to a 6 MW copper billet (Allen et al, 1994). Arc furnaces that are used for processing metal can operate at fundamental frequencies of a few tens of hertz and waveforms can have significant harmonic components. Fields of a few hundred microtesla have been measured within a few metres of this type of furnace (Cooper, 2002).

There is a range of other industrial sources that produce power frequency magnetic fields. Pulsed magnetic fields are used commercially to permanently magnetise small ferromagnetic components. The procedure often relies on charging and discharging through a coil, and the pulse produces an infinite range of frequencies. Peak fields of several millitesla can be produced at the coil, falling to several hundred microtesla within a few centimetres (Cooper, 2002).

Crack detection systems use AC or DC power supplies, and the latter can produce a rectified current that may contain harmonics of the fundamental power frequency. The current is delivered continuously or in pulses. Some systems incorporate a demagnetising function whereby an alternating current is gradually reduced to zero amplitude over a period of time. Static and time-varying fields of a few tens of millitesla occur at positions occupied by personnel whilst operating the equipment (Cooper, 2002).

Tape erasers, or degaussers, typically use a magnetic field to erase data stored on tapes or other magnetic media. Maximum magnetic fields of a few millitesla can occur at a few centimetres from such devices, falling to a few hundred microtesla within a few tens of centimetres, and falling again to several tens of microtesla at the trunk of the operator (Allen et al, 1994).

The magnetic fields produced by electric motors can be quite large, occur over a wide range of frequencies, and exhibit great spatial variation. Magnetic fields up to a few millitesla have been measured at the surface of electric motors, falling to a few microtesla at distances of a few tens of centimetres (Maslanyj and Allen, 1998).

2.3 Exposure assessment

An exposure assessment might be carried out for a variety of reasons, including the testing of compliance with exposure guidelines, identifying a hazard in an occupational setting, or for research purposes. The main design aspects of an assessment are summarised in Figure 2.3.

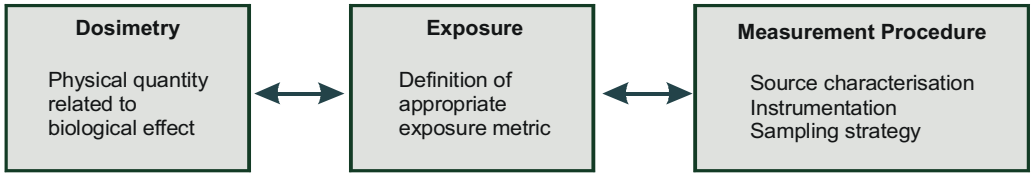

FIGURE 2.3 Main design aspects of an EMF exposure assessment

2.3.1 Dosimetry

The first consideration in an exposure assessment is to determine what physical quantity inside the body is of interest. Ideally this is the dosimetric quantity that can be related directly to a biological effect. Under existing guidelines from the International Commission on Non-Ionizing Radiation Protection (ICNIRP), the dosimetric quantity for EMFs is the induced current density (ICNIRP, 1998), although there is a growing view that the appropriate quantity should be induced electric field strength (NRPB, 2004). Numerical modelling methods are used to link the internal dosimetric quantity and external magnetic and electric field strengths. These external field quantities are relatively easily measured and used in the first stage of assessing compliance with maximum guideline values.

An exposure metric is a numerical quantity that is used to summarise a particular characteristic of EMF exposure. The metric may combine frequency, time and spatial parameters of the field to which people are exposed, to produce a single summary measurement. In the case of the ICNIRP guidelines the important exposure quantities are the unperturbed root-mean-square (RMS) electric and magnetic field strengths.

It is not known what aspect of exposure (if any) is most directly related to health outcomes, and various other exposure metrics have been used in epidemiological studies, the most common being the peak, cumulative and time weighted average (TWA) exposure – the RMS field value averaged over time. In relation to appliances, the peak exposure will depend on the proximity of the particular source in use, whereas the TWA will tend to reflect the extent and frequency of use. Alternative metrics include the median or geometric mean used to determine the background level when exposures are log normally distributed, time above threshold representing prolonged periods of high field exposure, and others reflecting short-term variations. Some of the alternative metrics may be related closely to the TWA but this is not always the case.

Modern instruments have enabled more sophisticated metrics to be evaluated, reflecting field characteristics such as frequency, harmonic content, ellipticity, direction in relation to the Earth's static field, time variation, rate of change with time (d$\mathbf{B}$/dt), and frequency and magnitude of transients.

Transients are rapidly changing signals that tend to appear at irregular intervals. They contain a broad range of frequencies that may extend into the megahertz range (NRC, 1997). Transients occur in all environments where switching of electrical appliances or equipment occurs and, because they are characterised by a high rate of change of field, they may induce large current densities within individuals (Kaune et al, 1997; Kavet, 1998).

Relatively few studies have assessed exposure to electric fields, partly because of the difficulty of making electric field measurements that properly take into account the perturbing effect of the bodies of subjects (Kaune and Gillis, 1981; Deno and Silva, 1984; Chartier et al, 1985; Kaune et al, 2002). In the UKCCS pilot study of electric fields, the measurements of field strengths were made in the absence of the perturbing effect of people (UKCCS Investigators, 2002).

2.3.2 Measurement procedures

2.3.2.1 Source characterisation

It is important to consider the source operating characteristics in the development of an exposure assessment. Knowledge of the source, and how it is used, facilitates the selection of appropriate instrumentation and the measurements that are necessary. Close to many sources the fields are inhomogeneous and assessing them may require complex measurement procedures and calculations. In some situations, particularly in occupational settings, it may be necessary to capture the waveform in order to identify the range of frequencies that is likely to be encountered. Information about the design and operation of appliances and equipment can usually be obtained from the manufacturer or operator.

2.3.2.2 Instrumentation

The development of sophisticated measurement instruments has played an important role in advancing exposure assessments in EMF research. Some of the meter parameters that need to be taken into account are frequency response, sampling rate, dynamic range, and accuracy. Meters are normally calibrated to a traceable standard and reliability checks may be required during the course of the assessment. Modern meters usually incorporate a digital computer and signal processing circuitry, which allow different metrics to be computed from time-series data, and information about waveforms may also be recorded (Bowman et al, 1998).

Electric field meters

Most electric field meters measure the induced current flowing between two electrically isolated metal plates or shells that are placed in the field. Modern sensors measure simultaneously the RMS orthogonal components of electric field strength, and these may be logged for a specific time interval.

Measuring electric fields presents unique difficulties and the measurements are also difficult to interpret (Kheifets et al, 1997). Common objects in the environment perturb the field. A personal exposure meter will reflect the perturbation of the field by the body. The field recorded is very dependent on where the device is worn, the posture of the subject, and the relative location of any sources. The meter itself perturbs the field and this has to be taken into account in the design, calibration and use of the meter.

Careful procedures are required to ensure that people carrying out the measurements do not affect the sensor.

Magnetic field meters

Magnetic fields are usually measured using coils that are shielded from the effect of electric fields. The field strength is normally determined by measuring the currents induced in three orthogonally mounted sensor coils. In contrast to electric fields, any field perturbation is minimal.

2.3.2.3 Sampling strategy

The main purpose of an exposure assessment in epidemiological studies is the classification of study subjects into groups with differing exposures. Exposure is most often characterised either by indirect surrogates of TWA field strength or by direct measures of the field averaged over periods of time typically from a few seconds to many hours (Kaune et al, 2001). In general, two broad measurement approaches are available: spot measurements, in which field meters are placed in locations to reflect those occupied by the subject, and personal monitoring, in which exposure meters are worn by the subject.

The use of spot measurements in epidemiological studies should be approached carefully. Whereas appropriate weighting of spot measurements for children has been found to yield good correspondence with personal exposure (Kaune et al, 1994), a similar approach in a study of adults has explained less than half the variability in the exposure data (Kavet et al, 1992).

Personal exposure meters integrate the general background field with the other fields that might be encountered by the subject whilst performing activities, such as using an electrical appliance. The time variation observed in the data is due both to the temporal variation of the field and to the spatial variation as the subject moves from one location to another. Careful account needs to be taken of the periods when the meter is not worn, the position of the meter on the body, and the variation of the field outside the record period.

A key challenge in most of the studies is how best to use contemporaneous measurements to assess the exposure incurred during some predefined period usually before the diagnosis of disease. An important question is whether a subject's behaviour or work pattern is likely to have changed as a result of the disease, and whether the measurements are likely to reflect previous exposures. Control subjects, if they are more active than cases, could have higher exposures recorded on personal meters, or alternatively cases could be assigned higher exposures if they are less active in a high field area. The measurements may be supplemented with information on lifestyle and working patterns, obtained from questionnaires, which can be used to refine the exposure measure.

One of the limitations of the measurements used in epidemiological studies is that they are acquired over a time interval that may not necessarily characterise the full range of field variation. Long assessment periods tend to increase the cost of the study, consequently a number of studies have utilised surrogates of exposure, relying on exposure conditions being predictable in certain environments. The wire-code surrogate, used widely in the USA (NRC, 1997), is based on power line conductor configuration and distance from the subject's home. The job title surrogate used in

occupational studies relies on the exposure being predictable for certain job types. A number of epidemiological studies have used the load-current data usually available for HV power lines to predict historical exposure.

2.3.2.4 Time variation of power frequency EMFs

An important aspect to consider in relation to the melatonin hypothesis, is the variation of magnetic field strength in time. The variation of the background magnetic field in homes generally follows a diurnal pattern related to electrical power consumption (Silva et al, 1989; Dovan et al, 1993; Kaune et al, 2001; Banks et al, 2002). In general, the highest field levels are observed in the evening and the lowest during the night (Figure 2.4). Banks et al (2002) carried out a detailed investigation of residential 60 Hz magnetic fields in two metropolitan areas of the USA. Measurements over 24 hours were repeated on a regular bi-monthly schedule over a period of one year. A systematic and appreciable diurnal effect was found, suggesting that evening spot measurements overestimate the long-term exposure by 20% or more. The study concluded that 24 hour measurements are likely to produce a fairly reliable estimate of exposure. However, because the study was based on spot rather than personal exposure measurements, the results apply strictly to people who are residentially stable. Ideally, assessments in the home should be made over at least 24 hours (Swanson, 1996). The detailed pattern is not always easy to predict because the background field arises mainly from net currents in the local supply circuits.

Magnetic field levels in homes are not necessarily low overnight, and there are a number of situations where high night-time exposure could occur:

(a) in homes close to electricity cables that supply night-time industry, or in areas where low tariff energy is used overnight,
(b) in homes where energy is consumed overnight through the use of night storage heaters or underfloor heating,
(c) through the overnight use of electric bed-warming appliances such as electric blankets.

An example of night-time elevation of the magnetic field in a home that uses night storage heaters and off-peak demand electricity is shown in Figure 2.5. Many underfloor heating systems draw current during the night, relying on off-peak electricity and the heat capacity of the floor to provide warmth during the day.

Kaune et al (2001) found that residential magnetic fields measured in bedrooms showed no strong weekday–weekend dependence; however, personal exposure data showed that adults at work or out of the house on weekdays incurred higher personal exposures than on weekends.

Seasonal changes in residential magnetic fields have been observed in a number of studies. Banks et al (2002) found a small but significant seasonal effect (−3% to +4%) in homes in the USA. Kaune et al (2001) found that bedroom magnetic fields, and to a lesser extent personal exposure, were substantially larger in winter than summer, suggesting that epidemiological studies should control for the date when measurements are made. The seasonal maximum fields usually coincide with the time of maximum electricity usage, normally in the winter. Deadman et al (1999) made 48 hour personal exposure measurements on 382 Canadian children in five provinces, and found that arithmetic mean exposures

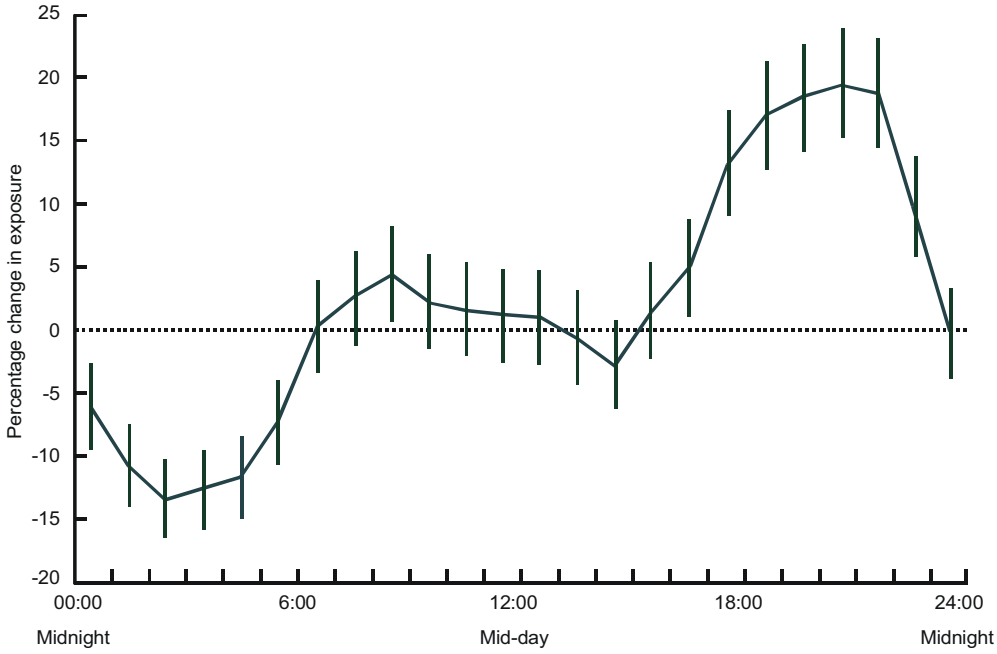

FIGURE 2.4 Typical diurnal variation of the power frequency magnetic field in a home. Values represent hourly arithmetic mean percentage change from the long-term exposure estimate and 95% confidence intervals. Estimates based on spot measurements of the magnetic field in 51 homes. After Banks et al, 2002

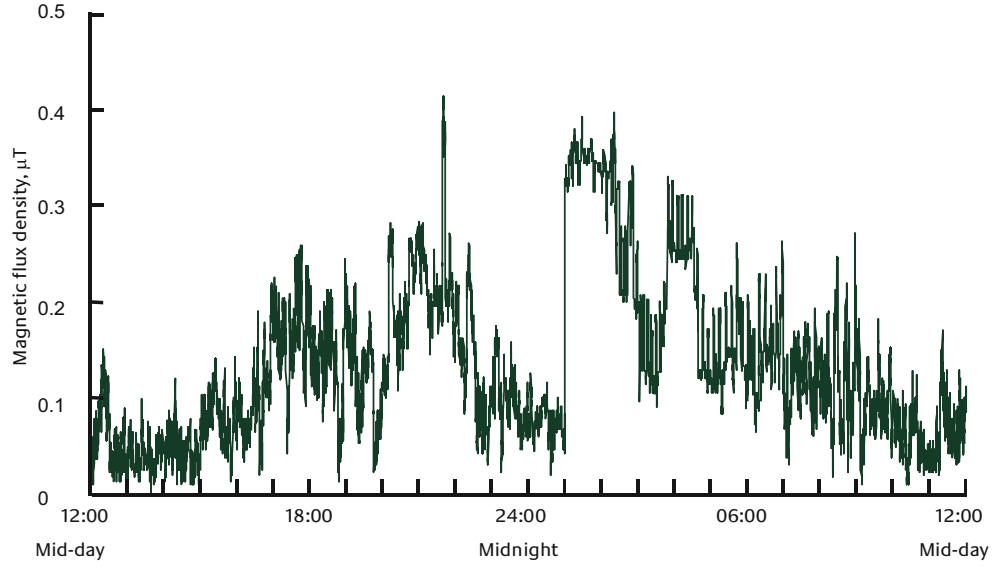

FIGURE 2.5 Variation of the magnetic field in a home that uses night storage heaters and low tariff electricity. The resultant field in the broadband frequency range (40–800 Hz) was measured in a bedroom away from operating appliances. Data from the UKCCS Investigators, by permission

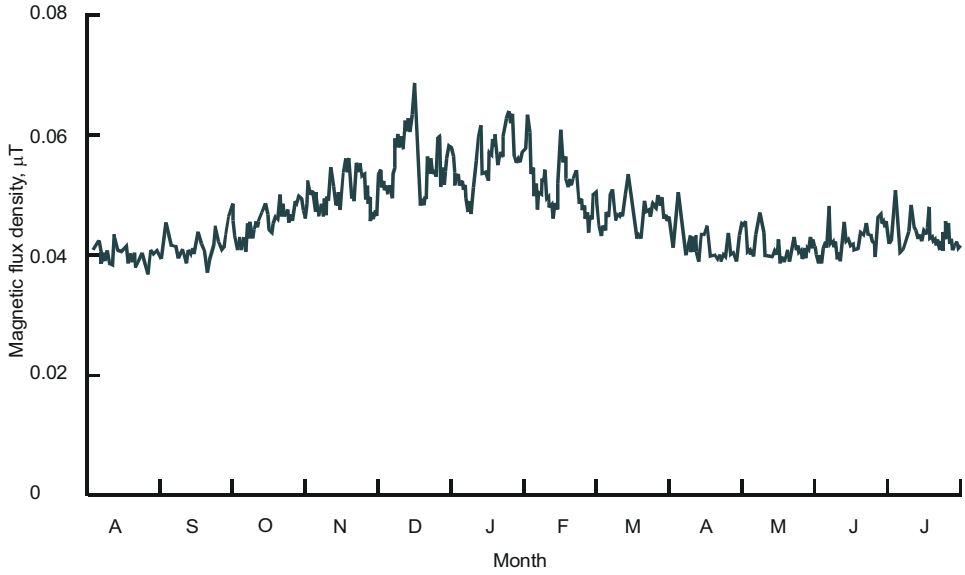

FIGURE 2.6 Seasonal variation of the background magnetic field in a UK home. Values represent the 24 hour average field over a period of one year. After Swanson and Renew, 1994

were higher in winter (November–March; 0.137 μT; 95% confidence interval, CI, 0.114–0.160 μT) than in summer (April–October; 0.109 μT; 95% CI 0.096–0.123 μT). In the UK, background fields in homes vary seasonally (Figure 2.6), broadly following the annual variation of load on the relevant 415 V distribution circuit (Swanson and Renew, 1994).

The load currents on HV power lines have been shown to exhibit diurnal, weekday–weekend, seasonal and other variations, suggesting that contemporaneous short-term measurements in homes near power lines are unlikely to provide good estimates for historical exposure (Kaune et al, 1998).

With the development of logging meters, there has been interest in the short-term temporal variability of magnetic fields and related exposure metrics. In the study carried out by Kaune et al (2001), the short-term variability in personal exposure data was attributable predominantly to the physical movement of a subject through a spatially varying field. The lowest variability in personal exposure was observed when a subject was sleeping, even when night–day temporal variability was similar. The study concluded that spot measurements were unlikely to provide reasonable surrogates for the temporal variability of exposure.

The temporal changes of electric fields in the home are less well documented. In general, because of the voltage stability of the power supply, electric fields vary less than magnetic fields. The changes that do occur depend on the use of specific appliances and circuits inside the home, which are difficult to predict. The average electric field levels in the UKCCS homes apparently varied little over the day, based on measurements of the vertical field component (UKCCS Investigators, 2002).

2.4 Exposure environments

2.4.1 Residential exposure

The background magnetic field levels encountered in UK homes typically fall in the range from 0.01 to 0.1 μT, and average residential exposures above 0.4 μT occur for about 0.5% of the population (AGNIR, 2001). The background field encountered in homes in most countries is usually less than 0.2 μT (Swanson and Kaune, 1999).

HV power lines can be a major contributor to long-term TWA magnetic field exposure (Kavet et al, 1992; Merchant et al, 1994; Forssén et al, 2002), raising exposure levels typically by an order of magnitude. However, averaged over the whole population, HV lines contribute only a small fraction of the collective exposure – for example, 5% of total population exposure in the UK (Swanson, 1999). In a recent study based on the EMF part of the UK Childhood Cancer Study (UKCCS Investigators, 1999; Maslanyj et al, 2005), less than 2.5% of homes had average exposures above 0.2 μT, and HV power lines operating at 275 kV and above explained only 9% of these exposures. Low voltage (LV) sources including currents associated with the supply, and sources inside the home including suspected wiring faults, accounted for most of the exposures. House wiring may produce elevated fields inside the home under certain conditions, for instance, if field cancellation is imperfect, either because live and neutral wires are not close together or because of imbalance between live and return currents.

The contribution of household electrical appliances to exposure is an important factor to consider in the design and evaluation of studies (AGNIR, 2001). Most domestic appliances will not give rise to appreciable additional TWA exposure, because the fields they produce are very localised and the periods of use are relatively short (Delpizzo, 1990).

The proportional contribution of appliances to TWA exposure varies depending on the background field. In the UK, Swanson (1999) found that appliances contribute on average up to one-third of exposure, and between 3% and 50% for the majority of homes. In a study conducted by Mezei et al (2001), mean magnetic fields from various domestic appliances correlated only weakly with mean daily exposure, suggesting that studies focusing on a single appliance or a small number of appliances are unlikely to give a good indication of TWA exposure. The same appliances, however, could be significant determinants of peak and above-threshold exposures.

Appliances can be the dominant source of exposure to body extremities (Mader and Peralta, 1986). However, in the absence of an organ-specific effect, exposure tends to be estimated from fields averaged over the entire body. Fields of a few tens of microtesla reduce to a few tenths of a microtesla or even a few hundredths of a microtesla when averaged over the whole body. The contribution of an appliance field to exposure is often further reduced when averaged over time. Hairdryers can produce intense magnetic fields at 10 cm from the head, compared with ambient levels; however, average use of a few minutes per day means they contribute less than 5% to the average daily exposure (Kaune et al, 2002). Home sewing machines produce magnetic fields in the abdominal area of the body that are elevated by a factor of nearly three over the ambient level; however, based on average daily use, they contribute only about 2% to the daily exposure (Kaune et al, 2002). Kaune et al (2000a) found no statistically significant differences between magnetic fields calculated at each distance for TV sets used for viewing programmes and TV sets used for playing video games. However, children's exposure levels were larger, by a factor of

three, when TV sets were used to play video games rather than watch TV programmes. The major reason for the difference was children sat on average about 75 cm closer to the sets while playing games.

In relation to the melatonin hypothesis, certain appliances, such as electric blankets, night storage heaters and underfloor heating, can contribute significantly to overnight exposure, depending on design and pattern of use (Delpizzo, 1990). Electric blankets operating at 240 V can produce magnetic fields averaged in the region of the body of 0.2 μT for underblankets, and 0.25 μT for overblankets, and the fields are likely to be 2 to 2.5 times higher for appliances designed to operate at 110 V. Underblankets are insignificant sources of cumulative exposure under most realistic conditions of use; however, overblankets designed for all-night use may result in substantial exposure. For water bed heaters, which are designed for all-year-round use, the exposure can vary greatly, depending on the size and position of the heating element. The heaters that are located directly under the user, common in the older designs, can produce body averaged fields of 0.4–0.5 μT (Delpizzo, 1990). Underfloor heating systems operate by way of electric coils that are embedded in floors and connected during off-peak periods, usually overnight. The magnetic fields associated with underfloor heating depend on the configuration and depth of the cables, and the current flowing in them. Typical magnetic fields up to 1.5 μT can occur at floor level falling to a few tenths of a microtesla at 1 m above the floor (NRPB, 2004). In the residential study reported by Delpizzo (1990), concrete slab coil heaters operating at 240 V produced whole-body average fields on a bed of 1–6 μT; a standing adult would be exposed to a whole-body average field of up to 6.5 μT, and a child playing on the floor would be exposed to an average field of 15 μT or more. Systems operating at commercial premises can give up to a few hundred microtesla at floor level falling to a few tens of microtesla at 1 m above the floor (ICNIRP, 2003). As with electric blankets, the TWA exposure will depend on pattern of use.

2.4.1.1 Other frequencies

The frequencies of the ambient magnetic fields in homes are usually the dominant 50/60 Hz component and odd-harmonics whose strengths grow progressively weaker with increasing frequency (Kaune et al, 2000a). For most residential sources, the strengths of the even-harmonic components are markedly smaller than those of the odd-harmonic components (Zafanella, 1993). Some appliances produce different frequency spectra from the ambient fields in homes. TV sets, in particular, contain even-harmonic components (Kaune et al, 2000a), notably the 120 Hz related to the vertical deflection coils and frequency components in the very low frequency range related to the horizontal deflection coils.

There has also been interest in the transient magnetic field events caused by electrical switching in residential and occupational environments. Transients, because of their high frequency content, induce considerably stronger instantaneous currents and electric fields inside the body than similar magnitude power frequency magnetic fields (Kaune et al, 1997). In a study of magnetic transients in the home reported by Kaune et al (2000b), the diurnal pattern of transient activity was similar to that observed for power frequency magnetic fields.

2.4.2 Workplace exposure

Exposure in the workplace has been considered in detail by AGNIR (2001). Most of this information comes from industries where high levels of exposure have been anticipated due to the presence of high

voltages or currents. Studies have also been conducted where health concerns have been expressed about particular appliances such as sewing machines (Kelsh et al, 2003). Most of the information available concerns magnetic fields.

Occupational magnetic field exposure can be more difficult to characterise than residential exposure. The sources may produce a range of field strengths and frequencies, and the extent and pattern of exposure can be complicated. The fields produced by industrial processes can be very large, vary in space markedly and change during operation. Spot measurements may be of limited use in assessing exposure in these situations and personal exposure measurements may provide a better alternative.

Sources such as transformers and electric motors generate fields that tend to decrease with the cube of the distance from the source. The exposure of some organs may be substantially different from that measured by a meter worn at a convenient position. Delpizzo (1990) showed that hip-worn meters consistently underestimate both whole-body average exposure and head exposure. Chest-worn meters were found to provide a generally better measure of whole-body exposure. Exposure may also be difficult to define unless the subject's position is reasonably well described by the task undertaken. Whilst there may be the potential for the maximum exposure of a working subject to be high, the TWA exposure will be determined to a great extent by actual working practice (Kelsh et al, 2000).

The magnetic fields produced by industrial equipment may have non-sinusoidal waveforms and different spectral components to the main power supply. Harmonics can be generated by transformer and motor cores operating under high load conditions, thyristor control of power to equipment, ill-matched oscillator circuits, and AC supply rectification. Pulsed magnetic fields and transients are difficult to measure: the wide frequency range associated with the signals might exceed the instrument bandwidth, and if the instrument's response time is greater than the duration of the pulse or transient, the instantaneous maximum field strength may be much greater than the indicated reading. In such environments it is important to characterise the waveforms and spectral content fully so that appropriate measurement instrumentation can be used (Chadwick, 1997).

Various surrogate schemes based on job titles or occupational task have been used for exposure classification purposes. Recent studies have tended to rely on measurement data linked to job titles, whereby measurements are summarised by occupational categories which represent similar groups of job titles (Kelsh et al, 2000). The measurement studies suggest that job title might not necessarily reflect the true exposure, and may be appropriate only for a few working practices found in certain industries. Large variations in exposure can occur within the same job category, probably due to specific differences in the processes and work practices (van Tongeren et al, 2004). In some cases the work environment is at least as important as the nature of the work. For instance, consistently high exposures might be encountered in a power distribution utility, regardless of the job concerned (Kelsh et al, 2000).

A number of exposure assessment studies of power industry staff have employed integrating exposure meters to estimate mean exposure. In the UK electricity supply industry, Merchant et al (1994) found that transmission staff at substation sites or working on lines encountered the highest fields (geometric mean TWA of 1.16 μT). Power station and distribution workers incurred exposures in the geometric mean TWA range 200–500 μT. Magnetic field exposure has been investigated for a range of industries in a study carried out as part of a UK adult brain tumour study (van Tongeren et al, 2004). Occupational

magnetic field exposure was found to be the main determinant of the overall TWA exposure, which included periods at home and travelling. In many modern office-based environments the average background levels might be similar to residential levels. However, field levels up to several microtesla can be encountered close to copy machines, and prolonged use of such equipment and of visual display units and fax machines can increase long-term exposure.

In relation to the melatonin hypothesis, very few studies have included comprehensive exposure assessments that take into account the timing of exposure. Consideration of exposure during shift work has rarely been considered.

2.5 Summary

Power frequency EMFs are encountered everywhere electricity is used, in and outside the home and at work. The electric field describes the force created by electric charges, and the magnetic field describes the force caused by moving charges in the form of electric current.

The electric fields encountered in homes normally range in strength between zero and several hundred volts per metre. Electric field strengths up to several kilovolts per metre can be encountered close to the surfaces of household appliances, light fixtures and supply wiring. The domestic background level away from appliances and electrical wiring is typically in the range 0–20 V m^{-1}. Maximum field strengths up to several kilovolts per metre can be encountered at ground level under high voltage lines; however, because electric fields are easily screened, levels inside homes are very much lower.

The background magnetic field levels encountered in UK homes mostly fall in the range from 0.01 to 0.1 μT and average domestic exposures at or above 0.4 μT occur for about 0.5% of the population. These generally arise from power lines, and net currents in supply circuits and conducting services. Electrical appliances give rise to the largest magnetic fields encountered in homes. Nonetheless, most appliances do not give rise to appreciable exposures because the magnetic field they produce is localised and the periods of use are short. Maximum fields up to a few tens of microtesla could occur beneath the largest power lines at maximum rating; however, because the lines normally operate below maximum rated conditions, the levels encountered rarely exceed more than a few microtesla.

Information on electric fields in occupational environments is sparse because most industrial processes rely on large currents rather than high voltages; thus most of the interest has been in magnetic fields. The industries most commonly associated with high magnetic fields are the electrical utilities, electrically powered transport systems, and those where the fields are produced directly as part of an industrial process such as in the electrochemical, welding and induction heating industries.

Accurate exposure assessment is one of the main challenges of EMF research. The field levels vary in time and space and exposure can be represented by a number of different metrics. The important dosimetric quantity in the ICNIRP exposure guidelines, is induced current density, and the related exposure metrics are unperturbed RMS electric and magnetic field strengths. It is not known what aspect of exposure (if any) is most directly related to health outcomes, and a range of exposure metrics have been used. Modern instruments allow more sophisticated metrics to be evaluated, reflecting field characteristics such as frequency, harmonic content, ellipticity, field direction, and time variation. An important aspect of power frequency magnetic fields in relation to the melatonin hypothesis, is the variation in time, which

broadly follows the pattern of electricity use, and certain appliances, such as electric blankets, night storage heaters and underfloor heating, can contribute substantially to overnight exposure, depending on design and pattern of use.

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3 The Pineal Gland and Melatonin

A vast amount of data exists concerning the physiology of the pineal gland and its principal hormone, melatonin. Aspects of this literature are summarised and reviewed below. In particular, the characteristics of melatonin production and the factors that affect the synthesis and metabolism of melatonin are described, with emphasis placed on effects of the light–dark cycle. The interactions of melatonin with other hormones, and possible mechanisms whereby melatonin may influence cellular physiology, are also described.

3.1 Basic physiology

3.1.1 Structure of the pineal

The pineal gland (epiphysis cerebri) is a small, unpaired central structure, essentially an appendage of the brain. Great variation in size and position is seen even within species. In humans the pineal weighs around 100 to 150 mg. It assumes a shape resembling a pine cone (hence pineal) and, again owing to its shape, has been referred to as the 'penis of the brain'.

The mammalian pineal gland is a secretory organ, whereas in fish and amphibians it is directly photoreceptive, and in reptiles and birds it has a mixed photoreceptor and secretory function. The extracranial parietal (parapineal, frontal) organ found in some lower vertebrates has been referred to as the 'third eye'. The principal cellular component is the pinealocyte, and elements of its photoreceptive evolutionary history remain in both structure and function. In some species, including humans, calcified lumps are frequently present in pineal tissue after puberty, although this calcification does not appear to be associated with a decline in metabolic activity except in the sense that activity declines in general with ageing. The gland is richly vascularised. Its principal innervation is sympathetic and arises from the superior cervical ganglion. In addition, good evidence has been presented for parasympathetic, commissural, and peptidergic innervation. Its primary function in all species studied to date is to transduce information concerning light–dark cycles to body physiology, particularly for the organisation of body rhythms. This information is encoded in the secretion patterns of the major pineal hormone melatonin (*N*-acetyl-5-methoxytryptamine) (Arendt, 1995; Klein et al, 1997; Korf et al, 1998).

3.1.2 Synthesis and metabolism of melatonin

Melatonin is synthesised within pinealocytes – cell types derived from photoreceptors – from tryptophan via the pathway shown in Figures 3.1 and 3.2. Most synthetic activity occurs during the dark phase, with a major increase (7- to 150-fold) in the activity of serotonin-*N*-acetyltransferase (arylalkylamine *N*-acetyltransferase – AA-NAT – commonly abbreviated as NAT). The rhythm of production is endogenous in that it is generated in the suprachiasmatic nuclei (SCN), the major central rhythm-generating system or 'clock' in mammals (the pineal itself is a self-sustaining 'clock' in some, if not all, lower vertebrates). The

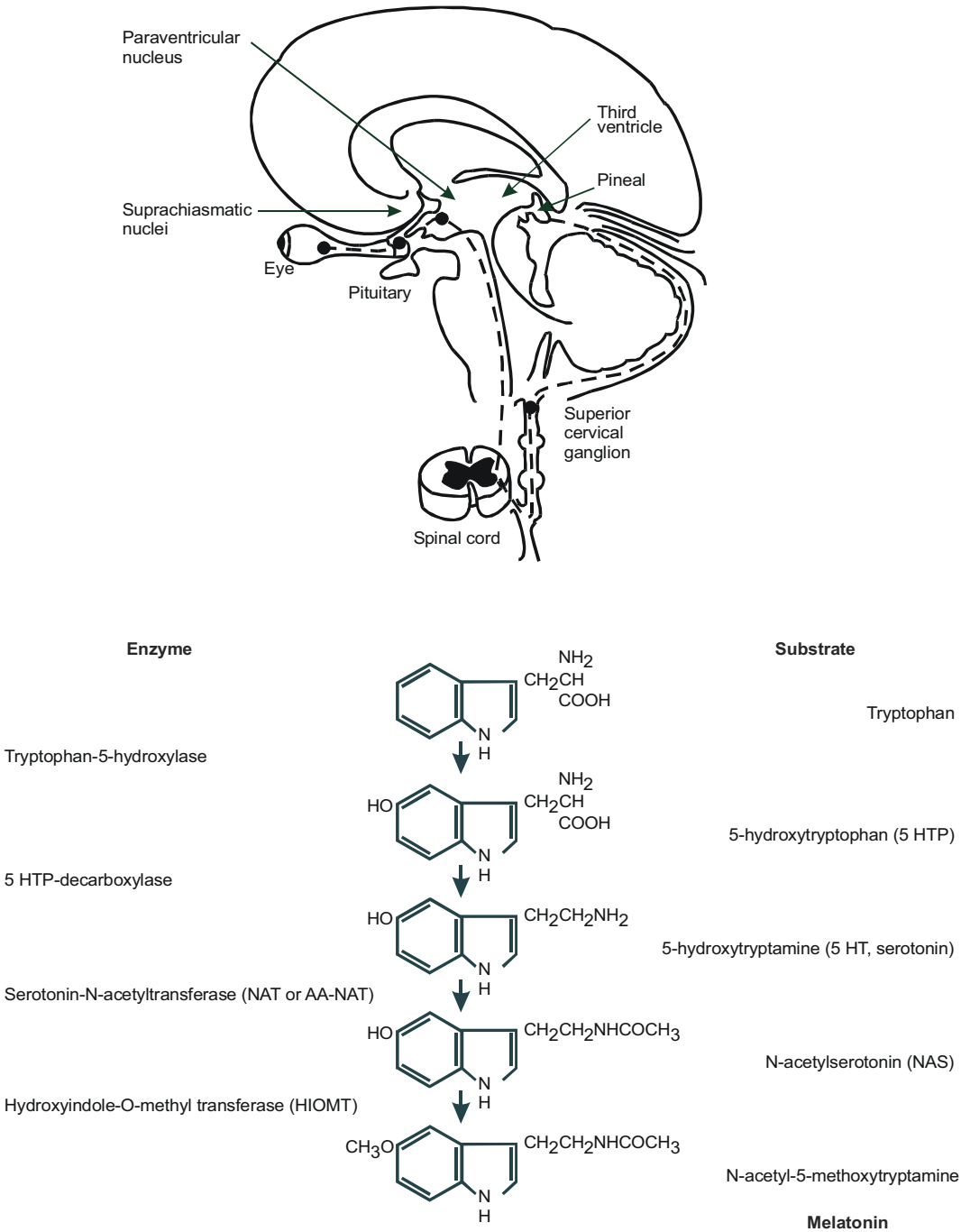
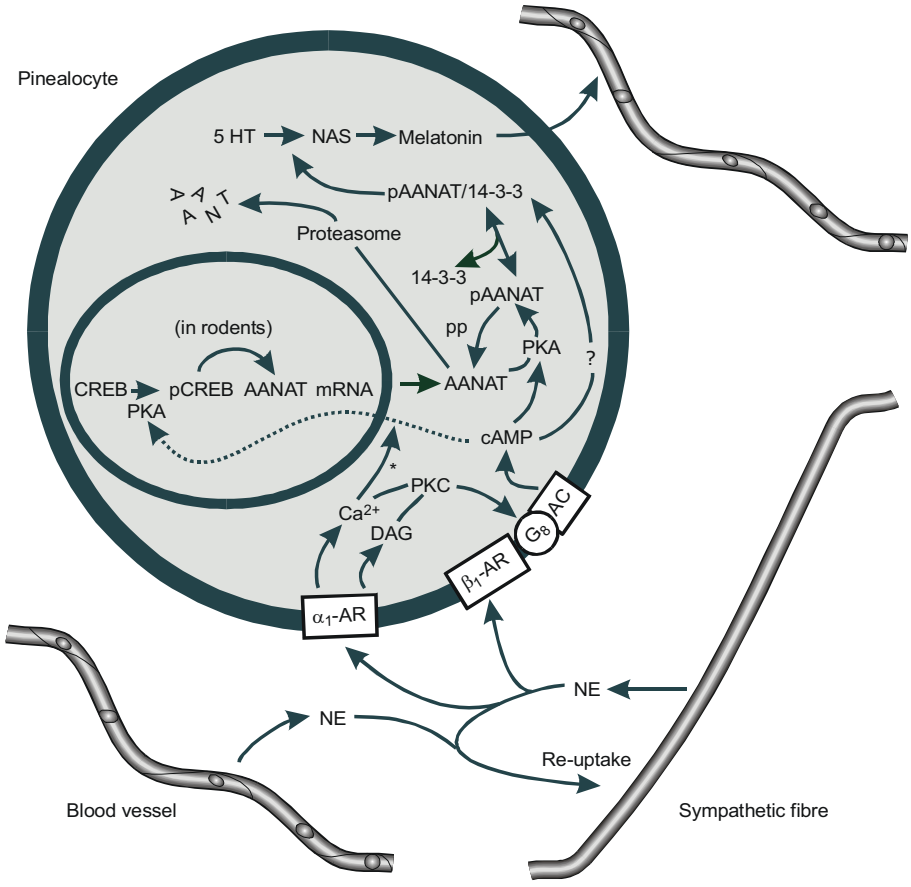


FIGURE 3.1 Diagram of the major sympathetic innervation of the pineal gland (top) and the synthesis of melatonin from tryptophan (bottom). From Tamarkin et al, 1985, by permission



5 HT	5-hydroxytryptamine
AC	adenylate cyclase
AR	adrenergic receptor
AANAT	serotonin-N-acetyltransferase (NAT)
cAMP	cyclic adenosine monophosphate
CREB	cAMP-responsive element-binding protein
DAG	diacylglycerol
NAS	N-acetyl serotonin
NE	noradrenalin (norepinephrine)
PKA	protein kinase A
PKC	protein kinase C
pCREB	phosphorylated cAMP-responsive element-binding protein
pAANAT	phosphorylated serotonin N-acetyltransferase
pAANAT/14-3-3	phosphorylated serotonin-N-acetyltransferase /14-3-3- protein complex

FIGURE 3.2 Sympathetic control of melatonin synthesis in the rodent pineal gland. From Ganguly et al, 2002, by permission

mammalian melatonin rhythm is generated by a closed loop negative feedback of clock gene expression in the SCN, *Clock* and *Bmal* being positive stimulatory elements, *Per and Cry* negative elements, subsequently influencing, clock-controlled genes (Figure 3.3). *Per* and *NAT mRNA* oscillate in the pineal, although post-transcription control is evident in some species. The rhythm is synchronised to 24 hours primarily by the light–dark cycle acting via the retina and the retinohypothalamic projection to the SCN. The cDNAs encoding both NAT and the *O*-methylating enzyme hydroxyindole-*O*-methyltransferase have been cloned, and studies of molecular regulation of melatonin production show some species differences. It is likely that the human enzyme is regulated primarily at a post-transcriptional level, whereas in rodents the key event appears to be cyclic adenosine monophosphate (cAMP)-dependent phosphorylation of a transcription factor that binds to the NAT promoter. Rapid decline in activity with light treatment at night appears to depend on proteasomal proteolysis of NAT following dephosphorylation and removal of a protective 14-3-3 protein (Klein et al, 2003; Simonneaux and Ribelayga, 2003).

Phosphorylation of the transcription factor CREB (cAMP-responsive element-binding protein) appears to be an important step in the signal transduction cascade that activates melatonin biosynthesis in the mammalian pineal organ. According to distribution studies of NAT mRNA, this enzyme is expressed in the pineal gland, retina and, to a much lesser extent, some other brain areas, the pituitary, and the testis, but, apart from the pineal, these structures contribute little to circulating concentrations in mammals. There is some evidence for high melatonin levels (one to three orders of magnitude higher than in plasma) and synthesis in bone marrow, the Harderian gland and the gut (Djeridane et al, 1998; Maestroni, 2000; Bubenik, 2002); however, it is possible that part of the melatonin found in these structures is derived from the pineal gland itself.

Within the rodent retina a self-sustaining 'clock' maintains rhythmic production of melatonin *in vitro* as it does in many lower vertebrates (Tosini and Menaker, 1996). Whether this pattern is true in humans remains to be seen.

Melatonin is metabolised primarily within the liver by 6-hydroxylation, followed by sulphate and/or glucuronide conjugation (Kopin et al, 1961; Arendt, 1995; Skene et al, 2001) (Figure 3.4). The principal metabolite in humans is 6-sulphatoxymelatonin (aMT6s), accounting for 50–80% of melatonin produced. A number of minor metabolites are also formed through ring splitting, cyclisation of the side chain, or demethylation (see Arendt, 1995, for a bibliography). In humans and rodents, exogenous oral or intravenous melatonin has a short metabolic half-life (20–60 minutes, depending on the author and species), with a large hepatic first-pass effect and a biphasic elimination pattern. In ruminants, longer half-lives are seen after oral administration.

3.1.3 Neural control of melatonin synthesis

In mammals, pineal denervation, or ganglionectomy, abolishes the rhythmic synthesis of melatonin and the light–dark control of its production. Noradrenalin is clearly the major transmitter and acts via β_1-adrenoceptors with potentiation by α_1-stimulation, but the role of neural serotonin is probably not negligible. A day–night variation is seen in pineal noradrenalin, with the highest values at night, approximately 180 degrees out of phase with the pineal serotonin rhythm. cAMP acts as a second messenger and stimulates NAT activity. β-adrenergic receptor-binding sites in the rat pineal vary over a 24 hour period, the lowest number being found toward the end of the dark phase and increasing shortly after lights on. A recent comprehensive review addresses this field (Simonneaux and Ribelayga, 2003).

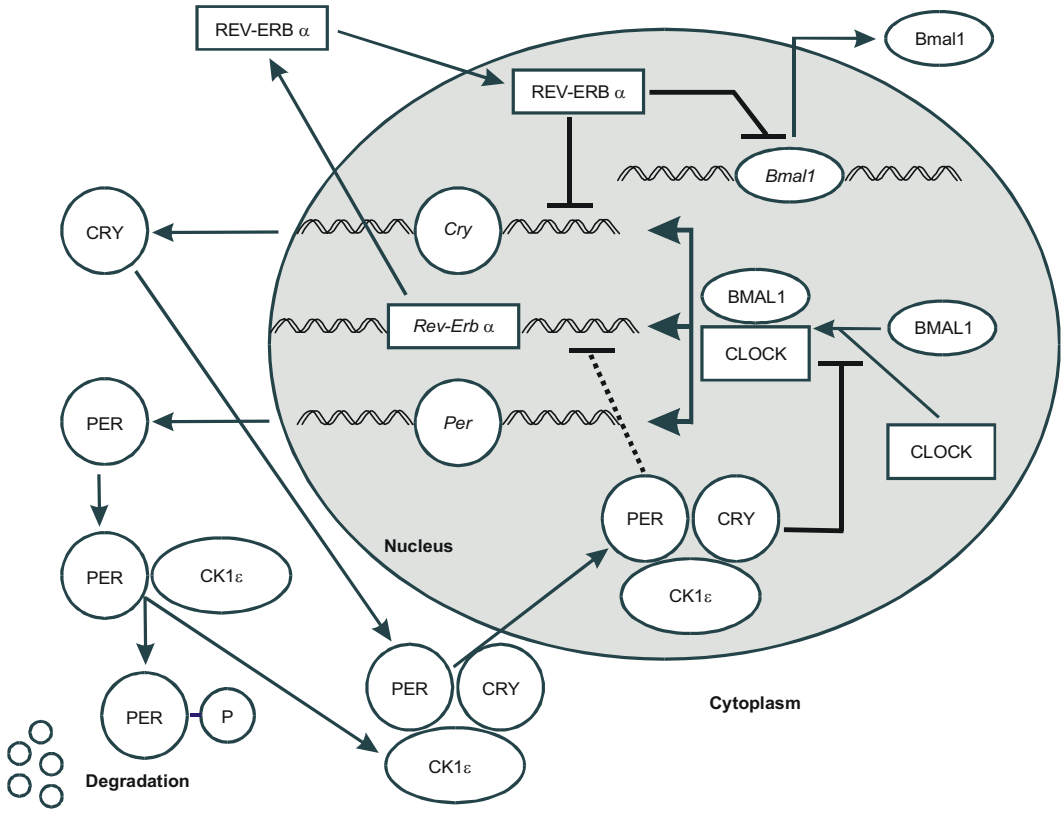


FIGURE 3.3 Generation of circadian rhythms: clock gene feedback loops. A simplified model of the molecular clockwork of the circadian clock in mammals. A general feature is feedback of clock gene proteins on clock gene promoters. Inhibition of transcription of RNA stops the circadian clock, and inhibition of translation of proteins stops the circadian clock. BMAL1 and CLOCK proteins together activate the expression of clock genes (and clock-controlled genes) in the promoter of these genes. Both mRNA and protein products of *Period (Per1, 2, 3)* and *Cryptochrome (Cry1, 2)* genes oscillate over the 24 hour cycle. Simultaneous activation of the *Per, Cry* and *Rev-Erb α* promoters by CLOCK and BMAL1 is followed by transcription of mRNA. mRNA transcripts are transported from the nucleus into the cytoplasm, bind to ribosomes and initiate translation of proteins which accumulate in the cytoplasm. PER proteins bind to CRY proteins in different combinations, casein kinase Iε (CKε) binds to and phosphorylates the PER-CRY dimers. Coordinated nuclear entry of CKI+PER+CRY trimers and binding to CLOCK and BMAL1 abolishes their transcriptional promotion. *Per* and *Cry* mRNA and proteins are short lived and following phosphorylation (P) and degradation the cycle begins again with *Per* and *Cry* transcription. A recently described clock gene product REV-ERB α protein represses BMAL1 and CLOCK gene expression and also represses CRY1 gene expression. The products of clock-controlled genes exert output functions. Some 2–10% of all mammalian genes are clock-controlled genes (Fu and Lee, 2003). The light–dark cycle dictates the timing of oscillations, whereby BMAL1 is high and PER and CRY are low at the beginning of a circadian day. CLOCK does not oscillate. Solid lines, direct regulation; dashed lines, indirect regulation. Redrawn and adapted from Fu and Lee, 2003. See Lakin-Thomas, 2000, and Fu and Lee, 2003, for reviews

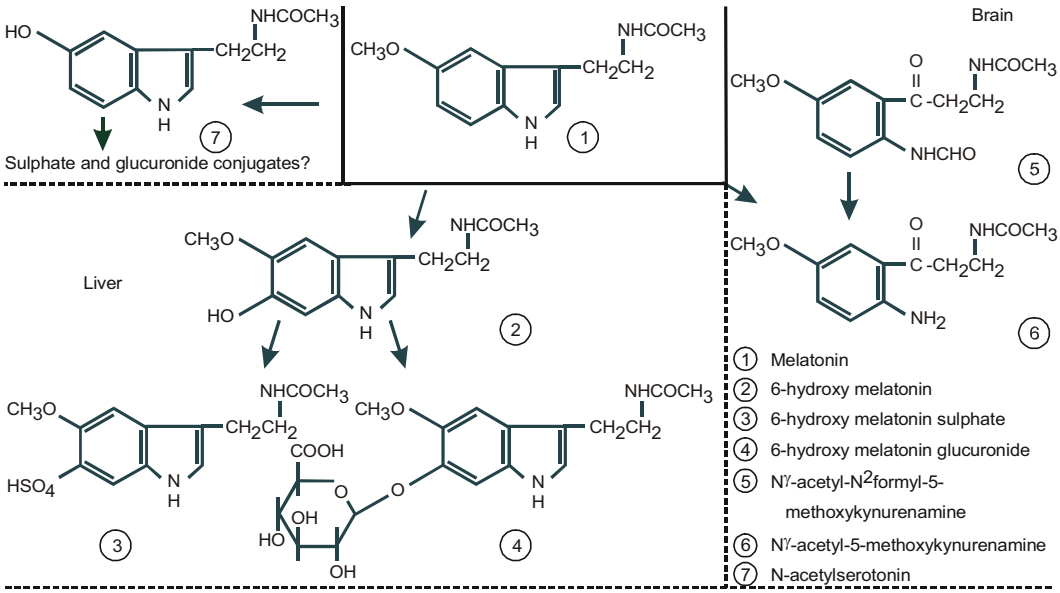


FIGURE 3.4 Metabolism of melatonin. From Arendt, 1995, by permission

3.1.4 Other control mechanisms

The pineal contains very large numbers of other neuromodulators, neuroreceptors and hormone receptors: evaluation of their physiological importance *in vivo* is in its infancy. They include vasoactive intestinal peptide, pituitary adenylate cyclase activating peptide, peptide histidine isoleucine, neuropeptide Y, vasopressin, oxytocin, somatostatin, substance P, calcitonin gene-related peptide, secretoneurin, hypocretin, delta-sleep inducing peptide, natriuretic peptide, angiotensin, opiate peptides, LHRH, and nitric oxide synthase. Current findings have been summarised by Simmonneaux and Ribelayga (2003) and most of these peptides appear to have modulatory effects on the primary noradrenergic stimulation of melatonin synthesis.

3.1.5 Miscellaneous factors influencing melatonin production

Melatonin production declines with age. This was one of the most consistent observations in the field (see Arendt, 1995); however, some controversy has arisen since in a small number of healthy human subjects this was not observed in cross-sectional studies (Zeitzer et al, 1999). Production of aMT6s declines with age (Bojkowski and Arendt, 1990) and, as yet, this is not a controversial observation. There are no consistent observations regarding melatonin production with respect to sex or weight. Numerous drugs are reported to influence melatonin. Substances that influence serotonin and catecholamines by changing either their availablility or their receptor activity are prominent. In particular, beta-adrenergic receptor antagonists, a commonly used anti-hypertensive medication, suppress melatonin. A number of anti-depressant serotonin and catecholamine re-uptake inhibitors, some anti-psychotics, and possibly caffeine, increase melatonin and/or change the timing of secretion. Some benzodiazepines and

non-steroidal anti-inflammatory drugs, alcohol and nicotine are reported to decrease circulating melatonin. Substances that compete for metabolic pathways influence plasma levels of melatonin and urinary levels of its major metabolite, aMT6s. Gonadal steroids may influence both metabolism and production, and there is evidence that the use of contraceptives influences circulating melatonin (Kostoglou-Athanassiou et al, 1998; Wright and Badia, 1999; Simmoneaux and Ribelayga, 2003; Thapan et al, unpublished observations; Thapan, 2001). Table 3.1 provides an overview of substances that affect melatonin production, secretion and metabolism.

TABLE 3.1 Various factors influencing melatonin secretion; reviews are cited where appropriate. Most references prior to 1995 can be found in Arendt, 1995

Factor	Model	Effect(s) on melatonin	Comment	References
Light	Humans	Suppression (>30 lux broad spectrum white in controlled studies) intensity required depends on previous light exposure	Maximum effective wavelength 460–480 nm	Zeitzer et al, 2000 Thapan et al, 2001 Brainard et al, 2001 Owen and Arendt, 1992 Hebert et al, 2002
Light	Animals	Suppression (intensity depends on species and environment)	Maximum effective wavelength 480 nm in mice	Foster and Hankins, 2002
Light	Humans	Entrainment: with scheduled sleep/darkness <200 lux Without other time cues >200 and <1000 lux	Short wavelengths more effective than white	Zeitzer et al, 2000 Wright et al, 2001 Middleton et al, 2002 Warman et al, 2003 Lockley et al 2003
Light	Animals	Entrainment (intensity depends on species and environment)		Elliot, 1981
Timing of sleep or rest–activity cycle	Humans Animals	Changes in timing (and sometimes amplitude after abrupt phase shift), eg shift work, jet lag in humans	Partly a secondary effect, sleep modifies light–dark exposure	Barnes et al, 1998 Rajaratnam and Arendt, 2001 Turek and Van Reeth, 1988 Fevre-Montange et al, 1981
Timing of light–dark cycle	Humans Animals	Changes in timing (and sometimes amplitude after abrupt phase shift), eg shift work, jet lag in humans		Deacon and Arendt, 1996 Illnerova and Sumova, 1997
Posture	Humans	Increase on standing up after a period of recumbency at night	20 min required to stabilise, may not affect timing	Deacon and Arendt, 1994 Voultsios et al, 1997
Exercise	Humans	Increase at night, induces phase shifts	Very hard exercise	Buxton et al, 2003
Adrenergic β-receptor antagonists	Humans Animals *In vitro*	Decreased synthesis, can be almost completely suppressed overnight	Anti-hypertensives	Arendt et al, 1985a,b Simmoneaux and Ribelayga, 2003 Klein et al, 1997

TABLE 3.1 Continued

Factor	Model	Effect(s) on melatonin	Comment	References
Some serotonin (5HT) uptake inhibitors	Humans	Increase with fluvoxamine	Antidepressant (probably metabolic effect)	Hartter et al, 2001 Skene et al, 1994
Noradrenalin uptake inhibitors	Humans	Increase/change in timing	Antidepressants	Checkley et al, 1986
MAO α inhibitors	Humans Animals	Increase, may change phase	Antidepressants	Arendt, 1989
Alpha-adrenoceptor antagonists	Humans	Decrease with alpha-1, increase with alpha-2		Palazidou et al, 1989a,b
Benzodiazepines	Humans Animals *In vitro*	Varied, eg decrease with diazepam, alprazolam, not triazolam, temazepam, zopiclone *In vitro* and *in vivo* results not necessarily similar	Probably via GABA related mechanisms	McIntyre et al, 1993 Monteleone et al, 1989 Niles, 1991 Copinschi et al, 1990 Mann et al, 1996 Cardinali and Golombek, 1998
Vasopressin	*In vitro*	Increase	Potentiates noradrenergic stimulation	Simmoneaux and Ribelayga, 2003
Dopamine	*In vitro*	Possible effects both inhibitory and stimulatory		Simmoneaux and Ribelayga, 2003
Acetylcholine	Animals *In vivo* microdialysis and *in vitro*	Inhibition of synthesis		Simmoneaux and Ribelayga, 2003
Glutamate	*In vitro*	Inhibition of synthesis	Inhibits noradrenalin stimulation	Simmoneaux and Ribelayga, 2003
GABA	*In vitro*	Inhibition of synthesis	Inhibits noradrenalin stimulation	Simmoneaux and Ribelayga, 2003
Nitric oxide	*In vitro*	Inhibition of synthesis		Simmoneaux and Ribelayga, 2003
Testosterone	Animals Humans	Stimulation	Castration reduces melatonin synthesis Treatment of male hypogonadism decreases melatonin, hyperandrogenic women have increased melatonin	Simmoneaux and Ribelayga, 2003 Puig-Domingo et al, 1992 Luboshitzky et al, 1997
Oral contraceptives	Humans	Increase	Possible metabolic effect	Wright and Badia, 1999 Simmoneaux and Ribelayga, 2003 Kostoglou-Athanassiou et al, 1998
Progesterone		Controversial		

TABLE 3.1 Continued

Factor	Model	Effect(s) on melatonin	Comment	References
Oestradiol	*In vitro*	Inhibition of synthesis		Simmoneaux and Ribelayga, 2003
Oestradiol	Animals	Decrease (inconsistent)	Ovariectomy increases melatonin in rats and sheep (transient) but inconsistent between species Possible metabolic effect by competition for the CYP1A2 metabolic pathway?	Simmoneaux and Ribelayga, 2003 Cheng, 2001
Oestradiol	Humans	Decrease (inconsistent, insufficient data)	Oestradiol treatment for delayed puberty lowered aMT6s in a case report No effect of ovarian suppression in precocious puberty Menopause may be associated with increased melatonin	Arendt et al, 1989 Berga et al, 1989 Okatani et al, 2000
Oestrous cycle	Animals *In vivo* microdialysis	Inconsistent in whole animals No differences	Pro-oestrous decline in rodents	Simmoneaux and Ribelayga, 2003
Menstrual cycle	Humans	Inconsistent and conflicting reports	Never properly assessed in constant routine conditions in humans	Arendt, 1995
Oestradiol, Progesterone	DMBA-treated rats	Decrease		Simmoneaux and Ribelayga, 2003
Nicotine	Humans	Possible increase, insufficient data		Tarquini et al, 1994
Alcohol	Humans	Decrease, dose dependent		Ekman et al, 1993
Caffeine	Humans	May delay clearance, ie increase		Shilo et al, 2002 Hartter et al, 2003
Some non-steroidal anti-inflammatory drugs	Humans	Decrease		Murphy et al, 1996
Chlorpromazine	Humans	Increase	Antipsychotic Metabolic effect	Ozaki et al, 1976 Smith et al, 1977 Beedham et al, 1987
Luteinising hormone	*In vitro*	Increased production	Few data	Simmoneaux and Ribelayga, 2003
Benserazide	Animals Humans	Decrease No amplitude change in Parkinson patients, possible phase change	Aromatic amino-acid decarboxylase inhibitor	Arendt et al, 1981 Fertl et al, 1991 Ho and Smith, 1982
Calcium channel blockers	Humans	Possible effects		Escames et al, 2004

3.2 Light–dark control of melatonin synthesis in animals and man

3.2.1 Darkness hormone

In all mammalian and other vertebrate species studied to date, whether nocturnal or diurnal, melatonin is normally synthesised and secreted during darkness. Remarkably, even the unicellular alga *Gonyaulax* appears to produce melatonin during the dark phase, and it appears to be present in higher plants (see Arendt, 1995). Melatonin production is clearly a highly evolutionarily conserved phenomenon. In most vertebrates the rhythm is endogenous, ie internally generated, and in higher vertebrates it is driven by the central pacemaker, the suprachiasmatic nuclei (SCN). In birds and lower vertebrates the pineal is a self-sustaining oscillator itself. The rhythm persists in the absence of specific time cues (zeitbegers) and becomes 'free-running', in general assuming a period deviating slightly from 24 hours, and is thus a true circadian rhythm. Lesions of the SCN lead to loss of the vast majority of circadian rhythms such as locomotor activity, sleep, behaviour, hormones including melatonin, and urinary constituents (Moore and Eichler, 1972; Stephan and Zucker, 1972; Klein, 1985; Moore, 1996). Circadian rhythms are entrained (synchronised) to the 24 hour day primarily by light–dark cycles (Figure 3.5) acting via the retina. It should be noted that in spite of a publication (Campbell and Murphy, 1998) reporting an impact of light applied behind the knees on rhythms, this has been refuted by several further studies (Lockley et al, 1998; Wright and Czeisler, 2002). Many blind people with no light perception at all, ie lacking the light–dark time cue, show free-running melatonin and other rhythms (eg sleep, cortisol, core temperature) in a normal environment (Lewy and Newsome, 1983; Arendt et al, 1997; Lockley et al, 1997a). In addition to entraining the rhythm, daylength (photoperiod) determines the duration of night-time secretion both by direct suppression of melatonin and by determining the length of the signal emitted by the SCN (Lincoln et al, 1985; Arendt, 1986; Illnerova and Sumova, 1997). Factors (zeitgebers) other than light–dark cycles that are involved in entrainment include behavioural imposition such as forced activity and rest, social and nutritional (rhythmic feeding) cues, temperature variations, knowledge of clock time, certain drugs, possibly EMFs, and melatonin itself.

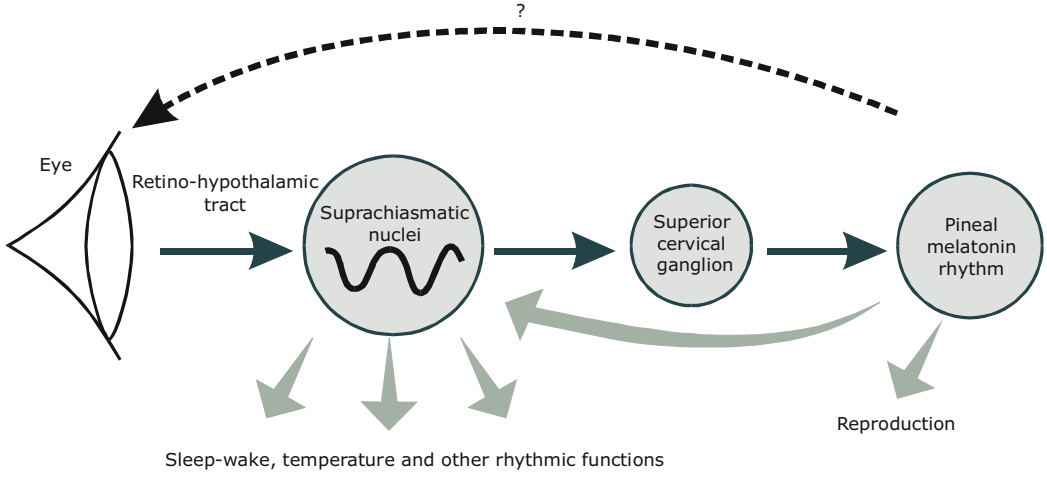


FIGURE 3.5 Mammalian circadian rhythms are generated in the suprachiasmatic nuclei, and entrained by light with contributions from non-photic time cues. Melatonin provides a closed loop to this system

3.2.2 Melatonin secretion in relation to daylength

In most species, melatonin secretion is related to the length of the night: the longer the night, the longer the duration of secretion. This phenomenon has been particularly well demonstrated in sheep, in which melatonin levels rise within a few minutes of lights off and, in photoperiods of more than around 14 hours of light, do not decline until lights on (Arendt, 1986) (Figure 3.6).

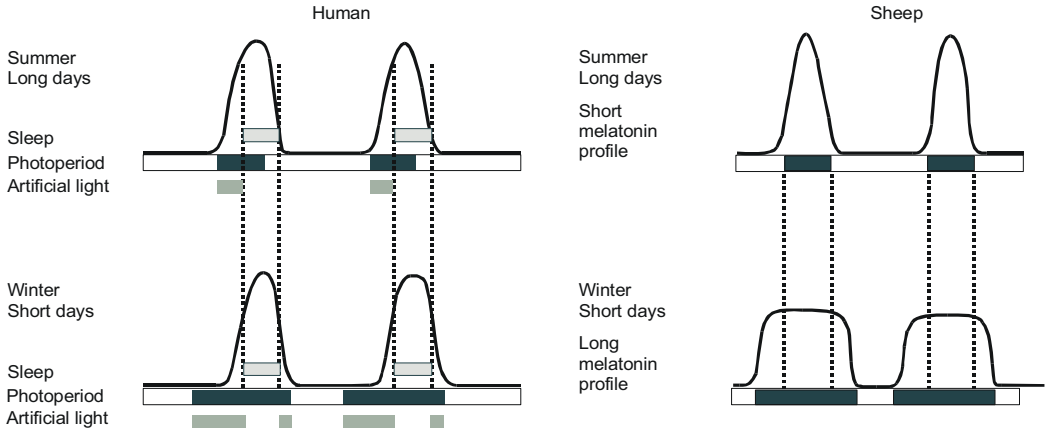


FIGURE 3.6 Winter and summer melatonin profiles in sheep and humans. Note the changed duration from winter to summer in sheep with primarily a change in timing – earlier in summer than in winter in humans

The most consistent observation in humans is that melatonin profiles show a phase change from winter to summer, with earlier secretion in summer than in winter (see Arendt, 1995). However, if humans are kept strictly in darkness for 14 hours per day for a period of 2 months, the melatonin secretion pattern expands to cover almost the entire dark period and, concomitantly, in extended periods of 16 hours of light, the rhythm contracts to less than 9 hours, with accompanying changes in body temperature and sleep (Wehr et al, 1993, 2001). Small changes in duration have been seen between winter and summer in high latitudes. Short-term imposition of artificial short days leads to a change in the timing of endogenous melatonin (Vondrasova-Jelinkova et al, 1999; Rajaratnam et al, 2003). Many clinical studies have not controlled sufficiently for exposure to natural or artificial light, making interpretation of data difficult.

3.2.3 Light suppression of melatonin secretion

Even brief exposure to light of suitable intensity, duration and spectral quality, suppresses melatonin production at night; short wavelengths (around 465 nm in humans) are most effective (Brainard et al, 2001; Thapan et al, 2001; Foster and Hankins, 2002) (Figures 3.7 and 3.8). Short wavelengths are also more effective than white light for entrainment of the rhythm to 24 hour (Lockley et al, 2003; Warman et al, 2003). Since the action spectrum derived from irradiance response curves does not correspond to either scotopic or photopic action spectra, a new photoreceptor(s) system has been

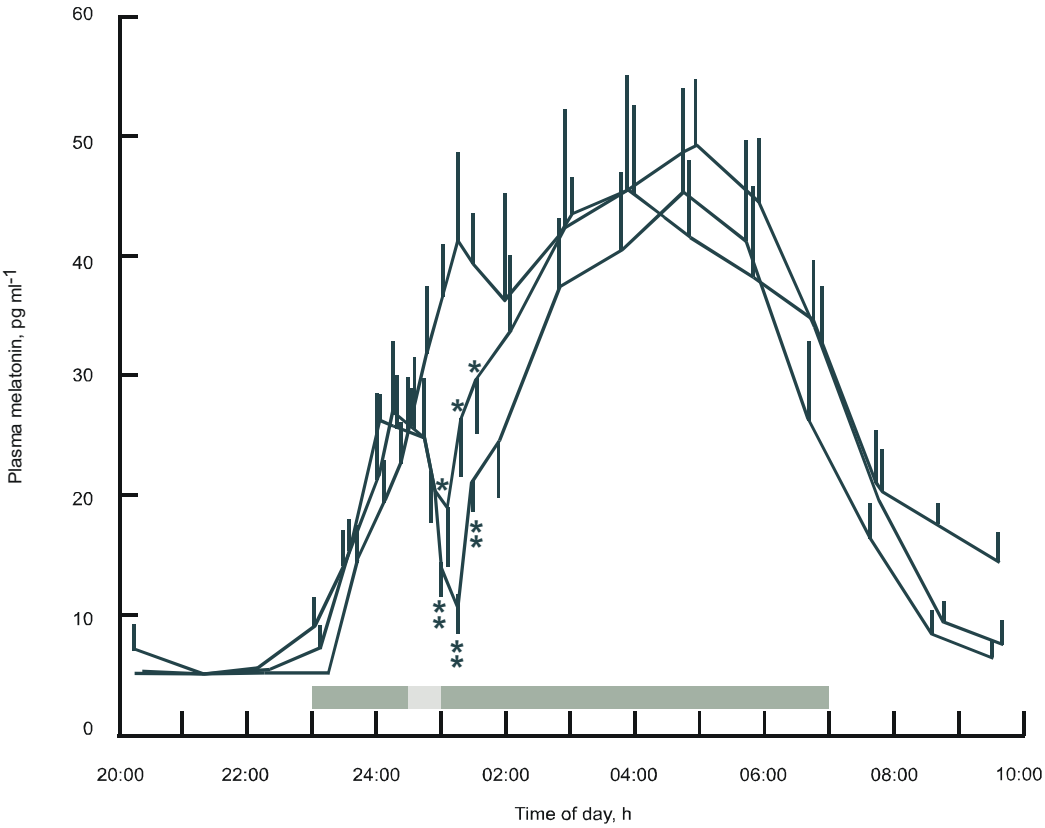


FIGURE 3.7 Melatonin suppression in humans by 2500 lux broad spectrum white light (double asterisks) and partial suppression by domestic intensity light (300 lux, single asterisks) from 00.30 to 01.00 hours, compared with control conditions in darkness. From Bojkowski et al, 1987a, by permission

invoked (Lucas et al, 1999). No sex differences have been noted with regard to entrainment in humans; however, the burdensome nature of these studies means that the number of subjects investigated is small. Moreover the tendency to use healthy, young men as volunteers reduces further the possibility of comparisons.

The amount of light required to suppress melatonin secretion during the night varies from species to species, with the time of night, and with previous light exposure. In humans, it was originally observed that 2500 lux broad spectrum white light (domestic light is around 300–500 lux) is required to completely suppress melatonin at night (Lewy et al, 1980), However, much lower 'domestic' intensity light will partially suppress and shift the rhythm in humans (Bojkowski et al, 1987a; Zeitzer et al, 2000). Thus artificial light at night is likely to modify melatonin production. These observations have been of very considerable importance for a general appreciation of the role of light in human physiology – in particular, its importance in the control of human rhythms and in the treatment of winter depression (seasonal affective disorder) (Rosenthal et al, 1984).

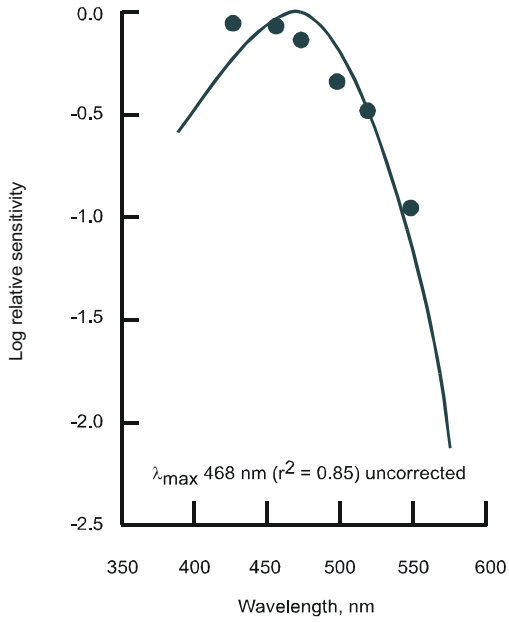


FIGURE 3.8 Action spectrum for melatonin suppression in humans. From Thapan et al, 2001, by permission

3.2.4 Light at night, melatonin suppression and risk of disease

The evidence that melatonin has some oncostatic activity has led to hypotheses regarding the increased incidence of breast cancer in female shift workers (see Chapter 5) and light suppression of melatonin in night shift workers. Little evidence exists for a decline in melatonin during night shift work, but it is likely that this can occur. In a light-induced forced nine hour phase shift, a clear decline in aMT6s production was noted (Deacon and Arendt, 1996). Unpublished work (Hall, English and Arendt) suggests that during a three day fast rotation shift schedule (three early shifts, three late shifts, three night shifts, rest days), the amplitude of aMT6s is significantly reduced (by approximately 30%) during the three night shifts (Figure 3.9). In an even faster (two day) rotation, significant changes were not observed (Costa et al, 1994). Actual light exposure at night needs to be evaluated carefully in field studies. Light at night has numerous other effects. The mere fact of frequent disruption of all circadian rhythms, not just melatonin, is effectively a physiological insult.

The behavioural effects of light at night include increased alertness and performance, and an increase in body temperature (Campbell and Dawson, 1990; Strassman et al, 1991; Myers and Badia, 1993; Phipps-Nelson et al, 2003). Noradrenalin and acetylcholine are decreased, and serotonin, GABA and dopamine are increased (Roberts, 2000). A number of neurotransmitters in the SCN are modified by light (Roberts, 2000). A recent review on light and immunomodulation suggests that light impacts on the immune system either by central neuroendocrine mechanisms or by skin-mediated responses (Carlberg, 2000). The treatment of neonatal jaundice by light is another example of a non-visual effect. There is good evidence that increased light exposure in the early morning (one hour, 05.00–06.00 hours) will increase

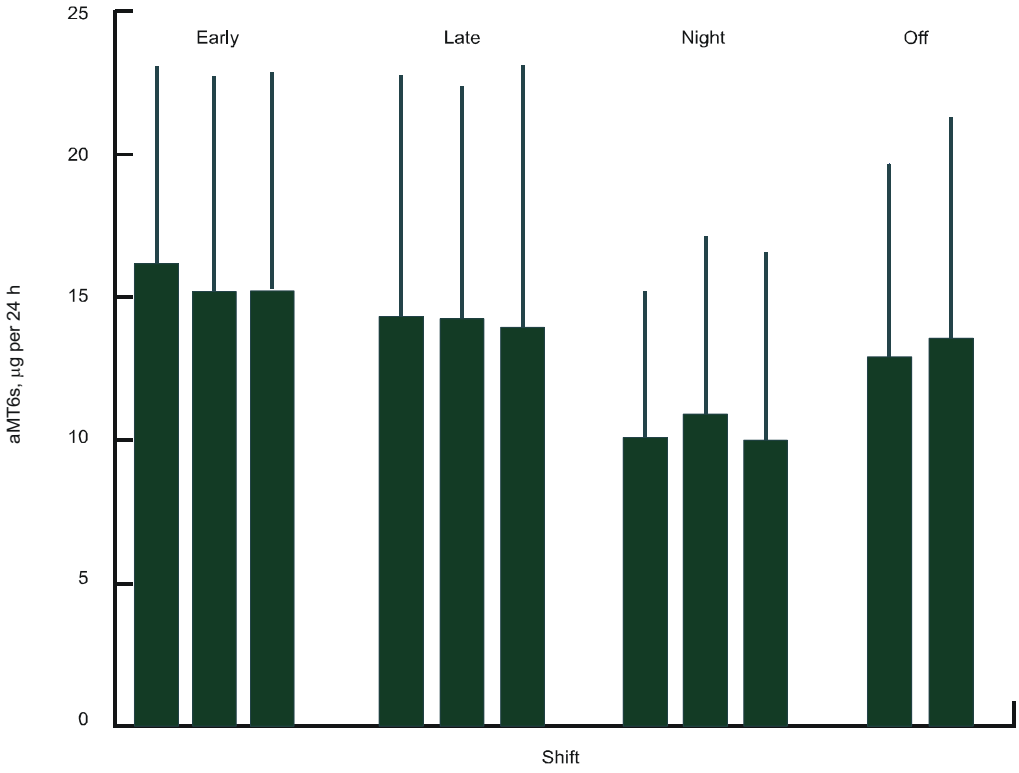


FIGURE 3.9 24 hour production of 6-sulphatoxymelatonin (aMT6s) (mean ± SD), during a three day shift rotation, onshore, N=15. Note lower production during night shift. From Hall, English and Arendt (unpublished data)

luteinising hormone (LH) secretion by 69% – it has even been proposed that this might improve fertility (Yoon et al, 2003). Cortisol secretion (in men) is increased by more than 50% by bright light exposure from 05.00–08.00 hours, but not by afternoon (13.00–16.00 hours) treatment (Leproult et al, 2001). Exposure to bright full spectrum white light is now a common treatment for seasonal affective disorder (albeit with a large placebo effect), but in spite of much effort it has not been possible to confirm a role for melatonin. Rather, serotonergic mechanisms are implicated (Partonen and Magnussen, 2001).

Impaired sleep as a result of shift work (Akerstedt, 1998) is evidently associated with light at night. Sleep deprivation and impaired metabolism at night are associated with a large number of problems including an increase in risk factors for major disease: higher circulating triacylglycerol, glucose intolerance, insulin resistance (Morgan et al, 2003). Sleep and sleep loss are also associated with immunomodulatory effects (Shephard and Shek, 1997; Redwine et al, 2000).

Most importantly, perhaps, light directly influences the expression of the clock gene feedback loops driving circadian rhythms (Reppert, 2000). Disruption of clock gene function is associated with increased risk of cancer in recent animal studies (Fu et al, 2002; Fu and Lee, 2003; Filipski et al, 2004). Moreover, a length polymorphism in the clock gene *per3* is associated with increased risk of breast

cancer in a very recent study (Zhu et al, 2005). Thus a more plausible hypothesis regarding the risk of breast cancer in subjects exposed to light at night would involve the whole circadian axis rather than just melatonin suppression.

3.2.5 Entrainment of the melatonin rhythm

A single daily light pulse of suitable intensity and duration in otherwise constant darkness is sufficient to phase shift and synchronise the melatonin rhythm to 24 hours in animals (Elliot, 1981). Phase shifting and entrainment have been demonstrated in humans with suitable intensity and duration of light treatment (Arendt and Broadway, 1986, 1987; Czeisler et al, 1986; Broadway et al, 1987; Shanahan and Czeisler, 1991; Middleton et al, 2002). However, the relative contribution of light to the entrainment of melatonin in a normal environment remains to be fully determined. Studies in Antarctica suggest that a structured social routine in a dim light environment (with no natural sunlight) suffices to synchronise melatonin to 24 hours (Broadway et al, 1987; Arendt, 1995). However, as many blind people with no conscious or unconscious light perception living in a normal social environment show desynchronised melatonin and other circadian rhythms, it is clear that at least some perception of light is of primary importance (Arendt et al, 1997; Lockley et al, 1997a,b; Hack et al, 2003).

3.3 Role of melatonin in photoperiodic seasonal functions in animals

3.3.1 Photoperiodism

Most species show seasonal variations in their physiology and behaviour, even humans. The reproductive cycle is timed so that environmental conditions are propitious for growth of the young, and variations in behaviour, pelage (coat growth and colour), appetite, body weight, and fat are such that survival in ambient temperature conditions is optimised and camouflage protects against predators (Hoffmann, 1979; Arendt, 1986; Goldman, 2001). When seasonal functions are primarily timed by daylength, species are referred to as being photoperiodic. Photoperiod is often critical for the timing of pubertal development (Foster et al, 1988). In general, puberty is reached only during the adult mating season. It is now clear that in photoperiodic mammals and marsupials, an intact innervated pineal gland is essential for the perception of photoperiodic change (Lincoln and Short, 1980; Arendt, 1986; Karsch et al, 1988; Lincoln and Richardson, 1998). Most information is derived from studies on reproductive function in hamsters and sheep.

3.3.2 Role of melatonin

Pinealectomy removes the vast majority of circulating melatonin in rodents, primates, and ungulates. It was therefore the first pineal hormone to be investigated as a pineal photoneuroendocrine transducer. It is possible to administer melatonin by daily infusion or feeding to generate at will circulating profiles with a duration characteristic of particular photoperiods in an intact or pinealectomised animal. In this way it has become clear that a particular melatonin duration is a necessary and sufficient condition for induction of a given seasonal response and is equipotent with a particular photoperiod. Long-duration melatonin is equivalent to short days, and short-duration melatonin is equivalent to long days.

Interpretation of the signal, as with daylength, depends on the physiology (for example, long- or short-day breeder) of the species in question. In sheep, the evidence is good that long days or short-duration melatonin can time the whole seasonal cycle, at least of reproduction, and act as a seasonal zeitgeber for a presumed endogenous annual rhythm. Animals become refractory to a specific duration of melatonin as they do to a particular photoperiod. For example, a period of long days (or a long-day melatonin signal) is required before a short-day melatonin signal advances the reproductive cycle in sheep (Arendt, 1986; Lincoln and Richardson, 1998; Goldman, 2001).

3.3.3 Puberty and development

The photoperiod via melatonin secretion determines the timing of puberty in some species, provided that a sufficient degree of physical maturity has been reached (Foster et al, 1988). Interestingly, photoperiod perception by the fetus is present before birth in rodents and ungulates and ensures a rate of development appropriate to environmental conditions (Davis and Mannion, 1988; Deveson et al, 1992). Melatonin crosses the placenta in a number of species, and melatonin injection in the mother can dictate the timing of postnatal reproductive development (Weaver and Reppert, 1986).

The laboratory rat is only marginally photoperiodic. Nevertheless, injections of melatonin during the late light phase, during a small window in the late dark phase, or even via continuous release implants, specifically during the period of pubertal development, delay reproductive maturity in both males and females (Sizonenko et al, 1985). Full sexual maturity is eventually achieved; thus the system is not permanently compromised. Moreover, *in vitro* melatonin inhibits gonadotropin-releasing hormone (GnRH)-induced LH release by cultured rat pituitary glands from prepubertal animals (Martin and Klein, 1976; Symons et al, 1985). These observations constitute the main evidence for a possible role of melatonin in the pubertal development of humans.

3.3.4 Mechanisms

The mechanism of action of melatonin with regard to seasonal variation in reproductive competence and the timing of puberty in animals is thought to involve an influence of melatonin on steroid feedback mechanisms in the brain, together with a direct influence on the pituitary gland via melatonin receptors (Goldman, 2001). For example, anoestrous sheep exposed to artificial short days in summer rapidly show increases in LH secretion and changes in the pulsatile pattern of secretion associated with onset of reproductive activity and oestrous (Bittman et al, 1985). There is clear evidence that the large seasonal changes in prolactin secretion in ungulates are mediated by a direct action of melatonin within the pars tuberalis (PT) of the pituitary via the MT1 receptor (Lincoln, 2002). Melatonin duration influences clock gene expression within the PT (Messager et al, 2000; Hazlerigg et al, 2001) but not within the SCN (according to Poirel et al, 2003). Figure 3.10 summarises melatonin actions with respect to seasonal and circadian rhythms.

Rhythmic clock gene expression in the anterior pituitary appears to depend on sensitisation of the adenosine A2b receptor occurring through activation of the melatonin (MT1) receptor (von Gall et al, 2002a,b). This observation leads to the possibility that melatonin widely influences peripheral gene expression.

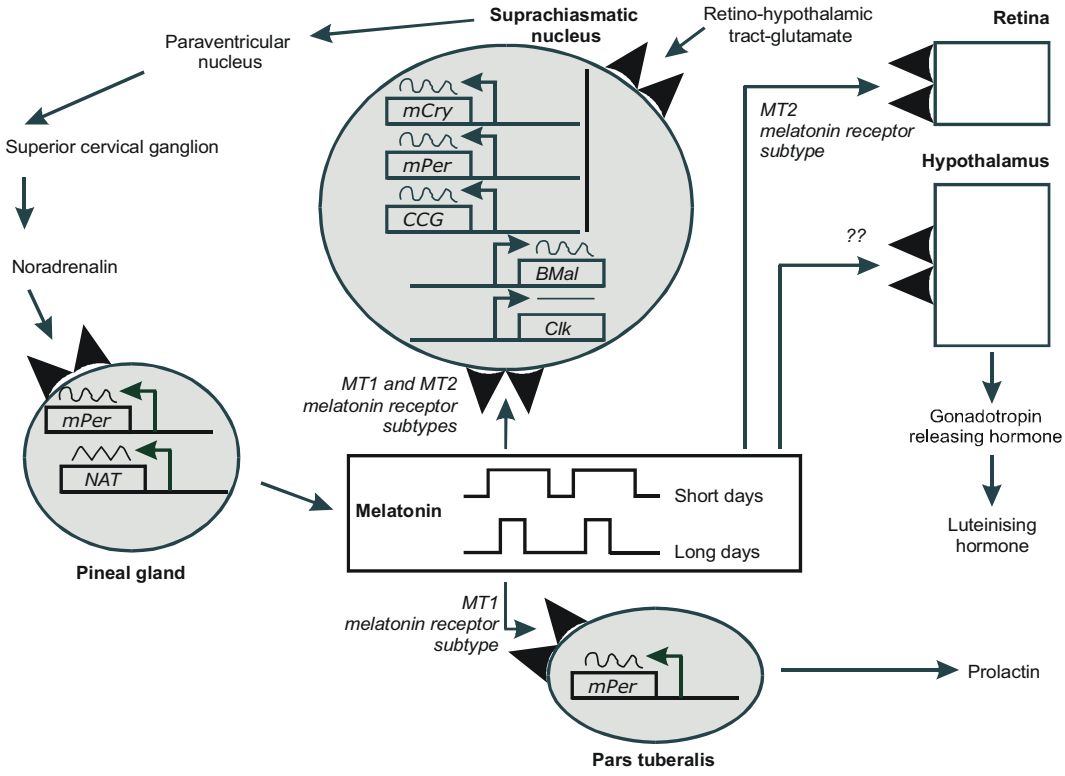


FIGURE 3.10 Control of production and the functions of melatonin with regard to seasonal and circadian timing mechanisms. The melatonin rhythm is generated by a closed loop negative feedback of clock gene expression in the SCN – *Clock* and *Bmal* being positive stimulatory elements, *Per* and *Cry* negative elements, and *CCG*, clock-controlled genes. *Per* and *NAT* mRNA oscillate in the pineal, although post-transcription control is evident in some species. Melatonin influences SCN activity via two or more receptors. MT2 appears to be the phase shifting receptor in rodents, whereas MT1 is associated with suppression of SCN electrical activity. The MT2 receptor was first characterised in the retina and influences dopamine release. Melatonin conveys photoperiodic information influencing the pattern of Per expression in the pars tuberalis for the control of seasonal prolactin variations via an MT1 receptor. Melatonin target sites in the hypothalamus influencing seasonal variations in reproductive hormones have yet to be fully defined. Based, with permission, on an original diagram by Dr Elisabeth Maywood, MRC Laboratory of Molecular Biology, Neurobiology Division, Hills Road, Cambridge, CB2 2QH, UK

3.3.5 Non-reproductive seasonal functions

The pineal gland via melatonin secretion probably plays a role in all photoperiod-dependent functions in mammals. Evidence exists to substantiate this statement with respect to behaviour, body weight, coat constitution and colour (for example, the white winter coat of some polar species), prolactin variations, antler growth, thyroid activity, appetite, thermoregulation, delayed implantation, embryonic diapause, and hibernation (see reviews by Arendt, 1986, and Goldman, 2001).

3.4 Role of the pineal in mammalian circadian rhythms

Until quite recently, opinion was that the pineal did not have an important role in the mammalian circadian system. Some strains of (healthy, prolific) laboratory mice have virtually no detectable melatonin. Pinealectomised rats show no obvious circadian abnormalities unless 'challenged'. Thus, in rats, pinealectomy increases the rate of re-entrainment to forced phase shifts of the light–dark cycle, and pinealectomy of hamsters in constant light leads to major disruption of the circadian system (Armstrong and Redman, 1985; Cassone, 1992). In hamsters, there is good evidence that maternal melatonin can influence the circadian timing of the fetus (Davis and Mannion, 1988). Melatonin is found in milk (of goats, cows, humans and rats) with increased levels at night. The hypothesis that melatonin in milk influences the circadian rhythms of the neonate has been refuted, at least in rats (Rowe and Kennaway, 2002). In humans it is likely that the phase shifting effects of light do not depend on melatonin suppression, but that the presence of endogenous or exogenous melatonin can, in some circumstances, modulate the effects of light on the circadian system (Arendt, 2003). It is quite possible to sleep out of phase with melatonin (although sleep is somewhat compromised). A substantial body of work implicates melatonin in circadian thermoregulation (see Badia et al, 1992, for a review). Many such effects may involve the thyroid gland. Most evidence for a circadian role of melatonin derives from timed administration.

3.5 Effects of timed administration of melatonin in animals

3.5.1 Behaviour, hormones, and temperature

In rats, daily melatonin injections synchronise free-running activity and temperature rhythms in constant darkness and are reported to partially or completely synchronise disrupted activity rhythms in constant light, although the latter observation is somewhat controversial (Redman et al, 1983; Chesworth et al, 1987). A phase–response curve to single injections of melatonin can be demonstrated with small phase advances of at most one hour during the late subjective day (Armstrong and Redman, 1985). Timed administration hastens adaptation of activity and melatonin production to forced phase shift and can change the direction of re-entrainment (Illnerova et al, 1989). Some, but not all, strains of adult hamster can be synchronised by melatonin administration, and fetal hamsters can be entrained by maternal injections of melatonin at 24 hour intervals in specific circadian phases (Davis and Mannion, 1988).

3.5.2 Gestation

In the rat, gestation length depends on the ambient light–dark cycle. Small advances or delays in parturition can be induced by daylengths shorter or longer than 24 hours, and the effect can partially be mimicked by timed melatonin administration (Bosc, 1987).

3.5.3 Oestrous cycle

The pineal is involved in circadian timing, so the presumption must be that it is concerned with timing of the LH surge and, indeed, with general oestrous timing. In rats, timed melatonin administration has been

demonstrated to mimic the effects of extending the light–dark cycle on timing of the LH surge. Observations of the melatonin rhythm itself show a decreased amplitude during proestrus in rodents, but with conflicting reports in other species (Rollag et al, 1979, and see Arendt, 1995, for a review).

3.5.4 Ageing and immune function

A fairly consistent observation in pineal research (but with some controversy) is the decline in amplitude of the melatonin rhythm in old age (see Arendt, 1995, for references). Pinealectomy is reported to accelerate the ageing process in animals; however; both acceleration and deceleration of ageing have been reported in shortened photoperiod (ie longer duration endogenous melatonin) (Holmes and Sugden, 1976; Armstrong and Redman, 1991; Aujard et al, 2001; Place et al, 2004). The possible anti-ageing effects of melatonin have generated considerable publicity (Trentini et al, 1992; Pierpaoli and Regelson, 1994; Reppert and Weaver, 1995; Arendt, 1996; Turek, 1996). Several hypotheses have been put forward to explain these sometimes flawed, insubstantial, but interesting observations. Melatonin, when appropriately administered, has (usually) stimulatory effects on aspects of the immune system, and longer survival with cancer has been claimed (Lissoni et al, 1993; Maestroni, 1999). One recent review considers that such effects are peripheral and primarily use nuclear melatonin receptors (RZR/ROR alpha and RZR beta) rather than the membrane receptors (MT1 and MT2) associated with rhythmic functions (Carlberg, 2000). Another explanation considers that appropriately timed daily melatonin administration optimises circadian relationships, especially of phase, and increases circadian amplitude (see Armstrong and Redman, 1991, and Arendt, 1995, for references). Such effects should lead to improved sleep, and thus enhanced immune function (Redwine et al, 2000).

3.5.5 *In vitro* phase shifts

The metabolic activity of the rodent SCN *in vivo* and the electrical activity of various *in vitro* SCN preparations can be modified by melatonin; it inhibits 2-deoxyglucose uptake into the nuclei in the late biological day with no effect at other times and inhibits electrical activity, also during the late biological day (Cassone et al, 1988; Shibata et al, 1989). There is very convincing evidence of the phase-advancing effect of melatonin on the circadian rhythm of electrical activity in cultured SCN neurons (McArthur et al, 1991). The effect was large, acute, and time dependent, with shifts of up to several hours being observed. Thus melatonin acts directly on a central biological clock to change its phase.

3.5.6 Retinal rhythms

Melatonin appears to function as a paracrine signal within the retina. It influences dopamine release (Dubocovich, 1983) and may enhance retinal function in low intensity light by inducing photomechanical changes and regulating turnover rates of the photoreceptive apparatuses of rods, cones, and the surrounding pigment epithelium (Reme, 1986; Iuvone and Gan, 1995; Iuvone et al, 2002). It is possible that retinal rhythmicity influences the central pacemaker. However, this question remains speculative.

3.5.7 Mechanisms

Using gene knockout technology and pharmacological manipulations, the results to date suggest that the phase-shifting melatonin receptor is MT2, whilst MT1 is associated with acute suppression of SCN electrical activity (Liu et al, 1997) in addition to its important actions within the pars tuberalis (Masana and Dubocovich, 2001; Ross and Morgan, 2002; Dubocovich et al, 2003). Several other physiological responses have been ascribed to MT1 and MT2 receptors, including (MT1) melatonin-mediated potentiation of adreneregic vasoconstriction and (MT2) modulation of dopamine release in the retina (Mahle et al, 1997; Doolen et al, 1998; Masana and Dubocovich, 2001). Genetic polymorphism has been identified within melatonin receptors and further investigation of these polymorphisms in relation to photoperioidism, human disease, sensitivity to melatonin, etc, is ongoing (Ebisawa et al, 2000; Migaud et al, 2002).

3.6 The pineal in human physiology and pathology

Clearly, the importance of the pineal in humans depends on the importance of light in human physiology. It is reasonable to assume that the pineal conveys information concerning light–dark cycles for the organisation of seasonal and circadian rhythms in humans as in animals. Pinealectomy in humans removes virtually all plasma melatonin (for review, see Arendt, 1995). Other consequences of the operation consist of diffuse neurological problems that do not add up to a consistent functional effect and may be more related to non-specific effects of the operation. Recent work suggests that melatonin is absent or very low in subjects with treated or untreated pineal germinomas (Murata et al, 1998), but the consequences remain to be defined. Interestingly, one study, as yet unpublished, suggests that seasonality is lost in pinealectomised humans (Macchi et al, 2002).

3.6.1 Measurement of melatonin and its primary metabolite 6-sulphatoxymelatonin

The most specific method of melatonin measurement is gas chromatography/mass spectrometry (GCMS) (Lewy and Markey, 1978). However, this technique is expensive, time consuming and has low throughput. Thus most investigators use either radioimmunoassay (RIA), which predates GCMS (Arendt et al, 1975), or enzyme-linked immunoassay (ELISA) (Middleton, in press). High performance liquid chromatography (HPLC) may also be used but does not have the throughput possibilities or, usually, the sensitivity of RIA and ELISA. The most reliable and specific RIAs and ELISAs are GCMS validated. Pre-extraction is required for most systems measuring plasma melatonin, although at least one GCMS validated assay operates successfully without extraction (Fraser et al, 1983a,b). By contrast, saliva measurements can usually be carried out without extraction (Vakkuri, 1985; McIntyre et al, 1987; English et al, 1993; Voultsios et al, 1997). The first urinary aMT6s assay to be developed was validated against GCMS measuring total urinary 6-hydroxyindoles rather than a specific GCMS assay (Arendt et al, 1985b; Aldhous and Arendt, 1988). To date, no specific GCMS assay exists for aMT6s.

Numerous commercial kits and reagents are available and a recent review addresses comparisons of different methods (Middleton, in press). In general, the sensitivity of assays has improved since the first publications, with analytical limits of detection for plasma and saliva melatonin now around 0.3 to

5.0 pg ml^{-1}, and for urinary aMT6s of 0.2 ng ml^{-1} or less. Reproducibility is reasonable, coefficients of variation are usually less than 15% and can be far less. These assays require care and attention to possible contaminants, usually listed by suppliers. For example, melatonin in powder form, opened in a laboratory, can contaminate a whole room which then requires in-depth cleaning. Direct measurements in saliva can be affected by food and drink, notably caffeine: subjects should wash their mouths out prior to sampling. Urinary aMT6s is robust and does not degrade at room temperature for 24 hours, at 4°C for 2 days and at −20°C for at least 2 years (Bojkowski et al, 1987b) and possibly as much as 15 years (Griefahn et al, 2001) without preservative.

If a 'pure' representation of melatonin production is required it is essential to control light exposure, posture and drug ingestion (see Table 3.1). The 'gold standard' for assessment of melatonin rhythms is the so-called 'constant routine' where subjects remain recumbent or semi-recumbent for at least 24 hours, in very dim light, awake, and with identical hourly snacks (Mills et al, 1978; Duffy and Dijk, 2002). This is evidently impossible in field situations. Here the use of urinary aMT6s enables long-term non-invasive monitoring of melatonin production. Ideally, light exposure and sleep times should be measured simultaneously (eg using an Actiwatch-L, Cambridge Neurotechnology Ltd).

Sampling frequency for melatonin measurement in plasma or saliva is usually hourly or half-hourly, depending on the degree of resolution needed. Very frequent sampling may lead to apparent episodic secretion (English et al, 1987; Claustrat et al, 1997). This may be in part an artefact of assay noise. However, the presence of two peaks of secretion has frequently been noted (Arendt, 1979, 1985; Wehr et al, 1995). There is undoubtedly a correlation between the total overnight melatonin production and the value obtained from a single early morning or 02.00 hours sample in normally entrained individuals (Arendt, 1978; Graham et al, 1998). However, a whole dimension of information (timing of the rise and fall, and the duration of secretion) is lost by using only a single measurement.

Sampling frequency for urinary aMT6s varies, but for field studies it is usually collected over 3 or 4 hour periods with a longer oversleep collection. A 24 hour urine collection will provide an estimate of overall production but not timing or duration of secretion (Bojkowski et al, 1987b). 'Overnight' total excretion has also been used, but small phase shifts (advance or delay) can lead to underestimation of production and if a subject has very delayed (or less commonly, advanced) circadian rhythms much of the peak production may be lost. A single morning urine sample in a normally entrained individual should provide values which correlate with overall night-time production. However, once again timing and duration are lost by this approach. Moreover, in subjects who are out of phase the relationship will no longer hold. Evidently the resolution of 3–4 hourly sampling will be less than 30 or 60 minute plasma or saliva melatonin measures. However, using these sampling intervals there is a very robust correlation with both the timing and amplitude of plasma melatonin (Arendt et al, 1985b; Bojkowski et al, 1987b; Ross et al, 1995). Naidoo (Naidoo and Arendt, unpublished observations) measured aMT6s in urine sampled at *hourly* intervals for 24 hours in 14 healthy volunteers. The parameters of acrophase (calculated peak time using cosine curve fitting techniques) and amplitude were derived using the hourly data and subsequently using the same data at 2, 3 and 4 hour intervals, with an 'oversleep' 8 hour interval inserted. The difference in calculated acrophase was on average 12 minutes between hourly data and 3 hourly data, although the significance of the cosine fit decreased with decreasing numbers of data points used (Naidoo, 1999). With 4 hourly sampling (and oversleep) intervals, a 48 hour collection period will provide significant cosinor fits in general.

It is possible to measure melatonin or aMT6s concentration in urine samples and relate the data to creatinine concentrations, in place of collecting the entire urine production for each collection period and measuring the volume. However, creatinine itself has a minor but significant circadian rhythm (difference between highest and lowest average values is reported to be from 6–29% of the average lowest value – Haus and Touiton, 1992), peaking during the early evening (Kanabrocki et al, 1988; Haus and Touiton, 1992). Thus the characteristics of the aMT6s or melatonin rhythms may be distorted by this method.

The timing and duration of melatonin secretion are its critical features with regard to physiological functions. The relevance of small changes in melatonin amplitude remains obscure, particularly since there is an enormous individual variation between normal healthy subjects (see Table 3.2).

Figure 3.11 indicates the markers used to characterise melatonin and aMT6s rhythms. Area under the curve, total 24 hour excretion (aMT6s) or cosinor derived amplitude are used to assess total secretion. At present there is no standard definition of onset/offset (and hence duration). The so-called dim light melatonin onset (DLMO) initially proposed by Lewy and Sack (1989) uses a specific plasma concentration

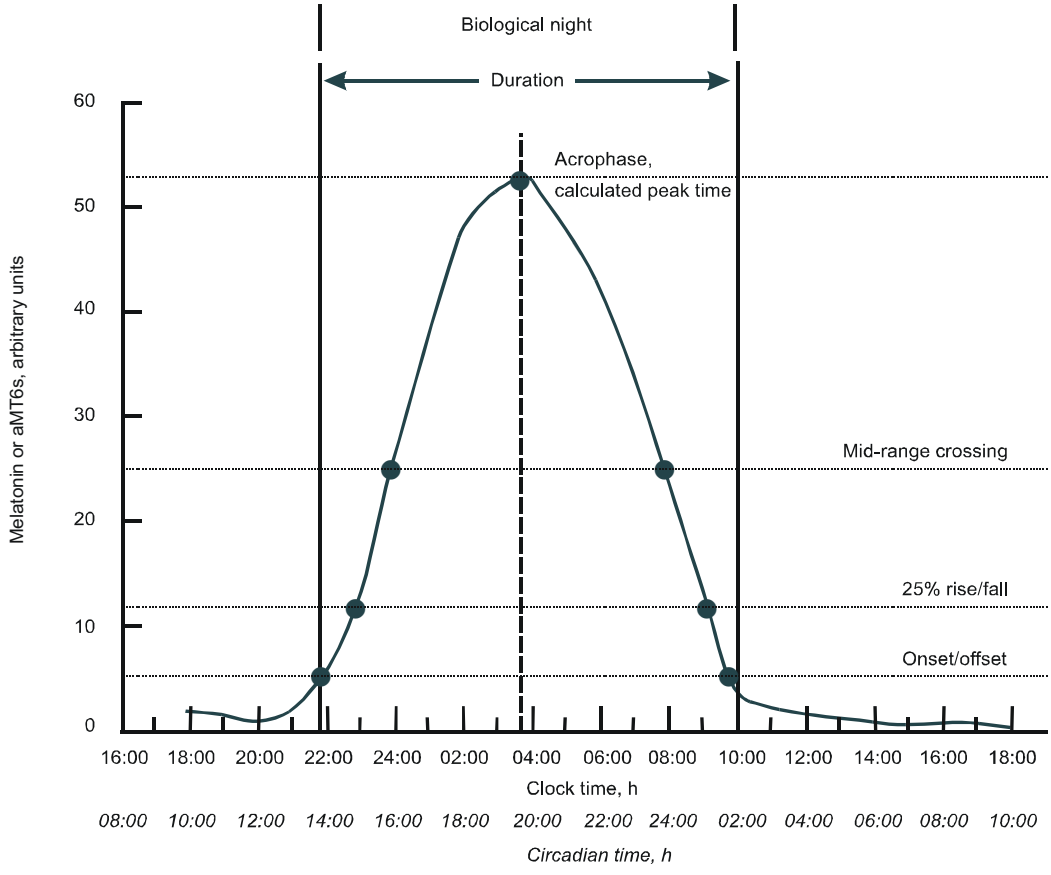


FIGURE 3.11 Phase markers for melatonin in plasma or saliva, or 6-sulphatoxymelatonin (aMT6s) in urine

above which melatonin secretion is considered to have started. However, in this case the amplitude of the rhythm influences the onset (and offset) time. Other definitions of onset/offset include values exceeding twice or thrice the standard deviation of mean baseline levels. With a noisy baseline the 'mid-range crossing' or 25% rise methods avoid problems (Figure 3.11). More sophisticated curve fitting techniques have been employed and appear to give reliable data (Brown et al, 1997; Gamst et al, 2004). One in particular bases the algorithm on the supposed switch on and switch off of melatonin synthesis (preceding the final drop to baseline in the morning) (Brown et al, 1997); another estimates the 'Synoff' or switch off of melatonin secretion using iterative regression fits (Revell et al, 2005). It is advisable to use all the methods available. Much depends on the assay in question since with different limits of detection onset and offset will vary. In recent studies Hoppen (Hoppen, Middleton, Stone and Arendt, unpublished observations) evaluated the stability of melatonin onset in saliva. Using values exceeding twice baseline and continuing to rise, melatonin onset in saliva was at 22.30 hours and with the mid-range crossing method was at 23.18 hours (12 young male subjects). When onset time was repeatedly assessed (five times) in the same 12 subjects at intervals of at least 2 weeks, the variation in onset time was 28±10 minutes and 24±9 minutes, X±SD, respectively, illustrating the reliability of the timing of melatonin production in free-living subjects (Hoppen, 2002) (see also the small variations found by Voultsios et al, 1997). The duration of melatonin secretion following 8 days of 16 hours of near-darkness, <5 lux, and recumbency, assessed recently in constant routine conditions was 9 hours ± 36 minutes (plasma, 8 young men) (Rajaratnam et al, 2003).

3.6.2 Human melatonin production

Both the amplitude and the duration of melatonin secretion in combination define production per 24 hours. Total urinary aMT6s per 24 hours provides an integrated measure of melatonin production in normal, healthy, unmedicated volunteers.

In a 'normal' environment, melatonin is secreted during the night in healthy humans as in other species. The rhythm is endogenous, with a period usually greater than 24 hours (Arendt et al, 1985a; Wever, 1989; Middleton et al, 1996; Wright et al, 2001). As is evident from studies in blind subjects, it is entrained primarily by the light–dark cycle with minor contributions of non-photic zeitgebers (Arendt, 2000). The average maximum levels attained in plasma in adults are around 60–70 pg ml^{-1} when measured with high specificity assays (see Arendt, 1995). Salivary melatonin reflects well the plasma profile but concentrations are approximately 30% of those in plasma. Mean maximum concentrations of aMT6s in plasma attain 80–100 pg ml^{-1}. Minimum concentrations of both compounds are usually below 10 pg ml^{-1}. Peak concentrations of melatonin in plasma and saliva normally occur between 02.00 and 05.00 hours. The onset of secretion is usually around 21.00 to 23.00 hours and the offset around 07.00 to 09.00 hours in adults in temperate zones (depending on the definition of onset and offset, see Figure 3.12). There is a strong correlation of both amplitude and timing of the melatonin rhythm in plasma and saliva with aMT6s in normal, healthy individuals, as previously noted. Drug treatment and disease may modify this relationship. The appearance and peak levels of aMT6s in plasma are delayed by 1 to 2 hours and the morning decline by 3 to 4 hours with respect to melatonin. Between 50 and 80% of exogenous melatonin is converted to aMT6s (Middleton et al, 1997) and most appears in the overnight sample (24.00 to 08.00 hours) in a normal environment. It is low, but rarely undetectable, in the afternoon and early evening.

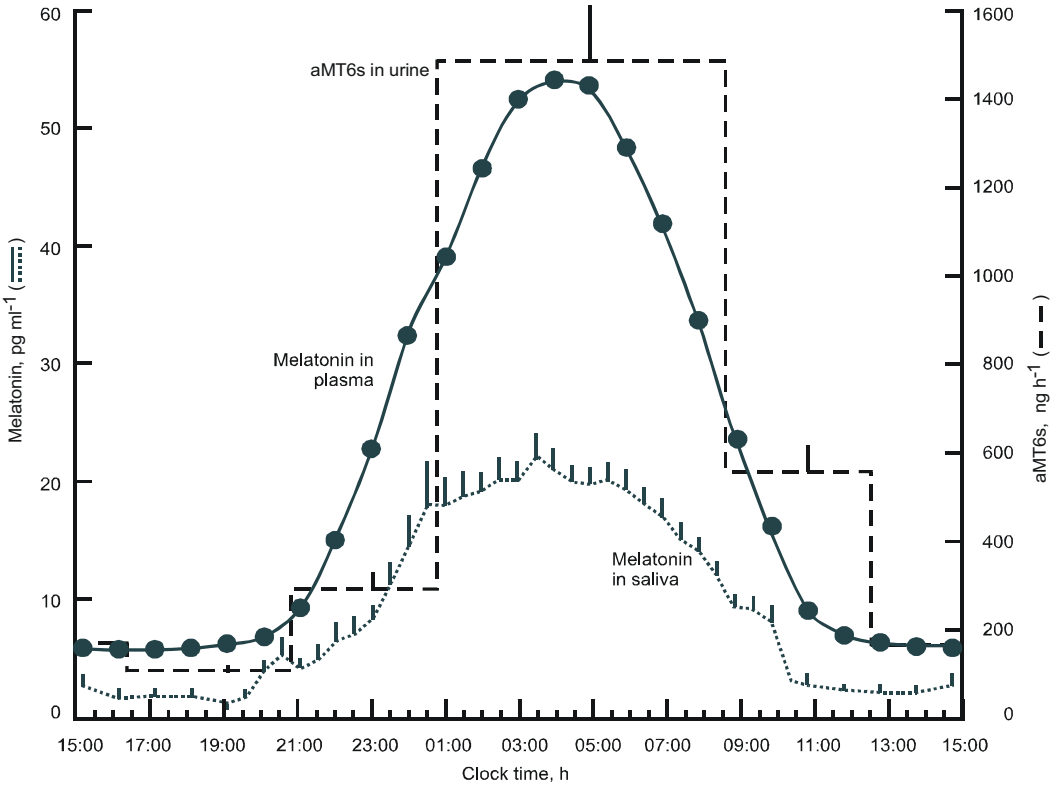


FIGURE 3.12 Mean concentrations (+ SEM) of melatonin in plasma (N=133, error bars within the symbol), saliva (N=28) and 6-sulphatoxymelatonin (aMT6s) in urine (N=88), healthy volunteers, all ages, M and F, all measurements by radioimmunoassay (Fraser et al, 1983a,b; Aldhous and Arendt, 1988; English et al, 1993)

Possibly the most striking characteristic of the normal human melatonin rhythm is its reproducibility from day to day and from week to week in normal individuals, rather like a hormonal fingerprint (Arendt, 1979, 1988; Bojkowski et al, 1987b; Voultsios et al, 1997; Klerman et al, 2002). This stability leads to the extensive use of melatonin in plasma or saliva, and aMT6s in urine, as marker rhythms for circadian phase – for example, in the investigation of sleep disorders, and evaluating adaptation to abrupt phase shifts as in shift work and jet lag. The very large inter-individual variations have been ascribed to the size of the pineal gland rather than to variations in enzymic activity, at least in sheep (Gomez Brunet et al, 2002) (Figure 3.13). A small number of apparently normal individuals have no detectable melatonin in plasma at all times of day.

In view of this inter-individual variation it is necessary to use subjects as their own controls in small studies. Table 3.2 shows some normal values for aMT6s, using the method of Aldhous and Arendt (1988), from young, healthy volunteers. There are no differences with sex in this series. A decline with age is apparent, as previously observed on numerous occasions (reviewed by Arendt, 1995). Importantly the timing of the rhythm is earlier with increasing age: a factor rarely taken into consideration. A power

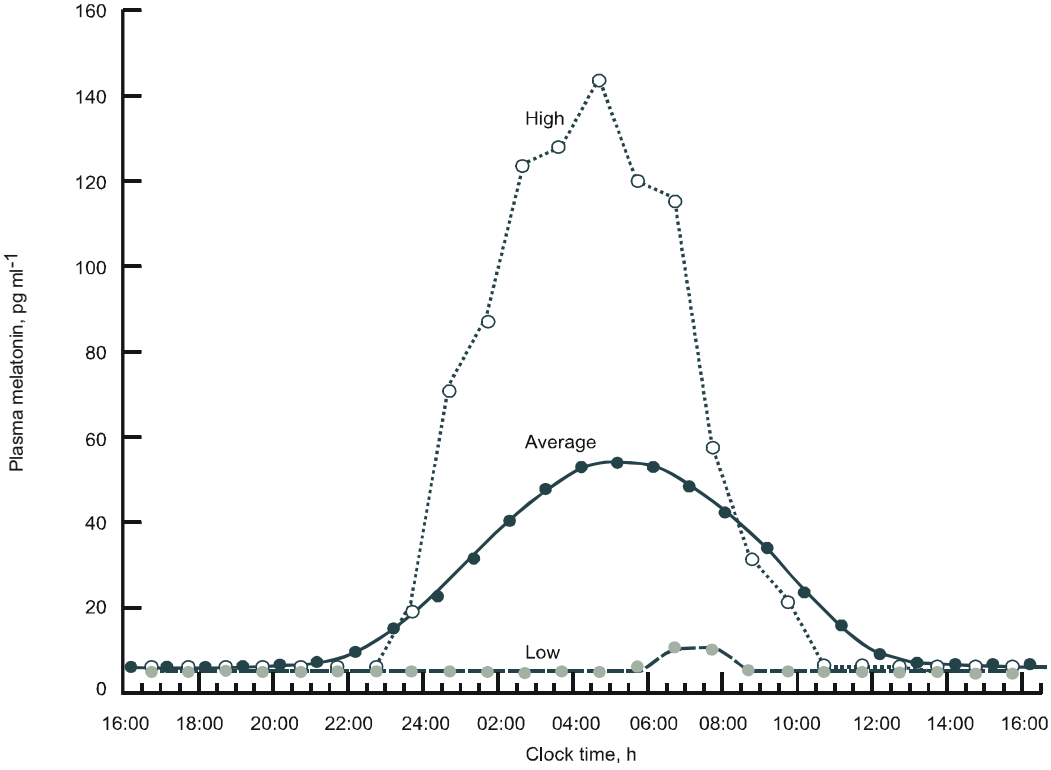


FIGURE 3.13 Examples of high (28 year old male) and low (30 year old male) melatonin plasma levels in normal, healthy volunteers compared with average data

TABLE 3.2 6-sulphatoxymelatonin (aMT6s) excretion in healthy, predominantly young male volunteers, N=85, examples of 'normal values' for total excretion per 24 hour and peak time. Note the very large range (maximum–minimum). Younger subjects (16–34) excrete more aMT6s than the older group and have later acrophases (ANOVA p=0.010). Previously reported normal values over the whole age range can be found elsewhere (Bojkowski and Arendt, 1990; Skene et al, 1990)

Subjects	Age (years), $\bar{X}$ ± SD	Total aMT6s excretion (ng per 24 h), $\bar{X}$ ± SD	Rhythm acrophase decimal (h), $\bar{X}$ ± SD, N	Rhythm amplitude (ng per h), $\bar{X}$ ± SD, N
All	26.4 ± 8.3	16072 ± 10898	4.4 ± 1.3	913 ± 776
Maximum	56	60536	8.1	4733
Minimum	16	540	0.9	70
All female (N=24)	28.21 ± 8.8	16827 ± 12741	4.55 ± 1.13	965 ± 943
All male (N=61)	25.66 ± 8.1	15774 ± 10185	4.38 ± 1.33	892 ± 707
16–24 years (N=49)	21.49 ± 2.1	16045 ± 10565	4.78 ± 1.2	904 ± 747
25–34 years (N=23)	28.5 ± 2.9	17856 ±14099	4.01 ± 1.3	1070 ± 1027
35–56 years (N=13)	43.4 ± 7.1	11387 ± 5908	3.95 ± 1.1	585 ± 385

calculation using the mean and standard deviation of aMT6s excreted per 24 hours indicates that for a 20% change to be found in a given population with 83% power and a significance level of p=0.05, 200 subjects must be studied.

Skene et al (1990) analysed 24 hour aMT6s production (6 hourly samples) in 160 healthy women, aged 40 to 69 years. These data are shown in Figures 5.2 and 5.3 in Chapter 5. They illustrate the age-related decline in 24 hour aMT6s production.

The amplitude of the melatonin rhythm appears to be an inherited characteristic, at least in sheep (Zarazaga et al, 1998), as may be the entrained phase (as a function of inherited periodicity). A polymorphism in the clock gene *per3* is strongly associated with extreme diurnal preference which in turn is related to long or short circadian period (Duffy et al, 1999; Gibertini et al, 1999).

Whilst many blind subjects with no perception of light (conscious or unconscious) show their intrinsic periodicity in a normal environment, ie they 'free-run', or have an abnormal phase of melatonin, the amount of melatonin or aMT6s produced is comparable to that of sighted individuals (Orth et al, 1979; Lewy and Newsome, 1983; Lockley et al, 1997a) and the duration of melatonin secretion does not differ from normal sighted subjects (Klerman et al, 2001). Only a small number of subjects have been investigated to date (Table 3.3). Light suppression of melatonin in the blind is of course only possible in subjects with (sometimes unconscious, unperceived) retained circadian light perception (Czeisler et al, 1995). These observations suggest that in a normal entrained environment, light exposure of sighted subjects, even in the evening, does not impact greatly on melatonin production. Healthy male subjects kept in very dim light (<5 lux) for periods up to four weeks show free-running melatonin rhythms, but the production of aMT6s is not different from that found in normal lighting (Middleton et al, 1996). It is worth noting that urban humans with artificial lighting are not normally exposed to substantial changes in photoperiod.

Possible decreased incidence of breast cancer in blind subjects has been addressed as potentially relevant to the role of melatonin in breast cancer aetiology (see Chapter 5). As discussed above, there is no

TABLE 3.3 6-sulphatoxymelatonin (aMT6s) production and intrinsic rhythm periodicity (tau) in sighted healthy subjects in a normal environment, in blind subjects, and in sighted subjects kept for 3–4 weeks in continuous very dim light (<8 lux). Published data (Middleton et al, 1996, 1997; Lockley et al, 2000; Hack et al, 2003) and normal laboratory values using reagents from Stockgrand Ltd, University of Surrey

Subjects	aMT6s (ng per 24 h), $\bar{X}$ ± SD	N	Intrinsic period decimal (h), $\bar{X}$ ± SD
Normal healthy sighted adults			
All ages, M and F	12601 ± 9528	182	24.00 entrained
Registered blind, no perception of light			
All ages, M and F	9350 ± 6380	30	24.49 ± 0.17 free-running
Registered blind, some light perception			
All ages, M and F	12650 ± 7450	19	24.00 entrained
Normal sighted young men, ages 20–30 years			
Normal environment	16410 ± 10020	65	24.00 entrained
Light <8 lux, partial temporal isolation	18900 ± 9120	12	24.33 ± 0.15 free-running

evidence that totally blind people have more melatonin than sighted people. However, distinctions are not always made between 'blind' subjects with no conscious perception of light but who may retain circadian photoreception, and the totally blind with no photoreception at all.

3.6.3 Seasonal variations

In temperate and polar zones, a delay in the timing of melatonin secretion in winter is often observed (Broadway et al, 1987; Bojkowski and Arendt, 1988; Midwinter and Arendt, 1991; Yoneyama et al, 1999). It is likely that decreased strength of the light–dark time cue in winter and a lengthening of the night lead to weak entrainment of the rhythm, since increasing light exposure (one hour bright white light in the early morning and evening) is sufficient to restore summer phase position (Broadway et al, 1987). There are also some inconsistent reports of an increase in duration of secretion and hence quantity of melatonin secreted in winter (Beck-Friis et al, 1984; Makkison and Arendt, 1991). Levels may also be increased in winter in high Arctic latitudes (Kauppila et al, 1987; Kivela et al, 1988), and it has been suggested that a reduced risk of cancer found in Arctic residents is due to greater melatonin secretion during the Arctic winter (see Chapter 5). There is certainly evidence for increased melatonin levels in the Arctic winter (which has been associated with anovulatory cycles (Kauppila et al, 1987). However, in Antarctic studies no difference in peak or daytime values was found between summer and winter, rather a change of timing of the rhythm (later in winter) was noted. If daytime melatonin appears to be increased, this may well in some cases be due to a delay in the timing of the peak levels and 'spill over' into the morning hours (Broadway et al, 1987; Midwinter and Arendt, 1991). This is particularly important to bear in mind if only a single morning sample (of plasma, saliva or urine) is taken.

Any delay or advance in melatonin secretion means that simply taking an early morning urine or plasma sample can give data suggesting increased or decreased production.

3.6.4 Association with core temperature

Many associations of melatonin with temperature exist in humans. The most striking is the reciprocal relationship in circadian profiles, where the temperature nadir correlates closely with the peak of melatonin (Figure 3.14). The increase in core temperature induced by light at night, and associated with melatonin suppression (see Table 3.1), could be at least partially opposed by replacement melatonin. Possibly half of the night-time decline in core temperature might be ascribed to the hypothermic effects of endogenous melatonin. A causal relationship is also indicated as exogenous melatonin can acutely depress body temperature in humans (Strassman et al, 1991; Cagnacci et al, 1992). The ovulatory rise in temperature during the menstrual cycle has been associated with a reported decline in the amplitude of melatonin, but the decline in melatonin is not a consistent observation.

3.6.5 Association with sleep

Obvious correlations are noted between melatonin production at night and sleep and, again, specific causal relationships may exist. However, sleep deprivation does not abolish the melatonin rhythm and, in very dim light, does not apparently affect secretion (Akerstedt et al, 1979). During sleep deprivation,

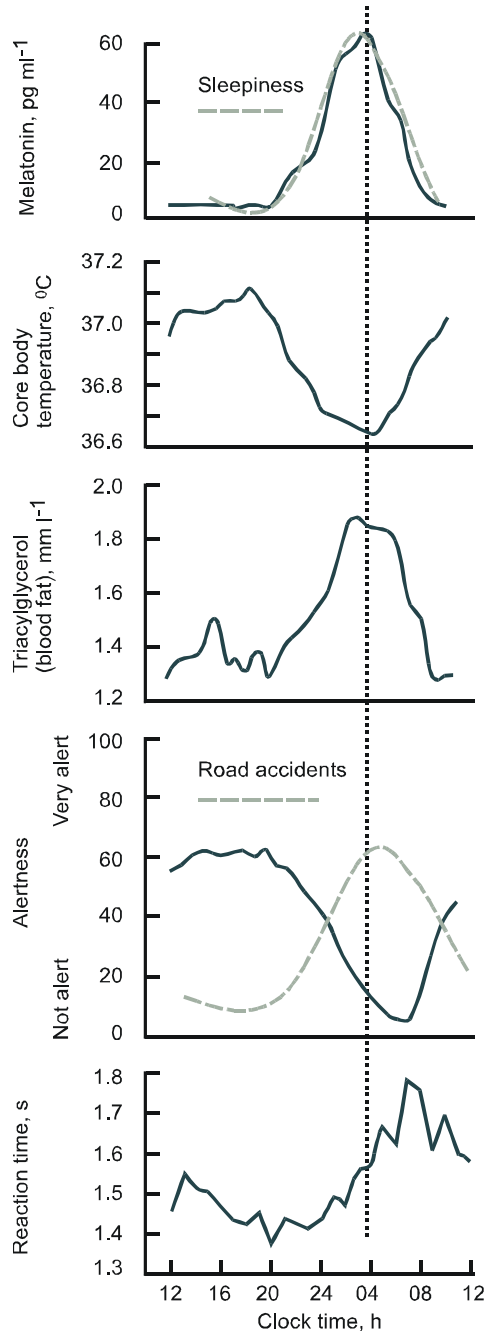


FIGURE 3.14 Relationship of plasma melatonin to other major circadian rhythms. Note the close correspondence between the core temperature nadir, the alertness nadir, the peak accident rate, slowest reaction time and fat metabolism with the melatonin peak. Reproduced from Rajaratnam and Arendt, 2001, by permission

self-rated fatigue exhibits a circadian rhythm that is closely correlated with plasma melatonin levels (Akerstedt et al, 1979). The close relationship between the evening rise of circulating melatonin and the evening increase in sleep propensity suggests a causal relationship (Wehr et al, 2001). There is a correlation between the timing of sleep spindles and certain other electroencephalographic (EEG) characteristics and the circadian phase of melatonin (Dijk et al, 1997). Careful observations in constant routine conditions of the profiles of melatonin, core body temperature, sleep propensity, sleepiness, cortisol, REM, etc, have led to a definition of biological night in humans (Wehr et al, 2001). This corresponds to the period during which melatonin is secreted, with an apparent switch process at 'dusk' and 'dawn' that is common to melatonin onset and offset, core temperature rise and decline, and increasing–decreasing sleep propensity. The so-called 'forbidden zone for sleep' or 'wake maintenance zone' occurs just prior to the onset of melatonin secretion (Shochat et al, 1997) and can be overcome by melatonin administration to advance the evening rise (Arendt et al, 1984, 1985a; Deacon and Arendt, 1995; Rajaratnam et al, 2004). The peak of melatonin secretion is also associated with the nadirs of alertness and performance (Carrier and Monk, 2000), together with increased blood lipid levels at night (Morgan et al, 1998) (Figure 3.14).

Patients suffering from non-24-hour sleep–wake disorder (largely blind subjects with no perception of light) intermittently secrete melatonin during the daytime. This is strongly associated with the presence of daytime naps (Lockley et al, 1997b). Smith-Magenis syndrome (caused by a deletion in chromosome 17p11.2) is associated *inter alia* with daytime melatonin production, poor night-time sleep, but with normal cortisol rhythms (De Leersnyder et al, 2001, 2003). Treatment of these patients with atenolol (a beta-adrenoreceptor antagonist) to suppress daytime melatonin was apparently successful in reducing daytime sleepiness, suggesting a contribution of the endogenous hormone to this phenomenon (De Leersnyder et al, 2001, 2003).

3.6.6 Association with posture

Changes in posture may affect circulating melatonin concentrations (Table 3.1). Abrupt transitions from recumbent to standing position increase melatonin, presumably because binding proteins retain this small molecule in blood whilst haemodynamic effects lead to diffusion of other unbound small molecules and water out of blood into the extracellular spaces, thereby concentrating bound molecules (Deacon and Arendt, 1994; Nathan et al, 1998). However, another study found no effect of posture itself or changes in posture on salivary melatonin (Voultsios et al, 1997).

3.6.7 Other associations

Obviously, any variable with a marked circadian rhythm shows correlations with melatonin – if necessary, displaced in time. Examples include cortisol, prolactin, thyroid-stimulating hormone, aspects of the immune system, and many others. Nocturnal exercise increases melatonin and can induce phase shifts (Strassman et al, 1989; Buxton et al, 2003). The relationships of stress and some other non-pharmacological interventions in modification of melatonin production are somewhat unclear and do not appear to play a major role in humans (see Table 3.1).

3.6.8 Development, puberty and ageing

Shortly after birth, very little melatonin or aMT6s is detectable in body fluids. A robust melatonin rhythm appears around 6 to 8 weeks of life (Kennaway et al, 1996). Whether in specific individuals this rhythm corresponds to the organisation and synchronisation of other circadian variables such as sleep remains a question of considerable interest. The plasma concentration of melatonin increases rapidly thereafter and reaches a lifetime peak on average at 3–5 years of age (for a review, see Arendt, 1995). The increment is much greater at night. Subsequently, a steady decrease is seen, with mean adult concentrations attained in the mid to late teens and the major decline occurring before puberty. Values remain relatively unchanged until 35–40 years of age (25–30 according to Kennaway et al, 1999), and a final decline in amplitude then takes place until (on average) low levels are seen in old age with the exception of one study using healthy elderly subjects (Kennaway et al, 1999; Zeitzer et al, 1999) Reports of an association of differences in secretion in adults with sex, height, or body weight are not consistent.

Although a lower melatonin concentration has been reported in children with precocious puberty and higher concentrations in those with delayed puberty and hypothalamic amenorrhea than in age-matched controls (reviewed by Arendt, 1995), these associations remain correlative and not causal. Ovarian suppression with a GnRH analogue in precocious girls was not accompanied by changes in melatonin secretion (Berga et al, 1989). However, in some case reports, induction of sexual development was associated with a decline in melatonin production (Arendt et al, 1989; Puig-Domingo et al, 1992).

Maternal melatonin appears to be transferred to the fetus in humans as it is in rats and hamsters (Okatani et al, 1998). Thus any manipulations of maternal melatonin levels may impact on the fetus. In photoperiodic species maternal melatonin influences timing of circadian phase and pubertal development (see Section 3.1). Moreover, circulating levels of plasma melatonin are reflected in the profile of melatonin in human milk (Illnerova et al, 1993). These observations imply that physiological 'insults' to the maternal circadian system (for example, by shift work or time-zone travel) may have effects on the progeny. However, in rats, manipulation of maternal milk melatonin does not appear to influence development (Rowe and Kennaway, 2002).

3.6.9 Menstrual cycle

Some of the very earliest reports on human melatonin described low preovulatory concentrations the morning before ovulation and suggested that low melatonin was facilitatory to the preovulatory LH peak. This observation is inconsistent, however, and more recent work indicates that neither the amplitude nor the phase of melatonin is altered in the course of the normal menstrual cycle (Brzezinski et al, 1988; Leibenluft et al, 1994; Parry et al, 1997; Wright and Badia, 1999). However, there is some evidence that oral contraceptives may increase melatonin (Kostoglou-Athanassiou et al, 1998; Wright and Badia 1999; Thapan, Arendt and Skene, unpublished data). In spite of numerous attempts made to relate melatonin to endogenous gonadal steroids in humans, little consistency emerges.

The effects of melatonin on core body temperature are reported to vary in the course of the cycle, and herein may lie a physiological function (Cagnacci et al, 1996, 1997). LH pulses are amplified in the early follicular phase by oral melatonin at 08.00 hours (Cagnacci et al, 1995). In very large doses (75–300 mg)

melatonin can suppress the ovulatory peak of LH secretion (Voordouw et al, 1992). However, attempts to develop melatonin as a contraceptive pill in combination with a synthetic progestin minipill have not been successful.

Very large doses (100 mg daily) of melatonin potentiate testosterone-induced LH suppression (Anderson et al, 1993b). A series of studies in males with and without hypogonadism has reinforced the perception that melatonin is essentially inhibitory to human reproductive function (see, for example, Luboshitzky et al, 1996, 1997, 2003; Luboshitzky and Lavie, 1999). Because humans appear to conceive more readily in long photoperiods, an explanation may reside in residual human photoperiodism (Roenneberg and Aschoff, 1990; Bronson, 2004). The results of these studies partially support the contention that melatonin, suitably administered, can inhibit human reproductive activity. Other data, however, indicate that over periods of eight days to several weeks, daily melatonin administration (low pharmacological doses) has no effect on a number of pituitary/gonadal hormones (Arendt et al, 1985a; Wright et al, 1986; Luboshitzky et al, 2000; Rajaratnam et al, 2003).

3.7 Effects of exogenous melatonin in humans

3.7.1 Early work

Enormous doses of up to 6.6 g (the daily production of about 200,000 people) in the daytime had no beneficial effects on Parkinsonism, Huntington's chorea, depression (which was worsened), and schizophrenia. Skin pigmentation was not affected: human pigment cells do not resemble amphibian melanophores in pigment migration phenomena. Small decreases in plasma LH and FSH were observed (see Arendt, 1995, for references). Large amounts of melatonin such as these may produce headache, abdominal cramps, and somnolence.

3.7.2 Acute effects

Much lower (2 mg intranasally, 0.3–240 mg orally or intravenously) doses of melatonin during the 'biological day', ie when endogenous melatonin levels are low, can induce transient sleepiness or sleep, and lower core body temperature, in suitably controlled circumstances. Posture is important; the greatest effects are seen with recumbent subjects in very dim light (see Arendt, 2003, 2005, for references). The initial evidence dates from 40 years ago when Aaron Lerner, who first isolated the substance, took 100 mg and described sleepiness after the dose. Early investigations used EEG characteristics to delimit an acute mild sedative and 'hypnotic' effect in both animals (cats, rats and chickens) and humans. Subsequently, a substantial body of literature, generally using much lower doses, has described advance shifts in the timing of sleep after early evening administration, transient sleepiness at several different times of day within two to four hours of the dose, time-dependent increases in sleep propensity, effects on the waking EEG comparable to but not identical with benzodiazepines, a lengthening of the first rapid eye movement episode after early evening administration, increases in the fast EEG frequencies after evening naps or night-time sleep, and 'beneficial effects' when taken at bedtime. The subject has been extensively reviewed recently (Zhdanova et al, 1997; and see *Sleep Medicine Reviews*, volume 9, 2004). A brief decline in performance but with no effect on memory, has

been reported after daytime melatonin administration (Lieberman et al, 1984; Graw et al, 2001). Acute oral doses of melatonin stimulate prolactin secretion (Wright et al, 1986; Waldhauser et al, 1987). This enhanced prolactin secretion may relate to the ability of melatonin in pharmacological amounts to inhibit some dopaminergic functions. Acute effects on other pituitary hormones are somewhat inconsistent, although recently a relationship between melatonin and vasopressin secretion has been established (Forsling et al, 2000). The acute pharmacological properties of melatonin in animals include sedation, hypothermia, anxiolysis, muscle hypotonia, decrease in locomotor activity with a rebound increase on increasing the dose, slight analgesia, slight protection against electroconvulsive shock, constriction of cerebral arteries, potentiation of noradrenalin-induced vasoconstriction, and very low toxicity (Guardiola-Lemaitre, 1997).

3.7.3 Phase shifting

In early work, daily feeding of low dose (2–5 mg) melatonin in the late afternoon advanced the timing of evening self-rated fatigue, the endogenous melatonin rhythm, and the morning decline in prolactin when compared with placebo. No significant effects were seen on self-rated mood or on LH, FSH, testosterone, cortisol, growth hormone, or thyroxine. No deleterious effects were reported by the subjects (Arendt et al, 1985a). Thus in low doses, melatonin has some chronobiotic effects in humans. Melatonin has rapid, transient, mild sleep-inducing effects and lowers alertness and body temperature during the 3 to 4 hours after low doses (0.5–5 mg) during the daytime, these effects being opposite to the acute effects of bright light given at night. In the same dose range it is able to shift timing of the internal clock to both later and earlier times when administration is appropriately timed (Figure 3.15). As for light, the appropriate timing can be predicted from a phase–response curve in subjects whose body clock phase is known (Lewy et al, 1998) (Figure 3.15). The phase–response curve to melatonin is essentially the reverse of that to light. Melatonin given approximately 8–13 hours before core temperature minimum will produce phase advances and, when given around 1–4 hours after core temperature minimum, will produce phase delays, although there is some controversy regarding the ability of melatonin to phase delay the circadian system. A recent, carefully controlled study (Rajaratnam et al, 2003, 2004) showed convincingly that daily administration of a 'surge sustained' release preparation (1.5 mg) of melatonin at 16.00 hours followed by recumbency and very dim light for 16 hours led to substantial phase advances of a number of circadian 'marker' rhythms and an advanced timing and redistribution of sleep during the dark phase. No increase in total sleep time was seen, reinforcing the view that melatonin acts on the timing mechanisms of sleep rather than being a 'hypnotic'. There were no deleterious effects on pituitary/gonadal hormones or daytime alertness. In this dose, reinforced by recumbency and dim light, melatonin is clearly very effective at shifting the circadian clock and sleep timing (Rajaratnam et al, 2003, 2004).

3.7.4 Entrainment

Melatonin can maintain entrainment to 24 hours in sighted subjects kept in a dim light environment conducive to free-running (Middleton et al, 1997). Most importantly perhaps, recent data have shown that timed melatonin administration (0.5–5 mg at 24 hour intervals, usually at desired bedtime) can fully entrain (or synchronise) the free-running circadian rhythms of most blind subjects exhibiting this

phenomenon, with a consequent improvement in sleep and daytime alertness. These observations, from two independent laboratories, indicate that melatonin can be as effective as light for circadian rhythm management, and is the treatment of choice for non-24-hour sleep–wake disorder of the blind (Arendt, 2000; Lockley et al, 2000; Sack et al, 2000; Lewy et al, 2001; Hack et al, 2003).

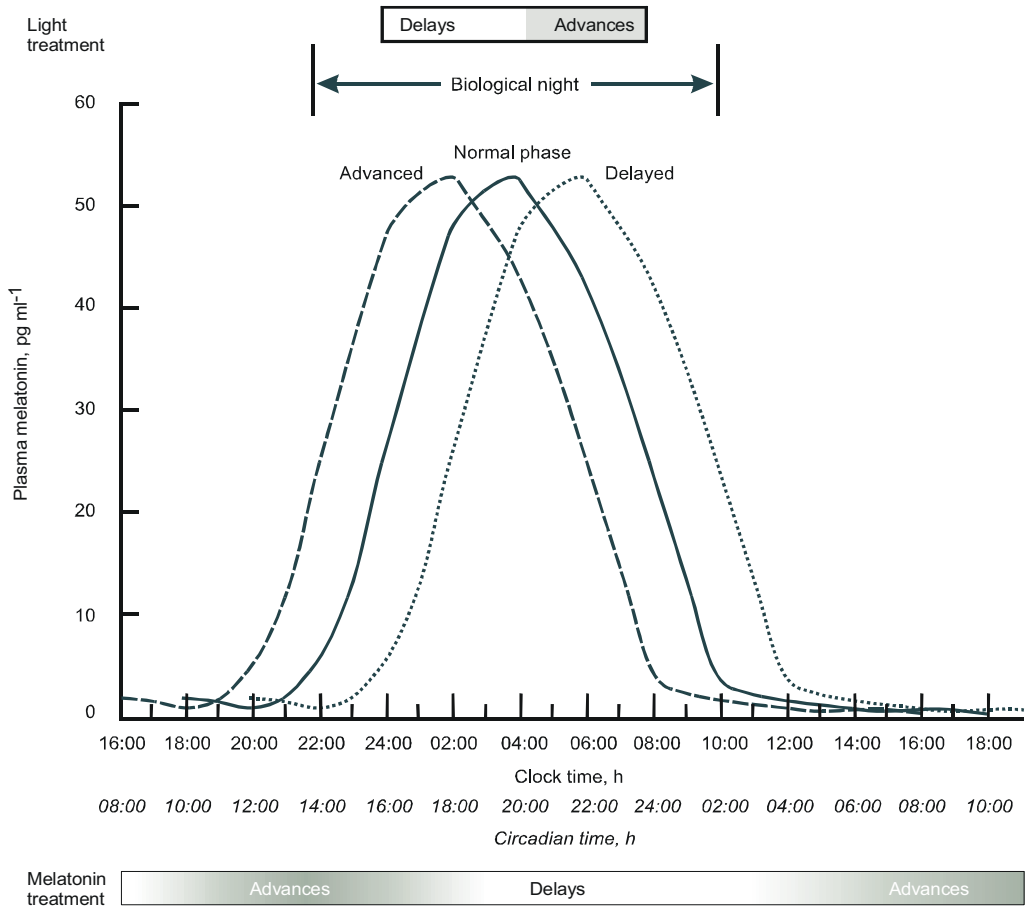


FIGURE 3.15 'Phase response curve' (PRC) to melatonin administration (from data in Zaidan et al, 1994; Middleton et al, 1997; Lewy et al, 1998). Melatonin (0.05–10 mg) given in the 'biological evening' advances the internal clock as evidenced by changes in timing of the endogenous melatonin rhythm, together with those of sleep, core body temperature, cortisol and other circadian rhythms assessed to date. In the 'biological early morning' delays are reported but remain controversial. Timed light treatment has essentially opposite effects on circadian rhythm timing and the PRC to light is approximately a mirror image of that to melatonin. Circadian time (CT) is a convention whereby the circadian period is divided into 'circadian hours' and referenced either to the melatonin onset (CT14) or to the core body temperature minimum (CT0). In the present illustration, circadian period in an entrained individual is 24 hours; however, the technique permits comparison of data from free-running individuals with periods differing from 24 hours

3.7.5 Jet lag and shift work

Melatonin treatment timed to induce phase advances and delays has been used in the alleviation of jet lag in at least 14 studies of real-life and simulation conditions, 11 of which reported beneficial effects. Field studies show that self-rated jet lag both westward and eastward can be reduced on average by 50% with appropriately timed treatment (Arendt et al, 2005). A recent Cochrane review concluded that melatonin was effective if used correctly to adapt to time-zone change (Herxheimer and Petrie, 2002). Exposure to bright light sufficient to suppress melatonin secretion during the night is clearly beneficial to night-shift workers in terms of alertness and performance (Czeisler et al, 1990, Bjorvatn et al, 1999). Preliminary work suggested improved sleep and increased daytime alertness in night-shift workers receiving melatonin at the desired bedtime (during the day) during a night-shift week as compared with placebo and baseline conditions (Folkard et al, 1993). A number of recent studies have successfully used melatonin to adapt to simulated or real shift work (Burgess et al, 2002), although it has to be said that several reports in the literature have shown no beneficial effects. Questions of posture, light environment and timing need to be resolved in field studies.

3.7.6 Blindness

Only a small number of subjects have been studied to date, but undoubtedly most blind/visually impaired subjects reporting sleep problems derive benefit from melatonin ingestion at the desired bedtime or specifically timed according to the circadian phase (Arendt et al, 1997; Arendt, 2000). Melatonin was strikingly effective at improving sleep and behaviour in multiply handicapped children with or without visual impairment (Jan et al, 1994).

3.7.7 Sleep problems of old age

Increasing numbers of positive reports now indicate that some elderly subjects will derive benefit from melatonin administration, including Alzheimer patients. It is possible that very low doses are more useful than 2–5 mg and that improvement is seen when an underlying rhythm disorder is present rather than non-specific insomnia. Dose timing and formulation remain to be optimised.

3.7.8 Delayed-sleep phase insomnia

Patients with delayed-sleep phase insomnia cannot sleep at the socially acceptable time of night; they delay sleep onset until the early hours of the morning and sleep through much of the day. This condition has been successfully treated with bright light in the early morning to induce phase advances of the clock. In others, evening melatonin (5 mg at 22.00 hours or 5 hours ahead of endogenous melatonin onset) also advances sleep time significantly. Judicious, timed application of both melatonin and bright light as time cues may well be the treatment of choice for such rhythm disturbances (Arendt and Skene, 2005).

3.7.9 Cancer

The preceding sections on the effects of melatonin have largely dealt with it as a chronobiotic, in line with its accepted physiolological properties. The approach to its possible use in cancer treatment has been more concerned with free radical scavenging and immunostimulant properties. However, chosen treatment times (usually early or later evening) are likely to induce phase advances and conceivably more optimal circadian phase, with consequent benefits for sleep and the functions related to sleep.

A few reports of positive effects of combination therapy – for example, melatonin and tamoxifen, melatonin and interleukin – require confirmation (Lissoni et al, 1996, 2000, 2003). Recently, the emphasis on the potential use of melatonin in cancer chemotherapy has moved to propose that it is effective at reducing the toxicity and increasing the effectiveness of existing treatments (Vijayalaxmi et al, 2002). Another consideration is the use of melatonin to 'optimise' circadian phase with regard to timed cancer treatment (chronotherapy). With the current striking data concerning the influence of clock gene function on cancer development (Fu et al, 2002; Fu and Lee, 2003) the potential influence of melatonin on clock gene function urgently needs further investigation.

3.8 Mechanisms of melatonin action

Melatonin, as described above, has been implicated in a diverse range of actions, and it implements these actions through a variety of mechanisms. The specific effect induced depends on the type of cell and the particular molecules that the cell possesses to interact with the hormone. Some cells possess receptors that bind melatonin but the hormone also has other routes of action.

3.8.1 Melatonin receptors

The development of 2-^{125}I-iodomelatonin as a high specific activity ligand has permitted the identification of high affinity (equilibrium dissociation constant, K_d, of 25 to 175 pM), saturable, specific, and reversible melatonin binding to cell membranes in the central nervous system, initially in the SCN (Vanecek et al, 1987) and the pars tuberalis of the pituitary (Morgan and Williams, 1989) and subsequently in many brain and other areas, including cells of the immune system, a number of cancer cell lines, the gonads, the kidney and, importantly, the cardiovascular system. The SCN shows clear binding in human postmortem tissue (Reppert et al, 1996). Species variation of melatonin-binding sites in the brain is of course apparent. The most consistent (but not universal) binding site between mammalian species is the pars tuberalis, primarily implicated in transduction of the effects of photoperiod, via melatonin, on seasonal variations in prolactin secretion in ruminants (Lincoln and Clarke, 1994).

Krause and Dubocovich (1990) have demonstrated a functional melatonin receptor initially in rabbit and chicken retina (inhibition of calcium-dependent dopamine release) that is localised in dopamine-containing amacrine cells in the inner plexiform, in the outer and inner segments in mice, and possibly in the pigmented layer in some mammals.

The interaction of melatonin with nuclear receptors (RZR/ROR alpha and RZR beta) and intracellular proteins, such as calmodulin or tubulin-associated proteins has also been reported. The transcription

factor RZR/ROR alpha may mediate a direct gene regulatory action of the hormone. It has been hypothesised that while the effects of melatonin on circadian and seasonal rhythms appear to use the membrane receptors, peripheral effects of melatonin (such as immunomodulatory effects) may largely be mediated by RZR/ROR alpha (Carlberg, 2000).

3.8.2 Melatonin receptor pharmacology

White et al (1987) initially demonstrated that melatonin-induced pigment aggregation in amphibian melanophores is a pertussis toxin-sensitive system and that melatonin inhibits forskolin-activated cAMP formation. Inhibition of cAMP production may be a general feature of melatonin receptors. Intensive investigation of the properties of the pars tuberalis-binding site has revealed that physiological doses of melatonin inhibit forskolin-activated cAMP production *in vitro* in a time- and dose-related manner (Morgan and Williams, 1989). Other studies have provided good evidence that most binding sites are coupled to G proteins (Reppert, 1997). Guanosine triphosphate analogues, which interfere with the regeneration of G_1-coupled receptors, decrease the affinity and sometimes the capacity of ^{125}I-melatonin binding in reptiles, birds and mammals.

Melatonin receptors have been cloned, and three subtypes were initially named Mel-1a, Mel-1b and Mel-1c (Reppert, 1996; Reppert et al, 1996). The Mel-1a receptor gene has been mapped to human chromosome 4q35.1. Its primary expression is in the pars tuberalis of the pituitary and the SCN. Mel-1b has been mapped to chromosome 11q21-22 and its main expression is in the retina and the brain. Mel-1c is not found in mammals. Two cloned mammalian receptors (Mel-1a and Mel-1b) have now been renamed MT1 and MT2 (Dubocovich et al, 1998, 2003; Masana and Dubocovich, 2002). They are a new family of G protein coupled receptors, have high affinity (K_d 20–160 pM) and inhibit forskolin-stimulated cAMP formation. Research is underway to investigate the possible relationships between polymorphisms in melatonin receptors and circadian rhythm disorders (Ebisawa et al, 2000).

3.8.3 Oestogen receptors

The effect of melatonin on oestrogen receptors is considered in Section 3.9.

3.8.4 Melatonin antagonists and agonists

Large numbers of putative and actual melatonin agonists together with some antagonists have now been described. It is expected that much new information in this area will be available shortly; however, none of these agents is available as yet as a registered medication in the UK.

3.8.5 Gap junction communication

Gap junction communication between adjacent cells plays a normal part in intercellular exchange of small molecules and has a vital role in regulating cell growth and differentiation. The loss or a decrease of this avenue of communication has been observed in many cancer cells or cells treated with carcinogenic agents. The role of gap junction intercellular communication has been reviewed by a number of authors (see, for example, Ruch, 1994; Sulkowski, 1999; Trosko, 2003).

There have been suggestions that melatonin may play a role in gap junction communication. Kojima et al (1997) found that melatonin markedly induced gap junction protein expression and gap junction intercellular communication, although this was achieved at a pharmacological concentration of melatonin (10^{-2} M). At lower concentrations, Cos and Fernandez (2000) showed that melatonin (10 μM or 1 nM) could increase the transfer of small molecules through gap junctions in the breast cancer cell line MCF-7. Similarly, Ubeda and colleagues showed that physiological levels of melatonin enhanced junctional transfer in mouse embryonic fibroblasts and hepatocytes (Ubeda et al, 1995; Blackman et al, 2001).

3.8.6 Free radicals

Free radicals are created by the transfer of an electron between molecules; the result is unstable, transitory and potentially damaging to biological systems, particularly if DNA is involved in the electron transfer. However, the production of free radicals is a normal occurrence – it is a process that occurs continuously in biological systems and for which there are very effective defence mechanisms.

Many studies have assessed the role of melatonin as a scavenger of free radicals. Being an easily oxidised molecule, melatonin does have antioxidant activity. It has been suggested that this activity was its primary evolutionary function in primitive species (Hardeland et al, 1995; Antolin et al, 1997). However, the quantities of exogenous melatonin required to generate significant antioxidant activity *in vivo* remain to be specified. It is evident that melatonin, at least at pharmacological levels, is an effective antioxidant and infers protection against the damage caused by production of free radicals (see reviews by Reiter, 1998, 2004, and Vijayalaxmi et al, 2002). Its role as a free radical scavenger at physiological concentrations and hence *in vivo* is less convincing, as there are many other cellular candidates such as glutathione, vitamin A, or vitamin E that are found in much higher natural concentrations than melatonin, and therefore potentially play a greater role in cellular defence. There is some evidence that the overall antioxidant activity of blood is correlated with melatonin content, at least in chicks (Albarran et al, 2001). Correlation does not of course establish causality. It is difficult to reconcile the fact that most melatonin produced is excreted as aMT6s, whereas if the molecule was oxidised other primary metabolites would be likely (eg *N*-acetyl *N*-formyl 5-methoxykynurenamine).

3.8.7 Immunology

Melatonin is thought to play an important enhancing role in the immune system. The status of the immune system is an important factor not only in combating infections but also with respect to the development of cancerous cells. Immunodepression can be induced experimentally in animals by the removal or suppression of melatonin synthesis and can be counteracted by melatonin supplementation. Natural fluctuations in the duration of melatonin production also occur and are related to the photoperiod. These seasonal variations have been linked to immune parameters (reviewed by Nelson et al, 1995) – for example, short daylength was shown to enhance immune function in laboratory studies. Therefore immunity appears to be linked to the season and mediated through melatonin production. One route of possible melatonin enhancement of the immune system may be mediated through T-helper cells. These cells express melatonin receptors on the cell membrane that once activated stimulate the release of cell cytokines to aid in the immune response. Melatonin may also stimulate the immune response by influencing specific progenitor cells such as natural killer cells, monocytes and pre-B cells. These studies have been reviewed elsewhere (Fraschini et al, 1998; Maestroni, 1999, 2001).

3.8.8 Other mechanisms

Several other mechanisms of melatonin action have been proposed. For example, it has been suggested that intracellular calcium is modulated by melatonin via its effect on the calmodulin-calcium ion signalling pathway (Dai et al, 2002). Mediavilla et al (1999) suggested that melatonin was involved in control of the cell cycle by inducing the expression of key proteins. Blask et al (1997) showed that glutathione was required for melatonin action; indeed the breast cancer line HS578T, which is normally insensitive to melatonin, could be made responsive by raising the intracellular concentration of glutathione.

3.9 Melatonin, other hormones and breast cancer

Substantial evidence exists that steroid hormones heavily influence the natural history of breast cancer. This is reviewed below together with the observation that melatonin may mediate or interact with the mechanism of hormone action.

3.9.1 Hormones and breast cancer

There is compelling evidence that hormones, in particular oestrogens, both increase risk of developing breast cancer and maintain the continued growth/progression of established tumours.

3.9.2 Role of hormones in the development of breast cancer

Many aetiological factors associated with breast cancer have a hormonal component (Miller, 1996). Thus, enhanced susceptibility to breast cancer appears linked to increased oestrogen exposure whether from endogenous (Henderson et al, 1982; Toniolo et al, 1995; Berrino et al, 1996; Dorgan et al, 1997; Thomas et al, 1997a,b,c; Hendersen and Feigelson, 2000) or exogenous sources (Steinberg et al, 1991; Agarwal and Judd, 1999; Ursin, 2002). Conversely, oestrogen deprivation protects against the disease (Feinleib, 1968; Trichopoulos et al, 1972). For example, ablation of ovarian function before the age of 35 years reduces subsequent appearance of breast cancer by two-thirds (Feinleib, 1968). The mechanisms by which this is achieved are still not completely understood, but there are several possibilities:

(a) oestrogens can increase the proliferation of breast epithelial cells which leads to genetic mistakes and results in a transformed cellular phenotype (Fiegelson et al, 1996),
(b) oestrogens can accelerate the growth of occult cancers leading to increased incidence of overt disease (Miller, 1996),
(c) there is also accumulating evidence that oestrogen metabolites are genotoxic (Yager and Liehr, 1996); in particular, metabolism of oestrogens via catechols (2- or 4-hydroxyestradiol or hydroxyestrone) may produce reactive quinones, which can directly interact with and mutate DNA, initiating carcinogenesis (Roy and Liehr, 1999; Santen et al, 1999; Raftogianis et al, 2000).

The enzymes responsible for this metabolic pathway may be raised in women at risk of breast cancer (Fiegelson et al, 1996; Santen et al, 1999; Raftogianis et al, 2000; Yue et al, 2003).

3.9.3 Oestrogens and established breast cancer

Ablative procedures that either destroy ovarian function in premenopausal women or reduce circulating oestrogens in postmenopausal women may cause the regression of established breast cancer (Luft et al, 1952; Huggins and Dao, 1953; Dao and Huggins, 1955; Pearson and Ray, 1959; Beatson, 1896; Newsome et al, 1997). Consequently, drugs that inhibit either the synthesis or action of hormones occupy a central role in the therapy of women with breast cancer (Jensen et al, 1967; Horwitz et al, 1975; McGuire, 1978).

Given these associations between reproductive hormones and the natural history of breast cancer, it is important to examine relationships between melatonin and these hormones – in particular, whether melatonin might influence levels of hormones or their signalling pathways.

3.9.4 Melatonin and steroid hormone levels

One of the initial theories suggesting that a diminished function of the pineal gland might promote the development of breast cancer hypothesised that melatonin suppression may lead to increased levels of reproductive hormones, particularly oestradiol, thereby stimulating the growth and proliferation of hormone-sensitive cells in the breast (Cohen et al, 1978; Stevens, 1987).

Lifetime changes in patterns of steroid hormones and melatonin differ considerably. Thus whilst oestrogens and other steroid hormones fall dramatically at the menopause with cessation of ovarian function (Miller, 1996), this general pattern is not seen in melatonin secretion (see Section 3.6) and there are no dramatic changes in melatonin levels at the menopause.

In contrast, within women of the same menopausal status there is some evidence of an inverse relationship between steroid hormones and melatonin. For example, a significant negative correlation has been observed between peak serum melatonin concentrations and those of 17-beta-oestradiol in premenopausal women (Okatani et al, 2000). Additionally, administration of melatonin reduces oestrogens in women. For example, melatonin (2 mg daily for 6 months) significantly reduced oestradiol concentrations in postmenopausal women aged between 64 and 80 years (Pawlikowski et al, 2002). (These observations would be consistent with the proposed action of melatonin in down-regulating hormones of the neuroendocrine axis leading to a decrease in circulating levels of steroids.) Conversely, daily oral administration of oestrogen (conjugated oestrogen, 0.625 mg) suppressed nocturnal melatonin (Okatani et al, 2000). There has also been a report of a relationship between plasma, nocturnal melatonin concentrations and the oestrogen receptor status of breast cancers (Tamarkin et al, 1982). Thus, women with oestrogen receptor positive (ER+) breast cancers had significantly lower concentrations of melatonin than patients with oestrogen receptor negative (ER-) breast tumours and healthy women.

Definitive studies in which melatonin and other circulating hormones have been measured and related to risk of breast cancer are comparatively few in number and have not produced conclusive evidence that melatonin levels are associated with breast cancer. Most notably, the limited data currently available from prospective studies are inconclusive on whether women who subsequently developed breast cancer had different melatonin levels from those who did not. Interestingly, previous studies derived from the same or similar populations of women had shown increased levels of circulating oestrogens in women who subsequently developed breast cancer (Thomas et al, 1997a,b; Hankinson et al, 1998).

3.9.5 Interaction of melatonin with hormone signalling

Evidence of a direct anti-oestrogenic effect of melatonin on breast cancer cells has come from *in vitro* studies. Most investigations have used the MCF-7 breast cancer cell line, which possesses oestrogen receptors (Soule et al, 1973) and whose growth appears dependent upon oestrogen, both in culture (Lippman et al, 1977) and when grown as xenografts in immunosuppressed animals (Soule and McGrath, 1980). In these experimental models cell proliferation induced by oestrogen was inhibited by melatonin at concentrations close to those found in nocturnal human plasma. More recently, results have been presented which suggest that these oncostatic properties of melatonin are dependent upon a viable oestrogen response pathway. Such data include the observations that melatonin:

(a) inhibits proliferation only in cells expressing ERα (Cos and Sanchez-Barcelo, 2000) (although only MCF-7 cells were inhibited at physiological concentrations); melatonin had no effect on oestrogen insensitive breast tumour cell lines,
(b) both blocks the mitogenic effects of oestrogen and antagonises oestrogen-induced invasion (Cos et al, 1998),
(c) potentiates the sensitivity of MCF-7 cells to anti-oestrogens (Wilson et al, 1992),
(d) inhibits the expression of oestrogen-regulated genes such as pS2 or cathepsin (Molis et al, 1995).

Whilst melatonin does not bind to the oestrogen receptor or interfere with the binding of oestradiol to its receptor (Molis et al, 1994), it decreases the expression of ERα and inhibits the binding of oestradiol-ER complexes to oestrogen response elements on DNA, suppressing oestrogen receptor gene transcription and decreasing the levels of oestrogen-receptor mRNA expression (Molis et al, 1994). The molecular basis of such interactions has not been completely elucidated but cAMP and other protein kinase activators appear to enhance ER-mediated transcription by mechanisms involving phosphorylation of the oestrogen receptor itself and receptor co-activators (Kiefer et al, 2002). It is thus relevant that melatonin, after binding to its membrane receptor, has been reported to decrease cAMP, thus potentially reducing co-activation of the oestrogen receptor pathway (Kiefer et al, 2002).

Conversely, there is a report that melatonin interacts with growth factors such as insulin and epidermal growth factor (EGF) to modulate the transcription of oestrogen receptor in the absence of oestrogen, an effect abolished by the addition of an anti-oestrogen (Cos and Blask, 1994; Ram et al, 1998). This suggests that melatonin and EGF co-operate to transactivate the oestrogen receptor, an effect that is not immediately compatible with melatonin's growth suppressive properties.

Whilst these data are persuasive of an effect of melatonin on oestrogen receptor signal transduction, which may translate into growth inhibitory effects in hormone sensitive cell lines, it should be noted that this has only been observed in model systems comprising a limited number of breast cancer cell lines. Furthermore, the literature is not completely consistent and others failed to show a reduction of oestrogen-stimulated cell growth by melatonin (see Table 5.1). Thus Cos and Blask (1994) and Baldwin et al (1998) found no inhibitory effect of melatonin when oestrogen was present and Bizzarri et al (2003) reported that melatonin blocks oestrogen stimulation of cell growth but only in the absence of fetal calf serum. These observations raise questions about the *in vivo* importance of melatonin as an oncostatic agent, especially in the presence of other naturally occurring hormones or growth factors.

Another potential interaction between melatonin and oestrogen has been reported in respect of oxidative damage to DNA. Thus melatonin has been observed to prevent oestrogen-induced DNA

damage and to synergise with oestrogen in reducing lipid peroxidation in liver and kidneys (no measurements were made in breast) (Karbownik et al, 2001).

Further research into these basic molecular mechanisms and their relevance to the clinical material is required before definitive conclusions can be reached.

3.9.6 Other hormones, growth factors and breast cancer

Although the links with the natural history of breast cancer are greatest for sex steroids, other hormones and growth factors such as prolactin and the insulin-like growth factor system have been associated with increased risk and progression of breast cancer.

Prolactin is a mitogen in the mammary gland in rodents (Reiter, 1980; Brzezinski, 1997) and also has a primary involvement in the development of mammary cancer in such animals (Vonderhaar, 1984, 1987). Whether prolactin has similar influences in humans is less clear. There are data that show:

(a) the presence of specific receptors for prolactin on breast cancer cells, (Welsch and Nagasawa, 1977; Codegone et al, 1981; Peyrat et al, 1981; Biswas and Vonderhaar, 1987),
(b) about 90% of primary breast cancers also express mRNA for prolactin receptors (Bonneterre et al, 1982),
(c) the growth of breast cancer cells may be stimulated by prolactin (L'Hermite-Baleriaux et al, 1984; Vonderhaar, 1987, 1998),
(d) melatonin may block the prolactin-induced growth of breast cancer cell lines (Malarkey et al, 1983),
(e) melatonin acutely increases prolactin according to several reports (Vonderhaar and Biswas, 1987; Lemus-Wilson et al, 1995).

However, whilst the majority of breast cancer biopsies contain prolactin-like material and express specific prolactin receptors, no consistent correlation is evident between prolactin/prolactin receptors and the aetiology/prognosis of breast cancer (Love and Rose, 1985; Wang et al, 1986; Ingram et al, 1990; De Placido et al, 1990; Love et al, 1991; Holdaway et al, 1997). Furthermore, it has been difficult to demonstrate tumour regression in breast cancer patients treated with prolactin-inhibiting drugs (Henson et al, 1972; Pearson and Manni, 1978; Manni et al, 1989; Anderson et al, 1993a).

Growth hormone is involved in the development of normal breast and has been hypothesised to be involved in the development and progression of breast cancer (Waters and Conway-Campbell, 2004). However, these influences may be mediated through insulin-like growth factor (IGF-1) and a direct role in breast cancer development is still unproven. Furthermore the effects of melatonin on growth hormone levels in women are variable and usually insignificant (Waldhauser et al, 1987; Forsling et al, 1999; Rajaratnam et al, 2003).

There are both epidemiological and biological data to link the IGF system with risk of breast cancer. Thus the majority of studies in premenopausal women have shown that high circulating levels of IGF-1 and IGFBP-3 increase risk of developing breast cancer; however, there was no consistent effect in postmenopausal women (Fletcher et al, 2005). Laboratory studies also demonstrate that the insulin-like growth factor system may stimulate neoplastic growth through mitogenic and anti-apoptotic effects on breast cells (Schairer et al, 2004; Voskuil et al, 2004). It has also been suggested that the oncostatic

effects of melatonin on breast cancer cells may be mediated through IGF-1 (Kajdaniuk et al, 2002). This is based on

(a) a negative correlation between circulating levels of melatonin and IGF-1 in women with breast cancer (Kajdaniuk et al, 2002),
(b) differences in seasonal variation of IGF-1 and melatonin between women with breast cancer and controls (Holdaway et al, 1997),
(c) melatonin therapy having clinical benefits in patients with advanced breast cancer, an effect associated with decreases in IGF-1 levels, these being significantly higher in responding tumours (Lissoni et al, 1995).

The immediate relevance of these studies, which involve small numbers of women with established breast cancer, to risk of the disease may be questioned.

3.10 Summary

The pineal gland is a photoneuroendocrine transducer organ, converting information about daylength (and possibly light intensity) into a hormonal signal: melatonin.

Melatonin is normally secreted during the night (the dark phase) in all species whether nocturnal or diurnal, and the duration of its night-time secretion indicates the length of the night. One definition of 'biological night' is the period of the 24 hour cycle for which melatonin is above baseline (usually daytime) concentrations. In seasonal mammals the profile of melatonin secretion provides an essential time cue for the organisation of seasonal activity (such as reproduction, winter or summer coat growth).

In so far as human physiology is dependent on daylength, melatonin is likely to serve as a photoperiodic signal. Its role within the circadian system of mammals including humans appears to be the reinforcement of 'night-time' physiology (for example, changes in core body temperature or sleep propensity), the modulation of the circadian phase shifting response to light, and in general to serve as an endogenous zeitgeber. Most is known of its actions on the central nervous system. Being highly liposoluble, it penetrates all tissues and body fluids and it therefore has the potential to influence peripheral oscillators. Evidence exists for an effect on clock gene expression in the pars tuberalis (the major site of melatonin receptor MT1). The presence of melatonin did not appear to be essential for maintained circadian function in a normal environment in early animal studies. However, pinealectomy is associated with increased incidence of cancer (chemically induced and spontaneous), hypertension, metabolic abnormalities and an abnormal response to abrupt phase shifts in rats. Endogenous melatonin probably serves to reinforce 'coupling' and to optimise phase within the circadian system, and thus influence the multitude of systems governed by circadian oscillators.

In mammals, melatonin is a 'hand' of the circadian clock rather than part of the clock mechanism. It is extensively used as the 'best' marker of the timing of the circadian clock either as the endogenous hormone or its major metabolite 6-sulphatoxymelatonin (aMT6s). There are very large individual variations in the concentrations recorded in plasma or saliva and in the urinary content of aMT6s. Thus for cross-sectional studies of changes in melatonin secretion, large study populations are required. A single sample, be this of morning urine, morning or night-time plasma or saliva, does not provide sufficient

information to define the characteristics of its secretion. There is an age-related decline in secretion and an earlier timing of the rhythm with age. Thus age-matched control populations or age-adjustment are required for comparative purposes. Light exposure must be controlled if the acute effects of light are to be avoided. Numerous drugs and other substances influence its secretion.

Light suppresses melatonin synthesis at night and timed exposure to light of suitable intensity and spectral composition shifts the melatonin rhythm and all other circadian rhythms investigated to date. Night-shift work and time-zone change may be associated with lower melatonin production, light at night being one of several possible causal factors. Few data address this point as yet.

Exogenous melatonin phase shifts and entrains circadian rhythms. It is successfully used in the treatment of circadian rhythm disorders. It possesses free radical scavenging and (in some reports) immunostimulatory properties, usually in 'pharmacological' doses. Whether these prove to be clinically useful remains to be seen.

Melatonin has a wide range of actions; the mechanism of melatonin action is via an effect on other biological molecules, although the particular mechanism depends on the type of cell. The actions can be mediated through binding to specific melatonin receptors or by modifying the influence of other molecules, such as inhibiting the binding of the oestrogen receptor to DNA. An area that has received much interest and speculation is the role of melatonin as a scavenger of free radicals. Melatonin is an effective antioxidant at least when used at pharmacological levels; however, it is one of many biological antioxidants and its role at physiological levels is not so clear. Several other mechanisms of melatonin action have also been demonstrated and they highlight the diversity of effects caused by this hormone and the complexity of its action.

While there is compelling evidence that oestrogens increase the risk of breast cancer, the hypothesis that suppression of melatonin may lead to increased levels of reproductive steroid hormones which in turn stimulate either the proliferation of breast cells or the growth of breast cancers is still hypothetical and without a substantial body of supportive evidence. Similarly, the case that melatonin may increase breast cancer risk through changing the levels of growth factors or hormones such as prolactin is not convincing. Data are limited and relate to small groups of women with established breast cancer.

Some *in vitro* data suggest that melatonin may have direct anti-oestrogenic effects on breast cancer cells. However, this has only been observed using a limited number of breast cancer cell lines, and other studies have failed to show any reduction of oestrogen-stimulated cell growth by melatonin.

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4 Effects of EMFs on Melatonin

4.1 *In vitro* studies

In vitro studies of exposure to EMFs divide into two types of investigation: effects on the production of melatonin by cells from the pineal gland; and effects on the action of melatonin on cells.

4.1.1 Effects on melatonin production *in vitro*

There are only a few studies that have investigated the effect of magnetic fields on melatonin production *in vitro.* All used rodents as the source of pineal gland cells but there are marked differences in their methodology. Most used power frequencies (50 or 60 Hz), but the field strength (50 μT – 1 mT) and duration (1 hour – 12 hours) differ between the studies.

Both direct and indirect measures have been used to show cellular responses to melatonin. Direct measures include melatonin content or melatonin release from cells. Indirect measures can be made from the activity of *N*-acetyltransferase (NAT), an enzyme involved in the synthesis of melatonin, or of hydroxyindole-*O*-methyltransferase (HIOMT), an enzyme responsible for methylation and hence release of melatonin from the cells. Most of the studies have stimulated pharmacologically the production of melatonin in the isolated glands by the addition of noradrenaline (NA) or isoproterenol. NA is naturally released from the nerve endings in the pineal gland in the intact animal at the onset of the darkness and initiates the synthesis of melatonin. Isoproterenol (isoprenaline) is a beta-adrenergic receptor agonist and will mimic NA through its stimulation of cyclic adenosine monophosphate (cAMP) production.

Lerchl et al (1991) exposed pineal glands from young rats, removed during the day-light period, to a combination of a static field (44 μT) and a low frequency magnetic field (44 μT at 33.7 Hz). These are the theoretical conditions for cyclotron resonance of the calcium ion. One experiment was performed. The glands were stimulated by the addition of NA (10^{-6} M): seven were exposed to the field for 2.5 hours, and a further seven were used as controls. Exposure caused a reduction in NAT activity, melatonin production and melatonin release into the culture medium. As the authors commented, this result was surprising as calcium normally promotes NAT activity.

Rosen et al (1998) also used pineal glands from the rat, but this study was different to the other studies in that the pineal gland was separated into individual cells. The isolated cells were exposed for 12 hours to a 50 μT, 60 Hz field and melatonin production was stimulated by the addition of NA at 10^{-6} M; the total amount of melatonin released from the cells was measured. The overall experimental protocol was well designed with two matched exposure systems both capable of being active or sham; sham–sham experiments were also run. One potentially interesting aspect of the protocol was to run some experiments blinded and compare these results with the non-blinded results. In the blinded experiments, three of the five did not show a reduction due to field exposure, and one even had a 31% increase.

However, the five blinded experiments combined showed a 26% reduction, although the claim by the authors that this was a significant reduction is statistically dubious and certainly could not be regarded as robust. The authors pointed out that in these three particular experiments there was a poor response to NA stimulation and hence no increase in the level of melatonin production by the cells. Thus there was a failure to stimulate production rather than a failure of the magnetic field to suppress melatonin synthesis. The overall result of the ten experiments was that magnetic field exposure caused a statistically significant 46% reduction in stimulated melatonin release (although the standard deviations quoted in the publication for the individual experiments are erroneous).

Chacon (2000) used rat pineal glands to study NAT activity. The glands were isolated during the rats' dark cycle; the procedure was carried out in dim red light to avoid exposing the animals and glands to white light. The NAT activity was predicted to be at its natural peak at this time; no pharmacological stimulation was given. Four experiments were performed which investigated three magnetic field strengths (10, 100 and 1000 μT) at 50 Hz and sham exposure; six test and six control glands were used in each experiment. The enzyme activity decreased by approximately 20% after a one hour exposure to the highest field strength tested (1000 μT) but was not significantly altered by field strengths of 10 or 100 μT. The interpretation of the result may be complicated by the removal of the pineal gland during the rats' dark period, which may have had an effect on melatonin synthesis and a confounding effect on the result.

A study by Brendel et al (2000) used pineal glands from the Djungarian hamster. It also differed from the previous studies in that the glands were maintained in a flow system, so that changes of melatonin released from the glands could be monitored throughout the duration of the experiment. The experimental protocol appears to have been well designed with random allocation of exposure or sham to identical exposure systems and the experiments run blind. The glands were removed during the normal light period, and hence avoided the possible confounding factors faced by the study by Chacon. The pineal glands were exposed for eight hours to a field strength of 86 μT at either 50 or 16.67 Hz (a frequency generated in some railway systems), using a rectangular waveform. The glands were stimulated for 30 minutes with isoproterenol (10^{-7} M) to increase melatonin production. Melatonin release was assessed at hourly intervals. Although an apparently well-conducted study, the interpretation of the results was not convincing. The authors concluded that EMF exposure inhibited melatonin production in both the 50 and 16.67 Hz experiments. However, there was only one time point in one of four experiments using 50 Hz where the release of melatonin was statistically different from the sham-exposed values. A similar single point difference was obtained for the 16.67 Hz experiments. From the graphs of the data, it is equally tempting to conclude that the sham-exposed values of melatonin release have varied at these time points in comparison with the pattern of the other experiments and the pattern of melatonin release is unchanged between the experiments. There is no convincing evidence in this study that EMF exposure causes inhibition of melatonin production.

Lewy et al (2003) used rat pineal glands isolated in the morning and hence during the 12 hour light period. The glands were exposed for four hours to a 50 Hz magnetic field at 1 mT. The activity of enzymes NAT and HIOMT was measured, as well as the release of melatonin into the incubation liquid. Two exposure systems were used that could be set to either sham or exposure. The glands were stimulated with NA (10^{-6} M), which caused a modest increase in both enzymes that was not suppressed by the exposure to the magnetic field. However, exposing the glands to the field for 30 minutes prior to the NA enhanced the stimulation (10–25%) compared with simultaneous exposure to NA and the

magnetic field. Addition of NA markedly increased the release of melatonin into the incubation liquid. Field exposure given simultaneously with NA or 30 minutes prior to NA administration caused a significant increase (approximately 50%) in melatonin release. This result is in contrast to the other studies where if an effect was found, it was a decrease. There was no change in melatonin release due to field exposure in glands that had not been stimulated by NA.

A study by Tripp et al (2003) was similar to the Brendel et al investigation in that it used a flow system to detect changes of melatonin release during the course of the exposure. The glands were obtained during the animals' light period and were exposed or sham exposed in identical systems. The exposure was for 4 hours to a circularly polarised 50 Hz magnetic field at 500 μT. Samples were taken every 30 minutes; the process used remote collection to avoid potential artefacts involved in manual collection. The glands were not stimulated pharmacologically and no field-dependent changes in melatonin release were detected.

To reconcile the various results above and find a common pattern of response to magnetic fields is difficult (Table 4.1). The 'cyclotron resonance' study of Lerchl and colleagues is not directly comparable to the studies using power frequency fields as the frequency was slightly lower at 33.7 Hz and the static field was set at 44 μT: it is the only study to have applied such fields to isolated pineal glands.

TABLE 4.1 Studies of the effects of magnetic fields on melatonin production

Exposure	Effect	Reference
'Ion-cyclotron resonance' for calcium	NA stimulation of melatonin production and release reduced	Lerchl et al, 1991
50 μT, 60 Hz for 12 h	NA stimulation of melatonin release reduced	Rosen et al, 1998
1 mT, 50 Hz for 1 h	NAT activity decreased	Chacon, 2000
86 μT,50 Hz or 16.67 Hz for 8 h	Isoproterenol stimulation of melatonin production reduced	Brendel et al, 2000
1 mT, 50 Hz for 4 h	NA stimulation of melatonin release increased	Lewy et al, 2003
0.5 mT, 50 Hz for 4 h	No effect on melatonin release	Tripp et al, 2003

Five of the studies reviewed used power frequency fields. The study by Tripp and colleagues used relatively high magnetic field strengths (500 μT) for four hours, the glands were removed during the light period, so the NAT activity would be low and the cells were not pharmacologically stimulated; no effect of EMF exposure was found. This is in agreement with the Lewy et al study where exposure for four hours at 1 mT produced no effect in cells that had not been stimulated by NA. The study by Chacon also used non-stimulated cells; however, exposure to 1 mT for one hour caused a decrease in NAT activity. These glands were isolated during the dark cycle and NAT activity was expected to be at its highest, which may be equivalent to pharmacological stimulation in other authors' studies. Based on these three studies, it could be suggested that NAT activity needs to be high, either achieved naturally during the dark cycle or artificially by chemical stimulation, to see an effect of EMF exposure of field strengths between 500 and 1000 μT. However, there is no agreement in which direction the effect is seen; Lewy et al found an increase in NAT activity and melatonin release, whereas Chacon showed a reduction in NAT activity. These contradictory findings cast doubt on whether the reported effects are real.

In the studies using lower field strengths (86 and 50 μT) both Brendel et al and Rosen et al used relatively long exposures – 8 or 12 hours. Both stimulated the cells with chemicals and achieved reduced levels of melatonin after field exposure. Chacon also used similar field strengths (10–100 μT) but field exposures of one hour did not cause a change in NAT activity. A possible explanation could be that, to see an effect, the NAT activity needs to be high and combined with a relatively long duration if the field strength is low. However, this postulation of an effect of low field strengths would be essentially based on two studies, neither of which in isolation could be regarded as robust.

4.1.2 Effects on the action of melatonin *in vitro*

The main interest in this area was caused by the claim that exposure to magnetic fields can block the inhibitory effect of melatonin on growth of breast cancer cells. The original work was reported by Liburdy et al (1993) in a study using a human oestrogen-responsive breast cancer cell line (MCF-7). They found that the proliferation of MCF-7 cells can be slowed by the addition of physiological concentrations of melatonin (1 nM). However, if the cells are simultaneously exposed to a 60 Hz, 1.2 μT magnetic field, then the effect of melatonin on the rate of proliferation is reduced. The effects are fairly small and can only been seen after seven days in culture. They suggested that the magnetic field disrupted either the ligand/receptor interaction or the subsequent signalling pathway. The authors found no effect at a magnetic field strength of 0.2 μT and suggested a threshold between 0.2 and 1.2 μT. No effect was seen using field exposure alone.

A similar effect of a 60 Hz field was reported by Harland and Liburdy (1997) but using tamoxifen (100 nM) rather than melatonin to bring about the initial inhibition. The effect has been reported in other cell lines, namely a second breast cancer cell line, T47D (Harland et al, 1998), and a human glioma cell line 5F757 (Afzal and Liburdy, 1998). All of these studies come from Liburdy's group.

There has been some criticism of the initial study. A report from the National Institute of Environmental Health Sciences (NIEHS, 1999) made the following comments: 'There was some concern about the experimental design of these studies' and 'because the effect was small, the importance of these findings for human health is not clear'. Previously, AGNIR (2001) endorsed the NIEHS concern about the robustness of the effect and felt that the changes were small (10–20% growth over seven days).

Until the paper by Blackman et al (2001) there have been two cited independent replications of the Liburdy et al findings. These appeared as abstracts at the Bioelectromagnetics Society Annual Meeting in Florida 1998. Neither has appeared in peer-reviewed journals as a full paper. Blackman et al (2001) is the only peer-reviewed, full paper that sets out to replicate the Liburdy et al findings, although a similar effect has been shown using slightly different conditions (Ishido et al, 2001).

The Blackman et al study used MCF-7 cells and the experimental criteria supplied by Liburdy. However, the protocol appears to have been modified and improved on the original. Two incubators were used, each containing an exposure system. Both melatonin and tamoxifen effects were investigated. The melatonin part of the study was fairly weak, with poor controls, small sample size, exposures not random or blinded, and no test with magnetic field but without melatonin. The melatonin caused a 17% inhibition in cell numbers compared with controls; this was not statistically significant. The design of the tamoxifen study was more rigorous. The protocol was not an exact replication, however, since the

authors added only 25% of the tamoxifen concentration used by Liburdy. There were nine separate experiments, and in each experiment there was a total of 48 petri-dishes. Each incubator had 24 dishes, 12 with and 12 without tamoxifen. The assays were blinded, and incubator allocation was random. Tamoxifen caused a 25% inhibition in cell numbers, which was reduced to a 13% inhibition by exposure to a 60 Hz magnetic field at 1.2 μT. This result confirmed the Liburdy et al findings, in which a 40% inhibition was reduced to 25% by EMF exposure. However, it should be noted that a 25% inhibition of cell numbers by tamoxifen implies that the experimental protocol was not optimal as a greater inhibitory effect of tamoxifen would be expected.

The effects of stronger magnetic fields were studied by Leman et al (2001) in three breast cancer cell lines that had, according to published reports, different metastatic capabilities: MDA-MB-435 cells were considered to have 'high' metastatic capabilities, MDA-MB-231 cells were considered 'low', and MCF-7 cells were considered 'non-metastatic'. Only their 'low' and 'non-metatastic' cells responded to melatonin and optimum inhibition was achieved at 1 mM concentration of melatonin (a million-fold higher than used in the Liburdy et al study). Cell growth was not affected in their culture conditions by exposure for 1 hour to a pulsed field at 300 μT (pulse duration 20 ms with a repetition rate of 2 Hz) repeated for 3 days. The cells' invasive properties were evaluated in an *in-vitro* assay; MCF-7 cells had a low invasion rate which was not affected by addition of melatonin or EMF exposure. (The 'non-metastatic' definition of MCF-7 cells was based on the study by Shafie and Liotta (1980) which found the incidence of liver, lung and spleen metastases in athymic, ovariectomised nude mice inoculated with MCF-7 cells and supplemented with oestrogen to be 40–60% after 5–7 weeks.)

Ishido et al (2001) also mainly used higher magnetic fields strengths. MCF-7 cells (supplied by Liburdy) were exposed to 0, 1.2 or 100 μT at 50 Hz for 7 days. Melatonin at concentrations of 10^{-9} M or higher induced inhibition of intracellular cAMP which was blocked by exposure to a 50 Hz field at 100 μT. These authors also found that DNA synthesis was reduced 20% by melatonin at 10^{-11} M (a concentration 100-fold less than used by Liburdy et al and not effective at causing cAMP accumulation in their system) and this reduction was blocked by exposure to a magnetic field at 1.2 μT. This result was presumably only obtained from a single experiment, as the number of repeat experiments is not mentioned. The result is consistent, however, with the Liburdy et al findings.

All the experimental studies mentioned above were performed with the breast cancer cell line MCF-7; however, studies using these cells can be problematic. This cell line has been in existence for many years and its characteristics continue to change. This was recognised by Liburdy et al (1993) who commented that MCF-7 cells display heterogeneity to their response to melatonin and that sub-clones may change sensitivity to melatonin as passage number increases. This finding was endorsed by two of the groups who attempted to replicate the work of Liburdy et al. Morris et al (1998) had difficulty obtaining a melatonin responsive cell line, despite being supplied with cells from Liburdy; the cells also had different growth characteristics. Luben and Morgan (1998), who also had cells supplied by Liburdy, found the effects of magnetic fields variable and dependent on the source of cells, and the number of times the cells had been cultured, and suggested that the effects were specific to individual clone phenotypes. Although the MCF-7 cell line has undoubtedly provided a useful model to investigate effects on isolated breast cancer cells, it is only one possible model in cells that have been separated from their natural environment and therefore its implication for breast cancer in general is limited.

Other research groups, working on subjects unrelated to EMFs, also have had problems finding a consistent MCF-7 cell response to melatonin. Baldwin et al (1998) concluded that melatonin did not inhibit oestrogen stimulated MCF-7 cell growth, whereas Molis et al (1994) claimed inhibition. Ram et al (2000) found significant differences in the responsiveness of various stocks of MCF-7 cells to the growth inhibitory effects of melatonin (see Chapter 5).

Notwithstanding these limitations, the effect of EMFs on the inhibition of MCF-7 cell growth by melatonin has been independently replicated, and thus merits some attention. Although it must be emphasised that the effect is in isolated cells and thus does not imply an effect in a whole organism. Therefore, the inference that EMF exposure may have a detrimental effect on cancer growth in humans via a suppression of the action of melatonin or tamoxifen is not justified without more appropriate studies. Also it should be noted that a biological change is not the same as a health effect.

The importance of independent replication of the effect of EMF exposure on melatonin inhibition of MCF-7 cell growth lies not just with the biology, but also with our understanding of the physics. At present there is no generally accepted physical mechanism that can predict or explain how such weak magnetic fields can interact with biological systems.

Several studies have looked at the effect of EMF exposure on gap junction communication. In general, most of the studies show an inhibitory effect of EMF exposure on cell–cell communication (Schimmelpfeng et al, 1996; Chiang et al, 1999; Hu et al, 2002; Lohmann et al, 2002; Yamaguchi et al, 2002; Marino et al, 2003). However, the exposure conditions were not consistent between studies and varied in magnetic field strength, frequency and duration. These studies have not been replicated or, where replication has been tried, the inhibitory phenomenon was not shown (Griffin et al, 2000). A few studies have investigated the combined effect of melatonin and EMF exposure on gap junction communication. Physiological levels of melatonin enhanced transfer of a fluorescent dye between cells and this enhancement was blocked by 50 Hz EMF exposure at 160 mT (Ubeda et al, 1995).

The effect of a magnetic field on the rate of radical pair recombination is a well-established phenomenon in chemistry. The effect depends on the influence of magnetic fields on the recombination probability of the radicals in the first nanoseconds of their creation. It is a static magnetic field phenomenon but, because the recombination times are so short (nanoseconds), the power frequencies of 50 or 60 Hz would be essentially static for that fraction of a second. Although the effect of magnetic fields greater than 1 mT has been established, the effect of much weaker fields on biological systems is less certain and is still the subject of research (Brocklehurst and McLauchlan, 1996; Brocklehurst, 2002). A further complication is the geomagnetic field, which is typically about 50 μT, but will vary markedly in the presence of some metal objects. The variation in the geomagnetic field due to walking near a steel object such as a filing cabinet or travelling in a lift, for example, will probably be much larger than that due to the presence of 50 or 60 Hz power frequencies.

4.1.3 Summary

Overall, the evidence that EMF exposure causes changes in melatonin production or release in isolated pineal glands is not convincing. This is despite the EMF field strengths used in the experiments being considerably higher than those usually encountered in the environment. Therefore a direct effect of EMF

exposure on isolated pineal glands or pineal cells is doubtful. However, this does not exclude an effect on animals as it may require an intact circadian system or input via a sensory organ, ie the eyes. Relatively few *in vitro* studies have been undertaken and some have serious limitations. All the studies have used cells from rodents and hence the results may not be directly applicable to human cells. There is scope for well-designed studies to investigate further the possibility of direct EMF effects on isolated pineal cells or glands.

The evidence that EMF exposure interferes with the action of melatonin on breast cancer cells *in vitro* is intriguing (Table 4.2) and there appears to be some supporting evidence in terms of independent replication using MCF-7 cells. However, this particular effect seems to be elusive: it requires a specific sub-clone of the MCF-7 cell line, and even this can transform and so lose its responsiveness. Overall, these results cannot not be regarded as a robust field-dependent effect. In view of these limitations, the significance of the biological findings is of doubtful relevance to other breast cancer cell lines, and of dubious significance to human health.

TABLE 4.2 Studies of the effects of magnetic fields on responses of cells to melatonin or tamoxifen

Exposure	Effect	Reference
1.2 μT, 60 Hz, for 7 days	EMF exposure partially blocked melatonin (10^{-9} M) inhibition in MCF-7 cells. Similar result with tamoxifen (10^{-7} M)	Liburdy et al, 1993 Harland and Liburdy, 1997
1.2 μT, 60 Hz for 7 days	EMF exposure partially blocked tamoxifen ($2.5\ 10^{-8}$ M) inhibition of MCF-7 cells	Blackman et al, 2001
1.2 or 100 μT, 50 Hz for 7 days	Inhibition of DNA synthesis by melatonin (10^{-11} M) partially blocked by 1.2 μT EMF exposure. 100 μT blocked cAMP inhibition by melatonin (10^{-9} M) in MCF-7 cells	Ishido et al, 2001
0.3 mT pulsed for 20 ms at 2 Hz for 1 h repeated over 3 days	Growth of MCF-7 cells unaffected	Leman et al, 2001

4.2 *In vivo* studies

Various laboratory studies have investigated the effects of EMFs on melatonin rhythms in animals. Most of these have used rats, although some studies have used mice. Other studies have used seasonal breeders, such as Djungarian hamsters and sheep, and a few studies have used cattle or non-human primates.

4.2.1 Rodent studies

Attention was first focused on the potential effects of electric fields, before interest turned to magnetic fields. Early studies (Wilson et al, 1981, 1983, 1986; Reiter et al, 1988) reported that the exposure of rats to electric fields significantly suppressed pineal melatonin and the activity in the pineal gland of an enzyme (NAT) important in the synthesis of melatonin, and that this effect was transient, appearing within three weeks of exposure but recovering within three days following the cessation of exposure.

A similar suppression of pineal melatonin was reported following the prenatal and neonatal exposure of rats to power frequency electric fields; no simple dose–response relationship was apparent. However, a later study from the same laboratory (Sasser et al, 1991) briefly reported that they were unable to reproduce the reduction in pineal melatonin. Another laboratory (Grota et al, 1994) also reported that exposure to power frequency electric fields had no effect on pineal melatonin levels or NAT activity in rats, although serum melatonin levels were reduced. These studies are summarised in Table 4.3.

TABLE 4.3 Studies of effects of exposure to 60 Hz electric fields on melatonin levels in Sprague-Dawley rats

Assay	Exposure	Result	Reference
Pineal NAT and melatonin (male)	1.7–1.9 kV m^{-1}, 20 h per day, 30 days	No change in night pineal NAT, reduction in night melatonin ($p<0.05$)	Wilson et al, 1981, 1983
Pineal NAT and melatonin (male)	39 kV m^{-1}, 20 h per day, for 1, 2, 3 or 4 weeks	Significantly reduced melatonin and NAT activity after both 3 and 4 weeks exposure, withdrawal of the fields returned night melatonin and NAT to normal	Wilson et al, 1986
Pineal melatonin (male and female)	10, 65 or 130 kV m^{-1}, *in utero* and 23 days after birth	Reduced pineal melatonin levels ($p<0.001$), melatonin rhythm phase delayed by 1.4 h	Reiter et al, 1988
Pineal melatonin and NAT, serum melatonin activity (male)	35 kV m^{-1}, 20 h per day, for 30 days	No change in night pineal or melatonin levels, reduced night-time serum melatonin levels	Grota et al, 1994
Pineal melatonin (male and female)	20 h per day, for 30 days	No significant effect	Sasser et al, 1991

The difficulties in repeating the results of some of the earlier studies and the possible use of unreliable techniques have been noted by Brady and Reiter (1992). In particular, Reiter (1993) questioned whether the reported effects were an artefact of the method of melatonin measurement or some other methodological procedure.

More recent work has concentrated on studies of the effect of exposure to power frequency magnetic fields. These studies are summarised in Table 4.4. An extensive series of tests has been carried out by Kato and colleagues of the effects of exposure to circularly or linearly polarised power frequency magnetic fields on pineal and serum melatonin levels in male rats (Kato et al, 1993, 1994a–d, summarised in Kato and Shigemitsu, 1997). However, there is a major difficulty with the interpretation of many of these studies: the sham-exposed group was sometimes treated as a 'low dose' exposed group because the animals were exposed to stray magnetic fields (of less than 2%) generated by the exposure system. Thus statistical comparison was sometimes made with historical controls. Such procedures fail to allow for the inter-experimental variability that was reported in replicate studies by Kato and Shigemitsu (1997). Kato and colleagues seem to have taken these decisions *post hoc*, since the concurrent sham-exposed groups were treated in two different ways. This will also have increased the number of statistical comparisons made (based on the multiple use of Student's t-test), increasing the possibility of false positives.

TABLE 4.4 Studies of effects of exposure to magnetic fields on melatonin levels in rats

Assay	Exposure	Result	Reference
Pineal and serum melatonin levels in Wistar-King rats	50 Hz, circularly polarised, 1, 5, 50 or 250 μT, for 6 weeks	Night-time and some daytime reductions in serum and pineal melatonin	Kato et al, 1993
Serum melatonin levels in Wistar-King rats	50 Hz, circularly polarised, 1 μT, for 6 weeks	Night-time melatonin levels reduced ($p<0.05$); returning to normal within 1 week	Kato et al, 1994a
Pineal and serum melatonin levels in (pigmented) Long-Evans rats	50 Hz, circularly polarised, 1 μT, for 6 weeks	Night-time pineal and serum melatonin reduced ($p<0.05$)	Kato et al, 1994b
Serum melatonin levels in Wistar-King rats	50 Hz, horizontally or vertically polarised, 1 μT, for 6 weeks	No significant effect	Kato et al, 1994c
'Antigonadotrophic' effect of melatonin on serum testosterone levels in Wistar-King rats	50 Hz, circularly polarised, 1, 5 or 50 μT, for 6 weeks	No significant effect	Kato et al, 1994d
Night-time serum melatonin levels and pineal NAT activity in Wistar rats	50 Hz, 1, 10 or 100 μT for 12 h (once) or 18 h per day for 30 days	Reduced melatonin and NAT activity after 100 μT (acute) ($p<0.05$) and 10 and 100 μT (chronic) ($p<0.05$)	Selmaoui and Touitou, 1995
Night-time excretion of melatonin urinary metabolite in Wistar rats	50 Hz, 1, 5, 100 or 500 μT for 24 h	No significant effects compared to baseline pre-exposure controls	Bakos et al, 1995, 1997, 1999
Night-time pineal melatonin levels in Sprague-Dawley rats (not DMBA treated)	50 Hz, 10 μT for 13 weeks	No significant effect	Mevissen et al, 1996a,b
Night-time serum melatonin, prolactin and oestradiol levels in Sprague-Dawley rats	50 Hz, 100 μT for 1 day, 1, 2, 4, 8 or 13 weeks	No consistent effects on melatonin; no effects on serum prolactin or oestradiol	Löscher et al, 1998
Night-time excretion of melatonin urinary metabolite in Sprague-Dawley rats	60 Hz, 1 mT continuous for 10 days or 6 weeks, or 1 mT intermittent for 2 days	No significant effect	John et al, 1998

The first study (Kato et al, 1993) found that night-time pineal and serum melatonin levels were significantly reduced following six weeks exposure to circularly polarised power frequency magnetic fields of up to 250 μT compared with levels in historical controls. In contrast, there was no difference between values in the exposed and concurrent sham-exposed groups. These results can be regarded as rather equivocal for the reasons outlined above. However, in a subsequent study (Kato et al, 1994a) a reduction in serum melatonin levels was observed in animals exposed to a circularly polarised magnetic field compared with sham-exposed animals. The next study (Kato et al, 1994b) reported the night-time suppression of serum and pineal melatonin in a different (pigmented) strain of rat in exposed animals compared with both sham-exposed animals and historical controls. In contrast to these results, a fourth study (Kato et al, 1994c) found that six weeks exposure to horizontally or vertically polarised power

frequency magnetic fields had no effect on pineal or serum melatonin compared with sham-exposed animals and historical controls. The reason for this difference between the effects of circularly polarised and horizontally or vertically polarised fields was not clear. In a final paper (Kato et al, 1994d) the idea was tested that a reduction in serum melatonin might be correlated with an increase in serum testosterone, given that melatonin is thought to have an 'antigonadotrophic' effect in some seasonally breeding animals. However, animals exposed to circularly polarised 50 Hz magnetic fields were found to have similar serum testosterone levels to their sham-exposed counterparts.

Other studies investigating the effects of magnetic field exposure on serum and pineal melatonin levels in rats have produced mostly negative results. Nevertheless, Selmaoui and Touitou (1995) reported that the acute exposure of rats to horizontally polarised power frequency magnetic fields significantly depressed night-time serum melatonin levels and NAT activity in the pineal gland; chronic exposure could produce a similar effect using a lower flux density (Touitou et al, 2002), suggesting a relationship between the effects of field intensity and duration of exposure. Sensitivity to magnetic fields may also depend on the age of the animals (Selmaoui and Touitou, 1999) as serum melatonin concentration and pineal enzyme activities in older animals were unaffected by magnetic field exposure. Bakos et al (1995, 1997, 1999, 2002) reported that exposure to a vertical or horizontal power frequency magnetic field had no consistent effects on the circadian excretion of the major urinary metabolite of melatonin. As part of a larger study of EMF effects on DMBA-induced mammary tumours and pineal function, Mevissen et al (1996a,b) found no effect of magnetic field exposure on pineal melatonin levels in rats not treated with DMBA. In addition, Löscher et al (1998) were unable to identify any consistent effects of power frequency magnetic field exposure for up to 13 weeks on night-time serum melatonin levels. Further, John et al (1998) reported that the exposure of rats for up to six weeks to power frequency magnetic fields under a variety of conditions had no effect on the circadian excretion of the major urinary metabolite of melatonin. Fedrowitz et al (2002) reported that exposure of rats to a magnetic field for two weeks had no significant effect on melatonin levels measured directly in the pineal or mammary tissues.

In addition, a few studies have been carried out using mice exposed to power frequency fields, and most have not reported any consistent field-dependent effects. A large-scale study (McCormick et al, 1995) briefly reported that exposure to continuous or intermittent magnetic fields had no effect on serum or pineal melatonin. Similarly, no effects on night-time plasma melatonin levels were observed in a small study using continuous, long-term exposure to a variable magnetic field (de Bruyn et al, 2001). As a part of a tumour promotion study, Heikkinen et al (1999) found no effect of chronic exposure to magnetic fields of varying intensity on the night-time excretion of a urinary metabolite of melatonin in mice exposed to ionising radiation at 4 Gy. However, Picazo et al (1998) described a significant reduction in the night-time serum melatonin levels of mice exposed up to sexual maturity for four generations.

4.2.2 Seasonal breeders

Several laboratories have investigated the effects of power frequency magnetic exposure on pineal activity, serum melatonin levels and reproductive development in seasonal breeding animals. These studies are summarised in Table 4.5.

Three laboratories examined these effects in Djungarian hamsters, in which the duration of melatonin secretion during the shortening days of autumn and winter inhibits reproductive activity. The most

TABLE 4.5 Studies of effects of exposure to magnetic fields on melatonin levels in seasonal breeding animals

Assay	Exposure	Result	Reference
Night-time pineal and serum melatonin levels	60 Hz, 100 μT for 15 min, 2 h before dark period	Reduced and delayed night-time peak ($p<0.05$); effects not replicated	Yellon, 1994
Night-time pineal and serum melatonin levels	60 Hz, 100 μT for 15 min, 2 h before dark period	Reduced and delayed night-time peak ($p<0.05$); effects diminished	Yellon, 1996
Night-time pineal and serum melatonin levels; adult male reproductive status	60 Hz, 100 μT for 15 min, 2 h before dark period for 3 weeks	No effects on pineal and serum melatonin; no effects on melatonin-induced sexual atrophy	Yellon, 1996
Night-time pineal and serum melatonin levels; male puberty, assessed by testes weight	60 Hz, 100 μT for 15 min, 2 h before dark period from 16–25 days of age	Reduced and delayed night-time peak ($p<0.05$); not replicated; no effect on puberty	Truong et al, 1996
Night-time pineal and serum melatonin levels	60 Hz, 10 or 100 μT before or after dark onset or intermittent 100 μT, 15 or 60 min	No significant effects	Truong and Yellon, 1997
Night-time rise in pineal and serum melatonin levels; testicular weight	60 Hz, 100 μT in complete darkness; 15 min per day for up to 21 days	No significant effects	Yellon and Truong, 1998
Night-time pineal and serum melatonin levels; testis cell numbers	50 Hz, 450 μT (peak) sinusoidal or 360 μT (peak) rectangular field, 56 days	Increased cell number and night-time serum melatonin after rectangular field exposure ($p<0.05$)	Niehaus et al, 1997
Night-time pineal melatonin levels, serum prolactin levels and testis and seminal vesicle weights in short day (regressed) animals	60 Hz, 100 or 500 μT; CW and/or intermittent, starting 30 min or 2 h before onset of darkness; for up to 3 h for up to 42 days	Reduced pineal melatonin after acute (15 min) exposure ($p<0.01$); reduced gonad weight ($p<0.05$) but not melatonin after 42 day exposure	Wilson et al, 1999
Night-time serum melatonin levels and female puberty, detected by rise in serum progesterone	60 Hz, 6 kV m^{-1} and 4 μT fields, for 10 months	No field-dependent effects, strong seasonal effects	Lee et al, 1993, 1995

complete data come from a series of studies by Yellon and colleagues. In the first study, Yellon (1994) found that acute exposure to a magnetic field two hours before the onset of darkness reduced and delayed the night-time rise in serum and pineal melatonin, but that this effect was diminished in a subsequent replicate study and absent in a third replicate study. Similarly, variable results on pineal and serum melatonin were reported by Yellon (1996) and Truong et al (1996). In addition, both studies found that magnetic field exposure had no effect on reproductive development, even in reproductively repressed hamsters on 'short day' (winter) schedules, which might be thought to be sensitive to reduced and delayed night-time melatonin elevation. A fourth study (Truong and Yellon, 1997) found no effect on the night-time melatonin levels of different magnetic field exposure parameters to those used in the previous experiments. Finally, Yellon and Truong (1998) reported that a brief exposure to a

magnetic field prior to the night-time rise in pineal and serum melatonin levels had no effect, even in complete darkness.

In contrast to the work of Yellon and his colleagues, Niehaus et al (1997) reported that the chronic exposure of Djungarian hamsters on 'long day' (summer) schedules to 'rectangular' power frequency magnetic fields resulted in increased testis cell numbers and night-time levels of serum melatonin, whereas exposure to sinusoidal power frequency magnetic fields had little effect. The authors concluded that the *in vivo* effects of magnetic fields may be dependent on their waveform, and that the rapidly changing waveform of the rectangular fields was a more effective biological stimulus. However, the results are not easy to interpret; increased duration of melatonin levels in the Djungarian hamster are usually accompanied by decreased testicular activity.

Wilson et al (1999) investigated the effects of exposure to magnetic fields on pineal melatonin levels, serum prolactin levels and testicular and seminal vesicle weights in Djungarian hamsters moved to a 'short day' light regimen in order to induce sexual regression. Night-time pineal melatonin levels were reduced following acute exposure but this effect diminished with prolonged exposure. In contrast, induced sexual regression, as indicated by the testicular and seminal vesicle weights, seemed to be enhanced rather than diminished by prolonged magnetic field exposure, suggesting a possible stress response.

Another set of studies investigating the effects of power frequency magnetic field on seasonal breeders concerned Suffolk sheep: these animals have a long gestational period and become reproductively active in the autumn, as daylength shortens. In two replicate studies (Lee et al, 1993, 1995), Suffolk ewe lambs were exposed outdoors to the magnetic fields generated by overhead transmission lines for about ten months. No field-dependent effects were reported on serum melatonin levels or on the onset of puberty.

4.2.3 Cattle

The effects of 60 Hz fields on melatonin levels in pregnant dairy cows have received some attention. No field-dependent effects on nocturnal melatonin levels were reported by Burchard et al (1998) following continuous exposure to a combined electric and magnetic field (10 kV m^{-1} and 30 μT) for several weeks. Melatonin levels were analysed from blood samples obtained every 30 minutes for 14 hours starting at 17.00 hours on day 25 of exposure. In a subsequent study, Burchard et al (2004) reported that continuous exposure to an electric field for four weeks was not associated with any changes in circulating levels of progesterone, melatonin, prolactin and insulin-like growth factor. Inconsistent changes in melatonin levels were noted, however. Blood samples were collected twice a week from catheters inserted into the jugular vein.

4.2.4 Non-human primates

Only two studies have investigated the effects of exposure to magnetic fields on melatonin rhythms in non-human primates. Rogers et al (1995a) reported that the chronic exposure of male baboons to power frequency EMFs had no effect on night-time serum melatonin levels. However, a preliminary study

(Rogers et al, 1995b), based on data from only two baboons, reported that a three week exposure to an irregular, intermittent sequence of a combination of electric and magnetic fields in which switching transients were generated, resulted in a marked suppression of the night-time rise in melatonin.

4.2.5 Summary

The possibility that melatonin rhythms are affected by exposure to power frequency EMFs has been investigated in a variety of mammals. Some, but not all, studies with rats report that exposure to power frequency EMFs results in a suppression of pineal and serum melatonin levels. The results of the early studies using electric fields could not be replicated and may have suffered from technical difficulties. The evidence from a series of studies using circularly polarised magnetic fields suggests that exposure suppresses night-time melatonin levels, but this result was sometimes weakened by inappropriate comparisons between exposed animals and historical controls. The data from other experiments were equivocal but mostly negative.

The evidence for an effect of exposure to power frequency EMFs on melatonin levels and melatonin-dependent reproductive status in seasonally breeding animals is mostly negative. A series of studies by one group reported reduced and delayed night-time peaks in pineal and serum melatonin in Djungarian hamsters. These effects could not be successfully replicated. No effects were seen on changes in reproductive status. Another study reported that 'rectangular' power frequency magnetic fields increased night-time serum melatonin levels. Testis cell numbers were increased, which is contrary to the expected inhibitory effect of melatonin in this species. A third group reported that sexual regression in male hamsters, induced by a short daylength, was enhanced rather than inhibited by magnetic field exposure. Finally, another group found no effect on serum melatonin and the onset of puberty in sheep.

Too few data exist to make any firm conclusions regarding the effects of magnetic fields on melatonin levels in cattle or non-human primates, although a preliminary study with baboons reported melatonin suppression in response to an irregular and intermittent exposure.

4.3 Human experimental studies

It is well established that exposure to light at night acutely suppresses melatonin in humans with conscious light perception (Lewy et al, 1980; Bojkowski et al, 1987; Zeitzer et al, 2000; Thapan et al, 2001), and subsequently causes shifts in the phase of circadian rhythms (Arendt and Broadway, 1986; Czeisler et al, 1986; Boivin and Czeisler, 1998; Warman et al, 2003b). These effects are comprehensively reviewed in Chapter 3.

Stevens (1987) proposed that magnetic fields might act in a similar way to light at night, suppressing the production of melatonin from the pineal gland, and that this reduction in night-time melatonin could result in an increased incidence of breast cancer. As a direct result of the melatonin hypothesis, the majority of studies that have been conducted to investigate the potential effects of magnetic fields in relation to light in humans have used melatonin suppression as a marker. Wever (1979) provided the first indication that exposure to low frequency EMFs may also adjust the human biological clock. He used a temporal isolation unit, shielded from external EMFs, to which controlled fields could be applied (or not)

during lengthy experiments (weeks). He reported that a 10 Hz square wave electric field at 2.5 V m^{-1} could possibly act as a zeitgeber with respect to free-running rhythms. The field was either continuous or switched on for half of each 'circadian day' and periods of exposure were compared to periods with no exposure. Lighting was either continuous illumination (intensity not given but probably around 100–200 lux), or self-selected illumination. The lighting (and other electrical equipment operating within the unit) may have produced ambient 50 Hz electric fields of about 10–100 V m^{-1}. The experimental conditions varied to the extent that grouping of subjects was difficult to justify. Nevertheless there was consistent evidence that the field exposure (continuous or intermittent) could shorten free-running period, reduce the variability between individuals in free-running period, and perhaps prevent 'internal desynchronisation' of the circadian system. On the strength of these data, Wever proposed that the circadian system provided a sensitive model with which to test the effects of low frequency fields on biology. This would manifest itself as a change in the timing of the melatonin rhythm if treatment were to occur at an appropriate phase. However, the potential neuroanatomical pathways by which magnetic fields could exert an action on melatonin (suppression and/or phase-shifting) in humans (and other animals) remain obscure.

4.3.1 Experimental studies

Studies in humans investigating the effects of exposure to magnetic fields on melatonin rhythms, both in and out of the laboratory, have generally failed to provide consistent support for a field-dependent effect. While the results of a few laboratory-based volunteer studies suggest acute exposure may have an effect on the production or timing of the nightly melatonin rise, the majority of laboratory studies have not found any significant effects (Table 4.6). A similar pattern of results has been reported from epidemiology studies that have measured melatonin levels following chronic exposure at environmental levels: these studies are discussed in detail in Section 4.4.

It is particularly noteworthy that the most recent controlled data (Griefahn et al, 2001, 2002; Warman et al, 2003a) showed no acute effect of exposure to magnetic fields on amplitude or timing of the melatonin rhythm. Griefahn et al (2001, 2002) used partial constant routine conditions (see Chapter 3), generally considered to be the 'gold standard' for rhythm amplitude and phase assessment. In the Warman et al (2003a) study, correct sham controls were developed, baseline measures of melatonin levels were taken during the night preceding treatment, posture and light were controlled, and magnetic field exposure was carefully characterised. No significant effects of acute exposure (two hours) at 200–300 μT were found on the timing or absolute levels of plasma melatonin (Figure 4.1). In addition, the timing of the core temperature rhythm was not affected. Statistical analysis of variance in melatonin onset data indicated that the protocol possessed 87% power to detect a change in the timing of melatonin onset as small as 20 minutes. In spite of this, no significant alteration in individual melatonin onset times was detected, and there was no significant effect of circadian time of exposure on the observation of a response relative to the sham data.

Correct sham controls require bifilar coil windings. Magnetic fields and sham fields may thus be created by unidirectional and bidirectional flow of current respectively to eliminate the confounding effects of vibration, electric fields, noise and heat. Exposure conditions must be monitored. Although Wood et al (1998) used correct sham controls, the apparent lack of control of light exposure seriously compromised their conclusions.

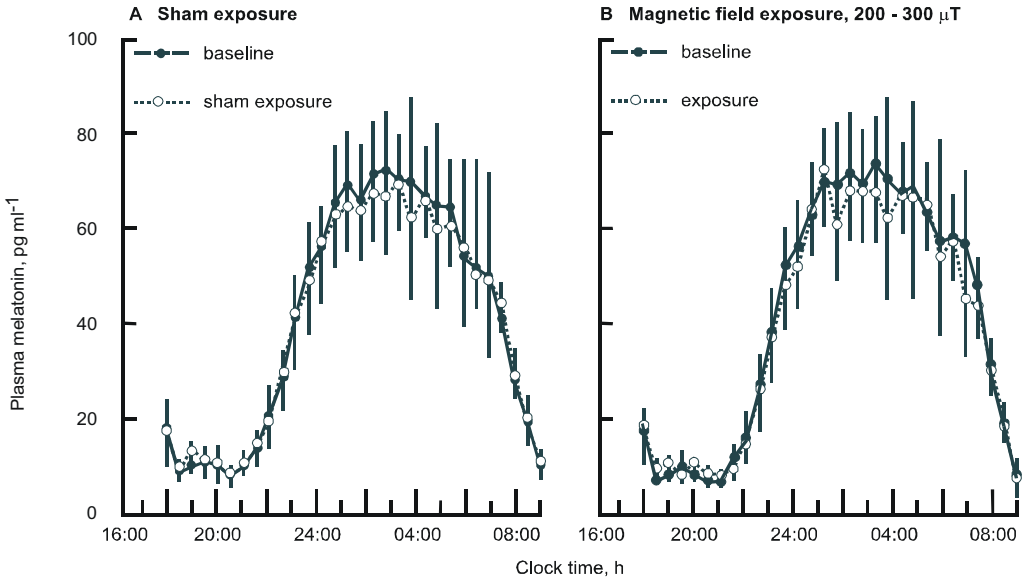

FIGURE 4.1 Mean melatonin profiles (N=19, mean ± SEM) of subjects from 18.00 to 10.00 hours with sham exposure (A) or magnetic field exposure (200–300 μT) during the melatonin rise (B). Closed circles denote the mean profile on baseline nights, open circles denote the mean profile on the sham (A) and exposure (B) legs. From Warman et al, 2003a

Two groups (Graham et al, 1996; Karasek et al, 1998) initially reported some positive effects of exposure to magnetic fields on melatonin. Graham and colleagues did not replicate their initial finding of an effect in a low melatonin subgroup in a subsequent experiment (Graham et al, 1997). Karasek and colleagues used a different exposure condition for their second attempt (Karasek et al, 2000) in which no field-related effects were found. The impressive series of studies by Graham and colleagues consistently used circularly polarised 60 Hz magnetic fields and convincingly demonstrated a lack of detectable effect in men or women, young or more elderly, with either continuous or intermittent exposure. It is possible that initial positive findings were due to chance with relatively small numbers of subjects.

Few early studies were conducted in a manner similar enough to allow direct comparisons to be drawn between them. Moreover in using melatonin as a marker, some researchers have neglected to bear in mind that it is an output from the circadian clock and as such its normal regulation is complex, time varying and extremely sensitive to light. As detailed in Chapter 3, the phase of the circadian clock can be altered by relatively modest amounts of light exposure (Boivin and Czeisler, 1998; Warman et al, 2003b) or by changes in behaviour (Buxton et al, 2003), and the time relative to the phase of the clock that a stimulus is administered can influence whether that stimulus has an effect or not (Khalsa et al, 2003). Standardisation of circadian phase such that the timing of the melatonin profile of all subjects is known prior to and during experimentation is thus essential to avoid confounding. Common methodological problems in studies with magnetic fields include single sampling, lack of appropriate baseline study nights, and lack of control for, or monitoring of, circadian phase (eg sleep–wake timing) and light exposure of subjects.

TABLE 4.6 Human experimental studies of magnetic field exposure

Reference	Exposure type	Design	Variable measured	Effect	Field strength
Wilson et al, 1990	Electric blankets 8 wk	Subjects' own controls N=28, F	aMT6s in overnight urine	7 showed decline and rebound post exposure with CPW	0.66 μT (max) conventional vs CPW blankets
Schiffman et al, 1994	MRI 01.00–02.00 h pulse sequence for imaging darkness	Subjects' own controls N=8, M	Plasma melatonin and cortisol	No effect	1.5 T control – darkness or light
Graham et al, 1996	CPMF, 60 Hz 23.00–07.00 h 1 h on, 1 h off <10 lux	Subjects' own controls N=33, M	Plasma melatonin hourly	No effect overall, lower in low melatonin group	1, 20 μT sham – field off
		Subjects' own controls N=40, M, low melatonin	Plasma melatonin hourly	No effect	20 μT sham – field off
Selmaoui et al, 1996	LPMF, 50 Hz continuous and 1 h on, 1 h off <30 lux	16 control, 16 test N=32, M, 20–30 y	Serum melatonin hourly aMT6s 3 hourly, 08.00–23.00 h, 9 h overnight	No effect	10 μT sham – field off
Graham et al, 1997	CPMF, 60 Hz, 23.00–07.00 h continuous <10 lux	Subjects' own controls N=40, M, 18–55 y	Plasma melatonin hourly	No effect in low or high melatonin group	20 μT sham – field off
Karasek et al, 1998	40 Hz, 20 min, 5 d per wk, 3 wk, 10.00 h or 18.00 h	Subjects' own controls N=12, M, middle-aged low back pain	Serum melatonin profiles	Lowers serum melatonin rhythm amplitude	2.9 mT pre-exposure (1 day) control
Wood et al, 1998	CPMF, 50 Hz during melatonin rise time 1.5–4 h light?	N=44, M, 18–49 y	Plasma melatonin 20–30 min hourly overnight	22 subjects had delayed melatonin rise time 12% lower peak	20 μT sham – bidirectional flow of current
Akerstedt et al, 1999	LPMF, 50 Hz 23.00–07.00 h continuous	Subjects' own controls N=18, M, 24–49 y	Plasma melatonin hourly	No effect on melatonin PRL, GH, cortisol, testosterone	1 μT
Graham et al, 2000a	CPMF, 60 Hz, 23.00–07.00 h 4 nights, 1 h on, 1 h off, <1 lux	Subjects' own controls N=30, M, 18–35 y	Melatonin and aMT6s AM urine aMT6s vs creatinine	No effect, less consistent on 4th night	28.3 μT vs <0.2 μT
Crasson et al, 2001	LPMF, 50 Hz continuous 30 min, intermittent on/off 15 s, 30 min, 50 lux 13.30 and 16.30 h	Subjects' own controls N=21, M, 20–35 y	Plasma melatonin hourly 20.00–07.00 h, urine volume recorded 19.00, 23.00, 07.00 h	No effect	100 μT sham – field off

TABLE 4.6 Continued

Reference	Exposure type	Design	Variable measured	Effect	Field strength
Karasek et al, 2000	200 Hz, Quatronic MRS 2000 8 min at 08.00 and 13.00 h, 3 wk, 5 d per wk	Subjects' own controls N=7, M, 32–42 y	Serum melatonin profile	No effect	25–80 μT
Hong et al, 2001	50 Hz electric blankets, 3 wk pre-exposure 11 wk exposure 2 wk post-exposure	Subjects' own controls N=9, M, 23–37 y	Melatonin in 5 urine samples daily 2 d, mid- week each condition	No effect	0.7 μT head 8.3 μT waist 3.5 μT feet
Graham et al, 2001a	CPMF, 60 Hz 23.00–07.00 h intermittent 1 h on, 1 h off <10 lux	Subjects' own controls N=53, F, 19–35 y 1 month apart 18–24 y match cycle days	Plasma melatonin and oestradiol hourly	No effect, no relation of melatonin to oestradiol	28.3 μT sham – field off <0.2 μT
Graham et al, 2001b	CPMF, 60 Hz 23.00–07.00 h continuous, intermittent 1h on, 1 h off <1 lux	Subjects' own controls N=24, M, 19–34 y	Melatonin and aMT6s AM urine aMT6s vs creatinine	No effect	127.3 μT sham – field off <0.2 μT
Graham et al, 2001c	CPMF, 60 Hz 23.00–07.00 h <10 lux	Subjects' own controls N=22 M and 24 F, 40–60 y	Melatonin and aMT6s AM urine aMT6s vs creatinine	No effect on melatonin, immune system, haematology	28.3 μT sham – field off <0.2 μT
Griefahn et al, 2001	16.7 Hz 18.00–02.00 h continuous <30 lux	Subjects' own controls N=7, M, 16–22 y	Constant, routine, saliva melatonin hourly, core body temperature, heart rate	No effect	200 μT sham – field off
Griefahn et al, 2002	16.7 Hz 18.00–02.00 h intermittent 15 s, <30 lux partial constant routine	Subjects' own controls N=12, M, 18–25 y	Saliva melatonin hourly, core body temperature, heart rate	No effect on melatonin or core body temperature, heart rate delayed	200 μT sham – field off
Kurokawa et al, 2003	LPMF, 50 Hz 23.00–07.00 h	Subjects' own controls N=10, M	Plasma melatonin hourly	No effect on melatonin, GH, PRL or cortisol	20 μT sham – field off
Warman et al, 2003a	CPMF, 50 Hz 2 h during melatonin rise time <10 lux partial constant routine	Subjects' own controls N=19, M, 18–35 y	Plasma melatonin hourly/half hourly 17.00–10.00 h, core body temperature	No effect	N=8, 200 μT N=11, 300 μT sham – bidirectional flow of current

Abbreviations: CPMF, circularly polarised magnetic field; CPW, continuous polymer wire; F, female; GH, growth hormone; LPMF, linearly polarised magnetic field; M, male; MRI, magnetic resonance imaging; PRL, prolactin

Although light is the factor that most significantly affects the clock, there are several non-photic stimuli that are known to influence circadian phase or apparent circulating levels of melatonin (as discussed in Chapter 3). Among these are posture (Deacon and Arendt, 1994), activity (Buxton et al, 2003), temperature (Dewasmes et al, 1994) and possibly even meal timing and content (Krauchi et al, 2002). A lack of standardisation of exposure to non-photic factors such as these may also produce changes in the melatonin profile, which can be falsely attributed to field exposure. However, the most important factor is lighting. Because relatively modest amounts of light commonly encountered in the home and laboratory setting can affect the circadian system greatly (Boivin and Czeisler, 1998; Warman et al, 2003b), wherever feasible, constant low levels (<10 lux) of light should be maintained throughout studies to avoid inadvertent light-induced phase shifting. In their study, Wood et al (1998) did not control for postural and lighting effects, and while they conducted baseline nights, they were not carried out immediately prior to each treatment as would be appropriate. Furthermore, different subjects appear to have received exposures of substantially different lengths (1.5–4 hours) and there are no data on whether exposure duration was correlated with observation of an effect. Overall, many of the earlier experimental studies investigating the effects of exposure to magnetic fields on melatonin profile suffer from a paucity of correct sham controls, a lack of explicit control of non-field-related factors, and inadequate field characterisation. Such studies are inadequate to assess the effects of magnetic fields on the melatonin profile.

The various factors that influence melatonin production and metabolism are listed in Table 3.1. Briefly, the most important are light exposure, circadian phase, sleep timing, posture, recent shift work or travel across time zones, beta-adrenergic antagonist and anti-depressant drugs. Most good studies also control for caffeine consumption, any medication including minor analgesics (eg non-steroidal anti-inflammatory drugs), use standardised meal times and meal content, and use subjects without extreme diurnal preference.

Single sampling to assess melatonin levels has been proposed as a cost-effective means of monitoring melatonin suppression in field-based studies (Cook et al, 2000). However, single sampling is innately risky due to the fact that one sample does not provide any temporal resolution of what is a time-varying process. Thus, without accurate personal monitoring of sleep–wake timing and light exposure of subjects prior to a single sample, and control of this timing, it is almost impossible to determine whether a difference in melatonin concentration in a single sample on different occasions is due to suppression or to a phase shift, or whether it is due to field exposure or to inadvertent exposure to light or changing sleep schedules. If a phase shift has occurred, a single sample can indicate an apparent increase or decrease in melatonin production when the overall effect is negligible (see Figure 4.2).

Some laboratory-based studies involve exposure of subjects to a sham condition on a separate occasion from the field exposure, suggesting circadian phase of the subjects could change between subsequent legs of the study. Without strict adherence to routine sleep–wake cycles between the legs of the study and without accurate assessment of circadian phase of subjects on entry to each leg (ie a baseline night), any changes in the timing or level of melatonin measured during the study cannot be attributed to field exposure.

If magnetic fields were a true zeitgeber and were able to phase shift the melatonin rhythm, the direction and magnitude of any shift would be expected to depend on the circadian timing of administration. Limited support for this hypothesis can be found in the data of Wood et al (1998), which suggested an

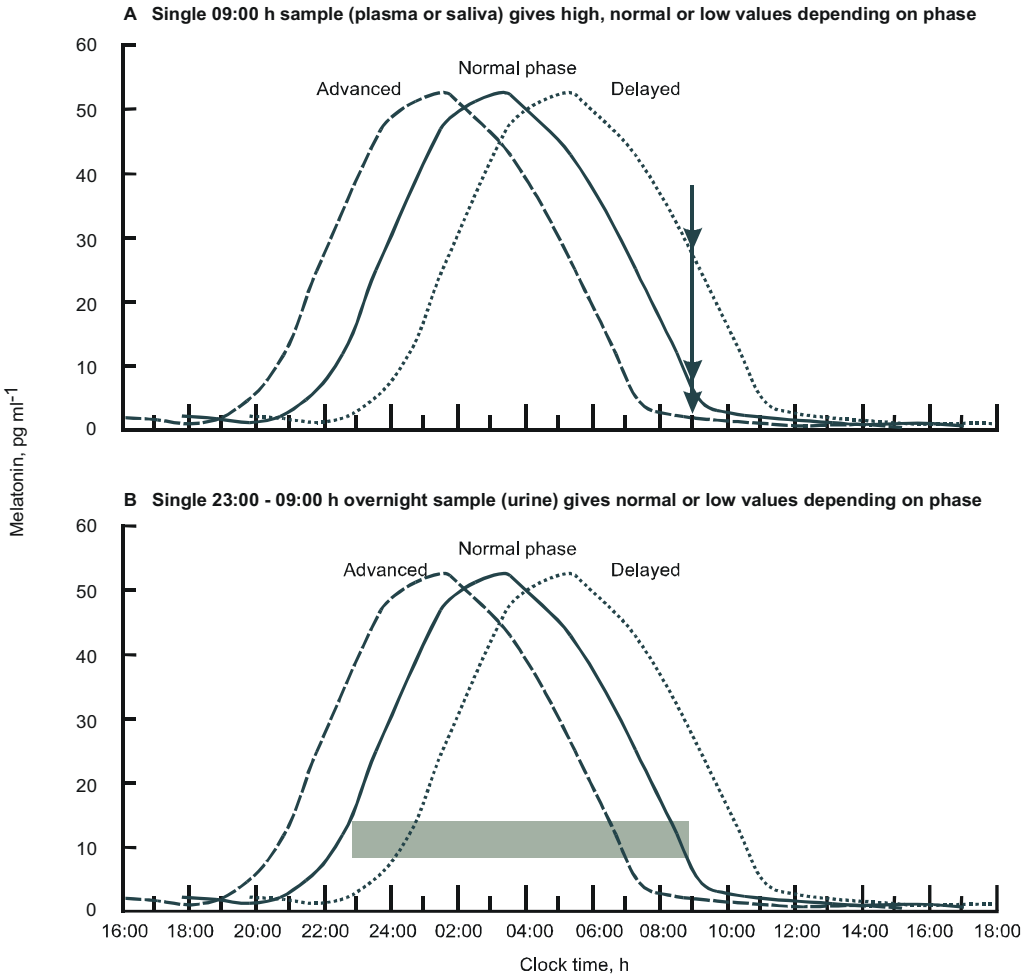

FIGURE 4.2 Differences in melatonin levels due to phase changes, not amplitude changes, using a single morning saliva or plasma sample (A) or a single overnight urine collection (B)

effect of magnetic field exposure on the timing of the melatonin rise (a 'phase shift') in a 'responsive subgroup' treated (20 μT, 1.5–4 hours) prior to or during the nightly melatonin onset, but not after it. In addition, Warman et al (2003a) noted that the variability of the timing of melatonin production was greater after field exposure than after sham exposure.

Although recent careful studies show no acute effects of EMFs or magnetic field alone on the melatonin profile, there are hints from the work of Graham et al (2000a), that longer term controlled exposure may eventually show some effect. They reported that intra-individual urinary measurements of melatonin and 6-sulphatoxymelatonin (aMT6s) were highly consistent over four exposure nights in the control condition. However, repeated nightly EMF exposure was associated with significantly reduced

consistency, suggesting a possible cumulative effect of EMF exposure on the stability of individual melatonin measurements over time. Moreover Griefahn et al (2002) reported that the rhythm of heart rate was delayed after acute exposure. Since, in general, all endogenous circadian rhythms are coordinated through the central circadian clock in the SCN, it is quite surprising that no effect was seen on the melatonin rhythm in this case. It is possible that the heart rate rhythm is influenced differently from melatonin downstream of the SCN.

Importantly, perhaps, sleep structure is modified by magnetic field exposure in a number of controlled studies (Akerstedt et al, 1999; Graham et al, 2000b) without concomitant effects on melatonin. Abnormal sleep is associated with a number of health problems, including compromised immune, hormonal and metabolic functions (Irwin, 2002; Spiegel et al, 2003).

Although most attention has been focused on investigating effects on melatonin, there have been a limited number of studies looking at circulating levels of other endocrine systems including the pituitary, thyroid, adrenal cortex and reproductive organs (see Table 4.6). As is the case with the melatonin studies, no conclusive effect of magnetic field exposure has been shown. In contrast, many reports of the effects of light on the timing of the circadian component of hormone rhythms exist, and most recently acute exposure to bright full spectrum white light in the early morning has been reported rapidly to increase production of both luteinising hormone and cortisol in young men (Yoon et al, 2003; Thorn et al, 2004).

4.3.2 Summary

Laboratory-based studies in humans investigating the effects of exposure to magnetic fields on melatonin rhythms have not provided consistent support for a field-dependent effect. While a few studies suggest acute exposure may have an effect on the production or timing of the nightly melatonin rise, the majority of studies have not found any significant modification of the rhythm. The lack of a consistent effect on the human melatonin profile may be due to inappropriate study designs, confounding, or the lack of an effect. The possibility remains that magnetic fields may affect the circadian clock in the long term and/or at a more subtle level.

4.4 Epidemiology

Observational epidemiology has considerable deficiencies compared with controlled trials as a method to investigate short-term effects of EMF exposure, and the epidemiological literature on EMFs and melatonin must therefore be considered cautiously, especially where data from volunteer trials are available. Three studies have been published of melatonin levels in relation to residential EMF exposures in women (Davis et al, 2001; Levallois et al, 2001; Youngstedt et al, 2002), one of occupational exposures in women (Juutilainen et al, 2000), and three of occupational or total exposures in men (Pfluger and Minder, 1996; Burch et al, 1998, 1999; Touitou et al, 2003). The studies including men have the complexity of interpretation that it is not certain to what extent factors affecting male levels would have the same effect in women (although for known factors so far, both sexes manifest similar effects).

4.4.1 Residential studies of women

Levallois et al (2001) found no relation of morning creatinine-adjusted urinary 6-sulphatoxymelatonin (aMT6s) concentration to proximity of residence to power lines or to measured 36 hour magnetic or spot residential electric fields, after adjustment for potential confounders. There were, however, significantly stronger relations of aMT6s to age and obesity (out of five variables for which the authors investigated effect modification) in women who lived close to power lines than in those who lived more distantly.

Davis et al (2001) assessed night-time urinary aMT6s concentrations normalised to creatinine in relation to nine magnetic field exposure variables derived from bedroom measures plus personal 24 hour metering. For one of these variables, bedroom magnetic field level, there was a borderline significant (depending on the potential confounders for which adjustment was made) association with aMT6s, significant in the subgroup of women who had used certain medications and a significant association at the time of year with fewest hours of darkness.

Youngstedt et al (2002) examined 24 hour urinary aMT6s excretion in 242 adults (226 women, 16 men*) aged 50–81 years in relation to 60 Hz magnetic field exposures levels in their beds, monitored over one week (but with no measurements of exposures in the rest of their lives, at home or occupationally). Fifteen per cent of the subjects had affective disorders at the time of observation (depression can affect melatonin levels) and some had had breast cancer. Regression analyses took account of several illumination variables, gained from 24 hour measurements, as well as age and usage of certain medications. All but six of the subjects had mean exposures of <0.5 μT. No significant associations were found between measures of magnetic field exposure (mean, maximum, intermittency and variability) and several measures of aMT6s excretion including 24 hour mean and amplitude, and timing.

4.4.2 Occupational studies of women

Juutilainen et al (2000) analysed total aMT6s excretion over Thursday night and over Sunday night, and calculated the ratio of aMT6s concentrations in the two samples, in garment workers (who were stated to have high EMF exposures) and in office workers employed elsewhere. The Thursday/Sunday ratios for each group were close to unity, suggesting that several days of work did not affect aMT6s levels. Average Thursday night aMT6s excretion was significantly lower in exposed workers than in office workers, after allowing for confounders, but there was no dose–response relation.

4.4.3 Studies of occupational or total exposures in men

A comparison between electric train drivers (average exposure 20 μT) and less-exposed railway workers (average exposure 1 μT) found significantly reduced evening aMT6s concentrations (probably creatinine standardised) on work days compared with leisure days for the heavily-exposed but not the less-exposed men (Pfluger and Minder, 1996). This was not seen for morning levels, nor was there any dose–response relation. The two groups differed in their occupational night exposure to light, and it is not clear that adequate adjustment for this was made in the analyses.

* Although in principle it seems undesirable to combine both sexes, there were so few men that the results presumably apply essentially to women.

A study of electrical utility workers found no association between post-work aMT6s concentrations and mean occupational magnetic field exposures, but a significant reduction, after adjustment for confounders, in creatinine-adjusted aMT6s concentrations in men in the highest quartile compared with the lowest, for a measure of stability of exposure on the 2nd and 3rd days of the working week (Burch et al, 1999). There was a significant interaction with the effect of occupational light exposure, such that the magnetic field effect was only present for men with low light exposure. A study of 24 hour magnetic field exposure measures in the same men in relation to overnight aMT6s concentrations and total aMT6s excretion (Burch et al, 1998), with adjustment for confounders, found no relation for magnetic field intensity, intermittence or cumulative exposure. There was, however, a relation of stability of fields with aMT6s concentration (significant for 4th *cf* 1st quartile for residential fields) but not with total excretion.

In another group of male electrical utility workers, Burch et al (2000) found trends of lower nocturnal creatinine-adjusted urinary aMT6s concentration and lower overnight total urinary aMT6s excretion with greater occupational 60 Hz magnetic field exposure, adjusted for workplace light exposure, but this was only present for one subgroup of workers – those working in substations or three-phase environments for more than two hours per day, and not for those working in these environments for shorter times than this, or working for short or long hours in one-phase environments. The restriction to three-phase and substation environments rather than one-phase was stated to be explicable if circular or elliptical polarisation affects melatonin more than linearly polarised fields.

A study of a further group of male electrical utility workers by the same authors (Burch et al, 2002), found reduced nocturnal creatinine-adjusted aMT6s concentration and overnight aMT6s total excretion in men with high compared with low or medium workplace 60 Hz exposure, adjusted for light exposure at work (but not a consistent gradient with degree of exposure), restricted to analyses within men with high occupational mobile phone use (ie not present in those with medium or no such phone use).

A French study (Touitou et al, 2003) compared plasma melatonin and creatinine-normalised urinary aMT6s between 15 men who worked at (and lived 'near') high voltage substations in Paris and 15 white collar workers, without occupational magnetic field exposures, from the same company. The former group had had chronic occupational exposure to 50 Hz magnetic fields for 1–20 years, with weekly geometric mean exposures for the individual men (measured continuously over 7 days and nights) ranging from 0.1 to 2.6 μT. The white collar workers had individual exposures ranging from 0.004 to 0.092 μT. Considerable care was taken to avoid confounding differences between the two groups: for instance, all were non-smokers of a restricted age range, who did not do night work, and who were asked not to use electric razors or hair dryers in the 24 hours before samples were taken, and lights were turned off from 22.00 to 08.00 hours on the night of sample collection.

Blood samples were taken hourly from 20.00 to 08.00 hours and overnight urine collected. The hourly profile of plasma melatonin was similar between exposed and unexposed men. The aMT6s concentration in the first morning urine was not significantly different between the two groups. There were also no significant differences in plasma melatonin between the most highly exposed workers and the unexposed group. It was stated that the exposed men had not been 'on call' in the 48 hours before the samples were taken, but it is unclear whether this meant they had had no occupational exposures during that period, or indeed how recently before the samples were taken they had been occupationally or substantially exposed, and thus to what lag period after exposure the results relate.

4.4.4 Summary

In aggregate, the epidemiological studies of the relation of melatonin levels to EMF exposure do not give convincing evidence that EMFs affect the secretion of melatonin in humans. Although each of the published studies, except those by Youngstedt et al (2002) and Touitou et al (2003), has found some significant findings, usually in a subset of the data, there has been no consistency in the subgroup for which significant results were found, and indeed, in general, the significant results have not been re-examined for the same subgroup in subsequent studies. The authors of each of the positive studies proffered (apparently *post hoc*) reasons why effects might be detectable only in the particular (but very different) subgroup(s) identified in their studies. The positive findings have been in subgroups, however, for which in general there is no obvious *a priori* reason why there should have been such a restriction of effect. Hypotheses about why effects were found only in individuals who, for instance, had low light exposure or had used certain medications or had high phone use, would only carry conviction if subsequent studies examined the same subgroup and found that risks were present and restricted to this subgroup. Otherwise there must be concern that the significant finding could simply have been due to chance.

A further difficulty of interpretation is that as well as subgrouping in the above sense, there have also been many different measures of EMFs and of melatonin secretion used in different studies, and often several within one study (for instance, stability of fields, average fields, measures at home and at work, measures of melatonin on different days of the week, or ratios between days). It is not obvious *a priori* why the particular measures for which significant results have been found should alone have given significant relations.

The studies have measured melatonin in two different body fluids (plasma and metabolites in urine) and the urinary measures have been of two different types (creatinine-adjusted concentrations, and total excretion over 24 hours or overnight).

In most studies, melatonin measures have been made for one point in time or period of the day, rather than for 24 hours. Such measures are potentially highly susceptible to confounding by factors that alter the phase of the circadian clock, notably light exposure and sleep–wake timing (eg waking at a different time at weekends than during the week). Adjustment for (relatively crude) measures of such confounders could still leave considerable residual confounding (see Table 3.1 for further detail on confounders).

Finally, the studies published to date have mainly used urinary measures of melatonin*, which might lead to low response rates and hence potential selection bias with respect to the individuals who took part. In general, the studies either have reported low response rates or have not reported the response rate.

For these reasons, unless and until the specific significant findings in the published studies can be backed up by biological evidence or by replication in independent epidemiological studies, they can be regarded as no more than hypothesis-generating.

* Touitou et al (2003) also used plasma measures, but in addition to urinary measures.

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5 Melatonin and Breast Cancer

5.1 *In vitro* studies

In vitro studies of melatonin and breast cancer have focused on the possible oncostatic properties of melatonin. More than 60 studies have been published, with the vast majority of the work being undertaken on the breast cancer cell line MCF-7. Surprisingly few studies have used other breast cancer cell lines.

The MCF-7 is an oestrogen receptor positive cell line derived from a pleural effusion from a breast carcinoma (Soule et al, 1973) and, as described in Chapter 4, it has been used extensively as a cellular model of breast cancer.

Several authors have commented on the heterogeneity of the MCF-7 cells and that cellular responses may vary depending on the sub-clone and the conditions under which the cells are grown (Osborne et al, 1987; Liburdy et al, 1993; Ram et al, 2000). One reported response, namely that physiological concentrations of melatonin inhibit the growth of MCF-7 cells, may be of particular relevance here. There is not complete agreement that the growth of MCF-7 cells is affected by treatment with melatonin – some researchers (Shellard et al, 1989; Bartsch et al, 1992; Panzer et al, 1998; Papazisis et al, 1998) find no inhibitory effect of melatonin at physiological concentrations. However, there are a sufficient number of reports from a variety of independent laboratories showing inhibitory effects of melatonin on MCF-7 cells that these effects must be accepted, in at least some of the sub-clones (Blask and Hill, 1986; Cos and Sanchez-Barcelo, 1995; Karasek and Pawlikowski, 1999; Rato et al, 1999; Scott et al, 2001; Czeczuga-Semeniuk et al, 2002). Some researchers express the view that it is only a sub-clone of MCF-7 that is responsive in this way and that this inhibitory effect is not a general phenomenon of all breast cancer cells (Panzer et al, 1998). Since the majority of these studies have used MCF-7 cells, it is difficult to judge how common this inhibitory effect might be in other mammary cancer cells, but at least one other cell line has been shown to be responsive. Bizzarri et al (2003), working on a rat mammary cancer cell line (RM4), found that melatonin inhibited proliferation if 17-beta-oestradiol was present, although 1,25-dihydroxyvitamin D_3 was more potent.

The mechanisms by which melatonin inhibits the growth of cells have been investigated. There are suggestions that it acts on the binding of the oestrogen receptor to DNA and therefore blocks the stimulatory effect of oestrogen (see Chapter 3 for further details).

At higher than physiological levels of melatonin, other mechanisms have been proposed. Scott et al (2001) suggested a receptor-modulated pathway of cytotoxicity and an uncoupling of oxidative phosphorylation in cells treated with 100 nM melatonin. At high concentrations ($>10^{-5}$ M) melatonin is an effective scavenger of free-radicals (Baldwin and Barrett, 1998) and will protect the cell from damage and exert an oncostatic effect. However, this protective effect is achieved at pharmacological levels of melatonin; at physiological levels its role as a free-radical scavenger may not be significant.

5.1.1 Summary

The bulk of the *in vitro* studies of breast cancer have been undertaken on one particular cell line: the MCF-7 (Table 5.1). Some sub-clones have been demonstrated to be sensitive to physiological levels of melatonin in that growth can be inhibited under certain conditions. The cell line has proved useful in providing some insights into the potential mechanism of action. However, little work has been undertaken with other breast cancer cell lines. It is therefore of concern that the oncostatic action of melatonin may be confined to a sub-clone of MCF-7 cells and not applicable to breast cancer cells or cancer cells in general.

TABLE 5.1 Summary of *in vitro* studies of the effects of melatonin on mammalian cells

Results	Reference
Melatonin (10^{-9} – 10^{-5} M) inhibited MCF-7 cell proliferation only in absence of oestrogen	Baldwin et al, 1998
Melatonin (10^{-9} M) inhibited proliferation in rat mammary cancer cell line (RM4) in presence of 17-beta-oestradiol, but had no effect when serum was included in culture medium	Bizzarri et al, 2003
Melatonin (10^{-11} – 10^{-9} M) inhibited MCF-7 cell proliferation, and blocked oestradiol stimulation, but required serum in the culture medium	Blask and Hill, 1986
Melatonin inhibited proliferation to a varying extent in three breast cancer cell lines, but only MCF-7 cells responded to physiological concentrations (10^{-9} M). No effect on oestrogen-insensitive cells	Hill et al, 1992
Inhibitory effect of melatonin (10^{-11} – 10^{-7} M) varied between stocks of MCF-7 cells	Ram et al, 2000
Melatonin (10^{-5} M) inhibited proliferation of MCF-7 cells, but had no effect on the stimulatory action of oestradiol	Czeczuga-Semeniuk et al, 2002
Melatonin (10^{-9} M) only inhibited proliferation in fast growing MCF-7 cells	Cos and Sanchez-Barcelo, 1995
Melatonin inhibited proliferation in MCF-7 cells	Karasek and Pawlikowski, 1999
Melatonin (10^{-9} M) inhibited proliferation in MCF-7 cells	Liburdy et al 1993
Melatonin (10^{-13} – 10^{-3} M) showed hardly any effect of inhibiting the growth of the six human cancer cell lines tested, including MCF-7 cells	Bartsch et al, 1992
No inhibitory effect of melatonin (10^{-13}– 10^{-7} M) in MCF-7 cells, nor in three other non-breast cancer cell lines	Panzer et al, 1998
No inhibitory effect of melatonin (10^{-11} – 10^{-9} M) on cell proliferation in breast cancer cell lines MCF-7 and T47D	Papazisis et al, 1998
No inhibitory effect of melatonin (10^{-10} – 10^{-8} M) on MCF-7 cells, nor in three other non-breast cancer cell lines	Shellard et al, 1989

5.2 *In vivo* studies

The potential of melatonin to modulate the incidence and growth of mammary tumours has been determined using various animal models of breast cancer. Most studies have assessed the effects of exogenous melatonin treatment on the growth of chemically induced mammary tumours. However, a few studies have investigated effects on transplantable tumours, while others have used normal and transgenic mouse strains that express a high spontaneous incidence of mammary tumours. Further

studies have investigated the effects caused by inducing changes in pineal function either through pinealectomy or by altering the photoperiod.

Early studies found that treatment with melatonin had inconsistent effects on the growth of various tumours in rodents, with both stimulatory, inhibitory and null effects being reported (see Pawlikowski et al, 2002). However, Bartsch and Bartsch (1981) suggested that the effect of melatonin on tumour growth was dependent on the photoperiod and the time of administration of the treatment: application of melatonin in the morning caused an increase in tumour growth, whereas exposure in the evening caused an inhibition of growth. A similar differential effect of time of treatment on tumour development was reported by Wrba et al (1986) and Chatterjee and Banerji (1989), prompting the suggestion that (mammary) tumours may exhibit a diurnal rhythm in sensitivity to melatonin (Blask, 1997). The majority of subsequent studies have found that melatonin administration in the late afternoon and early evening has an inhibitory effect on a wide variety of tumour types in rats, mice and hamsters: these data have been reviewed by Anisimov (2003), Brainard et al (1999), Cos and Sánchez-Barceló (2000), Pawlikowski et al (2002) and Sánchez-Barceló et al (2003). The discrepancies between studies have been largely attributed to differences in treatment times and also to complex differences in tumour types and to differences between the animal species used.

5.2.1 Transplantable tumours

Two studies have investigated the effects of melatonin on transplantable mammary tumours. Anisimov et al (1973; quoted from Anisimov, 2003) reported that daily subcutaneous (sc) injection of melatonin caused a large decrease in the size of the mammary tumours in female mice. Similarly, Karmali et al (1978a,b) reported that injection of melatonin significantly inhibited the growth of R3230AC mammary tumours in female Fisher rats. These studies are summarised in Table 5.2.

TABLE 5.2 Effects of melatonin on transplantable mammary tumours

Model	Treatment	Result	Reference
RSM carcinoma in C3HA mouse	Daily sc injection, 50 μg per mouse per day	Decrease in tumour size ($p<0.05$)	Anisimov et al, 1973
R3230AC tumour in the rat	1 mg, daily sc injection, beginning 2 days before transplant and continuing for 4 weeks	Reductions in tumour weight ($p<0.05$) and size	Karmali et al, 1978a,b

5.2.2 Chemically induced tumours

Many animal studies (see Pawlikowski et al, 2002) have reported that treatment with melatonin reduces the growth of a variety of chemically induced tumours, including colon cancers induced by 7,12-dimethylbenz[a]anthracene (DMBA) in rats (Anisimov et al, 1997a, 2000) and skin papillomas induced by benzo(a)pyrene in mice (Kumar and Das, 2000).

More than a dozen studies (summarised in Table 5.3) have investigated the effects of exogenous melatonin treatment on mammary cancer induced by DMBA or *N*-nitroso-*N*-methylurea (NMU) in female rats. Some of these studies also investigated the effectiveness of combined melatonin treatment with drugs such as tamoxifen or raloxifene, but these particular results are not considered here.

TABLE 5.3 Effects of melatonin on chemically induced tumours in female rats

Model	Carcinogen treatment	Melatonin treatment	Photoperiod (light:dark) h	Result	Reference
Sprague-Dawley rat	30 mg DMBA by gavage at 50 days old	100 μg by sc injection daily in morning from 43 to 243 days old	12:12	*Increase* in tumour incidence ($p<0.005$)	Hamilton, 1969
Sprague-Dawley rat	25 mg DMBA at 60 days old (route not specified)	200 μg by sc injection twice a week at 17.00 h from 60 to ≈135 days old	12:12	Significant decrease in latency ($p<0.001$). Incidence of tumours reduced by 25–30%	Aubert et al, 1980
Sprague-Dawley rat	15 mg DMBA, by gavage, at 50 days old	2.5 mg kg^{-1} by ip injection daily at 16.00 h, from 50 to 140 days old	12:12	Highly reduced incidence of tumours at 190 days old ($p<0.002$)	Tamarkin et al, 1981
Holtzman rat	10 mg DMBA by gavage, at 55–60 days old	500 μg by ip injection daily in late afternoon, from 52 to 145 days old	10:14	Incidence of tumours reduced by 38%	Shah et al, 1984
Sprague-Dawley rat	5 mg DMBA by iv injection, at 55 days old	250 μg by injection daily in late afternoon (from 16.00 to 18.00 h), from 76 to 181 days old	12:12	Slight, non-significant reduction in tumour incidence. Significant inhibition observed in combination with underfeeding ($p<0.05$)	Blask et al, 1986
Holtzman rat	20 mg DMBA by gavage, at 55 days old	100 μg per rat per day, in drinking water, from 20 to 140 days old	10:14	Reduced incidence of tumours at 180 days old ($p<0.05$)	Kothari, 1987
Holtzman rat	10 mg DMBA by gavage, at 55 days old	200 μg per rat per day, in drinking water, from 48 to 62 days old (initiation); or from 62 days old for 26 weeks (promotion)	10:14	Reduced incidence of tumours at 244 days old (initiation $p<0.05$; promotion $p<0.0025$)	Subramanian and Kothari, 1991a
Sprague-Dawley rat	3 doses of 10 mg* DMBA by gavage, on day of age 45, 50 and 55	20 μg ml^{-1} (0.45 mg per rat) in drinking water, from 15.00 to 08.00 h, 4 alternate days per week, from 33–37 days old for 26 weeks after DMBA treatment	12:12	Reduced incidence and frequency of tumours per group ($p<0.001$)	Môciková-Kalická et al, 2001
Wistar: Han rat	1.44 Gy γ-rays (^{60}Co) over 15 days from 44–46 days old. 3 doses of 10 mg DMBA, by gavage from 52–58 days old	100 μg ml^{-1} in drinking water, available continuously, from irradiation for 26 weeks	12:12	Total tumour volume decreased ($p<0.05$) but no significant effects on tumour incidence, frequency or latency	Môciková et al, 2000

* Dosage of DMBA as given by Môciková-Kalická et al (2001) in their abstract: the value of 10 μg given in their main text seems unlikely.

TABLE 5.3 Continued

Model	Carcinogen treatment	Melatonin treatment	Photoperiod (light:dark) h	Result	Reference
Sprague-Dawley rat	2 doses of 50 mg kg^{-1} NMU by iv injection, at 50 and 60 days old (initiation only) or at 50 and 57 days old	500 μg by sc injection daily in late afternoon, from 37 to 60 days old (initiation); from 85 days old for 16 weeks (delayed promotion); or from 57 days old for 19 weeks (promotion)	12:12	Initiation: no significant effects. Delayed promotion: slight, non-significant effects on tumour incidence and latency, number of tumours reduced ($p<0.05$). Promotion: tumour incidence and number reduced ($p<0.05$), no effect on latency	Blask et al, 1991
Sprague-Dawley rat	50 mg kg^{-1} NMU by ip injection, at 50 days old	200 μg per rat per day in drinking water, available continuously, for 180 days following surgical removal of primary tumour at ≤6 months	Not known	No effect on tumour incidence but latency of second generation tumours increased ($p<0.001$)	Kothari et al, 1995
Sprague-Dawley rat	50 mg kg^{-1} NMU by ip injection, at 50 days old	200 μg per rat per day in drinking water, available continuously, for 300 days	12:12	No effect on tumour incidence but latency increased ($p<0.001$)	Kothari et al, 1997
Sprague-Dawley rat	2 doses of 50 mg kg^{-1} NMU by ip injection, 7 days apart from 46–57 days old. 20 mg DMBA by gavage, at 50–54 days old	500 μg per rat daily in drinking water from 15.00 to 08.00 h, from 34–45 days old for 24 weeks	12:12	Non-significant changes in incidence and latency, except for decrease in mean tumour volume with DMBA ($p<0.05$)	Kubatka et al, 2001a
Sprague-Dawley rat	2 doses of 50 mg kg^{-1} NMU by ip injection, 7 days apart from 43–54 days old	110 μg per rat daily in drinking water, from 15.00 to 08.00 h, from 31–42 days old for 26 weeks	12:12	Non-significant decrease in incidence of tumours by 19%. Decrease in tumour frequency per group ($p<0.05$)	Kubatka et al, 2001b
Sprague-Dawley rat	2 doses of 50 mg kg^{-1} NMU by ip injection, 7 days apart from 51–58 days old. 3 doses of 10 mg DMBA by gavage, at 45–59 days old	500 μg per rat daily in drinking water, from 15.00 to 08.00 h, from 7 days before NMU treatment for 28 weeks, or 4 days before DMBA treatment for 14 weeks	12:12	Increased tumour growth over last 10 weeks with NMU ($p<0.01$). Decreased incidence of tumours ($p<0.05$) with DMBA. Other inhibitory effects not significant	Kubatka et al, 2002
LIO	50 mg kg^{-1} NMU by iv injection, at 2 months old	20 mg l^{-1} per day in drinking water, from 18.00 to 09.00 h for 3 days starting 2 days before NMU injection	14:10	Reduced number of malignant tumour bearing rats and reduced numbers of tumours per rat	Musatov et al, 1999

In an early study, Hamilton (1969) found that morning administration of melatonin resulted in an increase of DMBA-induced mammary tumours. However, subsequent studies have reported that late afternoon or evening treatment with melatonin generally inhibited chemically induced mammary tumorigenesis, with a reduced incidence and multiplicity of tumours and an increased latency period for tumour appearance (Aubert et al, 1980; Tamarkin et al, 1981; Shah et al, 1984; Blask et al, 1986, 1991; Kothari, 1987; Subramanian and Kothari, 1991a; Kothari et al, 1995, 1997; Musatov et al, 1999; Môciková et al, 2000; Kubatka et al, 2001a,b, 2002; Môciková-Kalická et al, 2001). Melatonin treatment also brought about consistent changes in the morphology of the mammary gland compared with control animals, with much reduced ductal branching with few terminal end buds and alveolar buds. However, melatonin had inconsistent effects on food intake or weight gain, although some studies reported significant decreases in weight and in food and water intake.

Despite there being many methodological and procedural differences between these studies, the overall results are comparable, and these studies indicate that the repeated, long-term treatment of female rats with high (supraphysiological) doses of exogenous melatonin can inhibit the growth and development of mammary tumours induced by chemical carcinogens. There is some evidence to suggest that higher doses of melatonin may be less effective at inhibiting mammary tumorigenesis than more physiological levels: for comparison with the values given in Table 5.3, Kubatka et al (2001a) estimated that, in the Sprague-Dawley rat, a dose of about 0.23 μg melatonin produces a concentration of 1 nM. Further, it would seem that melatonin may act through mechanisms associated more with inhibiting effects on tumour promotion rather than with inhibiting tumour initiation.

5.2.3 Spontaneous tumours

A handful of studies have investigated the effects of melatonin on the development of mammary tumours using mouse models expressing a high incidence of spontaneous mammary cancers: these are summarised in Table 5.4. Two studies have used inbred strains. Subramanian and Kothari (1991b) found that prolonged, oral melatonin treatment caused a significant suppression of the development of mammary tumours in female C3H/Jax mice. About 65% of these animals spontaneously develop mammary tumours by eight to nine months old. At one year of age, 23% of mice treated with melatonin had developed adenocarcinomas as opposed to 63% of control animals ($p<0.02$). Treatment with melatonin also resulted in significant reductions (in three month old mice) in serum 17-beta-oestradiol levels ($p<0.05$) and in DNA synthesis in the mammary gland ($p<0.02$). As part of a study investigating the effects of melatonin on long-term health and tumour development in adult, female CBA mice, Anisimov et al (2001) found that intermittent, oral treatment with melatonin at night did not affect the incidence of spontaneous mammary tumours.

Other studies have used transgenic mice over-expressing genes involved in mammary cancer. Mediavilla et al (1997) injected young female mice carrying the N-*ras* proto-oncogene with melatonin or vehicle late in the evening, five times a week. After five months of treatment, the mammary glands were dissected and examined for histological and immunochemical changes. Compared to vehicle-treated animals, the animals given melatonin had a lower density of hyperplastic alveolar nodules (HANs), an absence of epithelial dysplastic cells and weak immunostaining of the N-ras protein. In addition, none of the treated animals developed mammary carcinomas.

TABLE 5.4 Effects of melatonin on spontaneous mammary tumours

Model	Treatment	Photoperiod (light:dark) h	Result	Reference
CH3/Jax mouse	25 μg per mouse per day in drinking water from 21 to 44 days old, and 50 μg per mouse per day from day 45 until 12 months old	10:14	Significantly reduced incidence of tumours (adenocarcinomas) ($p<0.02$)	Subramanian and Kothari, 1991b
CBA mouse	20 mg l^{-1} at night in drinking water, 5 consecutive days per month, from 6 months old	12:12	No significant change in incidence of tumours (adenoma or adenocarcinoma)	Anisimov et al, 2001
MMTV-LTR/N-*ras* transgenic mouse	Subcutaneous injection, 200 μg per mouse per day for 5 days per week for 5 months from 28 days old, 2 hours before lights off	14:10	Reduced incidence of premalignant lesions and adenocarcinomas. Reduced expression of N-ras protein on focal hyperplastic lesions ($p<0.001$)	Mediavilla et al, 1997
TG.NK/c-*neu* transgenic mouse	50–200 mg kg^{-1} daily by gavage for 30 weeks from 28 days old	12:12	Significant delay in appearance of palpable tumours ($p<0.05$)	Rao et al, 2000
HER-2/*neu* transgenic mouse	20 mg l^{-1} at night in drinking water from 2 months to 9–13 months old. Calculated nightly dose of 0.076 mg per mouse	12:12	Significantly decreased incidence ($p<0.0003$) and size ($p<0.05$) of tumours. Expression of HER-2/neu mRNA reduced by 2.5 fold. Reduced life span ($p<0.05$)	Baturin et al, 2001 Anisimov et al, 2003

Rao et al (2000) reported that melatonin delayed the appearance of palpable mammary tumours and reduced the growth of these tumours in TG.NK female transgenic mice (which express the c-*neu* breast cancer oncogene under the control of mouse mammary tumour virus (MMTV) promoter). The animals were gavaged using a corn oil vehicle containing melatonin at 50, 100 or 200 mg per kilogram of body weight. These doses were administered no more than two hours before the start of the dark cycle each day for 30 weeks, starting at four weeks of age. A significant dose-related decrease was found for the incidence of tumours, and for the multiplicity of tumours per mouse. The authors noted that mortality was increased in the group treated with the high dose of melatonin, and suggested an interaction with the stresses associated with repeated gavage.

Anisimov and colleagues (Baturin et al, 2001; Anisimov et al, 2003) gave melatonin in drinking water at night (from 18.00 to 09.00 hours) to female FVB/N mice transgenic for the oncogene HER-2/neu from two to nine months of age. This treatment decreased the incidence and size of mammary adenocarcinomas, although the mean number of tumours per mouse was not changed. Treatment resulted in a reduction in the expression of HER-2/neu mRNA in mammary tumours. A reduction in life span in treated animals was attributed to melatonin-induced kidney insufficiency. Keeping the animals under constant light conditions was found to modulate the observed responses (see below) while interrupted treatment with melatonin (administered on only five nights per month, so the total dose of melatonin was relatively much smaller) increased the number of tumours per tumour-bearing mouse.

Overall, these studies suggest that treatment with exogenous melatonin has a generally inhibitory effect on mammary tumour development in mice. These effects appear dependent on factors such as the total dose of melatonin, dosing schedule, and the age of the animals.

5.2.4 Induced changes in pineal function

Some studies have indirectly assessed the potential of melatonin to affect the development of mammary carcinogenesis by causing changes in pineal function, either by manipulating the photoperiod or following pinealectomy. These studies have been reviewed recently by Brainard et al (1999) and Anisimov (2002, 2003).

Overall, these studies (Table 5.5) suggest that inhibition of pineal function with pinealectomy or with exposure to constant light (400–800 lux) stimulates chemically induced mammary tumorigenesis in female rats (Khaetski, 1965 (cited by Anisimov, 2002); Hamilton, 1969; Aubert et al, 1980; Tamarkin et al, 1981; Kothari, 1987; Kothari et al, 1982, 1984; Shah et al, 1984; Subramanian and Kothari 1991a; Anisimov et al, 1994, 1997; Travlos et al, 2001) and the induction of spontaneous mammary tumours in normal (Jöchle, 1964) and transgenic mice (Baturin et al, 2001). Exposure to constant light has also been reported to result in increased incidence of other spontaneous tumours and leukaemias in CBA female mice (Anisimov et al, 2004).

In addition, Blask et al (2002, 2003) reported that in ovary-intact, non-oestrogenised female nude rats bearing a xenograft of human MCF-7 breast cancer cells exposure to constant light produced an immediate and sustained increase in the growth rate of the tumour (Figure 5.1). After 15 days, the tumours were estimated to be significantly heavier than those of animals housed under a diurnal lighting schedule ($p<0.05$). Results suggested that this was mediated via a suppression of melatonin production (exposure of nude rats to constant light for five weeks was observed to suppress completely the nocturnal peak in circulating levels of melatonin), leading to an increased tumour update of linoleic acid and its metabolism to the tumour mitogen, 13-hydroxyoctadecadienoic acid (13-HODE). However, group sizes used were very small. Nevertheless, comparable results were also obtained using a tissue-isolated NMU-induced rat mammary tumour model as well as a rat hepatoma 7288CTC model (Blask et al, 2002), suggesting that inhibition of fatty acid metabolism may represent a common mode of action of melatonin.

Conversely, there is some evidence (summarised in Table 5.6) that treatments that stimulate pineal function, either through light deprivation or following surgical blinding, inhibit chemically induced mammary carcinogenesis (Chang et al, 1985; Anisimov et al, 1994, 1997b; Jull, 1996; see also Blask et al, 1988, and Sanchez-Barcelo et al, 1988). Anisimov (2002) cites early work by Kuralasov in 1979 that first demonstrated the inhibitory effects of light deprivation on the growth and development of transplantable (RMK-1) and DMBA-induced mammary tumours in rats. Survival of rats bearing tumours induced by DMBA was also 55% longer than controls.

A few of these studies have reported inconsistent or paradoxical results, possibly reflecting that the susceptibility of mammary tissues to carcinogenic insult is a complex response modulated by various environmental and other factors, including the level of endogenous melatonin.

TABLE 5.5 Effects of exposure to constant light (LL), pinealectomy (Px) or combined treatments (PxLL) on chemically induced, tissue-isolated xenografts, or spontaneous mammary tumours in female rats and mice

Model	Carcinogen treatment	Treatment	Result	Reference
Outbred rat	1.5 mg DMBA by 5 weekly iv injections	LL (4 weeks after last injection)	Significant increase in tumour multiplicity and growth rate; tumour latency reduced	Khaetski, 1965
Sprague- Dawley rat	30 mg DMBA by gavage, at 50 days old	LL	Tumour incidence increased ($p<0.002$)	Hamilton, 1969
Sprague-Dawley rat	25 mg DMBA at 60 days old	LL, Px or PxLL (Px at 58 days old)	Tumour latency *increased* (for LL, $p<0.02$)	Aubert et al, 1980
Sprague-Dawley rat	7 mg DMBA by gavage, at 50 days old	Px (Px at 20 days old)	Significantly increased incidence of tumours at 240 days old ($p<0.002$)	Tamarkin et al, 1981
Holtzman rat	10 mg DMBA by gavage, at 55–60 days old	LL or PxLL (Px at 1 day old)	Significantly increased incidence of tumours and significantly reduced latency period	Shah et al, 1984
Holtzman rat	20 mg DMBA by gavage, at 55 days old	LL, Px or PxLL (Px at 1-2 days old)	Increased incidence of tumours, and reduced latency, at 180 days old with LL and PxLL (in all cases, $p<0.05$). No effects with Px	Kothari et al, 1982, 1984; Kothari, 1987
Holtzman rat	10 mg DMBA by gavage, at 55 days old	LL, Px or PxLL (Px neonatally)	Increased incidence of tumours at 244 days old (during initiation or promotion)	Subramanian and Kothari, 1991a
Outbred rat	50 mg kg^{-1} NMU iv injection, 3 times weekly from 55 days old	LL	Increased incidence of tumours, and *reduced* latency (in both cases, $p<0.05$)	Anisimov et al, 1994, 1997b
Fischer 344 rat	50 mg kg^{-1} NMU ip injection, at 50 days old	Px	No significant effects	Travlos et al, 2001
Inbred nude rat	Tissue-isolated human MCF-7 breast cancer xenografts, grown in BALB/c nude mice	LL on day 40 after implant	Significant increase in tumour growth rate ($p<0.05$); tumour weight doubled on day 55 ($p<0.05$)	Blask et al, 2003
C3H-A mice	Spontaneous tumours	LL	Reduced latency of tumours.	Jöchle, 1964
HER-2/*neu* transgenic mouse	Spontaneous tumours	LL	Tumours multiplicity increased ($p<0.02$); mean latency *increased* ($p<0.05$)	Baturin et al, 2001

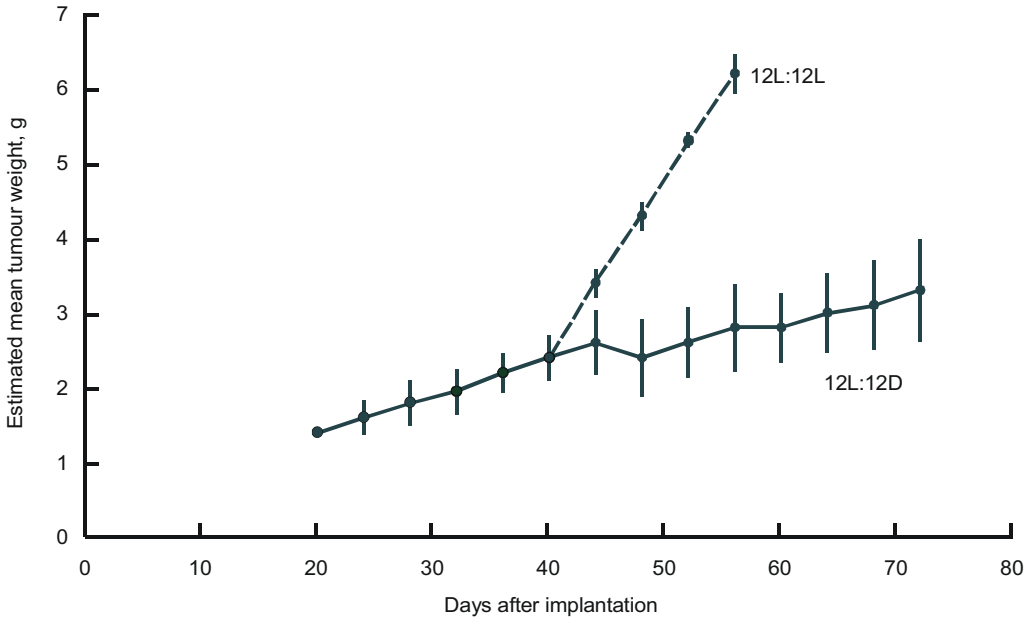

FIGURE 5.1 Effects of diurnal light (12L:12D) or constant light (12L:12L) on tumour growth in ovary intact, non-oestrogenised female nude rats bearing tissue-isolated MCF-7 human breast cancer xenografts. All rats (N=7) were maintained on 12L:12D until day 40 after implant at which time three rats were transferred to 12L:12L while the other four rats remained on 12L:12D. Each time point represents the mean (± SD) estimated tumour weights for both light treatment groups derived from serial measurements of each tumour over the course of the growth period. Tumour growth rates (slopes of the regression lines) between L:L and L:D groups were significantly different ($p < 0.05$). Redrawn from Blask et al, 2003, with permission

TABLE 5.6 Effects of light deprivation (DD) or surgical blinding (XX) on chemically induced mammary tumours in female rats

Model	Carcinogen treatment	Treatment	Result	Reference
Sprague-Dawley rat	20 mg DMBA by gavage, at 55 days old	DD and LL	3% and 87% of DD and 44% and 82% of LL animals developed fibroadenomas and adenocarcinomas respectively	Jull, 1966
Sprague-Dawley rat	3 x 5 mg DMBA by gavage, from 55–69 days old	Superior cervical ganglionectomy (GNx), or blinding plus olfactory bulbectomy (BA)	Generally reduced tumorigenesis: incidence of tumours reduced (GNx at 12 weeks, BA at 15 weeks, $p<0.05$); no. of tumours per rat reduced (GNx, $p<0.05$). No sham-operated controls	Chang et al, 1985
Outbred white rat	50 mg kg^{-1} NMU iv injection, 3 times weekly (DD) or single iv injection (XX) from 55 days old	DD or XX (at 30 days old)	Tumour incidence decreased by both treatments ($p<0.05$)	Anisimov et al, 1994, 1997b

5.2.5 Summary

A consistent pattern of results has emerged from studies investigating the effects of melatonin on mammary tumours in animal models. Treatment with melatonin in the evening, or manipulations that should increase the production of melatonin by the pineal gland, not only lead to an increase in the latency of chemically induced or spontaneous mammary tumours but generally also cause reductions in the incidence of tumours. In addition, melatonin may possess the greatest oncosuppresive potential during the promotional phase of tumorigenesis. Conversely, pinealectomy or exposure to constant light usually engenders the opposite effects, and causes increases in the incidence of tumours and acceleration of the growth of tumours. These responses on mammary carcinogenesis have been observed using a number of different models of breast cancer and using differing doses of melatonin, dosing regimes, and routes of administration. While these differences make detailed comparisons between the studies difficult, it suggests that the responses in animals generalise to some extent across different experimental conditions and are not limited to any specific circumstances. Nevertheless the importance of the time of treatment relative to the endogenous melatonin rhythm (or photoperiod) must be acknowledged. Other factors, including seasonal effects, may also modulate these observed responses, and these may account for some of the discrepancies between studies. While it might be possible to criticise the relevance of those studies where melatonin had been administered to animals at pharmacological doses, this cannot apply to those studies that manipulated the photoperiod. Further, the little evidence that exists suggests that physiological doses of melatonin are more effective at inhibiting tumorigenesis than doses at pharmacological levels.

5.3 Epidemiology: breast cancer risk in relation to melatonin levels

Studies in which melatonin has been measured prospectively in women who subsequently either developed breast cancer or remained disease free are rare. However, in a prospective nested case–control study amongst British women melatonin levels were not strongly associated with risk of breast cancer (Travis et al, 2004). In this study concentrations of 6-sulphatoxymelatonin (aMT6s) were measured by radioimmunoassay in 24 hour urine samples collected from women enrolled into a prospective investigation in which evidently normal women without any evidence of breast cancer were followed up for cancer incidence. Levels in 127 patients diagnosed with breast cancer during follow-up were not significantly different from 353 control subjects (not developing cancer) matched for age, date of recruitment, menopausal status and day of the menstrual cycle for premenopausal women or number of years past the menopause in postmenopausal women (comparing patients who had aMT6s levels in the highest third of measured values with patients who had levels in the lowest third, the odds ratio, OR, for breast cancer = 0.99, with a 95% confidence interval, CI, 0.5–1.70). This study is important in that it is prospective and well conducted. There has been some criticism of the investigation because the sample size did not allow exclusion of a modest association between urinary aMT6s and risk. The investigation also focused on total daily production of melatonin and did not measure either peak nocturnal levels or the timing of the nocturnal peak. The latter would only be pertinent if cancer risk were mediated through circadian patterns rather than absolute levels (Hrushesky and Blask, 2004).

Brief results from a case–control study nested within the US Nurses Health Study II cohort have very recently been presented at a conference (Schernhammer and Hankinson, 2005). As only a one paragraph

abstract is available, we cannot evaluate this study. The study compared urinary aMT6s levels in first morning voided urine between 190 women who subsequently developed breast cancer during four years of follow-up and 376 controls matched on several variables. After adjustment for other breast cancer risk factors, the risk in women with the highest quartile of urinary aMT6s compared with those in the lowest quartile was borderline significantly reduced (RR 0.59, 95% CI 0.34–1.00). We have noted in Chapter 3 the difficulties in interpretation of single urine specimens rather than 24 hour collections.

There are a large number of case–control studies that have compared melatonin levels in patients with breast cancer and in women without the disease. However, it is difficult to interpret findings in patients with breast cancer because the disease itself, treatment and stress/changes in behaviour occurring after diagnosis or treatment may influence melatonin concentrations in the blood. Additionally, the hormone/growth factor milieu may have changed radically from the time of initiation of malignancy to

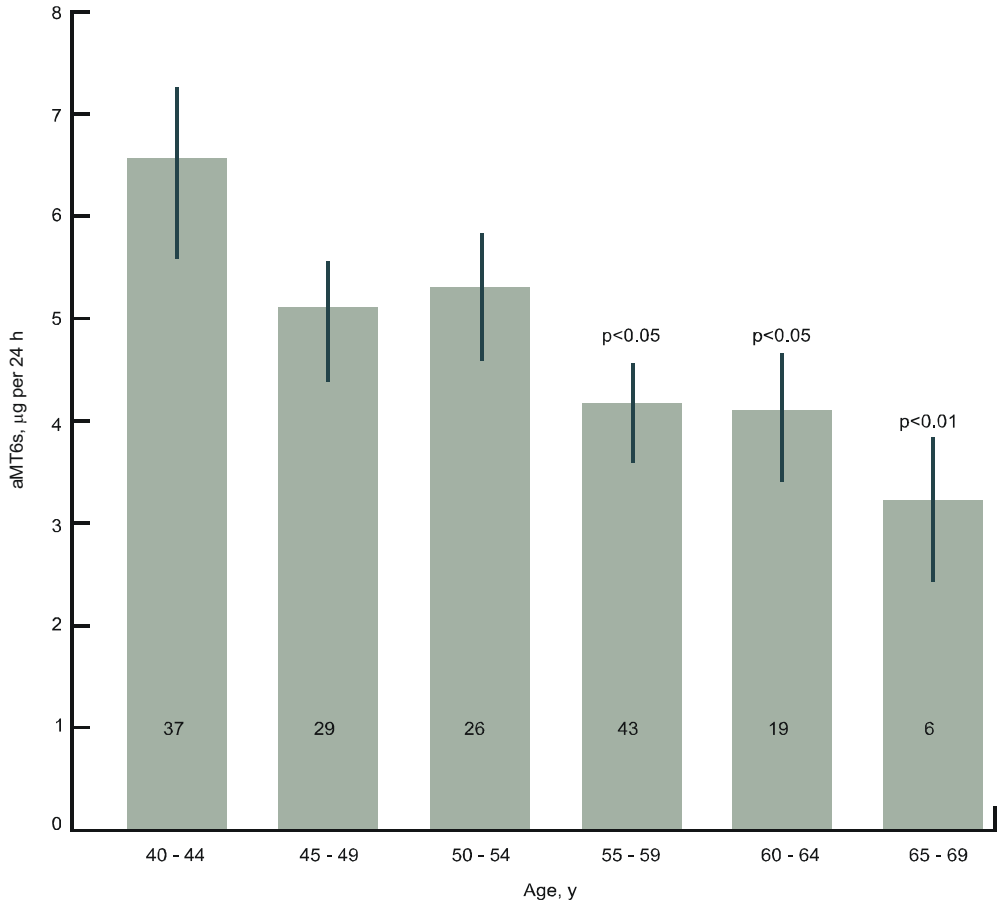

FIGURE 5.2 Age-related decline of 24 hour urinary 6-sulphatoxymelatonin (aMT6s) production (6-hourly samples) in 160 healthy women (results of ANOVA shown, compared to the 40–44 age group, negative correlation between age and aMT6s excretion, −0.269, p<0.001). From Skene et al, 1990, by permission

diagnosis of its presence; hence prospective investigations of cohorts of individuals who are apparently 'normal' but subsequently go on to develop cancer (or not) are much more informative.

These retrospective case–control studies have yielded conflicting results. Several have indicated lower concentrations of melatonin or metabolites of melatonin in the blood and/or urine of patients with breast cancer compared with measurements in breast-cancer-free women (Bartsch et al, 1981, 1989, 1991, 1997). Other case–control studies have found: the daytime serum concentration level of melatonin was higher in patients with breast cancer than in healthy control subjects (Lissoni et al, 1987, 1990); no association between the risk of breast cancer and the mean daytime nadir and night-time peak concentrations (Danforth et al, 1985); or the amounts of aMT6s excreted in urine samples (6-hourly for 24 hours) from women with malignant breast tumours (N=10) were similar to those in women with benign breast disease (N=14) and in a control group of 160 healthy age-matched women (Skene et al, 1990). There was, however, a highly significant decline in aMT6s with age, underlining the importance of age-matched controls (see Figures 5.2 and 5.3).

Additionally, most studies have failed to control for factors such as posture and environmental light that affect melatonin secretion and these may confound associations.

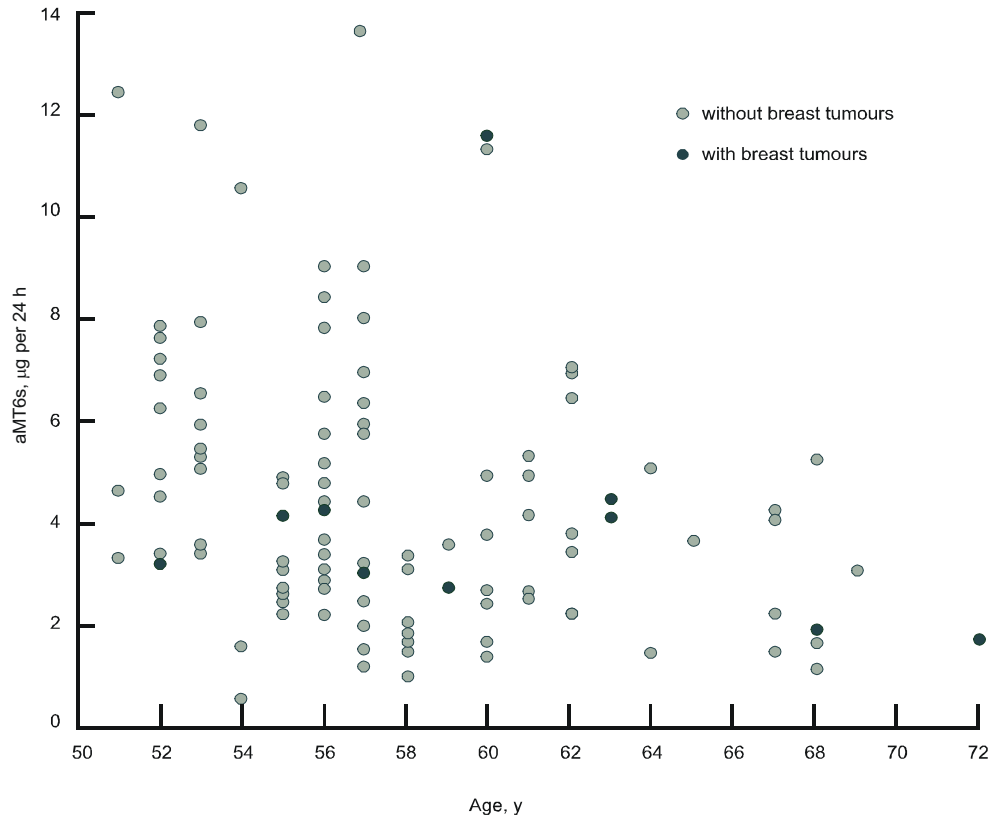

FIGURE 5.3 Individual 24 hour urinary 6-sulphatoxymelatonin (aMT6s) production from 160 healthy women without breast tumours together with that from 10 women who developed malignant breast tumours. Note the very large individual differences. From Skene et al, 1990, by permission

5.4 Epidemiology: breast cancer risk in relation to light exposure

Epidemiological studies of several groups thought to have unusual timing or extent of light exposure – for instance, blind women and shift workers – have been considered as giving evidence that might be relevant to the melatonin hypothesis, although in several instances this was not the objective of the studies. None of the studies has furnished any data directly on melatonin levels, and hence they provide only indirect evidence concerning the melatonin hypothesis. Therefore, consideration of the results in the present context needs to deal with both the extent to which the studies show a relation between shift work, blindness etc and risk of breast cancer, and also whether, if there is such a relation, melatonin is the likely explanation.

5.4.1 Breast cancer risks in blind women

Risk of breast cancer in blind women has been assessed in three cohort studies from the Nordic countries (Feychting et al, 1998; Verkasalo et al, 1999; Kliukiene et al, 2001) and two case–control studies from the USA (Davis, 1991; Hahn, 1991).

In Norway (Kliukiene et al, 2001) the standardised incidence ratio (SIR) for breast cancer in visually impaired women overall was close to unity (1.02) and there was no trend of risk with degree of visual impairment, although for totally blind women there was a non-significantly reduced SIR (0.64, 95% CI 0.21–1.49). The SIR was lower for women who became totally blind over the age of 65 years than for those who became totally blind at younger ages, although neither alone was significant. Based on small numbers, the SIR was lower for ever-married (0.42) than for never-married women (0.97) who were totally blind.

In Sweden (Feychting et al, 1998), too, there was a non-significantly reduced risk of breast cancer in women who were totally blind (0.82, 95% CI 0.47–1.34), but not in those who were severely visually impaired but not blind (1.06, 95% CI 0.92–1.21). In Finland the SIR for visually impaired women overall was not appreciably reduced (0.96, 95% CI 0.80–1.15), but there was a gradient of decreasing cancer risk with increasing visual impairment across five categories (p=0.036) (Verkasalo et al, 1999). The SIR for totally blind women was again non-significantly reduced (0.47, 95% CI 0.01–2.63).

A US case–control study based on diagnoses recorded on hospital discharge records (Hahn, 1991) compared the frequency of a diagnosis of profound visual impairment in both eyes between women who had breast cancer and women who had coronary heart disease or stroke diagnoses. Individuals discharged with diabetes were excluded from the study. Unfortunately the analyses were based on discharge episodes, not individual people, because no record linkage was available. As a consequence, the same individual could count more than once in the analysis. The use of cardiovascular disease patients as controls was unsatisfactory and a potential source of bias – for instance, such patients would be atypical with respect to menopausal status and smoking, which are related to cardiovascular disease risks. The analyses were not stated to have been age adjusted, so confounding by age may have occurred. (Stratified analyses were presented by very broad age groups, but these do not satisfactorily overcome this.) Also there was no information on the duration of blindness, so that onset of blindness in the subjects could even have occurred *after* the breast cancer developed. The odds ratio for breast

cancer in blind women was significantly reduced (0.57, 95% CI 0.35–0.92) and the author stated that there was little evidence that this was confounded by marital status. The effect of blindness was only present in women aged under 65, not older women.

A second US case–control study (Davis, 1991) attempted to replicate the study by Hahn, but with the advantages that record linkage was used to avoid duplicate inclusion of the same individual, and that a second (but not entirely satisfactory) control group, consisting of several cancer diagnoses, was used. The number of subjects was smaller than in Hahn's study, however. Breast cancer risk was not appreciably diminished using the same control diagnoses as Hahn had used (0.95, 95% CI 0.4–2.2), nor using the cancer controls (OR not presented).

5.4.1.1 Discussion

The methodologically superior cohort studies from the Nordic countries each showed no decrease in breast cancer risk for visually impaired women overall and a non-significantly reduced SIR for totally blind women. The larger US case–control study was the only one to show a significantly reduced risk, but it had too many serious methodological flaws to carry weight as evidence. The studies did not (with the exception of that by Hahn, 1991) publish results by age or menopausal status, but the data appear largely to relate to postmenopausal ages. Although the Finnish study showed a gradient of decreasing breast cancer risk with increasing visual impairment, this was not supported by the Norwegian and Swedish data. Overall, therefore, the evidence for an association between complete blindness and decreased risk of breast cancer is appreciable but not conclusive. Furthermore, there are several reasons why even if there is an association, it is far from obvious that it is due to melatonin levels or indeed any endocrinological consequence of blindness, rather than due to confounding by factors that might affect breast cancer risks in these women, or bias.

Numerous confounders might affect comparison of blind with sighted women, and almost none of these has been taken into account in the analyses discussed above. Reproductive factors might well differ between blind and sighted women, and indeed Kliukiene et al (2001) noted that in Norway the proportion of blind women who were nulliparous (89% of those born from 1935 onwards) was far greater than the proportion of women in the general population (39% of women born in the same period). The only, very partial, approach to addressing this problem was sub-analyses by marital status (and hence, by implication, by likelihood of having had any children) in two studies (Hahn, 1991; Kliukiene et al, 2001). Greater prevalence of nulliparity in blind women, however, would be expected to lead to *raised,* not reduced, risks of breast cancer.

Exercise levels could reasonably be lower in blind than sighted women, and Pukkala et al (1999) cite expert opinion that this is so. If this is the case, however, it would again be expected to lead to *increased* rather than decreased risk of breast cancer.

Age at menarche and age at menopause might differ between blind and sighted women, and Hahn (1991) cites evidence of earlier menarche in the blind, which would again bias the study in favour of finding an increased risk of breast cancer in blind women. Hormone replacement therapy and oral contraceptive use might differ between blind and sighted women, as might diet and alcohol intake. Such lifestyle-related factors might differ both because of the effects of blindness on lifestyle and also because blindness might affect employment opportunities, with consequent socio-economic effects. Pukkala et al

(1999) cite expert opinion that blind women do not drink more alcohol than others, but have worse diets (in terms of lack of fruit and high fat intake).

The diagnoses that lead to blindness might be associated with altered breast cancer risk – in particular, diabetes is a frequent cause of blindness and might be related to raised risk of breast cancer (Weiderpass et al, 1997; Wideroff et al, 1997). Also, macular degeneration risk is raised in smokers, and it is notable that the two studies that analysed it (Feychting et al, 1998; Pukkala et al, 1999) each found non-significantly raised standardised mortality ratios (SMRs) for lung cancer in totally blind women. Smoking, it has been suggested, may alter (increase or decrease) the risk of breast cancer (MacMahon et al, 1982; Gammon et al, 1998; Band et al, 2002).

Methodological features of the studies might also have affected risks. For instance, in the Finnish dataset (Pukkala et al, 1999) the blindness register was more complete in low social class geographical areas than higher social class areas, whereas the expected numbers were taken from the whole of Finland; as breast cancer risks tend to be lower in low social class areas, this would have given a bias toward finding a low risk of breast cancer in blind women in the cohort, although the authors state that adjustment for geography did not materially affect the conclusions. In the Swedish study (Feychting et al, 1998), inclusion of self- and relative-reported blindness as well as physician reports may have led to misclassification, but unless it was differential for cases as compared with non-cases, this would be expected to dilute and not exaggerate the effects found.

Finally, as discussed in Chapter 3, the limited available evidence does not suggest that melatonin secretion is increased in blind subjects.

5.4.2 Breast cancer risks in airline cabin staff

Breast cancer risks in female airline cabin staff have been assessed by cohort studies of cancer incidence in the Nordic countries (Pukkala et al, 1995; Lynge, 1996; Haldorsen et al, 2001; Rafnsson et al, 2001) and California (Reynolds et al, 2002), a cohort study of cancer mortality in Germany (Blettner et al, 2002), and a collaborative cohort study of cancer mortality in eight countries (Zeeb et al, 2003)*. Each study found somewhat raised breast cancer risk for airline cabin workers overall, with relative risks in the range from 1.1 to 1.9, although only in the Finnish (Pukkala et al, 1995) and Californian (Reynolds et al, 2002) studies was this significant. Risk in relation to duration (years) of exposed work was assessed in the Finnish, Norwegian, Icelandic, German and international cohorts, with no substantial trend of increasing risks with longer exposure, and in the Californian cohort, where there was a non-significant indication of greater risk after longer service. A nested case–control analysis in the Icelandic cohort, after extension of the follow-up and adjusting for reproductive variables, found risk was borderline significantly related to years of employment for the cohort overall, and was significantly related for employment before 1971, when jet aircraft were introduced on Icelandic international routes (Rafnsson et al, 2003). In the international

* In addition: (a) a small cohort study has been published from Greece with only two breast cancers observed (two expected) (Paridou et al, 2003) and (b) a US retrospective cohort study of female cabin staff, published as a letter, reported a raised risk of breast cancer (Wartenberg and Stapleton, 1998). There was almost no description of the methods in the US study, however, and there was apparently a serious bias from omission of cohort members who died, and perhaps omission of cancers, because of reliance on membership of a retirement association for inclusion of subjects in the study (Wartenberg and Stapleton, 1999).

mortality study (Zeeb et al, 2003), risks were non-significantly greater for women employed only before 1971 than for other female cabin crew.

The Finnish study considered risk by duration after recruitment: all breast cancers occurred at least 15 years after recruitment, but no trend was seen of increasing risks subsequently. In Iceland, all but 2 of 26 cases occurred at least 15 years after recruitment.

In Norway the extent of employment at a young age was considered, but was not related to risk. In California, there was a non-significant indication of greater risk for women who started this work younger, and also for those who flew international rather than domestic routes in 1997.

5.4.2.1 Discussion

The extent to which airline cabin staff are exposed to light at night and to time shifts would depend greatly on the type of airline work undertaken – for instance, short haul flights within Europe would entail little, if any, time shift. No direct information was available in any of the studies, however, to categorise risks in relation to the extent of these variables. (Limited information was available (at one point in time) on domestic versus international routes from the USA (Reynolds et al, 2002), but these overlap considerably in length and time shift.)

Numerous confounders might affect risks of breast cancer in airline cabin staff, and in general adjustment had not been made for these within the published studies. Airline cabin staff would be expected less often to be parous than women in the general population, which would lead to greater breast cancer risk. Pukkala et al (1995) cite data showing that in Finland 20% of female cabin staff aged 35 years and over are nulliparous compared with 12% of the general population, and that cabin staff have fewer children on average than the general population. They note, however, that these differences in reproductive factors would not entirely explain the level of raised breast cancer risk found in their cohort. In Iceland parity was lower and age at birth of first child was later for cabin staff than the general population (Rafnsson et al, 2001). In the Norwegian study (Haldorsen et al, 2001), adjustment was made for parity and age at first birth, and in these analyses there was no trend of risk with duration of cabin employment (the adjusted analyses did not compare cabin staff with the general population); in the Icelandic nested case–control study, however, there was evidence of an effect of duration of employment after such adjustment.

A second potential confounder is ionising radiation exposure due to cosmic radiation at high altitudes. Blettner et al (2002), however, calculate that the doses from such exposures in cabin crew generally would only lead to a relative risk of 1.0 to 1.1 for breast cancer, based on BEIR V calculations of the effect of ionising radiation (BEIR, 1990).

A further factor that has been shown to be greater in airline staff than others is alcohol consumption (Hansen, 2001), which would be expected to lead to raised risk of breast cancer. Other factors that have been noted to differ between airline cabin staff and the general population (Blettner et al, 2002) are passive smoking, pesticide exposure on board, and EMF exposure, although there is not good evidence that any of these factors would affect breast cancer risk. In a more general sense, airline work might be associated with a relatively high social class lifestyle; it has been noted in two studies (Pukkala et al, 1995; Lynge, 1996), however, that the breast cancer SIR for cabin attendants was greater than that for social

class 1 in the same country, giving some evidence that the raised risk was not simply due to socio-economic lifestyle factors.

In summary, there is appreciable evidence that breast cancer risk is raised in female airline cabin attendants, but in part this may well reflect their reproductive status, and beyond this there may be other confounding factors. We know of no data reporting decreased melatonin levels in airline cabin staff, although one study suggests greater variability in melatonin production assessed by aMT6s excretion, and another reports shifts in the timing of the rhythm but does not provide data on amplitude (Roach et al, 2002; Grajewski et al, 2003). Thus the evidence is weak that there is a raised risk specifically due to melatonin-related aspects of airline cabin work.

5.4.3 Shift work and risk of breast cancer

The relation of shift work to risk of breast cancer has been investigated in four published investigations, two of cohort design and two case–control studies. Tynes et al (1996) published a cohort study of 2619 Norwegian women who had been certified to work as radio and telegraph operators during 1920–80*. Of these women, 98% had worked at sea on merchant ships. Follow-up was exceptionally complete, with only 41 women lost plus 103 who had emigrated. The degree to which the women had worked shifts was imputed from histories of the work patterns over time on the merchant ships, which had been collected from seamen's records. It thus appears that work patterns were not known for each individual directly. 'Shift work' was categorised as 0, 1, 2 or 3, but it is unclear what these levels mean or indeed what defined shift work in this study. In nested case–control analyses matched on year of birth, there was no association between shift work and breast cancer risk at ages under 50 years, but a significant (p=0.01) trend in risk for ages older than this. The relative risk of breast cancer for the top category in this analysis (compared with no shift work) was 6.1 (95% CI 1.5–24.2). There was a strong correlation between the extent of shift work and duration of employment, and duration of employment was itself significantly associated with risk of breast cancer. After adjustment for duration of employment, the effect of shift work was present but not significant. Analyses adjusting for fertility were very limited because they were based on few cases.

The other cohort-based analysis derived from the US Nurses Cohort Study, in which ten years of follow-up were available for a cohort of 78,562 US nurses in relation to exposure variables originally ascertained by questionnaire (Schernhammer et al, 2001). The study is large, detailed, and has been highly influential, although follow-up has been somewhat incomplete (90% in 1994 – Hankinson et al, 1998). The questionnaire had included an enquiry about the number of years in total that the nurses had worked on rotating night shifts with at least three nights worked per month, in addition to days or evenings in that month. Risk of breast cancer increased significantly (p=0.02) with number of years working rotating night shifts, defined in this way, with an SIR for the longest category of exposure (≥30 years) compared with never working such shifts of 1.36 (95% CI 1.04–1.78). The trend and the elevation of risk in the highest exposure category were significant in postmenopausal women, and although there were results in the same direction in premenopausal women, these results were not significant. There was evidence that the relation to shift work was specific to oestrogen receptor positive cancers. The analyses were adjusted for

* The authors have recently published a second paper about this cohort (Kliukiene et al, 2003), but this does not contain analyses of shift work.

a large number of potential confounders including age at first birth, parity, oral contraceptive use, body mass index, alcohol consumption, postmenopausal hormone use and menopausal status.

In a case–control study in Seattle, USA, Davis et al (2001) compared risk factors between 813 population-based cases of breast cancer and 793 controls identified by random digit dialling. Information on 'graveyard' shift work, defined as eight hours work between 19.00 and 09.00 hours, was obtained by interview of the cases and controls. The odds ratio for breast cancer in relation to ever-work on the graveyard shift in the ten years before diagnosis was 1.6 (1.0–2.5). There was a significant relationship of risk to number of hours worked per week on the graveyard shift in the ten years before diagnosis, with a greatest relative risk of 2.3 for the top quartile in this analysis compared with no such shift work. The risk in relation to number of years of shift work was borderline significant, depending on the analytical method. The analysis was adjusted for four breast cancer risk factors, but these did not include menopausal status, age at menarche or age at first pregnancy.

The other published case–control study was conducted by record linkage in Denmark (Hansen, 2001). Over 7,000 women diagnosed with breast cancer at ages 30–54 years, who were identified from the Danish Cancer Registry, were compared with matched controls taken at random from the Central Population Register. Employment histories were gained from a national pension scheme with compulsory membership, and occupations were counted as night work if, in a 1976 survey, 60% or more of respondents in this occupation had 'night-time schedules'. Occupations were counted as not night work if less than 40% of respondents to this survey had reported night-time schedules. The odds ratio for breast cancer in women who had worked for at least half a year in an occupation deemed to be night work from the 1976 survey, compared with work in occupations that were deemed not to be night work, was 1.5 (1.2–1.7)*. Adjustment was made for a small number of potentially confounding variables, not including age at menarche, menopausal status or alcohol consumption. Only four occupations met the criterion to be considered night workers in this study, and each of them separately showed a relative risk greater than unity for breast cancer. It was also noted, however, that each had an alcohol consumption in the 1976 survey of more than three times the median for female employees overall. There was said to be a positive trend of risk of breast cancer with number of years of work at night, but it was not stated whether this trend was significant.

5.4.3.1 Discussion

Each of the four published studies on shift workers has found significant results, often with dose or duration–response effects, for breast cancer risk in relation to shift work. It should be noted, however, that the studies differed in the aspects of shift work/night work for which such relations were found, and it is not clear that each was evaluating a comparable variable with respect to effects on melatonin. Furthermore, the Norwegian shipping cohort (Tynes et al, 1996) may have involved time-zone shift as well as shift work, and it is possible that the extent of time-zone shift differed between shift workers and non-shift workers.

The studies also varied in the menopausal status group for which an association was found. Tynes et al (1996) found a relation for postmenopausal but not premenopausal women. Schernhammer et al

* 1.5 (1.3–1.7) in a table.

(2001) found an effect of similar magnitude for both premenopausal and menopausal women (although based on larger numbers, only significant for the latter). The other two studies, which did not analyse their data by menopausal status or age, were in one instance probably largely composed of postmenopausal women (ages 20–74 years – Davis et al, 2001), and in the other probably mainly premenopausal (ages 30–54 years – Hansen, 2001). There is no obvious reason why an effect of shift work via melatonin should apply only to premenopausal, or only to postmenopausal, breast cancer. However, the general epidemiology of breast cancer gives several indications that aetiology may differ between premenopausal and postmenopausal tumours, so a difference for shift work is not inherently implausible.

While the study by Schernhammer et al (2001) adjusted for a large range of potential risk factors, and thus appears unlikely to be confounded by known risk factors for breast cancer, the other three studies had partial if any such adjustment and therefore may have been confounded by other known risks factors for breast cancer. (The Tynes et al (1996) study, however, might be a special circumstance where confounding was minimal because all cohort members were from similar backgrounds and lived in similar conditions.) A notable potential confounder, because it was found to be raised in shift workers in the study by Hansen (2001), is alcohol consumption. Ever-use of oral contraceptives has also been found more common in shift workers as, slightly, has nulliparity (Davis et al, 2001). There might be confounding by exercise levels, both occupationally and outside the workplace. There is also a possibility that genetic factors might influence which individuals find night-shift work manageable, and hence there might be genetic differences between night or shift workers and other workers. For instance, it has been found that 'morning types' have more difficulty in adapting to night work, and are more likely to give it up, than 'evening types', and have different melatonin profiles (Griefahn et al, 2002). Furthermore, given the lack of complete knowledge about breast cancer aetiology, it is possible that there are other, as yet unknown, risk factors for breast cancer that are confounding – because shift work is strongly associated with many aspects of behaviour and lifestyle there is considerable scope for this to occur. As well as differences between shift workers and non-shift-workers within an occupational group, there could also be confounding differences between those occupations in which shift work occurs frequently and those in which it does not.

Methodological issues can be noted in certain of the shift worker studies that lead to some uncertainty about their results, but in general these were well-conducted studies and the results are not obviously the consequence of bias or errors in methodology. The study by Tynes et al (1996) leaves it unclear what shift work means as a variable within the study, and has no individual data on whether subjects were shift workers, but has the strength of an apparently well-conducted and complete cohort study. The Nurses Health Study asked only about rotating shift work, so that non-rotating (permanent) night workers would count as 'unexposed'. The study by Davis et al (2001) has the potential selection and reporting biases of a case–control study – in particular, that random digit dialling might have given bias in the types of individual included in the controls. Finally, the Danish record linkage study (Hansen, 2001) has the deficiency that no information was available for the individuals in the study concerning their shift work status, and this was deduced simply from a separate survey at one point in time. Overall, however, the studies can be regarded as supportive of an association of shift work with risk of breast cancer, although not conclusively so, but they are more unclear on whether an association, if one exists, is causal or a consequence of confounding.

Furthermore, even if shift work is causally associated with breast cancer risk, it would remain to be demonstrated whether effects on melatonin were the pathway for this risk. Although there is evidence that recent shift work is associated with diminished melatonin levels (Schernhammer et al, 2004; and see Chapter 3), this could be because melatonin is affected by shift work, or because women with lower melatonin levels choose to do shift work. It is very likely that work on the night shift with sufficient illumination will lead to suppression of melatonin secretion (see Chapter 3), although this remains to be demonstrated. It may, however, be more appropriate to consider the disruption to the whole circadian system as a result of rapid change of time cues. Animal work suggests that such disruption is associated with vulnerability to cancer progression (Filipski et al, 2004). Furthermore, oestradiol levels have been found raised in long-term shift workers (Schernhammer et al, 2004), giving an alternative potential pathway for causality.

5.4.4 Breast cancer risks in inhabitants of the polar regions

It has been hypothesised that winter darkness in the Arctic might lead to raised cumulative melatonin levels during these months, and hence to altered breast cancer risk (Erren, 2002). Limited data address this point. The Antarctic winter leads to a change in *timing* (delay) but not the amplitude of melatonin secretion and other circadian rhythms (Broadway et al, 1987, Yoneyama et al, 1999). One report describes free-running melatonin and other rhythms without apparent change of amplitude or duration of melatonin in four subjects (Kennaway and Van Dorp, 1991) and the available evidence from Northern Europe (summarised in Chapter 3) indicates an increase in melatonin levels on specific days of the menstrual cycle and in daytime in winter compared with summer (Kauppila et al, 1987; Kivela et al, 1988).

Low incidence of breast cancer has been found in Alaskan Indians (SIR 47) (Lanier et al, 1980) and Alaskan, Canadian and Greenland Eskimos (SIRs about 20–50) (Lanier et al, 1980; Miller and Gaudette, 1996). These data are ecological, however, relating to populations not individuals, and as such are weak evidence. Furthermore, in addition to severe potential for confounding by behavioural factors and exposures, there may be genetic differences between Arctic inhabitants and persons elsewhere that may influence cancer risks.

5.4.5 Light at night and risk of breast cancer

As well as analysing risks in relation to shift work, the case–control study by Davis et al (2001) discussed above examined breast cancer risks in relation to reported degree of light exposure at night. Breast cancer risk increased significantly with reported greater number of nights per week on which the subject was not asleep between 01.00 and 02.00 hours (stated to be the period of peak melatonin levels – we would expect it to be two hours or so later), and number of years of such a sleep pattern, after adjustment for a limited number of potential confounders.

Cases and controls did not differ significantly, however, in the extent of getting up and turning the light on at night, and in the lightness of the bedroom at night. The study analysed several variables, and even for those that were significant the results were not entirely consistent.

5.4.6 Summary

There is appreciable, but not conclusive, evidence for a relation of blindness to decreased risk of breast cancer and of airline cabin work and shift work to raised risk. Confounding is a potential explanation in each instance, but even if there are true associations it is far from clear, and there is no direct evidence, that the associations involve melatonin. None of the studies included data on melatonin levels.

The uncertainty is compounded by a lack of knowledge on which melatonin parameters (eg 24 hour total secretion, peak levels, circadian timing) might be relevant to cancer aetiology. Most epidemiological studies have assumed, if only implicitly, that 24 hour melatonin secretion is associated with their study variables. For blind women and airline cabin staff there is no evidence that cumulative melatonin secretion differs from that in normal women. For shift workers, there is some inconsistent evidence of reduced cumulative melatonin secretion (see Chapter 3) during night work in the short term. It is unclear, however, whether any long-term changes are a cause of taking up and remaining in shift work, or a consequence of it.

A further difficulty is that the exposure variables examined in these studies have often been ascertained in ways likely to lead to considerable misclassification (eg recall of sleep patterns over many years), and they were ascertained for particular time periods (eg the last ten years) that may not be those most relevant to aetiology, if there are any. Thus if there are true effects, they are likely to be greater than those shown by the studies.

Overall, the results on breast cancer risks in relation to blindness, airline cabin work and shift work are intriguing and deserve further study, but do not, at present, provide strong evidence for the melatonin hypothesis.

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6 Effects of EMFs on Breast Cancer

6.1 *In vitro* studies

AGNIR (2001) reviewed the evidence from cellular studies linking EMF exposure with carcinogenesis, which included breast cancer. It was concluded that there was no convincing evidence that EMFs at the levels likely to be encountered in everyday life could be directly genotoxic or bring about the transformation of cells in culture. Exposure to EMFs was therefore unlikely to initiate carcinogenesis and there was no clear evidence that EMFs could affect biological processes. Since completion of the AGNIR report, some *in vitro* studies specifically relevant to breast cancer have been published. These and other studies are reviewed here.

In their study of gene expression in MCF-7 cells, Dees et al (1996) found no field-dependent changes after exposure to 60 Hz fields at 1.2, 100 or 900 μT for exposure periods of 2–30 hours, the time depending on the particular experiment.

Ding et al (2001) also investigated gene expression and found no change in MCF-7 cells exposed to a 60 Hz field at 5 mT for 4, 8 or 24 hours. However, if a 24 hour exposure was given after x-rays (at 12 Gy), there was a significant decrease in apoptosis, but this effect was not seen at 72 hours, suggesting, at most, a transient suppression of x-ray-induced apoptosis.

Loberg et al (2000) investigated several breast cancer lines, including MCF-7 and normal breast epithelial cells. They found no evidence that exposure to a 60 Hz field at 1 mT for 72 hours altered cell viability or growth. This work complemented their earlier study (Loberg et al, 1999) on human breast epithelial cells which reported no effects on early gene expression from exposure to a 60 Hz field at 10–1000 μT for 24 hours.

Supino et al (2001) exposed MCF-7 cells and normal human fibroblasts to a 50 Hz field at 20 or 500 μT for 1 or 4 days. They found no effect on growth or viability of the cells at either field strength or for either duration.

Tofani et al (2001) investigated MCF-7 cells along with two other cell types (human adenocarcinoma and embryonic lung fibroblasts). In both transformed cell lines, but not in the non-transformed lung fibroblasts, there was increased cell death after exposure to magnetic fields strengths greater than 1 mT. The effect was independent of frequency but increased when static and 50 Hz fields were superimposed.

Yoshizawa et al (2002) exposed five cell lines derived from human tumours (including MCF-7) to magnetic fields for 3 days. Both 50 and 60 Hz were studied, as were linearly polarised, circularly polarised and elliptically polarised fields. The field strengths used were 2, 20, 100 and 500 μT rms for the linearly polarised and 500 μT rms for the rotating fields. No effects of any exposure on cell growth were found.

The consensus of the power frequency studies is that exposure to magnetic fields in the range 1.2 μT to 5 mT for durations up to 4 days has no effect on gene expression or cell growth in the breast cancer cell

line MCF-7 (Table 6.1). What has not been answered by these studies is whether there is a co-promotional effect of EMF exposure or whether there is an effect of long-term exposure. In general, these newer studies give no cause for concern; they provide some further reassurance regarding the lack of direct effects of EMF exposure on breast cancer cells.

TABLE 6.1 *In vitro* studies of magnetic field exposure and breast cancer

EMF exposure	Effect	Reference
1.2, 100 or 900 μT, 60 Hz for 2 to 30 h	No change in gene expression in MCF-7 cells	Dees et al, 1996
5 mT, 60 Hz for 4, 8 or 24 h	No change in gene expression in MCF-7 cells	Ding et al, 2001
1 mT, 60 Hz for 72 h	No effect on viability or growth in several breast cancer cell lines including MCF-7 cells	Loberg et al, 2000
20 or 500 μT, 50 Hz for 1 or 4 days	No effect on viability or growth of MCF-7 cells	Supino et al, 2001
>1 mT, frequency independent	Increased cell death in cancer cell lines, including MCF-7 cells	Tofani et al, 2001
2, 20, 100 or 500 μT, 50 or 60 Hz, for 3 days	No effect on cell growth in five tumour cell lines including MCF-7 cells	Yoshizawa et al, 2002

6.2 *In vivo* studies

The induction of mammary tumours in female rats has long been used as a standard assay in the investigation of potential carcinogenesis, often using a chemical carcinogen such as 7,12-dimethyl-benz(a)anthracene (DMBA) as an initiator in the two-stage initiator/promoter model of carcinogenesis. A number of studies have used this model to explore the potential of magnetic fields to cause tumour promotion. However, there are limitations in using carcinogen-induced rodent models. Thus, the mammary glands of rodents differ from the human breast in terms of structure, developmental stages and sensitivity to mutagens and hormones (Short and Drife, 1977; Tsubura et al, 1991; Anderson 1998; Cardiff and Wellings, 1999). In addition, the DMBA model has limitations in terms of being morphologically dissimilar to most breast cancers, being highly vascular and encapsulated, having a particular sensitivity to prolactin, and rarely forming metastases.

6.2.1 EMF effects on mammary tumour growth

In the first study to explore the effects of magnetic fields on the growth of induced mammary tumours, Beniashvili et al (1991) found that while chronic exposure to magnetic fields for 0.5 hour per day caused no effects on rats, exposure for 3 hours per day significantly increased the incidence of chemically induced mammary tumours and decreased their latency. The exposed animals also had a higher total number of large malignant mammary tumours (adenocarcinomas) than the control group. However, the experimental details were only briefly summarised. In particular, a detailed description of the exposure

system was not provided, the strain and background mammary tumour incidence was not given, and the methods of counting tumour incidence and assessing tumour latency were not clearly described.

Subsequently, a series of medium-term studies of magnetic field effects on mammary tumour incidence were carried out by Löscher, Mevissen and colleagues (Löscher et al, 1993, 1994, 1997; Baum et al, 1995; Löscher and Mevissen, 1995; Mevissen et al, 1993a,b, 1996a,b, 1998). These authors attempted to control for possible confounding factors. The evidence from a full histopathological analysis of mammary tissue showed that, under two different exposure conditions (0.3–1.0 μT and 100 μT), there was no statistically significant effect on tumour incidence. These studies are summarised in Table 6.2.

TABLE 6.2 Effects of long-term exposure to power frequency magnetic fields on DMBA-induced mammary tumours in female Sprague-Dawley rats

Exposure	Result	Reference
50 Hz, 0.3–1.0 μT for 13 weeks	No effect on visible or histologically identified tumour incidence	Mevissen et al, 1993a,b
50 Hz, 10 μT for 13 weeks	No effect on incidence of visible tumours at autopsy	Mevissen et al, 1996a
50 Hz, 50 μT for 13 weeks	Increased incidence of visible tumours at autopsy ($p<0.05$)	Mevissen et al, 1996b
50 Hz, 100 μT for 13 weeks	Increased incidence of visible tumours ($p<0.05$), no effect on histologically identified incidence, increased malignancy ($p<0.05$)	Löscher et al, 1993 Baum et al, 1995
50 Hz, 100 μT for 13 weeks	Increased incidence of visible tumours at autopsy ($p<0.05$)	Mevissen et al, 1998
50 Hz, 100 μT for 27 weeks	Increased mammary tumour incidence in exposed group ($p<0.05$)	Thun-Battersby et al, 1999
50/60 Hz, 100 or 500 μT for 13 or 26 weeks	No significant effect. Sham tumour incidence very high in two of three experiments	Anderson et al, 1999 Boorman et al, 1999 NTP, 1999
50 Hz intermittent fields of 250 or 500 μT for 21 weeks	No significant effect	Ekström et al, 1998

In contrast, the incidence of palpable tumours (detected during exposure) and, more particularly, macroscopically visible tumours (detected during post-mortem examination) was significantly increased following exposure to 50 μT (Mevissen et al, 1996b) and to 100 μT (Löscher et al, 1993). Indeed, the percentage increase in the incidence of macroscopically visible tumours compared with their concurrent sham control showed a highly linear dose–response relationship over the flux density range 0.3–1.0 μT up to 100 μT (Löscher and Mevissen, 1995) but not up to 30 mT (Mevissen et al, 1993a). Thus, Löscher and Mevissen (1995) argued that magnetic field exposure did not alter the incidence of (neoplastic) mammary lesions but accelerated tumour growth, so that higher numbers of tumours were macroscopically visible when the rats were sacrificed. In addition, Baum et al (1995) reported that in rats exposed to 100 μT, compared with sham-exposed animals, there was a statistically significant increase in the number with mammary gland adenocarcinomas. However, the total number of malignant tumours in the exposed group was not statistically significantly increased (AGNIR, 2001).

A particular difficulty with the interpretation of these studies is that there is considerable variation between experiments in the tumour incidence in the sham-exposed groups (AGNIR, 2001). For example, in the first 100 μT experiment (Löscher et al, 1993; Baum et al, 1995) there was an unusually low incidence of visible tumours in the sham-exposed group compared with the overall mean sham incidence in the four studies from which the linear dose–response was derived (Löscher and Mevissen, 1995). In addition, contradictory results were seen in replicate studies carried out at 30 mT. These observations suggest the possibility that the authors actually lacked control over a factor or factors that influenced the outcome of their experiments.

In an analysis of these data, Löscher et al (1997) noted the possibility of seasonal influences on mammary tumour incidence. In particular, the low sham incidence of 34% coincided with exposure during autumn and early winter, in contrast to the other experiments. A replicate 100 μT study (Mevissen et al, 1998) carried out at a different time of year to the original study reported that the incidence of macroscopically visible tumours in the sham-exposed group was 62%, almost double the incidence in the earlier study. In addition, significantly more (83%) of the exposed animals had developed macroscopically visible tumours. A re-analysis of all of these data still revealed a statistically significant linear correlation between flux density and increase in tumour incidence (Mevissen et al, 1998). However, this analysis does not account for the variability seen in the sham-exposed data. Thun-Battersby et al (1999) reported a significantly increased incidence of mammary tumours following 10 μT exposure for 27 weeks following initiation by only 10 mg DMBA. This effect was attributed to a field-induced increase in the proliferative activity of the epithelial cells in the mammary tissue (Fedrowitz et al, 2002).

Independent replication of these results is clearly of importance. An attempted repeat and extension of the original study using the same outbred Sprague-Dawley strain of rat has been reported by one laboratory: the NTP study (Anderson et al, 1999; Boorman et al, 1999; NTP, 1999). This found no evidence that magnetic field exposure was associated with an earlier onset or an increased multiplicity of mammary tumours, nor was the mammary tumour incidence significantly increased in the one experiment (of three) in which an increased incidence could have been detected.

There were, however, clear differences in the responsiveness of the rats used in the NTP study to DMBA compared with those used by Löscher and colleagues and other differences in experimental protocol (Anderson et al, 2000); use of 20 mg DMBA in the first 13 week NTP study (Anderson et al, 1999) or 10 mg in the 26 week study (Boorman et al, 1999) resulted in a high incidence of mammary tumours in the sham-exposed groups, diminishing the sensitivity of these experiments. However, the second 13 week study, using only 8 mg DMBA, resulted in an incidence of around 40–50% in the sham group (Anderson et al, 1999). Ekström et al (1998) found no effect on DMBA-induced mammary tumour incidence in the same rat strain following prolonged exposure to intermittent power frequency magnetic fields. There were no statistically significant differences in the number of tumour bearing animals and no differences in the total number of tumours between the different groups. In addition, the rate of tumour appearance was the same in all groups. However, the description of the experimental protocol, statistical analysis and results was somewhat brief.

The issue of differential responsiveness of different stocks of the same strain of animals to magnetic fields was examined by Fedrowitz et al (2004). They reported that one substrain of Sprague-Dawley rat exhibited a significantly increased sensitivity to magnetic fields in the DMBA model compared with

another substrain. Thus genetic background was considered to play a crucial role in effects of magnetic field exposure.

6.2.2 EMF effects on melatonin levels and tumour growth

Other studies by Löscher and colleagues have attempted to correlate field-induced changes in the incidence and growth of DMBA-induced mammary tumours in rats with changes in the nocturnal levels of serum melatonin. In two experiments (Löscher et al, 1994; Mevissen et al, 1996a) it was reported that exposure for three months to a power frequency magnetic field, which had no significant effect on the incidence of DMBA-induced mammary tumours in female rats, was associated with significantly decreased nocturnal melatonin levels. Subsequently, a significantly increased incidence of DMBA-induced mammary tumours in female rats was reported (Mevissen et al, 1996b, 1998); however, nocturnal melatonin levels were not significantly affected. Thus, EMF-induced changes in DMBA-induced mammary tumour incidence were not correlated with changes in nocturnal melatonin levels.

6.2.3 Summary

The effects of magnetic fields on mammary tumours have been investigated in animals using the DMBA model of breast cancer, but the evidence concerning field-dependent effects is equivocal.

Two laboratories have reported an increased incidence of chemically-induced mammary tumours in female rats exposed to power frequency magnetic fields. However, potential problems exist with these studies. The work of one laboratory was inadequately described, making it difficult to judge how well the study had been carried out, and the experiments from the other laboratory suffered from inter-experimental variability, suggesting some sort of experimental confounding.

In contrast, another two laboratories have reported a lack of effect of power frequency magnetic field exposure on chemically induced mammary tumours. One of these laboratories could not replicate the positive effects reported above, and the other found no effects following exposure to intermittent power frequency magnetic fields. However, potential problems also exist with these studies.

It has been suggested that the genetic background of animals may play a pivotal role in sensitivity to magnetic fields in the DMBA model of breast cancer. This may contribute to the observed inconsistency in outcomes between studies.

6.3 Epidemiology

Studies on the relation of EMF exposure to risk of breast cancer have been reviewed previously by AGNIR (2001) and elsewhere (Ahlbom et al, 2001), and therefore will not be considered in detail here. In brief, there have been several studies of breast cancer risks in women in relation to residential proximity to electricity transmission power lines. There have also been studies of breast cancer risk in women in relation to the use of electric blankets, and studies of breast cancer risks in occupations with potential high exposure to magnetic fields; the latter occupations tend to be male dominated, and as a

consequence many of these studies have been of breast cancer in men. There have been some studies showing raised risks for men (based on small numbers) (Tynes and Andersen, 1990; Demers et al, 1991; Matanoski et al, 1991; Feychting et al, 1998; Pollan et al, 2001) or for women mainly at premenopausal ages (Coogan et al, 1996; Feychting et al, 1998; Forssén et al, 2000; Kliukiene et al, 1999, 2003, 2004). A few studies have included data on both residential and occupational EMF exposures in women (Forssén et al, 2000; Kliukiene et al, 2004). These found some evidence for an association with breast cancer in women below 50 years of age. Overall, however, the literature does not provide substantial support for an association (AGNIR 2001; Ahlbom et al, 2001; McElroy et al, 2001; van Wijngaarden et al, 2001; Davis et al, 2002; Kabat et al, 2003; London et al, 2003; Schoenfeld et al, 2003; Forssén et al, 2005).

Few of the studies have included data on dose response, and the results of these have been inconsistent – most have not shown evidence favouring a relation (Brainard et al, 1999). There has generally been a lack of data on confounders, and only indirect indicators of exposure. Thus the studies provide only weak evidence on the possibility of an association.

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7 Conclusions

The present report by the independent Advisory Group on Non-ionising Radiation considers scientific research investigating the possibility that chronic exposure to power frequency electromagnetic fields (EMFs) may increase the risk of breast cancer via a reduction in melatonin secretion from the pineal gland. The report covers cellular, animal and human volunteer studies as well as epidemiological investigations. It also summarises the sources and extent of exposure to EMFs in the home and at work, and reviews the basic physiology of the pineal gland and the secretion of melatonin. The risk of cancer from exposure to extremely low frequency EMFs was the subject of a comprehensive review by the Advisory Group in 2001; this report extends and updates that information relating to breast cancer.

7.1 Sources and exposure assessment

Power frequency EMFs are encountered everywhere electricity is used, in and outside the home and at work.

Maximum electric field strengths of up to several kilovolts per metre can be encountered at ground level under high voltage lines in the UK; however, because electric fields are easily screened, ambient levels inside homes are usually very much lower, typically in the range 0–20 V m^{-1}.

Magnetic fields of up to a few tens of microtesla can occur beneath the largest power lines; however, the levels normally encountered rarely exceed more than a few microtesla. Magnetic fields are not easily screened and the levels inside homes usually arise from the external electricity supply circuits, with contributions from the wiring and electrical appliances inside the home. The background fields in UK homes typically fall in the range from 0.01 to 0.1 μT; average residential exposures above 0.4 μT occur for about 0.5% of the population

The industries most commonly associated with high magnetic field exposure are the electrical utilities, electrically powered transport systems and those where magnetic fields are produced directly as part of an industrial process such as welding or induction heating.

Characterisation of exposure is complex because of the wide range of measures that are necessary to describe fully the fields and the diversity of possible sources. It is not known what aspect of exposure, beyond the field strength values that form the basis for exposure guidelines, might most directly relate to health outcomes in human populations. However, instrumentation has improved and modern meters allow more flexible approaches, which include logging over time and details of exposure characteristics such as frequency, polarisation, spatial orientation and wave shape. It is also important to consider that power frequency magnetic fields vary with time broadly following the variation in use of electrical power.

7.2 The pineal gland and melatonin

The pineal gland is a photoneuroendocrine transducer organ, converting information about daylength (and possibly light intensity) into a hormonal signal: melatonin.

Melatonin is normally secreted during the night (the dark phase) in all species whether nocturnal or diurnal, and the duration of its night-time secretion indicates the length of the night. One definition of 'biological night' is the period of the 24 hour cycle for which melatonin is above baseline (usually daytime) concentrations. In photoperiodic mammals (where seasonal changes in physiology depend upon daylength), the profile of melatonin secretion provides an essential time cue for the organisation of seasonal activity, such as reproduction or coat growth.

In so far as human physiology is dependent on daylength, melatonin is likely to serve as a photoperiodic signal. Its role within the circadian system of mammals including humans appears to be the reinforcement of 'night-time' physiology (for example, changes in core body temperature, or sleep propensity), the modulation of the circadian phase shifting response to light, and in general to serve as an endogenous zeitgeber (time cue). Being highly liposoluble it penetrates all tissues and body fluids and it therefore has the potential to influence peripheral oscillators, including gene expression. Endogenous melatonin probably serves to reinforce 'coupling' and to optimise phase within the circadian system and thus influence the multitude of systems governed by circadian oscillators.

In mammals, melatonin is a 'hand' of the circadian clock rather than part of the clock mechanism. It is extensively used as the 'best' marker of the timing of the circadian clock as either the endogenous hormone or its major metabolite 6-sulphatoxymelatonin (aMT6s). There are very large variations between individuals in the concentrations recorded in plasma or saliva and in the urinary content of aMT6s. Thus for cross-sectional studies of changes in melatonin secretion, large study populations are required. A single sample, be this of morning urine, or morning or night-time plasma or saliva, does not provide sufficient information to define the characteristics of its secretion. There is an age-related decline in secretion (in most studies) and an earlier timing of the rhythm with age. Thus age-matched control populations (or age-adjustment) are required for comparative purposes. Light exposure must be controlled if the acute effects of light are to be avoided. Numerous drugs and other substances influence its secretion.

Light suppresses melatonin synthesis at night and timed exposure to light of suitable intensity and spectral composition shifts the melatonin rhythm and all other circadian rhythms investigated to date. Night-shift work and time-zone change may be associated with lower melatonin production, light at night being one of several possible causal factors.

Exogenous melatonin phase shifts and entrains circadian rhythms. Melatonin has a diverse range of actions implemented through a variety of cellular mechanisms. Some actions are mediated via specific receptors for melatonin, whereas other effects are more general – for example, its ability to scavenge free radicals is related to the hormone's chemical structure.

7.3 Effects of EMFs on melatonin

In vitro studies

The evidence that EMF exposure changes melatonin production in isolated rodent cells or glands is not convincing, despite the exposures being higher than those encountered in the environment.

The idea that EMF exposure can interfere with the cellular action of melatonin is intriguing and there is some independent supporting evidence. However, the effect is fairly small, not robust and has doubtful significance to human health.

In vivo studies

Many, but not all, studies using rats, mice and hamsters indicate that exposure to magnetic fields does not result in a consistent suppression of nocturnal melatonin levels. The best evidence for a field-dependent effect comes from studies with rats exposed to circularly polarised magnetic fields, although these results are not definitive. Too few data exist to make any firm conclusions regarding the effects of magnetic fields on melatonin levels in non-human primates. The results of existing studies using electric fields are unreliable.

Human experimental studies

While a few laboratory-based studies suggest that acute exposure of volunteers may have an effect on the production or timing of the nightly melatonin rise, the majority of studies have not found any field-dependent effects. The lack of a consistent magnetic-field-dependent effect on the human melatonin profile may be due to inappropriate study designs, confounding, or the absence of a real effect. The possibility remains that magnetic fields may affect the circadian clock at a more subtle level, and long-term effects have not been properly evaluated as far as melatonin is concerned.

Epidemiology

In aggregate, the epidemiological studies of the relation of melatonin levels to EMF exposure do not give convincing evidence that EMFs affect the secretion of melatonin in humans. Although most of the published studies have found some significant results, usually in a subset of the data, there has been no consistency in the subgroup for which significant results were found, and indeed in general the significant results have not been re-examined for the same subgroup in subsequent studies. The studies have varied in the measures of EMF exposure and of melatonin secretion that have been used, and the melatonin measures have often been unsatisfactory as markers of 24 hour total excretion. At present, therefore, the studies overall can be seen only as hypothesis-generating rather than giving clear evidence for an association.

7.4 Melatonin and breast cancer

In vitro studies

The oncostatic action of melatonin has been demonstrated mainly in one breast cancer cell line (MCF-7) of which only some sub-clones were responsive to physiological levels of melatonin. In the light of these limited findings there is concern that the oncostatic action may not be applicable to other breast cancer cells or cancers in general.

In vivo studies

Under the appropriate experimental conditions, treatment with exogenous melatonin in the late afternoon or early evening can result in well-defined oncosuppresive effects in animals with mammary tumours. Melatonin appears to have the greatest effect on the promotional phase of tumorigenesis. Conversely, manipulations that decrease endogenous melatonin levels, such as light-at-night, may increase the incidence and growth of tumours (which is consistent with the hypothesis that light-induced suppression of melatonin rhythms may be a risk factor for human breast cancer). A few studies have reported inconsistent or paradoxical results, possibly reflecting that the susceptibility of mammary tissues to carcinogenic insult is a complex response modulated by various environmental and other factors, including the level of endogenous melatonin.

Epidemiology: breast cancer risk in relation to melatonin levels

Two cohort studies have assessed the risk of subsequent breast cancer in women from whom data were available on urinary melatonin metabolite levels. The numbers of breast cancers included in each were not large, and the currently available results are inconclusive. Several case–control studies have compared melatonin levels in patients with breast cancer to those in women without this disease, but because the levels might have been altered by the presence of breast cancer, its treatment, and changes in behaviour as a consequence of these, the results do not allow conclusions on melatonin as a potential causal factor for breast cancer.

Epidemiology: breast cancer risk in relation to light exposure

There is appreciable, but not conclusive, evidence for a relation of blindness to decreased risk of breast cancer, and of airline cabin work and shift work to raised risk. Confounding is a potential explanation in each instance, but even if there are true causal associations it is far from clear, and there is no direct evidence, that the associations involve melatonin. The results currently available are intriguing, but do not provide strong evidence for the melatonin hypothesis.

Melatonin, other hormones and breast cancer

The hypothesis that decreased exposure to melatonin leads to increased levels of reproductive steroid hormones which in turn stimulate either the proliferation of breast cells or the growth of breast cancer remains speculative and is without a substantial body of supportive evidence. Similarly, the case that melatonin may increase breast cancer risk through changing the levels of growth factors or other hormones such as prolactin is not convincing: data relate to small groups of women with established breast cancer. However, there is an interesting and increasing body of evidence to suggest that melatonin may have direct anti-oestrogenic effects on breast cancer cells.

7.5 Effects of EMFs on breast cancer

In vitro studies

An earlier Advisory Group report found no convincing evidence of genotoxicity or transformation in cells due to exposure to EMFs. The studies undertaken since that report provide further evidence of a lack of a direct effect of exposure on breast cancer cells.

In vivo studies

The effect of magnetic fields on mammary tumours has been investigated in rats using the DMBA model of breast cancer. However, potential problems exist with these studies and the evidence concerning field-dependent effects remains equivocal: some studies report effects while others do not. Significant differences in sensitivity to the chemical carcinogen used in these studies have been observed in the same strain of rat, and this may reflect genetic differences between different stocks of these animals. It has been suggested that the genetic background of animals may play a pivotal role in sensitivity to magnetic fields in the DMBA model of breast cancer, and this may have contributed towards the inconsistency in experimental results so far obtained.

Epidemiology

Although some epidemiological studies of breast cancer risk in relation to EMF exposure have had positive findings, the literature overall does not support an association. The studies have generally only collected indirect data on EMF exposures, and have lacked data on confounders. They therefore provide only weak evidence on the possibility of an association.

7.6 Overall summary and conclusions

EMF exposure is ubiquitous in modern life. The hypothesis that chronic exposure to power frequency EMFs may increase the risk of breast cancer via a reduction in melatonin secretion was first made almost 20 years ago, and has led to a great deal of research. To review this hypothesis, the Advisory Group has addressed in this report evidence on three questions: whether EMFs affect melatonin production or action; whether melatonin affects risk of breast cancer; and whether EMFs affect risk of breast cancer.

Investigations using cells, animals and humans have not given consistent or convincing evidence that EMF exposure affects melatonin production or action. However, there are deficiencies in the existing research, which leave open the possibility of an effect.

There is stronger evidence that melatonin can inhibit the growth of cancer cells both *in vitro* and in animals. Data on the possible relation of melatonin levels to risk of subsequent breast cancer in humans are limited and inconclusive. Studies investigating the effect of light exposure (which affects melatonin) on breast cancer risk in humans have given some evidence for an association, but left it unclear whether, if there is an association, it is causal in nature.

There is no consistent evidence, from research using cells, animals and humans, that EMF exposure is a cause of breast cancer, nor has any mechanism for such an association been demonstrated.

Overall, the evidence that melatonin, and the timing and extent of light exposure, may affect breast cancer risk is intriguing but not conclusive. In aggregate, the evidence to date does not support the hypothesis that exposure to power frequency EMFs affects melatonin levels or risk of breast cancer.

8 Research Recommendations

The following recommendations are made for further research into possible relations between exposure to power frequency electromagnetic fields (EMFs), melatonin and the risk of breast cancer. Separate proposals are made regarding the effects of exposure to EMFs on melatonin, the effects of melatonin on breast cancer, and the effects of EMFs on breast cancer. Specific recommendations are made for laboratory investigations using cells, animals and humans, and for epidemiological research.

8.1 Effects of EMFs on melatonin

In vitro studies

There is scope for well-designed experiments on the effects of EMF exposure on isolated pineal glands, especially during a period of elevated melatonin synthesis.

In vivo studies

Generally, additional animal studies exploring the effects of EMFs on melatonin rhythms are not recommended, due to the low probability of finding consistent field-dependent effects. However, in order to confirm and characterise the effects of circularly polarised fields more fully, additional studies with these fields should be undertaken. These studies should aim to replicate the reported effects, but using concurrent sham-exposed animals, and not using historical control groups.

Human experimental studies

The lack of a consistent short-term effect on the major features of human melatonin profile does not eliminate the possibility that magnetic fields may affect the circadian clock at a more subtle level. Clarification of this issue will rely on the analysis of more sensitive indicators of changes in the rhythm-generating system, such as changes in clock gene expression in the suprachiasmatic nucleus or in peripheral oscillatory systems. In addition, although difficult to accomplish, longer-term controlled studies are required. A compromise is possible, which would avoid the difficulties of long-term controlled experiments. This would be the evaluation, in constant routine conditions, and controlling for all relevant factors, of melatonin phase and amplitude, and detailed profiles, in subjects whose magnetic field exposure has been carefully monitored over a designated time period. More importantly, however, other circadian rhythms should be taken into account. Any effect on melatonin amplitude or timing is likely to involve the whole circadian system.

Epidemiology

The existing literature does not suggest that further epidemiological study of the possible relation between EMFs and melatonin should be a priority, especially as human volunteer studies are in general methodologically preferable for investigating this issue. If further epidemiological studies are to be conducted, they need to include better information on circadian factors, especially light exposure, and

need to re-examine apparent positives from the existing literature rather than solely to examine new subgroups or analytical categories.

In addition, careful consideration should be given to the characterisation of the EMF environment and the way in which the exposure assessment is conducted. The ability to define more carefully the relevant exposure of interest seems to be a crucial challenge for future studies. Exposure assessments should include comprehensive breakdowns by periods at home, work and elsewhere, and separate exposures during sleep and work, including shift time. The potential for diurnal and seasonal variability in the exposure data also needs to be taken into account.

8.2 Melatonin and breast cancer

In vitro studies

Melatonin may have direct anti-oestrogenic effects on breast cancer cells. Further research is required to determine the physiological relevance of these results (which have been derived from relatively artificial model systems, such as MCF-7 cells) and whether the observations will also be apparent in primary breast cancers.

In vivo studies

Studies with rodents have indicated that melatonin may possess oncostatic properties. Therefore further animal studies are suggested to establish and characterise these possibilities further. In this respect, the use of transgenic models which over-express genes associated with mammary cancer offer promise since there is no need for additional treatment with a chemical carcinogen, and hence there is the elimination of any variables associated with that process. In addition, application of melatonin in animals' drinking water is considered the least stressful route for administration for the animals, especially compared to repeated, long-term gavage or injection (although the latter may offer the potential for a greater control and consistency of dose). The effects of exposure to constant light are also considered important to explore further, and studies using the tissue-isolated, MCF-7 breast cancer xenograft preparation in nude rats are recommended in particular to confirm and extend the original observation.

Epidemiology: breast cancer risk in relation to melatonin levels

Case–control studies comparing melatonin levels in patients with breast cancer and in women without the disease are difficult to interpret because cancer itself, treatment and stress or behavioural changes associated with diagnosis and treatment may influence hormone concentrations. Furthermore, during the time interval between initiation and appearance of cancer, the hormone/growth factor milieu may have changed markedly. Prospective investigations of cohorts of individuals who are apparently 'normal' but subsequently go on to develop cancer or remain disease-free are much more informative. However, these need both to be well conducted and to take account of potential confounding factors. Large numbers of subjects will be required, as can be seen from previous investigations into the role of oestrogens in risk of breast cancer. The cost and labour involved in establishing such research simply to compare melatonin levels do not seem to be justified. However, this should not preclude the inclusion of melatonin measurements in sub-protocols of studies investigating risk of breast cancer for other primary reasons.

Epidemiology: breast cancer risk in relation to light exposure

The results that suggest the relation of blindness, airline cabin work and shift work to risk of breast cancer, give a strong case for further research to clarify these potentially important associations. Future epidemiological studies need to gain better data on potential confounders and, for airline and shift work, need to gain more detailed information on the occupational factor: ideally these breast cancer studies would include data on melatonin and other hormone levels. It would also be of value to investigate further the relation of blindness, airline cabin work and shift work to hormone levels.

8.3 EMFs and breast cancer

In vitro studies

There is some uncertainty about EMFs having co-promotional effects on breast cancer cells, and of possible long-term effects of exposure; however, both of these questions would be better answered by *in vivo* studies.

In vivo studies

Studies using the DMBA model of breast cancer have suggested that different substrains of animals may exhibit different sensitivity to magnetic fields. Therefore further studies are suggested to establish whether differing sensitivities exist, and if so then genomic studies are recommended to identify the genetic factors involved. It should be relatively straightforward, although time consuming, to use DNA microarrays or similar modern technologies to identify and characterise the genetic basis for these differences between substrains.

Epidemiology

The existing epidemiology leaves uncertainty on whether there is a relation of EMF exposure to risk of breast cancer. If further investigation of this is undertaken, it needs to include better exposure assessment, data on potential confounders, and analysis of dose and duration response relations.

Glossary

Acrophase The time of the peak of a rhythm, usually the peak time of the best-fitting mathematical function approximating the data.

Amplitude The amount of variability due to a given rhythm, usually defined as equal to one-half the peak-to-trough difference.

Biological/subjective day/night Biological/subjective night is often considered as the period of time during which melatonin is secreted in humans. In general, with no light and dark time cues biological/subjective night refers to the time during which night-time phenomena are manifest, eg running activity and peak melatonin secretion in nocturnal rodents, sleep and peak melatonin secretion in humans.

Change in timing A regularly occurring phenomenon which occurs earlier or later than usual

Circadian Occurring or recurring about (Latin – *circa*) once per day (Latin – *dies*). Biological circadian rhythms are internally generated and, in humans, have an intrinsic period (in the absence of time cues) which is usually slightly longer than 24 hours, other terms include circannual: about 1 year, ultradian or pulsatile: with a period shorter than 20 hours.

Entrainment An 'entrained' rhythm is synchronised with the correct phase with respect to a specific time cue, eg melatonin production at night.

Free-run/free-running A rhythm displaying its intrinsic period. Human free-running rhythms are usually slightly longer than 24 hours.

Gavage Direct delivery of solution (eg drug, nutrient) into the stomach using a small tube via the mouth; also known as intragastric (ig) intubation.

Intrinsic period (tau, τ) The period of a biological rhythm manifested in the absence of time cues.

Period The duration of one complete cycle of a rhythmic variation.

Phase A distinct stage in a process of rhythmic change, eg a circadian rhythm, the dark phase or the light phase of the day.

Phase shift An abrupt change in the timing of time cues maintaining circadian synchrony, eg after time zone change or starting night shift work

Photoperiod Strictly the length of the light phase of a particular light–dark cycle, but may be used to describe the whole light–dark cycle.

Photoperiodism The response of an organism to changes in the lengths of the daily periods of light.

Synchronisation The process by which a free-running rhythm is timed to coincide with a specific time cue or cues. A synchronised rhythm having the period of a specific time cue (eg 24 hours) does not necessarily have the 'correct' phase, eg melatonin peak production during the daytime.

Vehicle An inert carrier in which a chemical or other active agent is administered.

Zeitgeber, time cue or synchroniser A periodic stimulus capable of determining the timing, with respect to clock hour or calendar date, of a given endogenous rhythm.

Appendix

Publications of the independent Advisory Group on Non-ionising Radiation

1 Electromagnetic fields and the risk of cancer. Report of an Advisory Group on Non-ionising Radiation. *Doc NRPB,* **3**(1), 1–138 (1992).

2 Electromagnetic fields and the risk of cancer. Summary of the views of the Advisory Group on Non-ionising Radiation on epidemiological studies published since its 1992 report. *Doc NRPB*, **4**(5), 65–9 (1993).

3 Health effects related to the use of visual display units. Report of an Advisory Group on Non-ionising Radiation. *Doc NRPB,* **5**(2), 1–75 (1994).

4 Electromagnetic fields and the risk of cancer. Supplementary report by the Advisory Group on Non-ionising Radiation (12 April 1994). *Doc NRPB,* **5**(2), 77–81 (1994).

5 Health effects from ultraviolet radiation. Report of an Advisory Group on Non-ionising Radiation. *Doc NRPB*, **6**(2), 7–190 (1995).

6 Use of sunbeds and cosmetic tanning. Statement by the Advisory Group on Non-ionising Radiation. *Radiol Prot Bull,* No. 218, 11–15 (1999).

7 The solar eclipse. Statement by the Advisory Group on Non-ionising Radiation. Chilton, NRPB Information Services Leaflet P8/99 (1999).

8 ELF electromagnetic fields and the risk of cancer. Report of an Advisory Group on Non-ionising Radiation. *Doc NRPB,* **12**(1), 1–179 (2001).

9 Possible health effects from terrestrial trunked radio (TETRA). Report of an Advisory Group on Non-ionising Radiation. *Doc NRPB,* **12**(2), 1–86 (2001).

10 ELF electromagnetic fields and neurodegenerative disease. Report of an Advisory Group on Non-ionising Radiation. *Doc NRPB,* **12**(4), 1–24 (2001).

11 Health effects from ultraviolet radiation. Report of an Advisory Group on Non-ionising Radiation. *Doc NRPB*, **13**(1), 1–276 (2002).

12 Health effects from radiofrequency electromagnetic fields. Report of an independent Advisory Group on Non-ionising Radiation. *Doc NRPB*, **14**(2), 1–177.

13 Particle deposition in the vicinity of power lines and possible effects on health. Report of an independent Advisory Group on Non-ionising Radiation and its Ad Hoc Group on Corona Ions. *Doc NRPB*, **15**(1), 1–55 (2004).

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Documents of the Health Protection Agency
Series B: Radiation, Chemical and Environmental Hazards
RCE-1
February 2006
ISBN 0 85951 573 7
£30.00

This report is printed on chlorine-free paper