1. Endnotes

This section consists of notes that describe the background information of some of the aspects of biology and epidemiology central to the understanding of the ELF fields effect science.

Note 1: The role of calcium In biological systems

The flow of calcium ions (Ca^{+2}) across the cell membrane is an important means of transmitting signals from the exterior to the interior of the cell in all organisms. "Pumps" in the membrane maintain a 10,000-fold difference between extracellular (about 10-3 molar) and intracellular (about 10^{-7} molar) free calcium ion concentrations. Most of the intracellular calcium is normally bound to other molecules or otherwise sequestered. Calcium is also present bound to the membranes to be released in the event of an appropriate triggering signal. Component molecules of the membrane (primarily those called called glycoproteins) have charged ends that attract and hold a layer of ions, principally calcium and hydrogen ions competing for the available sites. This layer of protein ends and ions (an ionic bilayer) is a major element that modulates the action of drugs and other chemicals.

Note *2:* Hormones

Hormones which are produced by glands of the endocrine system play a major role in regulating the numerous functions of the body. The four general functions of the endocrine system are: maintenance of the intricately balanced biochemical and conditions in the body; response to everyday demands of physiologic and psychologic stress; integration of growth and development in the appropriate time sequence; and processes controlling sexual reproduction and fetal and newborn nourishment.

For some functions a multiplicity of hormones have to act in concert. For example, the sequence of hunger, and appetite production are controlled by a system of six hormones acting in sequence arising from different glands in the body. On the other hand, there are some hormones such **as** insulin, parathyroid hormone and epinephrine each of which performs multiple actions.

Because of their complexity, it is not simple to extrapolate observations of hormonal changes induced by ELF fields in in vitro cellular experiments to conclusions about end effects in the whole body. The effect of a hormone in the body depends on its interaction with **a** receptor which in turn depends on the circulating level of hormone present. There are basal and stimulated levels of secretion of hormones and the levels are also regulated by various feedback mechanisms. In vitro observation cannot simulate all the above processes that act together in the body.

The three major classes of hormones are; steroid, peptide and aminoacid. Cortisol which regulates appetite, and is an example of a steroid hormone while PTH and ACTH are peptide hormones. Catecholamines are aminoacid hormones. The chemical nature and structure of the hormone determines how the hormone operates biochemically. All hormones interact with receptors on the cell membrane, and the combination initiates a transmembrane message which for most hormones is an activation of adenylate cyclase, an enzyme that is bound to the inner surface of the cell membrane. Adenylate cyclase

then "sends" cyclic **AMP** (cAMP) which can widely circulate through the cell and is "the second messenger" **that activates and regulates other** enzymes through an intermediate enzyme called protein kinase. One of these, called protein kinase C, has been associated with the mechanism for cancer promotion. cAMP and calcium comprise the primary messenger system, both being released from stores on the cell membrane in response to messages.

Hormones that appear often in description of ELF effects are: the catecholamines, the serotonin 5-HT)- melatonin sequence, parathyroid hormone (PTH), ACTH, and growth factors (GF). These are briefly described below.

Catecholamines and the serotonin group belong to a class of hormones known as autacoids, or autopharmacologic agents and also as local hormones.

Catecholamines: Most prominent among the aminoacid hormones synthesized in the brain and the nervous system are the catecholamines dopamine, norepinephine and epinephine. As they are released from nerve endings during physiologic stimulation, they are called neurotransmitters. They have several functions and affect muscle, heart, liver, spleen, lung and brain physiology. They control blood pressure, heartbeats, some forms of headache, the basic metabolic rate, some psychic changes and several other rates. Deficient or excessive secretion of catecholamines or alterations of their action cause major physiological and psychological problems, Increased metabolism of catecholamines are also associated with neuroblastoma, a tumor originating in the neural crest, often during fetal development.

Serotonin, 5-hydroxytryptamine (5-HT) and Melatonin

Serotonin, first identified as a vasoconstrictor material, that appears in the serum when blood clots is the common name for the hormone whose active **part** is the complex 5-HT or 5-hydroxytryptamine: Serotonin is widely present in animal and plant tissue and has a wide array of regulatory action. 5-HT is synthesized **all** over the body, from tryptophan which is present in many foods, notably milk. Receptors for serotonin are present in cell membranes **all** over the body, Serotonin stimulates or inhibits many of the muscles and nerves, depending on the amount and the phase of the organ in its function. It can stimulate or depress heartbeat, contract blood vessels and change blood pressure. Serotonin prevents clotting, and provides reflexes such as coughing or hyperventilation.

In humans, serotonin also serves as a chemical transmitter in the brain. Serotonin and its product melatonin influences sleep, perception of pain, psychological depression and social behavior.

Melatonin is also secreted by the pineal gland and is described in the endnote on circadian rhythms.

Parathyroid hormone (PTH)

PTH is a major hormone in the control of mineral (calcium, magnesium and phosphorus) metabolism. PTH and a group of other hormones, together with extracellular calcium, synergize and feedback on each other to regulate processes such as the growth and availability of calcium for conveying messages into the cells. Produced in the thyroid gland, PTH secretion in the cells is regulated primarily

by calcium and cAMP. Calcium concentration in the extracellular fluid is the primary variable that is controlled by PTH, which this determines the rate of resorption of Calcium in bones, kidney and intestine tissue.}

Adenocorticotropin (ACTH)

ACTH is a hormone produced by the pituitary gland and is responsible for stimulating the secretion of the steroid hormone cortisol and related steroids by the adrenal gland. Cortisol levels in turn govern ACTH levels in the blood, and further ACTH production. The ACTH level is a function of the presence of cortisol or analogous steroids such as cortisone and aldosterone, the circadian rhythm and stress. The circadian variation of ACTH regulates that of cortisol which governs hunger and appetite, control of hypertension, and other functions; cortisone, which governs inflammatory response; and corticosterone which governs stress response, sexual development and other function. The “biologic clock” and stress work through the central nervous system to stimulate ACTH. The stimulation of cortisol by ACTH is mediated by cyclic AMP.

Note 3: Stages In the formation of a tumor or cancer

[Cole 87, Guddon 81, Berenblum 75, Trosko 83, Trosko 85]

For convenience in understanding the origins and development of a tumor or cancer, oncogenesis or carcinogenesis[1] is usually thought of in terms of three stages : initiation, latent period and clinical manifestation.

Initiation is generally assumed to be a clear period in time when an agent damages the genetic material of a cell to produce changes that starts the tumor or cancer by changing the codes for production of essential biomolecules, or by inducing the production of molecules normally foreign to the organism at that stage of its life. As shown in Figure 1-1, reproduced from an article by Swenberg [Rice 79], this damage may be repaired depending on the repair mechanisms, the extent of damage and genetic susceptibility for repair.

During the latent period, the altered cells (also **called** “transformed’* or “neoplastic” cells) continue to grow and proliferate, to finally manifest themselves into detectable sizes as a ‘frank” or clinical tumor. Both external agents and epigenetic (or, extranuclear) events **maybe** important at this stage. An external agent may be a promoter, that is, it might enhance the effect of an initiator to further promote the transformation which may not otherwise not have been completed or for which the dose of the initiator was not enough.

The idea that tumor cancer development is **a** multistage process came from the pioneering experiments of Rous and his colleagues [Friedewald 44]. They found that virus-induced skin tumors that regressed after a period of time could be made to grow again if the skin is physically stressed or by application of irritants such as croton oil. Rous et al then concluded that the tumor cells could exist in a latent state and could be “promoted” by appropriate agents. They named these stages initiation and promotion. Berenblum then did an elegant series of experiments on mouse skin tumors showing clearly

[1] **"onco"** is a prefix denoting a benign or malignant tumor, while **"carcino"** denotes a cancer which may be a malignant tumor

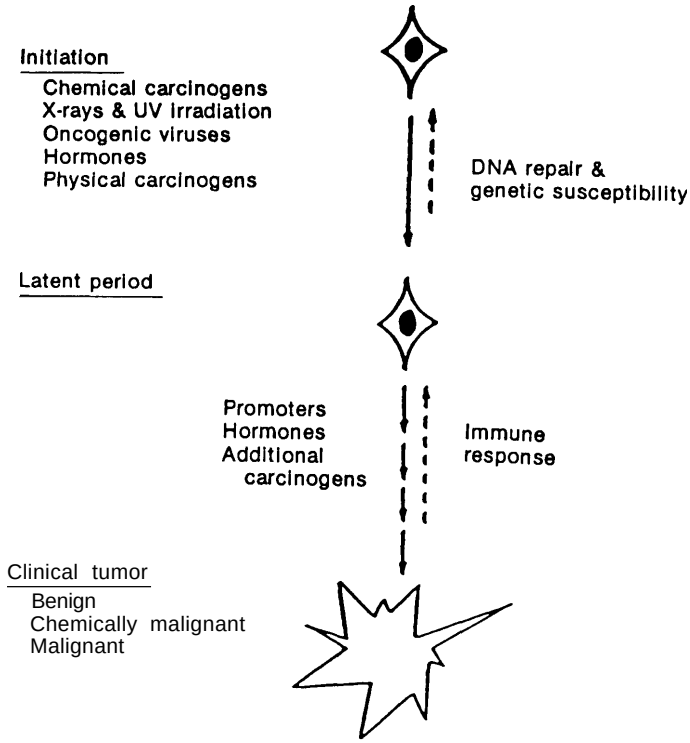

Figure 1: Stages in the development of a tumor or cancer [Rice 79]

that there were substances that could not initiate cancer but could promote its growth [Berenblum **41]. Although these experiments were performed almost forty years ago, and** vigorous research has continued since, we still do not have a clear delineation of the processes and mechanisms involved in the stages of oncogenesis or carcinogenesis.

Initiation and growth alone are enough to induce cancer. Promotion by a separate or the same agent is not necessary. A single appropriately high dose of certain carcinogens have been shown to induce cancer in animals.

Initiation and promotion has now been widely studied in chemical carcinogenesis after the identification of chemicals such as urethane which are primarily initiators and components of croton oil that have only **a** promoting activity. The **classic** promoter used as a standard because of its potency is a chemical isolated from croton oil, 12-o-tetradecanoyl phorbol-13-acetate, or, TPA.

External agents may also affect the growth of the cancer by enhancing cell growth and proliferation processes or by having the properties to enhance those of a transformed cell in preference to a normal cell. Epigenetic events **that maybe** important during the latent period in increasing the rate of growth are: the hormone and immunologic status of the body; the stage of tissue differentiation especially if the affected body is that of a fetus or neonate; and metabolic competence in general, or in particular with respect to certain agents that are promoters or growth enhancers.

Trosko and Chang have developed models of some aspects of multistage carcinogenesis and reviewed the subject in detail [Trosko 83, Trosko 85]. They have hypothesized that in some cases at least, promotion occurs when **a** cell escapes control by the normalizing regulating molecular signals from its communicating neighbors.

circadian regulation was a powerful selection agent in evolution. Submammalian vertebrates exhibit endogenous circadian rhythmicity. That is, a submammalian pineal separated from the organism, continues its cycle of function given the proper LD cycle. This is because in birds and other lower vertebrates, the pineal itself contains photoreceptive cells, a function that has been separated to the SCN in mammals.

The pineal contains a multitude of critical biochemical. Among the most studied of these are:

1. norepinephrine, serotonin and dopamine(neurotransmitters that transmit excitatory and inhibitory signals in the central and peripheral nervous system)
2. histamine (a chemical mediator found in connective tissues throughout the body that causes blood vessels to dilate facilitating the access of serum proteins, antibodies and white blood cells to the location as needed to respond to injury and infection),
3. melatonin (a hormone that regulates sexual function and development, among other things) which has been most widely studied, and other related indolamines.
4. the inhibitory neurotransmitter gamma amino butyric acid (GABA). In addition, there are a number of other pineal hormones.

It is evident from the above that an environmental agent that affects the pineal could have important biological effects. The most striking aspect of indolamine synthesis in the pineal is the marked circadian rhythms in the concentrations and therefore in the modulations of the synthetic processes. Light is the single most significant environmental stimulus guiding these. Pinealectomy experiments in fish, amphibians, reptiles and birds have established several interesting rules about the circadian rhythm-setting role of the pineal.

Pineal-ScN interaction in mammals is distinctly different from these other vertebrates and work on pinealectomy on mammals has provided only sparse and inconclusive data, The pineal in the mammal appears to be a feedback modulator of the circadian activity. Disorder of the pineal gland has been associated with a range of symptomatic disorders such **as** psychological depression and sexual dysfunction [Maurizi 84]. Low levels of melatonin have been found in people with certain types of cancer and melatonin has been administered to cancer patients to inhibit cancer growth.

Note 5: **Epidemiological Studies [Savitz 87, Rothman 86, Aldrich 85]**

Epidemiological studies have the notable advantage that they are studies of human populations and are, therefore their results, if clear-cut, should be directly applicable in public health decision-making without the animal to human extrapolation problems faced by experimental studies. However, because there are ethical and practical limitations on the extent to which epidemiological studies can be designed as experiments, their results are often inconclusive, the main problems being confounding effects, uncertainties about exposures and concurrent behaviors and exposures of the subjects, recall biases and, in the case of weak agents with non-specific effects, which maybe the case for ELF field exposure, that of getting enough numbers in the study to provide statistical significance or" power" to the study.

Epidemiology has really begun to emerge as a sound methodology only in the last three decades, with several epidemiological studies by Health departments in the U.S. such as the Framingham Heart Study initiated in 1949. The Heart study has been instrumental in providing much of our knowledge about cardiovascular disease and health habits and still continues to yield results., after 35 years. Other

Tumor promotion itself is likely to be a multistep process including some or all of the following :

1. Inhibition of the intercellular communication which normally restricts cell proliferation beyond the healthy regime for the organ,
2. Changing or blocking of normal regulatory processes within the cell, or,
3. Affecting gene expression (i.e., the manufacture of the appropriate proteins) through enzyme induction or inhibition. This may lead to the production of too much of the normally occurring proteins or that of altered proteins.

From the above, it is clear that the most obvious limitation of an in vitro result in studying mechanisms of carcinogenesis is the fact that carcinogenesis is an in vivo process that is a combination of physiologic factors, immune responses, pharmacokinetics, and metabolic effects.

Two facts about the cellular level experiments involving field exposure are worth noting. Ornithine decarboxylase production is increasingly being used as a biochemical marker of some types of cancers. [] It has also been noted that membrane modulators which control or inhibit calcium efflux or cause an internal redistribution of calcium will act as stimulants of promotion or as promoters of cancer. (Trosko85b)

Note 4: Circadian Systems and the Pineal Gland [Relter 84]

While all the biology of the circadian and other timing systems is not well understood as yet, research in the last two decades have brought considerable understanding of some of the elements of the system. It is believed that the system has several elements that work in some logical sequence. Prime among these are : transducers that sense cues of time; pacemakers which keep time; and, mediators that are neural and biochemical pathways which transfer appropriate information to the respective systems. Under general constant conditions, circadian rhythms are “free-running”, that is, they show periods close to 24 hours. Environmental stimuli can be used to re-synchronize or “entrain” these cycles to other periods within some limited range of periods. A stimulus that is usually used in experiments is the “light-dark” (LD) cycle, which can be used to entrain both periods and phases of biological activity

The specialized organs that have a major role in the organization of circadian rhythms in mammals are the suprachiasmatic nucleus (SCN) and the pineal gland . The SCN is a nucleus located in the hypothalamus (the region of the brain that is the main regulator of the endocrine system), close to the base of the optic nerve . The SCN receives direct input from the retina that conveys information about light and dark and continues receiving this information, independent of conscious light-dark perception. The pineal, so named for its pine cone-like structure, is a tiny gland, weighing only about 0.15 gram in the human, at the base of the brain. The pineal receives its regulatory signals directly from the SCN via a neural pathway. In mammals, when there is no light input, pineal rhythms still persist; but these are no longer entrained to the external LD cycle but rather to a free-running rhythm set by the SCN which is believed to be the only internal clock that drives the pineal in the mammal. Any signal that cues the SCN and therefore the pineal are termed “zeitgebers” or, time givers. The LD cycle is the most important natural zeitgeber for the pineal.

The pineal is one of the most important regulatory organs in the body, one that has existed through millenia of evolution. The pineal gland acts as a neurochemical transducer, that is, it converts information about the level of environmental lighting into neurochemical activity. Thus it plays an important role in controlling hormone systems, although its full function is not yet known. It is believed that the protection of certain biochemical processes from lethal solar ultraviolet radiation by confining them to the night via

triumphs of epidemiology have been in establishing the connection between smoking and health, low-level ionizing radiation and leukemia, and maternal ingestion of DES (diethylstilbestrol) and cancer in female offspring.

Because it is a science in its infancy, epidemiology still faces some disagreement and confusion about definition of measures. Considerable time and effort are needed to make the basic measurements in epidemiology which track the disease incidence. Getting data from people is often difficult, very time- and resource-intensive, and hampered by problems of non-cooperation, population mobility and privacy.

Rothman points out that the theoretical foundation of epidemiology lies in statistics but that some of the methods that have become incorporated into epidemiology are not theoretically sound in biological applications while they make sense in other applications in statistics. Statistical hypothesis testing is such a method that was evolved for agricultural and quality control experiments with very uniform and discrete results . The criteria for significance in disease definition are often arbitrary and the concept of significance not always meaningful.

Cause and Effect in Epidemiology

Many of the public health effects result from interaction of several factors in a biological system so that the “single cause’’-to-"observed effect” often used in the generalization of epidemiological studies to public health protection measures is too simplistic. “Sufficient cause” for **a** public health effect often consists of many components acting in concert or in a definite or random sequence. Strength of each cause, interaction among causes and proportion of disease due to specific causes all combine to produce an epidemiological effect. Often the biologic factors underlying any or all of these are unknown and this makes interpretation of associations difficult.

Because of the lack of knowledge of biological mechanisms and individual variation, when we are faced with a decision of making a public health decision about an agent we assign an average value of risk of a particular health effect from the agent to everyone in the exposed population while in reality there are some individuals who have no risk at all of contracting that particular effect from that agent. For example, smoking does not necessarily cause lung cancer in everyone who smokes. What we know is we face enough of a whole set of causes of lung cancer that removing smoking, the one known component cause makes sense from a public health point of view.

Types of Epidemiologic Studies

Epidemiologic studies may be designed experiments as in the case of chemical, field and community intervention trials; or, as is more often the case, they may be studies of selected populations who have been exposed to the agent of interest. Obviously, all studies involving exposures to environmental agents with potential or unknown harmful effects have to be of the latter type.

A major difference between follow-up and case-control studies is that the subjects are chosen according to some exposure of the agent of interest in follow-up studies and according to **a disease or** end point in case control studies. As a time interval is allowed to elapse as part of the study in the

follow-up case, these are called longitudinal studies. A case-control study is essentially a cross-sectional study, looking at a sample of the cross-section of diseased population at a particular point in time. Obviously, etiology of a disease is better studied with the longitudinal follow-up studies.

Measures of Effect in Epidemiology

Epidemiology may measure effects on the absolute level in terms of incidence rate , cumulative incidence or prevalence; and on a relative scale by several measures described below. When one potential cause is to be studied, a useful measure to work at is the attributable proportion for which the disease incidence can be said to have arisen from exposure to the agent as sufficient cause. Strictly speaking, attributable risk should be calculated only if the causation has been established.

The ELF field studies have all measured the possible effect in terms of the relative measures, Relative Risk (RR), Odds Ratio (OR), Proportional Mortality Ratio (PM R), and Standardized Mortality Ratio (SMR). A couple of early studies calculated Proportional Incidence Ratio (PIR) or Proportional Registration Ratio (PRR).

Relative measures essentially take the ratio of the incidence rate or difference in incidence rate in exposed population to ratio among unexposed or less exposed. If I_0 and I_1 are respectively the ratio of incidence among unexposed (or less exposed) and among exposed, the relative risk is simply the ratio I_1 / 1_0. The relative risk is also referred to as the relative incidence, or rate ratio.

In case-control studies, cases are those who became ill during a time period and the controls area fraction of exposed and unexposed (all undiseased) persons. If the sampling for controls is random and an equal fraction of exposed and unexposed persons have been sampled, the relative risk is the exposure odds ratio, that is, the exposure odds among cases to exposure odds among controls, usually called the Odds Ratio.

When death certificate or mortality statistics are used, the proportional mortality ratio or PMR is taken as a measure of the effect of an exposure. PMR is the ratio of the exposed subjects who died of a specific cause to the unexposed subjects who died of the same cause . PMR values have to be interpreted carefully for a specified cause because if the subjects are exposed to a spectrum of causes and other relevant agents, a high PMR might reflect the preventive effect of an agent in the unexposed population rather than the causative effect of the agent on the exposed population.

Instead of mortality statistics, if registration or other incidence data are available, Proportional Incidence ratio (PIR) or Proportional Registration Ratio (PRR) can be calculated.