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Bruce Rapley
37 Ferguson St. Palmerston North

THE DESIGN AND TESTING OF A MAGNETIC BIOSTIMULATOR

By

Bruce Ian Rapley.

A thesis presented in partial fulfilment of
the requirements for the degree of
Master of Philosophy.

Massey University
Palmerston North

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ABSTRACT

A commercial pulsed electromagnetic field therapy unit, typical of those used in current medical practice, is evaluated. The principle of operation is determined, and the magnetic field output quantified. The unit is trialed on a human subject to verify the manufacturer's claims regarding the physiological responses of both vasoconstriction and vasodilation. The results to not confirm the manufacturer's claims.

A programmable magnetic biostimulator is designed and tested. This approach is unique, featuring a transconductance amplifier to drive the stimulation coil. Significant increases in performance are obtained in comparison to standard voltage feedback amplifiers, particularly with rapid rise-time waveforms, such as square waves.

The magnetic biostimulator is trialed in a clinical setting on four experimental subjects to determine the claimed vasodilation response of pulsed magnetic fields. Two subjects are normal, healthy individuals, and two have been diagnosed as having Primary Raynaud's Disease, a disorder of peripheral circulation. Various responses are recorded and discussed in the text.

The magnetic biostimulator is trialed in a laboratory situation in order to determine the effect of magnetic fields on the cytogenetics of the broad bean, *Vicia faba*. No significant differences in the number of chromosome or chromatid breaks are recorded between the control and test groups. Significant differences at the 95% probability level between the control and test groups are recorded, however, for various stages of the cell cycle. This finding may imply that various forms of exogenous magnetic fields may affect the cellular mechanisms involved in mitosis.

The clinical and laboratory trials verify the effectiveness and practicality of the chosen design. In reviewing the performance of the magnetic biostimulator, suggestions for future implementations are discussed.

To Anne-Marie

Without her constant help, support and love,

the completion of this thesis would

never have been possible.

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~

"Men are deplorably ignorant with respect to natural things, and modern philosophers,as though dreaming in the darkness, must be aroused and taught the uses of things, the dealing with things; they must be made to quit the sort of learning that comes only from books, and that rests on vain arguments from probability and upon conjectures."

William Gilbert, "On the Loadstone".

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1

INTRODUCTION

CONTENTS

1.1 STATEMENT OF OBJECTIVES

1 To design and construct a multi-function biomagnetic stimulator which may be used to determine the effects of applied electromagnetic fields on various living systems.

2 To clinically trial the magnetic biostimulator by attempting to evaluate the potential use of pulsed electromagnetic fields as a vasodilator.

3 To trial the magnetic biostimulator in a laboratory setting to evaluate the cytogenetic effects of alternating magnetic fields on the Broad Bean: *Vicia faba.*

4 To evaluate the effectiveness of the magnetic biostimulator as a potential research and clinical tool.

1.2 HISTORICAL PERSPECTIVE

It has long been thought that magnetic fields have curative properties. The ancient Greeks are known to have mined magnetite (a magnetic form of iron oxide, Fe_3O_4) in the province of Magnesia in Asia Minor (now modern Turkey) as far back as 1000 B.C. The first Greek scientist, Thales of Miletus (624 - 565 B.C.), believed that loadstone (magnetite) was 'alive', attracting metal by 'animating' (exciting) it.

William Gilbert, 1545 - 1603, physician to Queen Elizabeth I, proclaimed that the Earth was a giant magnet. He compared magnetic forces with life forces.

Anton Mesmer, 1733 - 1815, an Austrian physician developed his theory of Animal Magnetism around 1770 based on the Greek notion that magnets were alive. Mesmer was the first researcher in modern times to develop a comprehensive model of the interaction of magnetic fields and living organisms. He postulated that a subtle, imperceptible 'fluid' flowed through the human body, the smooth uninterrupted flow of which constitutes health - blockage causing disease. His 'magnetic' therapy sessions became famous throughout Europe and were highly sought after.

Along with the discovery that rubbing magnets over the body often proved to be beneficial, Mesmer also invented 'group therapy' and discovered the placebo effect.

Magnetic therapy fell into disrepute following a vicious attack on Mesmer by the French Royal Academy of Sciences who hired such eminent people as Benjamin Franklin (then the American ambassador to France) and Antoine Lavoisier (considered to be the Father of Modern Chemistry). This austere commission declared Mesmer to be a quack, creating a cloud which persists to this day.

In the 20th century such scientists as Nicola Tesla and Lakhovsky persisted with experiments investigating the effects of magnetic fields on plants and animals, including humans. In particular Lakhovsky had great success in treating people with cancer, recording a number of spectacular 'cures' (Brown$_{13}$). Tesla attributed his long life to his practice of taking daily 'electrical baths' inside one of his giant solenoids (Cheney$_{14}$). However magnetic fields were still not generally accepted by the medical fraternity as legitimate forms of therapy due to the lack of a known scientific mechanism of action in the human body.

A mechanism began to emerge in 1957 with the publication of a paper by Fukuda$_1$, which showed that hydrated, living bone exhibited the piezo electric effect. This paper formed the basis of a theory which eventually lead to the clinical application of magnetic fields to treat bone non-union.

In the 1970's Robert O. Becker, then an orthopaedic surgeon at the Veteran's Administration Hospital in Syracuse, New York, developed the first modern treatment involving magnetic fields. This treatment is used today to treat recalcitrant fractures, (fractures which will not heal), and is to be found in most modern hospitals.

Research into further medical uses for magnetic field therapy is expanding exponentially world-wide. Considerable hope is held for new therapies which will treat soft tissue disorders as well as organic diseases such as cancer and A.I.D.S.

Parallel to the development of medical treatments using magnetic fields is the equally important research into the possible harmful effects of similar fields. Numerous publications relating potential dangers have literally flooded the literature (Carstensen[15], Polk & Postow[16], Wertheimer & Leeper[17], Feychting & Ahlbom[18], Olsen[19], Floderus[20]). Not restricted to scientific journals, the deluge receives much attention in the popular press, magazines and newspapers. Such publication of often ill researched findings has only led to great public confusion, and in some cases - panic.

1.3 POTENTIAL APPLICATIONS OF MAGNETIC FIELD THERAPY

1.3.1 Potential Benefits of Pulsed Electromagnetic Fields

The reported effects of electromagnetic fields are manifold. Since Fukuda's[1] landmark paper of 1957 detailing the piezo electric effect in hydrated living bone, research world-wide has embarked on an exponential growth curve. Contemporary research includes such diverse experiments as: "The effect of magnetic treated water on the growth, flowering and fruiting of glasshouse grown tomatoes", Pavlov[2] et al., 1983; "The effect of alternating magnetic fields (60 - 100 gauss, 60 Hz) on *Tetrahymena pyreformis*," Tabrah[3], et at. 1978; and Pulsing electromagnetic fields induce cellular transcription, Goodman[4], 1983.

Electromagnetic fields are now commonly used to stimulate enzyme activity in recalcitrant seeds, e.g. "Effects of magnetic seed treatment on yields of barley, wheat, and oats in Southern Alberta", Pittman[5], 1977; and "Effects of magnetic seed treatment on amylolytic activity of quiescent and germinating barley and wheat seeds," Pittman[6], 1979. While the use of various electromagnetic fields on seeds and plants offers considerable commercial opportunity, this thesis focuses on two studies: a) the effects of such fields on humans, with the emphasis on beneficial medical applications with respect to Raynaud's disease; and b) the effects of such fields on the DNA of beans.

All experimentation involves potential risk. In the experiments involving humans discussed in this thesis, every effort has been made to minimise the actual risk to subjects. Field exposures have been kept within the I.R.P.A. /

I.N.I.R.C.[21] established guide-lines, (also Appendix Four) and are generally similar to other experiments currently undertaken around the world. In all cases, the subjects involved in the experiments discussed in this thesis have been appropriately informed, are participating voluntarily without obligation, (having the option of discontinuing the experiments at any time for any reason,) and have signed a consent form detailing their involvement and understanding of the experimental protocol. Although the human experiments were conducted prior to the commencement date of this masterate, the author sought independent assessment of the protocols, presenting a proposal to the Human Ethics Committee at Massey University. The guide-lines suggested by the committee were adhered to. Subsequently a formal proposal was passed in principle by the Massey University Human Ethics Committee, although further experiments and have been conducted since the commencement of the this thesis.

It is now well established that electromagnetic fields modify the behaviour of calcium in calcified and non calcified tissues: "Pulsing electromagnetic fields: A new method to modify cell behaviour in calcified and noncalcified tissues", Bassett[7], 1982. To quote Bassett directly;

During the past 20 years, great strides have been made in defining bioelectric phenomena in the skeletal system. As a result, it is possible to treat, clinically, disordered function in these tissues. The success of these efforts and a rapidly expanding base of fundamental data suggest that many important advances can be made as new research endeavours characterise effects of weak pulsing electric currents on cell behaviour. Future endeavours will involve investigators versed in biochemical, biophysical, developmental, endocrinological, physical-chemical, and physiological aspects of the skeletal and other systems. Unfortunately, few of these individuals are equipped by past experience or training to deal easily with the principles and techniques of electricity or

electromagnetism. In such an environment, confusion of concepts and terminology is an ever present and real danger.

Of particular importance is Bassett's warning. Since 1982 hundreds of papers have been produced world-wide, many of which are of dubious quality. The technical descriptions are so poor and lacking in detail that duplication is virtually impossible in all but a few instances. Carstensen15 (pp237-251) tabulates 116 clinical trials concerning the effects of extremely low frequency magnetic fields on plants and animals. Of these, only 18 experiments have been replicated to date. Ten with positive results confirming the original finding, and 8 negative results questioning the original findings.

Despite this plethora of inferior papers, significant breakthroughs have been accomplished. The treatment of recalcitrant fractures with alternating electromagnetic fields is now commonplace in many western hospitals. The groundwork for such therapies was laid by such researchers as Becker and Bassett, e.g. Modification of fracture repair with selected pulsing electromagnetic fields, Bassett[8], et al. 1982. Bassett recounts a spectacular cure of osteonecrosis of the hips in a 30 year old mother in "Biomedical implications of pulsing electromagnetic fields", in Surgical Rounds, 1982.

Unfortunately the success of such innovative treatments has sparked the production of a number of dubious magnetic field treatment devices. It is claimed that such units claim to beneficial in the treatment of: arthritis; arthrosis; bronchitis; bruising; burns; diabetes; fractures; migraine; myosistis and prostatitis; nephritis and gastritis; neuralgia, both brachial and intercostal;

osteoporosis; retinitis; rheumatism; sinusitis; sleep disorders; spondylitis and Scheurmanns disease; tonsilitis; tooth extraction; ulcers, including chronic, gastric and varicose; and be useful in generally aiding wound healing.

Extravagant claims are supported by such statements as: "...based on clinical evidence..."; "...one of the most advanced and effective units available"; "Russian cosmonauts have proved that men cannot remain in space away from the earth's magnetic field (9.6 Hz) for more than 250 days without permanent damage to the intricate biological function of the human body". All too often such claims are not referenced to any specific scientific literature. Where references are provided, in many cases they do not directly relate to the kind of stimulation which the unit is capable of providing. A list of manufacturers appears in Appendix One.

While the various diseases and disorders listed above may one day submit to treatment using electromagnetic fields, the case is far from decided. Clinical trials conducted scientifically with adequate control conditions as well as adequate control over the electromagnetic variables need to be undertaken before public announcements are made. Researchers in this field may be spurred on by the results of Becker, Bassett, Pilla, and Smith.

One area of potential enquiry worth pursuing is that relating to the redistribution of microcirculation patterns in the human body. Warnke, in a seminal paper entitled: "The possible role of pulsating magnetic fields in the reduction of pain", (published in Pain Therapy, 1983 Elsevier Biomedical Press), recounts the widening of blood vessels as registered by 5 and 12 μm

thermography under the influence of pulsed magnetic fields in the range of 500 μT - 2 mT. Essentially Warnke used a 200 Hz carrier wave modulating this between 5 - 25 Hz for optimal stimulation. He does however report increases in skin temperatures with a 50 Hz carrier modulated at 30 Hz at 1.5 mT. There are two points worth noting from Warnke's paper: a) both large vessels and capillaries dilate under the influence of the applied magnetic field; b) pulsating magnetic fields applied to the head need the lowest induction intensity in order to achieve a reaction.

Warnke's paper is a milestone in that he was able to show a similar response in both humans and horses, thus avoiding the placebo effect. In addition, the increase in peripheral blood flow was accompanied by an increase in oxygen in the tissues which was indexed by the amount of oxygen diffusing out through the surface layers of the skin. It is without doubt that his experiments showed a genuine increase in oxygen in the tissues. Obviously this would be of use when attempting to treat ischaemic pain (pain caused through lack of oxygen in the tissues).

Warnke proposes a mechanism to explain his results: hyperpolarisation of the nerves of the sympathetic system thus causing a reduction in information. He believes that the eddy currents generated across the various cell membranes as a result of the pulsed magnetic fields, while insufficient in themselves to directly cause hyperpolarisation, are able to cause an 'adding-up' of electrical potential across the membrane. In this way, sufficient polarisation may be achieved by the adding up of stored electrical potential in the form of differential concentrations of ions across the membrane. He is quick to point out that this response is obviously time dependent.

His theory relies upon the fact that the time constants of the pre and post-synaptic membranes of the alpha receptors of the sympathetic nervous system are sufficiently long for pulsed magnetic fields of the order of 200 Hz modulated at 5 - 25 Hz to effect a response. The time constants of the beta receptor system are considerably shorter and have a tendency to be influenced only by metabolic stimulation, so it is unlikely that they would be affected by similar electromagnetic stimulation. Warnke concludes that the hyperpolarisation of the alpha receptors influence the rate of action potentials delivered to the muscles surrounding the blood vessels, (arteries and metarterioles). This reduced muscle tone allows the vessels to expand under normal blood pressure, thus delivering more oxygenated blood to the tissues. As a result, not only does the surface temperature of the skin increase, but the amount of oxygen diffusing out thought the skin surface increases.

This thesis will investigate the potential application of the magnetic biostimulator in clinical trials in an attempt to determine the possible use of pulsed electromagnetic fields as vasodilators which could then be used in the treatment of Raynaud's disease.

1.3.2 Potential Dangers of Pulsed Electromagnetic Fields.

Not all of the reported effects can be regarded as beneficial. Garcia-Sagredo and Monteagudo[11], 1991, report that the waveforms generally used to enhance bone regeneration caused an increase in chromosome breakages which were statistically significant. It is important to note that these increased aberrations occurred at the 4.0 mT level. No increased effects above the normal were observed with field strengths of 1.0, 2.0 or 3.0 mT. A significant point

concerning the geometry of the applied field is that all four exposure levels were obtained by placing the test tube in a predetermined position within a pair of Helmholtz coils. While the author states that the peak readings were, 1.0, 2.0, 3.0 and 4.0 mT respectively, the exact location of the test tube within the coil would not only determine the peak flux density, but also the orientation of the field in space. The resultant vectors would be considerably different for each location which might tend to nullify the results as each treatment would have two different variables rather than the single stated one: field strength.

Khalil and Qassem[12], 1991, exposed human lymphocytes to a 10 ms pulse with a repetition rate of 50 Hz producing a duty cycle 1.0. A pair of Helmholtz coils produced the applied field at 1.05 mT. They discovered that cell cultures exposed for 72 hours exhibited a significant reduction in cell proliferation rate and an increase in sister chromatid exchanges. They conclude that exposure to pulsed electromagnetic fields may induce a type of DNA lesion that could lead to chromosomal aberrations and death. Such lesions do not seem to increase the number of sister chromatid exchanges.

The literature contains numerous papers discussing the potential harmful effects of electromagnetic fields, particularly those associated with power distribution, i.e. 50 - 60 Hz. A list of such papers is to be found in the bibliography. To date, there has been no compelling evidence to prove beyond reasonable doubt that such fields are harmful. Epidemiological studies tend to suggest some correlation between exposure to magnetic fields from high voltage overhead power lines and various types of cancer.

1.3.3 Evaluation of the Effect of Some Alternating Magnetic Fields

In order to test the biomagnetic stimulator it was decided to attempt to evaluate the effect of electromagnetic fields on two biological systems.

The first involves the use of the biomagnetic stimulator as a medically therapeutic device. (The current uses of magnetic fields in medical treatment have been discussed in brief in Section 1.2, Historical Perspective, and Section 1.3, Potential Applications of magnetic field therapy.) This study focuses on the claim that certain electromagnetic fields cause vasodilation which in turn cause an increase in skin surface temperature, (as shown by Warnke, 1983).

In brief, alternating electromagnetic fields are applied to the base of the skull via a short solenoid. Skin temperatures recorded by a computer are analysed to determine if the applied fields caused an increase in temperature. If successful, this technique could be used to treat sufferers of Raynaud's disease. (See Chapter Five.)

The second approach involves investigating the effects of various alternating magnetic fields on DNA during mitosis (cell division). The Broad Bean, *Vicia faba*, was chosen because of the relatively small number of large chromosomes. Growing beans were subjected to various alternating magnetic fields for three days after which the root tips were excised and prepared for microscopic analysis. Essentially, the chromosomes were examined in Metaphase for any visible aberrations such as breaks and bridges. In addition, the ratios of cells in

each stage of the cell cycle were determined in both control and test plants in order to determine if the applied fields affect the rate of cell division. The results of these experiments are presented in Chapter Six.

1.4 SUMMARY OF THE THESIS

Chapter Two:

Chapter two details the analysis of a typical medical biostimulator, using the Magnafield 990 Multi-Rhythm treatment unit. This unit representative of devices currently on the market. Firstly an electronic analysis is undertaken by determining the exact type of magnetic field produced. This is achieved by using a storage oscilloscope to examine the output of a magnetic flux meter the probe of which is placed near the induction coil. An example of the output is displayed in Plate 2.4.

The Magnafield 990 is then tested in two clinical experiments to determine the physiological response of a human subject. Two trials are conducted, first one to determine if the unit can produce a vasodilation response, the second to determine if a vasoconstriction response can be initiated. The results do not support the claims made by Magnacare for the Magnafield 990. This leads to conjecture over the clinical efficacy of such treatment devices.

Chapter Three:

Chapter three details the design and construction of the biostimulator modules including: timebase generator; timebase generator computer interface; simple analogue switch; zero-crossing switch; pseudo random analogue switch; programmable analogue switch and transconductance amplifier. In addition the design of a low-pass filter is discussed, as this is required for later analysis. Full circuits diagrams and the printed circuit layout, where applicable, are detailed.

Chapter Four:

The valuation of the magnetic biostimulator designed in Chapter Three is outlined in Chapter four. The devices tested include: timebase generator; simple analog switch; zero-crossing switch; pseudo random switch; low-pass filter and transconductance amplifier. Due to the inability to source some integrated circuits in New Zealand, the computer interface for the timebase generator was not completed, hence its actual operation could not be evaluated within the time constraints of this project.

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Chapter Five:

In order to evaluate the clinical efficacy of the magnetic biostimulator it was decided to test the unit by attempting to stimulate a vasodilation response in human subjects. In order to determine if vasodilation occurred, it was necessary to devise some non in-invasive procedure. The measurement of surface skin temperature was chosen. Chapter Five details some mathematical modelling based on the work of Hertzman5-16. A generally held belief is that skin surface temperature and sub-cutaneous blood flow are linearly correlated between 25C and 35C. The results of the mathematical modelling provide a more complex answer in the form of a second order differential equation.

A brief discussion of Raynaud's Disease is undertaken as an introduction to the clinical trials. The thesis is to test the effect of pulsed low frequency magnetic fields on normal subjects, and compare these results with those obtained from subjects who have been diagnosed as having impaired physiological temperature control.

The magnetic biostimulator was clinically trialed on four volunteer subjects, two who suffered from Raynaud's Disease, and two normal, healthy controls. Some interesting results were obtained which may be suggestive of a magnetically induced vasodilation response in the normal subjects. The response of the Raynaud's sufferers is however more complex, and cannot be investigated further within the bounds of this project. The topic is worthy of further investigation.

Chapter Six:

To test the magnetic biostimulator in a scientific laboratory setting, it was decided to investigate the effect of various magnetic fields on the chromosomes of *Vicia faba*, the broad bean. The rationale for using *Vicia faba* is that it has a large genome (24.3 picograms of DNA in a 2C nucleus). It has a diploid number of 12 chromosomes which are large and morphologically distinct. Thus *Vicia faba* is an ideal organism to use for studying cytological events. The stages of mitosis are easily identifiable and any chromosome breakages can be readily detected. Furthermore this species can be easily cultivated under standard glasshouse conditions.

Chapter Six begins with a review of current theories of interaction between living systems and low frequency magnetic fields. The experimental protocol chosen involves detecting possibly harmful effects of various magnetic fields by examining the number of chromosome and chromatid breaks at metaphase. In addition, the number of cells in each stage of the cell cycle is determined for five experimental conditions. This procedure would enable any changes in the rate

of cell division to be determined, as the number of cells in each phase is proportional to the time spent in each phase.

While no significant differences in the number of chromosome or chromatid breaks is evident between the various experimental treatments, the time taken in each phase of the cell cycle appears to be significantly different for the different treatments. A number of different avenues for future research are discussed.

Chapter Six also details an evaluation of the biomagnetic stimulator as a scientific research tool and outlines possible future improvements.

Chapter Seven:

Chapter Seven contains a brief resume of conclusions which includes the following sections:

Analysis of a typical magnetic biostimulator;

The magnetic biostimulator as a clinical tool;

Pulsed magnetic fields as a human vasodilator;

The magnetic biostimulator as a scientific research tool;

The effect of magnetic fields on chromosomes;

The effect of magnetic fields on the cell cycle.

1.5 REFERENCES

1 Fukuda.E. I.Yasuda. On the piezoelectric effect of bone. Journal of the Physics Society of Japan, Vol 12, pp1158-62, October 1957.

2 Pavlov.P. The effect of magnetic treated water on the growth, flowering and fruiting of glasshouse grown tomatoes. Plant Physiology, Vol. 4, 1982. Bulgarian Academy of Sciences.

3 Tabrah. F. et al. Effects of alternating magnetic fields (60-100 gauss, 60 Hz) on Tetrahymena pyreformis. T.I.T. Journal of Life Sciences, Vol 8, pp73-77, 1978.

4 R.Goodman, C.A.L.Bassett, A.S.Henderson. Pulsing electromagnetic fields induce cellular transcription. Science Vol 220, pp1283-1284, 17 June 1983.

5 Pittman. U. Effects of magnetic seed treatment on yields of barley, wheat, and oats in southern Alberta. Canadian Journal of Plant Science Vol 57, pp37-45, January 1977.

6 Pittmann. U. et al. Effect of magnetic seed treatment on amylolytic activity of quiescent and germinating barley and wheat seeds. Canadian Journal of Plant Science , Vol 59, pp1007-1011, October 1979.

7 Bassett.C. Pulsing electromagnetic fields: a new method to modify cell behaviour in calcified and noncalcified tissues. Calcified Tissue International. Vol 34, pp1-8. 1982.

8 Bassett.C. Modification of fracture repair with selected pulsing electromagnetic fields. The Journal of Bone Joint Surgery, Vol 64-A, pp888-895, July 1982.

9 Bassett.C. Biomedical implications of pulsing electromagnetic fields. Surgical Rounds, pp22-31, January 1983.

10 U.Warnke. The possible role of pulsating magnetic fields in the reduction of pain. Pain Therapy. Ed: R.Rizzi and M.Visentin. Elsvier Biomedical Press, 1983.

11 Garcia-Segredo.J.M. and Monteagudo.J.L. Effects of low level pulsed electromagnetic fields on human chromosomes in vitro: analysis of chromosomal aberrations. Hereditas, Vol 115, No 1, 1991.

12 A.M.Khalil, W.Qassem. Cytogenetic effects of pulsing electromagnetic field on human lymphocytes in vitro: chromosome aberrations, sister chromatic exchanges and cell kinetics. Mutation Research. Vol 247, pp141-146, 1991.

13 Brown.T. The Lakhovsky Multiple Wave Oscillator Handbook. Borderland Sciences, California. 1988.

14 Cheney.M. Tesla: Man out of time. Dell Publishing Co. New York. 1983.

15 Carstensen.E. Biological effects of transmission line fields. Elsvier. 1987.

16 Polk.C. & Postow.E. CRC Handbook of biological effects of electromagnetic fields. CRC Press, Florida. 1986.

17 N.Wertheimer, E.Leeper. Adult cancer related to electrical wires near the home. International Journal of Epidemiology. Vol 11, No 4, pp345-355, 1982.

18 M.Feychting, A.Ahlbom. Magnetic fields and cancer in people residing near Swedish high voltage power lines. IMM - rapport 6/92. Karolinska Institutet, Stokholm, Sweden. 1992.

19 J.Olsen et.al. Residence near high voltage facilities and the risk of cancer in children. Cancer Registry of the Danish Cancer Control Agency, Copenhagen, Denmark. 1992.

20 B. Floderus et.al. Occupational exposure to electromagnetic fields in relation to leukemia and brain tumours: a case control study. National Institute of Occupational Health, Solna, Sweden. 1992.

21 I.R.P.A. / I.N.I.R.C. Interim guide-lines on limits of exposure to 50/60 Hz electric and magnetic fields. Health Physics,Vol 58, No 1, pp113-122, Jan. 1990.

2

Evaluation of a Typical Medical Stimulator

CONTENTS

2.1 OVERVIEW

As a result of the scientific research conducted in recent years a number of companies have produced electromagnetic stimulators for the medical market. A list of some of the companies is listed in Appendix One.

A survey of the specifications reveals a similarity in operating frequencies, field strength, and waveforms produced. There are two common induction coil configurations: a flat, single layer involute spiral; and a solenoid usually large enough to fit around the entire body. Both Magnacare Pty. Ltd. and Emmet-Glen utilise the flat involute spiral, while Elec-Western use both the involute spiral and the large solenoid. Elec-Western's *Centurian System* is shown in Plate 2.1. In addition, Elec-Western make therapeutic systems specifically for animals. Plate 2.2 shows their *Centurian System* being used on a horse.

Magnacare and Emmet-Glen are Australian based companies and their local agents allowed their units to be evaluated. In both cases the technical specifications are brief, and as the two instruments are almost identical in electrical design and function, a detailed description of only Magnacare's Magnafield Multi-Rhythm model 990 will be presented. An external view of the Magnafield 990 is shown in the top figure of Plate 2.3, together with an internal view in lower figure of Plate 2.3.

2.2 TECHNICAL EVALUATION OF THE MAGNAFIELD MULTI-RHYTHM MODEL 990

2.2.1 General

The Magnafield 990 is a portable unit designed for both clinical and home use. It is mains operated and comes with a cushioned vinyl treatment coil and test magnet. Controls consist of a power switch; start and stop button; and an analogue frequency control. The lamp is connected in parallel with the energising coil, and may be observed to flash at the lower pulse frequencies.

The Magnafield 990 electronics are mounted on a single printed circuit board, (PCB). A single toroidal transformer powers the electronic control circuitry and provides the voltage for the treatment coil, as shown in Figure 2 of Plate 2.3.

2.2.2 Published technical specification

Model	990
Input	120 / 240 VAC 50 / 60 Hz 0.5 A
Output	20 Volts RMS AC 1.5 A
Frequency	0.5 - 18 Hz
Timer	20 minutes

Magnetotherapy for Home Use by Elec - Western

MAGNETOTHERAPY

HOME SYSTEMS

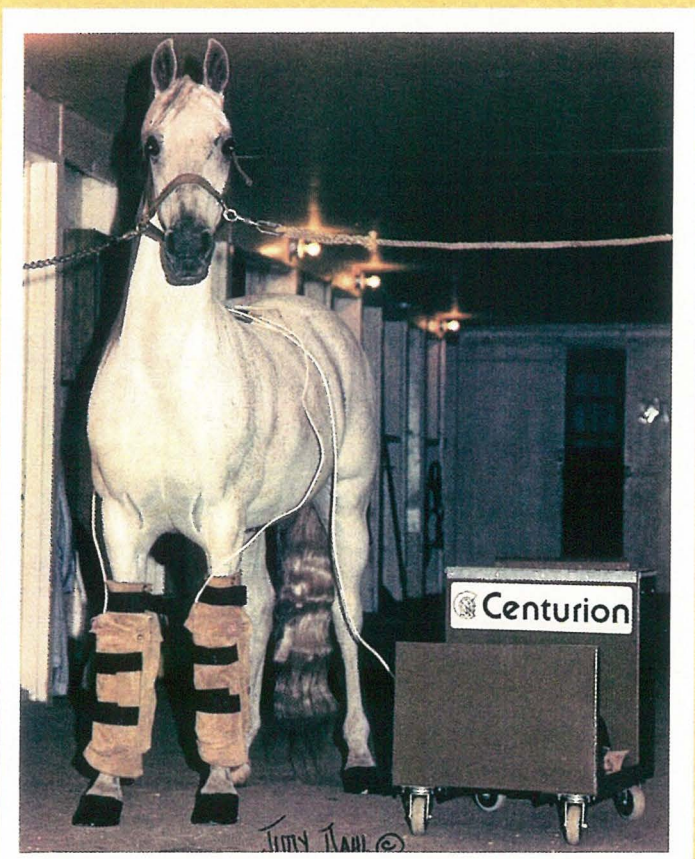
— USING LOW FREQUENCY
PULSATING MAGNETIC FIELDS
— DEVELOPED WITH THE UTILIZATION OF THE
MOST RECENT KNOWLEDGE IN SCIENCE

Centurion system

Plate 2 - 1

MAGNETOTHERAPY

— USING LOW FREQUENCY
PULSATING MAGNETIC FIELDS
— A SAFE, BROAD SPECTRUM, DRUGLESS
THERAPY



Centurion system

Plate 2 - 2

Magnafield 990 Multi Rhythm Magnetic Field Therapy Unit

Internal view

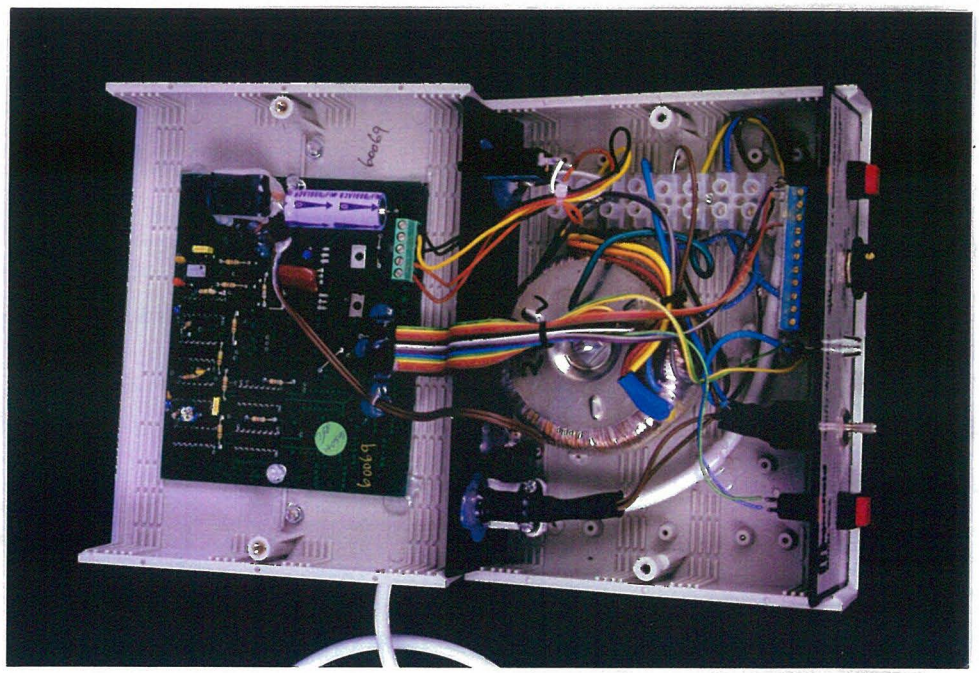


Plate 2 - 3

Magnafield 990 Magnetic field output from Labview

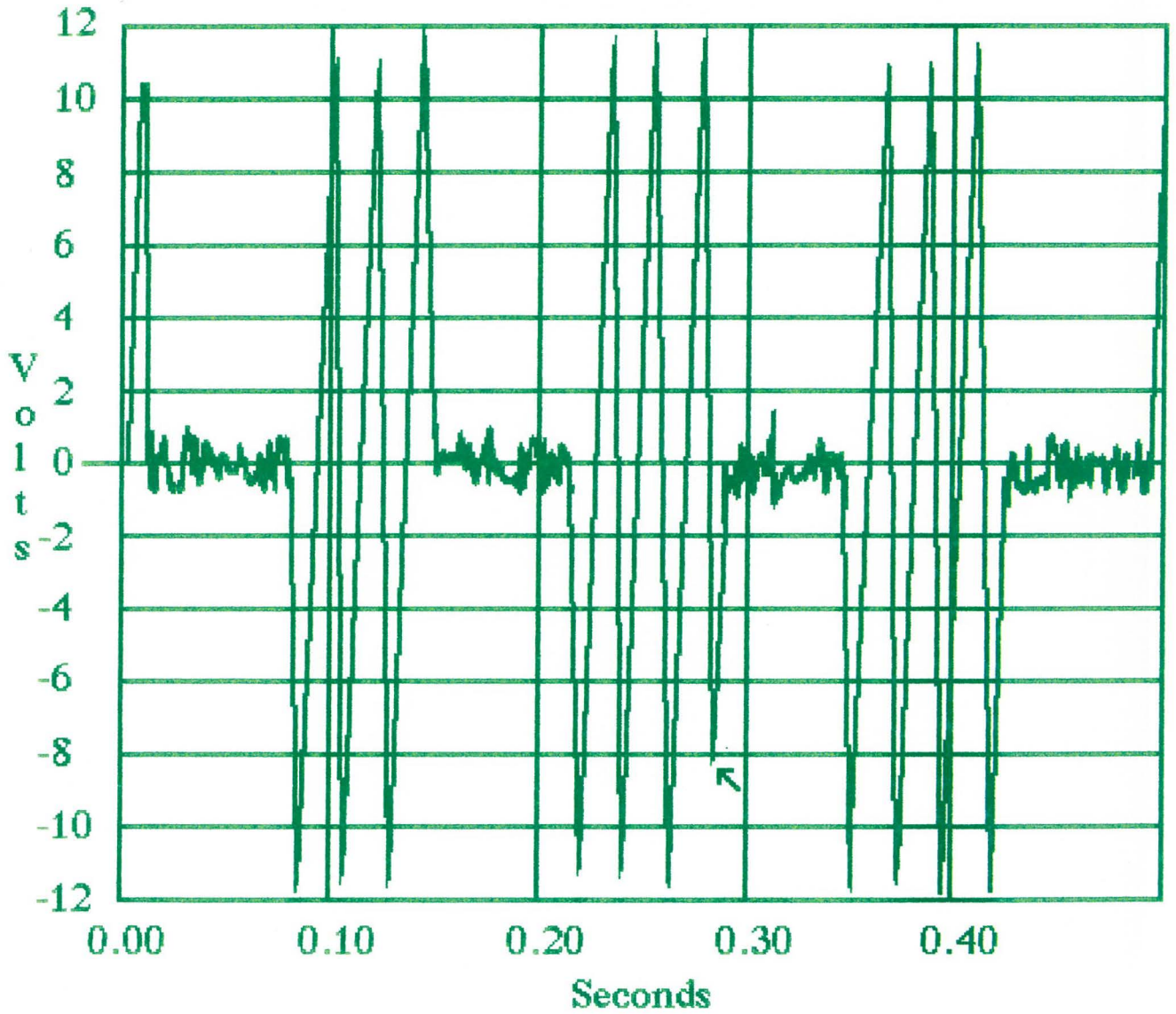


Plate 2 - 4

Appendix Two contains the publicity material supplied by Magnacare Pty. Ltd., including their description of the physiological mechanisms involved in pulsed electromagentic therapy. Also included is a brief operators guide with suggested treatment regimes.

2.2.3 Principle of operation

The signal produced by the Magnafield 990 was analysed by the F.W.Bell flux meter observing the magnetic field produced at the coil surface. The analogue output of the flux meter was connected to an oscilloscope which directly displayed the magnetic field produced in real time. The graphic output of the oscilloscope was connected to a MacIntosh II ci running LABVIEW. Recordings were made at the following frequencies: 0.5 Hz; 1 Hz; 2 Hz; 3 Hz; 4 Hz; 5 Hz; 8 Hz;10 Hz; 12 Hz; 15 Hz; and 18 Hz.

The analysis reveals that the Model 990 utilises the mains frequency as a carrier wave which is then chopped or pulsed at frequencies ranging from 0.5 Hz to 18 Hz with a 50% duty cycle. No attempt is made to control the amplitude of the signal applied to the induction coil. A sample of the LABVIEW output obtained in the above experiments is illustrated in Plate 2.4.

2.2.4 Flux density produced

The F.W.Bell flux meter was used to investigate the magnetic field produced at the surface of the applicator pad. A plot of the Z (vertical) axis magnetic field component is produced in Figure 2.1 .

Magnetic Field in Z Axis Across the Surface of the Magnafield 990 Coil

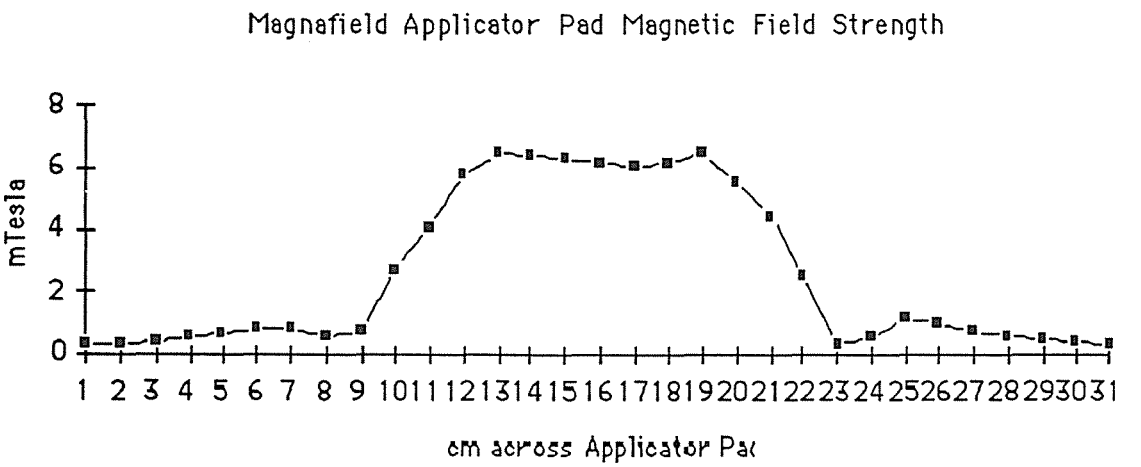


Figure 2.1

In the region of the actual wire, a relatively uniform field of approximately 6 mTesla is obtained at the surface.

2.2.5 Induction coil

The induction coil is mounted inside a rectangular foam-lined black vinyl case measuring approximately 25 cm x 35 cm. The case is heat sealed with a coiled lead exiting from one of the long sides. It was not possible to open the case to

examine the coil without damaging the demonstration unit. As Magnacare's N.Z. agent and the Australian General Manager both declined to give any details concerning the coil's construction, other than to say that is was designed by an academic at an Australian University, it was decided to X-Ray the intact unit.

The X-ray of the resulting shadow image was digitised with a standard Ikegami video camera and *Image* running on an Apple Macintosh Quadra 900. The resulting digital image was subjected to some image enhancement techniques before converting to a binary format for the word processor. The resulting binary image is shown in Figure 2.2 at a scale factor of 0.5454. Some aliasing problems are evident due to the pixel resolution of the screen image.

The involute spiral winding can be easily seen as a clear toroid against the black background, standard X-ray images being presented in negative format. The individual wires which were visible on the original x-ray are not visible in the digitised image due to resolution problems and scaling. The coil may be seen not to be a perfect circle, rather it has a noticeable kink visible at the lower left of the image. This is entirely accurate with respect to the original x-ray. The coil is somewhat roughly wound.

Digitised X-Ray of Magnafield 990 Coil

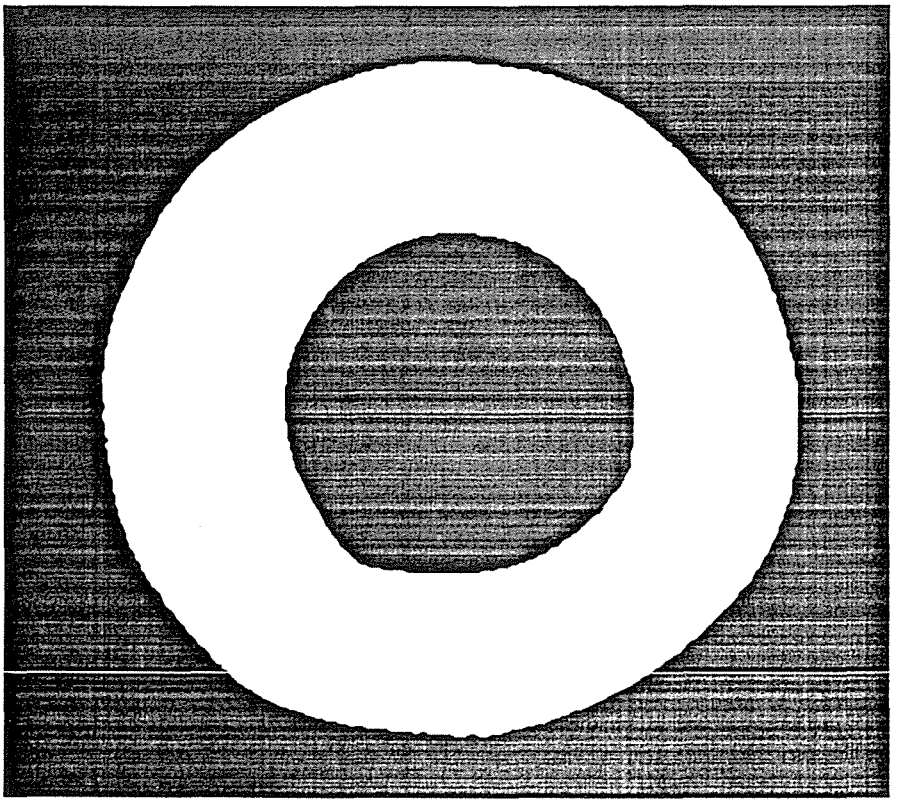


Figure 2.2

Figure 2.2, represents an involute spiral annulus of copper wire, approximately 160 mm in diameter, with an 80 mm internal diameter, giving a wire ring of approximately 40 mm width. There are approximately 50 turns of 0.8 mm copper wire. The diameter of the wire was determined using a calibrated eyepiece micrometer with an Olympus dissecting binocular microscope. This was verified by eye with a vernier micrometer viewed under the same microscopic conditions.

The D.C. resistance is 15 ohms. A Hewlett Packard LF impedance analyzer Model 4192A was used to determine the relationship between impedance

magnitude (Z), frequency (Hz), and impedance phase angle (Degrees), as shown in Table 2.1 and Figure 2.3 below.

Frequency vs Impedance & Phase Angle

f (Hz)	Z (Ω)	Angle (Degrees)
5	15.2	6.04
10	15.5	13.2
20	16.7	25.7
50	23.7	50.7
100	39.5	67.6
200	74.4	78.4
500	183	85.2
1000	366	87.5
2000	783	88.7
5000	1,970	89
10,000	5,130	87.3
20,000	43,300	-58.3
50,000	2,930	-86.6
100,000	1,140	-88.4

Table 2.1

Impedance vs Frequency for Magnafield 990 Coil

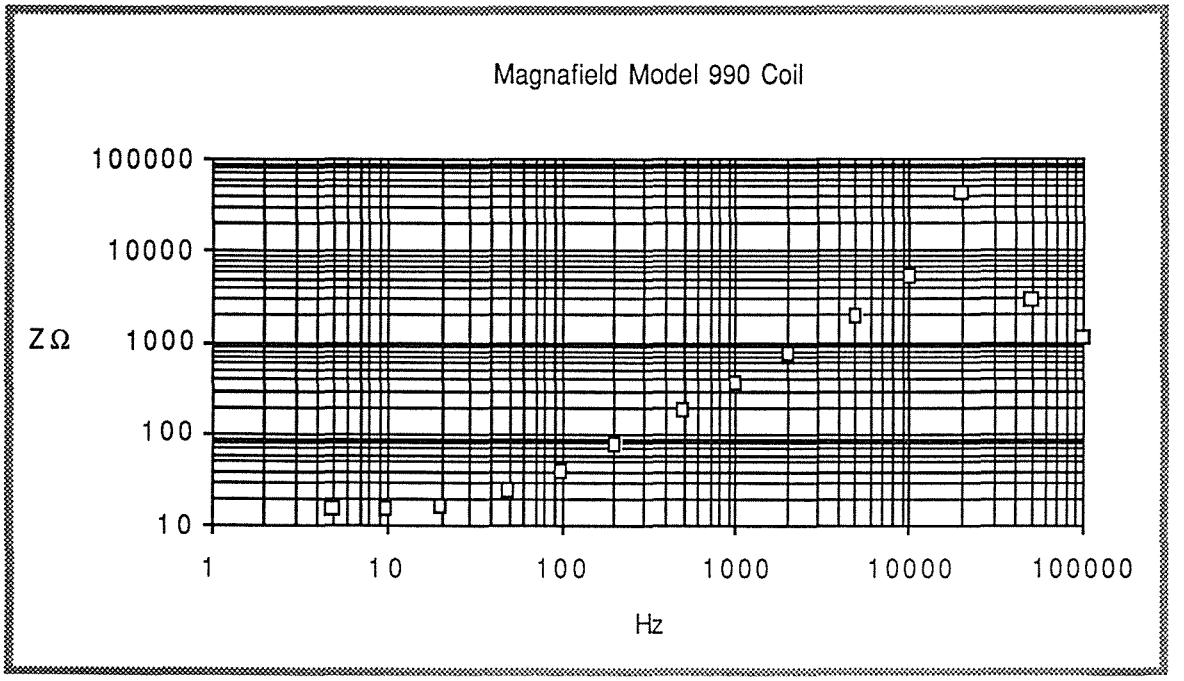


Figure 2.3

2.2.6 Determination of inductance and capacitive reactance

Due to an equipment fault during the short period of time that the Magnafield unit was on loan, it was not possible to measure the inductance and capacitance. The inductance and capacitance may be calculated however from the impedance data. The coil may be modelled as a resistor and an inductor in series, with a capacitor in parallel. This is shown in Figure 2.4.

Model of a coil

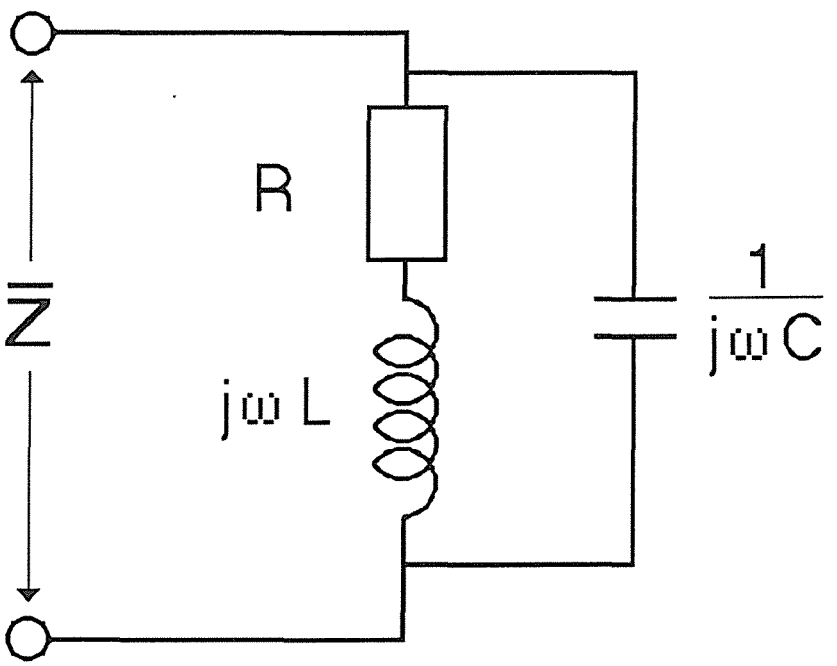


Figure 2.4

The impedance of the coil model above may be considered as two circuits in parallel: a resistor and an inductor in parallel with a capacitor, which is represented by the formula:

$$Z = (R + j\omega L) \,//\, \frac{1}{j\omega C}$$

Multiply top and bottom by jωC to remove fractional components.

$$Z = \frac{(R + j\omega L)\frac{1}{j\omega C}}{R + j\omega L + \frac{1}{j\omega C}} \times \frac{j\omega C}{j\omega C}$$

$$Z = \frac{(R + j\omega L)}{j\omega RC - \omega^2 LC + 1}$$

Rearrange to obtain:

$$Z = \frac{R + j\omega L}{(1 - \omega^2 LC) + j\omega RC}$$

Find the magnitude of Z^2 by taking the square of the real and imaginary parts of both top and bottom:

$$|Z|^2 = \frac{R^2 + (\omega L)^2}{(1 - \omega^2 LC)^2 + (\omega RC)^2}$$

$$Z^2\left[(1 - \omega^2 LC)^2 + (\omega RC)^2\right] = R^2 + (\omega L)^2$$

To determine the inductance and capacitance of the coil it is necessary to consider the above equation for Z1, Z2...Zn. Thus solving these equations as a nonlinear simultaneous set using the least squares as a measure of optimum fit yields the following values:

Inductance {L}=56 milliHenries Capacitance {C}=1.2nanoFarads.

In order to determine if these values are reasonable, it is desirable to see if they yield the same resonance point as the graph in Figure 2.4.

Starting with the formula for Z:

$$Z = \frac{R + j\omega L}{(1 - \omega^2 LC) + j\omega RC}$$

Make the denominator real by multiplying by the complex conjugate:

$$Z = \frac{(R + j\omega L)}{(1 - \omega^2 LC) + j\omega RC} \times \frac{(1 - \omega^2 LC) - j\omega RC}{(1 - \omega^2 LC) - j\omega RC}$$

Regroup to obtain:

$$Z = \frac{R(1 - \omega^2 LC) - j\omega R^2 C + j\omega L(1 - \omega^2 LC) + \omega^2 LRC}{(1 - \omega^2 LC)^2 + (\omega RC)^2}$$

Regroup real and imaginary parts to obtain:

$$Z = \frac{R + j(\omega L - \omega C(R^2 + \omega^2 L^2))}{(1 - \omega^2 LC)^2 + (\omega RC)^2}$$

At resonance $Z = Z_{max}$ so therefore there is no complex component.

$$\omega_0 L - \omega_0 C\left(R^2 + \omega_0^2 L^2\right) = 0$$

Thus:

$$L = CR^2 + \omega_0^2 L^2 C \Rightarrow \omega_0^2 = \frac{L - CR^2}{L^2 C}$$

So therefore:

$$\text{If} \quad CR^2 \langle L \qquad \therefore \qquad \omega_0^2 \approx \frac{1}{LC}$$

$$\omega_0 = \frac{1}{\sqrt{LC}} \quad \text{or} \quad f_0 = \frac{1}{2\pi\sqrt{LC}}$$

$$\text{Else} \quad \omega_0 = \frac{\sqrt{L - CR^2}}{L\sqrt{C}} \Rightarrow f_0 = \frac{\sqrt{L - CR^2}}{2\pi L\sqrt{C}}$$

Substituting in the values for L and C we obtain: **f_0 = 19,424.76 Hz.**

This is extremely close to the value of 20 kHz obtained from the data in Table 2.1.

(The use of either formula produces similar results to the third decimal place.)

From this we are able to conclude that the Magnafield 990 applicator coil has the following electrical characteristics:

DC resistance	= 15 Ω
Inductance {L}	= 56 mH
Capacitance {C}	= 1.2 nF
Resonant frequency {f_o}	= 19.424 kHz

The reader may be puzzled by such a high capacitance given the physical configuration of the coil: 50 turns of approximately 0.8 mm wire wound in an involute spiral (described above), however the majority of the capacitance is possibly contained within the 'curly-cord' which connects the coil pad to the instrument, rather than the coil itself. It is not possible to verify this without taking the coil assembly apart, something the company was not willing to allow. This high capacitance is however responsible for the low resonant frequency of approximately 20 kHz. This could have the effect of generating a significant electric field component as well as a magnetic component at some frequencies, however the medical significance of this remains to be demonstrated.

2.3 PHYSIOLOGICAL EVALUATION OF THE MAGNAFIELD MULTI-RHYTHM MODEL 990

2.3.1 General

In order to assess the clinical efficacy of the Magnafield Multi-rhythm Model 990, it was decided to use the instrument on a control subject. All the biomagnetic stimulator manufacturers claim significant results based on numerous clinical trials. They claim that from such a data base it is possible to draw conclusions and make generalisations about the various types of treatment which effect a particular result.

Magnafield, in particular, quotes specific physiological, clinical, effects for various frequencies which are presented in Table 2.2 below:

Table 2.2 Physiological effects of various specific frequencies.

Frequency	Clinical effect
0.5 - 4 Hz	Sedating effect.
	Reduced blood flow.
	Lymph drainage.
	Pain relief.
	Stimulation of the immune system.
2 Hz	Increased phagocyte and T-killer cell production.
	Stimulation of the immune system.
3 Hz	Lymph drainage.

5 Hz	Vasoconstriction.
8 Hz	Stimulation of A.T.P. {Adenosine triphosphate (energy)} production.
	Recharging of cell membrane potential.
	Muscle tissue repair.
10 Hz	Stabilizing (re-balancing) effect.
12 - 15 Hz	Increased blood flow - vasodilation.
	More oxygen, nutrients available to tissue.
18 Hz	Increased metabolic rate.

Table 2.2

2.3.2 Vasodilation trial

In line with the specific interest in vasodilation, (see Chapter Five), it was decided to attempt to cause vasodilation in the feet of a normal healthy subject. The subject was dressed in minimal clothing and lay recumbent under a brushed cotton sheet, (the same type of sheet was used on all subjects throughout all experiments). The stimulator, in this case the Model 990, was set to 12 Hz with the applicator coil pad placed vertically between the subjects feet. The stimulator was switched on after the 10th minute and off at the 30th, giving a standard 20 minute treatment as suggested in the clinical operator's manual supplied by Magnafield. In order to determine the amount of vasodilation, skin surface temperature was measured with LM35DZ temperature transducers taped to each foot. The temperatures were logged every 2 minutes by a computer data logger and the results are presented in Table 2.3. It is possible that the coils, while directly providing magnetic stimulus to the feet, may in fact stimulate effects in other areas. For this reason Table 2.3 presents all the various temperature sites logged by the computer so that physiological changes in the body could be monitored.

Table 2.3 Magnafield 990 - 12 Hz "vasodilation" trial temperatures.

Mins	Air	Chest	Core	R.H.	L.H.	R.Arm	L.Arm	R.Thi.	L.Thi	RFT	LFT	RFB	LFB
0	20.47	32.25	30.39	29.72	30.92	28.84	30.34	30.51	33.41	32.11	31.49	29.45	26.69
2	20.41	32.86	31.28	30.06	31.09	28.82	30.72	30.66	33.49	32.51	32.08	29.95	27.42
4	20.30	33.25	31.81	30.09	31.03	29.06	31.05	30.88	33.72	32.73	32.40	30.07	27.74
6	20.26	33.54	32.42	29.89	31.21	29.26	31.21	31.05	33.89	32.83	32.58	30.09	27.83
8	20.20	33.76	32.77	29.82	31.38	29.39	31.38	31.20	34.00	32.89	32.69	30.05	27.85
10	20.25	33.91	32.98	30.20	31.87	29.35	31.45	31.23	34.09	32.97	32.81	30.02	27.85
12	20.27	34.03	33.10	30.34	31.84	29.35	31.55	31.29	34.13	33.05	32.99	29.98	27.74
14	20.38	34.08	33.12	30.41	31.94	29.38	31.59	31.64	34.41	33.04	33.02	29.98	27.72
16	20.41	34.20	32.87	30.32	31.97	29.39	31.60	31.73	34.41	32.91	32.76	29.93	27.60
18	20.38	34.22	32.72	30.27	31.98	29.38	31.59	31.70	34.36	32.80	32.54	29.87	27.51
20	20.19	34.20	32.62	30.10	31.83	29.34	31.51	31.60	34.26	32.64	32.39	29.79	27.41
22	20.08	34.20	32.62	30.07	31.84	29.27	31.48	31.57	34.24	32.58	32.38	29.76	27.34
24	19.99	34.17	32.61	30.01	31.78	29.21	31.45	31.51	34.22	32.47	32.27	29.71	27.28
26	19.90	34.17	32.61	29.94	31.70	29.18	31.24	31.44	34.19	32.38	32.18	29.68	27.22
28	19.87	34.15	32.62	29.89	31.61	29.08	31.39	31.43	34.14	32.31	32.15	29.63	27.18
30	19.79	34.15	32.60	29.82	31.57	29.00	31.37	31.38	34.10	32.23	32.09	29.61	27.14
32	19.77	34.17	32.78	29.88	31.44	32.22	31.34	31.27	34.21	32.17	32.04	29.57	27.08
34	19.71	34.20	32.95	29.77	31.28	29.24	31.32	31.22	34.32	32.10	32.04	29.54	27.05
36	19.68	34.22	33.15	29.61	31.17	29.21	31.29	31.16	34.33	32.03	31.94	29.49	27.00
38	19.65	34.22	33.21	29.50	31.09	29.15	31.27	31.14	34.35	31.95	31.88	29.43	26.94
40	19.60	34.22	33.23	29.41	31.03	29.11	31.22	31.09	34.33	31.89	31.82	29.37	26.86
42	19.56	34.20	33.28	29.33	30.96	29.05	31.21	31.07	34.33	31.83	31.77	29.29	26.80
44	19.54	34.20	33.17	29.33	30.82	28.97	31.17	31.03	34.24	31.72	31.78	29.23	26.73
46	19.60	34.30	33.15	29.37	30.76	28.71	31.17	31.23	34.04	31.71	31.84	29.17	26.69
48	19.56	34.32	33.02	29.35	30.71	28.72	31.18	31.29	33.93	31.65	31.83	29.10	26.61
50	19.51	34.32	33.00	29.27	30.41	28.67	31.18	31.32	33.88	31.59	31.76	29.05	26.55
52	19.45	34.32	32.97	29.18	30.23	28.66	31.16	31.32	33.83	31.53	31.65	29.00	26.48
54	19.42	34.30	32.92	29.08	30.15	28.60	31.15	31.29	33.81	31.44	31.57	28.94	26.41
56	19.34	34.27	32.84	28.97	30.16	28.55	31.11	31.23	33.71	31.35	31.50	28.85	26.33
58	19.42	34.35	32.82	28.90	30.05	28.52	31.06	31.23	33.71	31.32	31.43	28.80	26.26
60	19.51	34.39	32.87	28.89	30.20	28.50	30.73	31.15	33.72	31.29	31.44	28.74	26.20
Min	19.12	29.39	31.03	28.63	28.99	27.72	31.46	26.94	28.46	27.09	28.02	22.75	24.15
Max	20.00	32.92	36.27	29..16	30.13	29.51	32.78	30.48	31.53	28.74	29.58	25.06	26.70
Mean	19.40	32.56	35.59	28.95	29.66	29.10	32.57	29.36	30.62	28.12	28.92	24.02	25.44
Diff	-0.10	0.45	0.95	-0.33	-0.17	-0.64	-0.10	1.79	1.57	-1.63	-1.49	-2.31	-2.35

Table 2.3

In addition to monitoring skin surface temperatures, the air temperature was also recorded. The air temperature is presented together with the chest and core estimate temperatures in Figure 2.5.

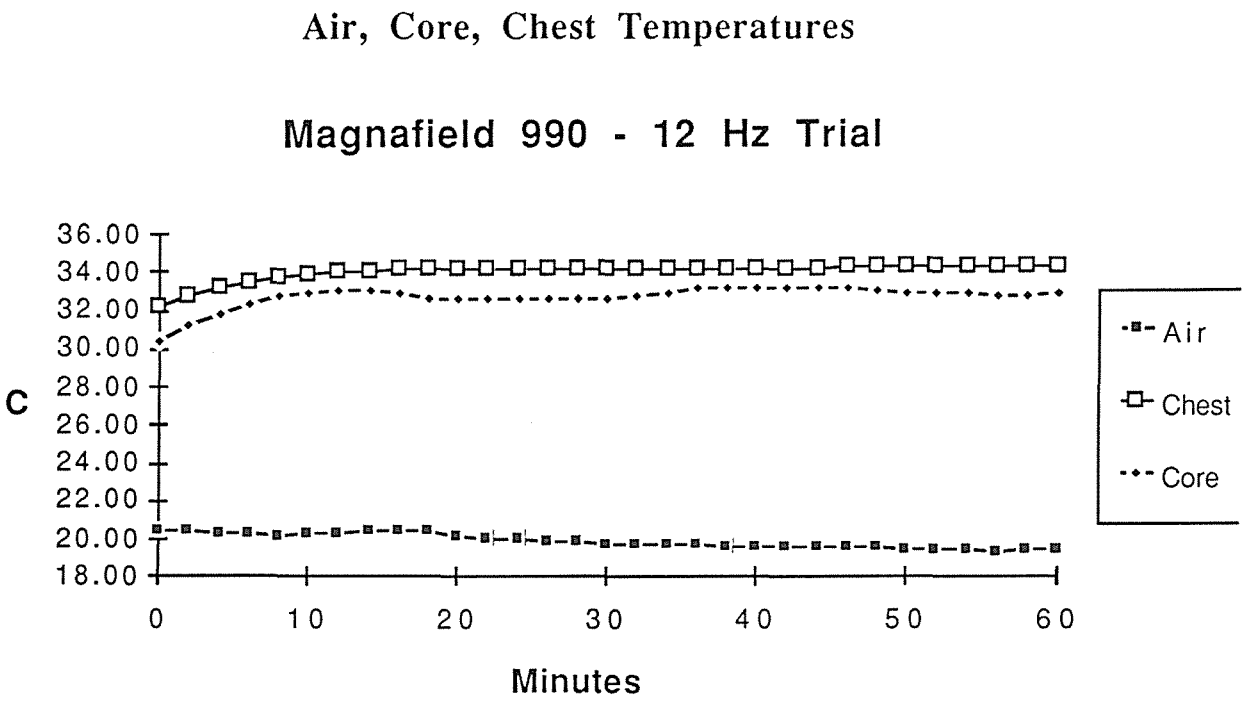


Figure 2.5

The room temperature varied little throughout the experiment with a mean of 19.4 C and a drop of only 0.1 C from the 10th to the 60th minute. The subject exhibits a period of equilibration during the first 10 minutes, undergoing the change from being active and upright to being passive in a recumbent position. Both the core estimate and the chest measurement demonstrate typical stability.

Peripheral sites temperatures

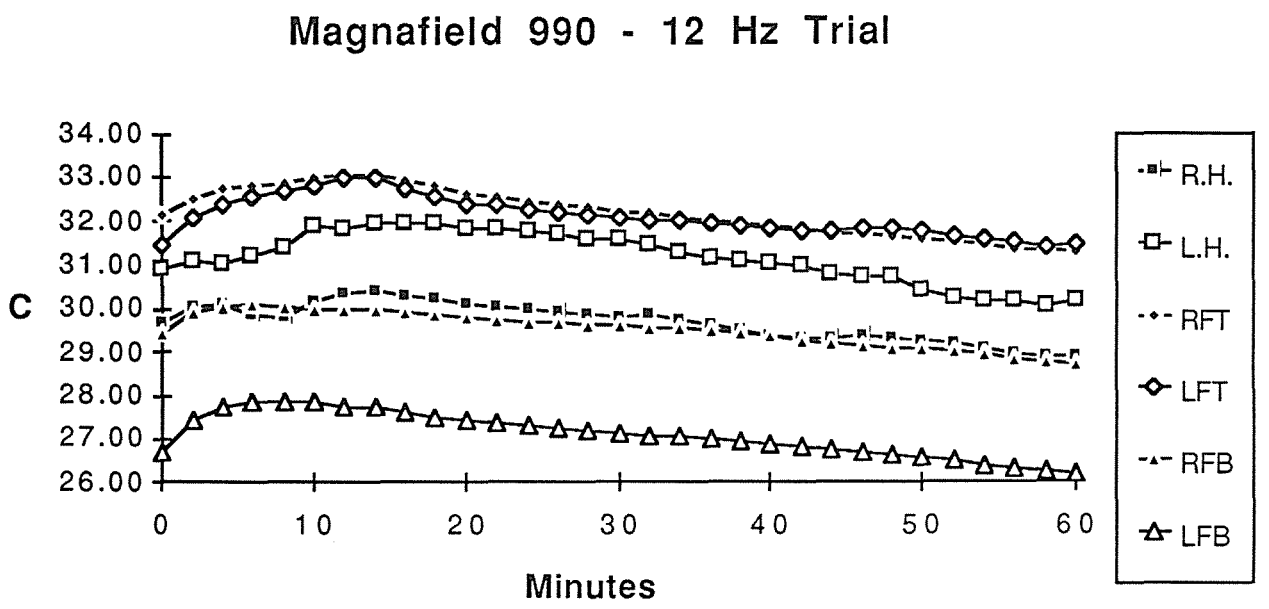


Figure 2.6

Figure 2.6 illustrates the temperature changes in the hands and feet of the subject. The first 10 minutes of the data should be disregarded as this represents the equilibration period during which time there is no stimulus. From the 10th to the 30th minute the stimulator is turned on without the subject's prior knowledge, the experiment being termed 'single-blind'. There is no increase in the temperatures of the feet, or indeed the hands which may be considered as a 'control within subject' for another peripheral area. The hands and feet may be considered 'peripheral' areas and are controlled differently to the main body core. Peripheral areas contain the temperature regulating artery to vein short circuit shunt, the arteriovenous anastomoses which are under the control of the autonomic nervous system. Figure 2.6 clearly shows a steady decline in temperature from approximately the 10 to the 60th minute.

The difference temperatures (temp. (C) at 60 mins minus temp. (C) at 10 mins) for all body sites are presented in Figure 2.7.

Difference temperatures - all sites.

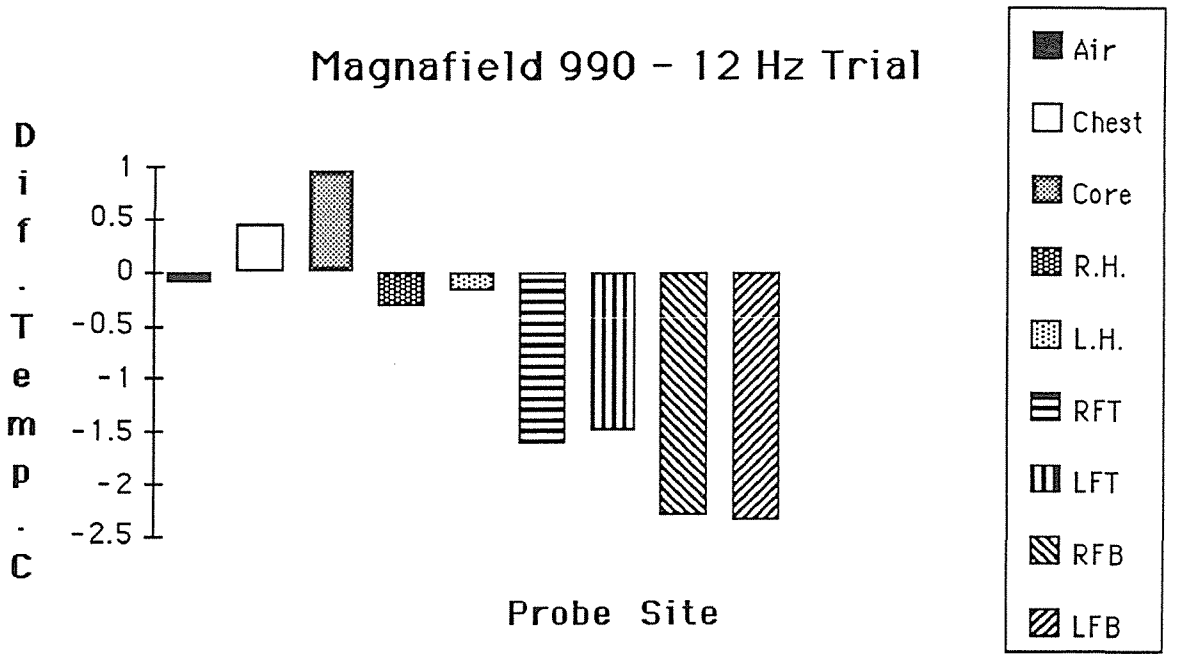


Figure 2.7

It can be concluded that the stimulation did not produce the claimed effect of vasodilation as there is no significant difference between the rate of decline of the temperature of the hands, (not being stimulated), and the feet, (being stimulated for vasodilation). Had the Magnafield 990's 12 Hz stimulation been effective at vasodilation, one would have expected an increase, or at least no decline, in the temperature of the feet, as opposed to the hands which should have slowly cooled down during the 50 minutes following equilibration.

2.3.3 Vasoconstriction trial

With the results of the 12 Hz trial more indicative of a vasoconstriction effect, it was decided to attempt a subject trial to evoke vasoconstriction, at 5 Hz, in accordance with the manufacturer's guide-lines. The same subject was used for conformity, and all conditions were identical to the 12 Hz "vasodilation" trial described in Section 2.3.2. The computer logged temperatures appear in Table 2.4.

The air, core estimate and chest temperatures are graphed in Figure 2.8, while the hand and foot temperatures are shown in Figure 2.9.

The ambient room temperature was almost identical to the first trial at a mean of 20.81 C. The chest temperature is within less than two degrees C of the previous trial, and well within normal limits at a mean of 34.46 C.

The difference temperatures are shown in Figure 2.10

Magnafield 990 - 5 Hz "vasoconstriction" trial temperatures.

Mins	Air	Chest	Core	R.H.	L.H.	R.Arm	L.Arm	R.Thi.	L.Thi	RFT	LFT	RFB	LFB
0	21.15	32.29	31.88	29.98	31.11	17.82	30.39	30.18	32.17	31.86	31.57	29.33	27.66
2	21.06	33.17	32.58	30.11	32.23	21.94	30.66	30.56	32.62	32.49	31.98	29.69	28.14
4	20.97	33.76	32.93	30.31	31.43	12.69	30.82	30.88	32.95	32.73	32.09	29.78	28.33
6	20.98	34.17	33.15	30.35	31.53	19.11	31.06	31.15	33.20	32.82	32.10	29.78	28.38
8	20.91	34.42	33.25	30.32	31.49	18.38	31.17	31.35	33.34	32.83	32.05	29.72	28.36
10	20.88	34.52	33.30	30.43	31.46	18.48	31.22	31.59	33.47	32.80	32.01	29.65	28.35
12	20.78	34.64	33.21	30.43	31.43	21.63	31.06	31.62	33.72	32.75	31.97	29.57	28.34
14	20.62	34.64	33.22	30.54	31.46	18.77	30.95	31.59	33.74	32.73	31.88	29.50	28.34
16	20.73	34.64	33.19	30.49	31.44	21.26	30.93	31.57	33.77	32.66	31.82	29.44	28.27
18	34.69	33.16	30.38	31.44	19.38	30.92	31.55	33.81	32.61	31.76	29.40	28.22	27.51
20	34.66	33.19	30.28	31.38	21.45	30.84	31.51	33.81	32.55	31.66	29.37	28.22	27.41
22	20.62	34.66	33.14	30.22	31.39	19.53	30.82	31.51	33.86	32.49	31.62	29.30	28.17
24	20.59	34.61	33.14	30.17	31.24	18.89	30.76	31.49	33.85	32.43	31.53	29.28	28.19
26	20.59	34.59	33.16	30.16	31.12	18.48	30.70	31.45	33.85	32.38	31.48	29.24	28.19
28	20.59	34.59	33.12	30.12	31.04	18.26	30.67	31.43	33.83	32.28	31.40	29.23	28.22
30	20.67	34.57	33.10	29.94	30.78	18.09	30.61	31.42	33.83	32.22	31.37	29.21	28.21
32	20.71	34.54	33.14	29.77	30.56	17.89	30.57	31.43	33.83	32.17	31.32	29.17	28.21
34	20.77	34.54	33.11	29.77	30.61	19.28	30.55	31.42	33.82	32.12	31.27	29.13	28.18
36	20.75	34.52	33.10	29.89	30.87	17.21	30.52	31.42	33.83	32.09	31.24	29.10	28.16
38	20.80	34.52	33.14	29.93	30.92	18.16	30.48	31.38	33.83	32.03	31.20	29.08	28.12
40	20.81	34.52	33.11	29.91	30.90	19.04	30.44	31.37	33.81	31.99	31.16	29.04	28.08
42	20.78	34.49	33.09	29.87	30.87	17.67	30.40	31.33	33.78	31.93	31.11	29.01	28.03
44	20.78	34.44	33.05	29.67	30.83	18.16	30.50	31.37	33.85	31.88	31.09	28.96	27.96
46	20.77	34.54	33.04	29.60	30.74	23.33	30.52	31.42	33.92	31.86	31.05	28.95	27.91
48	20.76	34.59	33.23	29.85	30.55	31.75	30.49	31.40	33.92	31.84	31.05	28.91	27.80
50	20.70	34.61	33.33	30.21	30.77	20.04	30.43	31.33	33.92	31.77	30.99	28.88	27.73
52	20.77	34.76	33.45	29.90	30.63	20.50	30.38	31.39	34.10	31.76	30.98	28.85	27.63
54	20.88	34.79	33.43	29.67	30.50	18.21	30.35	31.40	34.05	31.72	30.95	28.84	27.64
56	20.97	34.81	33.56	29.62	30.55	19.36	30.28	31.39	33.99	31.66	30.89	28.80	27.62
58	21.09	34.91	33.64	29.47	30.67	19.82	30.35	31.04	33.95	31.56	30.89	28.75	27.62
60	21.26	34.91	33.59	29.45	30.61	20.33	30.41	31.48	34.02	31.49	30.88	28.73	27.56
Min	20.59	32.29	31.88	29.45	30.50	12.69	30.28	30.18	32.17	31.49	30.88	28.73	27.56
Max	21.26	34.91	33.64	30.54	31.53	23.33	31.22	31.62	34.10	32.83	32.10	29.78	28.38
Mean	20.81	34.46	33.15	30.03	31.02	19.19	30.65	31.35	33.69	32.21	31.43	29.22	28.06
Diff	0.38	0.39	0.29	-0.98	-0.85	1.85	-0.81	-0.11	0.55	-1.31	-1.13	-0.92	-0.79

Table 2.4

Air, core, chest temperatures

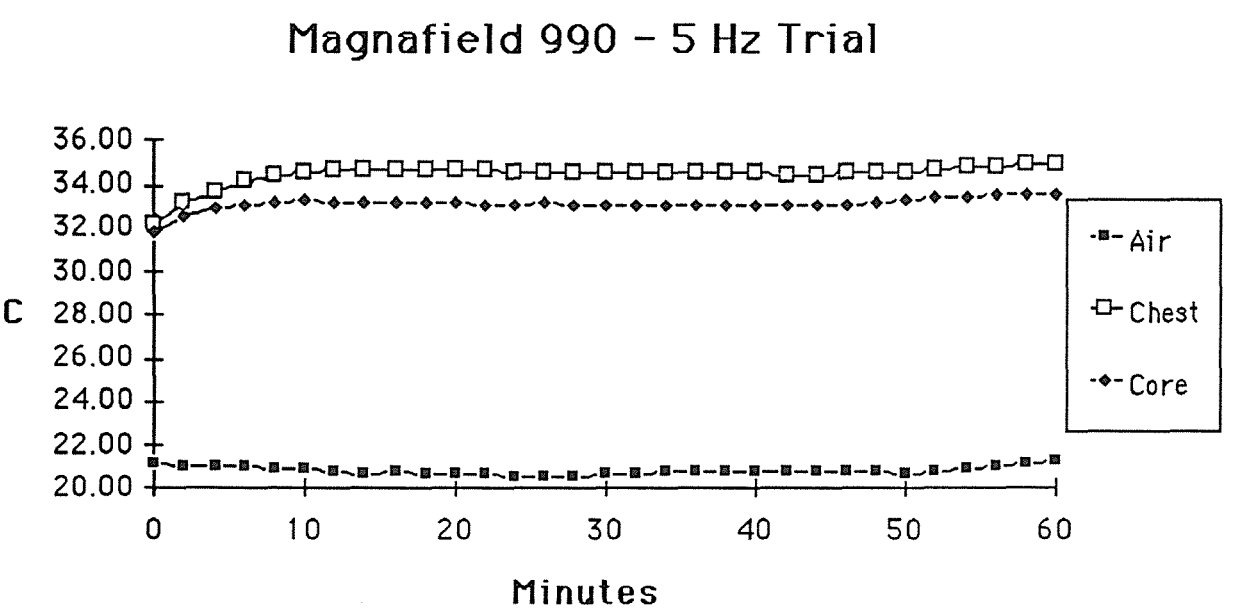


Figure 2.8

Peripheral site temperatures

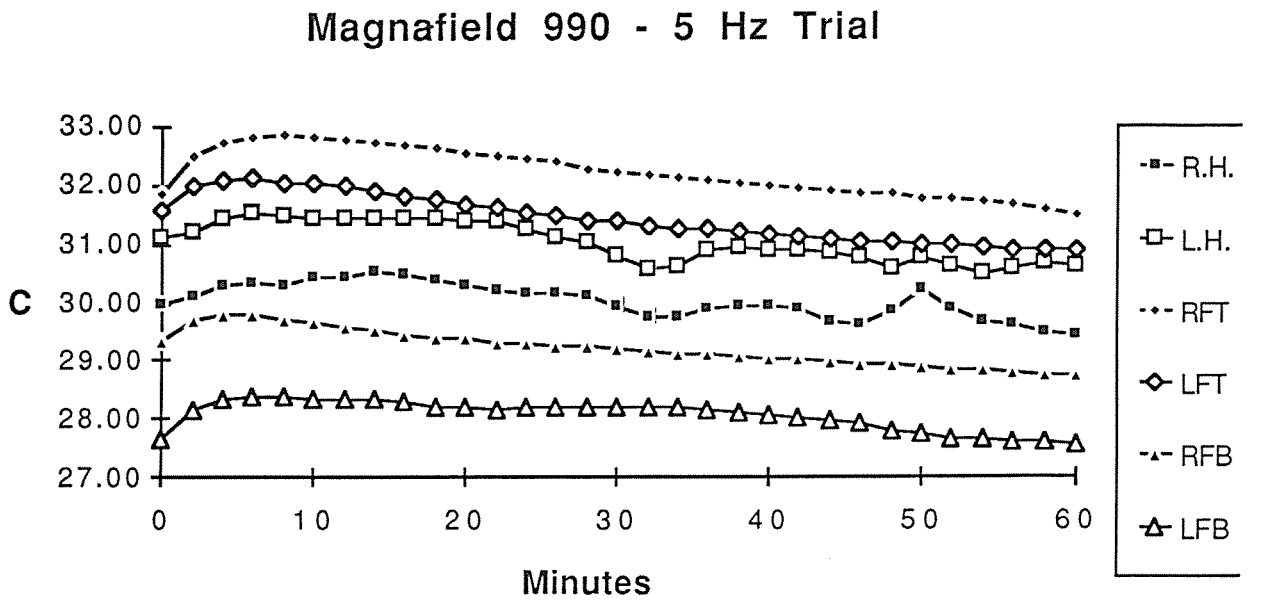


Figure 2.9

Difference temperatures - all sites

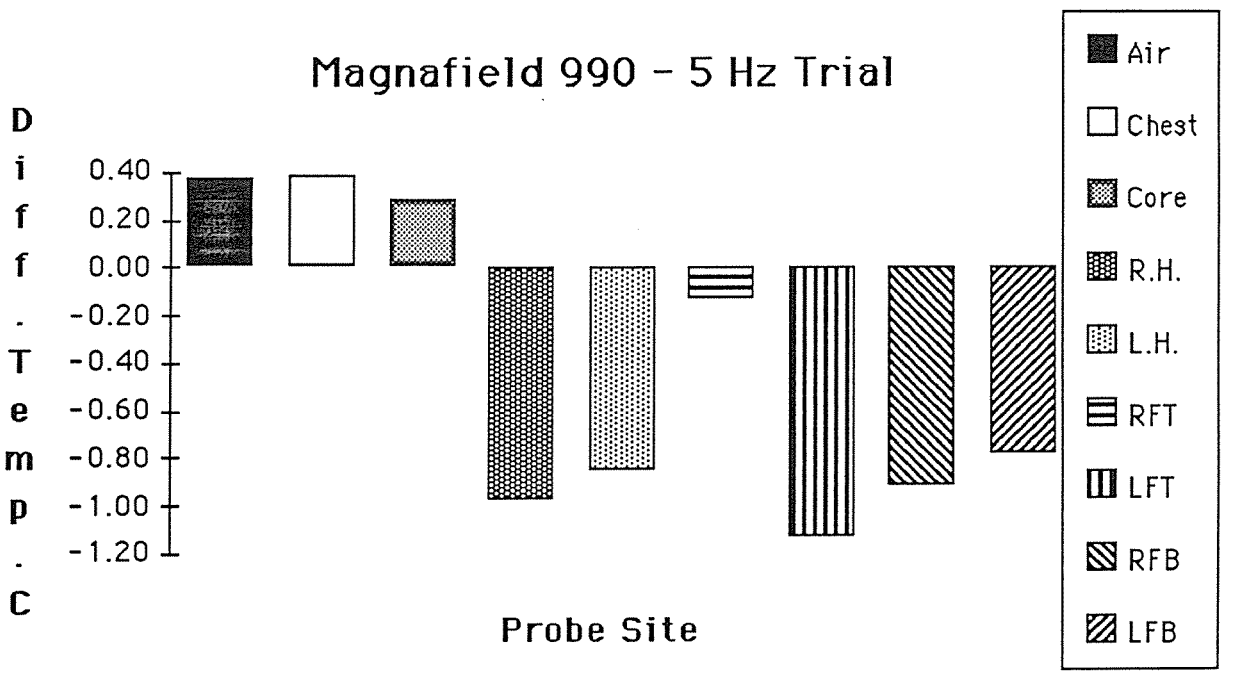


Figure 2.10

The results of the two trials are compared in Figure 2.11. The data presented are difference temperatures, i.e. $T_{60} - T_{10}$. (The first 10 minutes are used as an equilibration period as previously mentioned.)

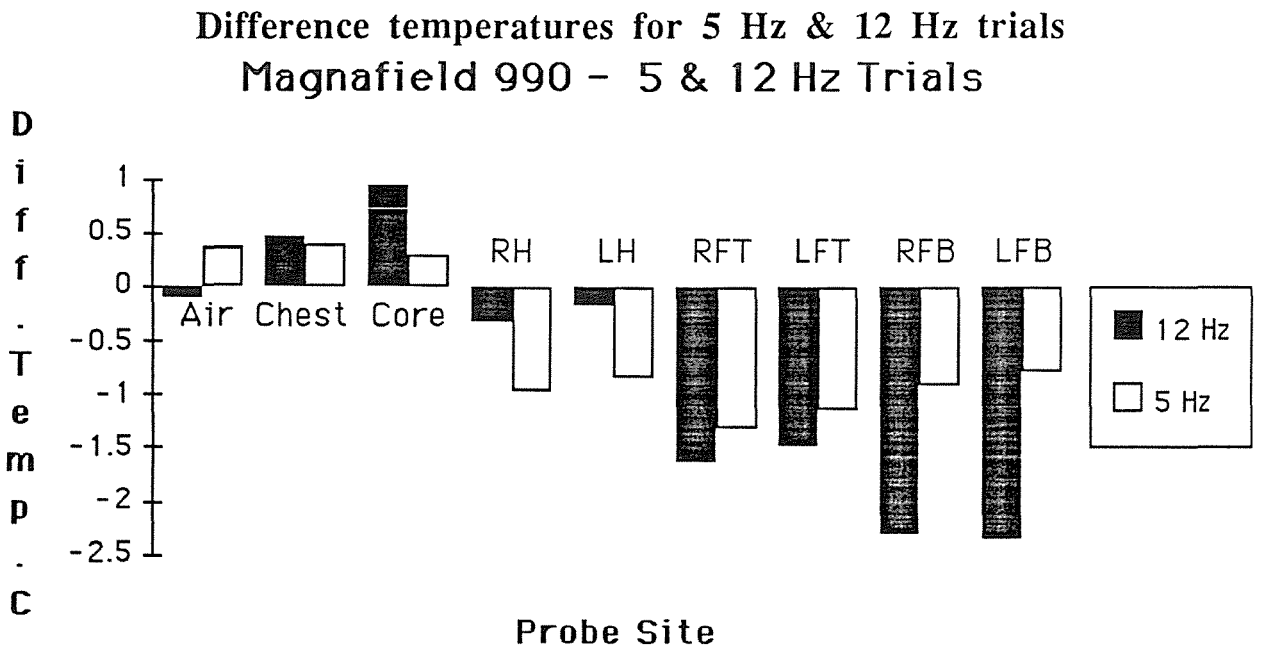


Figure 2.11

The ambient temperature appeared to differ little in both trials, the mean being 20.0 C for the 12 Hz trial, and 20.81 C for the 5 Hz trial. It can be concluded that these minor perturbations of ambient temperature are too small to influence the subject's thermal response while lying recumbent under a sheet.

The chest temperatures also illustrate this similarity being both extremely stable and virtually identical. It is suggested that the slight rise in both cases is due to a slightly longer equilibration period and the effect of the covering sheet. It must be remembered that the chest is the single largest body volume being measured

for surface temperature. It also contains the majority of vital organs, necessitating the most stable temperature control. The results tend to support this.

The core temperature differences, while more marked, are of little significance considering the difficulty in estimating this value from skin surface.

The hand temperatures are interesting as they are controlled as "peripheral sites", rather than body "core", but were not the area subjected to magnetic stimulation. In the vasodilation trial, 12 Hz, both hands became slightly colder, less than 0.5 C. However in the vasoconstriction trial, 5 Hz, both hands got considerably colder - of the order of 1 C. These values should be compared to the feet, the peripheral areas subjected to the magnetic field, and where one could expect most reaction.

The feet did in fact change more in temperature than any other part of the body, however the results are somewhat incongruous: The 12 Hz, vasodilation trial, yielded in the range of -1.5 to - 2.5 C. This is disturbing as the manufacturers claim that 12 Hz will have the effect of dilating blood vessels, thus bringing more blood into the tissue, thereby warming it. (For a more complete discussion of blood flow vs skin surface temperature, please refer to Chapter Five.) This was certainly not the case for these results. It must also be stressed that the Magnafield 990 was used in strict accord with the operating manual.

The 5 Hz, vasoconstriction trial results are somewhat puzzling, as while the temperatures of the feet still dropped, they dropped less than in the 12 Hz,

vasodilation trial. Could this be interpreted as evidence for vasoconstriction at the 12 Hz vasodilation condition, i.e. by getting 'less cold' than the vasoconstricting condition ? The manufacturers explanation of the specific effects of these treatments would be better served if this subject's results were 'reversed'. Unfortunately this is not the case, and there can be little evidence that the Magnafield 990 caused stimulation of the physiological responses claimed by the manufacturers, at least with this subject. It tends to support the theory that there is no single response for all subjects, given the same magnetic stimulation.

2.4 CONCLUSION

It is clear from Figures 2.10 and 2.13 that vasodilation did not occur in either experiment. In both cases the temperature of the feet dropped considerably. In the case of the 12 Hz "vasodilation" trial the mean temperature drop of the feet was 3.89 C. This is conclusive evidence that vasodilation did not occur. By contrast, in the 5 Hz "vasoconstriction" experiment, the mean temperature drop for both feet was only 1.04 degrees C. While it could be argued that vasoconstriction may have occurred, the temperature drop is significantly smaller than those recorded in the 12 Hz "vasodilation" experiment. It is as if the results have been 'reversed'. This, however, is not the case.

In the absence of further investigation, it can only be concluded that the Magnafield 990 did not stimulate vasodilation when used in accordance with the manufacturer's guide-lines, however some vasoconstriction may have occurred in the 5 Hz trial. It is not possible to draw further conclusions based on only two experiments.

While these results are unable to validate the manufacturer's claims, further tests would be required, using a number of different subjects, to determine the precise nature of the interaction of 50 Hz electromagnetic fields pulsed at 5 Hz and 12 Hz respectively. A reasonable hypothesis would be that different individuals would respond differently to applied magnetic field therapies, just as individuals respond differently to pharmaceutical drugs.

3

Description of the Magnetic Biostimulator Design Process

CONTENTS

3.1 SYSTEM OVERVIEW

The biomagnetic stimulator can be functionally described in three discrete modules: a function generator to provide the stimulus waveform; an amplifier to increase the signal magnitude; and an application (magnetic induction) coil, shown in Figure 3.1.

Basic Biostimulator Modules

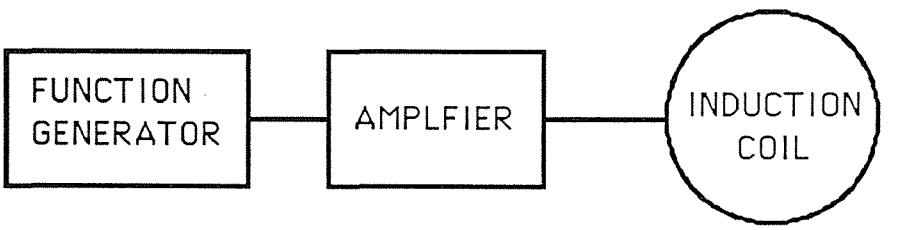


Figure 3.1

The function generator may in turn be sub-divided into three sub-modules: a function generator to provide the stimulus signal; an analogue switch to gate (pulse) the stimulus waveform; and a second function generator to drive the gate, shown in Figure 3.2.

Function Generator Biostimulator Modules

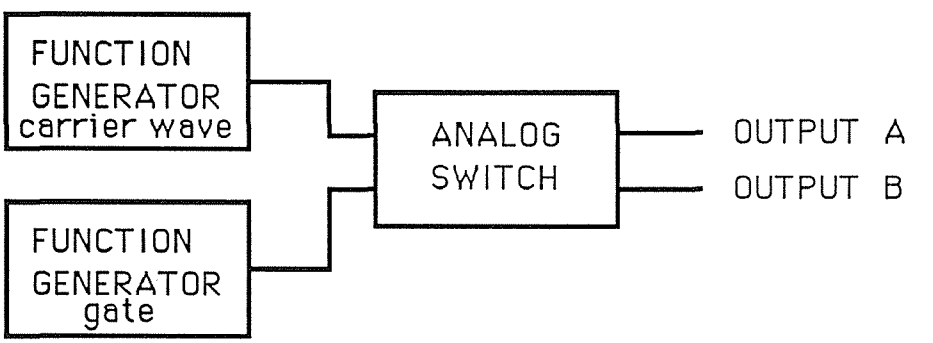


Figure 3.2

The output of such a system is a pulsed carrier wave. As the exact biological significance of the pulsed carrier wave is yet to be determined, four alternatives approaches have been considered: a simple analogue switch; a zero-crossing analogue switch; a pseudo-random analogue switch; and a programmable analogue switch.

The simple system described above requires manual setup and may be used to produce only one type of stimulus for any one situation. In order to provide for more flexibility it was decided to design a computer interface to allow the stimulus frequency to be rapidly changed automatically during a single experiment. The complete system is shown in Figure 3.3.

Complete Magnetic Biostimulator Schematic

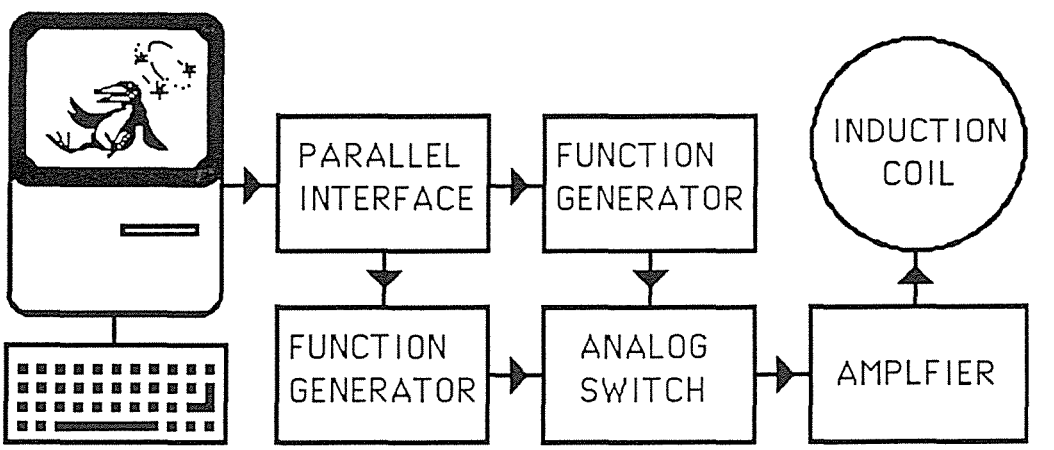


Figure 3.3

3.2 DETAILED TECHNICAL SPECIFICATION

Based on the investigation of a magnetic biostimulator (Chapter 2) the minimum specifications of a general purpose unit would be:

Carrier wave frequency	50 Hz
Modulation frequency	0 - 35 Hz
Field strength at the coil	6 mT
Waveform	Pulsed Sinewave

The carrier wave used in the treatment of recalcitrant fractures and osteonecrosis (bone death) however is 4,000 Hz, Bassett $_1$. The modulation frequency is either 15 or 72 Hz. Unfortunately the magnetic field strength is not quoted.

In order to design a research instrument capable of being used for a wide range of experiments, the range of both the carrier and modulation frequency need to be extended from the brief specification above. The extremely low frequency (ELF) region of the electromagnetic spectrum is generally defined as 30 - 300 Hz. However biological systems can respond to considerably higher frequencies, Polk & Postow$_2$. Microwaves modulated at E.L.F. frequencies also stimulate biological responses, (ibid. chapter 5). Currently, due to insufficient research, the upper limit of non-ionizing radiation frequencies which may illicit biological responses is not known. The setting of an upper limit is therefore somewhat arbitrary.

It was decided to set the frequency range for the carrier wave at 3 - 9999 Hz, (see section 3.3 on function generator for details). In order to achieve the widest practical specification the same range was chosen for the modulation frequency generator. This would however mean that the modulation frequency could be set to a higher value than the carrier wave, so care would need to be exercised when setting up the equipment for an experiment.

The field strength used clinically, according to several physiotherapists (personal communication) is somewhat arbitrary. There is some suggestion that as long as it is greater than approximately 1 mT, the exact value is somewhat less important.

The operation manuals of the two units investigated, (Magnafield 990 manufactured by Magnacare Pty. Ltd. of South Australia, and the Bi-Phasic Multi Rhythm instrument produced by Emmet Glen Pty. Ltd. of Brisbane, Australia) make no mention of the field strength to be used for any particular ailment. Both companies provide tables of treatments which include the pulse frequency and duration of treatment for a number of conditions, but fail to specify the field strength. The Magnafield 990 has a rotary gain control for adjusting the magnetic field magnitude, while the Bi-Phasic only offers a high / low switch. Nowhere in the documentation however do either company explain which field strength is appropriate for any particular condition. Nor do they explain how the therapist may determine the preferred setting for a particular patient.

In relation to instrument design, and in the absence for any further information, 5 mT was chosen as the target value.

The coil design is a more difficult issue to decide on as there are many different types in use ranging from the flat involute spiral used by Magnacare and Emmet Glen, to the 1 metre body solenoid of the Elec-Western. In order to maintain a field of 5 mT over a volume of the body fitting solenoid 900 mm in diameter and 600 mm long, a very high powered amplifier would be needed. Such an amplifier is well beyond the budget of this project, so it was decided to concentrate on: the flat involute spiral; the squat solenoid; and the Helmholtz pair.

While many different waveforms are possible, the present wave shapes chosen for the prototype stimulator are the sine and square wave. Both the Magnafield 990 and the Bi-Phasic instruments use a sinewave for the carrier signal, gated by a squarewave.

It is interesting to note that a sinewave consists of a single frequency with little harmonic content, while a square waves consists of a combination of many frequencies (harmonics). It should therefore be possible to design experiments to determine the effect of harmonics on biological systems.

3.3 TIME-BASE GENERATOR

3.3.1 Overview

The main components of the timebase generator are: a voltage controlled oscillator; a Phase-Locked Loop (PLL) programmable divider; a 1 megahertz crystal reference oscillator and fixed divider to provide a 100 Hz reference signal; a comparator; and an output filter. This is shown schematically in Figure 3.4. The reference signal is stable to within 25 ppm with an aging factor of approximately 2 ppm per year.

3.3.2 Principle of operation

The main elements in the phase-lock loop are a Voltage Controlled Oscillator (VCO), phase comparator; and an output filter. The 100 Hz reference signal feeds one input of the phase comparator, while the other input is derived from the output of the VCO through the programmable divider. The output of the phase comparator is then passed through a filter to produce the tuning voltage for the VCO.

If the frequency of the VCO is initially the same as the divider frequency, (i.e. the loop is locked), then there is no output from the phase comparator and the filter voltage is held at the constant level maintaining the VCO's frequency. If the ratio is suddenly changed via the digital-switches on the programmable divider, then the filtered output of the phase comparator will drive to change the VCO frequency to match the new value from the programmable divider.

A sinewave is generated from the squarewave output using a low pass tracking filter. This module utilises a fifth order capacitive filter with a cutoff frequency 100 times its clock frequency. A clock frequency of 100 times the output frequency is obtained by tapping-off the divider chain two decades above the main output. The resulting sinewave has less than 1% total harmonic distortion (THD) and operates over the range 3-9,999 Hz. The minimum frequency obtainable is 3 Hz due to timing requirements of the programmable divider chip which uses a minimum divide ratio of three.

The biostimulator employs two such timebase generators, one for the carrier wave, and one for the modulation of the gate as shown in Figure 3.2.

3.3.3 Schematic diagrams

The operation of the function generator is shown schematically in Figure 3.4.

Timebase Generator Schematic

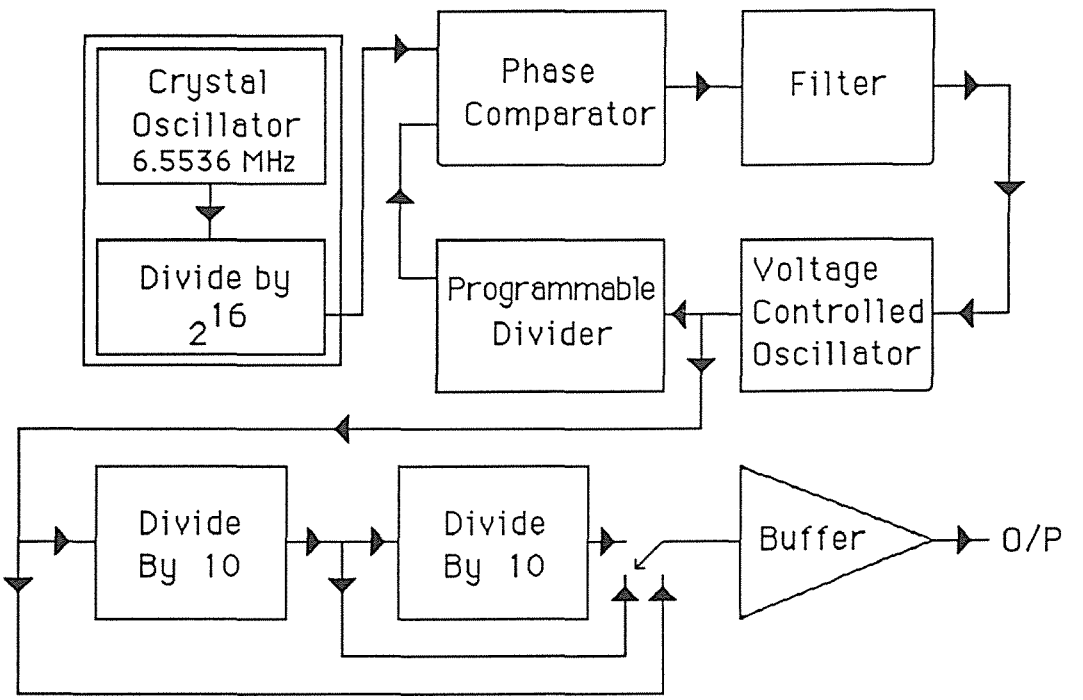


Figure 3.4

The full circuit diagram schematic is shown in Figure 3.5.

Circuit Schematic

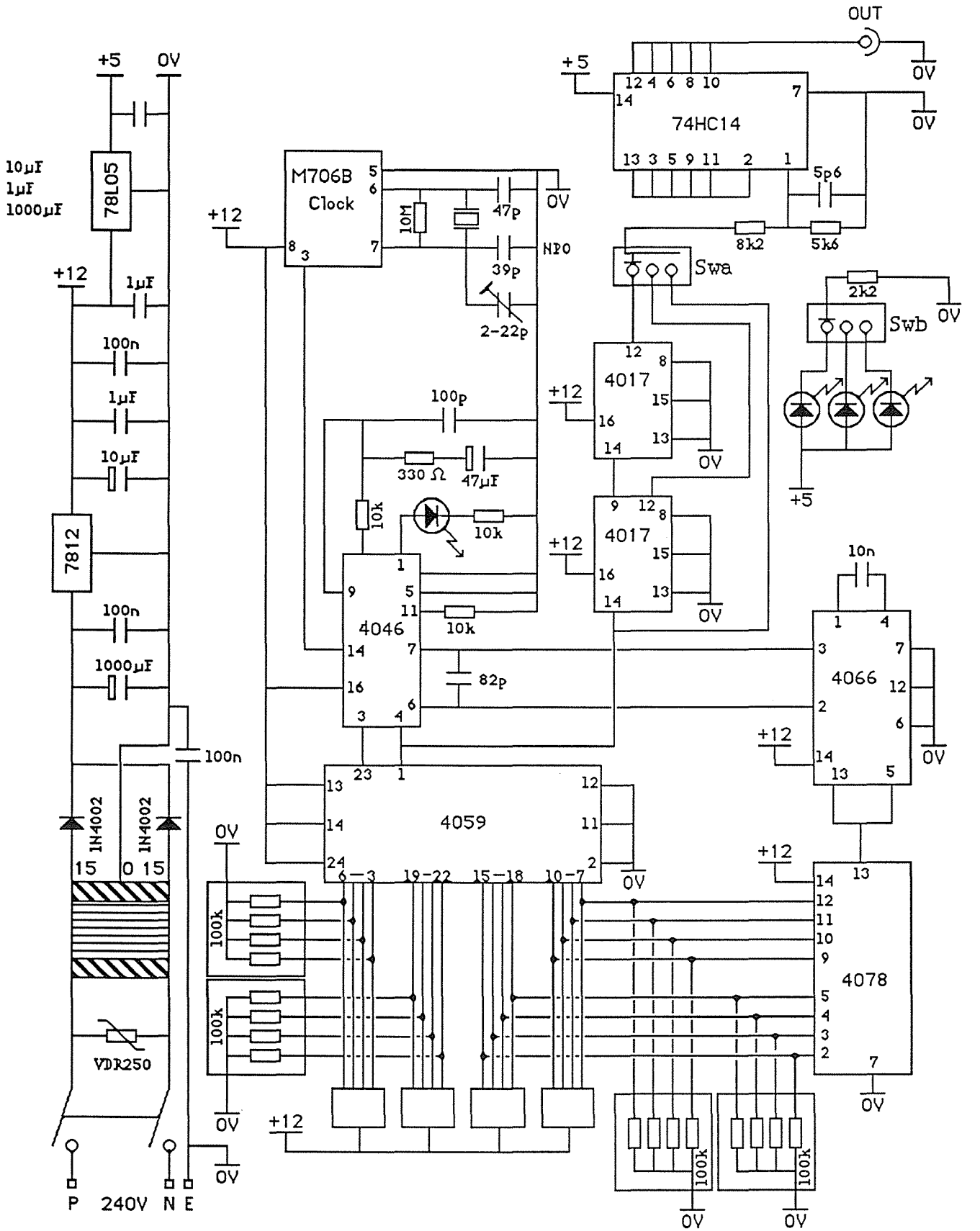


FIGURE 3.5

The function generator was constructed on a standard single-sided fibreglass printed circuit board. The negative is shown at approximately real size in Figure 3.6.

Printed Circuit Negative

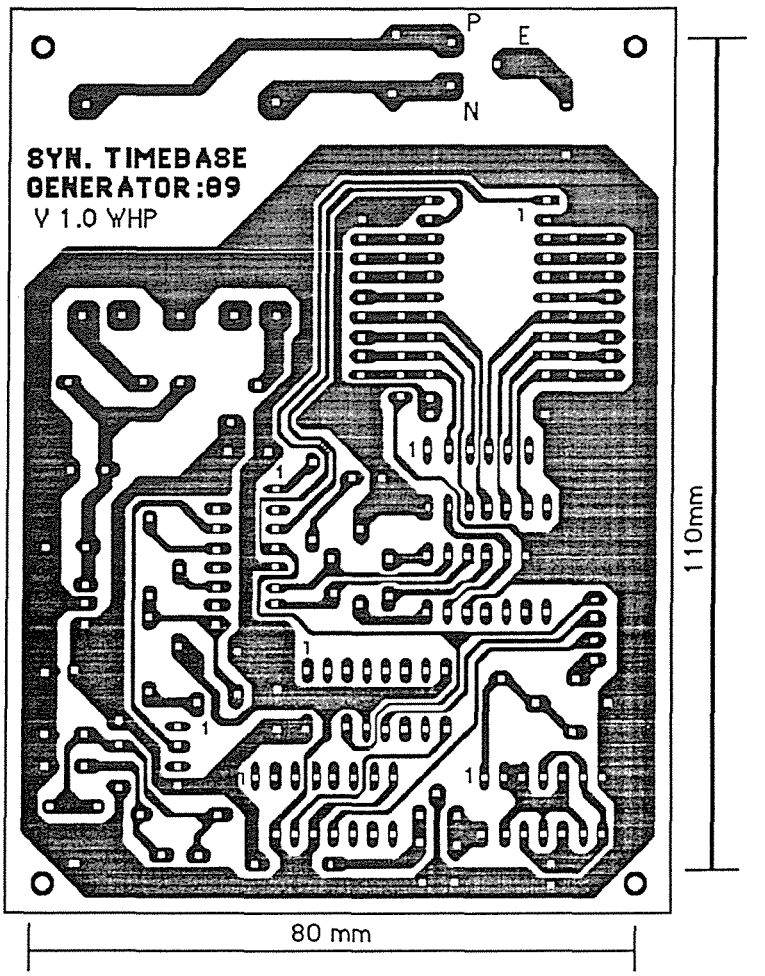


Figure 3.6

3.4 TIMEBASE GENERATOR COMPUTER INTERFACE

3.4.1 Overview

In some circumstances it may be desirable to alter the frequency of either the carrier or the modulation signal during the course of an experiment. Such an example may seek to test the effect of a number of frequencies on a particular system. It was decided that the most appropriate way to do this is have the timebase generator under computer control. The frequency of the timebase generator is manually set by four binary coded decimal thumb wheel switches. In principle then, it is a simple matter to provide the binary coded decimal output from a computer parallel port to substitute for the thumb wheel switches.

3.4.2 Principle of operation

An interface has been designed which will work with any standard parallel port, for example the Apple IIe and IBM compatible personal computers. Nine lines are required: the standby; seven I/O lines; and ground. An octal TTL compatible buffer (74HCT244) takes the output from a parallel port and converts it into two four-bit CMOS lines. Four lines are used for the address decoding (74HCT138), and four lines to code the four decades, (1, 10, 100, 1000) to set the frequency of the timebase generator (dual 4 bit latches 4508). A four-bit latch (74HC375) drives a quad analogue switch (4066) which is used to switch the range multiplier on the synthesized timebase generator giving: 1x; 10x; 100x; and 1000x.

3.4.3 Schematic diagrams

A functional schematic diagram of the computer interface is shown in Figure 3.7.

Timebase Generator Computer Parallel Port Interface Schematic

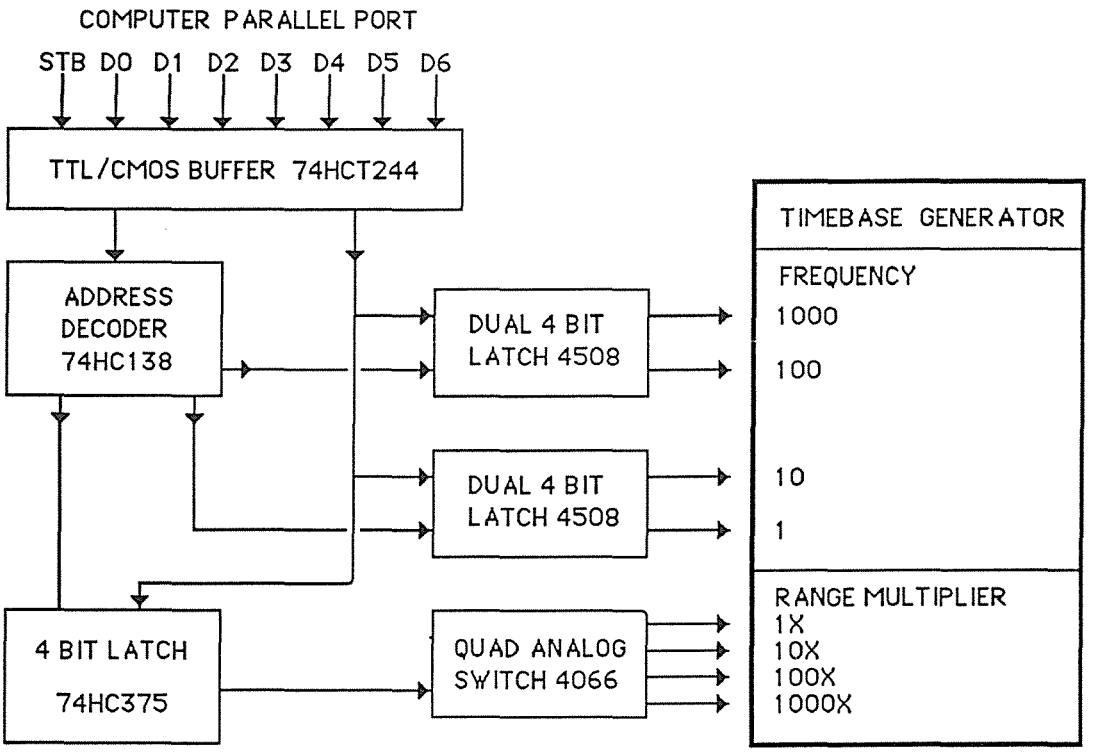


Figure 3.7

A full circuit diagram schematic is shown in Figure 3.8.

Timebase Generator Parallel Port Interface Circuit Schematic

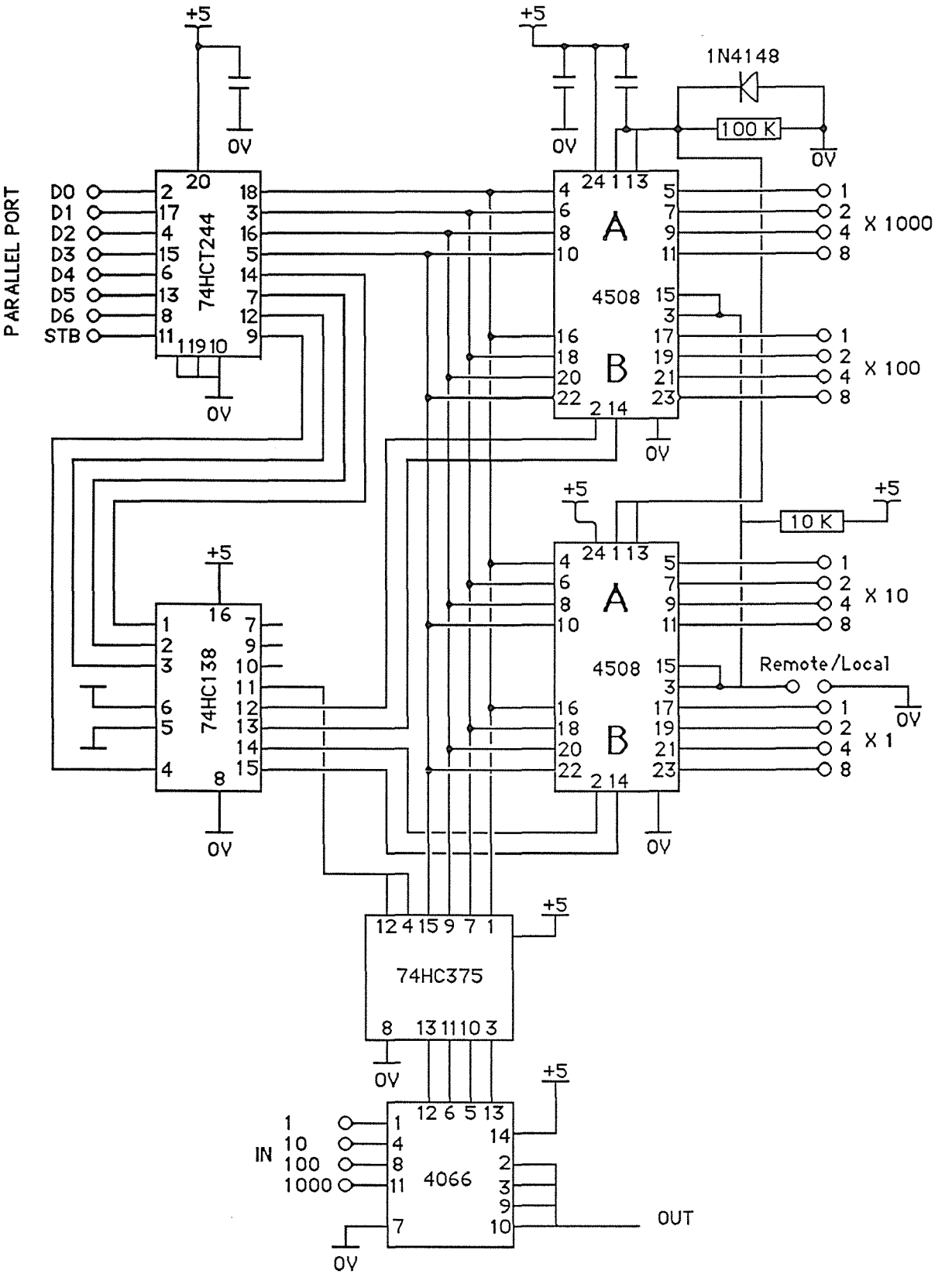


Figure 3.8

The computer parallel interface for the function generator was constructed on a standard single-sided fibreglass printed circuit board. It was designed identical in size to the function generator so that the two units may be stacked vertically. PCB pin connectors are used to connect the pads which would normally be connected to the BCD thumb wheel switches to the output of the two dual 4 bit latches. The two boards may be plugged together and 15 mm spacers inserted between the boards at each corner post. The printed circuit negative is shown approximately full size in Figure 3.9.

Function Generator Computer Parallel Port Interface Printed Circuit Negative

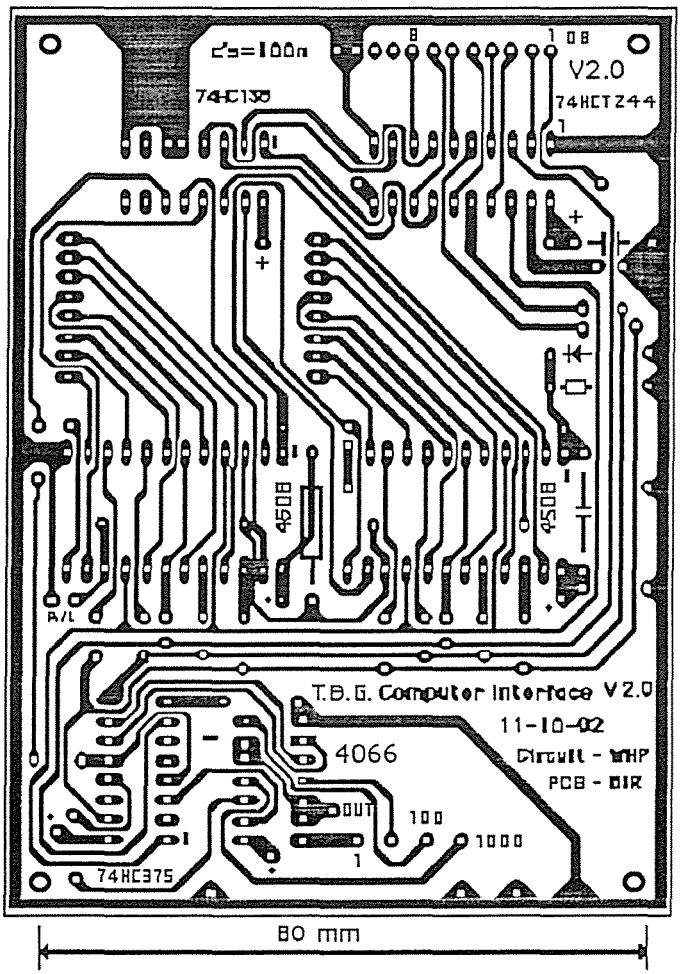


Figure 3.9

3.5 THE REQUIREMENT FOR A GATE

3.5.1 Overview

The waveform used to treat recalcitrant fractures, Bassett[3,4,5], may be described as a train of 3.5 kHz pulses gated at 15 Hz, E.Davies[6]. The stimulus for treating osteonecrosis (literally - bone death) is a stream of asymmetric 38 msec pulses repeated at 72 Hz, ibid. The precise biological requirements of such a pulse train, such as mark-space ratio, have not yet been adequately determined, however several clinically effective regimes have been determined.

The origin of the stimulus signals used is worthy of mention as they are based on in vitro experiments on hydrated living bone. Bone has been known to exhibit the piezo electric effect since Fukuda's landmark paper[7] in 1957. The semiconducting properties of bone are said to be attributed to the hydroxy apatite crystals locked in the collagen protein matrix. In addition bone contains calcium ions along with copper ions imbedded in the protein / hydroxy apatite matrix.. Becker[8]. When hydrated bone is subjected to physical stress it produces streaming potentials in its interstitial fluids.

Elizabeth Davies[6] in her historical review recounts the original experiments of Bassett which aimed at determining the endogenous waveforms. Shielded electrodes were attached to the surface of a bone, which was then subjected to mechanical loading, as part of a normal biological activity of weight bearing, and the electrical signals were observed on an oscilloscope.

Mechanical stimulation was then stopped and a coil of copper wire placed close to the bone was connected to a Taccusel PIT-20-2A potentiostat. The potentiostat was adjusted until the induced electric field picked up by the electrodes was identical to that produced by the mechanical deformation. Inductive coupling of the signal, via Helmholtz coils, avoided distortion evidenced by direct coupling, due to different dielectric properties of the many biological tissues involved in transmitting the signal. Variations of the signals were used to treat the damaged bones of live animals by a variety of investigators[1, 3, 4, 5, 8, 13, 14, 15].

By comparing the results of treated animals with controls given a placebo treatment, the most effective signals was determined. Such experiments lead Bassett and Becker to the two regimes mentioned above.

3.5.2 Principle of operation

In order to produce a pulse train, the signal from one of the timebase generators may be used to modulate or switch the signal of the other, via an analogue gate. Alternatively, the timebase generator output could be gated by some other type of signal generator. In order to more fully understand the requirement for a pulse train type signal, some brief discussion follows.

3.5.3 Neurophysiological function of the brain: a brief discussion

It is well known that the brain tracks changes in frequency of nerve impulses W.F.Ganong[9], C.A.Keele and E.Neil[10]. When an incoming signal attains a constant frequency over time, and supposing that such a signal is not indicative of a life threatening or dangerous situation, the brain may selectively ignore that individual input, Bini[11]. An example of this is to place a hand into slightly cold water. Initially the temperature sensing nerves relay a signal to the brain indicative of the new change in state, namely the hand is colder. If the hand is left in the water for some minutes, the temperature signal remains constant. The brain may decide to take some action, such as vasodilation, to reduce the degree of body cooling as a result, however this will not change the signal coming from the temperature sensing nerves close to the skin surface. Within a few minutes the brain will ignore the incoming temperature signals from the hand. There is no great danger so there is no need to take any further action. The body has now reached a new 'status quo' of dynamic equilibrium. The owner of the hand will have little or no sensation in the hand. Now if the hand is removed from the cold water the brain observes a change in state registered as a change in frequency of incoming nerve impulses. If vasoconstriction resulted from putting the hand into the cold water initially, that states will now reverse with vasodilation occurring over several minutes. The important point to note is that the brain may choose to ignore unchanging nerve signals in the absence of a life threatening situation.

For applied alternating magnetic fields to stimulate a biological response, the stimulus may need to be perceived as a constantly changing signal, thus the brain is forced to continually re-evaluate the situation. An alternative hypothesis may

be that the stimulus evokes some kind of response which requires a period of non-stimulation before further stimulation can have an effect. An example of this is the two-phase nature of photosynthesis: a light requiring phase followed by a non-light requiring phase.

In short, *the reason for the pulse train is not currently known.* It has simply been found to be a clinically effective form of treatment in some cases. Continual use of direct current fields has proved to be the cause of iatrogenic infections Bassett[13]. Pulsed d.c. and asymmetric a.c. were found to be as effective as constant dc for augmenting osteogenesis (bone growth) Bassett[13,14]. As a result, a quasi-rectangular waveform, shown in Plate 3.1 was proposed by Bassett and Pawluk[15].

In order to design a system capable of supplying a variety of pulse trains, three alternatives have been investigated:

Analogue Switch;

Pseudo-Random Switch;

and Programmable Interrupter or programmable pulse-width modulator.

The various alternatives will be discussed in Sections: 3.6; 3.7; 3.8; and 3.9.

3.6 SIMPLE ANALOGUE SWITCH

3.6.1 Principle of operation

In order to create a pulse train one timebase generator is used to switch the signal of another,via an analogue switch. The way in which this is achieved is displayed in Figure 3.10.

The first function generator produces a signal termed the carrier wave, either square or sinewave, while function generator two produces only a TTL compatible square wave. Both generators produce square waveforms in the frequencies in the ranges: 3 - 1,000 Hz by 1 Hz steps; 30 - 10,000 Hz by 10 Hz steps; and 300 - 100,000 Hz by 100 Hz steps. In addition both generators contain a 5th order filter which synthesizes a sinewave on the 3 Hz to 9999 Hz range. When the output of function generator two (V_2) is used to activate the analogue switch, the output signal from function generator one (V_1) is effectively turned on and off at the output with a 50% duty cycle, as shown in Figure 3.10. Also see Figures 3.13 & 3.14.

3.6.2 Schematic diagrams

The schematic diagrams for the simple analogue switch are presented overleaf:

Analogue Switch Operation

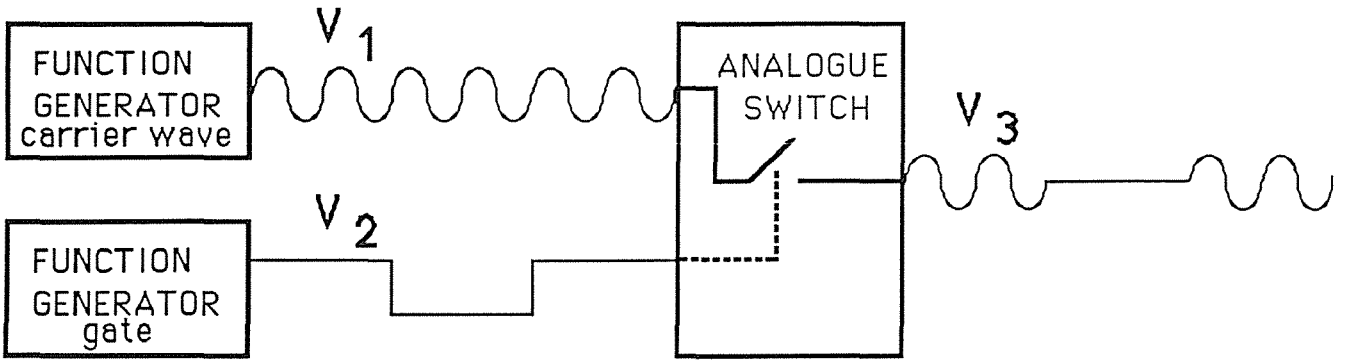


Figure 3.10

3.6.2 Schematic diagrams

Analogue Switch Schematic Overview

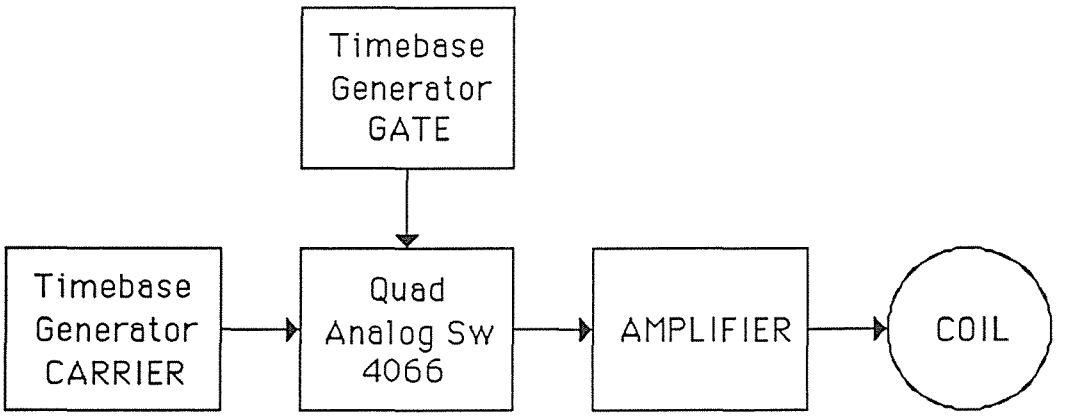


Figure 3.11

The design of the analogue switch module shown in the functional schematic in Figure 3.12.

Analogue Gate Functional Schematic

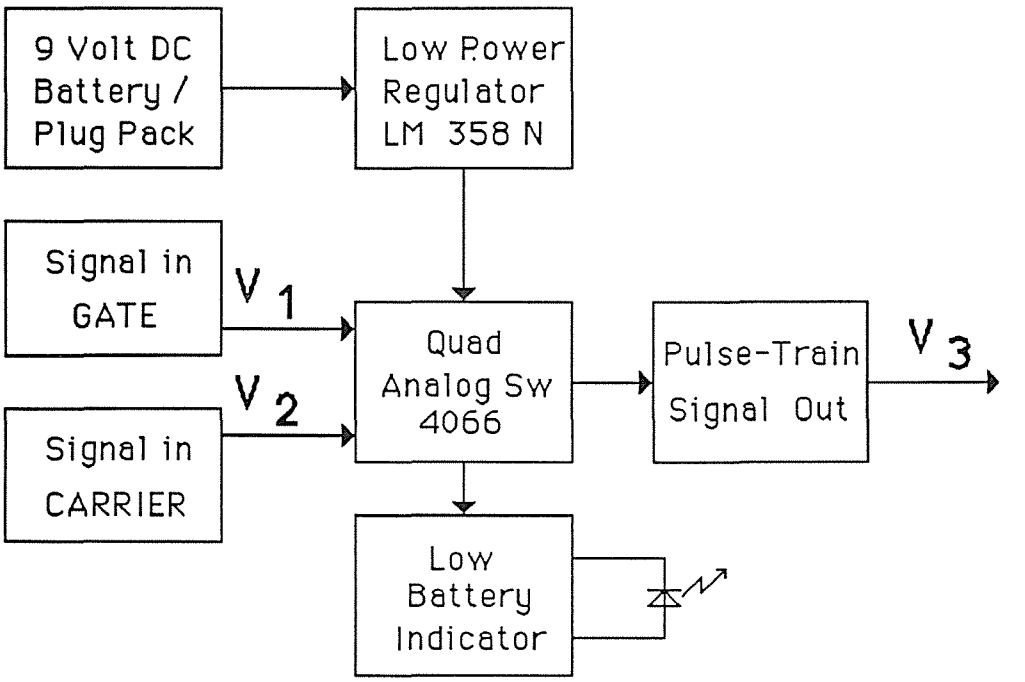


Figure 3.12

In order to switch on and off the carrier wave, it is necessary for the gate signal to be a square wave. The carrier wave can be any arbitrary bi-polar waveform, as the 4066 acts as a true analogue switch. An artificial centre zero is created from the input voltage. In this way a bipolar sinewave for example may be turned on and off at any desired frequency. The 4066 analogue gate is capable of switching a 40 MHz signal at up to 10 kHz.

The circuit schematic is shown in Figure 3.13

Analogue Gate Circuit Schematic

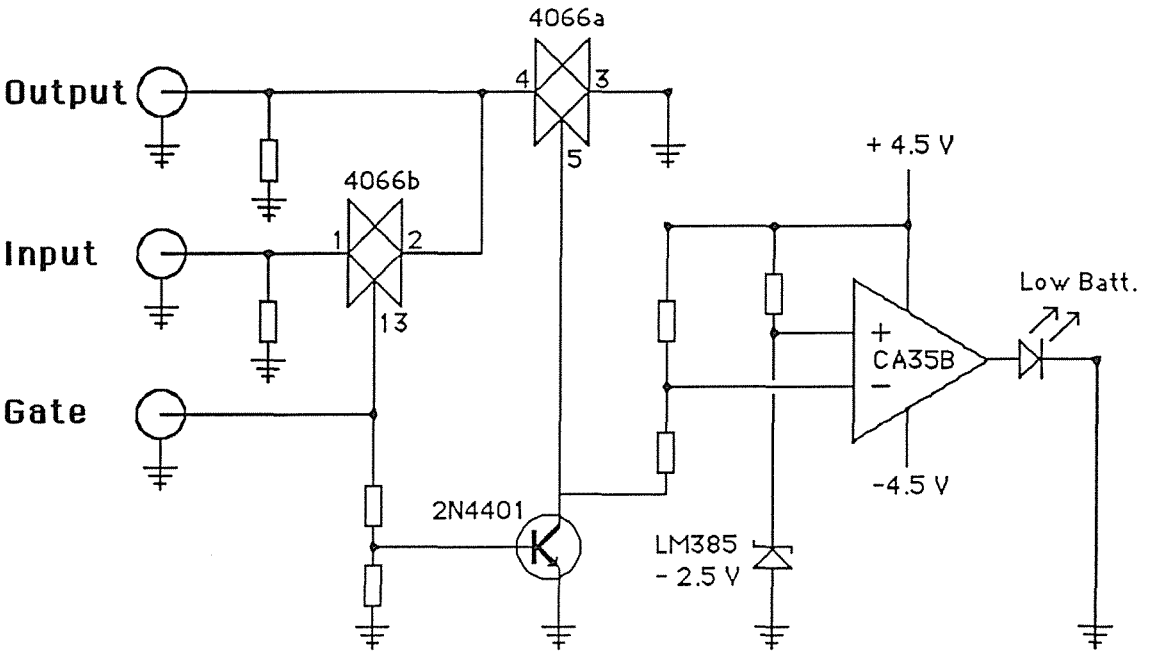


Figure 3.12

The analogue switch also makes use of a "rail-splitter" to allow the signal to swing both positive and negative with respect to zero volts, making it possible to handle truly differential signals. The circuit diagram is shown in figure 3.12.

Analogue Gate Circuit Schematic

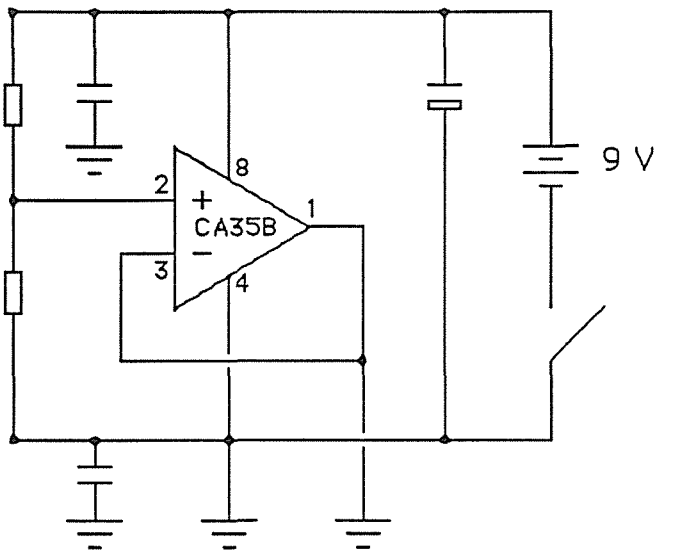


Figure 3.13

3.6.3 Zero crossing and reduction of harmonics

The circuit described does not necessarily operate the gate only at zero crossing with respect to the voltage waveform. This means that a carrier signal may be significantly above or below ground when being gated. This would result in a very sudden rise or fall, rather than the gentle gradient of voltage over time as in the sinewave example. The effect of this would be a sudden injection of harmonics which would not otherwise be present in the signal. A sinewave gated at zero crossing is shown in Figure 3.14. A possible example of non-zero crossing is shown in Figure 3.15.

Sinewave Gated at Zero Crossing

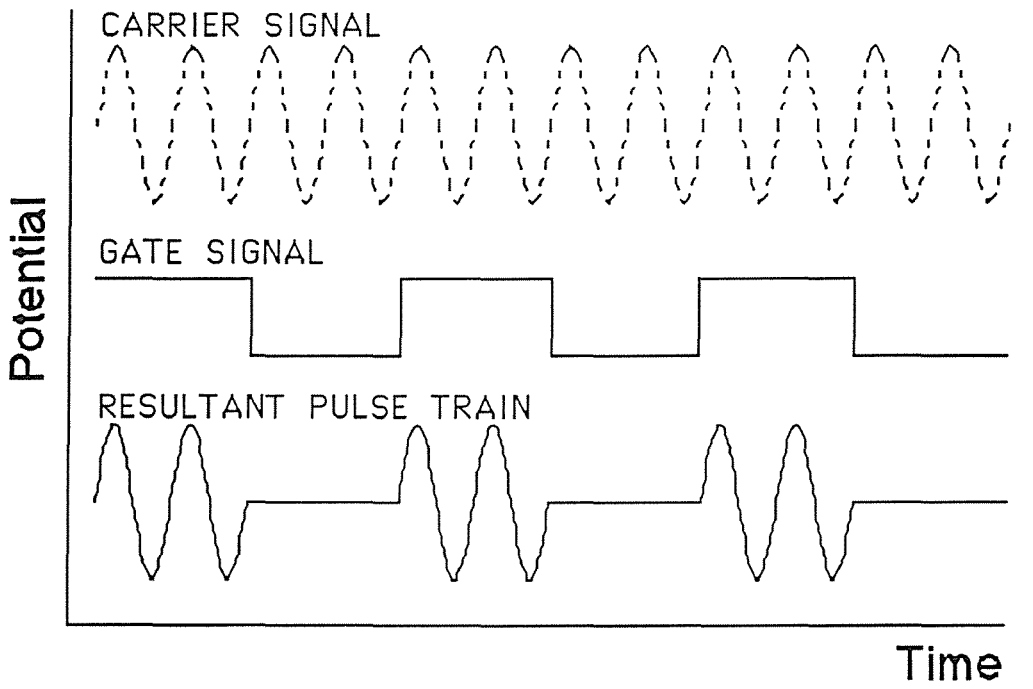


Figure 3.14

Sinewave Gated Non Zero Crossing

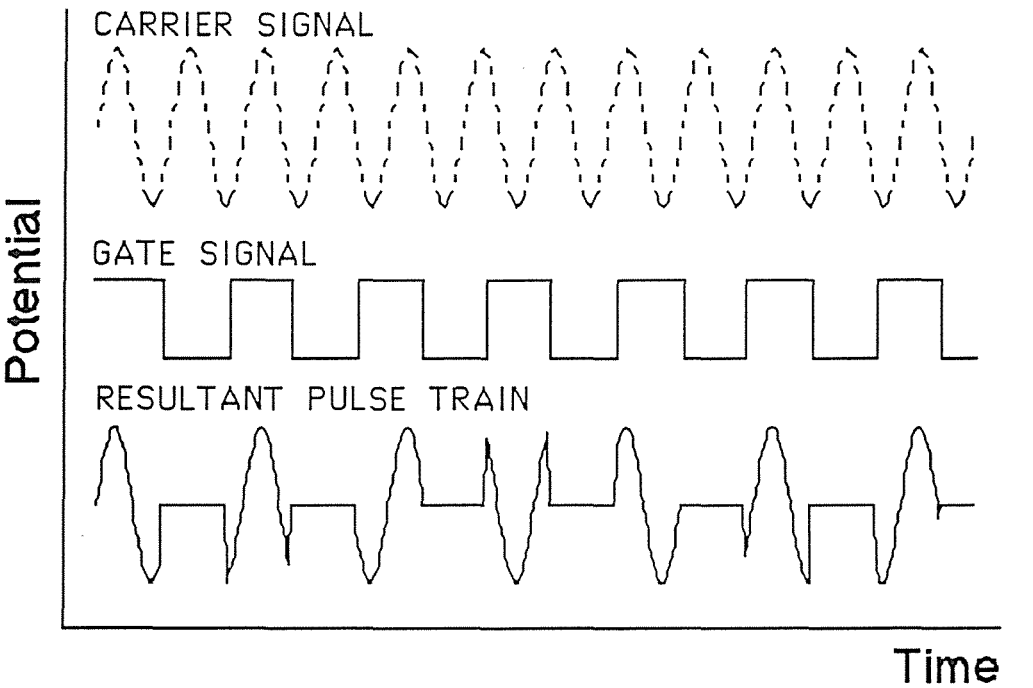


Figure 3.15

3.7 ZERO-CROSSING DETECTOR SWITCH

3.7.1 Principle of operation

In order to gate the carrier signal without generating additional harmonics it is necessary to switch the signal only when it is at zero volts. The circuit designed consists of a buffer amplifier, (TL071), with non-linear gain to allow for a wider range of input signals thus extending the range of the comparator. This is followed by a comparator, (LM311), with approximately 5% hysteresis to detect the zero crossing point. A transistor acting as an inverter selects either the rising or falling edge of the signal on which to trigger. A two-transistor network then shifts the level from 0 to 5 volts to +/- 7.5 volts for the analogue switch.

Signal generator two provides a TTL signal, either a 0 or 5 volt which then sets the dual D-latch to trigger on the next zero point from either a rising or falling (as selected) wave front. This then activates the analogue switches. If the TTL signal is zero, then the analogue switch (4066) is effectively open, and no signal appears at the output. In response to a 5 volt TTL signal, the analogue switch turns on at the next available zero point, gating the signal to the output, and hence to an amplifier.

3.7.2 Schematic diagrams

A functional schematic is shown in Figure 3.16 and the full circuit schematic in Figure 3.17.

Zero-Crossing Switch Functional Schematic

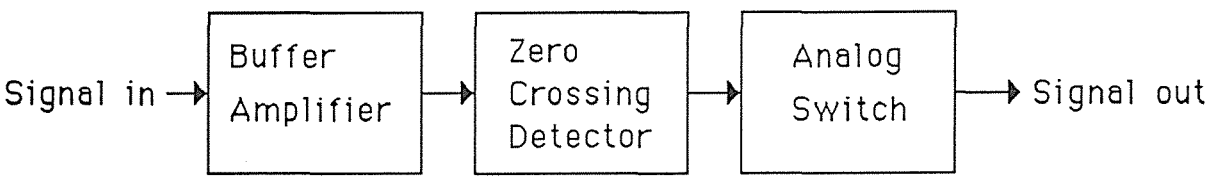


Figure 3.16

An additional feature has been incorporated which allows the signal to be gated on either the rising or falling edge. To eliminate noise, the input is effectively shorted to ground when the signal is not gated through. The 4066 contains effectively four analogue switches. These have been wired as two parallel pairs in order to reduce the effective gate resistance. The result is a low resistance electronic equivalent of a single throw, double pole switch. The output is thus gated to either ground, or the input signal, the change of state occurring only when the input signal approaches ground from either the positive or negative direction. The full circuit diagram schematic is shown in Figure 3.17.

Zero-Crossing Switch Circuit Schematic

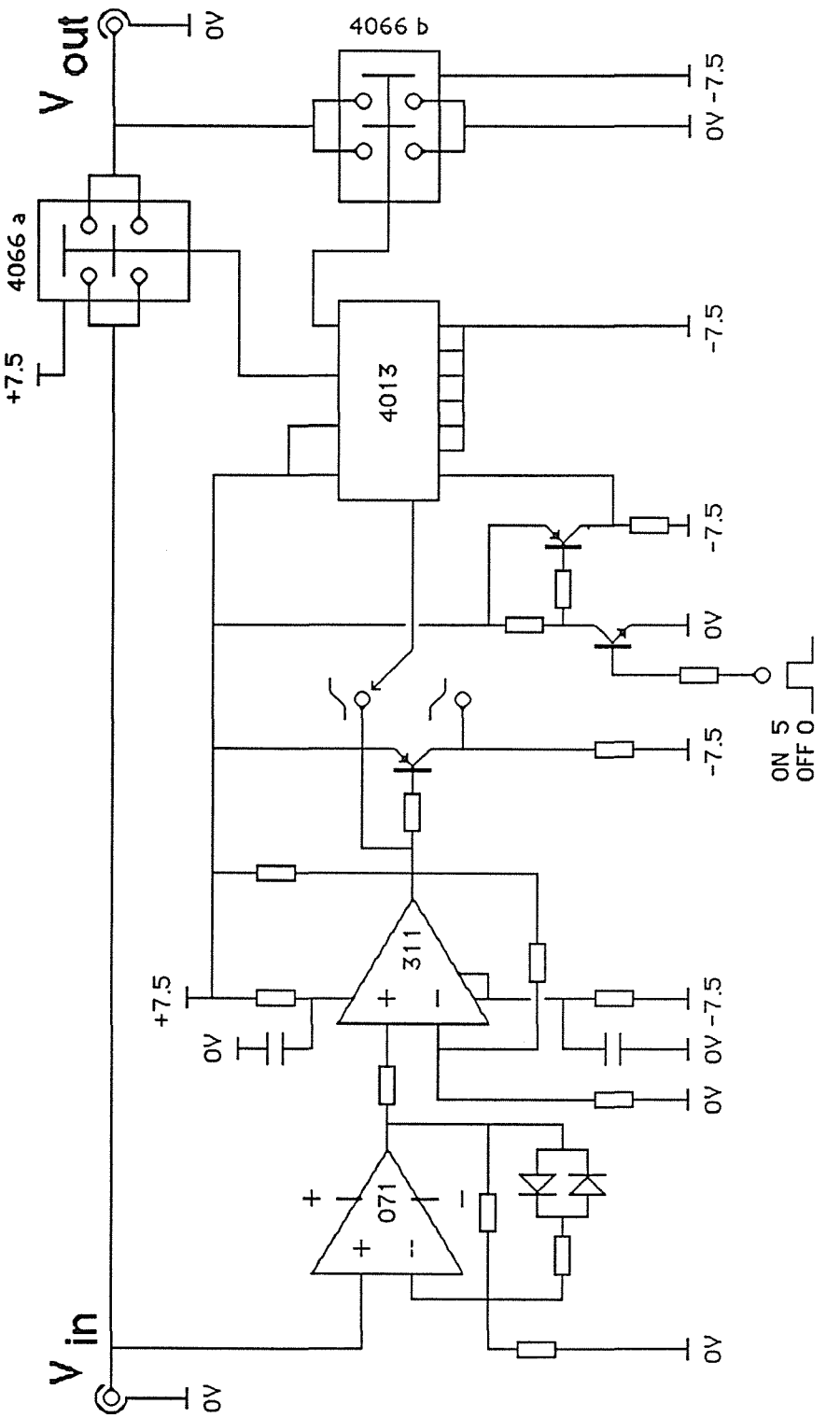


Figure 3.17

The zero-crossing switch was constructed on a standard fibreglass printed circuit board, the negative of which is shown in Figure 3.18. It was designed to be identical in physical size as both the timebase generator and the computer interface. This allows the final instrument to be constructed as a single stack of printed circuit boards.

Zero-Crossing Switch PCB

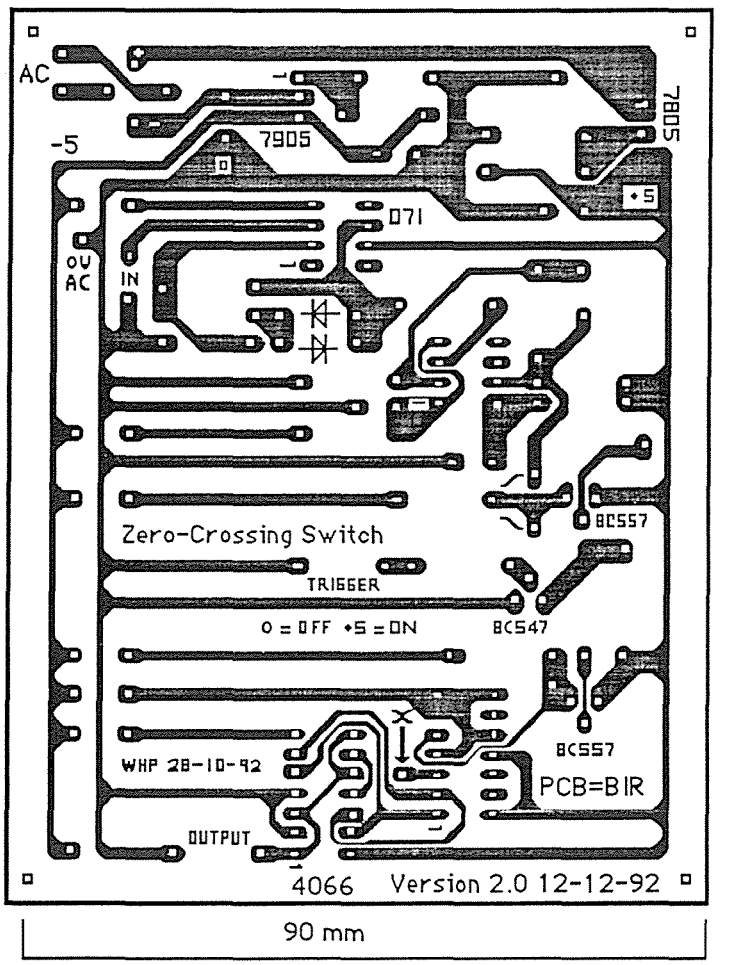


Figure 3.18

3.8 PSEUDO-RANDOM ANALOGUE SWITCH

3.8.1 Principle of operation

The second configuration utilises a pseudo-random number generator to activate the analogue switch. Two ranges have been chosen, 0.2 - 2.0 seconds, and 2.0 - 20 seconds. The signals from the function generators alternate between outputs A and B where the time in any one state is a random period in the range 0.2 - 2.0 or 2.0 - 20 seconds. This is shown diagrammatically in Figure 3.19. The logic state of the switch is shown below the two output signals to indicate the random period of the internal gating signal.

Pseudo-random Analogue Switch Operation

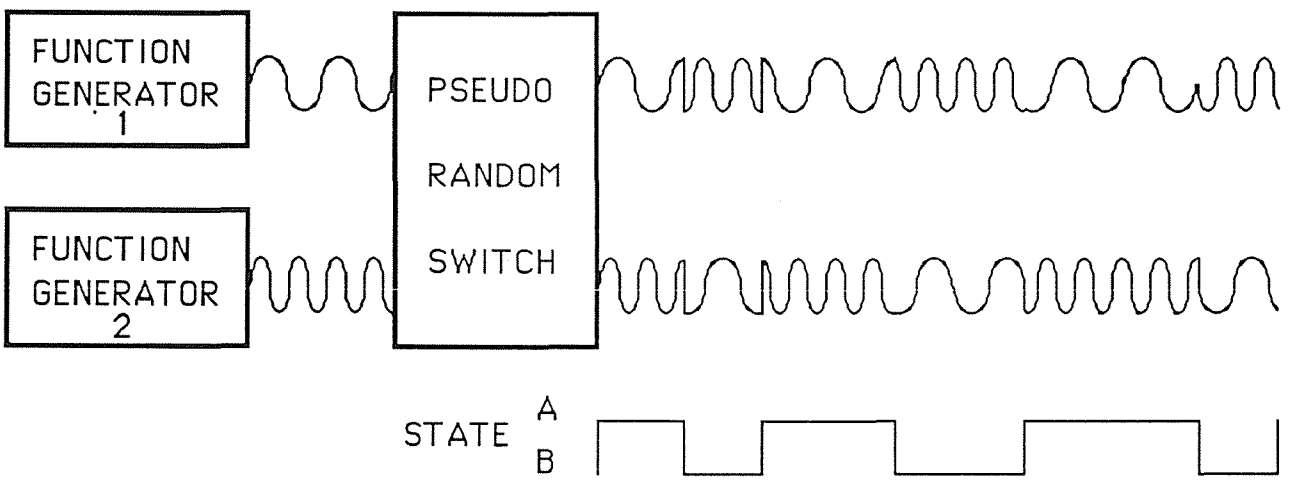


Figure 3.19

3.8.2 Schematic diagrams

The pseudo-random gate utilises a pseudo-random number generator the output of which is passed to a counter which in turn activates an analogue gate through which the carrier signal is being passed. This is shown in Figure 3.20.

Schematic Diagram of Pseudo - Random Gate

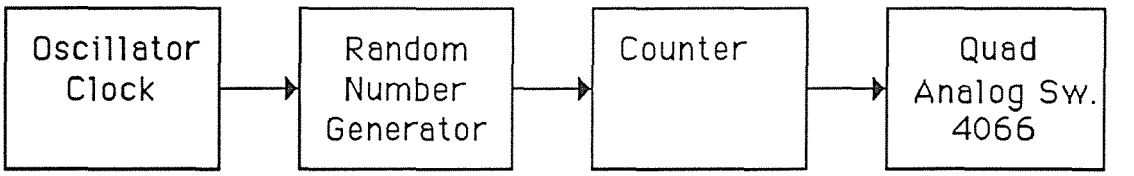


Figure 3.20

By altering the frequency of the clock driving the pseudo random number generator, the relative time that the analogue switch is in either state may be varied. Two ranges were arbitrarily chosen in the absence of any guidance from the literature: 0.2 - 2 seconds; 2.0 - 20 seconds. The effect of this is that the signal presented at the input of the analogue switch is gated on for a random period of time between 0.2 - 2.0 seconds on the low range, then off for another random period between 0.2 - 2.0 seconds. The same action occurs in principle on the high range where the period is between 2.0 - 20 seconds. The random number generator chip uses a seeded mathematical algorithm which means that in time, the sequence of random numbers will be repeated. On the low frequency range, 2.0 - 20 seconds, this will only occur after 19.7 hours, and 1.97 hours for the 0.2 - 2 second range.

In the final working system, two analogue gates were used in opposite states, one on while the other is off. This gives the possibility of inputting two signals which are mutually exclusive of each other, but there is always an output signal at each output port. Alternatively the same signal may be applied to both gates with the output being switched for random periods of time to either channel.

To explain it another way, in state one input A gates to output A while input B gates to output B. In the state two input A gates to output B while input B gates to output A. Flexibility of design was considered of prime importance.

Two LEDs indicate the state of the outputs. The green LED indicates A-A and B-B, while the yellow LED indicates A-B and B-A. The unit is powered by a commercial plug pack and has internal voltage regulation.

The complete circuit diagram of the pseudo-random gate is shown in Figure 3.21.

The prototype circuit was constructed on 0.1" veroboard rather than committing the design to a printed circuit board. As the periods chosen were somewhat arbitrary, it was hoped that biological experimentation would determine the preferred values. Such information may radically alter the approach taken to gate the carrier wave signals.

Circuit Schematic Pseudo Random Gate

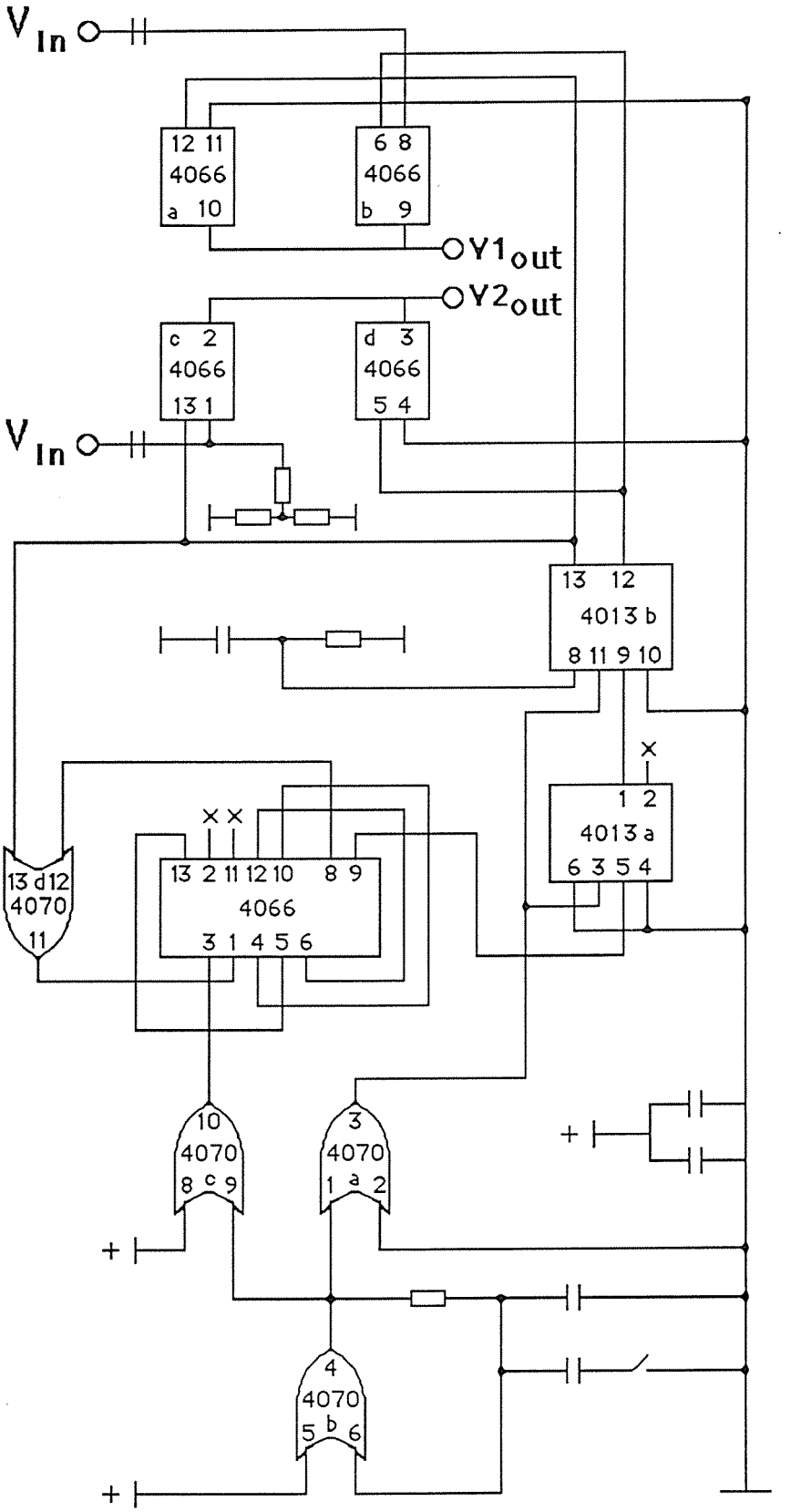


Figure 3.21

3.9 PROGRAMMABLE ANALOGUE SWITCH

3.9.1 Principle of operation

In the third configuration to produce various pulse train outputs from one of the timebase generators, it was decided to design a programmable gate with separate period and duty cycle controls. To achieve repeatability step, rather than analogue, controls were chosen. The range for the period of the gating cycle was chosen to be 0.5 to 5.0 seconds by 0.5 second steps. The duty cycle range was chosen to be 10% to 100% by 10% steps. (A 100% duty cycle means that the signal is continuous). The unit may accept two input signals which are gated alternately between outputs A and B at the chosen frequency and duty cycle.

A Schmitt trigger, (NE555), used in the astable squarewave oscillator mode, provides a clock signal for a decade counter, (4017). The frequency of the 555 clock is varied to provide the gate period, as detailed above. The decade counter drives a quad NOR gate reset/set latch, (4001), which toggles a quad analogue switch, (4066). The appropriate duty cycle is obtained by selecting which output of the decade counter is connected to the quad NOR R/S latch.

The unit may be powered from a 9 volt battery or plug pack. An LP 2951 micro-power regulator provides 5 volts internally for the i.c.'s allowing the unit to be battery operated with a reasonable life. A full circuit diagram is shown in Figure 3.24.

3.9.2 Schematic diagrams

A diagram to illustrate the principle of operation is shown in Figure 3.22. All signals are potential, or voltage waveforms.

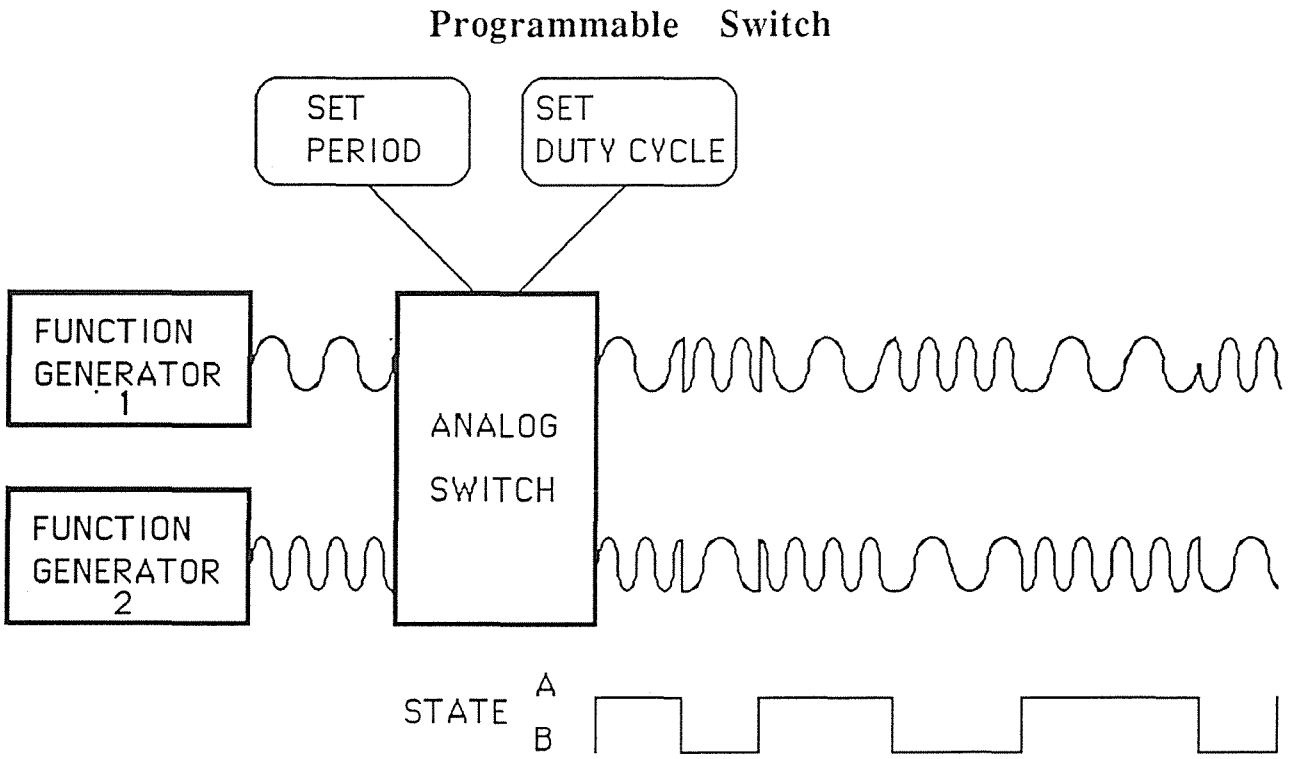


Figure 3.22

A functional schematic is shown in Figure 3.23, and a full circuit schematic in Figure 3.24.

Programmable Duty-Cycle Gate Schematic

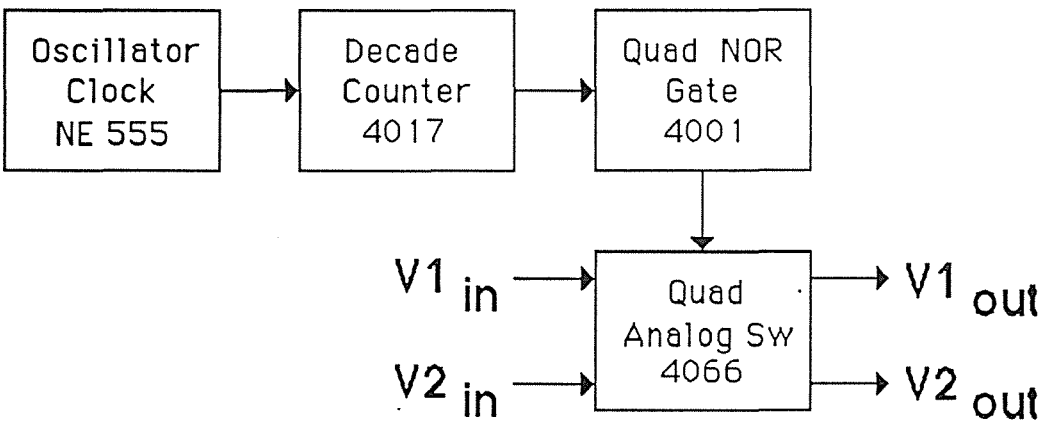


Figure 3.23

The full circuit schematic is shown in Figure 3.24

Programmable Duty-Cycle Gate Circuit Schematic

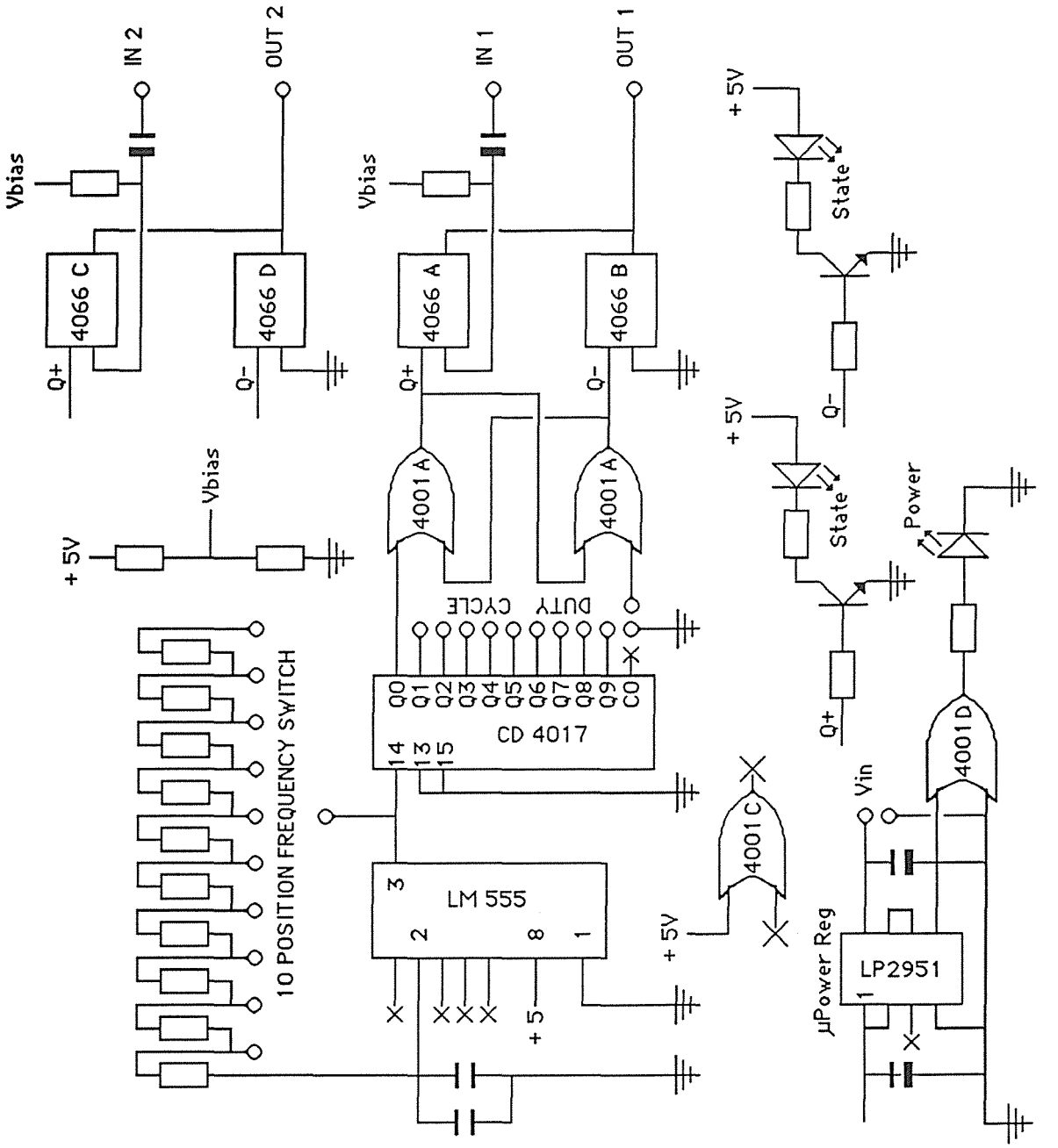


Figure 3.24

The effective wiring configuration of the 4066 analogue switch is shown in Figure 3.25.

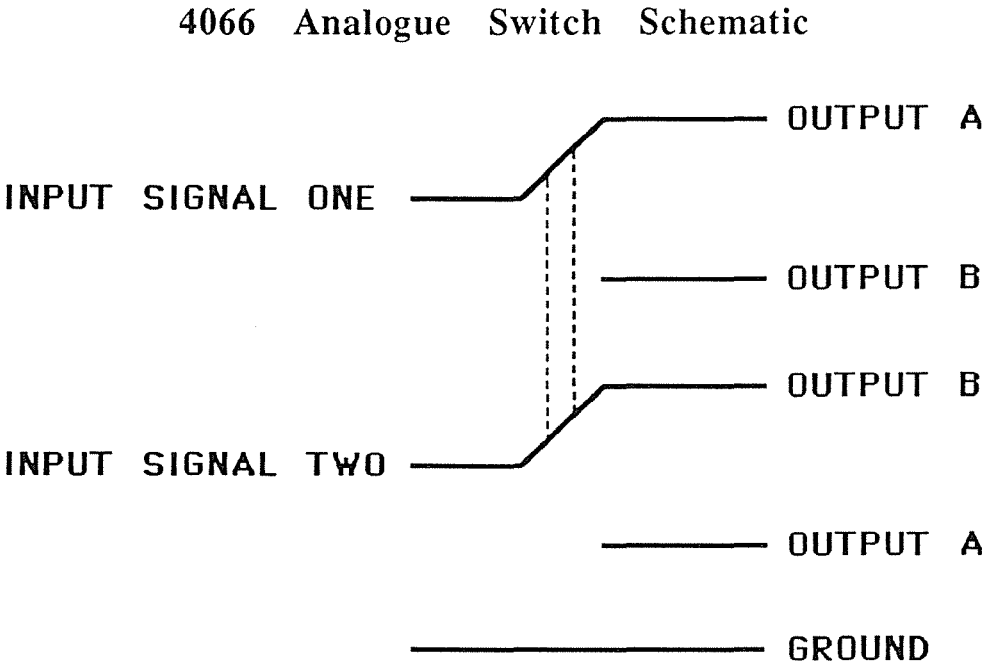


Figure 3.25

One advantage is that this device offers a number of configuration options. If a frequency is applied to one input only, then that frequency is effectively turned on and off at one output, (or the other), at the duty cycle chosen. Alternatively however, if a coil is connected to each output, then the signal alternates between the two coils. If a frequency is applied to each input, then several configurations are possible. A practical application of this would be to expose a person or system to two alternating frequencies e.g. 50 and 75 Hz. The other coil would produce the same frequencies in reverse order, 75 Hz / 50 Hz, and could be placed on another system or part of the body.

3.10 TRANSCONDUCTANCE AMPLIFIER

3.10.1 General overview

The amplifier module must faithfully reproduce the input waveform at levels which will produce magnetic fields in the region of 3.0 mT (30 gauss) in order to replicate the fields produced by $Warnke_{12}$, or 6.0 mT to produce similar fields to the Magnafield 990. The magnetic field produced by a coil is directly proportional to the number of turns and the current through the coil. Given this relationship, in order to produce a magnetic field corresponding to the input voltage waveform, it is necessary to generate a current, rather than voltage, waveform in the coil. An amplifier which produces an output current proportional to the input voltage is termed a transconductance amplifier. The gain (g_m) of such an amplifier is specified in volts per ampere.

A simple way of implementing a transconductance amplifier is to apply voltage feedback around a conventional amplifier where the sensed feedback signal is proportional to the output current. An implementation of this configuration is shown in Figure 3.26.

The voltage developed across the current sensing resistor (R_f) is fed back to the inverting input of the voltage amplifier. For an ideal voltage amplifier, the output voltage will increase until the feedback signal is equal to the input signal. Thus the output current will now be proportional to the input voltage.

General Transconductance Schematic

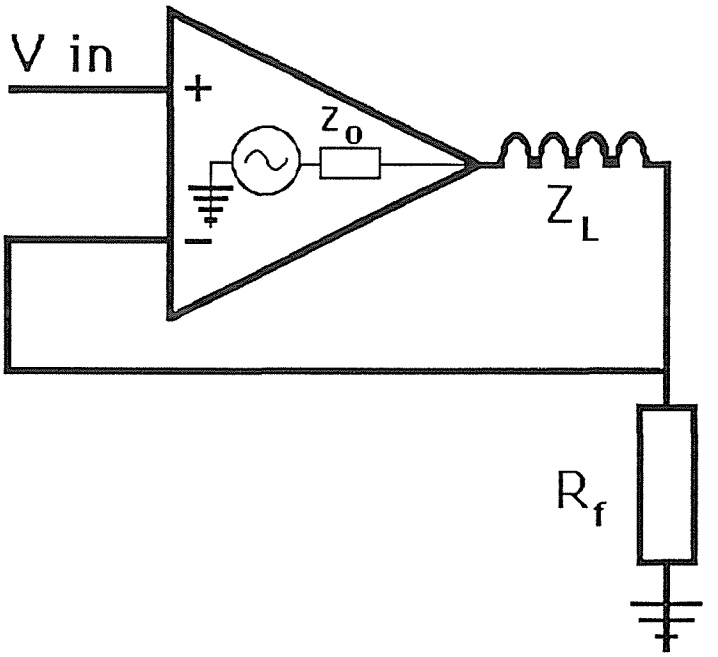


Figure 3.26

The following equation describes the gain of an ideal transconductance amplifier:

$$g_m = \frac{I_{out}}{V_{in}} = \frac{A}{(Z_0 + Z_1) * R_f * (1 + A)}$$

where:

A	=	the gain of the voltage amplifier;
I_o	=	output load current in amperes;
V_{in}	=	input signal in volts;
Z_0	=	open loop output impedance of the voltage amplifier in ohms;
Z_L	=	output load impedance in ohms;
R_f	=	resistance in ohms of the current sensing resistor.

For practical amplifiers, Z_0 will be much less than Z_L, and A will be very large. Under these conditions the gain equation reduces to:

$$gm = 1 / (ZL * Rf)$$

The ideal impedance of a coil, Z, is given by:

$$Z = \sqrt{(R^2 + (2\pi f L)^2)}$$

where:

R	=	d.c. resistance pf the coil;
L	=	inductance of the coil;
f	=	is the frequency of operation.

This equation shows that when 2π f L is larger than R, Z increases rapidly with increasing frequency. This implies that the gain of our transconductance amplifier will rapidly drop at high frequencies when driving an inductive load. Thus the output current will no longer follow the input waveform.

3.10.2 Idealised waveforms in voltage and current amplifiers

Figure 3.27 shows the output voltage and current waveforms for a voltage amplifier driving a coil with a squarewave. On the rising edge of the square wave, the current waveform no longer follows the input voltage, rolling off.

Voltage and Current Waveforms in a Coil driven by a standard voltage feedback amplifier

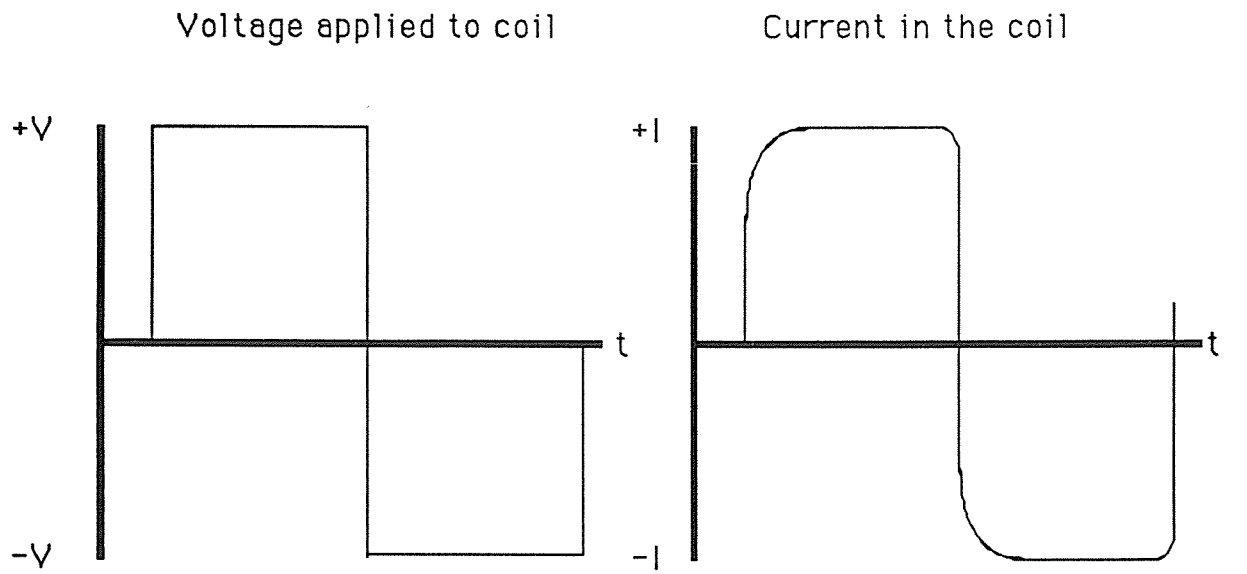


Figure 3.27

In the case of the current feedback amplifier, the input voltage is mirrored in the output current. In order to produce squarewave current in the coil, the output voltage increases rapidly on the rising edge of the squarewave. Thus compensating for the increasing impedance of the coil with increasing frequency. This is shown diagrammatically in Figure 3.27.

Voltage and Current Waveforms in a Coil driven by a Transconductance Current Feedback Amplifier

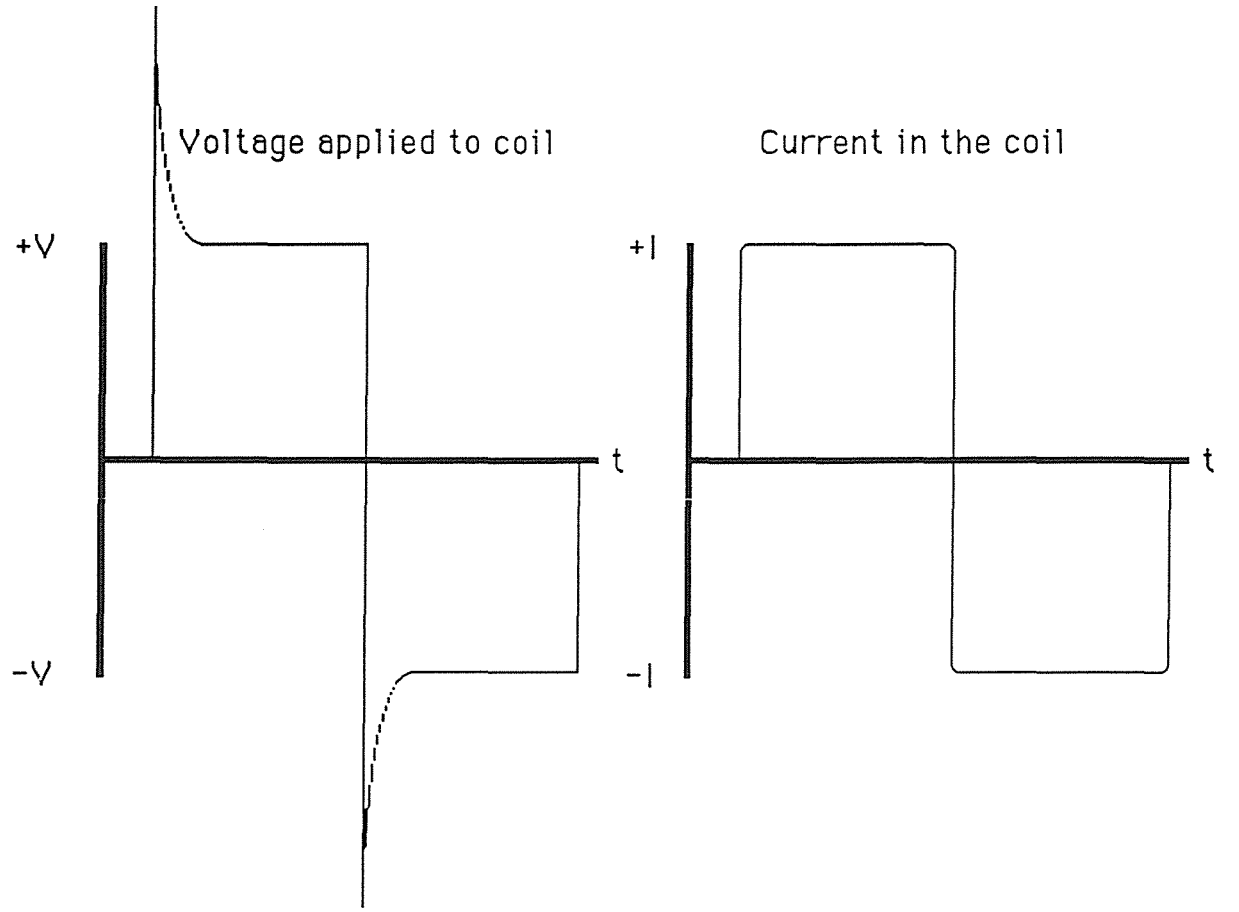


Figure 3.28

In practice it is impossible to obtain a perfectly square current waveform in the coil as this would require an instantaneous current change which in turn would require an infinite applied voltage. However it is possible using current feedback techniques to substantially reduce the round off error caused by the increasing impedance in the coil. Actual examples are given in Chapter Four, Evaluating the Amplifier.

Circuit Schematic of the Transconductance Amplifier

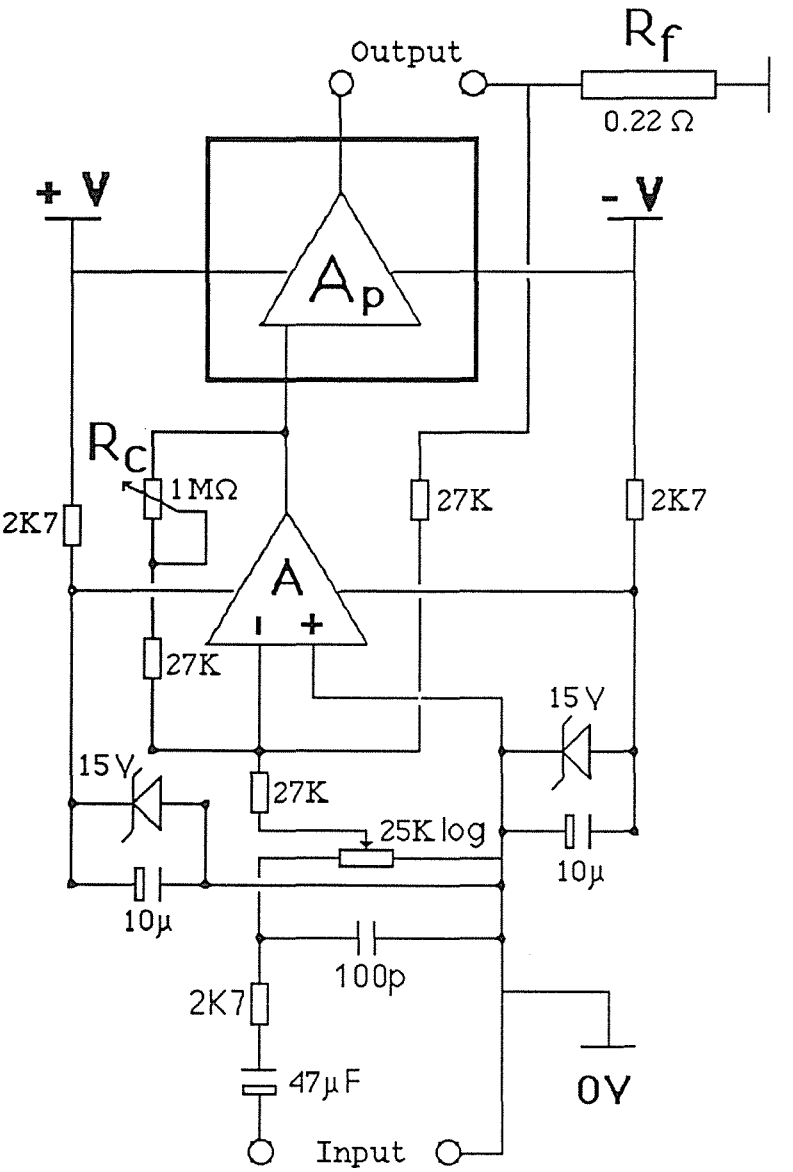


Figure 3.29

The above schematic diagram shows the implementation of the transconductance amplifier used in the experiments. A_p is a conventional voltage amplifier, ETI

480/50, rated at 50 watts into an 8 ohm load. The specifications are presented in Table 3.1

Specifications of the ETI 480 Amplifier

Output power	50 watts into 8 ohms.
Frequency Response	5 - 50 kHz
at rated power	(+0, -3dB)
Input sensitivity	500 mV
Signal to noise ratio	100 dB
Damping factor	25

Table 3.1

Full details of the ETI 480 may be found in Appendix Six.

The operational amplifier, **A**, in Figure 3.29, sums the input signal and the current voltage sensed in resistor $\mathbf{R_f}$. Since **A** is an inverting amplifier this summation subtracts the two signals. $\mathbf{R_c}$ controls the open loop gain of the op.amp./power amp. combination. Adjusting this control changes how closely the circuit approximates an ideal transconductance amplifier. This compensation control is necessary to optimise the circuit for various inductive loads. Over compensation may result in the amplifier oscillating, as the phase shift through the coil causes the current feedback signal to become positive.

3.11 Data Acquisition and Frequency Analysis

3.11.1 Overview

In order to evaluate the transconductance amplifier it is desirable to determine the frequency response under the condition of actually driving an inductive load with a square wave.

It was decided to employ the LABVIEW (**LAB**oratory **V**irtual **I**nstrumentation and **E**lectronic **W**orkbench) 40 kHz oscilloscope and fourier analysis package. While LABVIEW is extremely versatile, there are severe limitations imposed by the hardware interface facilities currently owned by the Department of Production Technology. With current hardware it is only possible to sample one channel at a time, at a maximum rate of 40 kHz. Until such time as a multi-channel input card is purchased, it is not possible to simultaneously sample the multiple outputs of the pseudo-random switch; programmable interrupter and zero-crossing switch.

3.11.2 Sampling theorem

The Nyquest Sampling Theorem dictates that in order to analyse a signal it is necessary to sample at least at twice the fundamental frequency. This would limit the maximum frequency of the input signal to the LABVIEW system to 20 kHz. However, in order to adequately sample a sine wave for example, it is desirable to have at least 10 samples during one period. This produces a maximum sample frequency of 4 kHz. If the incoming signal to be analysed has fast rise times, such as would be the case for a square wave, it is necessary to

have of the order of at least 100 samples per period in order to obtain even the fifth harmonic while still falling within the Nyquest criteria. In practice this would limit the incoming sample signal to 400 Hz. This value is suitable for testing the pseudo-random gate, programmable interrupter and zero crossing switch. It does not allow adequate sample of the programmable timebase generator over its full range.

3.11.3 The requirement for a filter

In order to determine the frequency component (spectrum) of the signal under analysis, the output from the LABVIEW 40 kHz oscilloscope is passed to a fast fourier transform routine which produces a graphical output. As mentioned above, the available hardware provides considerable restraint in terms of input frequency. Depending on the criteria chosen the maximum frequency of the signal to be analysed lies between 400 and 4000 Hz. For whichever value is chosen, it is necessary to limit frequencies above this limit to obtain accurate fourier plots. Frequencies above the chosen cut off point will effectively 'wrap around' and superimpose their component on lower harmonics. This will give artificially high values in the frequency plot because of this artefact.

To limit such frequency artefacts, it is necessary to include a filter in the input circuitry prior to the LABVIEW 40kHz oscilloscope. Essentially this must be a low pass filter, the ideal characteristics of which would pass all frequencies below the cutoff limit with a flat response, while passing none above. In practice this is impossible to obtain, however several types of filter have sufficiently step roll off to obtain an acceptable result.

3.11.4 Filter design

The most simple filter would consist of a resistor and capacitor, Figure 3.29.

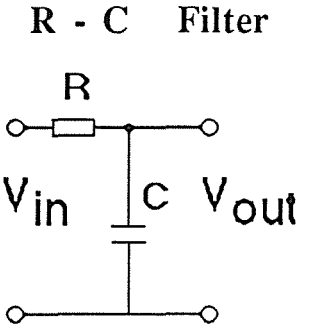


Figure 3.29

The response of such a filter would be a gently sloping from 0 Hz to the cutoff frequency, with a slope of 6 dB per octave above the cutoff point. The cutoff frequency for such filters is defined as the frequency -3 dB down from the input signal.

A better response may be obtained using a second order system which contains two R-C stages, Figure 3.30.

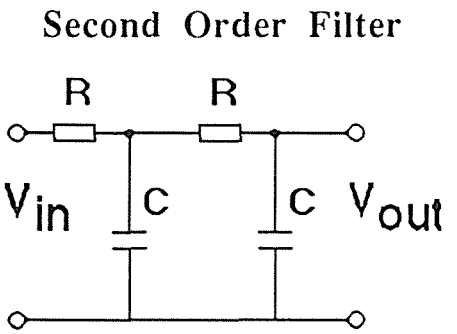


Figure 3.30

This gives a 12 dB slope per octave.

A second order filter may be used which incorporates an inductor in series with the resistor, Figure 3.31.

Second Order Filter with an Inductor

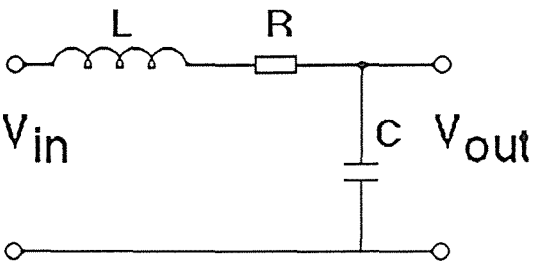


Figure 3.31

Inductors have two disadvantages however in that they are expensive, particularly if precise values are required, and they have a strong tendency to pick up magnetically induced hum. While mu metal screening can alleviate the inductive pick-up to some degree, this only adds to the cost of the system. Inductors are an inconvenient component to use. Such filters also have the disadvantage of increasing the response just prior to the cutoff frequency. This hump can also add artefacts to the fourier analysis.

A convenient solution to the problem of constructing an LCR filter is the operational amplifier, (op-amp) with an appropriate resistor-capacitor network. The classic Sallen and Key low-pass filter is an example of such an active filter which mimics the response of an LCR network, Figure 3.32.

Sallen and Key Filter

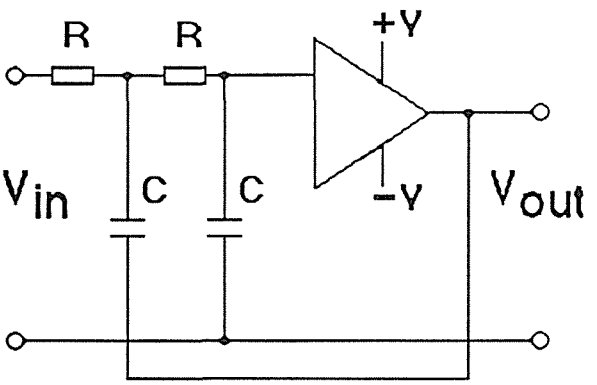


Figure 3.32

By varying the gain of the system, the Q may be determined to obtain the optimal response. The Q is determined by the equation : **Q = 1/3-A**

where A is the gain of the op-amp and Q = $1/\sqrt{2}$. The optimal response where the frequency response below the cut-off frequency is flattest corresponds to a gain of 1.6 and a Q of 0.7. This is known as the Butterworth response. Filters designed to these criteria are known as Butterworth filters.

3.11.5 Design of the low-pass filter

As described above the optimum filter would have a Q of 0.7 with a corresponding gain of 1.6. In order to obtain the sharpest slope after the cut-off frequency a 4th order design was chosen to give an effective slope of 24 dB per octave or 80 dB per decade. The schematic diagram is shown in Figure 3.33 and the printed circuit board layout in Figure 3.34.

Twin Butterworth Filter

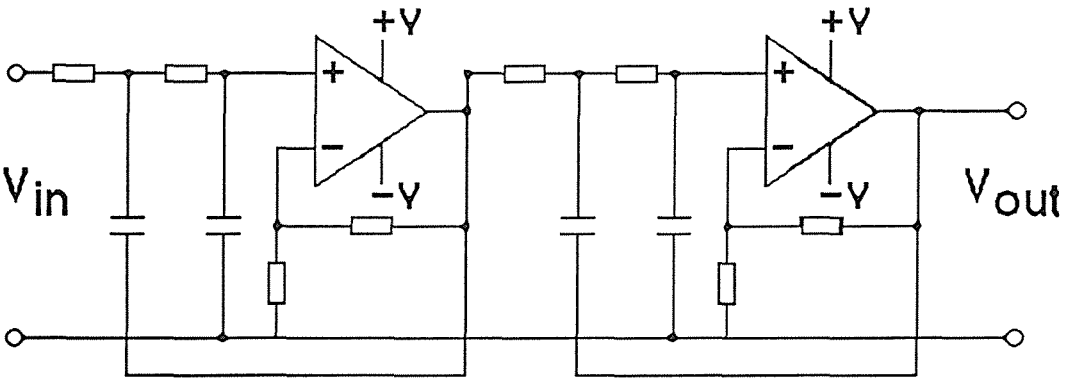


Figure 3.33

PCB Design 4th Order Butterworth Filter

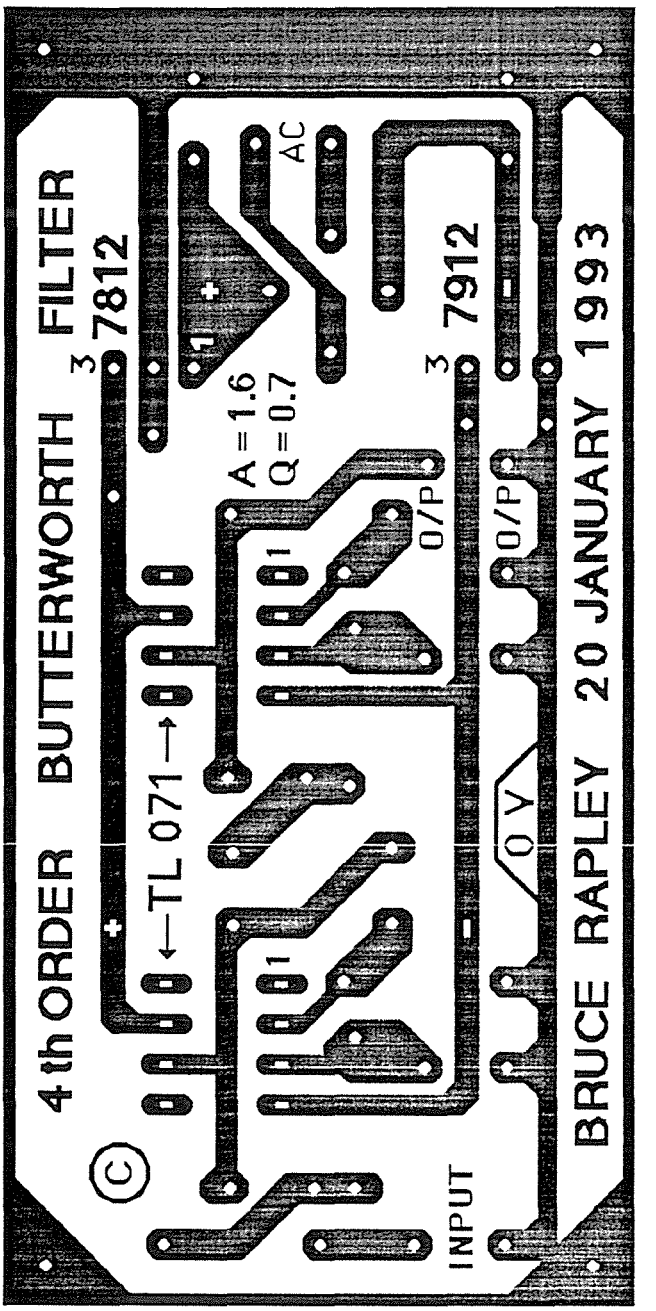


Figure 3.34

3.12 CONCLUSION

The preceding sections have outlined the basic modules which constitute the new magnetic biostimulator. The units have all been made, with the exception of the computer interface which is currently awaiting components. It appears that as of the time of writing, one of the components has been discontinued. If this is true, and stock cannot be obtained from overseas suppliers, some modification will be necessary to the printed circuit board as no pin compatible alternative exists.

The laboratory testing of the above modules is documented in Chapter Four.

3.13 REFERENCES

1 C.A.L. Bassett. Biomedical implications of pulsating electromagnetic fields. Surgical Rounds Vol 22, pp22-31, Jan 1983.

2 C.Polk & E.Postow, eds. C.RC. Handbook of biological effects of electromagnetic fields. CRC Press 1986.

3 C.A.L. Bassett, A.Pilla et al. A non-operative salvage of surgically resistant pseudoarthroses and non-unions by pulsing electromagnetic fields. Clinical Orthopaedics Vol 124, pp128-143, 1977.

4 C.A.L. Bassett, S. Mitchel et al. Treatment of ununited tibial diaphyseal fractures with pulsing electromagnetic fields. Journal of Bone Joint Surgery Vol 63(A), pp511-523, 1981.

5 C.A.L. Bassett, S.Mitchel et al. Pulsing electromagnetic field treatment in ununited fractures and failed arthroses.

6 E.Davies. The healing face of electromagnetic fields. Electronics World + Wireless World, April 1993.

7 E.Fukuda, I.Yasuda. On the piezoelectric effect of bone. Journal of the Physics Society of Japan, Vol 12, pp1158-62, October 1957.

8 R.O.Becker, G.Selden. The body electric - electromagnetism and the foundation of life. William Morrow N.Y. 1985.

9 W.F.Ganong. Review of medical physiology, Chapter 14, Neurocentres regulating visceral functions. Lange Medical Publications, 10 ed. 1981.

10 C.A.Keele and E.Neil. Samson Wright's applied physiology. Oxford University Press 1065.

11 G.Bini et al. Thermoregulatory and rhythm generating mechanisms governing the sudomotor and vasoconstrictor outflow in human cutaneous heme. Journal of Physiology, Vol 306, pp537-552, 1980.

12 U.Warnke. The possible role of pulsing magnetic fields in the reduction of pain. Pain Therapy, Elsvier Biomedical Press, 1983.

13 C.A.L. Bassett. Biological significance of piezoelectricity. Calcified Tissue Research, Vol 1, pp252-272, 1968.

14 C.A.L.Bassett. Biophysical principles affecting bone structure. In Bourne (ed): The biochemistry and physiology of bone, Vol III, pp1-76. Academic Press, New York, 1971.

15 C.A.L. Bassett, R.J. Pawluk. Effects of various direct and alternating currents on osteogenesis in vivo. In Conference on effects of diffuse electrical currents on physiological mechanisms with application to electroanaesthesia and electrosleep. University of Wisconsin Press, Milwaukee, 1967.

4

Performance Evaluation

CONTENTS

4.1 TIMEBASE GENERATOR

4.1.1 Frequency

The time-base generator is designed to a range of frequencies in three bands. The low band covers the range: low, 3-999 Hz; the medium band, 30-99990 Hz; the high band, 300-999900 Hz. The resolution of each band is four most significant digits, with the middle and high bands being activated by a range switch corresponding to 10x and 100x respectively. The frequencies produced by the time-base generator were determined with a Hewlett Packard frequency counter model 5381A. Brief specifications are presented in Table 4.2 below.

Table 4.2 Brief Specifications HP Frequency Counter 5381A

Frequency Range	10 Hz to 80 MHz
Display	7 digits
Input impedance	1 M Ω
Sensitivity	25 mV (RMS sinewave) 30 Hz to 20 MHz
	50 mV (RMS sinewave) 10 Hz to 80 MHz
Input attenuation	x1, x10, x100
Accuracy	+/- 1 Count +/- Timebase Accuracy
Gate times	0.1 second, 1 second, 10 seconds
Resolution	10 Hz at 0.1 second gate time
	1 Hz at 1 second gate time
	0.1 Hz at 10 second gate time
Time base	frequency 1 MHz
	aging < 0.3 ppm / month

The frequencies determined with the HP counter are shown in Table 4.3.

Time-base generator frequencies

Selected Hz Low Range	Measured Hz Low Range	Selected Hz Med Range	Measured Hz Med Range	Selected Hz High Range	Measured Hz High Range
3	3	30	30.0	300	300.0
4	4.0	40	40.0	400	400.0
5	5.0	50	50.0	500	500.0
6	6.0	60	60.0	600	600.0
7	7.0	70	70.0	700	700.0
8	8.0	80	80.0	800	800.0
9	9.0	90	90.0	900	900.0
10	10.0	100	100.0	1000	1,000.0
20	20.0	200	200.0	2000	2,000.0
30	30.0	300	300.0	3000	3,000.0
40	40.0	400	400.0	4000	4,000.0
50	50.0	500	500.0	5000	5,000.0
60	60.0	600	600.0	6000	6,000.0
70	70.0	700	700.0	7000	7,000.0
80	80.0	800	800.0	8000	8,000.0
90	90.0	900	900.0	9000	9,000.0
100	100.0	1000	1,000.0	10000	10,000.0
200	200.0	2000	2,000.0	20000	20,000.0
300	300.0	3000	3,000.0	30000	29,999.9
400	400.0	4000	4,000.0	40000	39,999.8
500	500.0	5000	5,000.0	50000	49,999.8
600	600.0	6000	6,000.0	60000	59,999.7
700	700.0	7000	7,000.0	70000	69,999.6
800	800.0	8000	8,000.0	80000	79,999.6
900	900.0	9000	9,000.0	90000	89,999.4
1000	1,000.0	10000	10,000.0	100000	99,999.4
2000	2,000.0	20000	20,000.0	200000	199,998.8
3000	3,000.0	30000	29,999.9	300000	299,998.1
4000	4,000.0	40000	39,999.8	400000	399,997.5
5000	5,000.0	50000	49,999.8	500000	499,996.8
6000	6,000.0	60000	59,999.7	600000	599,996.1
7000	7,000.0	70000	69,999.6	700000	699,995.6
8000	8,000.0	80000	79,999.6	800000	799,995.0
9000	9,000.0	90000	89,999.4	900000	899,994.3
9200	9,200.0	92000	91,999.5	920000	919,994.0
9400	9,400.0	94000	93,999.5	940000	939,994.0
9600	9,600.0	96000	95,999.4	960000	959,994.0
9800	9,800.0	98000	97,999.4	980000	979,993.8
9999	9,999.0	99990	99,989.4	999900	999,893.6

Table 4.3

The measurements shown in Table 4.3 were all made at a gate time 10 seconds for maximum resolution. These results are graphed in Figures 4.1, 4.2, and 4.3.

Time-base generator frequency test low range (x1)

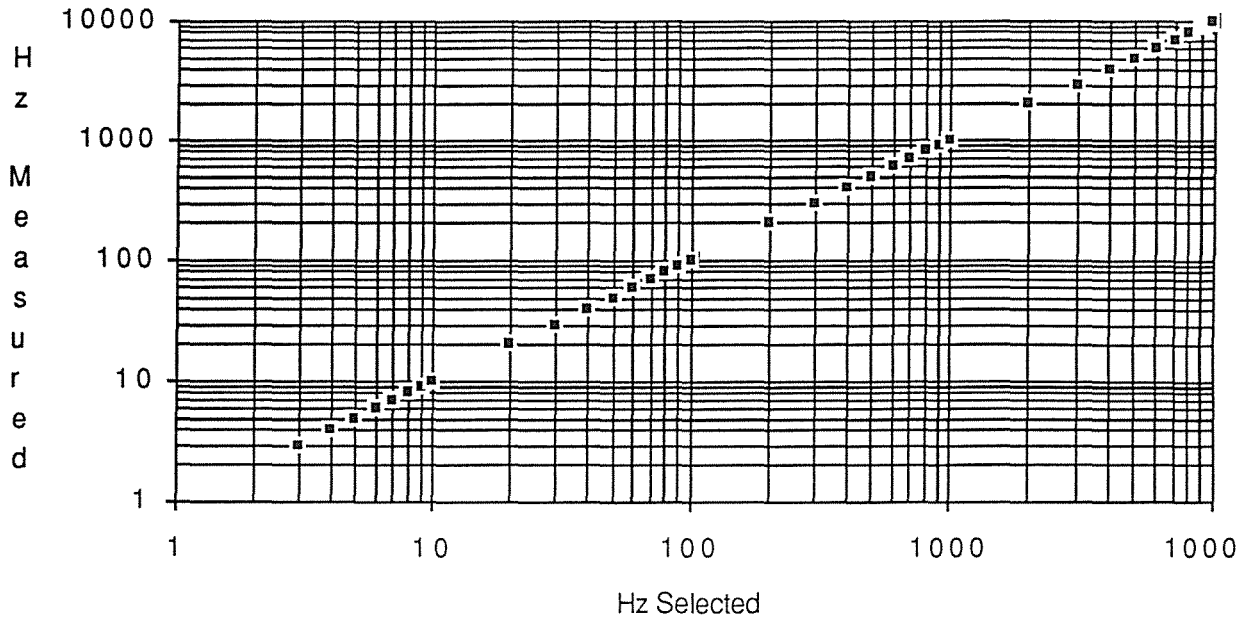


Figure 4.1

Time-base generator frequency test medium range (x10)

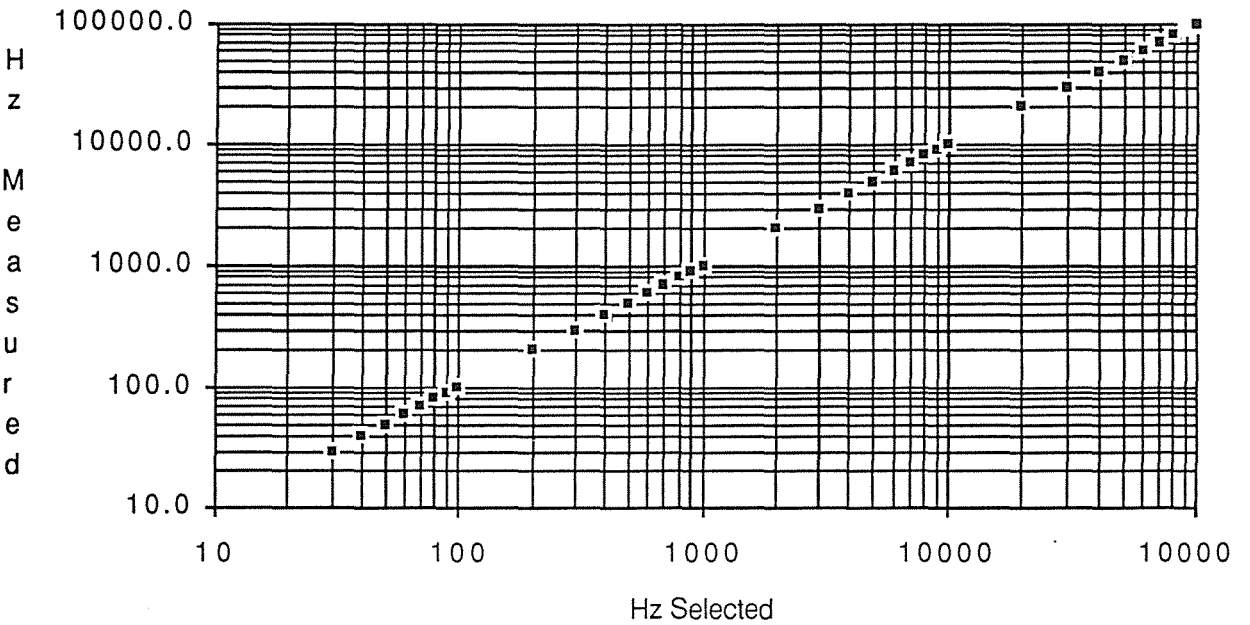


Figure 4.2

Time-base generator frequency test high range (x100)

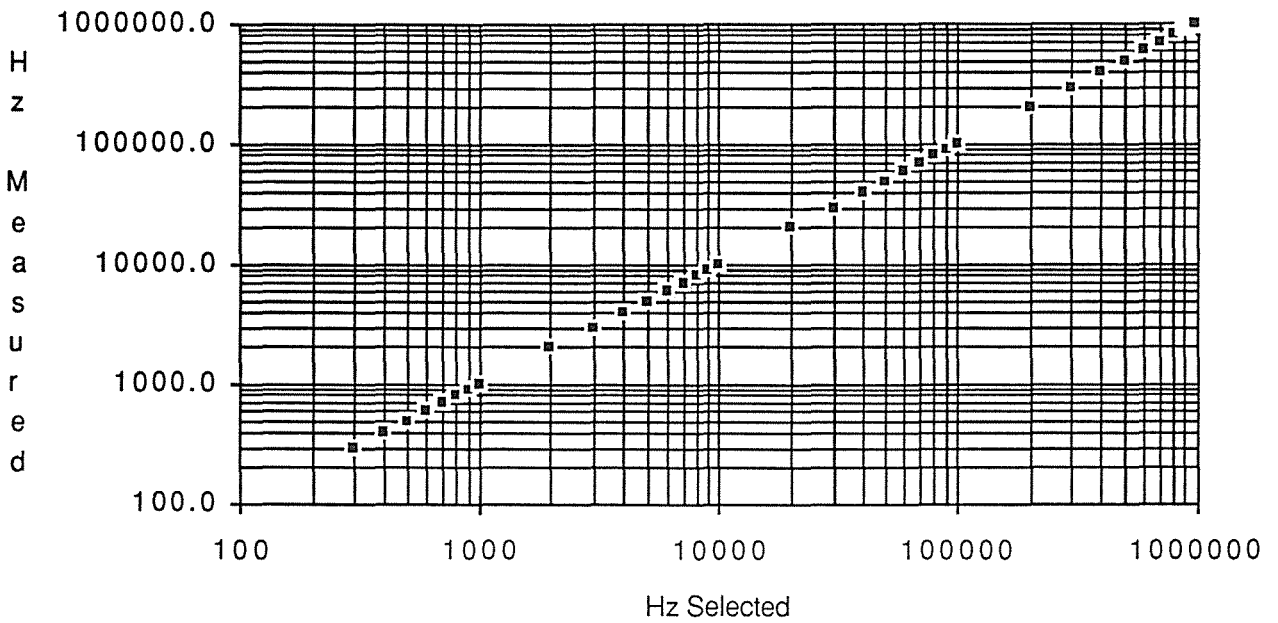


Figure 4.3

The HP counter was last known to be calibrated in 1976. With an aging factor of 0.3 ppm/month maximum this could give a possible error of +/- 25 Hz at 1 MHz. Applying this error to the maximum frequency of the time-base generator gives a range of 99,9975 to 1,000,025 Hz. The measured frequency at 999,900 is 999,893.6 Hz giving an absolute error of 6.4 x 10^{-4}%, or 6.4 Hz in 999,900. The measured value is within the absolute accuracy of the counter, given the time since it was last calibrated, therefore the error could conceivably be in the counter rather than the time-base generator. Without a more accurately calibrated counter it is not possible to determine the frequency of the time-base generator any more precisely.

4.1.2 Output Voltage

The output voltages were measured using a BWD model 539 B twin-beam DC coupled oscilloscope, and a Fluke model 23 volt meter. The oscilloscope was used to measure the output voltages across all frequencies, while the volt meter was able to reliably measure the outputs at only 50 Hz. The oscilloscope measurements are found in Table 4.4, and the volt meter readings in Table 4.5 .

Oscilloscope peak to peak voltage measurements

	Low range	**Medium range**	**High range**
TTL Square O/P	2.0 volts p/p	2.0 volts p/p	2.0 volts p/p
Sine O/P	2.8 volts p/p	N/A	N/A

Table 4.4

The oscilloscope readings were taken on the 0.5 volt per division setting which enables the value to be read to at least 1 decimal place. The input was DC coupled in calibrate mode. The timebase was varied to give at least two wavelengths on the screen for each frequency.

RMS voltage measurements

Output	**Low range**	**Medium range**	**High range**
TTL Square	1.156 volts	1.156 volts	N/A
Sine	0.948 volts	0.948 volts	N/A

Table 4.5

4.2 SIMPLE ANALOGUE SWITCH

To evaluate the operation of the simple analog switch, a 200 Hz 1.0 volt sine wave was connected to the carrier wave input, and a 25 Hz TTL square wave connected to the gate input. The resulting output was displayed on a Macintosh II ci running LABVIEW II's 40 kHz oscilloscope. The resulting waveform is shown in Figure 4.4. This waveform was chosen as it represents the Warnke protocol for the vasodilation response discussed in Section 1.3.

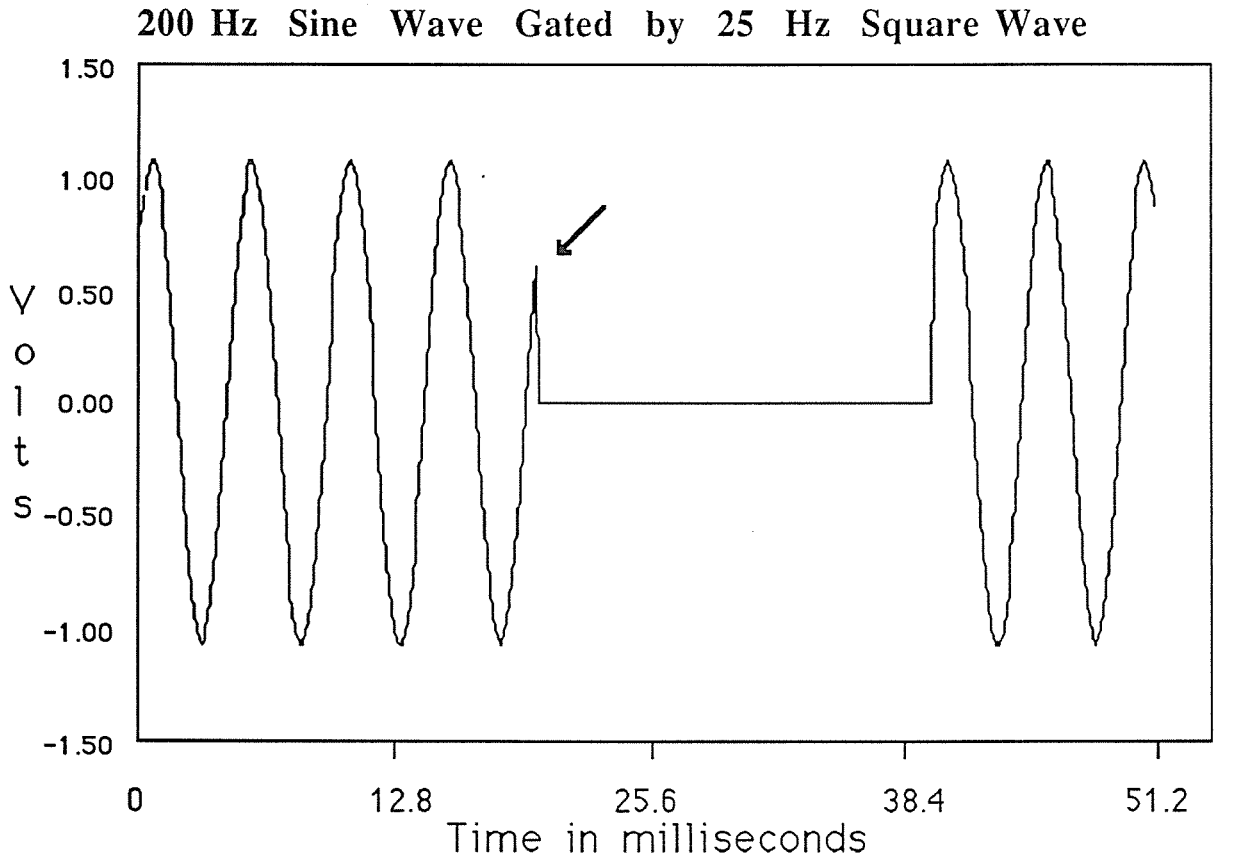


Figure 4.4

Note that the gating signal gates the carrier wave at precise time intervals independent of zero crossing, see arrow. The resulting cut off waveform, at approximately 20 mseconds on the above diagram, could result in the generation

of considerable harmonics as the voltage falls rapidly to zero in a coil. To further demonstrate this phenomenon, the sampling time period was doubled to approximately 100 milli seconds, by doubling the number of samples captured. The resultant waveform is shown in Figure 4.5. Note that the gating signal is not synchronised with the carrier wave, resulting in the on and off states not operating at precisely the same point in the carrier wave each time.

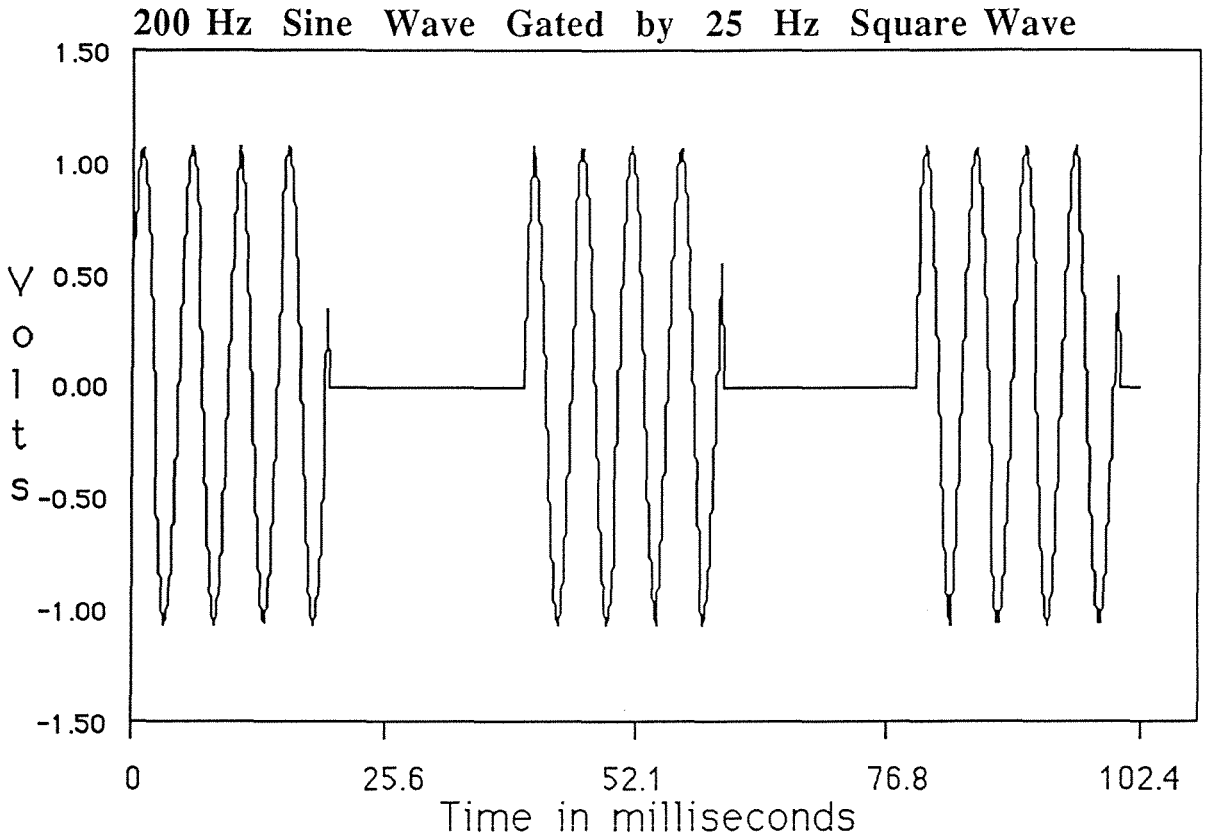


Figure 4.5

In addition, a 200 Hz carrier wave was combined with a 22 Hz gate signal to provide a graph where the gate frequency is not an even division of the carrier frequency. The resultant waveform is shown in Figure 4.6.

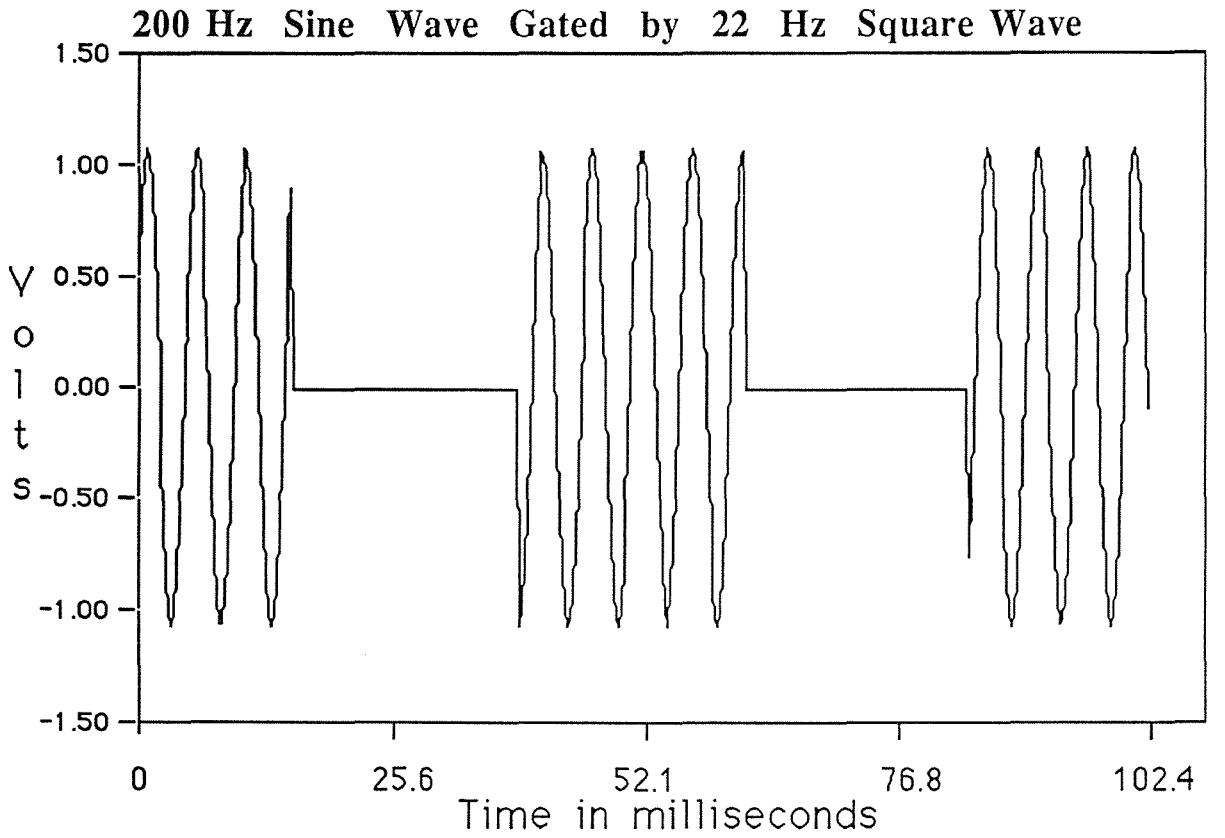


Figure 4.6

The arbitrary nature of the gating waveform is evidenced as clearly being unrelated to the zero crossing of the carrier wave. Gating may occur during either the negative or positive-going waveforms. The generation of harmonics from this rapid fall in voltage to zero when the carrier signal is significantly above, or below zero, was the reason that an alternative gating system was investigated and developed. (The uneven time (X) axis values are a result of the way in which Labview's oscilloscope samples the data, which is based on the actual number of samples collected by the analogue to digital card, rather than sampling for a set period of time.)

4.3 ZERO-CROSSING DETECTOR SWITCH

Identical test conditions to those used in Section 4.4 were used to evaluate the performance of the zero-crossing detector switch. The resultant captured waveform is shown in Figure 4.7.

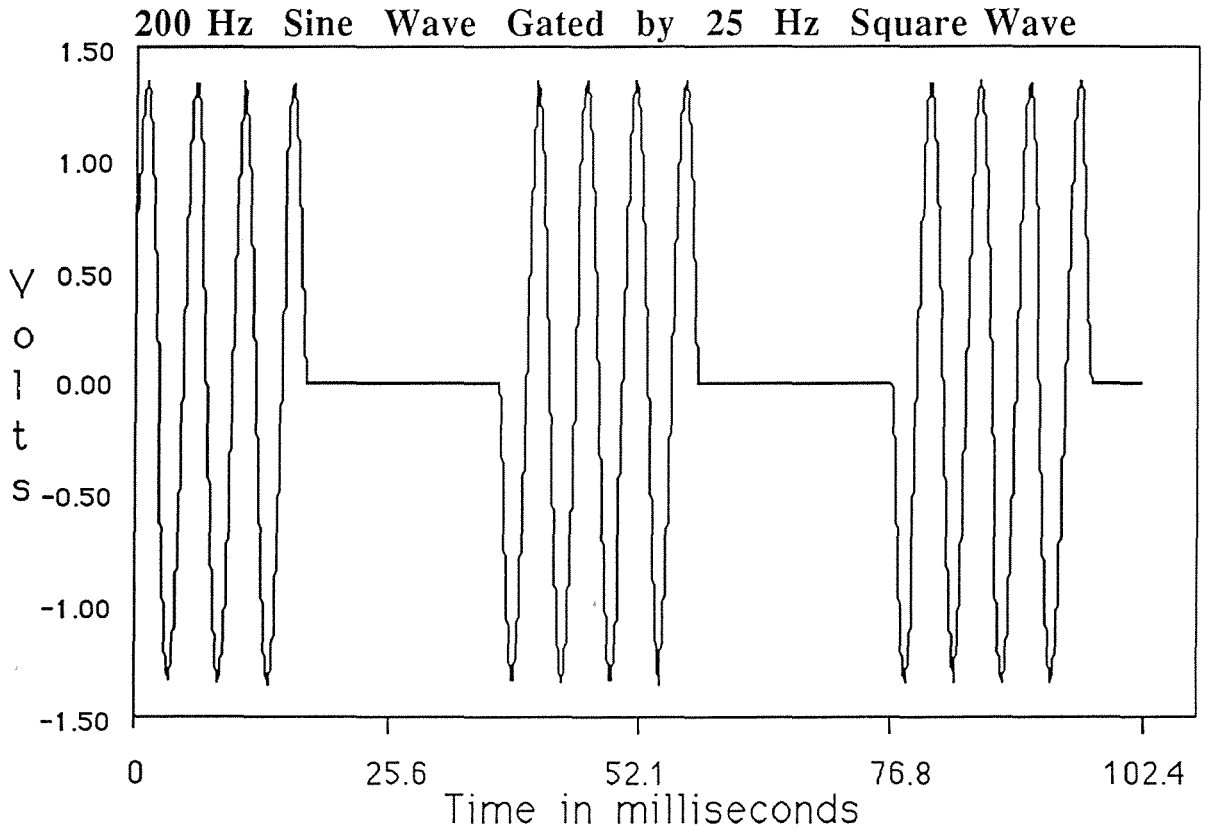


Figure 4.7

Note that the carrier wave signal is switched either on or off only when it crosses the zero line. The operation of the zero-crossing gate is such that when the gate signal indicates a change of state, the actual analogue switch is not activated until the carrier signal reaches zero volts. In this way minimal harmonics result from the switching operation initiated by the gating signal.

As with the simple analogue switch, an uneven multiple of the carrier wave signal frequency is chosen to test the operation of the zero-crossing switch. The results are shown in Figure 4.8. The resolution of the time (X) axis is increased to give a total sample period of the order of 50 milli seconds. This highlights the precise zero-crossing switch function. The results clearly show a totally satisfactory zero-crossing switch function.

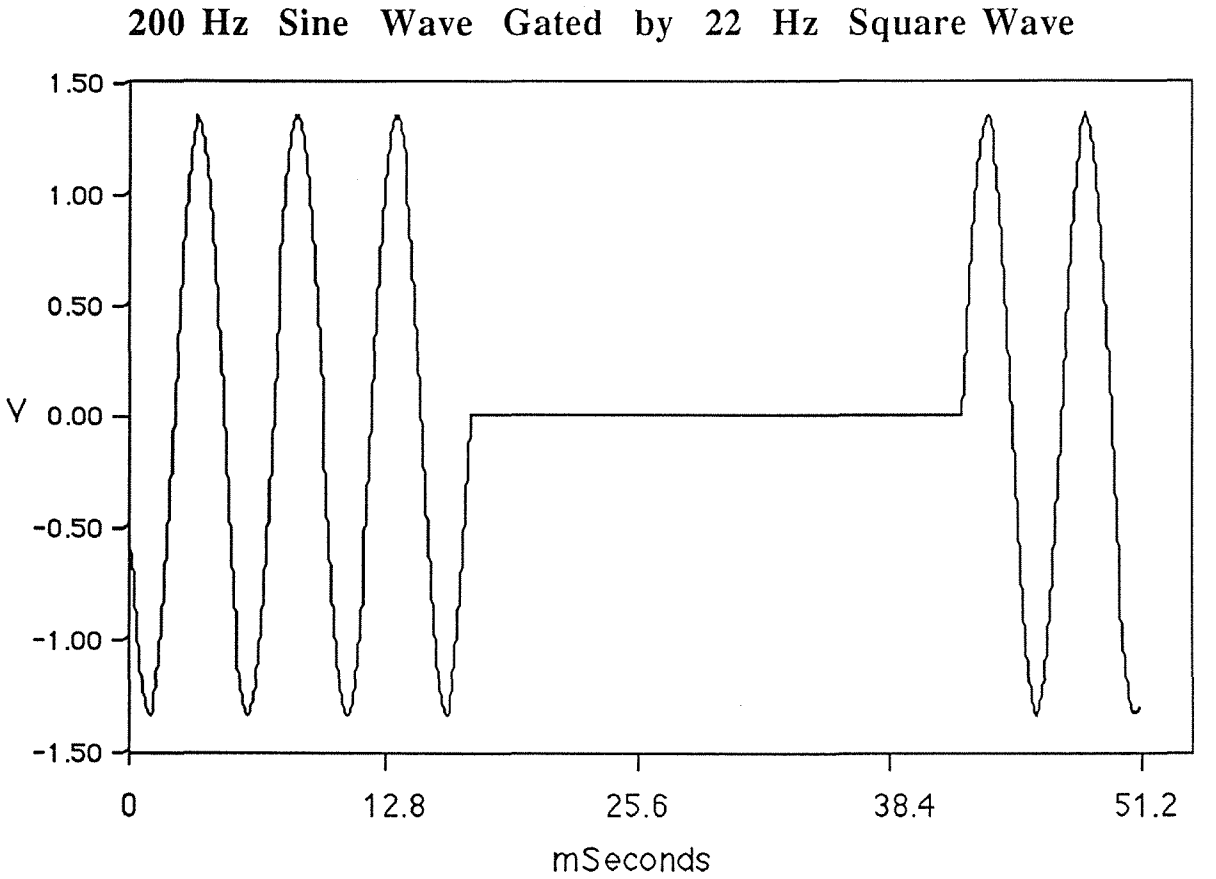


Figure 4.8

4.4 PSEUDO-RANDOM SWITCH

To demonstrate the function of the pseudo-random switch, a 100 Hz sine wave was chosen as the input frequency to be gated by the fast pulse mode. To adequately display the random function of the gating circuitry, it was necessary to choose a very large number of samples (10,000) to obtain the longest practical sample time. Because the 100 Hz sine wave has such a short period compared to the total sample time, it appears simply as a solid block.

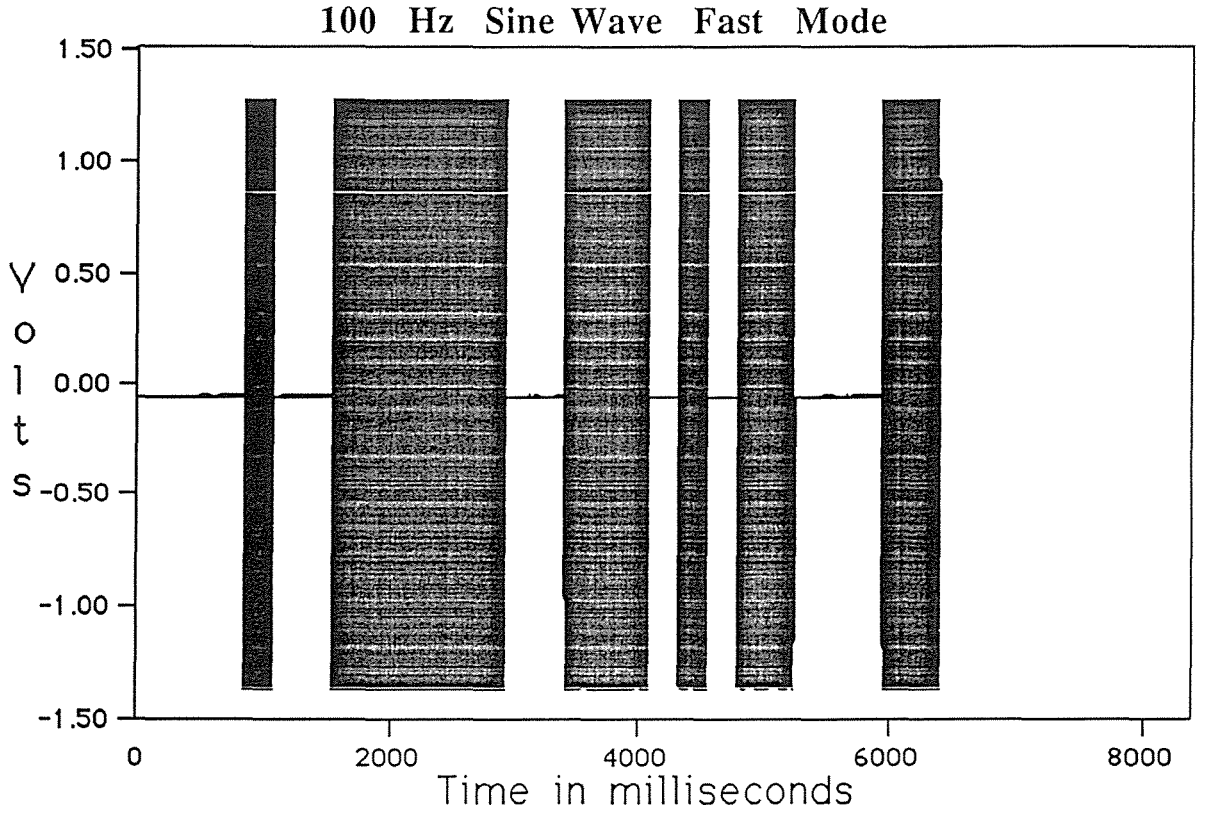


Figure 4.9

The random period for both the on and off states is clearly seen from the above figure. The slow mode is not presented here as the picture would effectively appear the same. The mode switch alters the internal clock speed only, and has no effect on the random mark-space ratio produced. The random periods are

produced by a seeded algorithm, and as such will repeat the sequence in approximately 1.97 hours for fast mode, and about 19.7 hours for slow mode, (refer to Section 3.8.2).

4.5 PROGRAMMABLE ANALOGUE SWITCH

The programmable analogue switch has 10 set periods ranging from 0.5 seconds to 5.0 seconds in 0.5 second steps. The duty cycle has 10 set levels ranging from 10% to 100 % in 10% steps. Refer to Section 3.9.1. Four examples will be presented. Figure 4.10 shows the Labview 40 kHz oscilloscope output for a 0.5 second period with a duty cycle of 50 %.

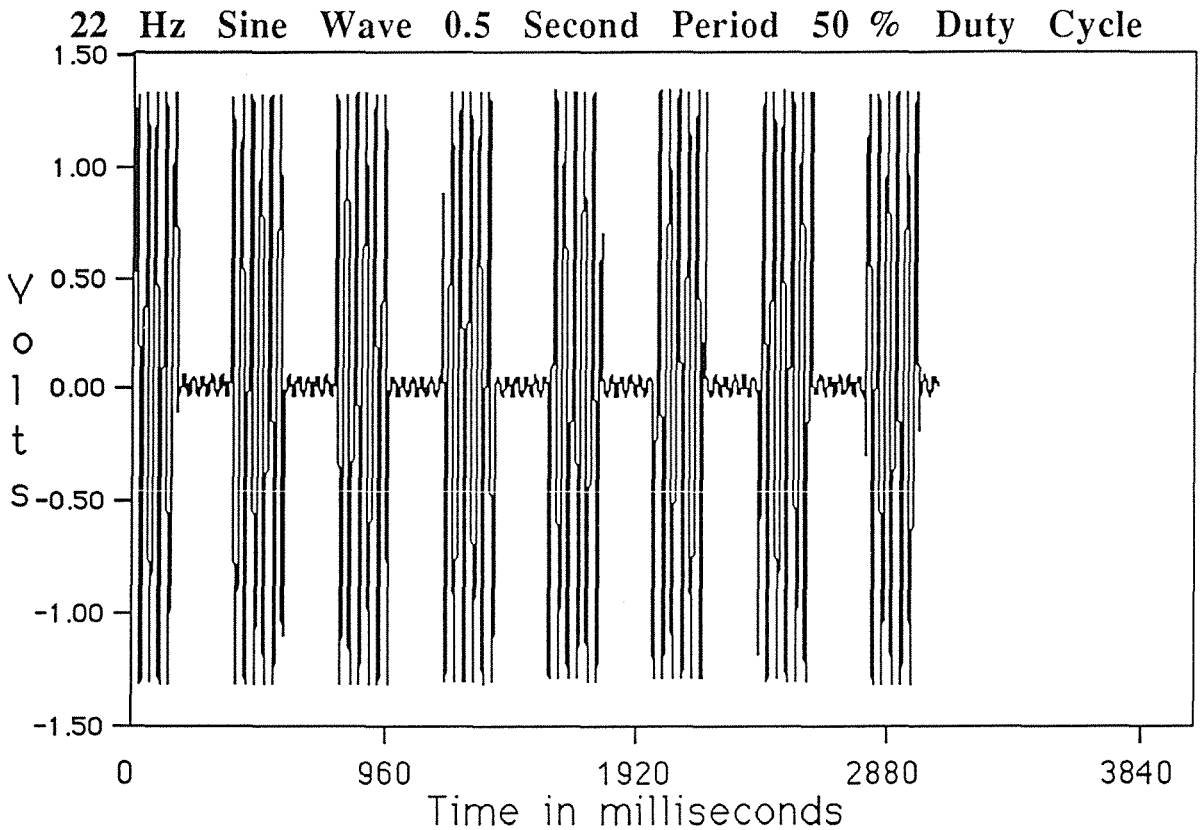


Figure 4.10

A period of 0.5 seconds means that the input signal will be gated on for 0.25 of a second then off for 0.25 of a second. The 50% duty cycle can clearly be seen from the above figure. The slightly irregular signal between the on states is due to noise derived from the input leads to the analogue to digital board and is an

artifact rather than a real output from the device under test. This was verified by use of a conventional oscilloscope.

An 80% duty cycle was evaluated under the identical conditions to the previous trial. The results are shown in Figure 4.11. The on state can clearly be seen to be four times as long as the off state.

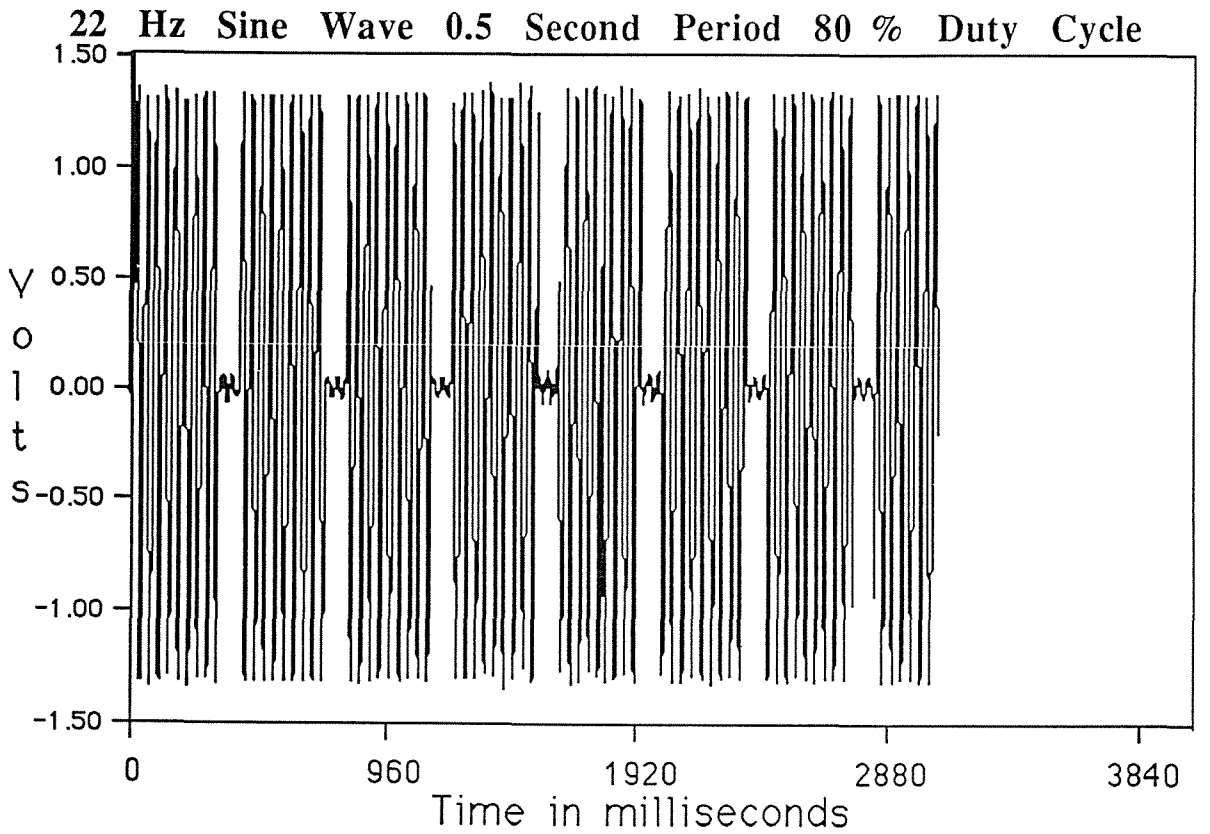


Figure 4.11

The inverse duty cycle, i.e. 20%, is shown for comparison in Figure 4.12.

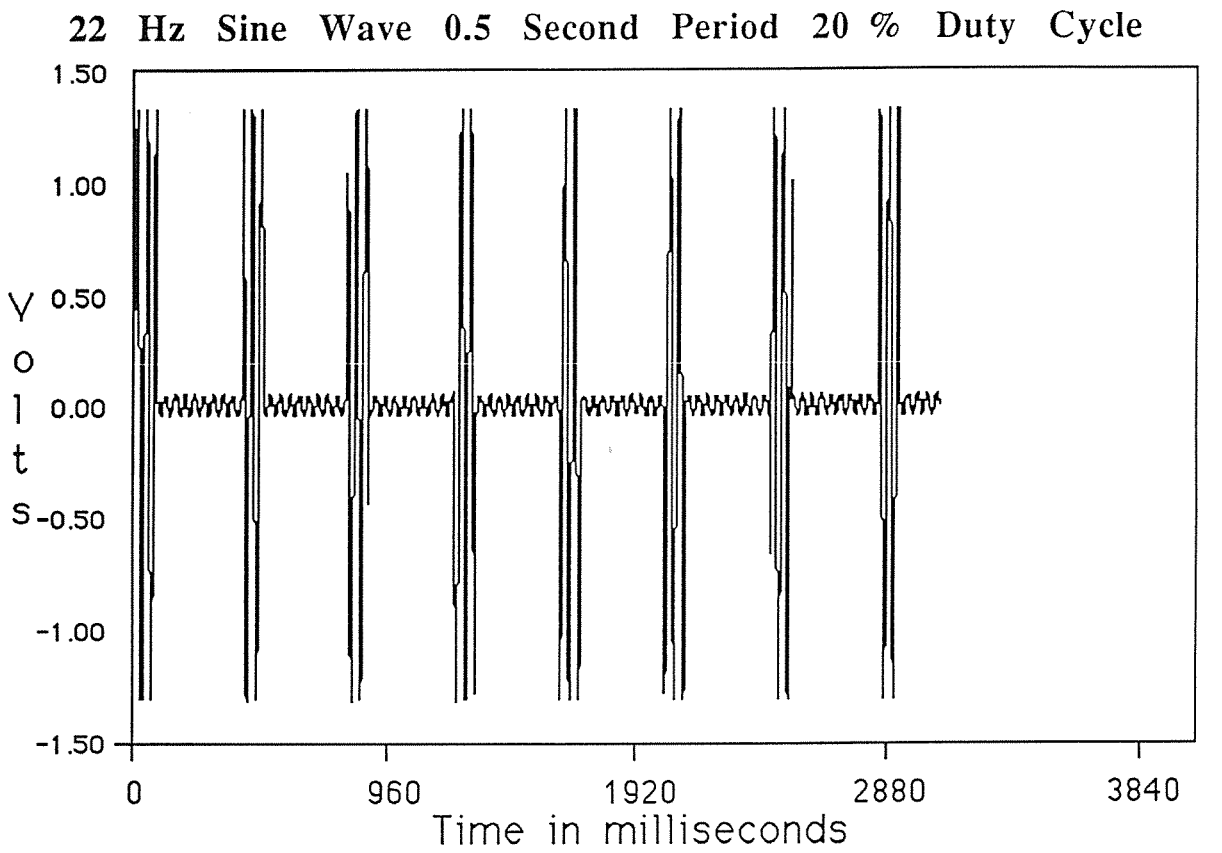

22 Hz Sine Wave 0.5 Second Period 20 % Duty Cycle
1.50
1.00
0.50
0.00
-0.50
-1.00
-1.50
Volts
0
960
1920
2880
3840
Time in milliseconds

Figure 4.12

4.6 LOW PASS ANTI-ALIASING FILTER

4.6.1 Evaluation of the Butterworth low-pass filter

In order to evaluate the filter's action, a 5.5 volt sine wave was fed into the input while the output was monitored by an oscilloscope. The frequency was varied from 500 Hz to 15 kHz. The results are reproduced in Table 4.1 and Figure 4.7.

Butterworth Filter Response

Hz	V out
500	5.5
1000	5.5
2000	5.5
3000	5.4
4000	4.0
5000	2.8
6000	1.75
7000	1.1
8000	0.7
9000	0.45
10000	0.3
15000	0.05

Table 4.2

Frequency response

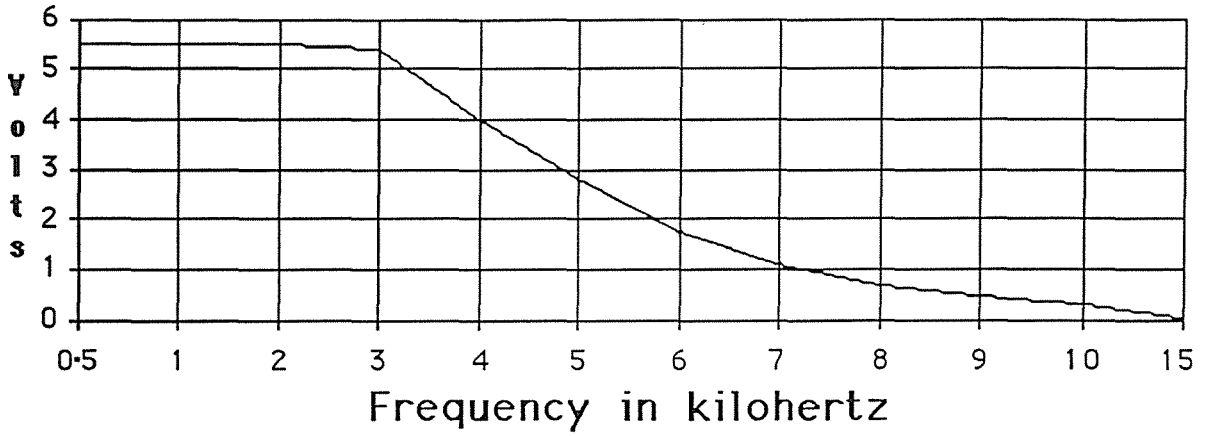


Figure 4.13

4.7 TRANSCONDUCTANCE AMPLIFIER

4.7.1 Waveforms: current and voltage

To test the transconductance amplifier, the time base generator was connected to the input with the applicator coil used in the vasodilation study across the output. In all test cases a 1 kHz square waveform was used. The Labview II 40 kHz oscilloscope was used to sample and display the results.

The amplifier has two sampling outputs specifically for the use of oscilloscopes for monitoring the output waveforms. One output provides the **voltage** waveform, the other the **current** waveform. Both will be utilised in this analysis. As the transconductance amplifier has variable current feedback, several scenarios will be investigated: minimal, optimum and maximum feedback.

The first trial involves the 1 kHz square wave signal with minimum current feedback. The voltage waveform is shown in Figure 4.14. Note the very rapid rise time as the amplifier attempts to produce the leading edge of the square wave in current waveform. The resultant current waveform is shown in Figure 4.15. The resulting current waveform is very unsatisfactory, resembling more of a triangle than a square waveform. The harmonic envelope will be analysed in Section 4.9.2, Fourier analysis.

1 kHz No feedback compensation

Voltage Waveform

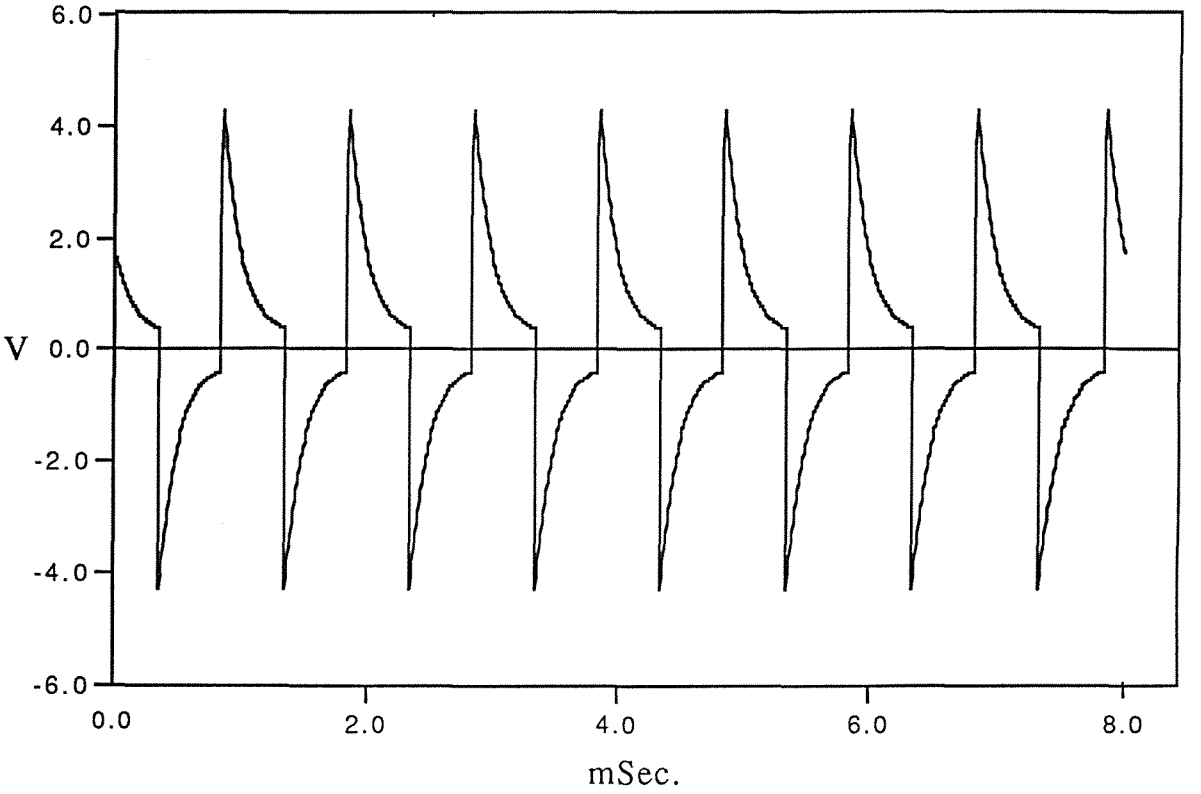


Figure 4.14

The voltage waveform of Figure 4.14 should be compared to the current waveform in Figure 4.15. The somewhat 'jagged' appearance of the waveform is a limitation of the screen display and hence an artifact, rather than an accurate representation of the actual signal. The screen resolution cannot be increased with the present configuration.

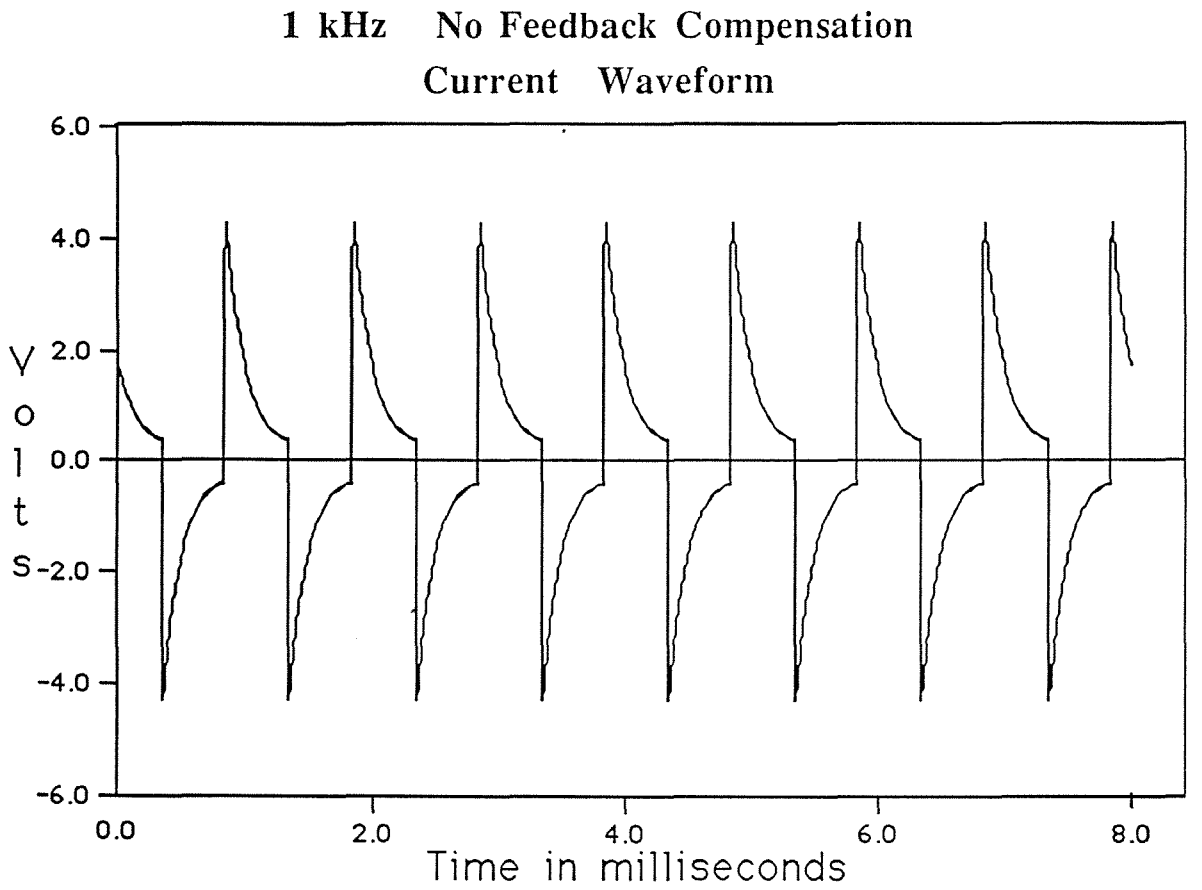


Figure 4.15

Optimal compensation was determined by trimming the current feedback control while observing the current waveform on a conventional oscilloscope, as the sampling and display function of the Labview oscilloscope proved to be too slow to operate in real time. This is the same procedure which would be adopted in the normal operation of the biostimulator. The voltage waveform is shown in Figure 4.16, and the resultant current waveform in Figure 4.17.

1 kHz Optimal Compensation
Voltage Waveform

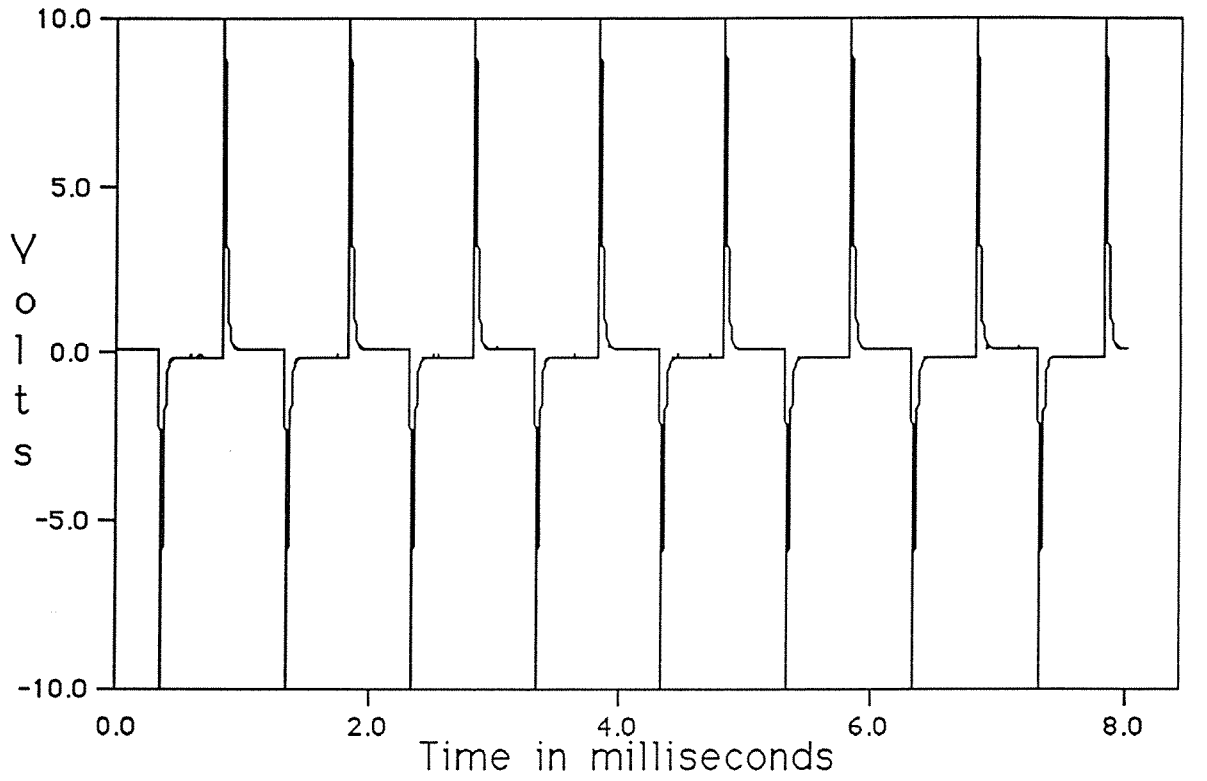


Figure 4.16

The voltage waveform under conditions which produce the best possible square wave appears as a symmetrical series of voltage spikes. This is necessary in order to obtain the most rapid rise time and hence the most 'square' waveform. The resultant current waveform is shown in Figure 4.17.

1 kHz Optimal Compensation
Current Waveform

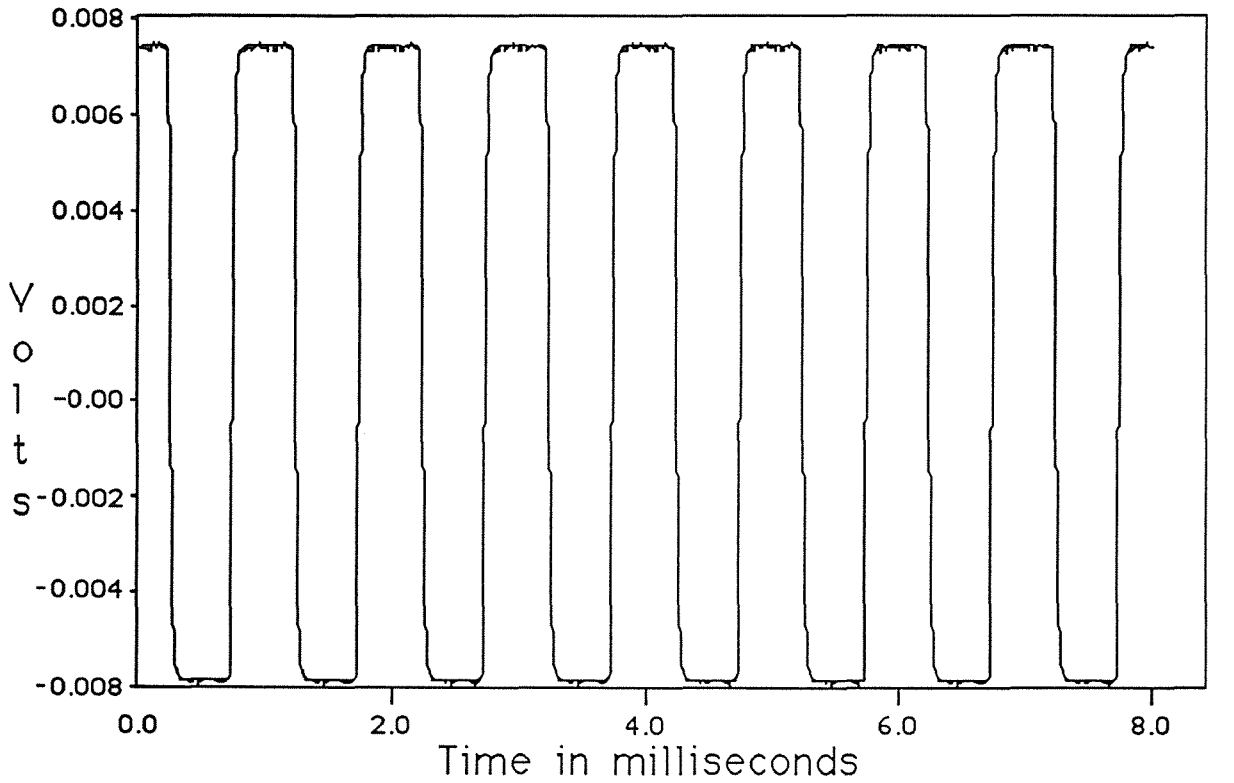


Figure 4.17

Again the problem of screen resolution prevents high definition observation of the leading and trailing edges. When compared to the current waveform produced from minimal feedback in Figure 4.15, the differences are both obvious and profound.

The analysis was repeated for maximum feedback, the results for the voltage and current waveforms are shown in Figures 4.18 and 4.19 respectively.

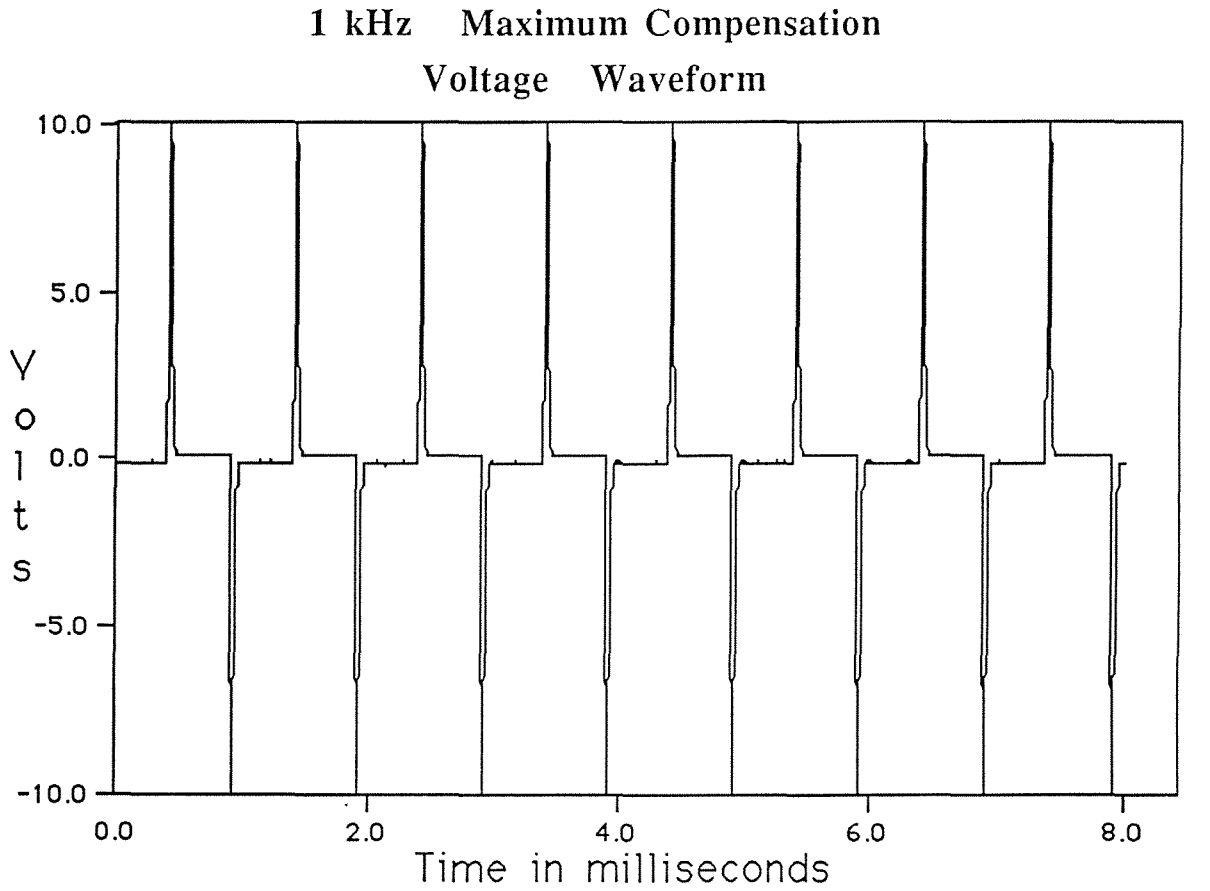


Figure 4.18

Little difference is observed between this figure and Figure 4.16, the optimal feedback condition. The same applies to the current waveforms Figures 4.15 and 4.17. While minor differences were observed on a conventional oscilloscope, the resolution of the LABVIEW display was unable to reproduce the required definition. (A conventional oscilloscope able to be linked to LABVIEW was not available).

1 kHz Maximum Compensation Current Waveform

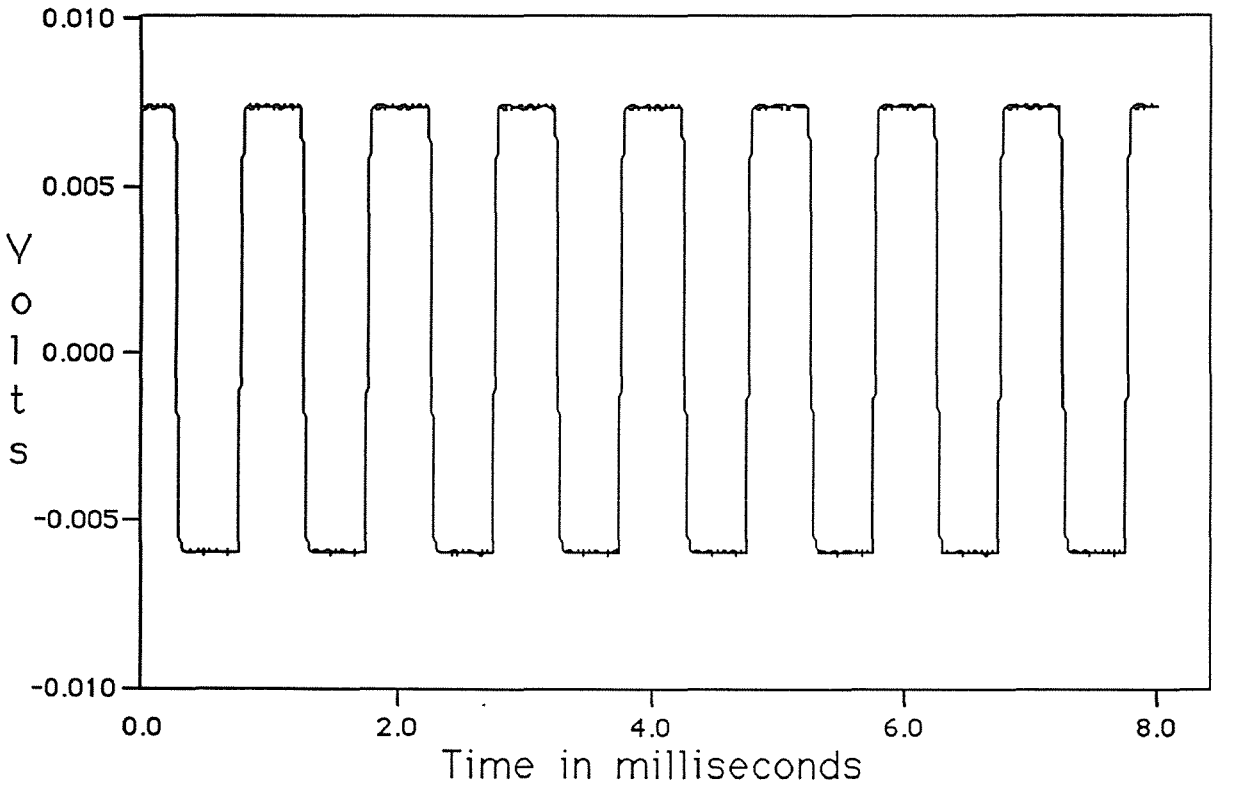


Figure 4.19

4.7.2 Fourier analysis

Fourier analysis was carried out on the three conditions used in the previous section: minimum; optimal and maximum feedback. The analysis was conducted twice, once with the low pass filter described in Sections 4.6, and once without the filter. Care should be exercised when examining the power spectrum of the unfiltered waveform as the input frequency exceeds the Nyquist criteria for sampling. This means that frequency components above 20 kHz (the oscilloscope samples at a maximum of 40 kHz) will be added to the values below this. In this way an artificially high dB value may be recorded below 20 kHz. The graphs of the Fourier analysis cannot be truncated at 20 kHz as the LABVIEW Fourier output is not user definable. The 'x' axis of the fourier plot is Hz, although Labview does not label it as such. Axis labels are not user definable functions.

Labview's output from the Fourier virtual instrument automatically displays the waveform sampled and the resultant power spectrum. The first case, 1 kHz unfiltered square wave with minimal current feedback compensation, is shown in Figure 4.22. The filtered case is shown in Figure 4.23.

1 kHz Minimal Feedback Unfiltered
1024 samples 10 μs gain = 1

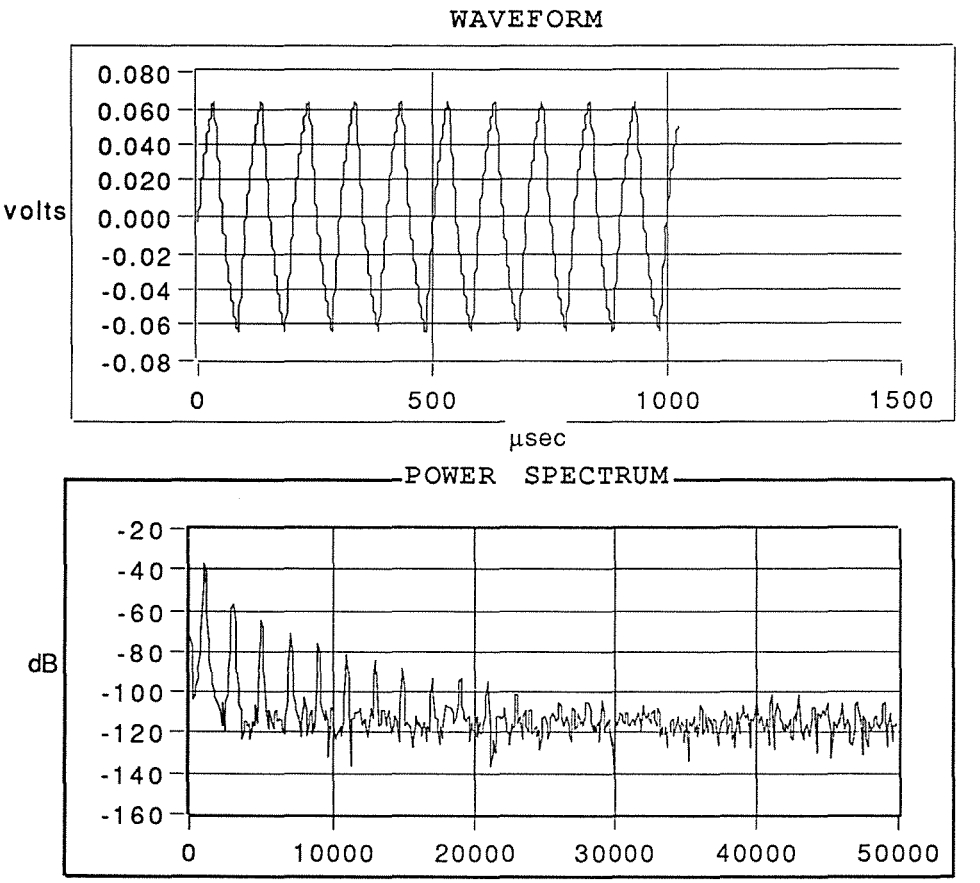


Figure 4.22

The peaks in the power spectrum correspond to: 1kHz (fundamental at -40 dB); 3 kHz; 5 kHz; 7 kHz ; 9 kHz; 11kHz; 13 kHz; 15 kHz; 17 kHz; 19 kHz; 21 kHz and 23 kHz. Above 23 kHz the power spectrum degenerates into noise. The values above 20 kHz are invalid however, due to violation of the Nyquist sampling theorem and should be disregarded.

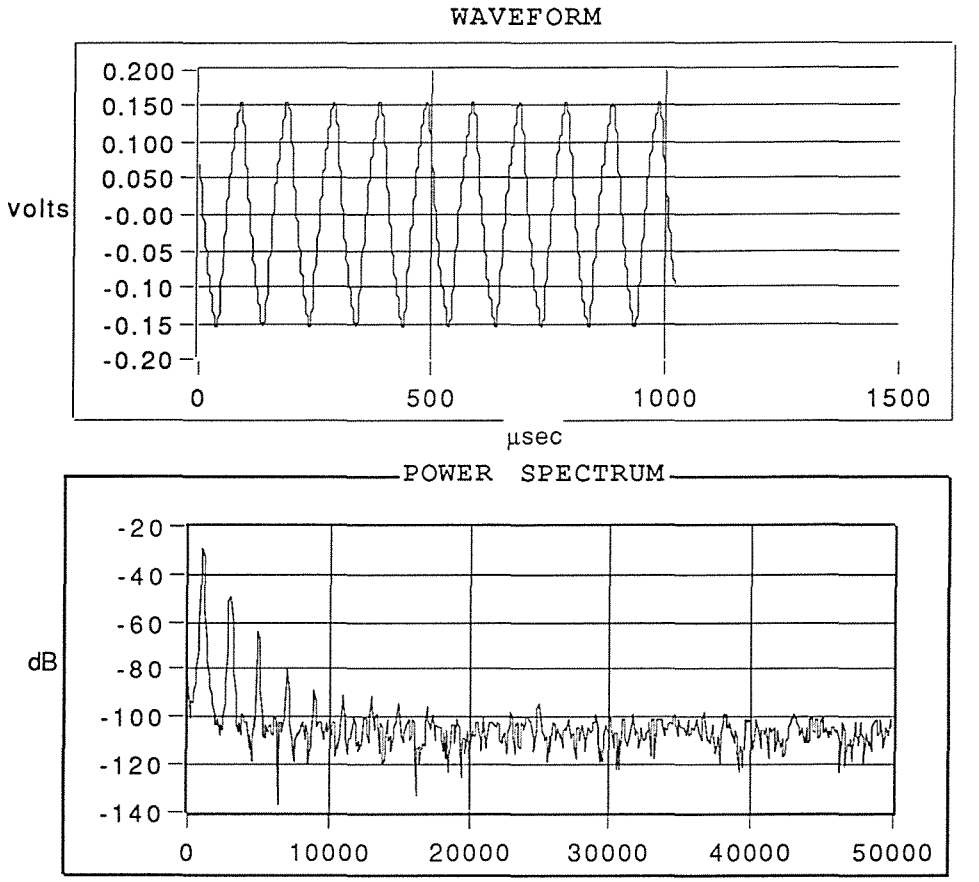


Figure 4.23

The filtered signal is probably a more accurate representation of the actual harmonic components of the signal, the peaks being significantly reduced in comparison to the unfiltered waveform.

The unfiltered current waveform and power spectrum for the 1 kHz square wave with optimum feedback are shown in Figure 2.24

1 kHz Optimal Feedback Unfiltered
1024 samples 10 μs gain = 1

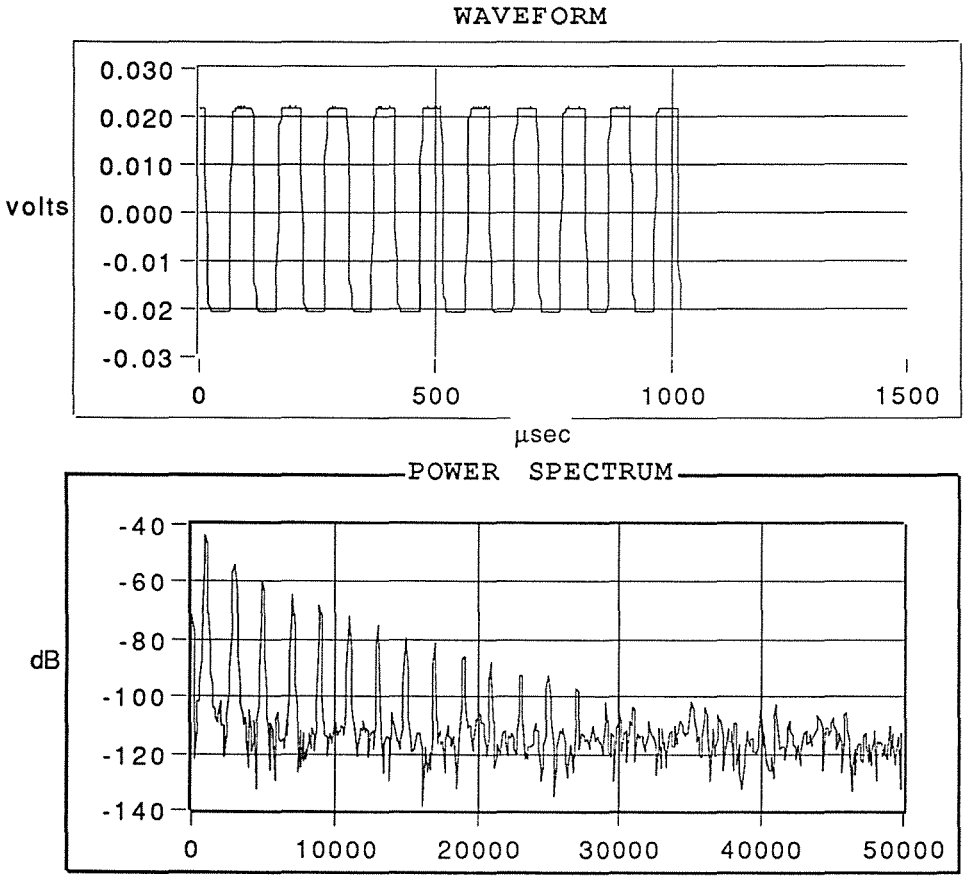


Figure 4.24

The unfiltered waveform produces an increased number of harmonics, at a much higher dB level, than the corresponding signal with minimum feedback, reference to Figure 4.22. This clearly demonstrates the justification of the use of a transconductance amplifier to produce a relatively square magnetic waveform from an inductive load i.e. a coil. Caution is still necessary with regard to frequencies above the Nyquist limit (20 kHz). For comparison, the results for the filtered signal are shown in Figure 4.25.

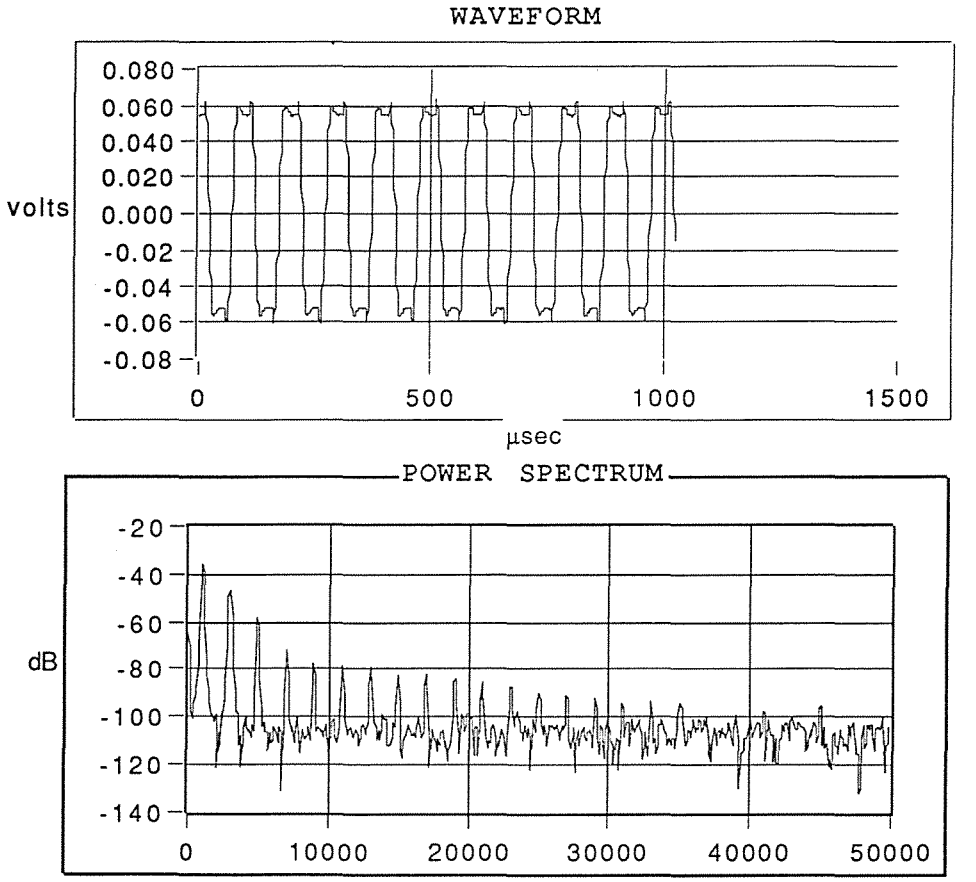


Figure 4.25

In a similar fashion to the paired results of the unfiltered waveform for the minimal feedback case, the optimum feedback filtered waveform contains significantly higher dB levels for harmonics than its minimal feedback, filtered signal counter part.

In an attempt to determine the effect of maximum current feedback, identical tests were performed, the results of which are shown for the unfiltered and filtered states in Figures 4.26 and 4.27 respectively.

1 kHz Maximal Feedback Unfiltered
1024 samples 10 μs gain = 1

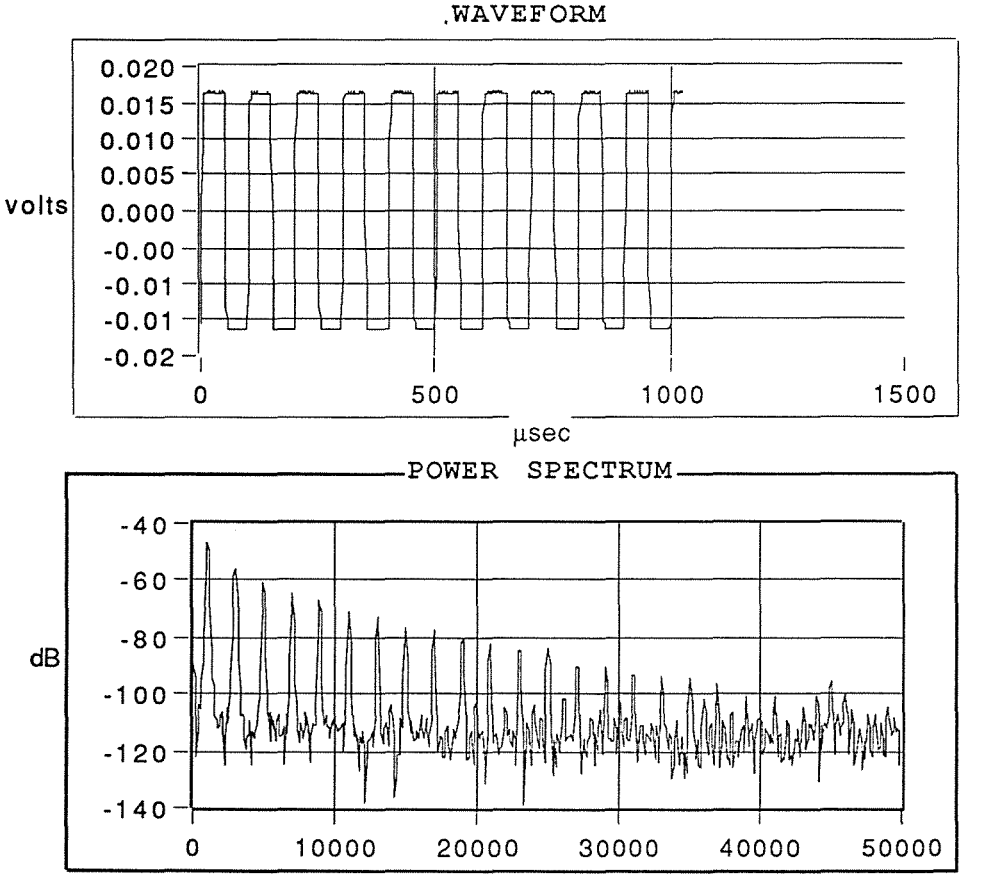


Figure 4.26

The results for the unfiltered waveform at optimal feedback produce an almost identical result to the maximum feedback case. Although the optimum feedback setting is well below the maximum possible setting, no significant increase in square wave performance is obtained. This is also borne out for the filtered case as would be expected. The filtered case is shown in Figure 4.27.

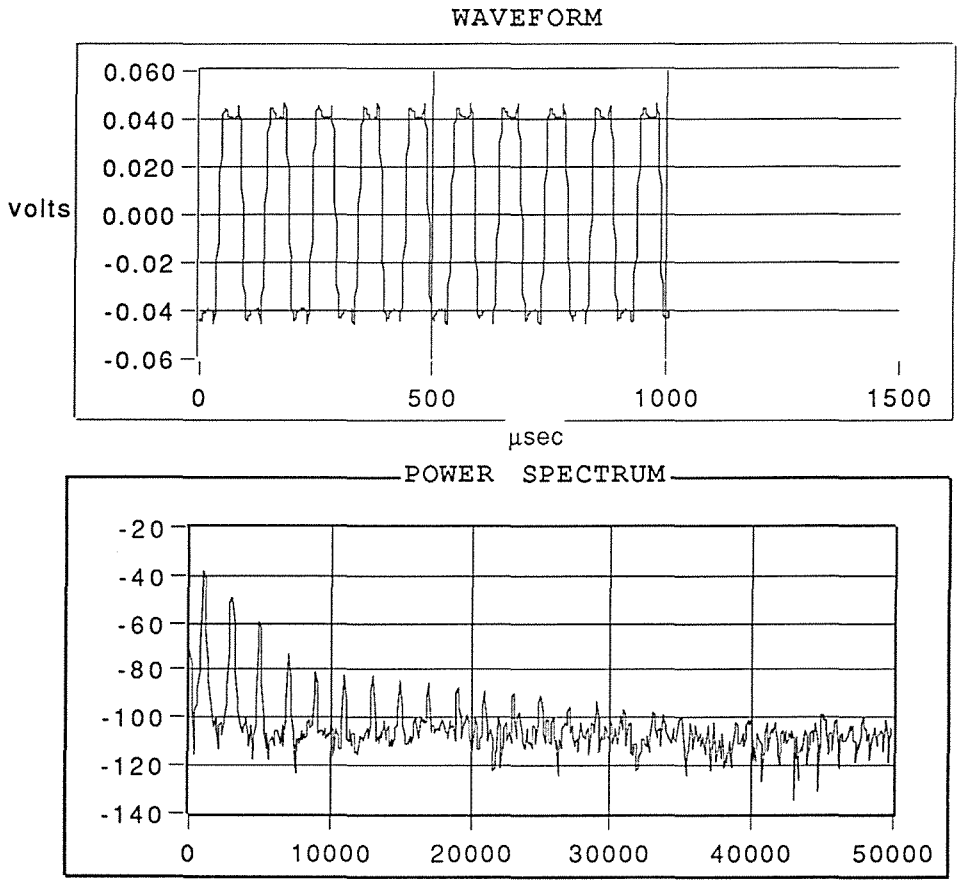


Figure 4.27

In order to further test the transconductance amplifier's performance, a 1 kHz sine wave was tested under circumstances identical to the square wave. The case for the maximum feedback condition was chosen which in fact also corresponds to the optimal condition. The results for the unfiltered trial are shown in Figure 4.28.

1 kHz Sine Wave Maximal Feedback Unfiltered
1024 samples 10 μs gain = 1

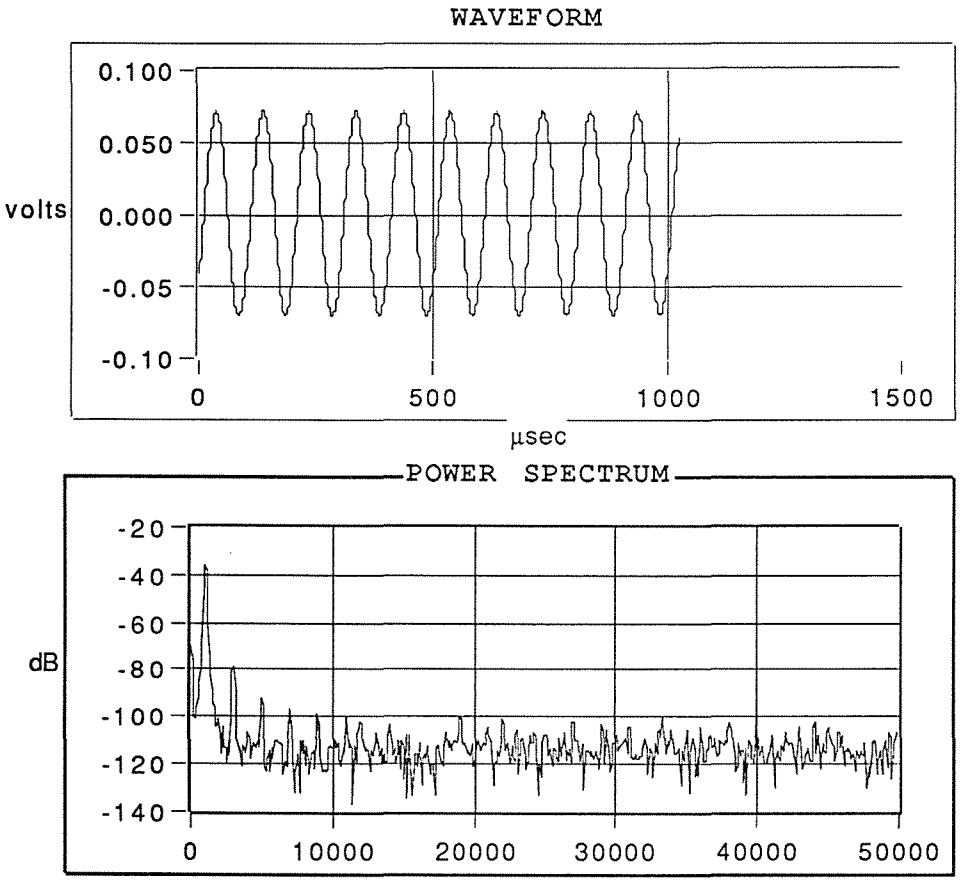


Figure 4.29

The harmonic envelope for a sine was is significantly different for the sine wave in comparison to the square wave, as would be predicted. Note that the harmonic peaks are even, rather than odd as for the square wave. It is interesting that the significant peaks are at: 1 kHz (fundamental); 3 kHz; 5kHz; 7 kHz and 9 kHz. Thereafter the signal in the power spectrum degenerates into noise. The harmonic peaks disappear into noise even more rapidly for the filtered case as is shown in Figure 4.30. This suggests that high frequency components are being added to the lower values, artificially raising them, as previously discussed.

1 kHz Sine Wave Maximal Feedback Filtered
1024 samples 10 μs gain = 1

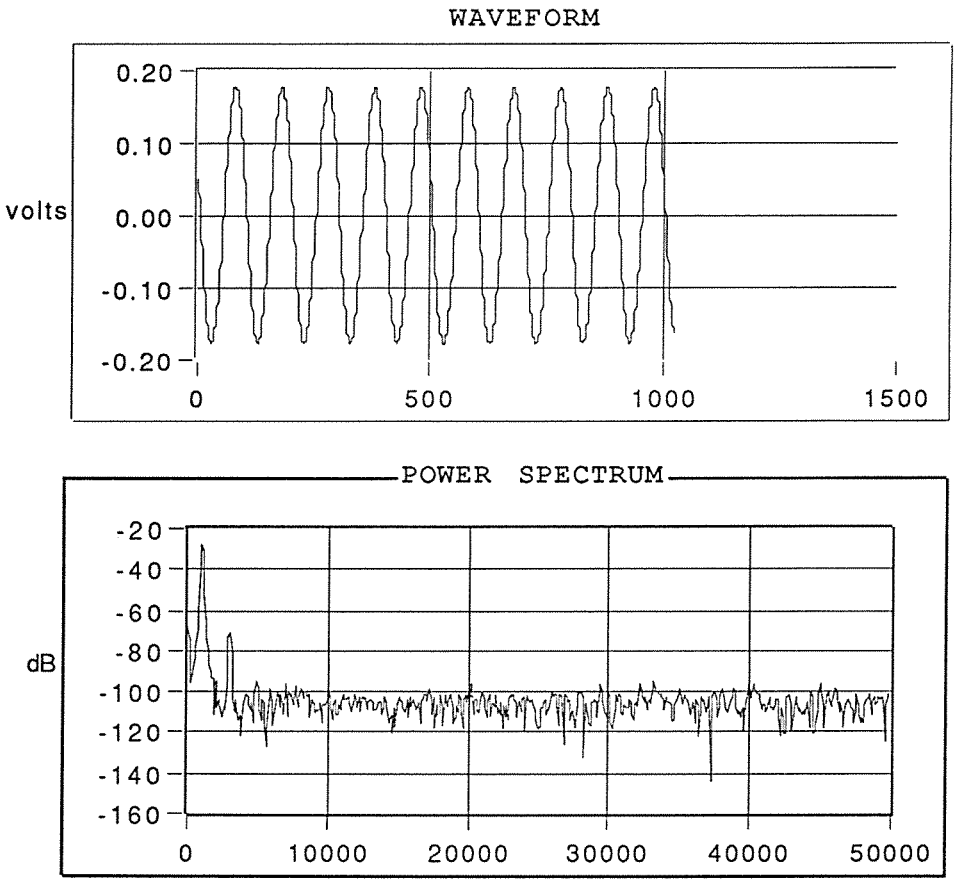


Figure 4.30

It is of biological significance that the sine wave produces far fewer harmonics than the square wave. This is particularly important if the phenomenon of frequency windows (or frequency dependent responses) is being studied. For example, if a physiological response peaks at 3 kHz, it might be expected that some response would also be initiated if a 1 kHz square wave were applied. This response would not, of course, be optimal, but there may be significant components of harmonics present in such a square wave to produce this sort of response. It is important therefore to investigate the effects of such harmonics on biologically responsive systems to determine the actual frequency response curve.

4.7.3 Harmonics: theoretical vs actual

In conclusion to the analysis of the transconductance amplifier, the actual versus theoretical harmonic envelope will be discussed.

A square wave may be considered as an infinite series of sine functions added together in a Fourier series of the following form:

$$f_{sq} = 1/3 \text{ Sine } 3\ \omega.t + 1/5 \text{ Sine } 5\ \omega.t + 1/7 \text{ Sine } 7\ \omega.t \ldots$$

The harmonics are all odd numbers in the case of the square wave, (whereas in the case of a triangle wave function the harmonics are all even numbers). The odd harmonics appear as the frequency term peaks in the Fourier analysis in Figures: 4.22 to 4.29.

Using the above formula the theoretical values for the first 13 harmonics were calculated and plotted in comparison to the actual values. The dB values for the actual harmonics were standardised for the fundamental being equal to zero. The plot thus shows the relative dB drop for each harmonic for both the theoretical and actual values. The dB plots of theoretical and actual are shown in Figure 4.31, and the values presented in Table 4.6.

Theoretical and actual harmonics

kHz	Theoretical dB	Actual dB
1	0.000	0.000
3	-9.55	-10.968
5	-13.98	-21.855
7	-17.08	-27.823
9	-19.09	-42.661
11	-20.82	-42.903
13	-22.27	-43.064

Table 4.6

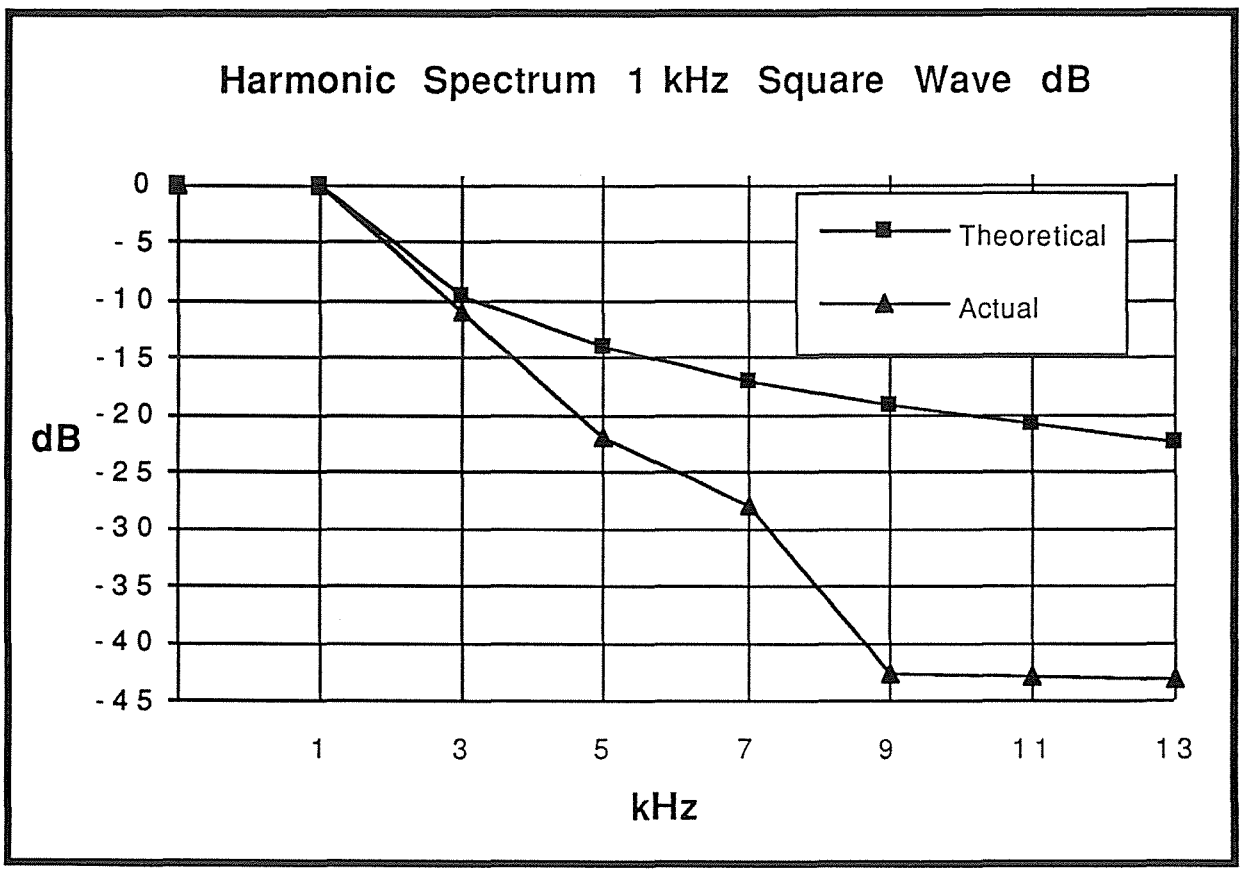


Figure 4.31

The actual harmonics are considerably lower than their theoretical values which leads to the conclusion that the transconductance amplifier is not a very good producer of square waves. The results are however considerably better than could be achieved with a conventional voltage amplifier which would behave in a similar fashion to the minimal current feedback condition. So while the transconductance amplifier is far from an optimal generator of square waves, it is significantly better than the available alternatives, thus justifying its inclusion in the magnetic biostimulator design.

To further explain the point, a graphic representation of theoretical versus actual harmonics is presented in terms of voltage in Figure 4.32.

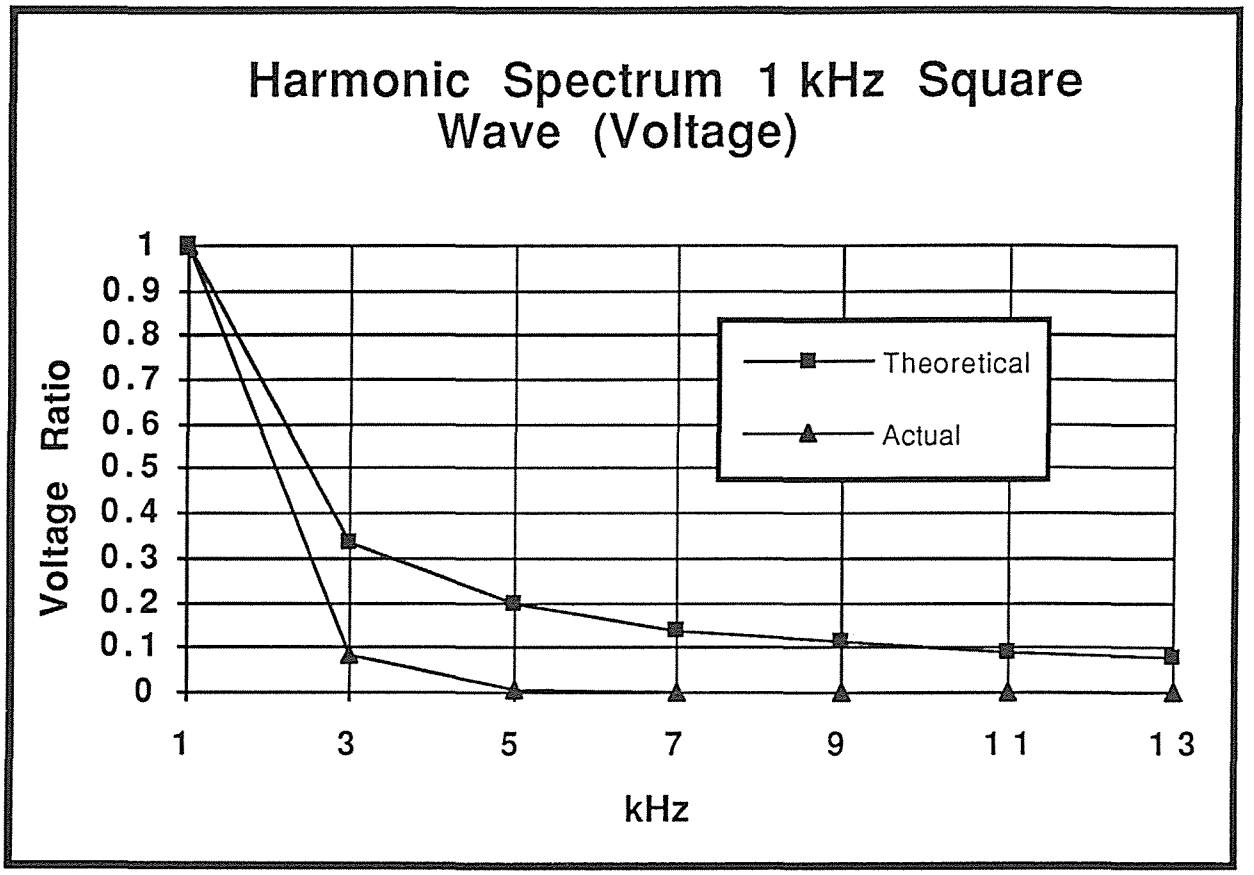

Harmonic Spectrum 1 kHz Square Wave (Voltage)
Voltage Ratio
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1
3
5
7
9
11
13
kHz
Theoretical
Actual

Figure 4.32

4.8 DISCUSSION AND CONCLUSION

The analyses detailed above show that the magnetic biostimulator performs to expectation. The accuracy of the timebase generator most probably exceeds the accuracy of the Hewlett Packard frequency counter used in the evaluation. The digital thumb wheel switches provide absolute accuracy in terms of frequency repeatability.

The analogue switches all perform to specification: the simple analog switch works precisely to the design expectations and has the advantage of working off a battery supply. The zero crossing switch gates precisely at the zero point, as shown in the figures of Section 4.3. The pseudo-random switch performs to specification, offering two ranges of clock speed.

The anti-aliasing low pass filter performs most adequately and was used extensively for analysing the squarewave capability of the transconductance amplifier.

The transconductance amplifier produces both sine and square waves at the rated power output discussed in Section 3.10.2. The squarewave is acceptable as referenced by the fourier analysis presented in Section 4.7.2. While not an ideal waveform, it is the best which can be achieved with such a basic system, and within the constraints of a limited budget. Future transconductance amplifiers may be able to produce faster rise times with greater power capability, however the cost will be substantially greater than this project's budget.

Together, the modules provide a very workable, practical instrument with much flexibility in terms of frequency, waveform, gating and power. Future developments may consider producing a unit with all the modules contained within a single case. This would increase the portability and ease of use.

The results obtained from both the clinical and laboratory studies provide testimony to a most useful, practical instrument with high accuracy and repeatability. Used in conjunction with MagneSim to simulate the magnetic flux density and distribution of symmetrical induction coils, a very scientific approach to magnetic field research may be attained.

5

Effects on Peripheral Circulation and Raynaud's Disease

CONTENTS

5.1 INTRODUCTION

In November of 1987 Dr Rochelle Wilson, a Palmerston North general medical practitioner, in response to reading Warnke's[15] paper in Pain Therapy, suggested to the author that patients with Raynaud's Disease would make ideal experimental subjects for determining if pulsed electromagnetic fields could increase peripheral circulation. It was decided to pursue this idea and guidance was sought from the Human Ethics Committee at Massey University. As a result of the joint cooperation of Dr. Wilson, Dr Geard (another Palmerston North medical practitioner), and Mr. Dunlop (a vascular surgeon), a number of subject trials were conducted between 1988 and 1990.

As a result of this pilot study, it was resolved that the author should undertake a post graduate research program to investigate the design and testing of a magnetic biostimulator. Although human clinical trials were carried out prior to commencement of the degree program, the results were so pivotal, in that they highlighted the requirement for a better biostimulator design, that it was decided that they should form part of the study, hence their inclusion in this thesis. A brief introduction to Raynaud's disease follows.

5.2 BACKGROUND

5.2.1 Maurice Raynaud

Maurice Raynaud[1], a Paris doctor, first identified a series of symptoms in 1862 including: white, blue, and red coloration of the skin; a feeling of severe cold; and associated pain. These symptoms manifest after exposure to cold or sudden swings in emotion. Raynaud described these symptoms together as "episodes of cold-induced digital vasospasm". The syndrome was restricted to the fingers, toes and, infrequently, the tongue. His findings formed the basis of his doctoral thesis at the Paris Institute. The arterial spasm common to both types of Raynaud's is shown in the upper Figure of Plate 5.1, with the lower Figure showing the worst case scenario of digital gangrene. In a few extreme cases, autoamputation of the digit may occur. More frequently surgical intervention occurs first in such cases.

5.2.2 Sir Thomas Lewis

Sir Thomas Lewis[2,3,], a prominent physician of the 1920's, after discovering that digital vasospasm attacks may be induced by interrupting the sympathetic nerves, concluded that there was a local fault in the sympathetic nervous system . He reasoned that there was a fault in the arterial wall that rendered the arterial wall over-responsive to the constrictive effects of cold.

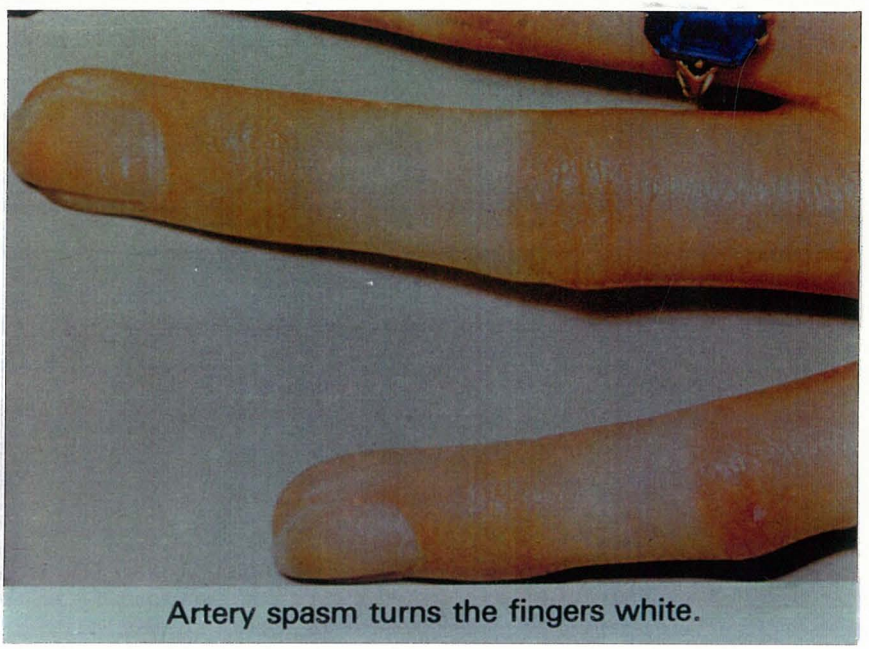
5.2.3 Raynaud's Phenomenon

Today 'episodes of cold or emotion induced vasospasm', in the absence of any other causal factor, are generally defined as **Raynaud's Disease**, or **Primary Raynaud's (Disease)**. Where these symptoms occur in combination with other disorders such as: lupus; arthritis; scleroderma; or arteriosclerosis, they are referred to collectively as **Raynaud's Phenomenon**, or **Secondary Raynaud's**.

5.2.4 Occurrence

There are no good indications of how prevalent Raynaud's Disease is in the community. Very few surveys have been conducted, probably because the disease is usually non-life threatening. In 1982 Heslop[4] conducted a survey of 520 patients in a Hampshire general practice, putting the incidence of Raunayd's at 17.6 % for women and 8.3 % for men. Heslop concludes that "These Figures may only suggest the tip of the iceberg however because the disease may only be reported when it reaches a serious stage". Holling[5] puts the Figure at 4 - 10% of the population while Spittell[6] reports the disorder to be nine times more common in women than men. Thus the vast majority of more minor instances may go unreported. Based on Figures from a Palmerston North practice, (personal communication), serious cases involving ulceration or autoamputation may be as rare as 1 in 10,000. With regard to more minor manifestations, the Figures may be as high as 50 % for women and 20 % for men. With respect to Raynaud's specifically in children, Duffey et al.[7] states "...the primary type is more common than was originally expected...".

Arterial spasm in the fingers of a Raynaud's sufferer



Artery spasm turns the fingers white.

Digital gangrene in a Raynaud's sufferer

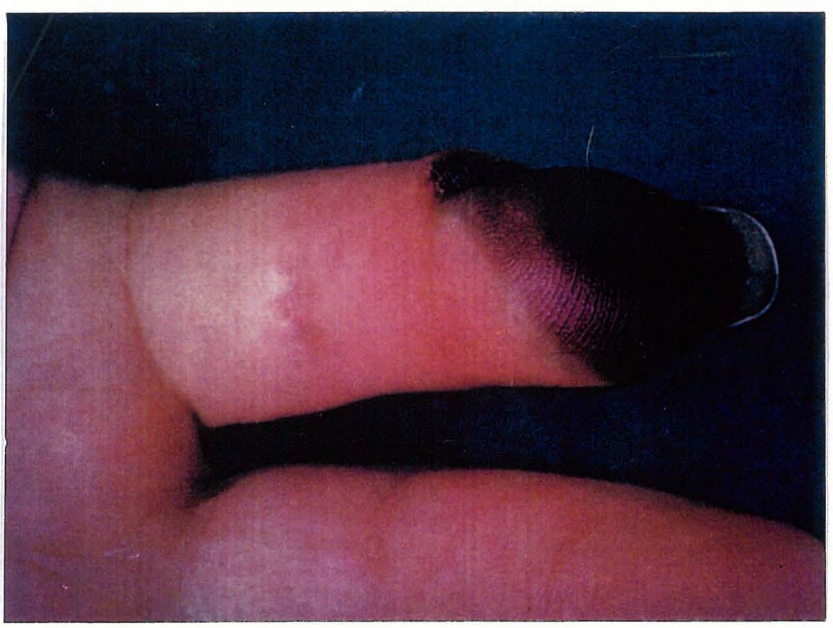


Plate 5 - 1

In a recent literature search spanning 1978 to 1993, only five epidemiological surveys were found: Hyslop[4] (mentioned above); Letz[8] et al.; Walker[9] et al.; Futatsuka[10]; and de Trafford[11] et al. Letz, Walker and Futatsuka all deal exclusively with vibration white finger disease, a version of Raynaud's Phenomenon, or Secondary Raynaud's, induced by holding vibrating objects such as hand tools. DeTrafford studied 1000 patients who had been pre-diagnosed as having Raynaud's Phenomenon, giving no indication of the incidence of Raynaud's in the general population. Hyslop is the only researcher to give any indication of the incidence of Raynaud's in the general population by examining 512 patients in a New Hampshire medical practice. No other surveys have been conducted in the last fifteen years relating to the occurrence of Primary Raynaud's in the general population.

5.2.5 Medical treatment

To evaluate the various medical treatments for Raynaud's, a computer data base search was initiated which covered the period 1978 to 1993. In all, 947 papers were selected by the key word, **Raynaud**. Many of these papers refer to vibration white finger disease, (or Secondary Raynaud's, otherwise known as Raynaud's phenomena or phenomenon) and consequently were dismissed. The current investigation will be confined to Primary Raynaud's Disease only. Some of the principal findings are discussed here:

Two scientists, Campbell and LeRoy[12], recently commented that "100 years on, Raynaud's still remains an enigma". Treatment has proved to be elusive. Mild cases tend to be treated with vasodilating drugs, however the effect is usually disappointing. Vasodilators tend to have a general effect, rather than localising to

the area of spasm, thus **all** vessels tend to dilate slightly. This results in a drop in overall blood pressure, which produces less blood flow through the affected capillaries because of their small diameter, (hence higher resistance).

Numerous other drugs have been tried over the years, but few have any real tangible benefits: while some drugs have some benefits, there is no universal cure-all. Attempts have been made to control the viscosity of the blood, the level of calcium associated with the cell membrane as well as fibrinolysis and red cell deformation. All have been largely to no avail. In some cases, there is a little improvement, but this is often transitory.

In severe cases of ulceration or where there is the danger of autoamputation, surgical techniques have been employed. This usually involves destroying the sympathetic nerves in a particular area either by injection of drugs, or by surgically severing the fibres. Cotton and Khan[13] in their "Raynaud's Phenomenon: a review" state that "sympathectomy fails in 81% of cases".

It is important to note here that the nomenclature of Raynaud's is by no means universal. The literature is littered with vague references to Raynaud's without specifying whether the primary disease or secondary phenomenon is meant. This makes interpretation of the literature a difficult task. In an attempt to summarise the current position regarding drug therapy, 25 key papers have been chosen which may be considered somewhat representative of contemporary medical opinion. It is clear that there is still great divergence of opinion regarding the efficacy of any particular drug treatment, hence Campbell and LeRoy's[12] comment regarding a

general lack of progress over the past 100 years. The papers are summarised in Table 5.1

Table 5.1 details the drug or drugs used for each trial. Where two drugs are listed this usually means the study was comparative of the two drug treatments. The Index Medicus unique identifier is listed for direct reference to the Appendix. The number of subjects is listed if known, and the experimental double-blind protocol is noted. Primary Raynaud's is denoted by "1^{o}", and secondary Raynaud's or Raynaud's phenomenon by "2^{o}", with the number of subjects per category listed. The presence of any side effects of the drug / drugs is flagged by a "√". The results have been summarised as follows:

? √	Some positive benefits noted in some subjects e.g. reduction in the number of attacks, or their severity.
√	Definite positive effects noted - this drug could be of considerable benefit to some subjects.
1^{o} √	Some benefits noted for Primary Raynaud's Disease.
2^{o} √	Some benefits noted for Secondary Raynaud's Phenomenon.
0	Null result - no difference in control vs treated, or between two drugs.
x	No benefits to the subjects at all. Drug not a suitable modality for Raynaud's.

Selected papers representative of contemporary drug trials.

Drug / Treatment	Unique ID	No. of Subjects	Double-Blind	1^o	2^o	Side Effects	Results
Nifedipine	87256034	?	?	?	?	?	? √
"	85171984	?	√	?	?	?	√
"	87254085	21	√	?	?	√	? √
"	87254086	16	√	8	0	?	√
"	89152134	15	√	?	?	√	x
"	89076709	56	√	?	?	?	x
"	87026447	34	√	28	6	√	√
"	89133132	22	√	22	0	√	x
Nifedipine+ Felodipine	90005600	16	√	?	?	?	0
Nifedipine+ Iloprost	89194528	23	√	?	?	√	N√ I√
Iloprost	88241213	12	?	0	12	√	? √
Nifedipine+ Ketanserin	89133131	28	?	?	?	√	K√, Nx
Ketanserin	88258206	13	√	?	?	?	? √
"	89064895	14	√	?	?	?	? √
"	89371790	222	√	?	?	?	√
"	89305940	29	√	?	?	?	√
Enalapril	91265247	21	√	21	0	?	x
"	88273377	17	√	9	8	?	1^o√, 2^ox
Prostaglandin	89234788	1	?	0	1	?	√
PEG	90096079	12	?	0	12	√	√
Prazosin	89214753	24	?	?	?	?	√
Omega 3	89116243	32	?	?	?	?	1^o√, 2^ox
Hexy Nicotin.	89076824	30	?	?	?	?	? √
Biofeedback	88320840	27	?	?	?	?	√

TABLE 5.1

With respect to the selected papers in Table 5.1, it is interesting to note that only 15 of the 24 experiments employed double-blind procedures, while 8 of the 23 drug trials noted significant side effects of the drug / drugs forcing some subjects to withdraw from the study. Interpretation of the results is further complicated as 16 of the studies do not specify whether the subjects suffered from either Primary or Secondary Raynaud's. This is a significant problem as subjects with Secondary Raynaud's would be expected to have the confounding variables of other disorders such as arthritis, which could markedly alter their response to any particular drug treatment.

When examining the efficacy of a particular drug therapy it is significant that the results are not consistent between studies. Nifedipine, considered by many the drug of choice, provides a very mixed response: two of the eleven studies provided some slight beneficial effects; five further studies produced no beneficial effects; and only four studies relate significant beneficial effects. Even considering the 4 positive results, the benefits were in fact only minor, e.g. a reduction in the number or severity of attacks, rather than a total lack of symptomology. At best nifedipine can be considered a help in some instances rather than a "cure".

It is interesting to note that the biofeedback experiment produced the best long term results without any side effects. Freedman$_4$ et al. discovered that feedback-induced vasodilation was attenuated by brachial artery infusions of propranolol in infused, but not contralateral hands, and this was not affected by digital nerve blocks. Their experiments revealed that quantitative measurement of finger blood flow demonstrated that vasodilation occurred in the arteriovenous anastomoses (shunts) in normal persons, and in the finger capillary beds of those subjects with Raynaud's disease. More important is the finding that **Raynaud's disease**

patients who received finger temperature feedback reported 80% fewer symptoms one and two years after treatment, and they retained the ability to increase finger temperature and capillary blood flow at these times. These positive results were not mirrored by patients given autogenic relaxation training. It would appear that biofeedback offers a unique approach to the treatment of Raynaud's disease without the accompanying side effects of most drug regimes.

5.2.6 Magnetic fields

In 1983, Dr. Ulrich Warnke[15] published a paper entitled, "The possible role of pulsating magnetic fields in the reduction of pain". Warnke discusses three indicators for the effectiveness of alternating magnetic fields which symbolise the possible therapeutic use of weak electromagnetic fields: active widening of the blood vessels; increase in the partial pressure of oxygen in the terminal tissues; and the local perfusion and velocity of capillary blood flow.

Using infrared cameras in the 5 μm and 12 μm ranges, he was able to show modification of blood flow with the application of 200 hertz alternating magnetic fields pulsed between 2 and 25 hertz at a strength of 3 mtesla or 30 gauss. The results were shown to be similar in both humans and horses.

Warnke goes on to suggest that certain types of pain are caused through oxygen deficiency, (ischaemia), of the tissues as a result of reduced blood flow. This reduced vascularisation may also result in the build up of metabolic by-products in

the cells. Thus any agent which could increase blood flow would potentially be able to reduce such pain.

Magnetic fields, Warnke suggests, may accomplish vasodilation by means of hyperpolarising the post synaptic junction. While an individual pulse may not be able to directly suppress nerve firing, hyperpolarisation of the membrane may be a way in which the energy from a series of impulses may be stored up over time, ultimately causing a reduction in firing rate. If this reduction of firing rate occurs in the nerves of the sympathetic arm of the autonomic system, one result that could be predicted would be a reduction in muscle tone in the metarterioles of the capillary bed. This would result in more blood flow through the capillaries which could be measured either by an increase of surface skin temperature, or an increase in evolved oxygen through the skin, and subjectively by a perceived reduction of pain by the subject.

To test Warnke's hypothesis it is necessary to find a noninvasive, clinical procedure for determining sub-cutaneous blood flow. Section 5.3 investigates the possibility of using surface skin temperature for this purpose.

5.3 DETERMINATION OF PERIPHERAL CIRCULATION BY SURFACE SKIN TEMPERATURE MEASUREMENT.

5.3.1 Procedural requirement

In order to determine the effect of alternating magnetic fields on peripheral circulation, it is necessary to devise a non-invasive procedure capable of being correlated to sub-cutaneous blood flow.

5.3.2 Assessment of peripheral circulation

Biologists are familiar with the problem of making direct measures of biological phenomena. It is not possible to directly measure blood flow in peripheral tissues without disrupting the very phenomena under measurement. Therefore it is necessary to employ a secondary indicator for peripheral circulation.

In most cases the human body functions in an environment significantly cooler than its internal blood temperature. In this situation, the body acts as a heat source with the environment as the sink. In modelling the system the body may be described as a heat source, the core, surrounded by an insulating layer, the skin, existing in a significantly cooler environment. Given that the internal core is maintained at a relatively constant temperature by metabolic activity, it is reasonable to assume that surface temperature measurement would be a good indicator of the flow of heated fluid, the blood, close to the surface. The degree of insulation will have a direct effect on the temperature displayed at the surface.

In order to make use of this information, it is necessary to have some better understanding of the precise relationship of skin surface temperature to peripheral

blood flow. Elizabeth Dean[16] writing in the Australian Journal of Physiotherapy states:

"...skin temperature and blood flow are related linearly between 20 C and 30 C. Disproportionately greater flow is required, however, to effect temperature changes of the skin in excess of 30 C. Despite these restrictions, skin temperature can be a useful tool in establishing an index of blood flow and of the reactivity of the peripheral blood vessels in response to changes in vasomotor state. Normally with body heating and inhibition of sympathetic tone of blood vessels, blood flow increases and skin temperatures may approximate blood temperature. Body cooling will normally produce vasoconstriction and skin temperatures close to room temperature."

Alrick Hertzman[17], writing in the International Review of Physical Medicine and Rehabilitation, August 1953, provides a somewhat different picture of skin temperature versus skin blood flow. Hertzman is in general agreement with Dean that a decrease in peripheral blood flow usually results in cooling of the skin, and that this statement holds true at room temperatures between 20 to 25 C under the conditions usually prevailing during observations on peripheral vascular behaviour. The implied relationship between skin temperature and peripheral blood flow requires precise measurement of skin surface temperature and control of the environmental conditions under which this relation was to be applied. The data from this measurement regime have been utilised frequently in various tests of vasomotor tone, vascular occlusion and therapy, Dean[19]. In many applications, semi-quantitative comparisons of changes in blood flow have been inferred from the recorded changes in skin temperature.

Hertzman summarizes the results of these studies as follows:

"At room temperatures (20 - 25 C) ordinarily employed, heat losses to the room from skin surface depend principally on the difference between the skin and room temperatures. Hence at constant room temperature (and in the absence of sweating) skin temperature must rise when heat delivery to the skin increases with greater blood flow, (heat loss to the room likewise increases) and fall when vasoconstriction occurs."

If this relationship was a simple linear one, it would be a trivial matter to calculate blood flows from skin temperatures as the flow would equal k * T_S where k is the required proportionality constant, and T_S is the temperature of the skin. However the actual relationship is far too complex for such a ready solution. Hertzman provides a graph of surface skin temperature versus sub cutaneous blood flow in litres / metre^2 / hour at 20 C ambient room temperature which is produced in Figure 5.1.

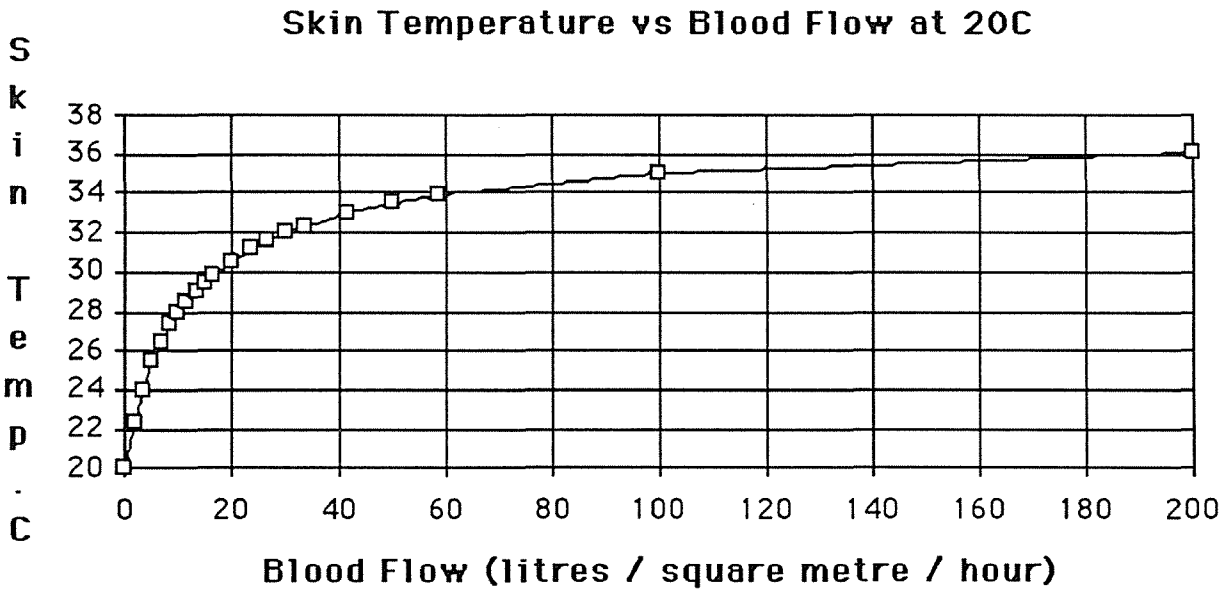


Figure 5.1

It is obvious that the relationship between skin temperature and blood flow is not linear. Matlab was used to model an equation to fit the data from Hertzman. The equation produced is a second order differential expression in the form:

$$T_{skin} = 37 - \left(7.69\, e^{-\frac{flow}{5.78}} + 9.31\, e^{-\frac{flow}{49.6}}\right)$$

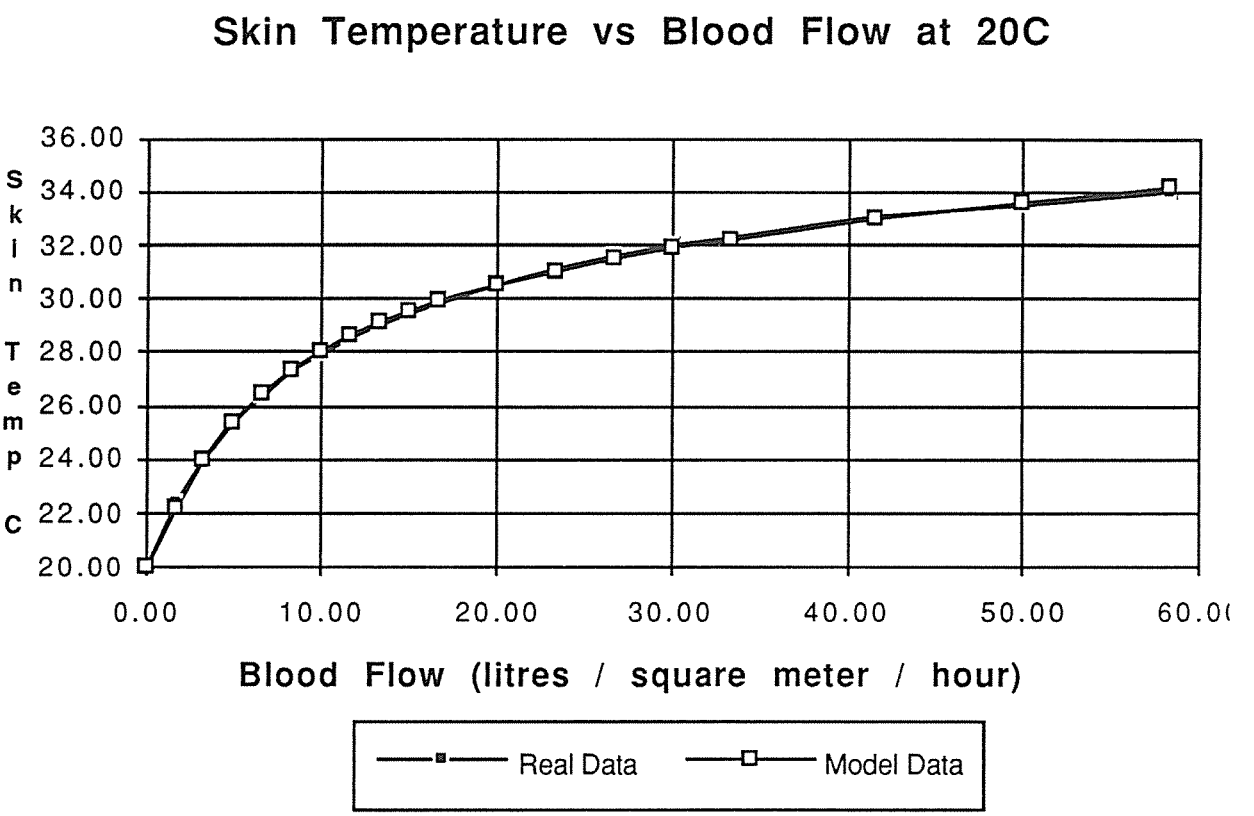


Figure 5.2

As can be seen from Figure 5.2, the model so closely matches the original data that it is not possible even to discern two distinct lines. To confirm this, the residuals of the sum of least squares used by Matlab to determine goodness of fit of the model are plotted in Figure 5.3.

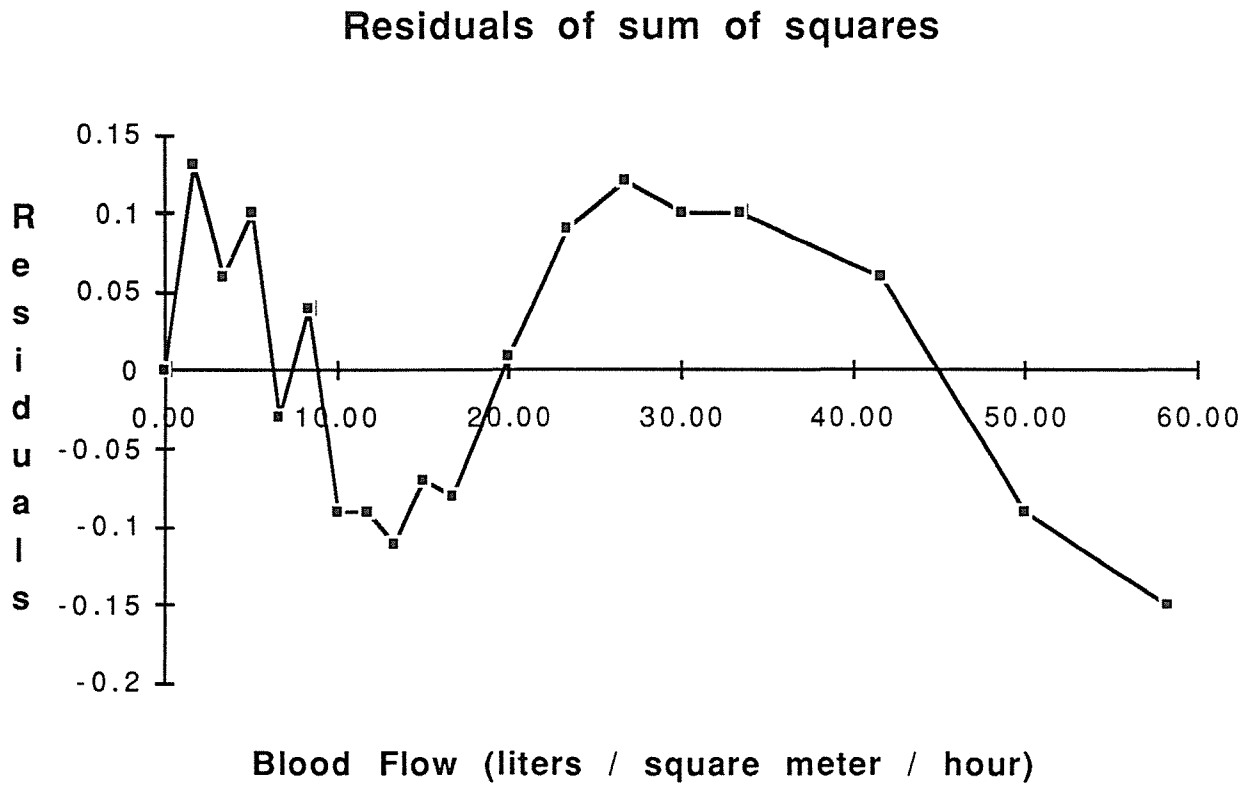


Figure 5.3

Although the relationship between skin temperature and blood flow is not linear, it is reasonable to use skin temperature data as an indicator of sub-cutaneous blood flow. While the above model describes the Hertzman data well, it must be remembered that this is only one data set from one experiment. There may exist significantly different data obtained by different researchers under various conditions which would undoubtedly be modelled by different equations. What this does show, however, is a relationship between blood flow and skin temperature which can be modelled mathematically.

While the relationship between skin temperature and sub-cutaneous blood flow is clearly not linear, skin temperature is certainly a good indicator for estimating blood flow and may be used as a non-invasive, clinical procedure.

5.4 Coil Model

Warnke[15] provides no information regarding the precise details of the coil used to produce the observed increases in skin temperature of either human subjects or horses. Therefore the exact field magnitude the hypothalamus experiences in his experiments with human subjects is not able to be determined. Basic assumptions were made to produce a simulation with MagneSim™.

Warnke states that the field magnitude at the coil surface is of the order of 2.5 -3.0 mT. This was used as the starting point for the simulation. Such considerations as the available power output of the amplifier, and the physical size limitations which would allow ease of use with human subjects resulted in the following model.

The physical dimensions chosen were 200 turns of 32 gauge wire wound in a tight circular section, short solenoid. The annulus has a mean diameter of 100 mm, with a cross sectional diameter of the winding of approximately 10 mm. The coil was tightly wound on a poly methyl methacrylate former, with the windings being varnished in an attempt to minimise sonic oscillation.

The magnetic characteristics were modelled using the MagneSim™ short solenoid option. The model of the resultant vectors is shown in Figure 5.4. The table of resultant vector directions in cartesian coordinates is shown in Table 5.2.

Resultant Vector Plot

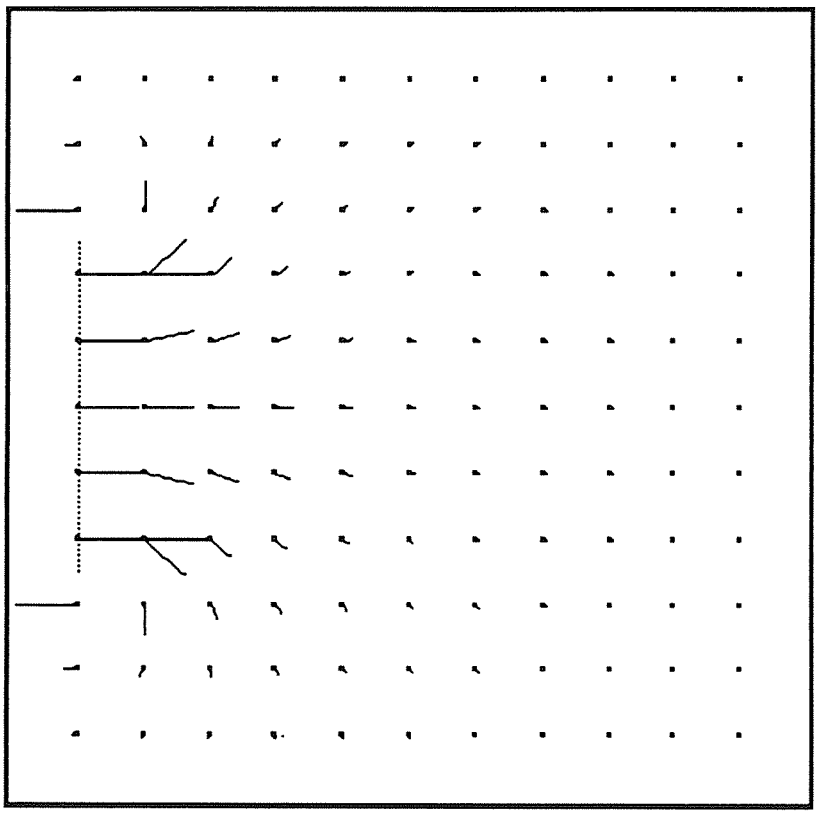


Figure 5.4

Direction of Resultant Vectors

180	140	110	90	76	66	58	52	47	42	39
180	125	93	74	62	54	47	42	38	34	32
180	92	68	56	47	41	36	32	29	26	24
0	41	41	36	31	28	24	22	19	18	16
0	14	18	17	16	14	12	11	10	9	8
0	0	0	0	0	0	0	0	0	0	0
0	346	342	343	344	346	348	349	350	351	352
0	319	319	324	329	332	336	338	341	342	344
180	268	292	304	313	319	324	328	331	334	336
180	235	267	286	298	306	313	318	322	326	328
180	220	250	270	284	294	302	308	313	318	321

Table 5.2

The magnitudes of the resultant vectors is shown in Table 5.3.

Magnitude of Resultant Vectors

2.167	2.078	1.810	1.459	1.132	0.865	0.662	0.509	0.396	0.311	0.248
5.326	4.658	3.413	2.381	1.663	1.178	0.851	0.627	0.471	0.361	0.281
26.761	12.698	6.407	3.733	2.340	1.541	1.056	0.748	0.545	0.407	0.311
56.725	22.430	9.840	5.207	3.037	1.894	1.245	0.855	0.608	0.447	0.336
28.685	21.164	11.587	6.245	3.554	2.153	1.381	0.929	0.652	0.473	0.353
25.133	20.117	11.967	6.594	3.742	2.248	1.430	0.956	0.667	0.482	0.359
28.685	21.164	11.587	6.245	3.554	2.153	1.381	0.929	0.652	0.473	0.353
56.725	22.430	9.840	5.207	3.037	1.894	1.245	0.855	0.608	0.447	0.336
26.761	12.698	6.407	3.733	2.340	1.541	1.056	0.748	0.545	0.407	0.311
5.326	4.658	3.413	2.381	1.663	1.178	0.851	0.627	0.471	0.361	0.281
2.167	2.078	1.810	1.459	1.132	0.865	0.662	0.509	0.396	0.311	0.248

Table 5.3

In order to determine the exposure conditions of the hypothalamus, the anatomical configurations of the skull were transferred to scale onto the resultant vector diagram. This anatomical model is shown in Figure 5.5 and should be compared to the resultant vector diagram, Figure 5.4.

Anatomical Model of Hypothalamus Exposure

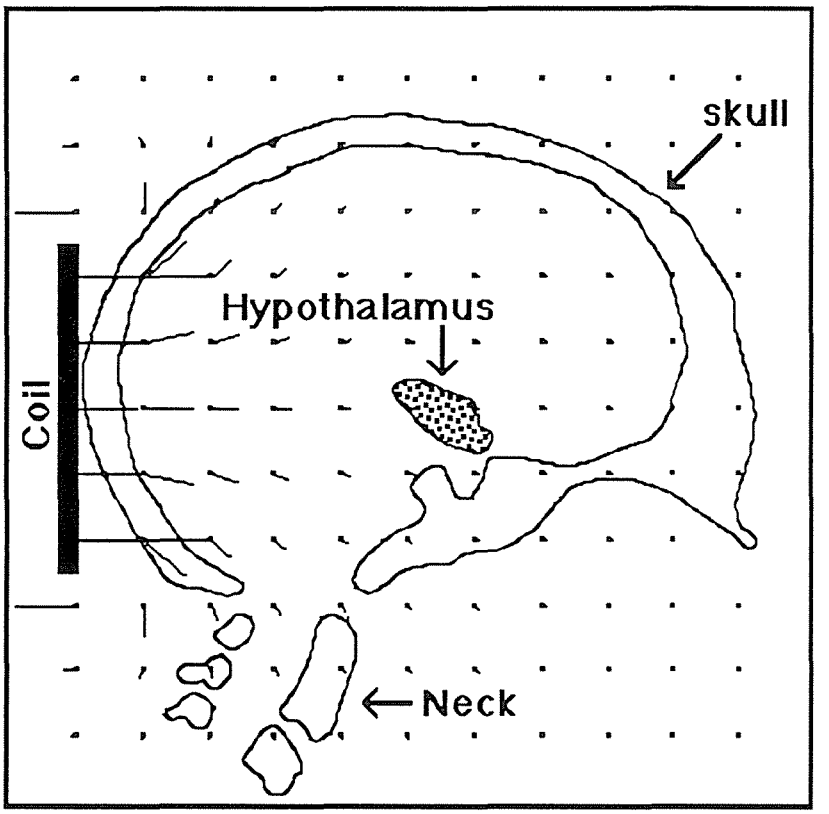


Figure 5.5

The direction vectors in the immediate vicinity of the hypothalamus extracted from Table 5.2 are presented below in Table 5.4

Directions of Vectors in the Hypothalamus

14	12	11
0	0	0
346	348	349

Table 5.4

The magnitudes of the corresponding resultant vectors in Gauss, (as MagneSim's output is in gauss) are presented in Table 5.5. (To convert gauss to mTesla, divide gauss by 10).

Magnitudes of Vectors in the Hypothalamus

2.153	1.381	0.929
2.248	1.430	0.956
2.153	1.381	0.929

Table 5.5

From Figure 5.5 and Table 5.5 it can be seen that the hypothalamus, the temperature control centre of the brain, is subjected to magnetic field strengths of the order of those which would be produced from commercial treatment units (Section 2.2.4), and probably the Warnke protocol. As previously stated, since Warnke gives no details of the coil system, the above calculations are at best an estimate of the exposure he used. Section 5.5 outlines the initial pilot study experiments using the system previously described above.

5.5 EXPERIMENTAL PROCEDURE

Warnke[15] has not established which component of the applied pulsed magnetic field initiates the response. The frequency of the carrier wave (200 Hz) could be critical, or alternatively, the frequency at which the carrier is chopped (22-25 Hz) could be the active component.

5.5.1 Pilot Study

In an attempt to isolate the vital component, it was resolved that a single frequency would be applied at similar field strengths to the Warnke experiment. Initially the frequency of the pulsed modulation was chosen - 22 Hz after personal communication with Dr. Warnke. This frequency was applied to several subjects in a similar manner to the Warnke protocol with little result. As a result of the lack of a clear response in this pilot study, it was decided to pulse the frequency on and off at random intervals varying from 0.2 to 2.0 seconds. This may have the effect of reducing the habituation response which is postulated by the author as the cause of the failure of some of the pilot study experiments to obtain the vasodilation response. The habituation response is discussed in Section 3.5.3, Neurophysiological functioning of the brain.

If the system was able to produce an habituation response, (that is, lock on to the signal and exclusively ignore it as it was not life threatening), the use of varying intervals of no stimulation could confuse the detection mechanism sufficiently to regard each additional pulse burst as a new and unique occurrence. It is reasonable to assume that the nervous system is able to lock on to a repetitive pulse train just as easily as a continuous stimulus, (as a continuous stimulus also produces a pulse

train of nerve impulses). This would however be dependent upon the period of the interval between stimuli. Therefore a continually changing stimulus should not produce habituation by definition. After consideration of the results of the pilot study, a major experiment was undertaken.

5.5.2 Experimental Subjects

Initially four patients suffering from Primary Raynaud's Disease were chosen along with four normal, healthy, controls. Each subject was asked to present for four trials. Two of the trials were to be controls where no magnetic stimulation occurred, the subjects experiencing 'sham exposure', while the remaining two involved magnetic stimulation. The experiments were run single blind, the subjects not knowing whether stimulation was being applied. Random numbers were used to determine whether a trial was of the control or test type on any particular day.

5.5.3 Magnetic Stimulation

Trials involved exposing the subject to an alternating magnetic field of 22 hertz at 25 gauss measured in the vertical 'Z' axis at the centre of the coil. (For a description of the coil see Section 5.4). The field was pulsed on and off randomly in the range of 0.2 - 2.0 seconds. The induction coil was wrapped in a towel and placed at the back of the subject's head with the coil centre approximately level with the hypothalamus.

5.5.4 Clothing

The subjects were all asked to present themselves for the trials wearing minimal clothing. They was then covered with a brushed polycotton sheet for the duration (60 minutes) of the experiment, the same sheet being used for all subjects throughout all trials.

5.5.6 Measurement

To determine the relative amount of vasoconstriction / vasodilation, surface temperature probes were placed on: the backs of the hands central to the palm; the upper arm midway between the elbow and shoulder; the base of the sternum on the chest; the middle of the upper thigh midway between the knee and hip; the tops of the feet approximating the junction between the cuboid and the scaphoid bones of the tarsus; and the soles of the feet at the junction of the third metatarsal (middle toe) and the fourth tarsus (forth exterior cunieform). An additional probe was placed in the armpit to estimate core temperature. To date, **no** papers have revealed a change in core temperature under the application of extremely low frequency magnetic fields. The ambient temperature of the room was also recorded. An optical probe placed on the ear lobe was used to determine pulse rate.

Plate 5.2 shows a recumbent model, (not a real experimental subject), with temperature sensors taped to the skin and connected to the computer controlled data acquisition system. Plate 5.3 show the optical heart rate probe.

Model subject with temperatures sensors in place

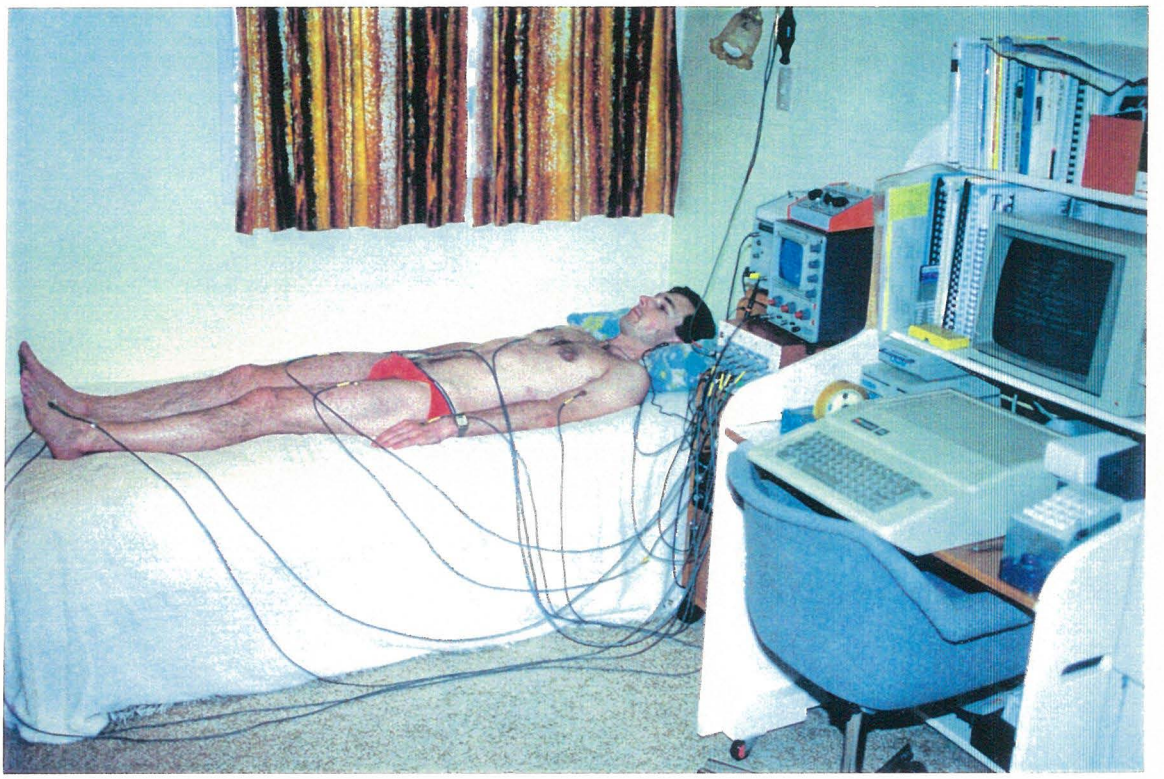


Plate 5 - 2

Model subject with optical heart rate sensors on earlobe

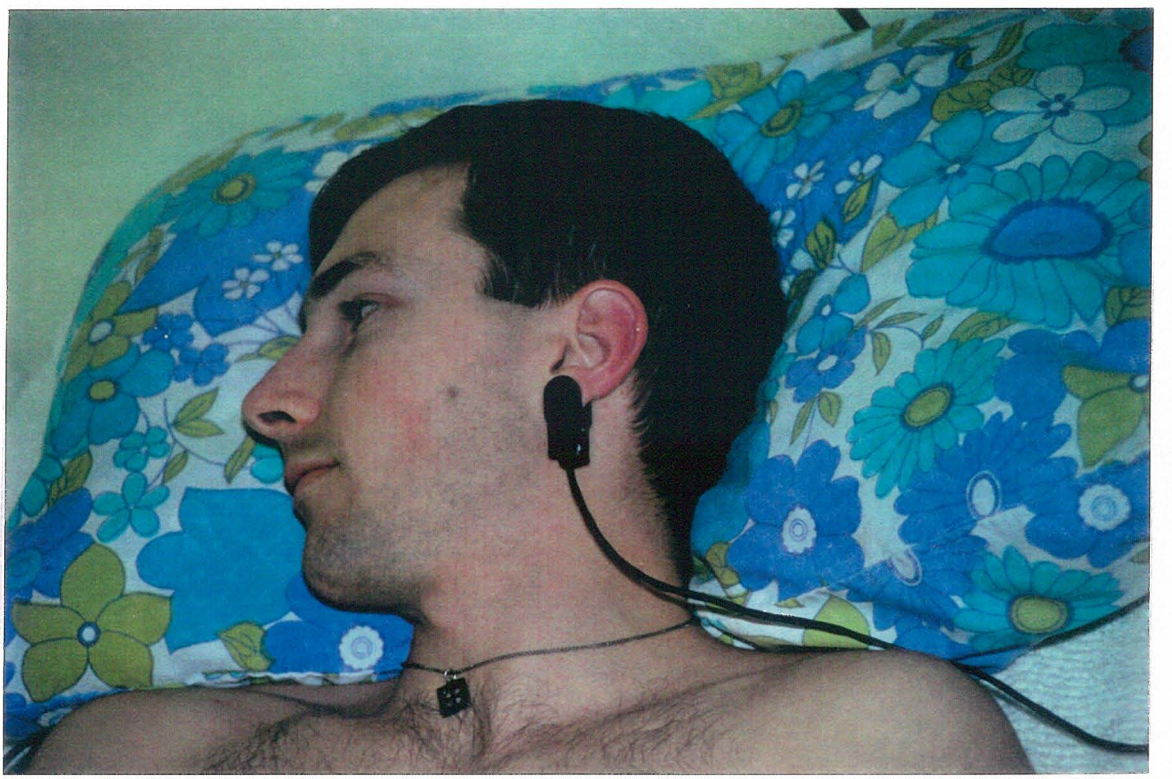


Plate 5 - 3

In test trials, the stimulation was turned on from the 10th minute and allowed to continue to the 60th minute. At the end of the 60th minute, the trial was terminated. Temperature readings were recorded by the computer every 120 seconds providing 31 data points (including time 0) per channel.

5.6 RESULTS

5.6.1 Grouped mean differences.

The minimum, maximum, mean and difference temperatures were determined for all probe sites for the four chosen subjects. The difference temperature is defined as the temperature at time 60 minutes, minus the temperature at time 10 minutes ($T_{60} - T_{10} = T_{diff}$). The means of the difference temperatures for all subjects are presented in Figure 5.4. "N" represents a normal, healthy subject, while "R" represents a Raynaud's subject, i.e. someone who has been diagnosed by a qualified medical practitioner as having Primary Raynaud's Disease.

The trends displayed in the grouped means may not be significant due to the considerable differences between subjects, indeed combining subject means is probably an invalid comparison due to the differences between individuals. The values are shown in Table 5.6 and presented graphically in Figure 5.6.

All Subjects Mean Difference Temperatures T_{60} - T_{10}

Probe Site	Normal Control	Normal Test	Raynaud's Control	Raynaud's Test
Air	0.033 -	0.03	-0.07	-0.55
R Hand	-0.06	-0.20	-0.01	0.116
L Hand	0.022	-0.14	0.269	0.092
R Arm	0.05	-0.02	0.017	-0.28
L Arm	0.03	-0.04	-0.15	-0.04
Chest	-0.03	-0.03	-0.06	0.04
Core	-0.02	0.029	0.021 0.	168
R Thigh	0.055	0.043	-0.13	-0.46
L Thigh	0.017	0.00	-0.02	-0.51
RFT	-0.15	-0.19	0.533	0.308
LFT	-0.11	-0.25	0.256	0.180
RFB	-0.12	-0.37	0.463	0.378
LFB	-0.10	-0.37	0.371	0.278

TABLE 5.6

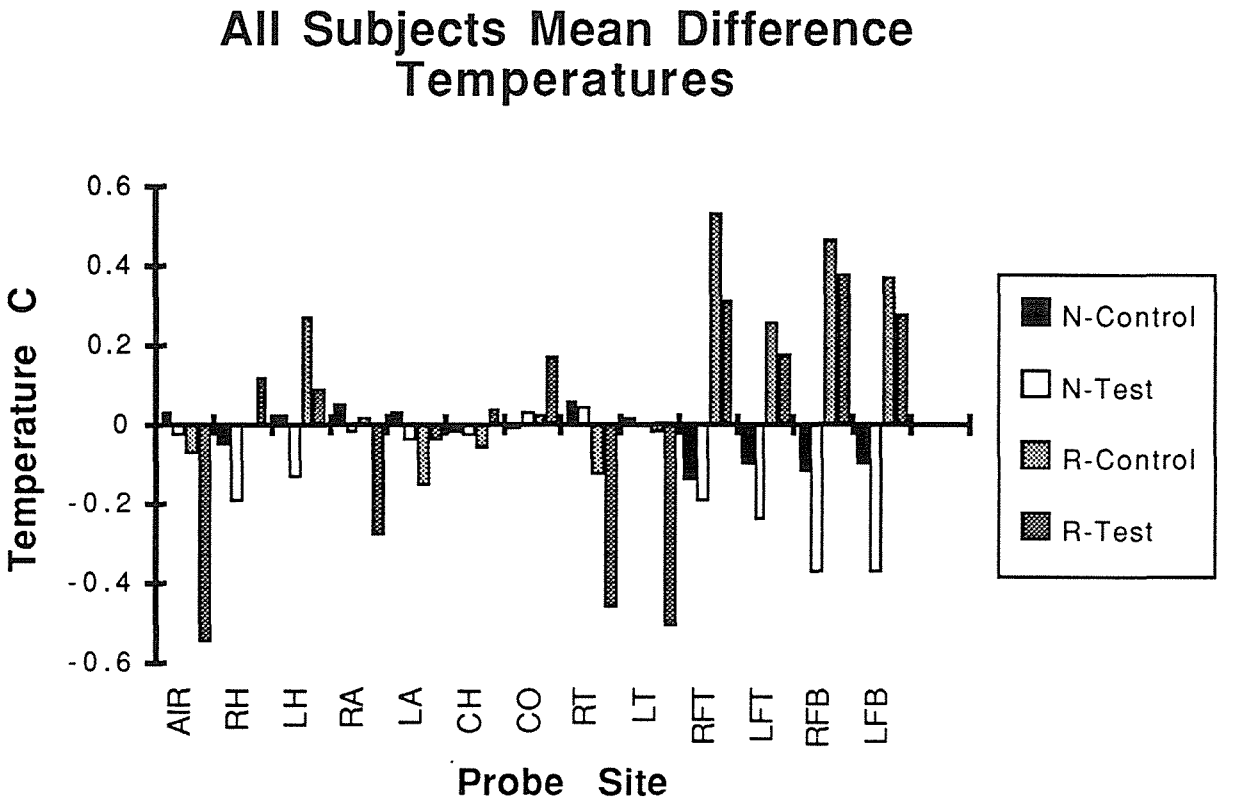


Figure 5.6

5.6.2 Normal control subjects.

Before discussing the effects of pulsed magnetic field therapy on Raynaud's sufferers, it is logical to examine the effects of such stimulation on normal subjects. The effects of pulsed electromagnetic fields on Raynaud's sufferers will be compared and contrasted with those of normal control subjects in Section 5.6.3. Because of the considerable differences between individuals, it is not valid to compare results between subjects, only between trials within each subject. For this reason, and in the interest of brevity, only two examples will be quoted which may be said to be somewhat representative of the general trend. The subjects are referred to by a two letter code to preserve their anonymity - all results being

strictly confidential in line with the Human Ethics Committee's suggested guide-lines.

5.6.2.1 Normal subject BF

The first control subject BF, a 25 year old male with no contraindications, was subjected to two control trials where no magnetic stimulus was applied, and two test trials where 22 Hz, fast random pulsed, magnetic fields were applied at 25 gauss to the back of the head. The subject was not aware of which experiments involved the use of magnetic stimulus - the protocol being 'single-blind'. The order of the trials, (placebo control and test) were randomly assigned by computer. The difference temperatures (T_{diff}) are shown in Figure 5.7.

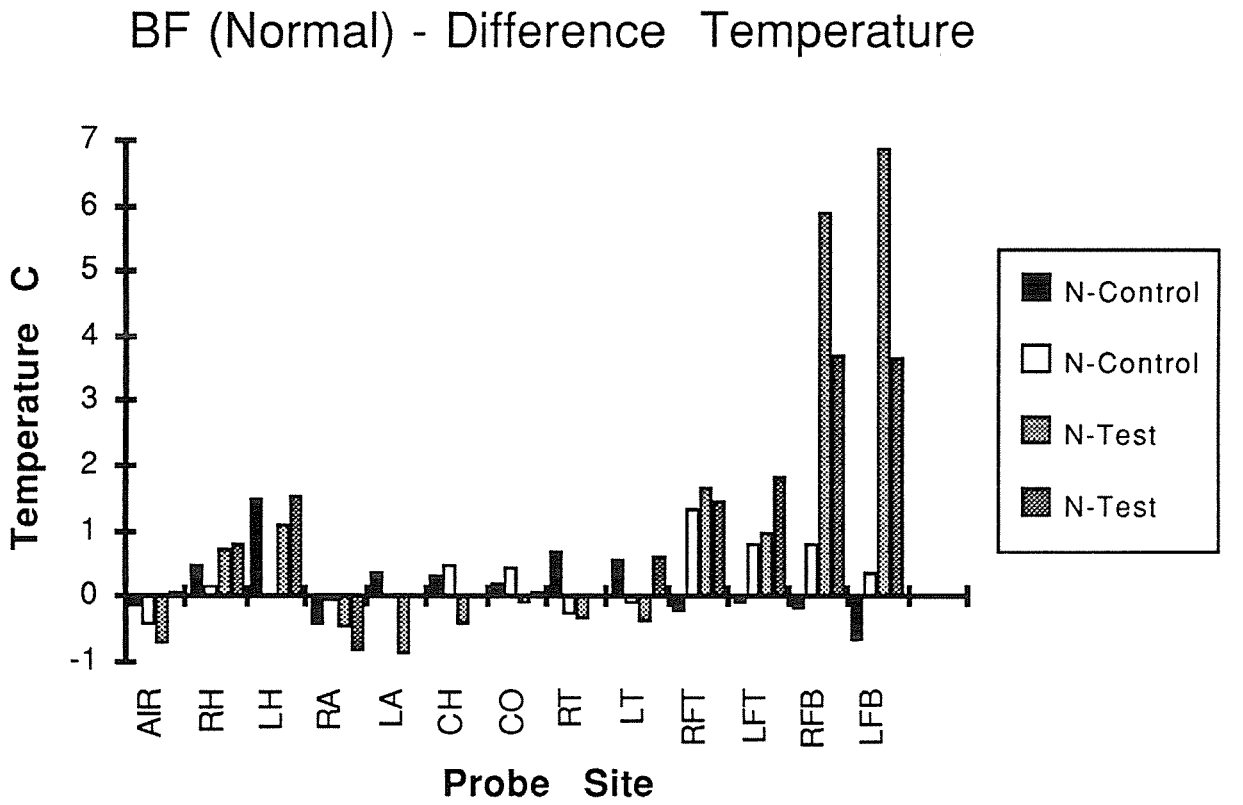


Figure 5.7

No significant increases in temperature are noted for the arms, thighs, chest and core temperatures. The hands show a mean increase of 1.05 C with magnetic stimulation, compared to 0.55 C without stimulation. The feet however show very significant increases: 0.54 C compared to 6.54 C with stimulation. These grouped means are shown in Figure 5.6.

The hands and feet are under the control of the autonomic nervous system and differ in their response to normal temperature stimulation in comparison with the arms, thighs and torso. (Torso is used here to refer to the main part of an animal body excluding the limbs, while trunk includes them). The first reaction to cold temperature stimulus of a normal individual is to reduce the blood flow in the

peripheral areas (hands and feet) instead shunting blood to the core (torso). The sympathetic half of the autonomic nervous system is said to be dominant. The inverse scenario occurs in response to warmer ambient temperatures when the parasympathetic system is said to be dominant. The results tend to indicate that pulsed magnetic stimulus centred on the back of the head, (hypothalamus) causes the balance to shift towards parasympathetic dominance.

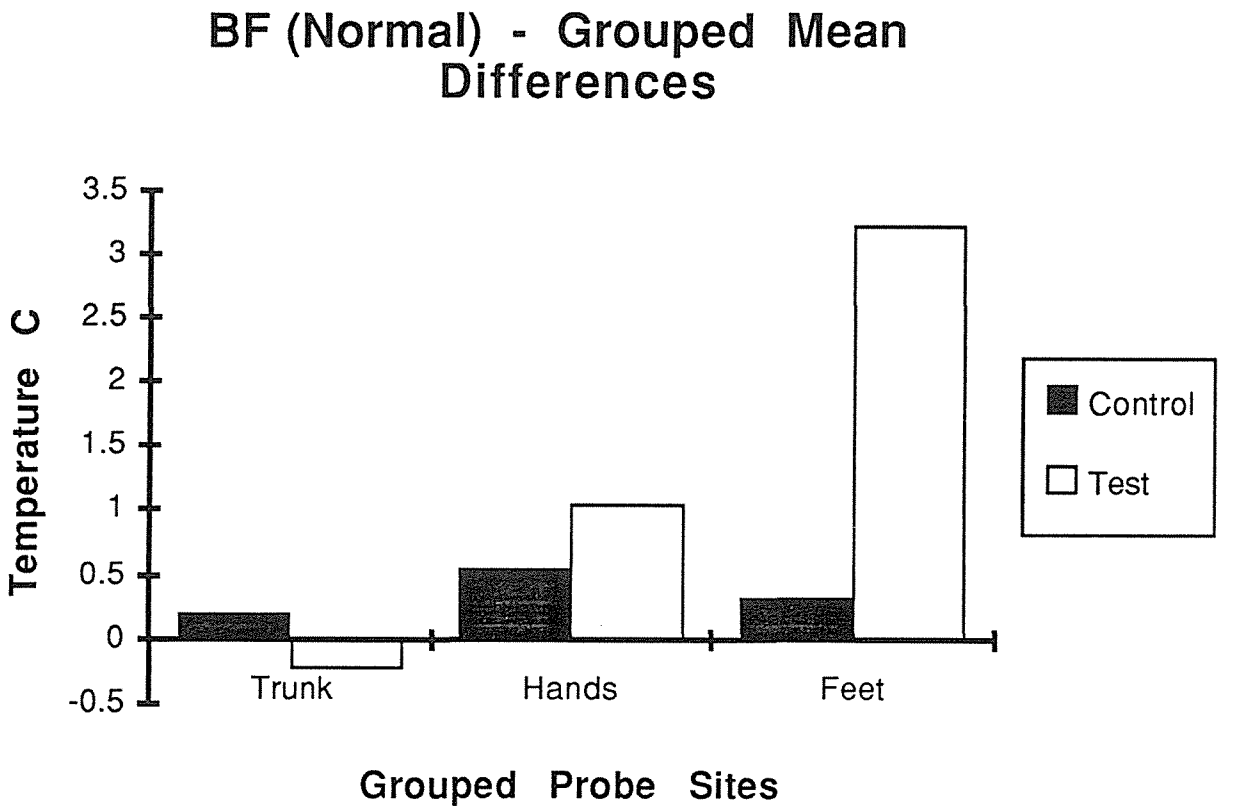


Figure 5.8

5.6.2.2 Normal Subject SL

The second control subject SL, a 25 year old female with no contraindications, was subjected to identical conditions to the first subject. The differences are shown in Figure 5.9.

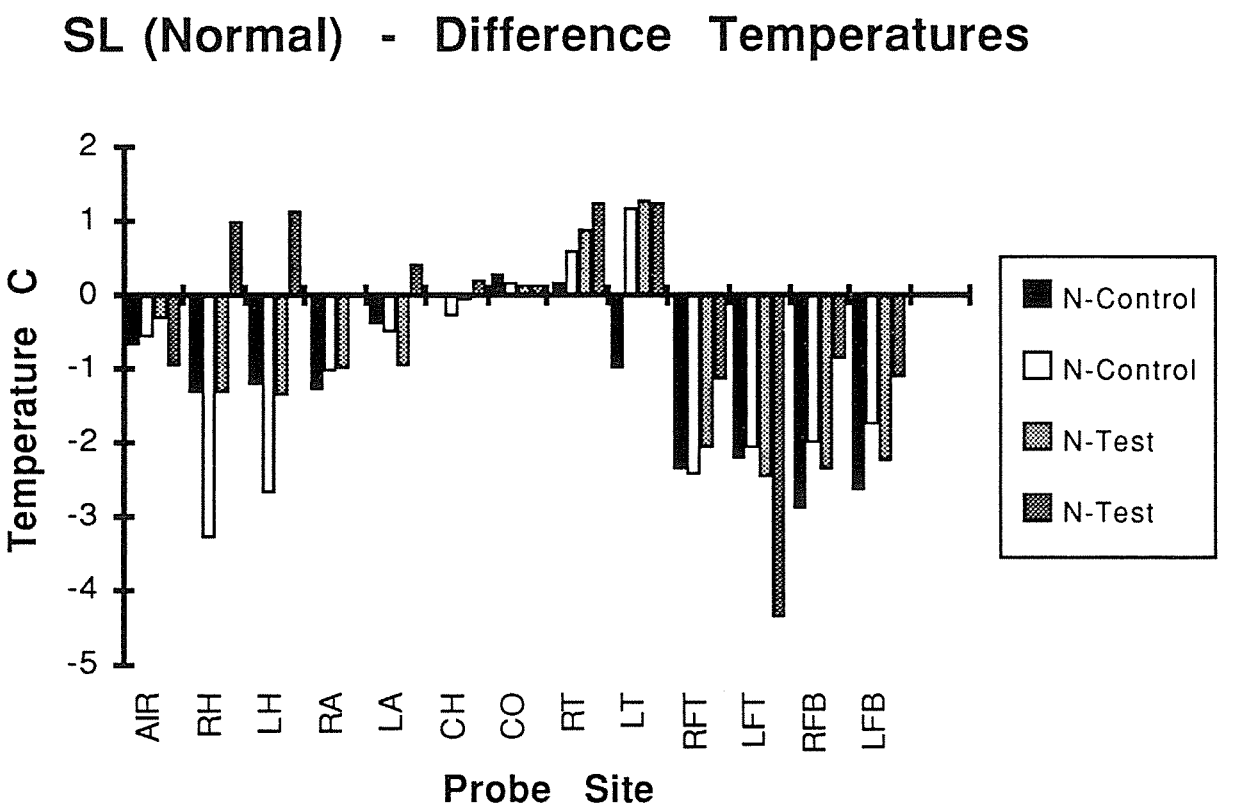


Figure 5.9

The grouped mean difference temperatures are shown in Figure 5.10

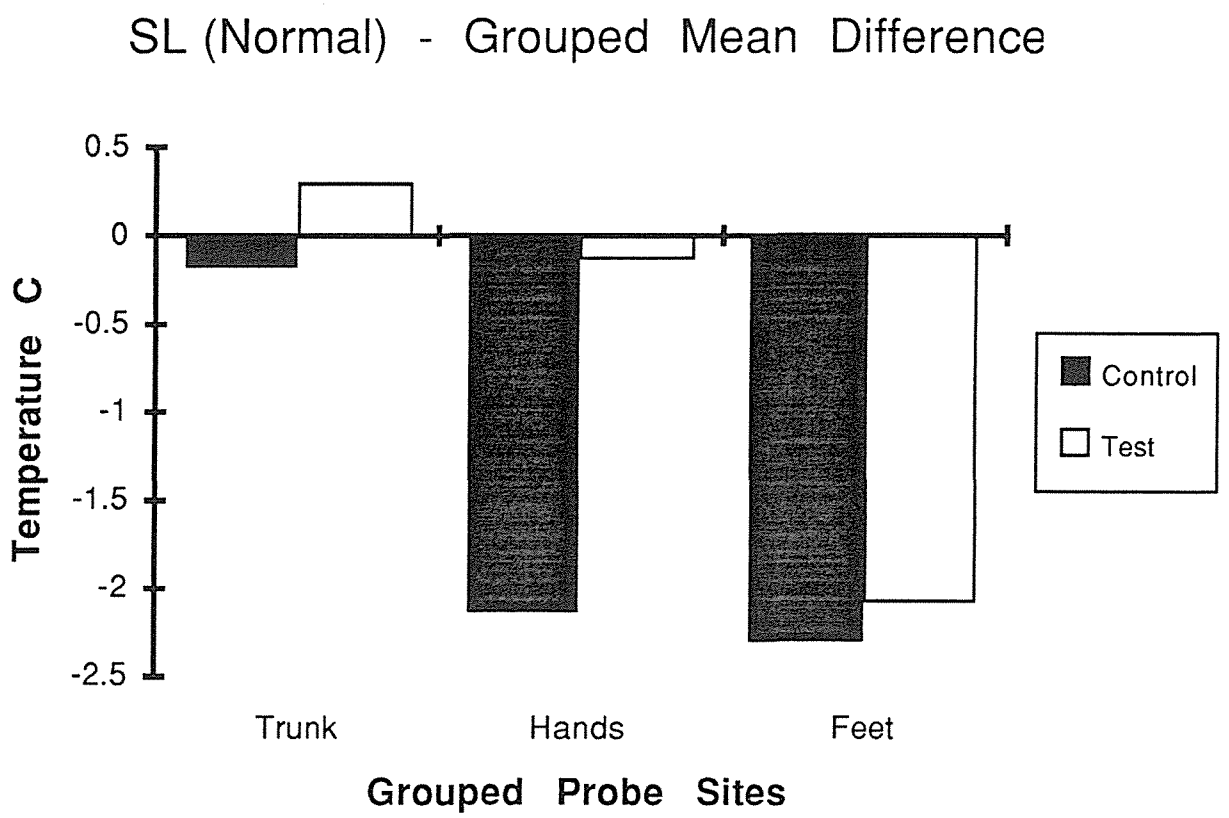


Figure 5.10

The trunk bears a marked similarity to BF in that there is insignificant temperature difference between the control and test trials. In contrast to BF, the second control subject shows a significant drop in temperature for the two placebo control trials for the hands and feet. It is significant to note that the hands do not follow this drop in temperature when the magnetic stimulus is applied, while the feet temperatures still fall by about 2 degrees.

The fact that SL is a female may be highly significant as women Raynaud's patients outnumber men at least 3 to 1, Lafferty$_{18}$. Women have a different temperature control system in that they have a lower stimulus point which causes blood to be

diverted from the limbs to the abdomen in response to the slightest drop in temperature, regardless of whether or not they are pregnant.

5.6.3 Raynaud's subjects.

Raynaud's subjects were obtained by direct referral from a medical practitioner or self referral (word of mouth). Those who contacted the investigator privately were required to verify recent diagnosis of Primary Raynaud's by a medical practitioner, as well as presenting no other medical symptoms. All subjects were advised to keep in contact with their general medical practitioner throughout the trials. All results from the study were made available to the participants and their medical practitioner. It must be clarified that all Raynaud's subjects were diagnosed as having Raynaud's Disease, or Primary Raynaud's, rather than the more complicated Raynaud's Phenomenon, or Secondary Raynaud's.

5.6.3.1 Raynaud's subject SW

The first Raynaud's subject SW, a 28 year old female, with no contraindications, was subjected to two control trials where no magnetic stimulus was applied, and two test trials where 22 Hz, fast random pulsed, magnetic fields were applied at 25 gauss to the back of the head. The subject was not aware of which experiments involved the use of magnetic stimulus - the protocol being 'single-blind'. The temperature differences are shown in Figure 5.11 and the grouped mean differences for the four trials shown in Figure 5.12.

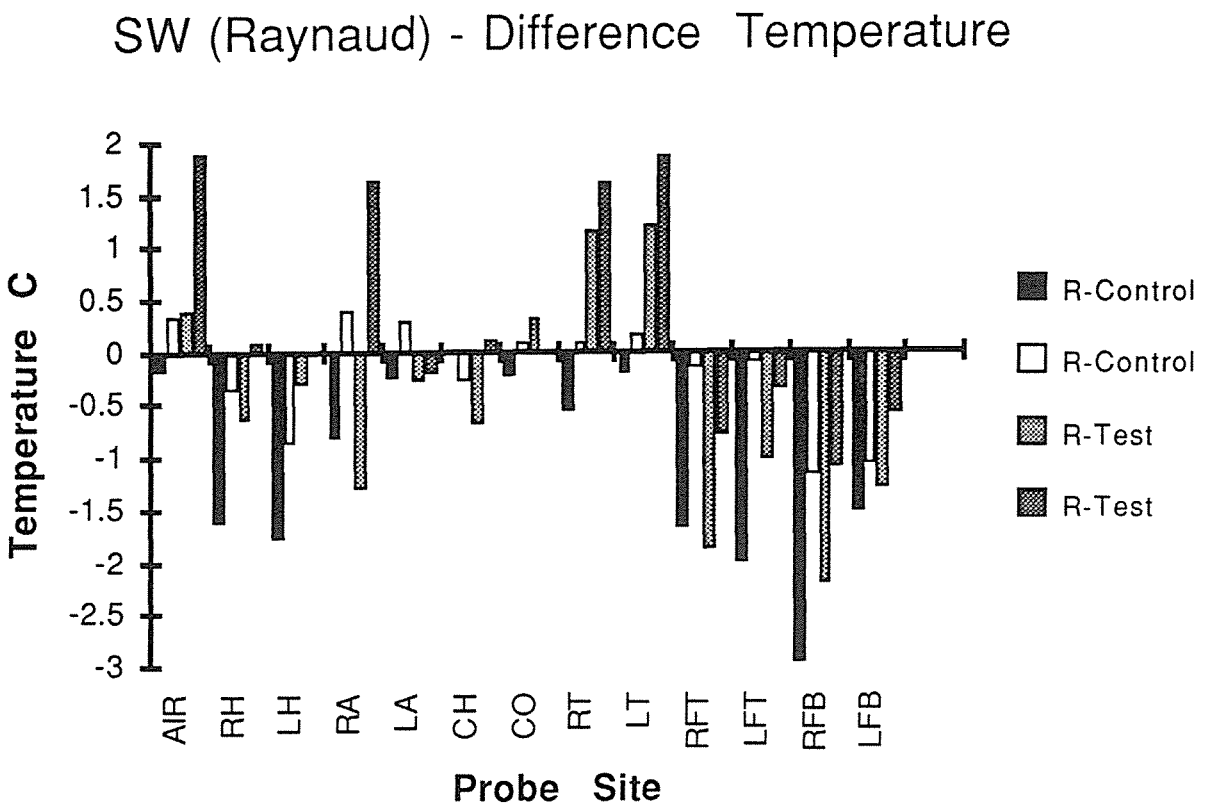

SW (Raynaud) - Difference Temperature
Temperature C
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2.5
-3
AIR
RH
LH
RA
LA
CH
CO
RT
LT
RFT
LFT
RFB
LFB
Probe Site
R-Control
R-Control
R-Test
R-Test

Figure 5.11

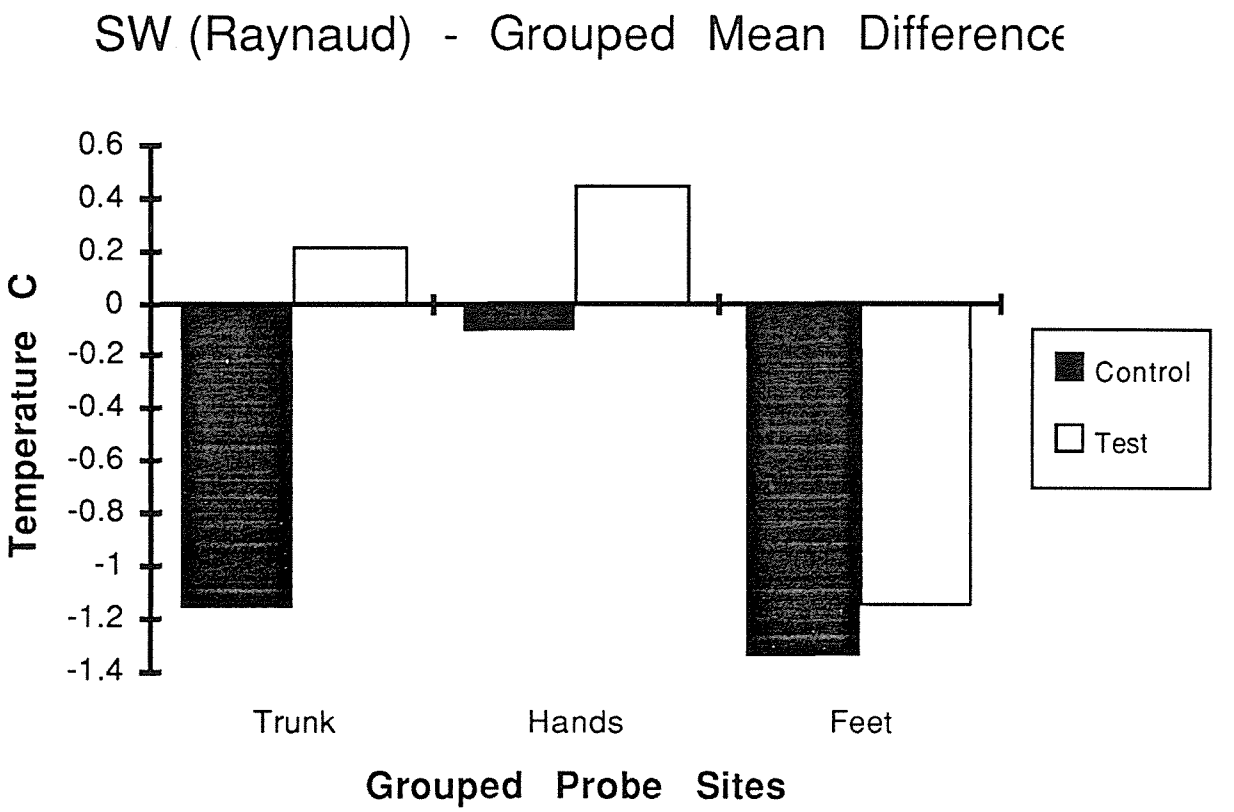


Figure 5.12

5.6.3.2 Raynaud subject MH

The second Raynaud's subject MH, a 65 year old female, with no contraindications, was subjected to two control trials where no magnetic stimulus was applied, and two test trials where 22 Hz, fast random pulsed, magnetic fields were applied at 25 gauss to the back of the head. The subject was not aware of which experiments involved the use of magnetic stimulus - the protocol being 'single-blind'. The differences are shown in Figure 5.13 and the grouped mean differences for the four trials shown in Figure 5.14.

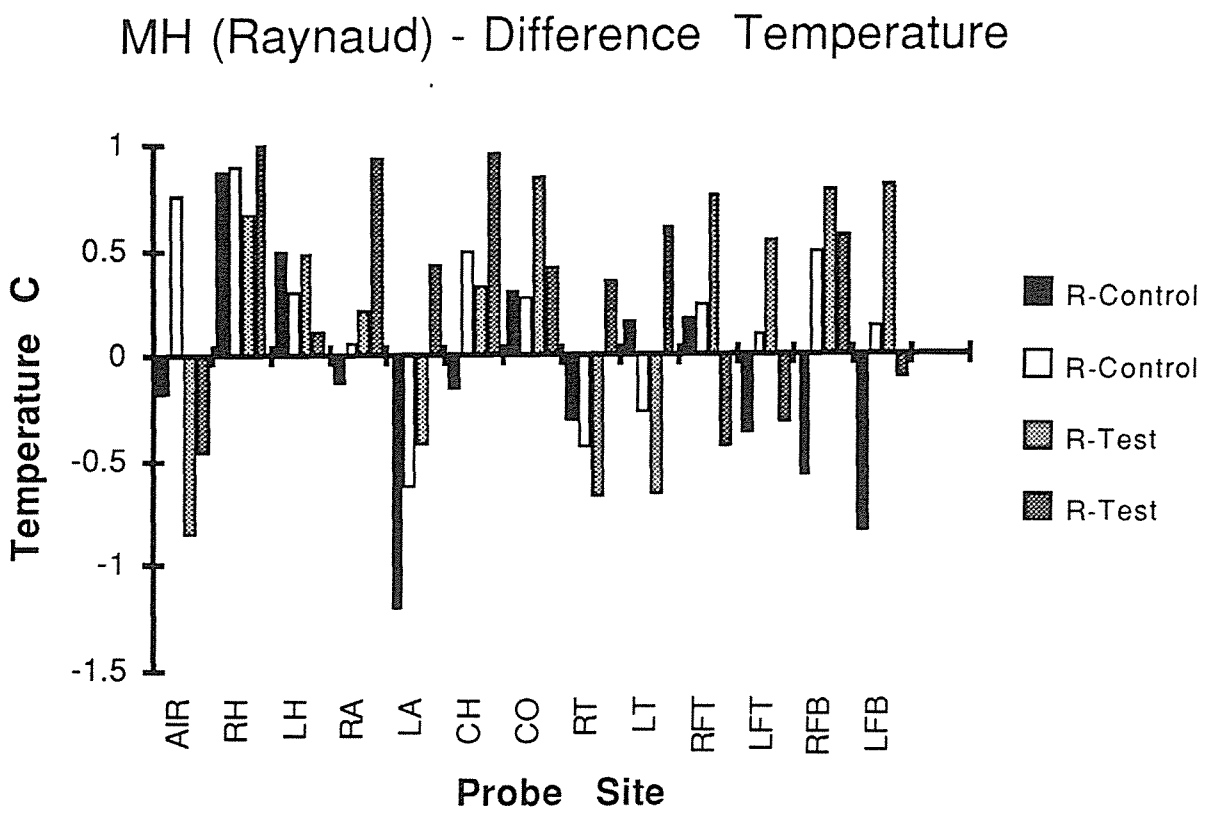

MH (Raynaud) - Difference Temperature
Temperature C
1
0.5
0
-0.5
-1
-1.5
AIR
RH
LH
RA
LA
CH
CO
RT
LT
RFT
LFT
RFB
LFB
Probe Site
R-Control
R-Control
R-Test
R-Test

Figure 5.13

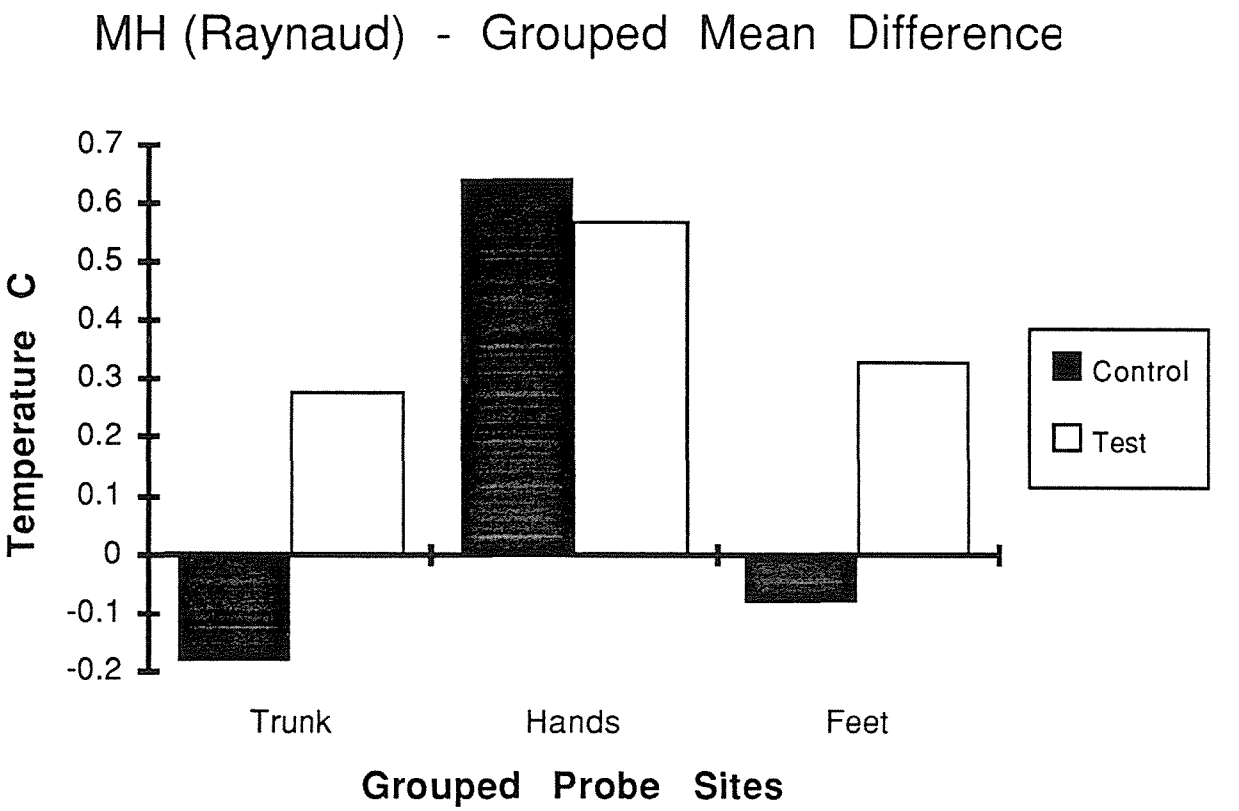

MH (Raynaud) - Grouped Mean Difference
Temperature C
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
Trunk
Hands
Feet
Grouped Probe Sites
Control
Test

Figure 5.14

5.7 CONCLUSIONS

5.7.1 Clarification of objectives

The aim of this exercise is to test the biomagnetic stimulator in a real clinical setting, where the results obtained are of interest only in so far as they represent four single case studies. These experiments were never intended to represent a comprehensive examination of the effects of magnetic fields on the peripheral circulation of either normal subjects, or subjects suffering from Raynaud's disease. The production of the equipment which includes both the biomagnetic stimulator detailed in Chapter 3, and the data collection hardware / software previously developed by the author, is simply being tested in a clinical setting on real subjects.

It is important to note that this study was brought before the Human Ethics Committee at Massey University and was approved provided that certain conditions were met. The guide lines were adhered to in this study.

The above comments not withstanding, it is interesting to take a brief look at the results obtained.

5.7.2 All subject mean differences

The groups' means show great variation which is a reflection of the differences between subjects rather that the effects of the applied magnetic fields. It is not valid to make comparisons between different subjects, or even different disease states, particularly with such a small number of subjects and trials per subject. The

number of subjects and trials also makes it virtually impossible to gain any statistically significant results.

5.7.3 Normal subject BF

The ambient room temperature, hands, arms, chest and core show no significant variations of temperature with regard to the T60 scores. The soles of the feet however do show an interesting trend where the two test trials show increases of the order of 3 to 7 degrees, see Figures 5.5 and 5.6. The control trials show no significant changes. This may lead to the conclusion that some effect has been manifested by the application of the test magnetic field. The subject also reported a feeling of warmth travelling up from his feet during the two test trials, although he was not told until after the series of experiments were concluded which were the sham in fact. This phenomenon has been reported by other subjects in the pilot study conducted in 1988 by the author.

5.7.3 Normal subject SL

The second control subject presents a more complex picture. It should be considered in light of the comments regarding females' genetic predisposition of a more sensitive thermal set point than is commonly found in males, as outlined in Section 5.6.2.

A difference between this subject and the normal control male, BF, is that the hands also show significant changes in temperature in addition to the feet. The hands and feet are of course both considered physiologically peripheral areas, both of which contain the artery / vein shunt, the **arteriovenous anastomoses**. In the control trials the temperature of both hands dropped in the order of approximately one to

three degrees, (refer to Figure 5.7) in comparison to a reduced drop in temperature in trial three, and an increase of the order of one degree in trial four.

It has been noted in the first pilot study that some subjects, while still experiencing a drop in temperature of the hands or feet during a control trial, experience a lesser drop during test trials. It may be that the magnetic field does not always produce a positive T60 score for peripheral sites, but may stimulate a lesser drop than may be normally expected. If this is the situation, it would tend to indicate a magnetically induced effect which is not providing optimum stimulus for the particular subject. A slight change in frequency or magnitude may illicit a more positive T60 score.

The feet of subject SL exhibit the precise response outlined above, where the test trials tend to lessen the temperature drop which would be otherwise produced in the control, sham exposure trials. It is understood that no definite conclusions may be drawn from such a small sample, however the results are sufficiently encouraging to suggest further research should be undertaken.

5.7.4 Raynaud's subject SW

The results from the first Raynaud's subject show a varied response in both control and test conditions, refer to Figure 5.8. The same phenomenon is again observed where the test trials show reduced T60 levels compared to the controls. It is as if the magnetic stimulation is not optimised for the particular subject. This subject also exhibits unusually large variation in T60 values for the arms and legs which are generally not considered to be so susceptible to the same temperature changes as are the hands and feet. The arms and legs tend more often to track body core or

chest reading than hands or feet. This may be a result of the Raynaud's overall phenomenon we are witnessing. No control subjects exhibited such wide variation.

5.7.5 Raynaud's subject MH

It is interesting to note that the same wide range of temperature differences are observed with the second Raynaud's subject. The results shown in Figure 5.10 exhibit similar T60 scores for the arms, legs, hands and feet. There is no distinct pattern observed, which again may be indicative of the Raynaud's condition. The hands for example, Figure 5.11, show a significant increase in T60 in the controls, with a smaller value in the test condition, while the feet were negative for T60 by approximately 0.1 degrees with approximately a 0.4 degree increase for the magnetic treatment.

It is important to stress the lack of statistical surety obtained from such a limited sample size, however there do appear to be tantalising differences between the normal control subjects and the Raynaud's patients. It would be of great value to pursue this topic further.

5.7.6 General conclusions

The number of subjects in these clinical evaluation trials is insufficient to provide any definite conclusions about the benefits of pulsed electromagnetic fields as a potential vasodilator for the peripheral circulation systems of human subjects. Interesting results however were obtained, particularly with the control subjects. These suggest that magnetic fields may influence the circulation system of human subjects, therefore adding weight to the claims of Warnke et al[15]. The far greater

range of results obtained from the Raynaud's subjects is interesting, and worthy of further investigation.

The current experimental protocol focussed on using the modulation frequency of 22 Hz as suggested by Warnke, but pulsed on and off in a random fashion to avoid the habituation response. This protocol is not the same as used by Warnke in his paper in *Pain Therapy* [15]. This may be the reason that a more significant result was not obtained.

5.7.7 Magnetic biostimulator assessment

The magnetic biostimulator performed its task effectively, proving to be a practical, non-invasive and comfortable way of magnetically stimulating human subjects. The coil proved to be absolutely silent, and did not develop any detectable heat which would enable the subject to determine if the field was present or not. The system proved to be easy to use with a minimum of setup time required of the operator. During the placebo trials, the equipment was turned on, but no signal was connected to the amplifier or coil. The subject was therefore given no clues as to which experimental protocol was running at any particular trial. This unit could be a practical addition to any therapist wishing to subject patients to pulsed magnetic field treatments, as well as an effective research tool for the scientist.

5.8 FUTURE WORK

The results presented here offer more questions than they answer. Future research might consider expanding the number of frequencies used on human subjects, along with a range of different magnetic field strengths. In addition, it would be worth experimenting with the distribution of the magnetic field in space. It would also be of interest to compare the effects of Helmholtz coil pairs with respect to solenoid designs, as well as the flat involute style of applicator used by Magnafield Pty Ltd. and Haines.

The Warnke protocol of a 200 Hz carrier sine wave modulated at 22 - 25 Hz by a square wave would be an obvious next step, as the current experiments focussed on only the modulation frequency. This alone may account for the lack of a more positive increase in peripheral temperatures.

A useful addition to the equipment for the research scientist would be to have a computer control system which would allow for the running of double-blind trials. The data acquisition system would need to be able to record the state of the stimulator in addition to the subject data, in such double blind trials.

5.9 References

1 These pour Le Doctorar en Medecine. De L'Asphyxie Locale Et De La Gangrene Symetrique Des Extremites. Rignoux, Imprimeur De La Faculte De Medicine, presentee et soutenue le 25 fevrier 1862.

2 T.Lewis. Experiments relating to the peripheral mechanism involved in spasmodic arrest of the circulation of the fingers, a variety of Raynaud's Disease. Heart, Vol 15, pp7-101, 1929.

3 T.Lewis, E.Landis. Observations upon the vascular mechanism in acrocyanosis. Heart, Vol 15, pp229-246, 1930.

4 J.Heslop et al. The prevalence of intermittent digital ischaemia (Raynaud's phenomena) in a general practice. Journal of the Royal College of General Practitioners, Vol 33, 1983.

5 H.Holling. Peripheral vascular disease: diagnosis and management. Ed. Holling. Lippincott, Philadelphia, 1972.

6 J.Spittell. Raynaud's phenomenon and allied vaso-spastic conditions, in: Allen - Barker - Hines' peripheral vascular diseases. Saunders, Philadelphia, 1972.

7 C.M.Duffy, R.M.Laxer, P.Lee, C.Ramsay, M.Fritzler, & E.Silverman. Raynaud syndrome in childhood. Journal of Paediatrics, Vol 114, No 1, pp73-78, 1989.

8 R.Letz, M.G.Cherniack, F.Gerr, H.Hershman, P.Pace, A cross sectional epidemiological survey of shipyard workers exposed to hand-arm vibration. British Journal of Industrial Medicine. Vol 49, No 1, pp53-62, Jan. 1992

9 D.D.Walker, B.Jones, S.Ogston, E.G.Tasker, A.J.Robinson. A study of white finger in the gas industry. British Journal of Industrial Medicine. Vol 42, No 10, pp672-7, Oct 1985.

10 M.Futatsuka . Epidemiological studies of vibration disease due to brush saw operation. International Archives of Occupational & Environmental Health. Vol 54, No 3, pp251-60, 1984.

11 J.C.de Trafford, K.Lafferty, C.E.Potter, V.C.Roberts, L.T.Cotton. An epidemiological survey of Raynaud's phenomenon. European Journal of Vascular Surgery. Vol 2, No 3, pp167-70, Jun 1988.

12 P.M.Campbell & E.C. LeRoy. Raynaud's Phenomenon. Seminars in Arthritis and Rheumatism, Vol 16, No 2, pp92-103, 1986.

13 L.T.Cotton, O.Khan, Raynaud's phenomenon: a review. International Angiology. Vol 5, No 4, pp215-36, Oct-Dec 1986.

14 R.R.Freedman, S.C.Sabharwal, P.Ianni, N.Desai, P.Wenig, M.Mayes. Nonneural beta-adrenergic vasodilating mechanism in temperature biofeedback. Psychosomatic Medicine. Vol 50, No 4, pp394-401, 1988.

15 U.Warnke. The possible role of pulsating magnetic fields in the reduction of pain. Pain Therapy. Ed: R.Rizzi and M.Visentin. Elsevier Biomedical Press, 1983.

16 E.Dean. Assessment of the peripheral circulation: an update for practitioners. The Australian Journal of Physiotherapy. Vol 33, No 3, 1987.

17 A.B.Hertzman. Some relations between skin temperature and blood flow. International Review of Physical Medicine and Rehabilitation. August 1953.

18 Lafferty.K. Raynaud's - a better understanding. Pub. The Raynaud's Association, Cheshire. 1984.

19 Dean.E. Assessment of the peripheral circulation: an update for practitioners. The Australian Journal of Physiotherapy. Vol 33, No 3, pp164-172. 1987.

6

Cytogenetic Effects

CONTENTS

6.1 BACKGROUND

A variety of biological effects have been attributed to the exposure of cells or organisms to extremely low frequency (ELF) electromagnetic (EM) fields. Some of these responses include: modification to ion transport across membranes; interference with DNA synthesis; alteration of mRNA transcription; disruption of normal cellular responses to hormones, neurotransmitters, growth factors, and changes in cancer cell kinetics. Such interactions are claimed for frequencies whose wavelengths are significantly longer than the target cell or organisms, and at power levels less than 10 mT - insufficient energy to cause any significant thermal effect, Goodman & Henderson$_1$.

The lack of consensus on a definite mechanism that can explain these effects has divided the scientific community. There are several factors contributing to this controversy:

Firstly, Teneford & Kaune$_2$, state that the amount of energy carried by ELF EM fields is considered to be too low to act through known physical mechanisms of heating, dielectric breakdown, particle displacement or electrophoresis. Secondly, it is unlikely that the mechanism of action is attributable to changes in transmembrane potentials, since the potentials from EM fields are much lower than membrane potentials, Goodman & Henderson$_1$, 1991. Thirdly, the diversity in experimental effects observed is large, suggesting multiple routes of activity. This situation is not helped by the vast array of experiments performed using electric and magnetic stimuli in a variety of frequencies and field strengths. Replication of any particular study is the exception rather than the rule.

6.1.1 Theory of free energy capture.

A significant contribution to the general understanding of this field was initiated by Westerhoff et.al.[3] who produced a paper in 1986 entitled: "How enzymes can capture and transmit free energy from an oscillating electric field". They showed that free energy from an alternating electric field can drive the transport of Rb^+ by way of the Na^+, K^+ - ATPase enzyme system. They further suggest that many transmembrane enzymes can be expected to absorb free energy from an oscillating electric field and transduce that to chemical work. In the theoretical analysis it is sufficient that (i) the catalytic process be accompanied by either net or cyclic charge translocation across the membrane and (ii) the stability of the enzyme states involved be asymmetric. Their calculations based on a four-state enzyme model reveal that free-energy transduction occurs with sinusoidal, square-wave, and positive-only oscillating electric fields and for all cases that exhibit either linear or exponential field-dependent rate constants. This also suggests that in addition to oscillating electric field-driven transport, the mechanism they propose can also explain, in part, the "missing" free energy term in cases in which ATP synthesis has been observed with insufficient transmembrane proton electrochemical potential difference.

The significance of this paper is that for the first time it has been shown that weak magnetic fields could, in theory at least, transfer energy to a living, biological system. In particular, the magnitude of energy could be significantly lower than was previously considered.

6.1.2 Cyclotron resonance and the thermal noise limit

Liboff[42] et al. studied the uptake of radioactive calcium-45 by human lymphocytes, (white blood cells), discovering that uptake was significantly increased by a 60 minute exposure to a magnetic field modulated at the cyclotron resonance frequency of calcium. Of significance was the finding that the frequency had to be reduced from the calcium-40 value by exactly the amount necessary to take account of the radioactive isotope's extra mass.

Cyclotron resonance refers to the observation that a charged particle moving in a magnetic field, for example between the poles of a magnet in an evacuated chamber, spirals around at a fixed frequency (known as the cyclotron frequency) while it receives regular accelerating pulses from an electric field applied at the same point in each orbit so that its energy increases in a resonant fashion. Liboff[43,44,45], offers this as the mechanism by which weak alternating magnetic fields may interact with the biochemical processes of living systems.

Sandweiss[46] counters this argument by stating that the radius of gyration required by calcium far exceeds the diameter of the cell. The radius of gyration is given by the simple formula:

$$r = (m \upsilon / q B)$$

Where $\mathbf{m}\upsilon$ is the ionic momentum as calculated from the ionic energy, **q** is the ion's charge, and **B** is the strength of the applied magnetic field in tesla.

The cyclotron frequency for calcium is approximately 16 Hz. With an applied magnetic field of 35 μT (approximately the earth's static field component) the radius of gyration is approximately 48 metres ! In addition, the conditions for cyclotron resonance require that the particle shall travel for tens of milliseconds without colliding with another particle, Male[47].

Male also states that ions in solution are bound, not free, often attracting a shell of water molecules around them. The effective increase in mass of the additional water molecules wound decrease the cyclotron frequency further. Either counter argument would strongly suggest that it is unlikely that cyclotron resonance alone could account for the observed increase in calcium-40 by human lymphocytes.

A further fundamental problem is that there is simply not enough energy available in an applied magnetic field of approximately 35 μT to compete with the random thermal energy of an ion in solution at normal temperatures. A magnetic field of 50 μT has an energy density of some 13 orders of magnitude less than the thermal energy of water at room temperature, (Male). In addition, the only force that a magnetic field can exert directly on an ion is the Lorentz force, which always acts at right angles to the ion's motion and therefore cannot add to the ion's energy. It is unlikely that direct energy transfer to the ion is the important factor.

A more attractive possibility is that the magnetic field initiates some kind of triggering process, which releases or redirects ordinary metabolic energy to produce the observed biological effects. Male and Edmonds[48] and Lednev[49] have proposed the theory that the magnetic field interacts not with a free ion in solution, but with a bound ion, and specifically with an ion protected within the binding site

of an ion-dependent protein. They propose that the interaction of the field with the ion is assumed to affect the probability that the ion remains bound to the protein which would affect the probability that the protein can carry out its ion-dependent biological function. In consideration of the fact that most enzymes are proteins, and that enzymes are responsible for most biological reactions at the cellular level, the implications are of increasing significance.

6.1.3 Larmor precession

Male[47] proposes the following mechanism: Consider an ion, calcium for example, loosely bound inside the "molecular cage" of a protein, e.g. calmodulin. The ion is free to vibrate about its equilibrium point under the influence of thermal energy. It will vibrate in this way at some frequency in the infra-red spectrum. For simplicity the ion will be considered to move in a simple manner, back and forth along a straight line termed the vibrational axis. If a steady magnetic field is applied at right angles to the simple harmonic motion described above, a basic physical theorem expounded by Larmor states that the vibrational axis will change its orientation. It will in fact precess about the field direction at a steady rate, known as the Larmor frequency. The Larmor frequency, like the cyclotron resonance frequency, depends on both the field strength and charge-to-mass ratio of the ion, but is smaller than the cyclotron frequency by a factor of two.

If the applied magnetic field is now amplitude modulated, the precession of the ion's vibration axis will speed up and slow down in synchronism. If the modulation is at the cyclotron frequency (twice the Larmor precession frequency), the speeding up and slowing down will always occur when the axis is aligned in the same direction. Thus the vibration will spend more time in the 'slow' direction and less time in the

'fast' direction at right angles to it. Male[47] hypothesizes that this vibrational pattern will affect the strength of the ion's binding to the protein, offering a plausible mechanism for the alteration of biochemical processes by applied modulated magnetic fields.

Landau[4] and Lifshitz[50], treat the ion's behaviour as a charged spatial oscillator. In such a system, a magnetic field effectively splits the oscillation into two sub-frequencies which are separated by an amount exactly equal to the cyclotron frequency. For electrons, this kind of splitting is the basis of the Zeeman effect which is observed as the splitting of atomic emission or absorption lines in the optical spectrum by strong magnetic fields. The field splits the atomic energy levels into several components associated with different quantized orientations of the total magnetic moment with respect to the field.

This approach forms the basis of Lednev's[49] more quantum mechanical approach. Lednev suggests that if the magnetic field is modulated at the frequency equal to the difference between the two vibrational sub-frequencies, (that is at the cyclotron resonance frequency), there is an increased probability that the ion will undergo a transition from its excited state to a lower energy state. Thus it would become more tightly bound to its respective protein. Lednev bases this suggestion, by analogy, on a physical process known as parametric resonance, in which a magnetic field disturbs the interference between very closely adjacent electronic energy states. This has been experimentally observed by Aleksandrov et al. as the light-scattering properties of optically excited atoms using magnetic fields of the order of 25 μT.

The above theories offer plausible mechanisms for the interaction of magnetic fields and living systems. Only considerable experimentation in the future involving the cooperative research of biologists, physiologists, biochemists and physicists, will determine the answer. Certainly, there is sufficient theory to justify further biological investigation.

6.1.4 Physarum - mitotic cycle length

Significant changes in the mitotic cycle length of *Physarum* (the common slime mould) were reported by Greenbaum[4]. Large plasmodia of Physarum polycephalum were formed from mixtures of micro-plasmodia grown in shaker cultures exposed to 2.0 Gauss (rms) at 75 Hz, with duplicate control cultures not being exposed. Exposed cultures grown continuously in the field displayed a longer mitotic cycle than controls. Mixed cultures display synchronous mitosis and a cycle length intermediate to the cycle lengths of exposed and control cultures. The cycle length of mixed cultures varied with the proportions of the mixture in a non-linear manner. Further experiments by: Goodman et al.[5], 1976; Marron et al.[6], have revealed that *Physarum* cultures exposed to a variety of different EM exposure conditions may produce: a longer mitotic cycle; a depressed respiration rate; and a slower rate of spontaneous protoplasmic streaming. It is perhaps significant that exposure to electric or magnetic fields alone produce similar, but less pronounced changes compared to exposure situations where both the electric and magnetic component are combined, 1975 Goodman et.al.[7].

6.1.3 Transcription in dipteran salivary glands.

Goodman et.al.[8], report changes in transcription in dipteran (lit. two-winged fly) salivary glands monitored with tritiated uridine in transcription radiography,

cytological nick translation, and analysis of isolated RNA fractions when two pulsed magnetic fields were applied in vitro. The fields chosen are commonly used in the clinical treatment of recalcitrant fractures and bone non-unions, [9,10,11,12]. One was an asymmetric 5 kHz square wave pulsed at 15 Hz, while the other was a 380 μsec asymmetric pulse repeated at 72 Hz. Goodman[8] reports different effects for each signal. The single pulse increased the specific activity of messenger RNA after 15 and 45 minutes of exposure while the pulse train increased specific activity only after 45 minutes of exposure.

6.1.4 Human chromosome aberrations.

Khalil and Qassem[16], exposed human lymphocytes (white blood cells involved in immune response) to pulsing electromagnetic fields (PEMF) of 50 Hz at 1.05 mT (10.5 gauss) for durations of 24, 48, and 72 hours. They report a statistically significant suppression of mitotic activity and a higher incidence of chromosome aberrations. In addition, the shorter exposure times of 24 and 48 hours did not cause a significant delay in the cell proliferation index (CPI), (also known as cell turnover), or an increase in the baseline frequency of sister chromatid exchanges (SCE). Exposure of the lymphocytes to the PEMFs for 72 hours, however, resulted in a significant reduction in the CPI and an elevation of SCEs.

Their results suggest that exposure to a PEMF may induce a type of DNA lesion that leads to chromosome aberrations and cell death, but not to SCEs, except probably at longer exposure times. If true, these findings would tend to support such epidemiological studies as: Wertheimer & Leeper[17] "Adult cancer related to electrical wires near the home"; Feychting et.al.[18] "Magnetic fields and cancer in people residing near Swedish high voltage power lines"; J.Olsen et.al.[19] "Residence

near high voltage facilities and the risk of cancer in children"; and Floderus et.al.[20] "Occupational exposure to electromagnetic fields in relation to leukemia and brain tumours: a case control study."

6.2 EQUIPMENT

6.2.1 Magnetic field requirements

It is difficult to decide on the specific protocol to use for the first investigation of the effects of magnetic fields on the cytogenetics of *Vicia faba*. Four parameters however need to be considered: field magnitude (strength); frequency; waveform; and field gradient.

6.2.2 Field magnitude

It was decided to expose growing root tips of *Vicia faba* to various magnetic fields in the region of 1 mTesla, that is, approximately 20 times the field strength of the static component of the earth's static magnetic field. Experiments performed by Greenbaum et.al.[4], and R.Goodman et.al.[8], as well as standard magnetic field therapies for the treatment of recalcitrant fractures and bone non unions, Bassett et. al.[9,10,11,12], J.S.Kort et.al.[13], W.J.W. Sharrard et.al.[14], and M.L.Sutcliffe et.al.[15], use field strengths up to 6 mTesla. The field strengths quoted however, are usually those at the coil surface, rather that the actual value in the tissue of interest. Insufficient details of the coil are recorded in these papers to allow calculation of the field in the area of the tissue. It can be assumed however that this will be of the order of at least 0.1 that of the value of the field at the coil, based on measurements taken during the evaluation of the Magnafield 990, Chapter 2. For this reason a value of 1.5 mTesla was chosen as the target value as a starting point for investigation. It is also an achievable value with the current equipment, particularly the transconductance amplifier.

It must be understood that there is a considerable range of field magnitudes used in experiments, from epidemiological studies involving fields substantially lower than background endogenous earth fields, to fields as high the 14 tesla used by Sperber and Dransfield[21] for their orientation of pollen tube growth experiment.

6.2.3 Frequency range.

It was resolved that in addition to a static field, three alternating frequencies would be trialed: 50 Hz; 60 Hz; and 75 Hz. Insufficient time precluded any further frequencies being added to the list. The rationale for the three frequencies chosen was based on the current debate regarding the effects of power frequency fields on human health. In particular the frequencies of 50 Hz and 60 Hz are those linked with cancer in epidemiological studies [17,18,19,20]. Therefore in the absence of any suggestions to the contrary, 50 Hz was chosen to align the results with other work. Similarly, 60 Hz is the other common frequency utilised by energy supply authorities in the United States, over which there is also much debate.

The third choice, 75 Hz was chosen to be a frequency close to the two main frequencies under suspicion of possible harmful effects, while not being a common harmonic of either. 100 Hz for example is the second harmonic of 50 Hz and may therefore be expected to have a somewhat similar effect as proposed for the fundamental frequency. 75 Hz seemed an appropriate choice, midway between 50 and 100.

6.2.4 Waveform

The choice of waveform was easily arrived at. In consideration of the vast literature on the effects of sine wave power frequency fields, sine waves appeared the obvious choice. In addition they have the advantage that they produce the least harmonics, as opposed to square waves which produce the richest harmonic envelope. It may be argued that the use of square waves could confound the results of any experiment attempting to determine a frequency specific response due to the excessively large number of odd harmonics present. For these reasons sine waves were the obvious choice, at least for the experiments conducted for this thesis.

Future work may include other waveforms such as square, triangle and ramp, as well as those waveforms used for medical purposes. Refer to Section 3.5.3.

6.2.5 Field gradient

The field generated by the proposed coil is required over the volume of a cylinder approximately 50 mm in diameter and 50 mm in height to accommodate the growing root tips. It was decided that the magnitude gradient should not be greater than 10% of the field strength over the defined volume of interest.

6.2.6 Physical considerations.

In order to incorporate a growing volume of approximately 50 mm diameter and 50 mm long, the space needed for the growing lateral root tips, the coil was designed as an almost 'square-Section' solenoid, 135 mm in diameter, and 123 mm

high, consisting of 480 turns of 1 mm enamelled copper wire in 4 layers. This configuration would be able to provide a growing environment commensurate with the field gradient of 10% variation outlined in Sections 6.2.2 and 6.2.5. The coil model calculations are presented in Tables 6.1 and 6.2.

6.2.7 MagneSim™ model.

The precise design of the coil was arrived at by successive approximation using the MagneSim™ magnetic field modelling program produced by B.Foster. This software is able to calculate the magnetic field vectors produced in circular: short solenoids; long solenoids; as well as Helmholtz and Maxwell coil pairs. MagneSim™ is able to calculate and display up to a maximum of 11 points in the horizontal axis and 22 points in the vertical axis. The output produced includes a 'lollipop' plot of the resultant vectors where the dot on the lollipop represents the origin of the vector, and the 'stick' length represents the vector magnitude, while the angle is displayed in Cartesian coordinate geometry. The 'sticks' are auto-scaled such that the maximum vector magnitude is represented by two units, with all other values scaled accordingly. The coil described is modelled in Figure 6.2, Tables 6.1 and 6.2 with the input control screen shown below in Figure 6.1.

MagneSim™ Input Screen

```
H = Helmholtz pair
L = Long solenoid
M = Maxwell pair
S = Short solenoid
Any other entry returns to the main menu
Enter your selection: L
Enter the number of horizontal points for the calculation: 11
Enter the number of vertical points for the calculation: 22
Enter the left coordinate for the calculation (in metres): 0
Enter the bottom coordinate for the calculation (in metres): 0
Enter the right coordinate for the calculation (in metres): .123
Enter the top coordinate for the calculation (in metres): .137
Enter the radius (in metres): .0685
Enter the current (in amperes): 2
Enter the horizontal coordinate of the left end of the coil (in metres): 0
Enter the vertical coordinate of the coil centre (in metres): .0615
Enter the length of the coil (in metres): .123
Enter the number of turns in the coil: 120
Enter the number of layers in the coil: 4
```

Figure 6.1

The magnitude and direction of the resultant vectors are shown in the form of a lollipop plot, Figure 6.2. MagneSim™ produces a table (Table 6.1) of vector angles in Cartesian coordinates which have a 1 to 1 physical mapping with the 'lollipop' plot Figure 6.2. The magnitudes of the resultant vectors are shown in gauss in Table 6.2. In addition MagneSim™ is capable of producing a table of vector magnitudes for both the radial and axial components, however these are not particularly relevant as the 'specimen in the coil' experiences only the effective resultant of these two sets of vectors.

Figure 6.2 shows an axial slice bisecting the coil which is depicted as lying at 90 degrees to the observer. In this way the coil may be thought of as a 'tube' of copper wire turns, lying on the table at right angles to the observer. The end turn is represented by a vertical dotted line. The three dimensional field can thus be

obtained by rotating the figure about its central horizontal axis, the field having rotational symmetry. MagneSim™ is only capable of calculating fields for coils with circular symmetry.

Cartesian Coordinate Plot of Resultant Vectors

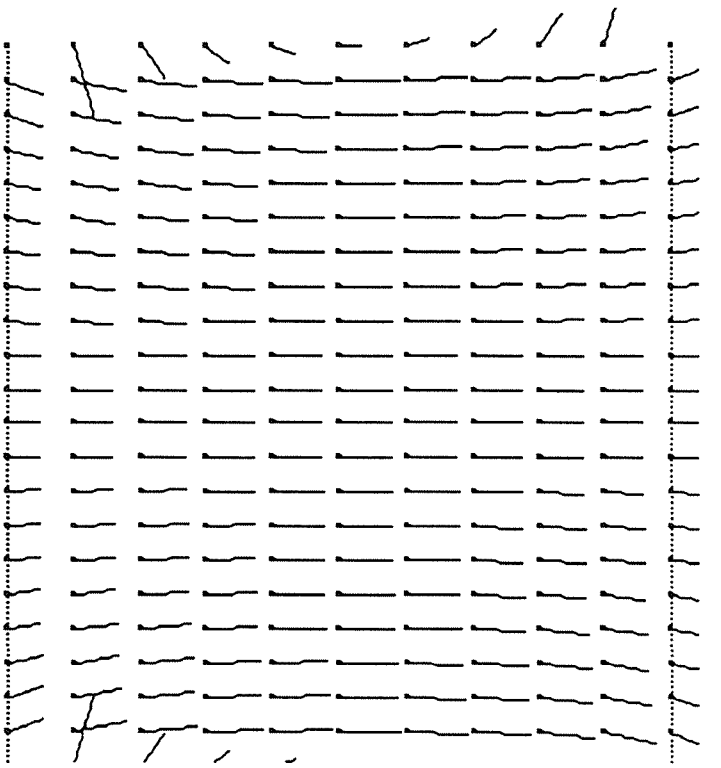


Figure 6.2

Table 6.1 shows the directions of the resultant vectors. Where a calculation value falls directly on a turn of the coil, MagneSim™ replaces the value with a "?". This is because the algorithm is unable to calculate the magnitude vectors as they approach infinity at the centre of the wire.

Cartesian coordinates of resultant vectors

?	278	288	303	326	0	34	57	72	82	?
321	340	349	354	357	360	3	6	11	20	39
329	341	349	354	357	360	3	6	11	19	31
335	343	350	354	357	360	3	6	10	17	25
339	345	351	354	357	360	3	6	9	15	21
343	348	352	355	358	360	2	5	8	12	17
347	350	353	356	358	360	2	4	7	10	13
350	352	355	357	358	360	2	3	5	8	10
353	355	356	358	359	360	1	2	4	5	7
356	357	358	359	359	360	1	1	2	3	4
359	359	359	360	360	360	0	0	1	1	1
1	1	1	0	0	0	360	360	359	359	359
4	3	2	1	1	0	359	359	358	357	356
7	5	4	2	1	0	359	358	356	355	353
10	8	5	3	2	0	358	357	355	352	350
13	10	7	4	2	0	358	356	353	350	347
17	12	8	5	2	0	358	355	352	348	343
21	15	9	6	3	360	357	354	351	345	339
25	17	10	6	3	0	357	354	350	343	335
31	19	11	6	3	0	357	354	349	341	329
39	20	11	6	3	0	357	354	349	340	321
?	82	72	57	34	360	326	303	288	278	?

Table 6.1

The magnitudes of the resultant vectors are shown in Table 6.2 below.

Magnitude of Resultant Vectors in Gauss

?	44.056	21.442	13.047	8.844	7.421	8.844	13.047	21.442	44.056	?
14.585	17.463	18.270	18.742	19.009	19.095	19.009	18.742	18.270	17.463	14.585
12.948	15.944	17.338	18.085	18.478	18.602	18.478	18.085	17.338	15.944	12.948
12.152	14.874	16.527	17.475	17.975	18.133	17.975	17.475	16.527	14.874	12.152
11.666	14.131	15.862	16.935	17.517	17.701	17.517	16.935	15.862	14.131	11.666
11.339	13.605	15.334	16.476	17.114	17.319	17.114	16.476	15.334	13.605	11.339
11.108	13.227	14.925	16.099	16.774	16.993	16.774	16.099	14.925	13.227	11.108
10.945	12.957	14.618	15.804	16.500	16.728	16.500	15.804	14.618	12.957	10.945
10.833	12.770	14.399	15.586	16.294	16.528	16.294	15.586	14.399	12.770	10.833
10.762	12.652	14.258	15.444	16.158	16.395	16.158	15.444	14.258	12.652	10.762
10.728	12.595	14.189	15.373	16.090	16.328	16.090	15.373	14.189	12.595	10.728
10.728	12.595	14.189	15.373	16.090	16.328	16.090	15.373	14.189	12.595	10.728
10.762	12.652	14.258	15.444	16.158	16.395	16.158	15.444	14.258	12.652	10.762
10.833	12.770	14.399	15.586	16.294	16.528	16.294	15.586	14.399	12.770	10.833
10.945	12.957	14.618	15.804	16.500	16.728	16.500	15.804	14.618	12.957	10.945
11.108	13.227	14.925	16.099	16.774	16.993	16.774	16.099	14.925	13.227	11.108
11.339	13.605	15.334	16.476	17.114	17.319	17.114	16.476	15.334	13.605	11.339
11.666	14.131	15.862	16.935	17.517	17.701	17.517	16.935	15.862	14.131	11.666
12.152	14.874	16.527	17.475	17.975	18.133	17.975	17.475	16.527	14.874	12.152
12.948	15.944	17.338	18.085	18.478	18.602	18.478	18.085	17.338	15.944	12.948
14.585	17.463	18.270	18.742	19.009	19.095	19.009	18.742	18.270	17.463	14.585
?	44.056	21.442	13.047	8.844	7.421	8.844	13.047	21.442	44.056	?

Table 6.2

The finished coil was constructed on a piece of 135 mm diameter PVC pipe 125 mm long, with a poly methyl methacrylate turned base inserted at one end. 240 turns of 1 mm diameter enamelled copper wire was wound round the outside of the tube in two layers in a tight format.

The finished coil has a DC resistance of 4.6 ohms, with an inductance of 20.6 mH and an inter-turn capacitance of approximately 3.0 nF. To determine the resonant frequency the coil was analysed with a Hewlett Packard LF impedence analyzer Model 4192A. The results are presented in Table 6.3.

Impedance vs Frequency Including Phase Angle

Hz	Impedance	Degrees	V	I (mA)
5	4.86	7.1	0.004	0.923
10	5.00	15.0	0.005	0.919
20	5.50	28.7	0.005	0.918
50	8.20	53.3	0.007	0.915
100	14.1	70.0	0.013	0.909
200	26.8	79.5	0.023	0.888
500	66.05	85.63	0.051	0.780
1,000	131.84	87.68	0.075	0.581
2,000	265	88.7	0.090	0.347
5,000	697	89.2	0.096	0.141
10,000	1,720	88.9	0.087	0.052
20,000	57,000	53.1	0.088	0.004
50,000	863	-83.4	0.097	0.092
100,000	293	-83.6	0.095	0.166

Table 6.3

The impedance plot is presented in Figure 6.3. It can be seen from Table 6.3 and Figure 6.3 that the coil impedance peaks at approximately 20 kHz, with the impedance dropping sharply thereafter. This corresponds well with the theoretical calculated value of 20,245 Hz, and is a result of the negative phase angle due to the inter-turn capacitance which has an increasing effect with frequency, as shown in Figure 6.4. At resonance the expected phase angle should be zero, From Figure 6.4 the interpolated value for resonance based on the zero phase angle

criteria would lie between 20 to 50 kHz. The graph however lacks sufficient resolution in this range to enable any more precise estimate. The figures are within the same order of magnitude which tends to indicate that they are approximately correct.

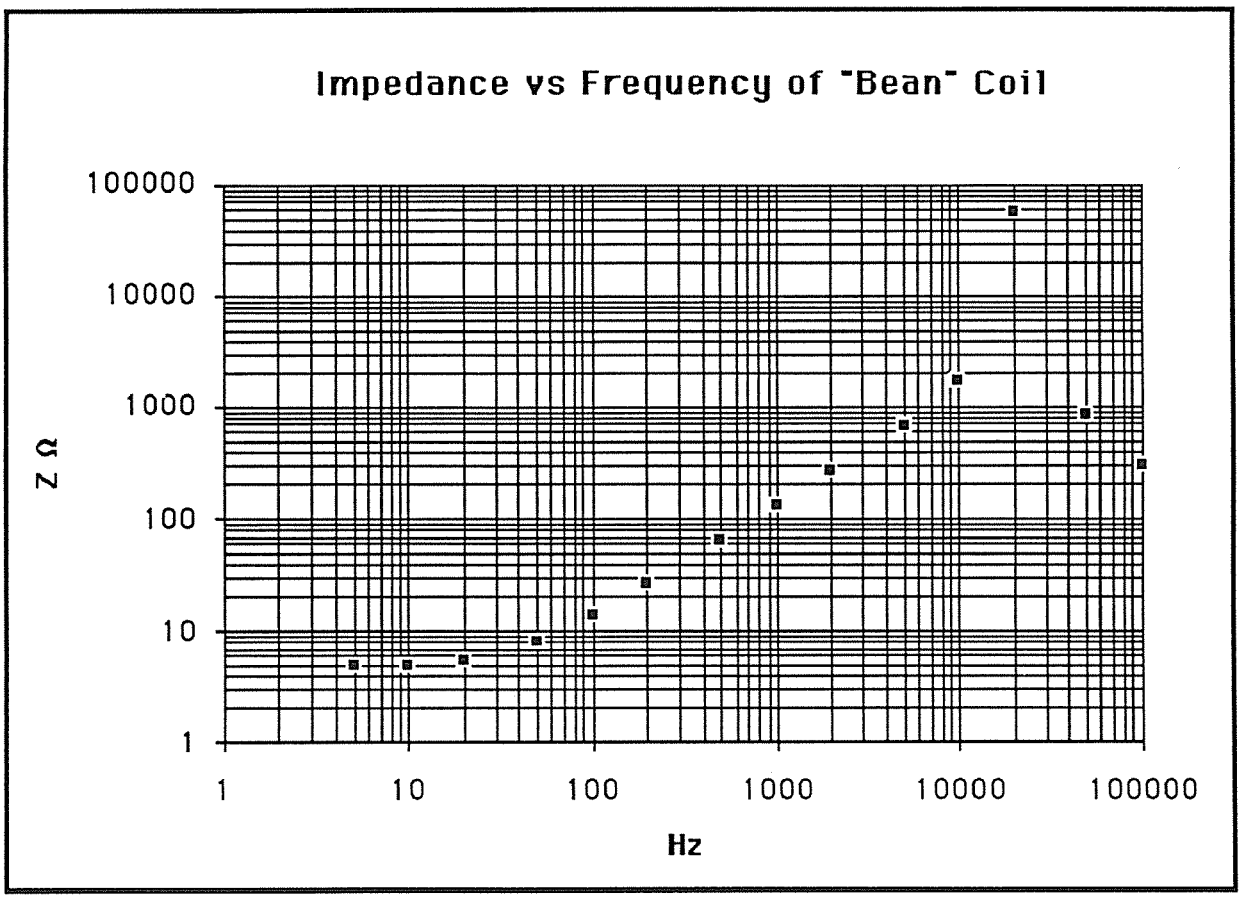


Figure 6.3

Because the coil possesses considerable inter-turn capacitance, the phase angle becoming **negative** above the impedance peak, it is necessary to show this on a separate graph, negative numbers not being able to be plotted on a standard log log graph in Excel 4.0 .

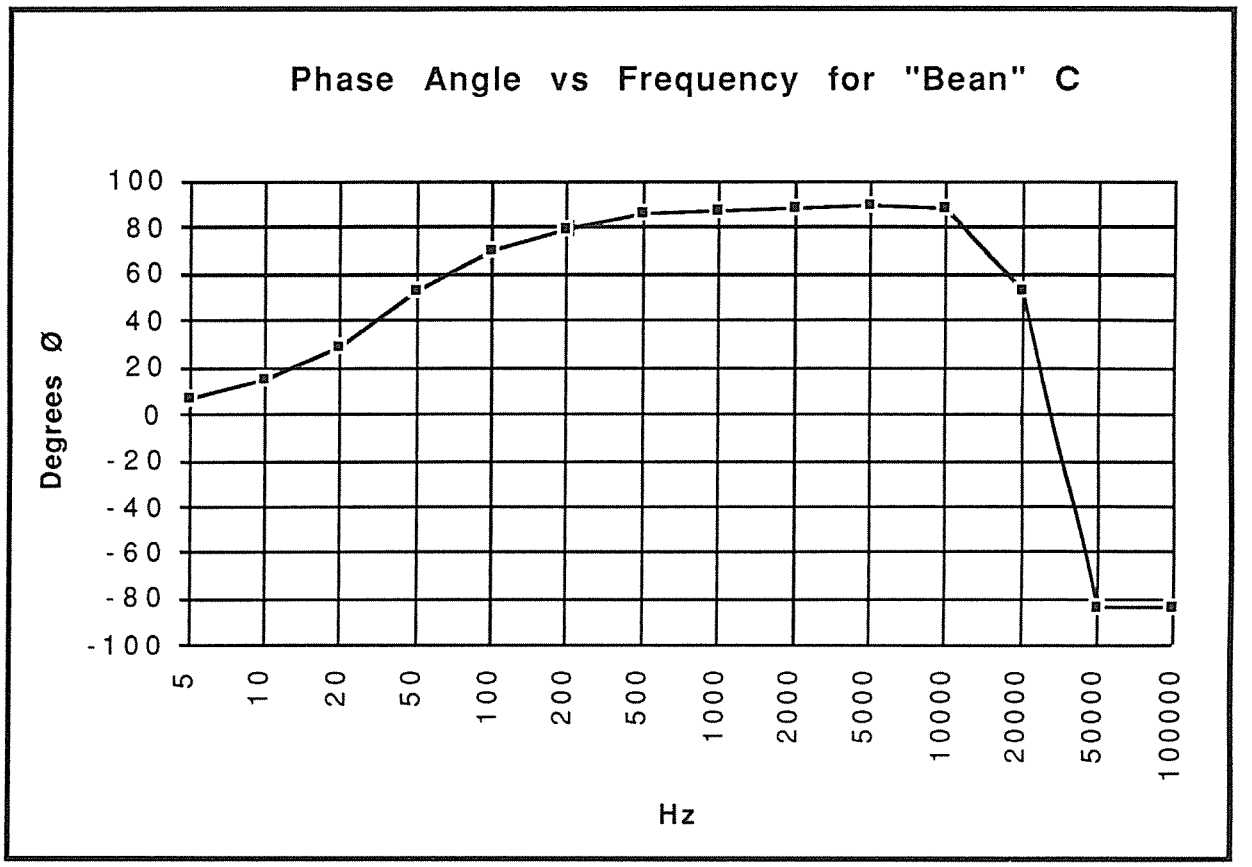


Figure 6.4

6.2.8 Microscopic imaging system

An Olympus Model K microscope was outfitted with the following lenses:

10 x	chromatic	na = 0.25
20 x	plan achromatic	na = 1.4
40 x	chromatic	na = 0.65
100 x	plan achromatic	na = 1.25

The standard light source, Olympus TE-II, was adjusted for Kohler illumination and fitted with two blue colour correction lenses. A trinocular head was fitted with an MTV -3 camera mount incorporating an FK 2.5 x camera adapter lens. An

Ikegami CCD camera, IDC 290, was connected to the camera tube without further optical filtering. The video signal from the camera was passed through the Analog Video Pre-processor designed by Wyatt Page of the Department of Production Technology, Massey University. This provided manual control of the contrast ratio to provide an optimum signal for the 16" Ikegami video monitor model PM-175 B Rev C.

The Ikegami camera proved to be particularly sensitive to the precise light level presented though the trinocular head. This resulted in the minor alterations to the variable a.c. light controller making large differences in the illumination, often outside the range of acceptable values. Minute increases in light level, barely detectable to the naked eye, resulted in excessive blooming or white out. Conversely minor reduction in light level resulted in black out for the camera. It became obvious very quickly that the analogue a.c. Olympus TE-II light controller was totally inadequate for controlling camera illumination.

To overcome this problem, a digitally controlled d.c. converting light controller was employed. This unit, constructed by the author, used as its input voltage the maximum output a.c. voltage from the Olympus TE-II. The a.c. was converted into variable voltage d.c. which was supplied directly to the microscope light source. Two 10 position thumb-wheel switches provided 99 digitally selectable, and repeatable, light levels. The microscope lamp is equally well served by either a.c. or d.c., and the repeatable settings made possible the use of the Ikegami CCD camera.

The magnification chosen for determining the cell cycle data utilised the 40x objective lens in combination with the 2.5x camera lens and video system described

above. The 2.5x camera adapter lens in the MTV -3 provides approximately half the diameter of the field of view observed through the 10x oculars. This magnification was chosen to provide maximum magnification through the video system while maintaining optimum resolution to allow accurate details of the mitotic stages to be clearly discernible. With an image of closely packed cells on the slide, approximately 100 nuclei could be identified.

One disadvantage of this combination of 40x objective and 2.5x camera lens is that the image is not plano, that is, not all of the field of view is in the same focal plane. This necessitates adjustment of the fine focus control in order to scan the entire field of view on the video monitor. Sufficient resolution is obtained to allow accurate determination of all the stages of mitosis. To use the 20x objective, which is a plano lens, would result in approximately 500 nuclei being in each field of view. The individual size of each nucleus at this magnification, however, is very small, often with too little resolution on the video monitor to determine the precise stage of mitosis. In addition it is very much more difficult to count 500 small nuclei, rather than approximately 100 nuclei of twice the optical diameter observable under the 40x objective. For these reasons the 40x objective is a compromise sacrificing the plano image for greater resolution, but a very workable one.

6.3 EXPERIMENTAL PROCEDURE

In an attempt to evaluate the magnetic biostimuator as a scientific research tool, it was resolved to test the following null hypothesis:

> **That there is no significant difference between the cell populations of the test conditions with respect to the controls.**

Vicia faba (broad bean) root tips were subjected to a variety of both static and alternating magnetic fields, *in vivo*, at similar field strengths and frequencies as those used by Khalil and Qassem[16]. The field strengths and frequencies tested consisted of the following:

	Expt	Hz	mT
(control)	0	0	0
(test)	1	0	1.0
(test)	2	0	5.0
(test)	3	50	1.5
(test)	4	60	1.5
(test)	5	75	1.5

6.3.1 General procedure

Vicia faba beans were presoaked in water overnight then planted in Vermiculite (a hydrated sheet silicate aggregate used as a growing medium). After seven days the main root tip of each bean seedling was excised in order to stimulate lateral root growth. Ten day old seedlings with emerging lateral roots were used for the magnetic field experiments. Three seedlings were planted in a one litre beaker filled with Vermiculite and left untreated as the control. A similar beaker, also containing three seedlings, was used for the test condition and placed inside the solenoid coil. The chosen field was then applied for 72 hours. This procedure was followed for each of the test conditions listed above. This length of time allowed the cells to pass through approximately three complete cell cycles. (The cell cycle time for dividing *Vicia faba* root tips is approximately 24 hours, personal communication: Dr. Rowland, Department of Plant Biology, Massey University).

6.3.2 Slide preparation

At the end of the 72 hour treatment, root tips from both the control and test plants were harvested. Some excised root tips were placed in 0.05% colchicine (aqueous solution) for three hours fifty minutes prior to fixation in 1:3 glacial acetic acid : methanol. These colchicine-treated root tops were used for metaphase break analysis. Other root tips were fixed directly in 1:3 glacial acetic acid : methanol immediately upon excision without colchicine pretreatment. These latter root tips were used for cell cycle analysis.

Fixed root tips were hydrolysed and stained by the standard Feulgen method, macerated in 45% acetic acid, squashed and the cover slips removed by the liquid nitrogen technique. After dehydration for 10 minutes in 100% ethanol and the

slides air dried, the cells were permanently mounted in DPX. The slides were examined by light microscopy.

6.4 ANALYSIS PROCEDURE

6.4.1 Chromosome breaks

Determination of chromosome breaks is particularly difficult to determine, requiring expert knowledge of the karyotype, hence the services of an expert were engaged.

The metaphase chromosomes from each experimental procedure were examined in collaboration with Dr. Rowland of the Department of Plant Biology and Biotechnology at Massey University. The number of chromatid and chromosome breaks were recorded. The results are presented in Table 6.4 of Section 6.6.1.

6.4.2 Determination of the cell cycle

Prepared (fixed) slides of *Vicia faba* which had not been treated with colchicine, (a metaphase blocker) were examined with the microscope imaging system. One hundred visual fields were scored for stages of the cell cycle. In this way 6 population samples were examined for the control (n=6), 5 for the DC field exposure, 7 for 50 Hz, 6 for 60 Hz and 5 for 75 Hz. The criteria for determination of the stages is outlined in Section 6.5.3. The number of population samples was limited by the number of prepared slides available. The results are presented in Section 6.6.

6.4.3 Cell cycle analysis protocols

The stages of the cell cycle were determined according to the following criteria:

(Diagrams representing each stage of mitosis may be found in Section 6.5.)

Prophase.

Nuclei were defined as being in prophase if the chromosomes were clearly visible as definite strands within the nuclear envelope. This includes the occurrence of very fine strands as well as the somewhat thicker chromosomes of late prophase. No attempt was made to discriminate between early, mid or late prophase.

Metaphase

Nuclei were classified as metaphase if the following two criteria were met: Firstly chromosomes appeared as short, thick, dark units where two chromatids were clearly visible, and secondly these paired chromatids were aligned approximately across the mid point of the polar axis of the cell. The procedure used to present the specimen on a microscope slide involves squashing the root tip, which may skew the alignment of the chromosomes at the mid point of the polar axis. Thus alignment could not be the sole determinant for identification of metaphase. Rather, clear definition of the somewhat thicker strands and chromatid development was used as the primary protocol. While this may lead to some difficulties in determining the exact point of transition from prophase to metaphase, the process is obviously continuous. This difficulty should only account for perhaps a $\pm$ 1% error. It will be shown in the results, Section 6.6, that the variation between treatments is

far more significant than this. The large number of nuclei counted, could be expected to nullify these errors. ±

Anaphase

Anaphase is the clearest and most easily definable stage. Here the criteria for determination is the appearance of "V" like structures as the paired chromatids are pulled apart. Anaphase includes chromosomes from the first point of 'unzippering' to the later stage where the individual chromatids are pulled to the poles of the now elongated cell.

Telophase

Telophase nuclei were defined as those where the individual chromatids appeared as two distinct, somewhat squared, clumps at either pole of the nucleus. The "V" shape of anaphase is no longer apparent and the chromatids are very tightly packed together. In addition the cell wall, and hence the cell membrane, must not show completion of cytokinesis (formation of two separate entities). The telophase definition states that the two clumps of chromatids must still be within the same cell wall. As the nuclear and cell membranes are not visible under the light microscope, they have not been included in the operational definition of the stags of mitosis.

Interphase

Interphase nuclei are easily distinguishable as large granular sphere-like structures and are clearly recognisable from all other mitotic stages.

6.5 Brief Background to The Cell Cycle

In order to understand the significance of the experimental procedure and results, a brief background to the cell cycle is presented.

When early microscopists first observed living cells under their rudimentary microscopes, they were intrigued to find that the considerable periods of apparent inactivity were occasionally broken by short periods of rapid activity, ultimately resulting in the production of two cells. During this period of high activity, the cell division, minute thread-like structures became visible, then dissolved back into the background of the two new cells. To describe the process these early pioneers borrowed **mitos** from the ancient Greek meaning thread; and **osis**, also from the Greek, meaning process. Thus the process of formation of thread like structures became: **MITOSIS.**

As technology improved the microscope, magnification and resolution increased. Parallel advances in differential staining procedures accompanied this developing hardware technology. It then became possible to determine, in more detail, the nature of thread like structures within the dark central body, the nucleus. Those minute threads we now know as chromosomes: from the Greek **chroma** for colour and **soma** meaning body. Clearly it was within this dark body, the nucleus, that exciting things happened which ultimately gave rise to new cells. It is relevant to understand that the term nucleus derives from the diminutive of the Latin **nux nucis**, a nut. Thus it was known from early times that as the nut held the secret of reproduction in the plant world, and so it was they reasoned, that the dark body in the centre of a cell must also contain its germ or seed.

It was not until biochemists investigated the period of inactivity between duplications in cells that we were to discover three distinct stages in the period known as interphase, undetectable to the naked eye, . These were latterly termed **G1, S** and **G2**: standing for Gap one, Synthesis, and Gap two. This is diagrammatically represented below in Figure 6.5. The approximate lengths of time are given in percent, as the actual times vary considerably between organisms, tissues, and physiological conditions. The direction of movement through the clock sectors is clockwise.

The Cell Cycle of Reproduction (mitosis)

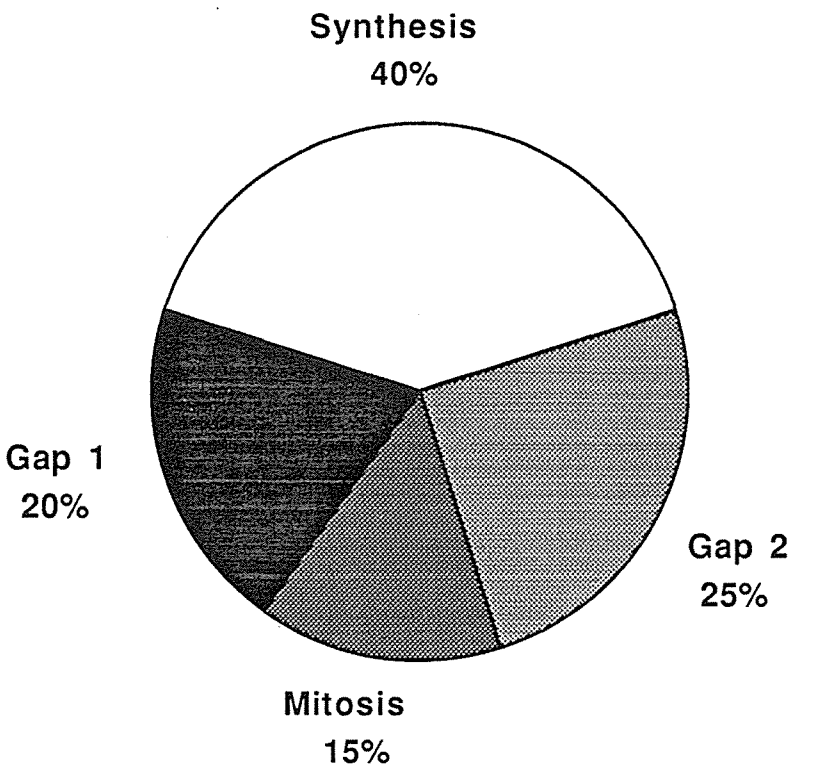


Figure 6.5

Gap 1 is where most cells remain at maturity. They no longer enter the S phase and hence on to mitosis via Gap 2. A few cells are thought to stop in G2 at maturity, although this is understood to be rare. For cells which continue on after G1, they enter S phase where all the precursors for the DNA are assembled. The chromosomes are duplicated into two identical sister chromatids in readiness for cell division. Later, at anaphase, the sister chromatids are separated to either pole which results in the production of two identical, "sister" cells. The two new cells are termed "sisters" as they are essentially identical, both containing the normal complement of chromosomes (termed **2n**). (Alternately, **meiosis** is the process by which a cell divides twice to produce four cells with only half the normal chromosome number, termed **n**. These cells are known as germ cells being either egg or sperm cells.)

With more powerful microscopes at their disposal, biologists began to unravel the mysteries of the nucleus at mitosis. Ultimately four separate stages were identified within mitosis: Prophase, Metaphase, Anaphase and Telophase.

The first, **Prophase**, (**pro** from the Greek meaning before, and **phasis** for appearance) is defined as the stage at which the chromosomes first become visible as individual threads or strands. The DNA exists in its 'unwound' state interphase, whereas in prophase the individual strands contract into more complex tertiary structures, becoming more visible in the process. The shortened chromosomes also stain more readily with certain dyes. The chromosomes are identifiable as consisting of pairs of identical chromatids in late prophase. Prophase also marks the beginning of spindle formation, the role of which becomes more evident in anaphase. The nuclear membrane begins to dissolve, although this is somewhat

more difficult to see without special microscopic techniques. A diagrammatic representation of late prophase is presented in Figure 6.6.

Prophase

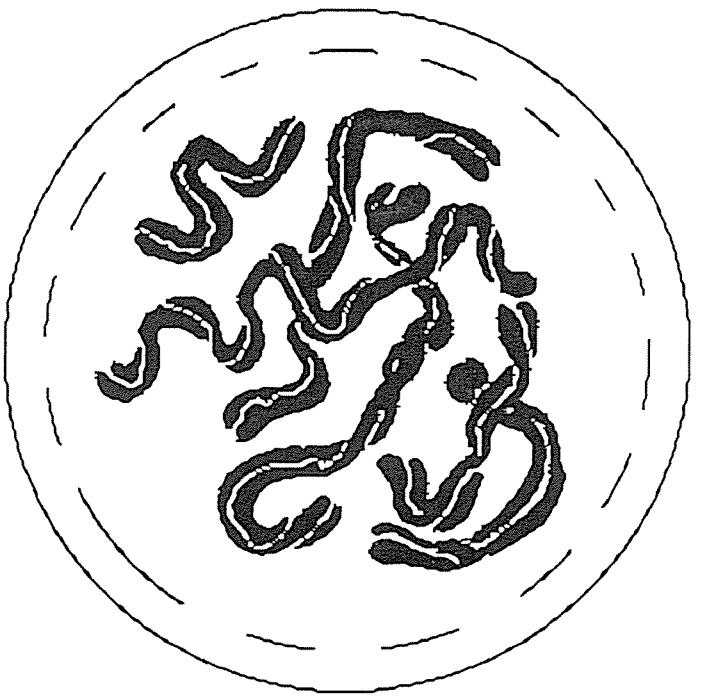


Figure 6.6

Metaphase, (**meta** from the Greek for 'after, occasion with the sense of change') is marked by the final condensation of tertiary structure of the paired chromatids and attachment of the centromere to the spindle apparatus. Visually the chromosomes now appear as the very thick well defined structures which we are most familiar with. The physical appearance of the chromosomes at this stage is referred to as the "karyotype". Indeed at this stage chromosome abnormalities may be determined, such as trisomy 21 in humans. The chromosomes have very distinctive morphologies specific to the species of origin. Another distinctive

feature of this stage is the physical orientation of the chromosomes within the cell. They tend to 'line up' across the diameter of the cell at right angles to the polarity of the spindle fibres. Metaphase is diagrammatically shown in Figure 6.7.

Metaphase

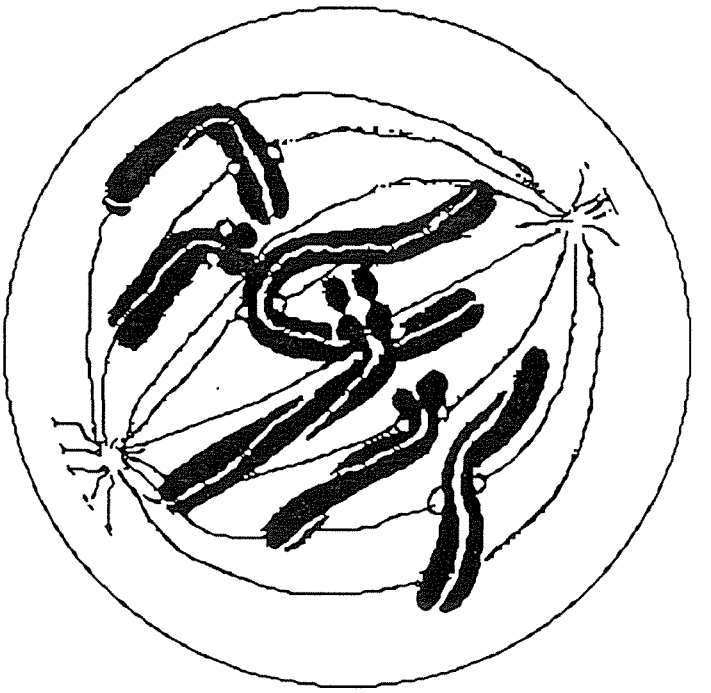


Figure 6.7

Anaphase is the most easily determined of all the stages. (It is interesting to note that **ana** comes from the Greek for 'up, back, again or new'.) It is now that we see the most dramatic change of all. The paired chromatids (of each chromosome) attached at the centromere to the spindle fibres *are pulled apart* (back) to either end of the cell. Visually it appears as if a zipper were being pulled open from the centre, the chromatids forming 'V' shapes as they are pulled to their respective poles. The precise mechanism which accounts for this pulling is not fully

understood, however it is known that the spindle fibres are constructed of microtubular bundles of the contractile protein, tubulin. These fibres generally consist of a tubular structure about 24 nm in diameter formed by the aggregation of α & β tubulin dimers, and a small amount of associated other proteins, in a helical array. They function as the 'skeletal' structure of the cell in addition to providing the pulling forces required for anaphase. Most investigators now believe that the spindle microtubules generate the poleward movement by a combination of controlled growth and active sliding movements. In this sense they are similar to the contractile proteins of muscle: actin and myosin. Anaphase is shown diagrammatically in Figure 6.8.

Anaphase

Figure 6.8

Telophase is the final stage of the mitotic process, (**telo**, Greek for end). The tubulin spindle fibres dissolve and the formation of a new nuclear membrane begins around each set of chromatids at the poles of the cell. At this stage, in *Vicia faba*, the chromatids form a very square structure which is particularly distinctive. The cell now undergoes cytokinesis: division of the cytoplasm and the formation of two separate cells, (daughter cells in the case of mitosis). The term cytokinesis is derived from the Greek kytos - a hollow vessel, and kinesis - motion. The DNA of the chromatids now starts to 'unravel', forming the granular mass typical of interphase. Telophase is diagrammatically shown in Figure 6.9. Note that cytokinesis is not complete.

Telophase

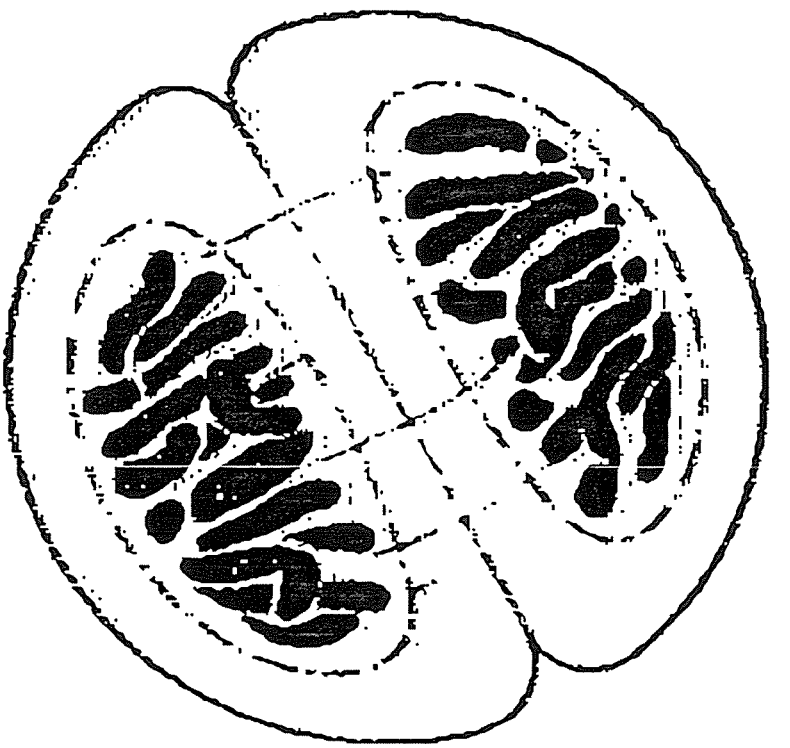


Figure 6.9

Microscope images of the phases of Mitosis in *Vicia faba*

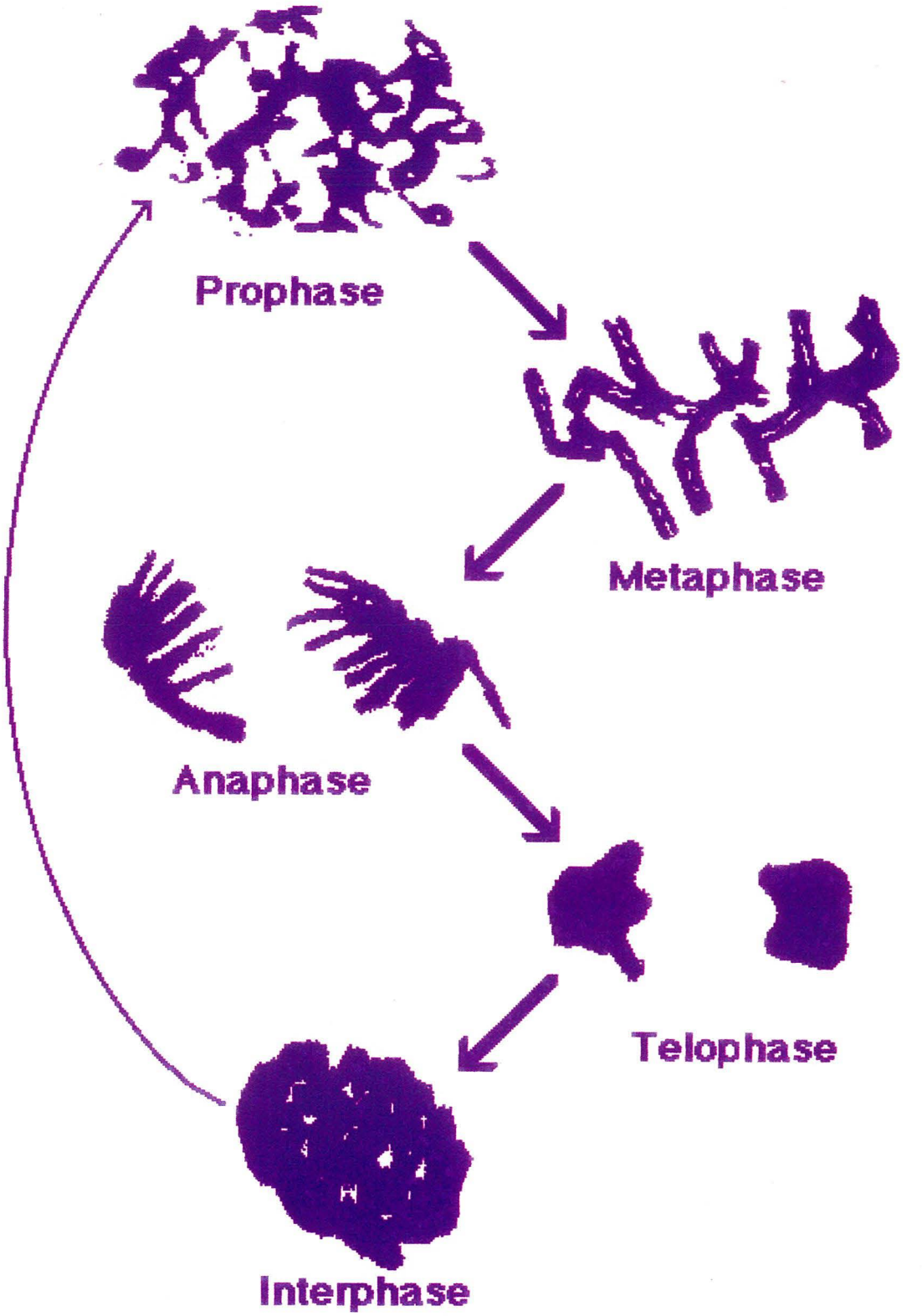


Plate 6 - 1

Real images of the nuclei of *Vicia faba* in all stages of the cell cycle were captured from the microscope imaging system described in Section 6.2.7, then subsequently processed by *Image* on a Macintosh Quadra 950. The resulting smoothed images were converted to binary format for presentation. These images are shown in Plate 6.1 tinted purple to resemble real Feulgen staining.

6.6 RESULTS

6.6.1 Chromosome breaks

The chromosome and chromatid breaks at mitosis for each experimental treatment are presented in Table 6.4 below.

Chromosome and Chromatid Analysis Results

Experiment	Hz	β mT	Chromosome Breaks	Chromatid Breaks	Total Cells Counted
Control	0	0	8	15	1879
1	0	1.0	3	9	998
2	0	5.0	5	8	885
3	50	1.5	3	11	1026
4	60	1.5	4	8	771
5	75	1.5	5	8	942

Table 6.4

The results in Table 6.4 are standardised to percentage breaks for each experimental treatment and presented in Table 6.5.

Percentage of Chromosome and Chromatid Breaks

Experiment	Hz	β mT	Chromosome % Breaks	Chromatid %Breaks	% Total Breaks
Control	0	0	0.426	0.798	1.224
1	0	1.0	0.301	0.901	1.202
2	0	5.0	0.565	0.904	1.469
3	50	1.5	0.292	1.072	1.364
4	60	1.5	0.519	1.038	1.557
5	75	1.5	0.531	0.849	1.380

Table 6.5

A Chi-square analysis, Table 6.6, was performed to determine if the number of chromosome and chromatid breaks were related to the experimental treatment. The results clearly show that there is no significant dependence (relationship) between breaks and treatment. The results for all data are summarised in Tables 6.7, chromatid breaks for all groups, and 6.8, basic statistics of root population samples.

Chromosome Breaks for all groups

Statistic	DF	Value	Probability
Chi-Square	5	1.584	0.903
Likelihood Ratio Chi-square	5	1.632	0.897
Mantel-Haenszel Chi-square	1	0.200	0.654

Table 6.6

Chromatid Breaks for all groups

Statistic	DF	Value	Probability
Chi-Square	5	0.740	0.981
Likelihood Ratio Chi-square	5	0.727	0.981
Mantel-Haenszel Chi-square	1	0.204	0.652

Table 6.7

6.6.2 Cell cycle effects

The root tips from one plant from each experiment were examined under the microscope and the stages of the cell cycle recorded for each nucleus. Details of the microscope imaging system are detailed in Section 6.2.7.

The basic statistics including minimum, maximum, mean and standard deviation for the combined results of each set of 100 visual fields per population per experiment are presented in Table 6.8. The number of root populations are represented by "n" for each treatment, the treatment being represented by "Expt".

Because the number of root populations of nuclei varied depending on the successful preparation of the microscope slides, further statistical analysis was based on the General Linear Models procedures within the statistical analysis package, SAS.

An "F-test" to compare the variances between the following populations (mitotic phases): prophase, metaphase; anaphase; telophase; and interphase was performed, for all experimental treatments. The statistic used in this instance is the number of cells in each phase, which is equivalent to the time spent in each phase. Any signficant variation in the number of cells in a particular stage may be directly correlated to the length of time taken to complete that phase. The results detailing the variable; degrees of freedom (DF); F value; and significance level (Pr>F), are presented in Table 6.9. The full printout of the SAS analysis is presented in Appendix Three.

Analysis of variance was conducted using Scheffe's test comparing treatments with each other for: prophase; metaphase; anaphase; telophase and interphase, a precis of the results being presented in Table 6.10. Comparisons of significance at the $\alpha = 0.05$ level are represented by "*" . The full printout of the Scheffe's analysis is presented in Appendix Three.

Basic Statistics of Root Population Samples

Expt	Variable	n	Min	Max	Mean	Std.Dev.
Control	Prophase	6	52	93	67.83	17.72
"	Metaphase	6	12	21	15.33	3.44
"	Anaphase	6	9	12	11.17	1.17
"	Telophase	6	15	32	23.33	5.82
"	Interphase	6	1942	2347	2110.67	138.55
O Hz 50 G	Prophase	5	98	149	118.60	21.07
"	Metaphase	5	29	41	34.60	4.62
"	Anaphase	5	15	25	20.00	4.80
"	Telophase	5	29	42	33.00	5.34
"	Interphase	5	3097	3483	3280.80	191.20
50 Hz 15 G	Prophase	7	116	152	133.14	15.14
"	Metaphase	7	28	44	37.29	6.37
"	Anaphase	7	19	45	30.00	7.92
"	Telophase	7	36	62	50.57	9.78
"	Interphase	7	3051	3409	3255.43	135.44
60 Hz 15 G	Prophase	6	103	138	120.5	14.27
"	Metaphase	6	43	61	50.00	6.23
"	Anaphase	6	24	39	32.83	6.68
"	Telophase	6	42	52	46.33	4.59
"	Interphase	6	3412	3804	3595.17	149.36
75 Hz 15 G	Prophase	5	125	142	135.40	6.88
"	Metaphase	5	46	68	57.40	8.23
"	Anaphase	5	22	31	26.80	4.44
"	Telophase	5	31	37	34.00	2.83
"	Interphase	5	3170	4107	3647.80	347.69

Table 6.8

F-Test on Prophase, Metaphase, Anaphase, Telophase, and Interphase for all data.

Dependent Variable	DF	F Value	Pr > F
Prophase	4	18.20	0.0001
Metaphase	4	41.39	0.0001
Anaphase	4	14.08	0.0001
Telophase	4	18.21	0.0001
Interphase	4	57.44	0.0001

Table 6.9

Scheffe's Test - Precis of Results

Expt Comparison	Prophase	Metaphase	Anaphase	Telophase	Interphase
75Hz vs 60Hz	0	0	0	0	0
75Hz vs 50Hz	0	*	0	*	*
75Hz vs DC	0	*	0	0	0
75Hz vs Control	*	*	*	0	*
60Hz vs 75Hz	0	0	0	0	0
60Hz vs 50Hz	0	*	0	0	0
60Hz vs DC	0	*	*	*	0
60Hz vs Control	*	*	*	*	*
50Hz vs 75Hz	0	*	0	*	*
50Hz vs 60Hz	0	*	0	0	0
50Hz vs DC	0	0	0	*	0
50Hz vs Control	*	*	*	*	*
DC vs 75Hz	0	*	0	0	0
DC vs 60Hz	0	*	*	*	0
DC vs 50Hz	0	0	0	*	0
DC vs Control	*	*	0	0	*
Control vs 75Hz	*	*	*	0	*
Control vs 60 Hz	*	*	*	*	*
Control vs 50 Hz	*	*	*	*	*
Control vs DC	*	*	0	0	*

Table 6.10

Further analysis was carried out using Dunnett's T test which compares each treatment with the control for each of the dependent variables: prophase; metaphase; anaphase; telophase; and interphase. The results are presented in precis in Table 6.11 where "*" indicates significance for $\alpha = 0.05$, and 0 indicates no significant difference.

Dunnett's T Tests - Precis of Results

Expt Comparison with Control	Prophase	Metaphase	Anaphase	Telophase	Interphase
75 Hz	*	*	*	*	*
50 Hz	*	*	*	*	*
60 Hz	*	*	*	*	*
O Hz	*	*	0	0	*

Table 6.11

6.7 DISCUSSION AND CONCLUSIONS

6.7.1 Chromosome / chromatid breaks

Analysis of chromosome breaks is a well established technique for detecting any damage to the genome by a harmful environmental agent. A close relationship exists between mutagenic agents, such as X-rays, and the incidence of chromosome breakages. From the analysis of chromosome and chromatid breaks in the present thesis it can be concluded that there is no significant, lasting effect of any of the five magnetic field treatments used in this study. This is not to say that other magnetic field exposures may not cause significant chromosome breaks, only that the combinations of frequency and field strength used in this study had no discernible effect.

As some 6501 mitotic nuclei were examined for chromosome breaks, it is reasonable to assume that if an effect exists, it should be revealed by such a large data sample. As cells in metaphase account for approximately 1% of the total population of meristematic (actively growing) tissue, approximately 650,000 nuclei were examined in order to discover only 87 breaks in total. One of the obvious difficulties is that the breaks in a chromosome or chromatid are visible only when they appear in metaphase. There may be significant numbers of breaks in other phases but they cannot be observed.

Another difficulty in using chromosome or chromatid breaks as the dependent variable for the experimental treatment is that the DNA comprising the chromosomes is almost constantly under the scrutiny of a sophisticated enzyme repair mechanism. This is especially so if the incidence of breakages is low.

Special proteins (ligases) travel along the DNA detecting incorrect bonding and breaks, repairing them as they pass. In this way incorrect adenine - thymine or cytosine - guanine bonding is detected.

The rules for DNA base pair bonding dictate that a purine (cytosine or adenine) may only bond with a pyrimidine (guanine or thymine). As the number of bonds is different for the two pairs of bases, adenine can only bond with thymine, and cytosine with guanine. If a mistake has occurred during DNA replication in S phase which has resulted in the purine base adenine bonding to the pyrimidine guanine for example, the extra unpaired electron bond will be detected, the errant nucleotide removed, and the correct one re-inserted.

It can be clearly seen that any potential breaks, possibly caused by some exogenous magnetic perturbation, may be corrected prior to the cell entering metaphase where the karyotype may be visualised and the break detected. Although the cell cycle is a continuous process in meristematic tissue, with all cells continually moving through each stage, and the the experiments conducted here were of sufficient duration to allow each cell to pass through the entire cell cycle process at least three times, any magnetic perturbation resulting in either chromosome or chromatid breaks may not be retained through the entire cycle to be viewed in metaphase.

The current results conclude that there is no significant net increase in the number of chromosome or chromatid breaks in the treated groups when compared with the number of naturally occurring, spontaneous, breaks observed in the control population. This does not imply that the magnetic stimulus failed to produce breaks at any stage of the cell cycle, only that the resultant numbers observed at

metaphase do not significantly differ from the normal background values of the control group.

6.7.2 Cell cycle ratio aberrations

The Null Hypothesis (Ho) stated: **There is no significant difference between the cell populations of the test conditions with respect to the control.**

An F-Test performed on the combined cell populations grouped by phase, i.e. prophase, metaphase, anaphase, telophase and interphase show significant differences between the groups, at the probability level of 0.0001. This is to be expected, as it is well established that the time spent in each of the stages of mitosis is significantly different, therefore it is expected that the numbers of cells in each stage will be significantly different. It is a well established principle that the number of cells present in each stage of the cell cycle, for an asynchronous growing population, is directly equivalent to the time spent in each stage. For this reason it is expected that the number of cells counted in each stage is significantly different. The population of *Vicia faba* cells used in these experiments may be considered normal, (in this sense). This is confirmed by Van't Hof_{26}. in the Handbook of Genetics Volume Two, "The duration of chromosomal DNA synthesis, of the mitotic cycle, and of meiosis of higher plants".

The analysis of variance carried out with Scheffe's Test compares each stage of the cell cycle for each experiment, with every other phase, for each group. This is a type of multiple T-Test, used to determine the statistical significance between a set of population means. Scheffe's test is used here with $\alpha = 0.05$. Fifty two of the

comparisons are significant, at this level, out of a possible 100. The interpretation of this large number of comparisons is particularly difficult to analyse, however, a few interesting points will be examined. The results discussed are shown in Table 6.10.

There appears to be no significant difference between 75 Hz and 60 Hz for any phase. Metaphase, telophase and interphase are different for 50 Hz and 75 Hz, therefore suggesting that 75 Hz may not be producing the same effect as 50 Hz, for these phases. This supports use of 75 Hz as an experimental variable where the frequency is close to those used for power distribution, but is obviously perceived as significantly different in the physiological responses it initiates. In one sense it may be considered an 'active' control, that is, a frequency control rather than the control simply being 'no field'. This data tends to support the notion for biological systems of a frequency 'window', or differential response, with respect to frequency.

Comparing 50 Hz and 60 Hz population samples, only metaphase is significantly different. For all other phases, no difference between the sample populations may be determined. This may be of great significance with respect to the fact that both these frequencies are used for power distribution which has been linked to various cancers: [27,28,29,30,31]. These results should be compared with the controls in order to obtain the real significance. For this reason, Dennett's T tests were performed on the four different magnetic field treatments, combining the populations for each phase of mitosis within each treatment group.

Dennett's T test is a test of variance which compares the control population's mean with the population means of the treated groups. This test controls the Type 1

experimentwise error for comparisons of all treatments against the control. That is, Dennett's T Test is less likely to commit a Type 1 error, which is the rejection of the null hypothesis (Ho) if it is correct. Analysis is carried out at the $\alpha = 0.05$ level. The results discussed here are shown in Table 6.11.

The Null Hypothesis is accepted for the anaphase and telophase stages for the d.c. 5.0 mT trial only. The Null Hypothesis is thus not accepted for any other phases or experiments, (where a = 0.05). This means that there is only a 5% chance that the results obtained for the experimental groups, (with the exception noted above), could have come from the same population as the control. **The obvious conclusion is that the magnetic stimulation applied to the experimental groups may have caused some physiological changes which could account for these statistical differences.** <u>It by no means proves it</u>. A number of other variables could also be responsible, some of which are noted below.

One possible confounding factor is that the root tip populations were drawn from different plants for each experiment, even though the root tips samples for each treatment were from the same plant. The F Test however compares the variance within and between groups, the result being a significant difference at the $\alpha = 0.0001$ level for all comparisons. This tends to suppport the conclusion that the magnetic field treatments may have had some influence.

6.7.3 Possible mechanisms of action

This section should be considered in conjunction with Section 6.1.2, Cyclotron resonance and the thermal noise limit, and Section 6.1.3, Larmor prescession.

Goldberg[27] considers 4 possible mechanisms of action for low frequency electromagnetic fields which are representative of current thinking:

- Disruption of cell communication.
- Modulation of cell growth via changes in calcium ion flux.
- Activation of specific (oncogene) sequences.
- Stress as a factor operating through disruption of hormonal and immune system tumour control mechanisms.

While Goldberg is concerned with stimulation of cancer in humans (with particular reference to points 3 and 4), the same basic mechanisms may be applied to plant systems. Each of these will be assessed in turn.

Disruption of cell to cell communication.

Cell to cell communication is known to occur in both plants and animals. This is largely achieved by two mechanisms: chemical (including hormone) and electrical, (including ion transport).

In the course of normal embryonic development in animals, the sorting out, (differentiation during morphological development), and adhesion of cells is quickly followed by the formation of more or less permanent intercellular junctions of various kinds. In contrast to the glycoprotein interactions responsible for initial

cell-to-cell recognition and adhesion, which occurs at resolving powers below the range of the electron microscope, the junctions formed as cells take up their permanent places in tissues involve extensive and easily visible rearrangements of the plasma membrane and the cell surface. These junctions which hold many of the tissues of multicellular animals (metazoans) together, also provide the means of communication between some specialised types of cells. These adhesion and communication links can be found throughout the entire life of the tissue, Wolfe$_{32}$. Junctions may be easily recognized in electron micrographs as the regions of membranes of two adjacent cells which are more closely opposed than the normal 20-30 nm interspace. In the closely opposed regions, electron-dense, fibrous material forms layers between the cells, or in the cytoplasm just inside the membranes. The layers are symmetrical so that the dense deposits of material look the same in each cell forming the junction.

The junctions are of three major types: adhesive; sealing and communicating. Communication junctions provide transmembrane channels allowing direct flow of ions and small molecules between the cells. Junctions of these types are widely distributed in the animal kingdom.

The existence of communicating junctions was hypothesised long before Loewenstein's$_{40}$ land mark research in 1975. Loewenstein used microelectrodes inserted into growing cells to determine that the resistance between two cells connected by the apparent gap (communicating) junction, often termed a **nexus**, had significantly lower resistance than between two cells with unbroken membranes. Further experiments involving the injection of fluorescent dyes proved that molecules certainly do pass through these gap junctions between cells.

Communication between plant cells has also been observed, Robards[41], 1975. Both primary and secondary plant cell walls retain minute openings called **plasmodesmata** which are analogous to the nexus of animal cells. Cytoplasm of adjacent cells passes through these openings and act as an obvious form of molecular communication. The openings are originally formed during the deposition of the cell plate, the new cell wall that forms between daughter cells, during the later stages of mitosis. As the cell plate thickens into the primary wall, and as the later thickenings convert primary to secondary walls, the plasmodesmata persist in their original numbers which may vary from 1 to 140 per square micrometer. At these levels, a mature plant cell may have as many as 1000 to 100,000 plasmodesmata connections to its neighbours.

The diameter of plasmodesmata vary from 25 nm to 0.2 μm. Often the larger diameter plasmodesmata contain a neck, or restriction, of some 30 nm. Cross sections also show a tubular inclusion in the centre which may have some controlling, as well as structural, role. It has been hypothesised that the tubular structure is actually an extension of the endoplasmic reticulum, the terminal cisternae of which closely approach the plasmodesmata on either side of the cell wall. These terminal cisternae may make connections between cells by way of the tubular structure. Flagg-Newton et al.[33] have determined that molecular communication does occur through these membranous pores having observed the passage of molecules with molecular weights up to 1800 daltons in insect cells. The gap junctions of the mammalian cells tested including, rat liver, human mammary and calf lens cells, pass molecules with an upper limit of 800 daltons, and are thus more restrictive. Ions are known to pass freely through membranes

which accounts for the low resistivity measured across cells which are connected via nexus (of animals) or plasmalemma (of plants).

Cell to cell communication does not just occur with respect to ions, but also larger molecules, as detailed above. It is obvious that molecules travel from cell to cell in all living systems. The transport of photosynthetic product in plants for example, or the digested food products in animals. In addition to this sort of food / energy molecule transfers, another system of specific communication molecules exists: **hormones.**

It is well known that animal systems rely on the production and transport of hormones for many biological processes. Such biochemicals as adrenalin and thyroxine are well known examples in animals. Plants however have a complex system of hormones which include: auxins, cytokinins; kinetins; gibberellins and ethylene. These substances control the overall growth and development of the plant, acting as hormones in the true definition.

Lubin$_{36}$ has conducted experiments concerning the effects of low-energy, low-frequency electromagnetic fields on bone and bone cells *in vitro* , namely the inhibition of responses to parathyroid hormone by low-energy, low-frequency fields. His results suggest a magnetic coupling to ion transport which interferes with this natural action of parathyroid hormone. This may support the work of Male$_{47}$.

Clearly the stage has been set for complex molecular interactions with low energy, low frequency, magnetic fields which have far reaching implications for both plants

and animals. It is possible that the magnetic stimulation applied to *Vicia faba* may have in some way interfered with the normal hormone mediated processes of growth. The current experiments do not conclusively show that such an interaction exists, however, they do provide ample encouragement for further research to fully elucidate the phenomenon.

Modulation of cell growth via changes in calcium ion flux.

Gap junctions are always present where cells have been shown to be electrically coupled. The passage of ions is freely observed where cell to cell electrical resistance is low. Typically the electrical resistance between cells through the plasmalemma is 50 times lower than if no plasmalemma or other membrane breaks exist. Calcium is the most resistant to passage but may be actively transported, Dreifuss[34] et al. Active transport of other ions is known to exist, such as sodium and potassium, and is ATP dependent. Blank[37] has shown that sodium, potassium ATPase function is affected by alternating electrical fields. This finding supports the work of Liboff[42], Goodman[1,5,7,8,37,38], Bassett[52,53], and Male[47].

Calcium ions are certainly involved in the repair of recalcitrant fractures by pulsed electromagnetic fields, Bassett[35] et al. It has further been suggested that the currents induced by pulsed magnetic fields may exert an electrochemical effect at the cell surface which, in turn, influences the membrane transport and intracellular concentration of calcium ions, Lubin[36], Goodman[37]. Because of the important role played by calcium ions in metabolism and growth regulation, this proposal deserves careful consideration in the context of extremely low frequency magnetic field effects at the cellular level.

Clearly a possible mechanism of interaction of extremely low frequency magnetic fields and living cells is interference in the normal concentrations of intra and extracellular calcium. Because calcium is involved in growth processes, it is possible that the differences observed in the experiments detailed above, concerning the ratios of cells in each stage of the cell cycle, could have been caused by direct effects on calcium efflux.

Activation of specific (oncogene) sequences.

The possible activation of specific genes is suggested by Goldberg[27]. Oncogenes, (genetic locus capable of transformation of the host cell into a cancerous one), are generally discussed in relation to humans or animals, but it is conceivable that such genetic loci also exist in plants. Of equal importance is the work of Reba Goodman[8] who has shown that pulsed electromagnetic fields can effect cellular transcription in dipteran salivary glands. In addition Goodman[38] has shown that both transcription and translation are affected by such fields, and that this effect is mediated by frequency, field strength and time-dependent windows. (**Transcription** is the process whereby sections of DNA are copied into messenger RNA molecules which pass out through the nuclear membrane where it undergoes translation. **Translation** is the process of changing the messenger RNA code into strings of amino acids, thus producing a protein.) The phenomenon of frequency windows adds weight to the finding that the 75 Hz and 60 Hz comparisons in Table 6.10 were both similar (i.e. no significant difference) yet 75 Hz showed significant differences to both 50 Hz and 60 Hz.

If magnetic fields are able to interact with and alter the growing phase of plant cells, it may be due to the manipulation of hormones and their receptor sites. This might

be achieved by electrically mediated changes to the complex tertiary structure of molecules, particularly at their binding site, see Male[47]. Proteins which are noted for their reliance on tertiary structure for successful binding could also be affected by external electromagnetic fields. This hypothesis would be supported by the work of Blank[37]. As most known enzymes are proteins, this could explain much about the varied results of applying low frequency, low magnitude, electromagnetic fields to cellular systems.

Stress as a factor operating through disruption of hormonal and immune system tumour control mechanisms.

Goldberg's fourth point is in many ways a restatement of the above three points. While his mechanism was originally stated in the context of animal cells, specifically mammals, the concept of stress can be equally well applied to plant systems. The plant hormone ethylene is known as the stress hormone, Hill[39].

Ethylene production is frequently stimulated by auxins, both natural and synthetic. Many effects of exogenous auxin appear to be mediated through their effects on ethylene production, Hill[51]. It has been shown that various types of stress, for example: wounding; exposure to ionizing radiation; disease or physical restriction, will all cause an increase in ethylene production, independent of auxin concentration. The mere bending of a plant in the wind is often sufficient to stimulate ethylene production which has the direct effect of stimulating the production of additional collenchyma cells (supporting tissue).

It is possible that if exogenous magnetic stimulation affects the balance of ions such as sodium, potassium or calcium across the membrane, then such action could result in the production of ethylene, the stress hormone. While it may be less likely to stimulate cancer in plants, it is conceivable that growth processes would be affected, e.g. collenchyma production. This may be reflected in the ratio of cells in the various stages of the cell cycle, as observed.

While the present experiments cannot determine the specific mechanism responsible for the observed significant differences between experimental groups, the above hypotheses offer considerable scope for future work in this dynamic and expanding field.

6.7.4 Evaluation of the biomagnetic stimulator

The cytogenetics experiments detailed above were undertaken to test the magnetic biostimulator in a realistic, scientific application. While the results of the break analysis were inconclusive, the cell cycle analysis experiments tend to indicate a differential effect on mitosis by the various magnetic field treatments. These latter results may be seen to validate the effectiveness of the magnetic biostimulator.

The magnetic biostimulator proved to be be able to produce the required magnetic field stimuli, performing flawlessly throughout the trials. The digital thumb wheels switches allowed the frequencyies to be set up with absolute repeatibility for all the trials. The transconductance amplifier produced the required field magnitude with a minimum of effort. Although the amplifier utilises an analogue gain control, the addition of a panel meter offered a quick method of setting the appropriate flux

density for each experiment. While not as repeateble and accurate as setting the frequency, in practice the flux density could be set within $\pm$ 2% without the need to resort to a flux meter.

Of more importance is the accurate positioning of the flux meter probe to be absolutely on the Z axis at the centre of the coil. Because the probe has a wide angle of acceptance of magnetic vectors, the only place where an accurate reading may be obtained is directly on the Z axis, where the lines of magnetic flux are parallel to the coils major axis. In practice this is achieved by inserting the flux meter probe in a wooden core, which is then inserted into the induction coil. The wood has no effect on the magnetic field, and allows for very accurate alignment.

6.7.5 Future biostimulator features

Future versions of the magnetic biostimulator may benefit from a digital panel meter to set the gain, increasing the speed with which settings may be reproduced.

If squarewaves are being used and whenever the frequency is changed, currently it is necssary to use an oscilloscope to set the current feedback control to optimise the waveform. It may be advantageous to find some alternative method of optimising squarewave signals.

Physical considerations such as incorporating all the modules into a single unit would increase both the ease of set up, and facilitate portability.

When a number of different frequencies are to be used for a single experiment, a computer interface controlling both frequency and timing functions would an advantage. Circuit modifications could be made to deal with the problem of unavailability of some integrated circuits, as mentioned in Section 3.4. In addition, a computer interface could be designed to control the gain and hence themagnetic flux density.

The above points notwithstanding, the present magnetic biostimulator has proved to be a most suitable configuration for producing accurate, defined and repeatable magnetic fields for both clinical and scientific research applications.

6.8 FUTURE WORK

6.8.1 Sister chromatid exchange

One process worthy of further investigation is the effect of magnetic fields on sister chromatid exchanges. It is known that identical sections of the DNA are exchanged between chromatids during mitosis. The first unequivocal demonstration of sister chromatid exchange in mitotic chromosomes of higher organisms was shown in 1957 by Taylor et al.[22], using autoradiographic techniques in plants. Following extensive studies of this phenomenon Taylor reached the following important conclusions:

- Sister chromatid exchanges can occur spontaneously.
- Each chromatid is composed of one DNA duplex.
- Rejoining of the subunits of a chromatid is not random, but strictly restricted to those having the same polarity.
- Sister chromatid exchange involves a double strand exchange.

This process of determining sister chromatid exchange is made possible due to the semi-conservative nature of DNA replication. One strand of the DNA is labelled with a dye which will effectively stain only one strand while leaving the other unaffected. In this manner any exchange of DNA between the chromatids will show up as an oppositely coloured band to the normal colour. It is important to note that although these exchanges involved breakage and reunion, they do not alter the overall morphology or function of the chromosome. Indeed it is speculated that this phenomenon may be another way that evolution ensures continued diversity of offspring. Alternatively it may be that this process is

actually the retention of a very early form of sexual reproduction from primitive prokaryotic or eukaryotic cells.

Whatever the reason for the existence of sister chromatid exchange, the process has stimulated intensive research since its discovery in the late 1950's. Sister chromatid exchange has become accepted as a sensitive means of monitoring DNA damage. In addition it provides an opportunity for cytological detection of DNA interchange. Another remarkable characteristic of the sister chromatid exchange phenomenon is its high sensitivity to some physical and chemical agents. Studies have also shown abnormalities in sister chromatid exchange formation in a number of heritable human diseases which are characterised by a putative DNA repair defect and predisposition to the development of cancer, $Sobti_{23}$ et al. 1991.

6.8.2 Effect of circadian rhythms on the cell cycle

The effect of circadian rhythms on the cell cycle has been well documented in the genus *Allium*, (onion and related species). In addition, circadian rhythms are also well known to affect many other genera. While circadian rhythms are not currently known to affect the cell cycle of *Vicia faba*, (Rowland, pers.comm.), future work could investigate this possiblity. If circadian rhythms were to affect cell replication, then the time of day that the root tips are harvested would clearly affect the results.

6.8.3 Larger sample size

One obvious line of investigation is to repeat the experiments presented here with an increased number of plants per treatment, and root tips. It would be of interest

to attempt to define the differences between plants, rather than simply between root tip populations. The difficulty of needing separate plants for the control and treated groups seems insurmountable, as there is no practical way that a single plant may be subjected to a magnetic field in one part of the root system, while another remains totally unaffected. It must be remembered that the plant responds as a whole, complex metazoan organism, with a complex internal structure and systemic hormone communication and control system. Therefore it is reasonable to assume that if one part of the structure is stimulated, then it may be possible for that influence to be transferred to another part. An analogy would be to inject a drug into the blood system of an animal and not want the chemical to travel to all cells throughout the body. This analogy is in no way trivial as it is well known that plants do have a complex circulation system involving both water, (xylem), and photosynthetic product, (phloem).

In addition is had been discovered that plants possess a real, active, chemical immune system, Ting$_{54}$. Such components as phenols and tannins, as well as proteins, are involved. On occasion these chemicals are exuded from the plant into the environment, such as is the case for the allelochemical of ferns, Ting$_{52}$, Salisbury & Ross$_{55}$. In addition, plants manufacture antibiotics for both procaryotes, and in some cases, against their own progeny. This means that plants, ferns for example, have the capability to exude chemicals into the surrounding soil which will inhibit the growth of similar plants from the same species. In this way they mark out a territory for themselves which is unencumbered by competing offspring.

Plants also have an active transport system which involves the use of hormones, that is, substances that are manufactured in minute quantities in one part of the organism which are then distributed throughout the entire structure where specific

target sites are recognised. For this reason alone, it could not be guaranteed that magnetic perturbations experienced in one part of the plant may not have an effect on some other distant part. In particular the roots are responsible for manufacturing a number of growth hormones, notably gibberellins and cytokinins. (In general terms gibberellins are associated with stem elongation, flowering, germination and utilisation of stored reserves. Cytokinins are concerned with promoting cell division, bud formation, delaying of senescence, and sometimes flowering and breaking of dormancy.)

For the reasons detailed above, it is not practical to use a single plant for both a control and treatment in a magnetic field exposure experiment. Some alternative methodology should be explored which will reduce the inter-plant difference. Larger numbers of plants could perhaps be used to determine the differences between individual plants.

6.8.4 Frequency and magnitude dependence

It is now accepted that response windows exist for biological systems: Jaggard[56]; Joines[57]; Wei[58]; Litovitz[59]; Joines[60] et al. Future work should be undertaken to determine the frequency response for the physiological responses reported here. In addition the magnitude of the stimulation required to produce the response should be studied further. Every biological system which responds to environmental stimuli does so within minimum and maximum limits. The mammalian eye for example is able to detect electromagnetic radiation in the form of photons of light in the range of 400 to 660 nm. The minimal energy required to stimulate nerve impulses from the retina, often called the absolute energetic limit, is of the order of 2.1 - 5.7 $x10^{-18}$ Joules, Mikhov[24] 1988. The range of illuminance response of the

human eye varies over an absolutely enormous range. Ganong's Review of Medical Physiology quotes Bell[25] et al. who states that the physical limits for visual perception range from 0.000,000,1 millilamberts for the threshold of dark adapted rod cells, to a maximum of 10,000,000,000 millilamberts with damage to the retina occurring over 1,000,000,000 millilamberts. It is obvious that the human organism at least, is capable of responding to electromagnetic stimuli over a vast range of magnitudes, (for the eye, some 16 orders of magnitude !). It is reasonable to hypothesize that the observed physiological responses of low frequency magnetic fields must not only have some frequency dependence, (or bandwidth), but also some magnitude thresholds. Future work should be focussed on determining some of these practical limits.

6.8.5 The need for more rapid identification of phases

If the ratios of cells in each stage of mitosis are to be used as a measure of the effects of magnetic fields on biological systems, particularly for experiments involving larger numbers of roots, a more rapid form of analysis needs to be employed for the practical reasons of time constraints. Each microscope slide of a root tip squash takes one person approximately 4 to 5 days to analyse, that is to classify and record the status of approximately 15,000 nuclei. While the author investigated the use of image processing techniques for two years prior to the presentation of these current results, to date, a fast, effective and accurate system has not been developed.

Considerable problems were encountered with respect to the uneven lighting across the microscope's field of view. While the possibility of taking a background image and subtracting this effect from each successive image, was investigated, significant

detail was still not attained due to the random variation in optical density of the microscope slide and mountant. These problems can lead to both false positive counts, and missed counts. In addition the present software is unable to distinguish between the various phases of mitosis. This means that the operator still has to identify the various stages, and subtract these from the total nuclei counted. For these reasons the present experiments which included the classification of 102,650 individual nuclei were carried out entirely manually. While this may be practical for a small number of root populations, the increase in variables would make this impractical within the available human resources.

6.8.6 Automation of identification of the cell cycle stages

The human resource / time problem detailed above could also benefit from some automated computer algorithm which would objectively identify each of the mitotic phases. The solution to this problem may lie in the use of texture analysis routines, however this has not yet been investigated by the author. In addition to the computer analysis routine, an automated scanning stage microscope could complete the system.

It is understood that considerable commercial value could be realised from the development of such a product. A high speed computer vision system capable of not only identifying stages of the cell cycle, including mitosis, could be of considerable value to scientific research. The author has already been approached by the New Zealand agents for Olympus microscopes and imaging systems: Thomas Hyde & Co. Collaborative research is a future possibility.

6.7 REFERENCES

1 R.Goodman, A.Henderson. Transcription and translation in cells exposed to extremely low frequency electromagnetic fields. Biochemistry and Bioenergetics Vol 25, pp335-355, 1991.

2 T.Teneford, W. Kaune. Health Physics Vol 53, pp585-, 1987.

3 H.Westerhoff et.al. How enzymes can capture and transmit free energy from an oscillating electric field. Proceedings of the National Academy of Sciences U.S.A. Vol 83, pp4734-4783, July 1986.

4 B.Greenbaum, E.Goodman, M.Marron. Magnetic field effects on mitotic length in Physarum. European Journal of Cell Biology Vol 27, pp156-160, 1982.

5 E.Goodman, B.Greenbaum, M. Marron. Effects of extremely low frequency electromagnetic fields on Physarum polycephalum. Radiation Research Vol 66, pp531-540, 1976.

6 M.Marron, E.Goodman, B.Greenbaum. Mitotic delay in Physarum polycephalum induced by low intensity 60 Hz and 75 Hz electromagnetic fields. Nature Vol 254, pp66-67, 1975.

7 E.Goodman, B.Greenbaum, M. Marron. Bio-effects of extremely low frequency electromagnetic fields: Variations with intensity, waveform, and individual or combined electric and magnetic fields. Radiation Research vol 78, pp485-501, 1979.

8 R.Goodman, C.A.L.Bassett, A.S.Henderson. Pulsing electromagnetic fields induce cellular transcription. Science Vol 220, pp1283-1284, 17 June 1983.

9 C.A.L. Bassett, R.J.Pawluk, A.A.Pilla, Science Vol 184, p575-, 1974.

10 H.Ito, C.A.L.Bassett. Journal of Bone Joint Surgery & Orthopaedics Transactions. Vol 5, p202-, 1981.

11 C.A.L.Bassett et.al. Journal of Bone Joint Surgery in America. Vol 64, p888-, 1982.

12 C.A.L.Bassett et.al. Journal of the American Medical Association. Vol 247, p623, 1982.

13 J.S.Kort et.al. Journal of the American Medical Association. Vol 247, p623-, 1982.

14 W.J.W. Sharrard et.al. Journal of Bone Joint Surgery Br. Vol 64, p189-, 1982.

15 M.L.Sutcliffe et.al. Clinical Orthopaedics. Vol 166, p45-, 1982.

16 A.M.Khalil, W.Qassem. Cytogenetic effects of pulsing electromagnetic field on human lymphocytes in vitro: chromosome aberrations, sister chromatic exchanges and cell kinetics. Mutation Research. Vol 247, pp141-146, 1991.

17 N.Wertheimer, E.Leeper. Adult cancer related to electrical wires near the home. International Journal of Epidemiology. Vol 11, No4, pp345-355, 1982.

18 M.Feychting, A.Ahlbom. Magnetic fields and cancer in people residing near Swedish high voltage power lines. IMM - rapport 6/92. Karolinska Institutet, Stokholm, Sweden. 1992.

19 J.Olsen et.al. Residence near high voltage facilities and the risk of cancer in children. Cancer Registry of the Danish Cancer Control Agency, Copenhagen, Denmark. 1992.

20 B. Floderus et.al. Occupational exposure to electromagnetic fields in relation to leukemia and brain tumours: a case control study. National Institute of Occupational Health, Solna, Sweden. 1992.

21 D.Sperber & K.Dransfield. Oriented growth of pollen tubes in strong magnetic fields. Naturewissenschaften Vol 68, No S. 40, 1981.

22 J.Taylor et al. Proceedings of the National Academy of Sciences U.S.A. Vol 43, pp 515-529.

23 R.Sobti et al. Sister Chromatid Exchanges, in: Eukaryotic Chromosomes: Structural and Functional Aspects.Narosa Publishing House and Springer-Verlag New York. 1991.

24 D.Mikhov. et al. The geomagnetic field as a low energetic factor with important biological significance, in Electromagnetic fields and biomembranes. Plenum New York 1988.

25 G.Bell et al. Textbook of Physiology and Biochemistry. 9th edition. Churchill Livingston, 1976.

26 J. Van't Hof. The duration of chromosomal DNA synthesis, of the mitotic cycle, and of meiosis of higher plants. Handbook of Genetics, Vol. 2. Plenum New York 1974. pp363-377.

27 R.Goldberg et al. A review of cancer induction by extremely low frequency electromagnetic fields. Is there a plausible mechanism ? Medical hypotheses, Vol 35 (3) pp265-74, July 1991.

28 R.Stern. Cancer incidence among welders: possible effects of exposure to extremely low frequency electromagnetic radiation (ELF) and to welding fumes. Environmental Health Perspectives, Vol 76, pp221-9 December 1987.

29 Floderus. B. et al. Occupational exposure to electromagnetic fields in relation to leukemia and brain tumours. A case control study. National Institute of Occupational Health, Solna, Sweden, 1992.

30 Olsen.J. et al. Residence near high voltage facilities and the risk of cancer in children. Cancer Registry of the Danish Cancer Control Authority. October 1992.

31 Feychting. M. Ahlbom, A. Magnetic fields and cancer in people residing near Swedish high voltage power lines. Report "IMM-rapport 6/92". Institute for miljomedicin, Karolinska Institutet. Stockholm, 1992.

32 S.Wolfe. Biology of the Cell. Wadsworth California. 1981.

33 Flagg-Newton.J. et al. Permeability of the cell-to-cell membrane channels in mammalian cell junction. Science Vol 205, pp404-407.

34 Dreifuss. J. Study of the propagation of excitation in rat ventricle in hypotonic solutions. Pflugers Arch. Vol 292, pp13-33.

35 Bassett.A. Acceleration of fracture repair by electromagnetic fields. Z surgically noninvasive method. Annals of the New York Academy of Science, Vol 238, 1974.

36 Lubin.R. et al. Effects of electromagnetic stimuli on bone and bone cells *in vitro*: inhibition of responses to parathyroid hormone by low-energy low-frequency fields. Proceedings of the National Academy of Sciences U.S.A. Vol 79, pp4180, 1982.

37 Goodman.R. et al. Pulsing electromagnetic fields induce cellular transcription. Science Vol 220, pp1283, 1983.

37 Blank. M. Na,K-ATPase function in alternating electrical fields. The FASEB Journal. Vol 6, pp2434-2438, April 1992.

38 Goodman.R. Transcription and translation in cells exposed to extremely low frequency electromagnetic fields. Bioelectrochemistry and Bioenergetics, Vol 25, pp335-355, 1991.

39 Hill.T. Endogenous plant growth substances. Studies in Biology No. 40. 2nd Edition 1980.

40 Loewenstein. W. Cellular communication by permeable membrane junctions. Cell Membranes. pp105-114. Ed. G.Weissman and R.Claiborne. H.P.Publishing. New York.

41 Robards. A. Plasmodesmata. Annual Review of Plant Physiology, Vol 26, pp13-29.

42 Liboff.A. et al. Ca^{+2}-45 cyclotron resonance in human lymphocytes. Journal of Bioelectricity, Vol 6, pp13-22, 1987.

43 Liboff.A. Cyclotron resoance in membrane transport. In Interactions between Electromagnetic Fields and Cells. Ed. Chiabrera, Nicolini & Schwan, pp281-296. Plenum Press, London, 1985.

44 Liboff.A. & McLoed.B. Kinetics of channelized membrane ions in magnetic fields - in review, quoted in Mechanistic Approaches to Interactions of Electric and Electromagnetic Fields with Living Systems. Ed. M. Blank & E. Findl, Plenum Press, New York & London, 1987.

45 McLoed.B. & Liboff.A. Dynamic characteristics of membrane ions in multifield configurations of low frequency electromagnetic radiation. bioelectromagnetics, Vol 7, No 2, pp177, 1986.

46 Sandweiss.J. On the Cyclotron Resonance Model of ion Transport. Journal of the Bioelectromagnetics Society, Vo. 11, No 2, 1990.

47 Male.J. Biological effects of magnetic fields: a possible mechanism ? Biologist, Vol 39, No 3, 1992.

48 Male.J. & Edmonds.D. Ion vibrational precession: a model for resonant biological interactions with ELF fields. Presented as poster #44 at the 12 Annual Meeting of the Bioelectromagnetics Society, San Antonio, Texas, June 1990.

49 Lednev.V. Possible mechanism for the influence of weak magnetic fields on biological systems. Bioelectromagnetics, Vol 12, pp71-75.

50 Landau.L. & Lifshitz.E. The clasical theory of fields. Plenum, New York, 1975.

51 Hill.T. Endogenous plant growth substances. Studies in Biology #40, Camelot Press, Southampton, Great Britain, 1980.

52 Bassett.C. Biomedical implications of pulsing electromagnetic fields. Surgical Rounds, Vol 22, January, 1983.

53 Bassett.C. Pulsing electromagnetic fields: a new method to modify cell behaviour in calcified and non calcified tissues. Calcified Tissue International. Vol 34, pp1-8. 1982.

54 Ting. I. Plant Physiology. Pub. by Addison & Wesley 1982.

55 Salisbury.F. & Ross.C. Plant Physiology. Wadsworth Publishing Co. California, 1978.

56 Jaggard.D. et al. Cellular effects: Millimeter waves and Raman Spectra - Report of a panel discussion. Proceedings of the I.E.E.E. Vol 68, No 1, Jan. 1980.

57 Joines.W. Frequency-dependent absorption of electromagnetic energy in biological tissue. I.E.E.E. Transactions on Biomedical Engineering. Vol BME-31, No 1. Jan. 1984.

58 Wei.L-X., Goodman.R., & Henderson.A. Changes in levels of c-myc and histone H2B following exposure of cells to low-frequency sinusoidal electromagnetic fields: evidence for a window effect. Bioelectromagnetics, Vol 11, pp269-272. 1990.

59 Litovitz.T., Montrose.C., Goodman.R., & Elson.E. Amplitude windows and transiently augmented transcription from exposure to electromagnetic fields. Bioelectromagnetics. Vol 11, pp297-312. 1990.

60 Joines.W., Blackma.C., & Hollis.M. Broadening of the RF power density window for calcium-ion efflux from brain tissue. I.E.E.E.E. Transactions on Biomedical Engineering. Vol BME-28, No 8, Aug. 1981.

7

Thesis Summary

CONTENTS

7.1 Brief Resume of Conclusions

7.1.1 Analysis of a typical magnetic biostimulator

The Magnafield Multi-Rhythm Model 990 is a typical example of the type of magnetic biostimulator being sold at the time of writing. The unit consists of a small portable mains powered console with a detachable applicator pad. The applicator pad consists of an involute spiral of varnished copper wire in a foam padded, vinyl pouch.

The unit offers a range of "pulse" frequencies from 0.5 Hz to 18 Hz, although some other units boast 35 Hz as the high frequency range. Many units supply a small vinyl covered bar magnet which the operator holds in close proximity to the applicator when the unit is operating, to verify the alternating magnetic field is present. Some would consider this the most useful function of the machine. The vibrating vinyl magnet is somehow, friendly, and reassuring.

What the manufacturers do not state in their literature, and in the case of Magnafield categorically deny, is that the stimulator simply pulses 50 Hz mains power through the coil. As such the unit is using the very frequency which is currently in debate as a potential cause of cancer. It is easy to understand why manufacturers may not want this information to 'leak out'.

In general, the claims made by manufacturers are extravagant, if not directly misleading. Claims are made for: stimulation of the immune system; lymph drainage; recharging of membrane potential; stabilising (rebalancing) effects and

an increase in metabolic rate. Each of these effects is correlated to a specific frequency or range of frequencies. Unfortunately the manufacturers provide little tangible scientific evidence to back up these claims. They do supply numerous testimonials and letters from satisfied patients and practitioners. Unfortunately there are no reputable double-blind scientific trials to support these claims. Indeed some claims seem to have no known physiological basis, e.g. 'rebalancing' of membrane potential. Membrane potential is a dynamic, changing phenomenon with very varied limits, based upon many factors and environmental conditions. There is no absolute 'balance' point.

The Magnafield 990 was clinically trialed to evaluate the effectiveness of its vasoconstricting and vasodilating ability, just two of the claims made in the operator's manual. The unit did not produce vasodilation as evidenced by no increase in surface skin temperature. The unit was then tested for its vasoconstricting capability. While there were minor reductions in skin temperature of the subject, these look remarkably like the first, vasodilation, trial. What this tends to indicate is that the unit had no effect at all. What the results show is the normal gradual cooling effect observed in all healthy subjects when they progress from an active, moving state, to a recumbent, passive state.

The Magnafield 990 costs in the region of $2000 and produces a 50 Hz mains based signal pulsed at various frequencies. There are dubious clinical effects which are not substantiated in the literature, or by the experiments conducted here. The unit is not even capable of producing the waveforms which have been scientifically established as beneficial in the treatment of recalcitrant fractures.

7.1.2 The magnetic biostimulator as a clinical tool

The magnetic biostimulator designed during this project was trialed on four human subjects in an attempt to determine its practical effectiveness as a clinical tool. It was resolved to use the 22 Hz component of the modulated waveform used by Warnke to produce vasodilation. The trials were not intended to be an exhaustive scientific study to determine the validity of the Warnke protocol, rather it provided a useful practical experiment to clinically test the system.

The biostimulator performed flawlessly throughout the trials, maintaining the stated frequency, waveforms and magnitudes. The biostimulator exists as a series of discrete modules which are connected together in the required configuration with adaptor leads. This was the only significant feature which could be improved upon. The ultimate clinical instrument would be better presented in a single console with switches rather than hook-up leads connecting the various functional blocks. This may provide some slight difficulties as the unit needs to house a transconductance amplifier of considerable current capacity. This necessitates adequate heat sinking which will get to hot to touch under normal operating conditions. The unit needs to be adequately shielded to protect the operator or client from burns.

7.1.3 Pulsed magnetic fields as a human vasodilator

With respect to the effectiveness of the trials, the first point is that it is inappropriate to compare the results between subjects. Each subject is a unique individual who responds to exogenous stimuli in different ways. For this reason it is reasonable to conclude that there is no one waveform which will have an identical effect in all subjects, just as no two people respond identically to the

same pharmaceutical drug. This is true also for the treatment of recalcitrant fractures by pulsed magnetic fields which have approximately an 80% success rate.

In comparing results within subjects, an interesting effect of some vasodilation does occur in one normal subject, BF, while a reduction in the cooling effect is exhibited in the other normal subject, SL. It could be argued that some vasodilation has occurred, or at least some increase in vasodilating effect on the metarteriolar nerves of the feet of normal subjects.

The Raynaud's subjects present a different case entirely. These two subjects exhibit far greater variation of skin temperature even in the control trials. A slight reduction in cooling of the feet is exhibited by SW, and a positive warming of the feet for MH. While these results are in no way conclusive evidence for a vasodilating effect of the pulsed 22 Hz magnetic field, it does offer sufficient enticement for further studies to be carried out.

7.1.4 The magnetic biostimulator as a scientific research tool

The biomagnetic simulator was trialed as a scientific research tool in experiments to determine the effect of various alternating magnetic fields on the DNA of the broad bean, *Vicia faba*.. The stimulator performed to specification throughout the trials. The same comment made in Section 1.4.2 is applicable here: the stimulator would be better presented in a single console where switches replace the hook-up leads. In addition, a more powerful amplifier would be an advantage, as experiments involving biological material often require large

application coil systems. Larger coils obviously necessitate considerably more power, proportional to the increase in volume.

The timebase generator with its digitally controlled frequency provides for extreme accuracy and repeatability of experimental variables. The accuracy is of the order of +/- 12 ppm, more than adequate for low frequency experiments. The ability to provide specific pulse modulation with variable duty cycle and period, in addition to random pulse modulation make this a very versatile unit.

The use of the transconductance amplifier is unique in the literature surveyed so far. No other research protocols detail such a system which offers control over the current, and hence magnetic flux in the coil. For studies which are concerned about the rate of change of magnetic flux, a transconductance amplifier is the only practical option, yet no other papers to date pay such attention to detail. Most are satisfied to produce the required frequency at the coil, with little or no mention of waveform with respect to time. A few papers show the voltage at the coil, but none provides information of the actual current, hence magnetic waveform produced. This may account for the very varied results in the literature and the general lack of reproducible results.

7.1.5 The effect of magnetic fields on chromosomes

The cytogenetics of *Vicia faba* provided a most suitable platform of experiments on which to test the scientific capabilities of the magnetic biostimulator. The results were rewarding, considering the protracted period of time taken to both carry out the experiments, and analyse the results.

With respect to the production of chromosome and chromatid breaks, no significant differences were detected in comparison to the control group. Some 650,000 nuclei were examined for metaphase chromosomes to find a total of 28 chromosome breaks and 59 chromatid breaks. There was no statistical correlation between the number of breaks and the treatment. It may therefore be concluded that the various magnetic field treatments had no effect on the net number of chromosome and chromatid breaks determined at metaphase. This is a useful finding in that no detrimental effects of the magnetic fields were found. With respect to magnetically induced damage, a negative result is a pleasing outcome.

7.1.6 The effect of magnetic fields on the cell cycle

The effects of the various magnetic fields on the cell cycle, including mitosis, revealed significant differences between the control and test groups at the $\alpha = 0.05$ level. This is strong evidence for an effect of alternating magnetic fields. The results, while extremely interesting, are not conclusive however. The fact that the root populations sampled came form different plants for different experiments means that the possibility of considerable differences due to the specific plant cannot be ruled out. The root population samples within each treatment group did come from the same plant however, ruling out one possible confounding variable. It is not possible however to both expose an intact growing plant to a magnetic field, and use part of it for a control with no field. Therefore it may not ever be possible to totally rule out this confounding variable. Significantly large numbers of plants should reduce the uncertainty however to acceptable limits.

The results show that there is only a 5% chance that we are incorrect in rejecting the null hypothesis (Ho) that there is no significant difference between the various root tip populations of the control and the various experimental conditions. The use of Dennett's T test is particularly good at reducing type I experimentwise errors and was used for this purpose. While these results indicate that the root tip populations of the various treatments are significantly different from the control, they do not prove that the magnetic fields are the cause. The results may be due to the different plants used in each condition, or perhaps some confounding effect of circadian rhythms or other growth conditions which were not adequately controlled by the experimental procedure. The task of determining the real reason for the difference between the root tips is a matter for future research.

If the differences between treatments are due to the exogenous magnetic stimulation, some interesting questions need to be asked. How precisely do magnetic fields interact with dynamic, living tissues ? Perhaps the differential effect on the various stages of mitosis gives us a clue to the mechanism. As totally different biochemical and cytological processes are occurring in each stage, the specific stages most affected should provide some clue as to the mechanism of interaction.

There are certainly significant differences between the groups at mitosis, with an apparent increase in time taken to pass through this stage which apparently correlates to frequency in a positive way: as frequency increases, so does the time taken to pass through the stage. Metaphase is dominated by the alignment of condensed chromosomes across the mid-plane of the cell, and attachment of them at the centromere to the spindle fibres. If exogenous magnetic fields do

affect the tertiary structure of proteins, this could conceivably affect the bonding process, hence metaphase would take longer to complete. Only further research could confirm this hypothesis.

Appendices

APPENDICES

APPENDIX ONE

Manufacturers of Magnetic Field Therapy Units.

Elec-Western Medical Devices Ltd.
120 - 58th Avenue, S.E.
2nd floor.
Calgary, Alberta. T2H ON7
Canada.

Emmet Glen Pty. Ltd.
47 Sebring Street.
Holland Park West.
Brisbane, Queensland, 4121.
Australia.

Haines.
R.D. 3, Pukeroro,
Hamilton,
New Zealand.
Ph. 07 827-5772

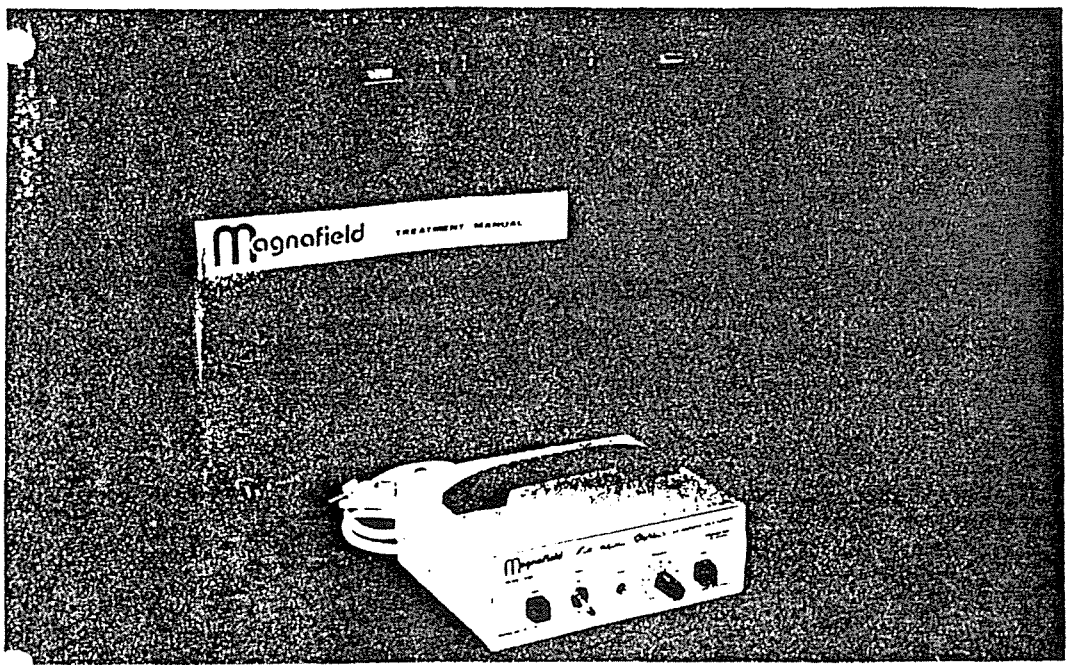
J.S. Inc. (Sell "Soltek")
Distributors of Electronic Devices.
1942 Addison Avenue East.
Twin Falls, Idaho 83301.
United States of America.

Magnacare Pty. Ltd. (Sell "Magnafield 990")
44 Kintore Avenue,
Prospect, South Australia. 5082.

APPENDIX TWO

Magnafield 990 Multi Rhythm Home Therapy Unit

Magnacare Pty. Ltd. Publicity material and Operators Guide



Magnafield Model 990 — Small Clinic or Home unit

★ *SIMPLY — A better way to reduce PAIN and improve HEALING*

Induced A.C. Magnetic Fields of specifically pulsed frequencies have proved therapeutically beneficial or useful in the treatment of the following: -

- PAIN — from migraine headaches, to troubled feet
- ARTHRITIS — all kinds, pain relief (temporary), and increased mobility
- RHEUMATISM — all kinds, also muscle spasms and cramps
- SPORTS INJURIES — Acute and chronic, also pre-sport and pre-exercise toning
- BACK ACHE — Sciatica, spondylitis, osteoporosis, neck and shoulder complaints
- CIRCULATORY PROBLEMS AND DISEASES — oedema, varicose conditions
- NEUROLOGICAL SYSTEM — disorders, diabetic neuropathy, paresthesia
- LEG ULCERS — burns, lesions, bed sores, all inflammation, P.I.D.
- TENDONITIS — R.S.I., bursitis, ankle joint swelling, ligament and muscle strains
- SKIN PROBLEMS — acne, scalp and hair growth problems, etc.
- TRAUMA RELIEF — asthma and bronchial spasm, rehabilitation

The model 990 is designed for very small Clinics,
and for use in the Home - under the direction of your therapist.

A valuable adjunct to any therapy

Theory of operation

PAIN?! INJURY?! DISCOMFORT?!

Almost every person experiences pain as a necessary part of life. Without pain, we would not be alerted to injury, burning, infection, hunger, etc. However, many people suffer pain beyond the useful or protective warning function, and the relief of such discomfort remains the most common demand on medical assistance.

Many methods are employed to reduce or block pain, including drugs that often could have unpleasant side effects or long term complications.

Among the non-drug modalities available, inductions of magnetic fields into the body areas have been used sucessfully by the medical profession over 50 years in Europe. Several makes and models have come onto the market — however, now using new technology, one of the most advanced and effective units currently available has been developed in Australia. It is the MAGNAFIELD MULTI-RHYTHM, model 990.

THE HOW & WHY OF MAGNETIC FIELD THERAPY

- The inducing of magnetic fields into body tissues causes bio-electric current activity which assists in normalising the electron flow in the body. This helps in **relief of pain** as well as the **repair of damaged tissues.**
- It has been established that **'pulsed alternating'** magnetic fields are many times **more beneficial** to the body than the direct magnetic fields normally used until recently.
- Resulting from recent research this modality has been developed even further with specifically shaped **multi-pulse bio-waveforms of alternating magnetic fields** that are complimentary to normal body rhythms and frequencies.
- These modified magnetic fields can influence the ion exchange at the cellular level, and have a powerful effect on **blood activity and flow.**
- At extremely low frequencies of 0.5-9Hz (pulses per second), there is a mild **constrictive effect** in blood flow, but **lymph drainage** is increased, particularly at 2-4Hz. This is the most important for treatment of acute stages of injury to reduce swelling and inflamation. This model 990 is one of the first to produce a multi-pulsing output of a 0.5Hz, which is now recognised to have additional beneficial results.
- However, at frequencies between 12-20Hz, the major blood vessels and the nutrient-carrying capillaries are **dilated**, allowing **increased blood flow** which is necessary for the secondary stages of healing and treatment of chronic conditions.
- These very-low-frequency and low power, biologically-compatible magnetic inductions subject nerve and cell tissues to changing electrical potentials, which **induce an analgesic effect and promote the healing of damaged tissues.**
- The effects of these magnetic fields include also
  - Increased vascularisation
  - Improvement of tissue oxygenation
  - Stimulus to lymphatic system
  - **Polarising of cell membranes**
  - Ionic transfer, calcium, potassium and sodium balance is restored
  - Balancing of energy flow in the body
- Particular effects have been noted at certain specific frequencies, including: muscle repair and toning; oedema reduction; immune system improvement (increased phagocyte cell production).
- Testimonials and extracts are available from many medical and scientific research papers on the bio-magnetic field effects on the body, and also from a wide range of professionals and beneficiaries of this therapy.
- The MAGNAFIELD MULTI-RHYTHM model 990 comes as a complete kit with one specially developed applicator pad enclosing electromagnetic wire wound coil and curled cord for convenience. A magnetic field tester and operator manual are included, and a carry case is provided for protection and mobility if necessary.
- This system features very low voltage with extra low frequency pulses for maximum safety.
  - **Treatment time** is 20 minutes. Buzzer indicates end of treatment.
  - **Frequency** in pulses per second (Hz) is the most important factor. These settings and recommendations are set out in the Operator Manual with each machine.
  - Clothes, plaster casts, bandages and metal implants etc., do not affect the magnetic field. It is non-invasive and there is no trauma for the patient or need of constant monitoring by the therapist. A mild thermal effect is usually the only sensation felt. The applicator does not have to be against skin as the magnetic field projects for several centimetres through the body.
- **Contra indication:** while there are no known side effects, commonsense suggests caution or avoidance of electro-therapy in the following cases:— pregnancy, viral infections, tuberculosis, mycoses, cancer, and pacemakers. Please consult an experienced electro-medical therapist, or the manufacturers if in doubt.
- Available to suit mains supply of 120, 220 or 240 volts A.C. 50/60 Hz.

MAGNACARE PTY. LTD. 44 Kintore Avenue, Prospect, South Australia 5082 or P.O. Box 20
Phone: (08) 344 6609, Fax: (08) 344 5965 Prospect S.A. 5082
[Overseas dial prefix (618) instead of (08)] Australia

DISTRIBUTOR

AGENT:

DESIGNED AND MANUFACTURED IN AUSTRALIA

Operators Guide

INDEX

The information in this booklet is based on experience in many clinics throughout the world.

While every care has been taken in obtaining, compiling and presenting the enclosed information, the manufacturers, distributors and agents cannot accept responsibility for individual problems or complaints arising from the use of this MAGNAFIELD Multi-Rhythm therapy.

The manufacturers reserve the right to update, revise or change this manual at any time.

MAGNAFIELD Multi-Rhythm Therapy Units are manufactured in Australia by

MAGNACARE PTY. LTD.
44 Kintore Avenue, Prospect, South Australia 5082
Phone (08) 344 6809 Fax (08) 344 5985
Overseas dial prefix (618) instead of (08)

MAGNETIC FIELD THERAPY

Magnetic Field therapy has a historical connection with China, as healing properties were claimed for lodestone (magnetic iron ore) in some early Chinese medical literature. However, it was not until 1530 that Paracelsus reported on the first treatments by magnetic fields at the University of Basle.

Thanks to work by such people as Franklin, Lavoisier, Galvani and Volta electo-magnetism and the birth of magneto-medicine began to materialise. Their investigations laid the foundations for the piezo-electric effect of bones and connective tissue as well as the bio-physical explanations for nerve and muscle function. Michael Faraday's work on electro-magnetic induction and in determining the magnetic properties of oxygen are factors that must be considered when searching for a rational explanation for the observed effects of magnetic field therapy.

Every day of our lives we are subjected to the effects of our magnetic energy. A force field of magnetic energy is created whenever a charged particle (e.g. an electron) moves. Even as you read this you are creating magnetic fields and likewise are subject to their effects. When the millions of electrons and other charged particles in your body move a multi-potential effect is exerted on you as a biological system.

BACKGROUND LEADING TO DEVELOPMENT OF MAGNAFIELD THERAPY
(Multi-Pulse Alternating Current Magnetic Field Induction Therapy)

Numerous different types of electro-medical therapy units developed over recent years are beneficial in the treatment of injuries and painful conditions of the body.

Among these, Magnetic Field Therapy has been used by the medical profession for over fifty years.

Recently much research worldwide into the effects of magnetic fields on body tissue has resulted in a better understanding of how and why magnetism is so important and essential for the balanced operation of every cell in the body. the magnetic emanations from the Earth also have a subtle but continuous effect on all living matter.

Several types, makes and models of electro-magnetic field therapy have become available over recent years, most of which have utilised the 'direct' or 'uni-directional' mode of electro-magnetism.

However, now with new technology available combined with extensive research and testing, the multi-pulsing A.C. MAGNAFIELD is one of the most advanced and effective units available.

A BASIC EXPLANATION OF THE EFFECTS OF MAGNAFIELD MAGNETIC THERAPY

The principle of this therapy is that extremely low frequency, low power, multiple-form pulses of alternating electro-magnetic forces in conjunction with a group of specific harmonics are introduced into the body subjecting nerve and other cell tissues to changing electrical potentials inducing an analgesic effect and promoting the healing of damaged tissues.

Many other associated effects have been noted from several thousand medical and scientific papers on this therapy including:-

* ionic transfer - calcium, potassium and sodium balance is restored
* protein synthesis is increased and they are absorbed more rapidly
* inflammation, swelling and pain are reduced
* cell regeneration and healing is improved
* blood flow may be increased (especially 12-15Hz)
* phagocyte cell production and auto-immune system may be stimulated (especially 0.5-4Hz)
* endorphin, enkephalin, seratonin and nor-adrenaline release for inhibition of pain
* increased production of neuro-transmitters
* synchronisation of dominant brain waves

Magnafield 990 Specifications

MAGNAFIELD MULTI-RHYTHM - Model 990

The 990 has been produced as a 'small clinic' or 'home' unit. It is designed to complement its stable mates the MAGNAFIELD Multi-Pulse 989 (Mark 3) and the MAGNAFIELD Multi-Rhythm 991.

Although the 990 has fewer features than the other models it is important to note that the nature and quality of the field produced is identical.

SPECIFICATIONS

MAGNAFIELD Multi-Rhythm A.C. Magnetic Therapy Unit

Model: 990

Input: 120/240 Volts A.C.; 60/50Hz; 0.5 Amperes

Output: 20 Volts r.m.s. A.C.; 1.5 Amperes

Frequency: 0.5 - 18Hz

Timer: 20 minutes

The unit is supplied with
- Applicator Pad
- Magnet Tester
- Operator Manual
- Carry Case

Note: The timer function may alter due to heavy current machinery or appliances turning on or off nearby.
Avoid close proximity to sensitive electronic equipment and recorded tapes or discs.

Instructions for use

INSTRUCTIONS FOR USE - MAGNAFIELD Multi-Rhythm 990

PLEASE READ CAREFULLY

Insert plug into normal outlet and turn wall switch on.

Set applicator pad adjacent to the area requiring treatment.
The pad may be placed in any position facing the area to be treated.
The field effect is equal on both sides of the pad.

The magnetic field is projected in an enclosed field and will treat right through the area of the body adjacent to the pad. Exact positioning is not critical with this type of applicator pad.

When ready;
1. Turn power switch ON, LED indicator will come on.
2. Set pulse frequency to required setting.
3. Press START button. Treatment is now operating.
   Treatment LED will flash indicating pulse rate.

The treatment will stop automatically. The end of cycle buzzer will sound and the LED will stop flashing.

The treatment can be stopped prematurely by pressing the STOP button.

At the start of each treatment the Magnet Tester should be held briefly over the applicator pad to check that it is operating.

Some warming of the body area being treated is common due to increased blood activity at the site. This is normally beneficial although not an intended part of the function of the magnetic field therapy.

If the unit is not going to be used soon after, then at the end of the treatment, turn power switch off. The LED indicator will go off.

NOTE: Do not bend the applicator pad.
The coil is specially designed to produce a uniform, penetrating field.

Treatment regimes

FREQUENCY - the key to effective treatment

MAGNACARE Pty. Ltd., in conjunction with its Research and Development group RANDA MEDICAL PRODUCTS, has for some time recognised the specific nature of the electro-magnetic signals natural to the normal operation of the body. These are characterised by low frequency, low power, A.C. signals with a group of associated, specific harmonics.

The MAGNAFIELD Therapy Units (all models) incorporate these properties in their induced fields.

Several years ago these products were the first in the world to reflect the added benefits gained from signals at 1-5Hz.

Now MAGNAFIELD has another world first.
Pulse frequency of 0.5Hz is a unique feature of this MAGNAFIELD. This lower frequency of 0.5Hz offers even greater benefits of low frequency electro-magnetic induction.

In the treatment of any condition with the MAGNAFIELD the frequency selection is the most important decision to be made. In general the following responses occur.

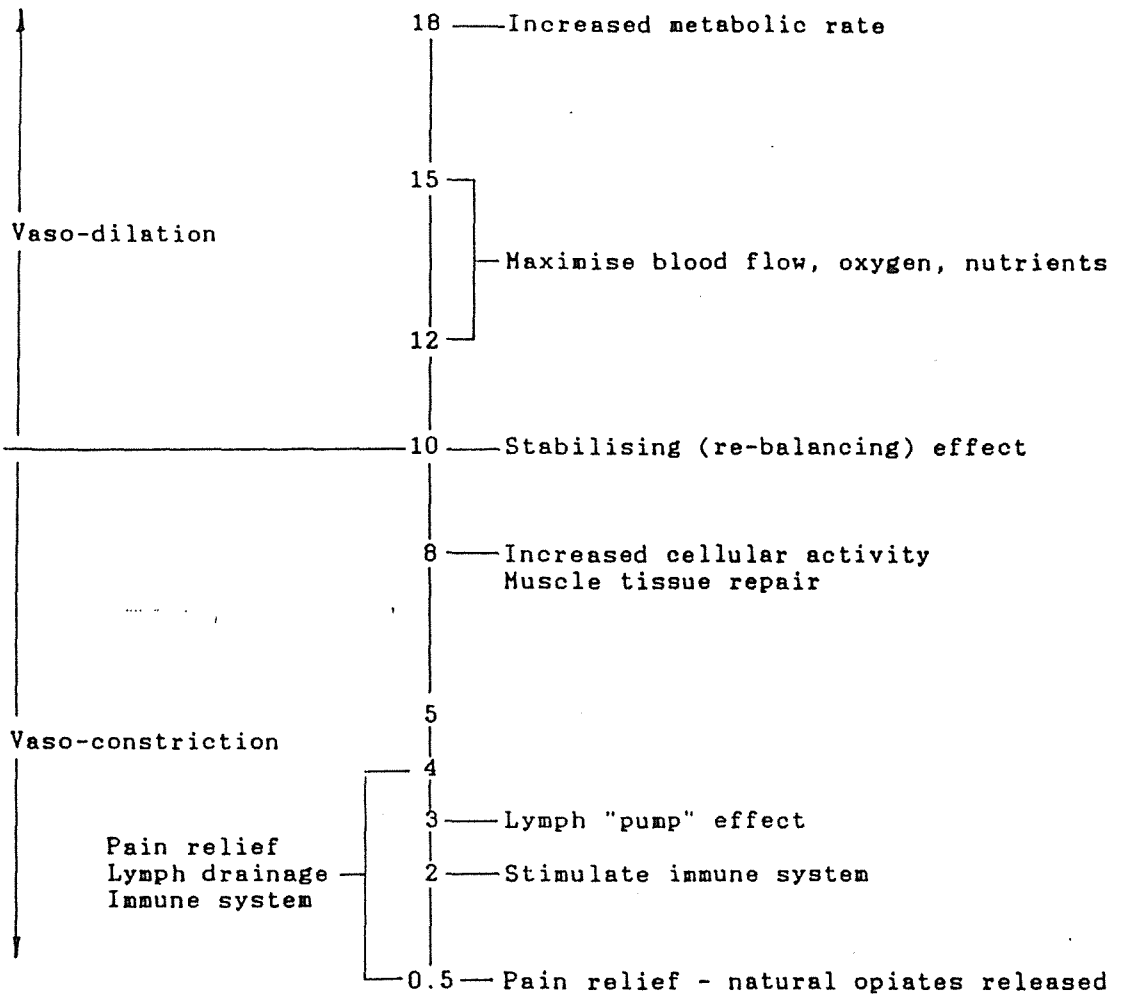
< 10Hz	Vaso-constrictive effect Increased lymph drainage Blood flow, inflammation, metabolism reduced
10Hz	Stabilising (re-balancing) effect
> 10Hz	Vaso-dilatory effect Blood flow increased Cellular activity, metabolism increased

At all frequencies there is a promotion of tissue repair.

Some specific frequencies to remember.

0.5-4Hz	Sedating effect Reduced blood flow Lymph drainage Pain relief Stimulation of the immune system
2Hz	Increased phagocyte and T-killer cell production Stimulation of immune system
3Hz	Lymph drainage
8Hz	Stimulation of A.T.P. (energy) production Recharging of cell membrane potential Muscle tissue repair
10Hz	Stabilising (re-balancing) effect
12-15Hz	Increased blood flow More oxygen, nutrients available to tissue
18Hz	Increased metabolic rate

The following schemata summarizes this information.



Trouble shooter's guide & suggested treatments for specific conditions

TROUBLE SHOOTER'S GUIDE

What frequency should I use for my problem?
When addressing specific symptoms the following guidelines may be applied.

Anxiety (Stress)		0.5-4Hz
Bleeding (e.g. open wound, blood nose)		0.5-4Hz
Healing - promotion of	(within each treatment)	4,8,12Hz
Infection		2Hz
Pain - general		0.5-4Hz
Relaxant - general muscle tension		0.5-4Hz
Swelling (e.g. sprained ankle)		3Hz

SUGGESTED TREATMENTS FOR SOME SPECIFIC CONDITIONS

All treatments should be approximately 20 minutes for maximum benefit. **
A break of at least 30 minutes is needed between treatments.
Benefit is increased with repeated treatments.

It is important to note that where a range of frequencies is given some experimentation may be necessary. Metabolic rate can vary from one individual to another and a number of contributing factors may be present.
In general, begin at low frequencies and work to higher frequencies.

NOTE: IF MORE THAN ONE CONDITION IS TO BE TREATED AND DIFFERENT FREQUENCIES ARE RECOMMENDED, SELECT THE LOWER FREQUENCY.
IF IN DOUBT CONSULT YOUR MAGNAFIELD AGENT.

		Frequency(Hz)
ARTHRITIS (including osteo and rheumatoid)		10
ARTHROSIS (knee and hip)		4
ASTHMA, BRONCHITIS	initially	3
	last few minutes of treatment	8
BACK PAIN (non-specific)	initially	0.5-4
after a few treatments	Acute	10
	Chronic	15
BURNS	initially	3
after a few treatments as with Treatment of Sports Injuries		

BURSITIS (knee)		5
DENTAL - pain relief		0.5-4
FEET (pains and circulatory problems)	initially	0.5-4
	after a few treatments	12-15
FRACTURES - see Treatment of Sports Injuries		
HARMONY - out of (jet lag, hangover, etc)		10
INSOMNIA		0.5-3
LUPUS	initially	3
	after a few treatments	8
MIGRAINE		0.5,3
MUSCLE INFLAMMATION (Myositis) - including strains, tears, ligament injury - see Treatment of Sports Injuries		
MUSCLE SPASM	injury related	0.5-4
	neural related	8
NEURALGIA	initially	0.5-4
	after a few treatments	12-15
NECK PAIN (non-specific or tension)	initially	0.5-4
	after a few treatments	12-15
OSTEOPOROSIS	initially	0.5-4
	after a few treatments	15-18
PSORIASIS (and other skin problems)	initially	3
	after a few treatments	8
RHEUMATIC CONDITIONS (chronic)		10
R.S.I. (repetitive strain injury)	initially	0.5-4
after a few treatments as with Treatment of Sports Injuries		
SCIATICA		3
SINUSITIS		3
SPONDYLITIS		3
TENDONITIS (and/or Tenosivitis)	initially	0.5-4
after a few treatments as with Treatment of Sports Injuries		
ULCERS	initially	0.5-4
	after a few treatments	12-15
	final healing	18

TREATMENT OF SPORTS INJURIES

In the treatment of any injury there are two phases to be considered.

1. Stabilize the traumatized area.
2. Facilitate the healing process.

1. TREATING A NEW INJURY

In treating a new injury the MAGNAFIELD can

a. reduce the blood flow to the site
b. remove waste materials from the site by relaxing the lymph system
c. relieve pain
d. stimulate the immune system.

The injury can be properly treated by following these simple steps.

1. Is there threat of infection?
   a) YES — Treat at 2Hz
   b) NO — Go to Qu. 2

2. Is there swelling?
   a) YES — Treat at 3Hz
   b) NO — Treat anywhere between 2Hz and 4Hz

This setting should be maintained until the injury has stabilized (normally 1 to 2 days).

2. TO PROMOTE HEALING

Once the injury has stabilized accelerated generation of healthy tissue can be achieved with the MAGNAFIELD by using combinations of frequencies.

NOTE: Times are approximate.
Variations of a few minutes will not have any detrimental effect on the treatment.

4 minutes on 4Hz	(assists the removal of waste materials from the tissue being treated)
8 minutes on 8Hz	(conditions and tones the tissue in the area being treated)
8 minutes on 12Hz	(dilates vascular system and pumps oxygen and nutrients into the tissue)

All treatments should be approximately 20 minutes for maximum benefit.
A break of at least 30 minutes is needed between treatments.
Benefit is increased with repeated treatments.

Magnacare PTY. LTD.

MANUFACTURERS OF MAGNAFIELD AND ACU-TREAT THERAPY UNITS

44 KINTORE AVENUE, PROSPECT, SOUTH AUSTRALIA 5082

February, 1991

WHY SUFFER UNNECESSARY PAIN?

Dear Practitioner and Therapist,

As you are well aware, 'PAIN', from whatever cause is the most common reason for patients to present for help or treatment.

Many are turning away from the dependence on analgesic drugs which in long term use can have serious side effects. Among several options available, one method of successful treatment - MAGNETIC FIELD THERAPY - is now gaining acceptance by all branches of medical and health professions in many countries around the world.

This modality has been used by doctors in Europe since 1938. However, it is during the past seven or eight years that medical and scientific research has shown the importance of magnetic fields in the balancing of activity at the cellular level. The analgesic effect and the promotion of healing of damaged tissue make this non-invasive and drug-free treatment worthy of your careful consideration.

A number of electro-therapy machines using magnetic fields have come onto the market in recent years - with mixed response. However, as the result of very intensive research and clinical tests, using the latest technology, we now have an updated system called the 'MAGNAFIELD'. The secret to success of this system is a multi-rhythm alternating current form of therapeutic electro-magnetic fields induced into body areas.

The Magnafield is available to you in two models. The model MF990 has proved to be the most successful unit, is just $1100-00 to you. The new deluxe model MF991 is basically similar to the MF990 but has touch sensitive controls, 2 displays and additional features for those who demand the best, and is being released internationally during March/April 1991 and is only $1300-00. Attractive leasing terms can usually be arranged through your bank or a finance company. When you realise what it can do for your patients and therefore your clinic, the investment is worth while. We all know that every dollar spent on equipment must prove beneficial and worth while. Please realise that there are some cheap copies and versions of magnetic field units about - your patients and your reputation deserve the best!

Please take the time to read the attached brochure and information data, and do not hesitate, either now or after you purchase, to enquire for additional information.

Thanking you in anticipation.

Yours faithfully,
MAGNACARE PTY. LTD.

Bob Grace / Peter Sadler.

APPENDIX THREE STATISTICAL ANALYSIS BY SAS

Key to experimental results.

EXPERIMENT	Numerical Title
Control	0
0 Hz @ 1.0 mT	1
0 Hz @ 5.0 mT	2
50 Hz @ 1.5 mT	3
60 Hz @ 1.5 mT	4
75 Hz @ 1.5 mT	5

BASIC STATISTICS OF VICIA FABA ROOT POPULATIONS *SAS

CONTROL EXPT

N Obs Variable	N	Minimum	Maximum	Mean	Std Dev
6 Prophase	6	52.0000000	93.0000000	67.8333333	17.7247473
Metaphase	6	12.0000000	21.0000000	15.3333333	3.4448028
Anaphase	6	9.0000000	12.0000000	11.1666667	1.1690452
Telophase	6	15.0000000	32.0000000	23.3333333	5.8195074
Interphase	6	1942.00	2347.00	2110.67	138.5462618

DC EXPT

N Obs Variable	N	Minimum	Maximum	Mean	Std Dev
5 Prophase	5	98.0000000	149.0000000	118.6000000	21.0665612
Metaphase	5	29.0000000	41.0000000	4.6000000	4.6151923
Anaphase	5	15.0000000	25.0000000	20.0000000	4.7958315
Telophase	5	29.0000000	42.0000000	33.0000000	5.3385391
Interphase	5	3097.00	3483.00	3280.80	191.1954497

50 Hz EXPT

N Obs Variable	N	Minimum	Maximum	Mean	Std Dev
7 Prophase	7	116.0000000	152.0000000	133.1428571	15.1374653
Metaphase	7	28.0000000	44.0000000	37.2857143	6.3695705
Anaphase	7	19.0000000	45.0000000	30.0000000	7.9162281
Telophse	7	36.0000000	62.0000000	50.5714286	9.7784993
Interphase	7	3051.00	3409.00	3255.43	135.4435395

60 Hz EXPT

N Obs Variable	N	Minimum	Maximum	Mean	Std Dev
6 Prophase	6	103.0000000	138.0000000	120.5000000	14.2653426
Metaphase	6	43.0000000	61.0000000	50.0000000	6.2289646
Anaphase	6	24.0000000	39.0000000	32.8333333	6.6758270
Telophse	6	42.0000000	52.0000000	46.3333333	4.5898439
Interphase	6	3412.00	3804.00	3595.17	149.3645429

75 Hz EXPT

N Obs Var iable	N	Minimum	Maximum	Mean	Std Dev
5 Prophase	5	125.0000000	142.0000000	135.4000000	6.8774995
Metaphase	5	46.0000000	68.0000000	57.4000000	8.2340755
Anaphase	5	22.0000000	31.0000000	26.8000000	4.4384682
Telophase	5	31.0000000	37.0000000	34.0000000	2.8284271
Interphase	5	3170.00	4107.00	3647.80	347.6905233

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General Linear Models Procedure
Class Level Information

Class	Levels	Values
EXPT	5	0 2 3 4 5

Number of observations in data set = 29

Dependent Variable: Prophase

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	4	17979.09918	4494.77479	18.20	0.0001
Error	24	5927.59048	246.98294		
Corrected Total	28	23906.68966			

R-Square	C.V.	Root MSE	Prophase Mean
0.752053	13.67812	15.71569	114.896552

Source	DF	Type I SS	Mean Square	F Value	Pr>F
EXPT	4	17979.09918	4494.77479	18.20	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
EXPT	4	7979.09918	4494.77479	18.20	0.0001

Dependent Variable: Metaphase

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	4	5885.665681	1471.416420	41.39	0.0001
Error	24	853.161905	35.548413		
Corrected Total	28	6738.827586			

R-Square	C.V.	Root MSE	Metaphase Mean
0.873396	5.53506	5.962249	38.3793103

Source	DF	Type I SS	Mean Square	F Value	Pr > F
EXPT	4	5885.665681	1471.416420	41.39	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
EXPT	4	5885.665681	1471.416420	41.39	0.0001

Dependent Variable: Anaphase

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1822.567816	455.641954	14.08	0.0001
Error	24	776.466667	32.352778		
Corrected Total	28	2599.034483			

R-Square	C.V.	Root MSE	Anaphase Mean
0.701248	23.29810	5.687950	24.4137931

Source	DF	Type I SS	Mean Square	F Value	Pr > F
EXPT	4	1822.567816	455.641954	14.08	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
EXPT	4	1822.567816	455.641954	14.08	0.0001

Dependent Variable: Telophase

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	3017.756979	754.439245	18.21	0.0001
Error	24	994.380952	41.432540		
Corrected Total	28	4012.137931			

R-Square	C.V.	Root MSE	Telophase Mean
0.752157	16.86247	6.436811	38.1724138

Source	DF	Type I SS	Mean Square	F Value	Pr > F
EXPT	4	3017.756979	754.439245	18.21	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
EXPT	4	3017.756979	754.439245	18.21	0.0001

Dependent Variable: Interphase

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	9069253.209	2267313.302	57.44	0.0001
Error	24	947371.481	39473.812		
Corrected Total	28	10016624.690			

R-Square	C.V.	Root MSE	Interphase Mean
0.905420	6.285564	198.6802	3160.89655

Source	DF	Type I SS	Mean Square	F Value	Pr > F
EXPT	4	9069253.209	2267313.302	57.44	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
EXPT	4	9069253.209	2267313.302	57.44	0.0001

SCHEFFE'S TEST OF VICIA FABA ROOT POPULATIONS * SAS

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General Linear Models Procedure

Scheffe's test for variable: Prophase

NOTE: This test controls the type I experimentwise error rate but generally has a higher type II error rate than Tukey's for all pairwise comparisons.

Alpha= 0.05 Confidence= 0.95 df= 24 MSE= 246.9829

Critical Value of F= 2.77629

Comparisons significant at the 0.05 level are indicated by '***'.

EXPT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
5 - 3	-28.409	2.257	32.923	
5 - 4	-16.813	14.900	46.613	
5 - 2	-16.323	16.800	49.923	
5 - 0	35.854	67.567	99.279	* * *
3 - 5	-32.923	-2.257	28.409	
3 - 4	-16.494	12.643	41.780	
3 - 2	-16.123	14.543	45.209	
3 - 0	36.173	65.310	94.446	* * *
4 - 5	-46.613	-14.900	16.813	
4 - 3	-41.780	-12.643	16.494	
4 - 2	-29.813	1.900	33.613	
4 - 0	22.430	52.667	82.903	* * *
2 - 5	-49.923	-16.800	16.323	
2 - 3	-45.209	-14.543	16.123	
2 - 4	-33.613	-1.900	29.813	
2 - 0	19.054	50.767	82.479	* * *
0 - 5	-99.279	-67.567	-35.854	* * *
0 - 3	-94.446	-65.310	-36.173	* * *
0 - 4	-82.903	-52.667	-22.430	* * *
0 - 2	-82.479	-50.767	-19.054	* * *

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General Linear Models Procedure

Scheffe's test for variable: Metaphase

NOTE: This test controls the type I experimentwise error rate but generally has a higher type II error rate than Tukey's for all pairwise comparisons.

Alpha= 0.05 Confidence= 0.95 df= 24 MSE= 35.54841

Critical Value of F= 2.77629

Comparisons significant at the 0.05 level are indicated by '***'.

EXPT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
5 - 4	-4.631	7.400	19.431	
5 - 3	8.480	20.114	31.748	***
5 - 2	10.234	22.800	35.366	***
5 - 0	30.035	42.067	54.098	***
4 - 5	-19.431	-7.400	4.631	
4 - 3	1.660	12.714	23.768	***
4 - 2	3.369	15.400	27.431	***
4 - 0	23.195	34.667	46.138	***
3 - 5	-31.748	-20.114	-8.480	***
3 - 4	-23.768	-12.714	-1.660	***
3 - 2	-8.948	2.686	14.320	
3 - 0	10.898	21.952	33.006	***
2 - 5	-35.366	-22.800	-10.234	***
2 - 4	-27.431	-15.400	-3.369	***
2 - 3	-14.320	-2.686	8.948	
2 - 0	7.235	19.267	31.298	***
0 - 5	-54.098	-42.067	-30.035	***
0 - 4	-46.138	-34.667	-23.195	***
0 - 3	-33.006	-21.952	-10.898	***
0 - 2	-31.298	-19.267	-7.235	***

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General Linear Models Procedure

Scheffe's test for variable: Anaphase

NOTE: This test controls the type I experimentwise error rate but generally has a higher type II error rate than Tukey's for all pairwise comparisons.

Alpha= 0.05 Confidence= 0.95 df= 24 MSE= 32.35278

Critical Value of F= 2.77629

Comparisons significant at the 0.05 level are indicated by '***'.

EXPT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
4 - 3	-7.712	2.833	13.379	
4 - 5	-5.444	6.033	17.511	
4 - 2	1.356	12.833	24.311	***
4 - 0	10.723	21.667	32.610	***
3 - 4	-13.379	-2.833	7.712	
3 - 5	-7.899	3.200	14.299	
3 - 2	-1.099	10.000	21.099	
3 - 0	8.288	18.833	29.379	***
5 - 4	-17.511	-6.033	5.444	
5 - 3	-14.299	-3.200	7.899	
5 - 2	-5.188	6.800	18.788	
5 - 0	4.156	15.633	27.111	***
2 - 4	-24.311	-12.833	-1.356	***
2 - 3	-21.099	-10.000	1.099	
2 - 5	-18.788	-6.800	5.188	
2 - 0	-2.644	8.833	20.311	
0 - 4	-32.610	-21.667	-10.723	***
0 - 3	-29.379	-18.833	-8.288	***
0 - 5	-27.111	-15.633	-4.156	***
0 - 2	-20.311	-8.833	2.644	

General Linear Models Procedure

Scheffe's test for variable: Telophase

NOTE: This test controls the type I experimentwise error rate but generally has a higher type II error rate than Tukey's for all pairwise comparisons.

Alpha= 0.05 Confidence= 0.95 df= 24 MSE= 41.43254

Critical Value of F= 2.77629

Comparisons significant at the 0.05 level are indicated by '***'.

EXPT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
3 - 4	-7.696	4.238	16.172	
3 - 5	4.011	16.571	29.131	* * *
3 - 2	5.011	17.571	30.131	* * *
3 - 0	15.304	27.238	39.172	* * *
4 - 3	-16.172	-4.238	7.696	
4 - 5	-0.655	12.333	25.322	
4 - 2	0.345	13.333	26.322	* * *
4 - 0	10.616	23.000	35.384	* * *
5 - 3	-29.131	-16.571	-4.011	* * *
5 - 4	-25.322	-12.333	0.655	
5 - 2	-12.566	1.000	14.566	
5 - 0	-2.322	10.667	23.655	
2 - 3	-30.131	-17.571	-5.011	* * *
2 - 4	-26.322	-13.333	-0.345	* * *
2 - 5	-14.566	-1.000	12.566	
2 - 0	-3.322	9.667	22.655	
0 - 3	-39.172	-27.238	-15.304	* * *
0 - 4	-35.384	-23.000	-10.616	* * *
0 - 5	-23.655	-10.667	2.322	
0 - 2	-22.655	-9.667	3.322	

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General Linear Models Procedure

Scheffe's test for variable: Interphase

NOTE: This test controls the type I experimentwise error rate but generally has a higher type II error rate than Tukey's for all pairwise comparisons.

Alpha= 0.05 Confidence= 0.95 df= 24 MSE= 39473.81

Critical Value of F= 2.77629

Comparisons significant at the 0.05 level are indicated by '***'.

EXPT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
5 - 4	-348.3	52.6	453.5	
5 - 2	-51.7	367.0	785.7	
5 - 3	4.7	392.4	780.1	***
5 - 0	1136.2	1537.1	1938.0	***
4 - 5	-453.5	-52.6	348.3	
4 - 2	-86.5	314.4	715.3	
4 - 3	-28.6	339.7	708.1	
4 - 0	1102.2	1484.5	1866.8	***
2 - 5	-785.7	-367.0	51.7	
2 - 4	-715.3	-314.4	86.5	
2 - 3	769.2	1170.1	1571.0	***
3 - 5	-780.1	-392.4	-4.7	***
3 - 4	-708.1	-339.7	28.6	
3 - 2	-413.1	-25.4	362.3	
3 - 0	776.4	1144.8	1513.1	***
0 - 5	-1938.0	-1537.1	-1136.2	***
0 - 4	-1866.8	-1484.5	-1102.2	***
0 - 2	-1571.0	-1170.1	-769.2	***
0 - 3	-1513.1	-1144.8	-776.4	***

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General Linear Models Procedure

Dunnett's T tests for variable: Prophase

NOTE: This tests controls the type I experimentwise error for comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 24 MSE= 246.9829

Critical Value of Dunnett's T= 2.619

Comparisons significant at the 0.05 level are indicated by '***'.

EXPT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
5 - 0	42.647	67.567	92.486	***
3 - 0	42.414	65.310	88.205	***
4 - 0	28.907	52.667	76.426	***
2 - 0	25.847	50.767	75.686	***

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General Linear Models Procedure

Dunnett's T tests for variable: Metaphase

NOTE: This tests controls the type I experimentwise error for comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 24 MSE= 35.54841

Critical Value of Dunnett's T= 2.619

Comparisons significant at the 0.05 level are indicated by '***'.

EXPT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
5 - 0	32.613	42.067	51.521	***
4 - 0	25.653	34.667	43.681	***
3 - 0	13.266	21.952	30.639	***
2 - 0	9.813	19.267	28.721	***

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General Linear Models Procedure

Dunnett's T tests for variable: Anaphase

NOTE: This tests controls the type I experimentwise error for comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 24 MSE= 32.35278

Critical Value of Dunnett's T= 2.619

Comparisons significant at the 0.05 level are indicated by '***'.

EXPT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
4 - 0	13.067	21.667	30.266	***
3 - 0	10.547	18.833	27.120	***
5 - 0	6.614	15.633	24.652	***
2 - 0	-0.186	8.833	17.852	

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General Linear Models Procedure

Dunnett's T tests for variable: Telophase

NOTE: This tests controls the type I experimentwise error for comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 24 MSE= 41.43254

Critical Value of Dunnett's T= 2.619

Comparisons significant at the 0.05 level are indicated by '***'.

EXPT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
3 - 0	17.861	27.238	36.616	***
4 - 0	13.269	23.000	32.731	***
5 - 0	0.460	10.667	20.873	***
2 - 0	-0.540	9.667	19.873	

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General Linear Models Procedure

Dunnett's T tests for variable: Interphase

NOTE: This tests controls the type I experimentwise error for comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 24 MSE= 39473.81

Critical Value of Dunnett's T= 2.619

Comparisons significant at the 0.05 level are indicated by '***'.

EXPT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
5 - 0	1222.1	1537.1	1852.2	***
4 - 0	1184.1	1484.5	1784.9	***
2 - 0	855.1	1170.1	1485.2	***
3 - 0	855.3	1144.8	1434.2	***

APPENDIX FOUR

The papers listed in this appendix are referenced in Table 5.1 of Section 5.2.5 of Chapter Five. They are intended to provide a basic review of the current forms of medical treatment used in the treatment of Raynaud's Disease and Raynaud's Phenomenon. While not exhaustive, they are representative of the general trends in drug based therapies.

These papers are drawn from a computer database search of Index Medicus, conducted at the Palmerston North Hospital by Ms. A. Kitchin at the author's request. Nearly 1000 papers were reviewed which cover approximately the last thirteen years. From this base, 24 papers were tagged as being either very representative of the current status of drug therapy, or as having some other special merit. They are grouped in Table 5.1 by therapy (drug), and referenced by their Index Medicus unique identifier. Their order in this appendix is the same as Table 5.1 for ease of reference.

Papers referenced in Table 5.1

87256034 Gush.R. et al. Acute effects of sublingual nifedipine in patients with Raynaud's. Journal of Cardiovascular Pharmacology. Vol 9, No 5, pp628-631, May 1987.

85171984 Malamet.R. et al. Nifedipine in the treatment of Raynaud's Phenomena. Evidence for inhibition of platelet activation. American Journal of Medicine. Vol 78, No 4, pp602-608, April 1985.

87254085 Wise.R. et al.. Acute effects of nifedipine on digital blood flow i human subjects with Raynaud's phenomena: a double blind placebo controlled trial. Journal of Rheumatology. Vol 14, No 2, pp278-83, April 1987.

87254086 Kellenberg.C. et al. Nifedipine in Raynaud's Phenomena: relationship between immediate, short term and long term effects. Journal of Rheumatology. Vol 14, No 2, 284-90, April 1987.

89152134 Joseph. B. et al. Effects of nifedipine therapy on pulmonary Raynaud's in primary Sjogren's syndrome. Clinical and Experimental Rheumatology. Vol 6, No 4, pp409-410, Oct-Dec. 1988.

89076709 Francis.J. et al. The effect of nisoldipine on whole blood platelet aggregation in patients with Raynaud's Phenomena. British Journal of Clinical Pharmacology. Vol 25, No 6, pp751-754, Jun 1988.

87026447 Waller D. et al. Clinical and rheological effects of nifedipine in Raynaud's Phenomenon. British Journal of Clinical Pharmacology. Vol 22, No 4, pp449-454, Oct. 1986.

89133132 Challenor.V. et al. Vibrotactile sensation and response to nifedipine dose titration in Primary Raynaud's Phenomenon. Angiology. Vol 40, No 2, 00122-128, Feb. 1989.

90005600 Schmidt. J. The clinical effect of felodipine and nifedipine in Raynaud's Phenomenon. European Journal of Clinical Pharmacology. Vol 37, No 2, pp191-192, 1989.

89194528 Rademaker.M. et al. Comparison of intravenous infusions of iloprost and oral nifedipine in treatment of Raynaud's Phenomenon in patients with systemic sclerosis: a double blind randomised study. British Medical Journal. Vol 298, (6673) pp561-564, March 4.

88241213 Yardunian. D. et al. Successful treatment of Raynaud's Syndrome with Iloprost, a chemically stable prostacyclin analogue. British Journal of Rheumatology. Vol 27, No 3, pp220-206, June 1988.

89133131 Codella. O. Controlled comparison of Ketanserin and Nifedipine in Raynaud's Phenomenon. Angiology. Vol 40,No 2, Feb. 1989.

88258206 Arneklo-Nobin. B. et al. Effect of long-term Ketanserin treatment on 5-HT levels, platelet aggregation and peripheral circulation in patients with Raynaud's phenomenon. A double-blind, placebo-controlled cross-ever study. International Angiology. Vol 7, No 1, Jan.-Mar. 1988.

89064895 Marasini.B. Ketanserin treatment and serotonin in patients with primary and secondary Raynaud's phenomenon. European Journal of Clinical Pharmacology. Vol 35,No 4, pp419-421, 1988.

89371790 Coffman, J. et al. Internal study of Ketanserin in Raynaud's Phenomenon. American Journal of Medicine. Vol 87, No 3, pp264-268, Sept. 1989.

89305940 Dormandy. J. The use of selective serotonin S2 receptor antagonist Ketanserin in the treatment of Raynaud's phenomenon. European Journal of Vascular Surgery Vol 2, No 6, 371-375, Dec. 1988.

91265247 Challenor. V. Subjective and objective assessment of Enalapril in Primary Raynaud's Phenomenon. British Journal of Clinical Pharmacology. Vol 31, No4, pp477-480, April 991.

88273377 Janini. S. et al. Enalapril in Raynaud's Phenomenon. Journal of Clinical Pharmacy and Therapeutics. Vol 13, No 2, pp145-150, April 1988.

89234788 Cohen. L. et al. Prostaglandin infusion therapy for intermittent digital ischaemia in a patient with mixed connective tissue disease. Case report and review of the literature. Journal of the American Academy of Dermatology. Vol 20, No 5 pt 2), pp893-893, 1989.

90096079 Langevitz. P. Treatment of refractory ischemic skin ulcers in patients with Raynaud's phenomena with PGE1 infusions. Journal of Rheumatology. Vol 16, No 11, pp1433-1435, Nov. 1989.

89214753 Wollersheim. H. et al. Dose-response study of prazosin in Raynaud's phenomenon: clinical effectiveness versus side effects. Journal of Clinical Pharmacology. Vol 28, No 12, pp1089-1093,Dec. 1988.

89116243 DiGiacomo. R. Fish-oil dietary supplement in patients with Raynaud's Phenomena: a double-blind, controlled, prospective study. American Journal of Medicine. Vol 86, No 2, pp158-164, Feb. 1989.

89076824 Bunker. C. the effects of topically applied hexyl nicotinate lotion on the cutaneous blood flow in patients with Raynaud's Phenomena. British Journal of Dermatology. Vol 119, No 6, pp771-776, Dec. 1988.

89080935 Freedman.R. et al. Nonneural beta-adenergic vasodilating mechanism in temperature feedback. Psychosomatic Medicine. Vol 50, No 4, pp394-401, Jul.-Aug. 1988.

Department of Health
TE TARI ORA

NATIONAL RADIATION LABORATORY

108 Victoria Street, Christchurch, New Zealand
P.O. Box 25099, Christchurch
[redacted]
Phone (03) 366 5059
Fax (03) 366 1156

19 October 1992 25/6

Mr Bruce Rapley
Department of Production Technology
Riddet Building
Massey University
PALMERSTON NORTH

Dear Bruce

Below are the present exposure limits to low frequency magnetic fields recommended by the National Radiation Laboratory:

Exposure characteristics	**magnetic flux density (microtesla) (rms)**
Occupational	
Whole working day	500
Short term (2 hours/day)	5000
Limbs	25000
General public	
Up to 24 hours/day	100
Few hours/day	1000

These limits are the same as those recommended by the International Radiation Protection Association. They were formulated after a careful examination of the health effects data, and are based on established health effects. They include a margin for safety. They can be applied for frequencies up to 3 kHz.

For static magnetic fields:

Exposure characteristics	**magnetic flux density (microtesla) (rms)**
Less than 2 hours/day	20000
2 - 5 hours/day	8000
Unlimited	2000

These limits are those recommended by the British National Radiological Protection Board (*Advice on the protection of workers and members of the public from the possible hazards of electric and magnetic fields with frequencies below 300 GHz*, NRPB, 1986). Again, they are based on established health effects and include considerable margins for safety.

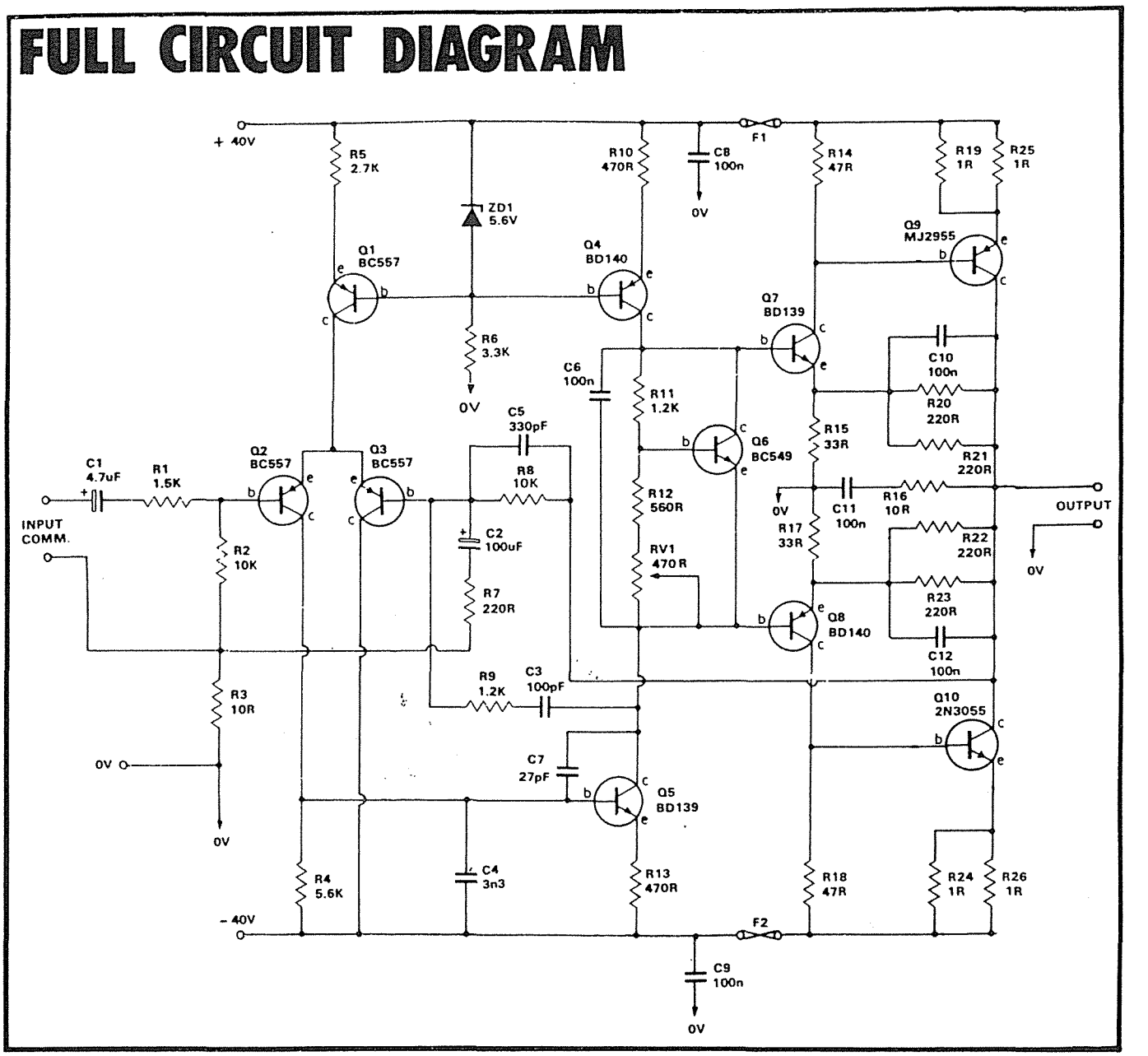
Yours sincerely

Martin Gledhill
for Director

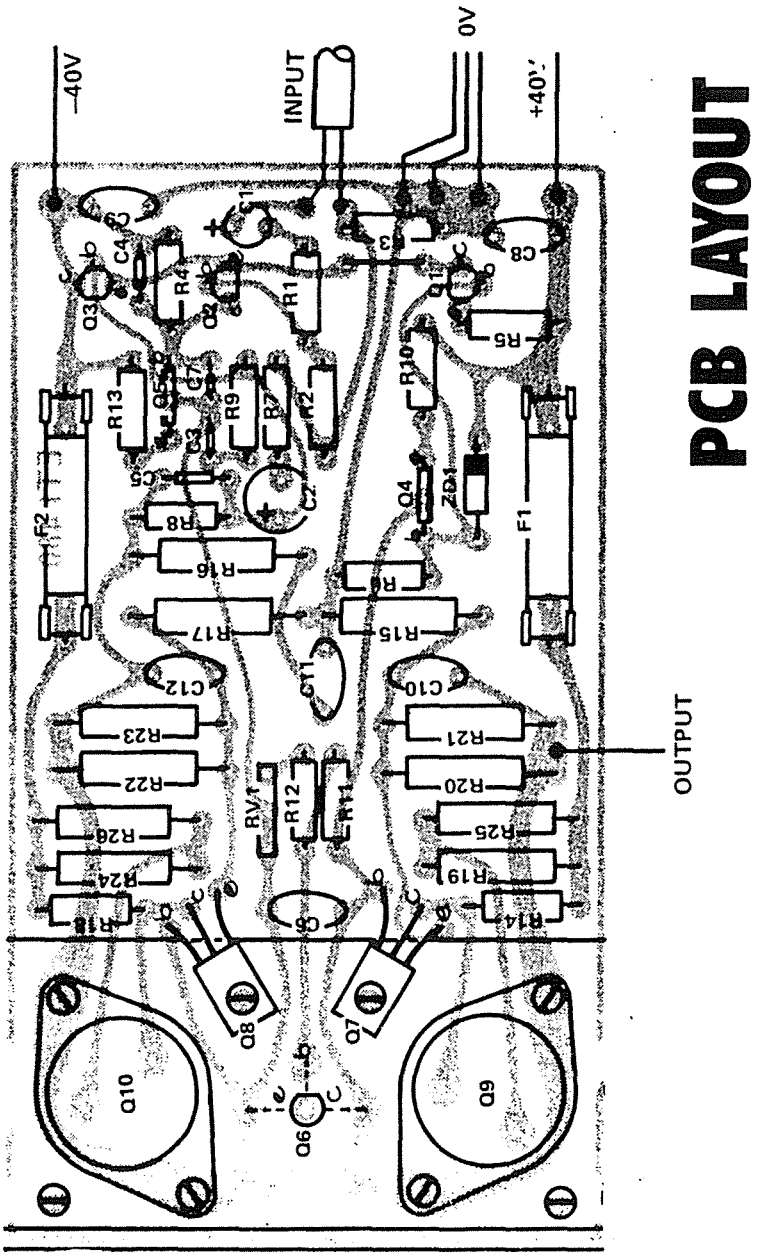
Encl
Health for all by the year 2000

APPENDIX SIX ETI 480 AMPLIFIER

Circuit diagram



Printed circuit layout



Finished printed circuit board