The measurement of small ionic currents in living organisms by means of sensitive magnetometry

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THE MEASUREMENT OF SMALL IONIC CURRENTS IN LIVING ORGANISMS

BY MEANS OF SENSITIVE MAGNETOMETRY

PhD thesis (pertaining to the disciplines of physics and biology)

submitted in August, 1984,

by Rosemary F. Lennard, MBChB (Sheffield, 1976).

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The design of equipment and the experiments described in this thesis form part of the work of the Biomagnetism Group, Physics Discipline, the Open University. Different members of the group contributed different skills at each stage, and it is not possible to demarcate clearly the different contributions of each member. All the experiments described in this thesis were the responsibility of R. F. Lennard. Where significant contributions were made by other individuals, this is acknowledged in the text.

Some early work on ionic currents in legs was described at the Fourth International Workshop on Biomagnetism and is published in the proceedings of that meeting\(^1\). The experiments on legs described in Chapter 5 of this thesis follow on from the experiments described in that paper.

Abstract: Recent research on developing and healing tissues suggests that small quasi-d.c. ionic currents (of magnitude 10–20µA) may play a controlling role in the initiation and organisation of growing tissues, but the difficulties of measuring such small currents has led to confusing results. Sensitive magnetometry provides a method of demonstrating, and, to some extent, locating such currents.

A novel SQUID magnetometer system has been built and used to investigate the magnetic fields around the uninjured human leg, and the developing chick embryo in ovo. Analysis of the magnetic fields around the human leg reveals the presence of macroscopic current loops (of magnitude up to 12µA) within the leg. These currents are broadly similar in all subjects, and show day-to-day reproducibility in individuals. They change predictably with time of muscle relaxation, and revert to the original signal on muscular exertion. These currents are of significance when considering the therapeutic use of injected current for the healing of non-union in bone.

The magnetic fields around eggs are detectable from day two of incubation, and increase in magnitude until day four or five, by which time there is a magnetic field pattern with a null line over or near the centre of the egg. After day five the magnetic field is reduced in size and of more complex pattern. The magnetic fields disappear if the egg is cooled, and reappear on rewarmling. Mathematical analysis suggests that the magnetic fields have a source deeper within the egg than the embryo itself, probably the extra-embryonic membranes.
ACKNOWLEDGEMENTS

In writing this thesis, I have been very aware of how much I have learned in the last three years, and how much I have enjoyed the research I have done. I am indebted to the members of the Physics Discipline, who gave me this opportunity and welcomed a non-physicist into their midst, and to the Open University for funding me during this time.

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of axial leg currents, and for his interest in the project.

Finally, I must thank the secretarial staff of the Physics Discipline for teaching me how to use the word processor, and accommodating me in their office while I finished the thesis.
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INTRODUCTION

'Wonderful as are the laws and phenomena of electricity when made evident to us in inorganic or dead matter, their interest can bear scarcely any comparison with that which attaches to the same force when connected with the nervous system and with life.'

Michael Faraday, 1839

This thesis describes a method of measuring small direct currents (of size a few microamps) in living organisms, and the application of this method to measure currents within the uninjured human leg and the developing chick embryo.

The majority of measurements of electrical phenomena in biological systems have been concerned with rapidly changing potential differences, and the subject of slowly changing or steady currents has only recently received more than occasional attention. For this reason, it seems necessary to justify the value of the measurement of small steady currents, as well as describing the methods and results of the research project.

With this in mind, I shall start this thesis (in Chapter 1) with a discussion of the possible relevance of such measurements, the difficulties in obtaining reliable results, and a review of key experiments in the field which
demonstrate the value of the data obtained. Chapter 2 describes how a SQUID magnetometer can be used to measure the small magnetic fields produced by electrical currents within living organisms, and Chapter 3 explains the novel way in which it is used in our laboratory to measure steady magnetic fields from direct currents. Chapter 4 discusses the principles by which the source currents of a magnetic field can be calculated. Finally, Chapters 5 and 6 and 7 describe the results obtained from such measurement of magnetic fields - around the human leg and chick eggs.
Chapter 1: Stable electrical fields and currents in biological systems - a brief review

Ever since Galvani showed at the end of the eighteenth century that a frog's leg twitched when a capacitor was discharged through the base of its spine (Geddes and Hoff, 1971), biologists have taken a keen interest in the electrical phenomena associated with living organisms. In practice, this has meant that they have used electrodes to measure the potential difference across some part of the organism. The results have been many and various but can be divided into two main groups - potential differences that change rapidly over seconds or milliseconds (action potentials) and those that are stable over minutes, hours, or days. In this thesis I shall be concerned entirely with stable or slowly-changing fields and currents.

Stable or slowly changing electric fields are found across membranes, whole cells and tissues. The best known example is the potential difference across cell membranes, the inside of a cell being electronegative to the outside by about 40mV in the average cell, and 80mV in excitable cells. Trans-membrane potential differences are maintained by active ion pumps in the membrane, which produce an imbalance in sodium and potassium concentrations in the intracellular and extracellular fluid.

The potential difference across organs and epithelial sheets
has been less well investigated. In some epithelia it is clearly the result of an asymmetrical distribution of ion pumps (often sodium pumps), which transport the appropriate ion across the epithelium. This has been well demonstrated in amphibia (Ussing and Thorn, 1973) and in the mammalian colon (Guyton, 1981), where the potential difference is apparently the consequence of a primary need for sodium absorption. In other systems, such as human skin, it is less clear why there should be a transepithelial potential difference, though there have been suggestions that it affects the movement of cells during wound healing (Barker, Jaffe and Vanable, 1982).

The stable potential differences I have just described are found in intact tissues, but their measurement may be confused by the potential differences created when tissues are damaged and the electronegative cell contents leak out into the extracellular space. In the literature, these tend to be called 'injury potentials' or 'injury currents' but their characteristics are not well defined. It is commonly assumed that they do not last more than about twelve hours after the injury has occurred. They are, of course, an important consideration when looking at potential differences around healing tissue using invasive measurement techniques, since experimentally induced cellular damage will alter the electrical fields in the region under investigation.
1.1 The importance of slowly changing electrical fields

Slowly changing potential differences have been considered far less than rapidly changing potential differences, partly because the use of electrodes favours the study of the latter, and partly because no important function had been ascribed to the former. However, over the last twenty years, some biologists (see below) have revived Lund's assertion, in the first half of this century (Lund, 1947), that slowly changing fields and currents across cells and tissues may play a controlling part in the growth, development and regeneration of tissues and organs.

This is an important suggestion. The way in which cellular proliferation and growth is organised to produce complex organisms is still not fully understood despite many years of research. It is not certain how and why an asymmetrical organism grows from an apparently non-polar ovum, developing pattern out of no pattern; we are not sure why epithelial cells migrate into a skin wound in the first stage of healing, nor why salamanders can regenerate an amputated limb, when no mammal does so; and no-one has with certainty identified the stimulus that causes osteocytes to appear and produce callus at the site of a bone fracture.

The answer to these questions would throw light on such emotive subjects as foetal malformations, cancer, and failure to heal, and might open up the field of organ regeneration in
humans. It is not, therefore, surprising that over the last ten years there has been a rapid increase in research aimed at finding a link between stable electrical fields and cell division and organisation. Unfortunately much of this research has been inconclusive, and the few examples of good studies have been done in such diverse disciplines as plant growth, embryology and clinical medicine, measuring such differing parameters as potential differences through tissues, potential differences along the surfaces of organs, and the volume currents that flow in their surrounding medium. This has made the information difficult to assimilate and compare.

In the following pages I will describe experiments which examine the association between electrical fields and growth, in a variety of biological systems. In doing so I will choose preferentially experiments which seem to me to be least invasive or unphysiological, or to have had a considerable influence on further experiments or clinical medicine. It is by no means an exhaustive review, though I have tried to include some experiments from each main line of research. My aim is to show that, although some results are confusing, there is evidence that small direct currents are associated with, and may influence, growth and regeneration, and it is therefore worth looking for better methods of investigating such currents, so as to obtain unequivocal results on direct current patterns.
1.2 Techniques used in the investigation of small steady electrical fields or currents in biological systems, and their limitations

There have been four main approaches to the study of steady bioelectric fields and currents. None of these techniques is problem-free, and the difficulties associated with each technique go some way to explain the confusions that abound in this area of electrophysiology.

(a) Direct measurement of the voltages within or across the system, with reference to an indifferent electrode.

The measurement of slowly-changing potential differences of only a few millivolts is not easy: metal electrodes have large tip potentials and show considerable base-line drift due to polarisation; salt solution electrodes may contaminate cells with the ions of their solution; tissue components may clog the tips of micropipettes; all point electrodes must cause local injury resulting in leakage of intracellular fluid into the extracellular space, with a concomitant alteration of local potential differences.

(b) Measurement of the potential gradients along the surface of a system. This is done by measuring the potential difference between two electrodes placed on the surface of an organ or organism.
In surface potential measurements the electrodes contact the surface via a contact solution that is usually different from the normal physiological environment, and recordings are made of the voltage between these two surface points. In aquatic organisms, this voltage is maximised by maximising the surface resistance, usually by drying the surface; in organisms with dry surfaces, the tissue must be wetted at the electrode contact. The resistance along the skin surface, and the hydration under the electrodes, varies with different experimental conditions, so that qualitative rather than quantitative results are often obtained. These inherent problems in technique probably serve to explain some of the very variable results gained from such experiments.

(c) Measurement of the currents driven through the surrounding medium by a biological system.

This method has the advantage of minimising the disturbance to an organism, providing it exists normally in a conducting medium. Jaffe has investigated external currents using a platinum black electrode, which vibrates through a distance of 10µm at a few hundred Hz (Jaffe and Nucitelli, 1974). The sinusoidal voltage differences, between this electrode and an indifferent electrode, are measured in the external conducting fluid. When combined with a measurement of resistivity of the medium, they yield the current density at this point, and, by moving the oscillating electrode, a current density map can be produced. Because these
measurements are non-invasive they form a large proportion of
the experiments reviewed in this chapter. However, the
measured volume currents can only be used to deduce the ionic
currents within fairly simple organisms or tissues, and their
use has tended to be restricted to developing ova.

(d) Perturbation of the normal field followed by
investigation of the effects on the system.

These experiments, which seek to alter rather than measure
electrical controls of development, have been many and
various. They make use of such techniques as voltage
clamping, current injection, changing electrical and magnetic
fields, and pulsed electromagnetic radiation. For instance,
in the field of bone growth, direct current, changing
magnetic fields and the insertion of teflon electret have all
been shown to stimulate callus formation. Unfortunately,
these results are difficult to interpret since little is
known about the electrical fields present in normally growing
bone, or in healing fractures. In general, these types of
perturbatory experiments have proved confusing because of
this lack of knowledge of the normal electrophysiology of the
systems concerned.

1.3 Experiments on single-celled organisms

Jaffe and his colleagues have used their extracellular
vibrating electrode, described above, to measure extra-
Figure 7.1. The pattern of pulses of current around a growing Pelvetia embryo (at the two cell stage). After Jaffe, 1979.
cellular currents around single cells (a review article by Jaffe and Nucitelli in 1977 describes all the experiments in this subsection except where other references are given).

They have investigated the current patterns around non-polar plant cells, which, at germination, develop a protuberance - one of the first signs of asymmetrical growth.

In Pelvetia and in lily-pollen grains, a relatively steady transcellular current begins a few hours before germination. Growth appears at or near to the point of current entry (Figure 1.1). The current density is calculated to be between 1 and 5μA/cm². In these experiments, current can be seen to both precede and predict growth.

It is possible to initiate growth from a particular point of the Fucus egg by shining unilateral light on it. In one experiment, Nucitelli reports having measured current entering from the illuminated side. He then reversed the illumination. The original current gradually died away as a new region of current entry was established on the newly illuminated side. Outgrowth eventually occurred towards the second light source, i.e at the second region of current entry. However, this experiment does not prove the causal association of current and growth since they may both be unrelated results of the unilateral light source.

The vibrating probe has also been used to investigate currents around animal ova prior to the first cell cleavage.
Figure 1.2. The arrangement of an *Acetabularia* segment in Novak and Bentrup's experiment (see text). The two cut ends of the stalk segment are in electrically insulated compartments. The potential difference between the fluid in these two end compartments reflects the difference in transmembrane potential difference at the two ends of the stalk segment.
In *Oryzias* fish eggs a current of 1-5μA/cm² enters the animal pole and leaves the vegetal pole after fertilisation. In frog eggs (*Xenopus*), and those of the sea-urchin (*Strongelocentrotus*), currents of 0.2-0.5μA/cm² enter the prospective cleavage furrow 10 mins before cleavage: after cleavage begins, the current reverses for about ten minutes. Although interesting, these results show little evidence of causality. It is not surprising that there are changes in membrane permeability just before cell division - cleavage must have a locally disruptive effect on the cell membrane. However, they have stimulated work on the ions responsible for these currents, and this may throw light on the control mechanisms of cell cleavage.

Novak and Bentrup (1972) have carried out a different type of experiment on the fields associated with cap regeneration in the enucleated stalk segment of *Acetabularia acetabulum*, a giant unicellular algae. They insulated the two cut ends of the stalk segment, and measured the voltage difference between the extracellular medium around these cut ends (Figure 1.2). They found the cell membrane at one end to be hyperpolarised by 5-15mV, about 15 hours prior to tip elongation (the first sign of cap regeneration). This hyperpolarisation predicted the end at which cap regeneration would occur. If the hyperpolarisation was reversed by voltage clamping, the cap regenerated at the artificially hyperpolarised end.
Although this experiment can be criticised for the unphysiological electrical insulation used, it is unusual in showing an electrical phenomena preceding and apparently controlling growth. Like other experiments showing a clear effect these results have stimulated further work on the same system. Goodwin et al (1979) have shown that the membrane hyperpolarisation is associated with tip elongation but not with cap and whorl formation.

1.4 Experiments on epithelial sheets

Many mature epithelia maintain a potential difference across themselves (Ussing and Thorn, 1973, quoted by Jaffe and Nuccitelli, 1977). One of the most extensively studied epithelium is human skin, a stratified squamous epithelium turning over once in about 21 days. Initial experiments were done purely on surface potential gradients, using saline electrodes on the skin. These measurements were performed principally by psychologists interested in the way that the surface voltages change with anxiety. The results were confusingly variable. Some of this variability can be explained by the effects on the surface potential gradients of skin hydration, dermal blood flow, number of hair follicles, and the number and activity of sweat glands.

Measurements across the skin (trans-epithelial), using surface electrodes referenced to a sub-epithelial electrode, have given more reproducible results (Barker, Jaffe and
Figure 1.3. The transcutaneous potential difference at the edge of a full thickness skin wound on the heel of a guinea pig. After Barker, Jaffe and Vanable (1982).
Variable y (1982). The subepithelial space is reported to be practically equipotential (±1mV) over the entire body, so that one sub-epidermal reference electrode can be used as the reference for all the surface electrodes. Barker et al. confirm that the transepithelial potential difference in guinea-pigs is related to hairiness, and varies from 30 to 100mV, inside positive. Similar measurements on humans reveal similar transepithelial voltages, with the highest voltages in non-hairy areas. Barker et al. have also measured the potential gradients at the edges of full thickness skin wounds of guinea-pig palmar skin, and report voltage gradients of 100-200mV/mm within 0.3 mm of the wound edges (see Figure 1.3). They calculate that 1μA flows out of each millimetre of the wound edge, and suggest that the electric field may be the signal guiding the migration of epithelial cells during wound healing. Although the existence of potential differences and currents has been demonstrated, their influence on cell division or growth is unproven, but, once again, these well-documented results pave the way for further research. In particular this area would seem suitable for therapeutic trials of the use of injected current in non-healing skin wounds.

Embryological epithelia also show potential differences between their two surfaces, though they tend to be smaller than in the adult - about 3-15mV (Jaffe and Nucitelli, 1977). Jaffe and Stern (1979) have measured the electrical field around the growing chick embryo in vitro. At about 24 hours
Figure 1.4. Cross-section through the chick embryo at the pre-streak stage. The epiblast and hypoblast are well formed, and are separated by an intra-embryonic space.

Figure 1.5. The pattern of current flow in the early chick embryo (seen from above). After Borgens, Vanable, and Jaffe. (1979).
of incubation the embryo consists of two flat epithelial sheets separated by a narrow intra-embryonic space (Figure 1.4) The upper sheet (the epiblast) contains a 1-2mm long groove (the primitive streak), through which epiblast cells migrate to form mesodermal tissue (tissue between the epiblast and hypoblast).

Jaffe and Stern found that steady currents of approximately 100μA/cm² leave the groove and return elsewhere into the upper sheet with current densities of 10-20μA/cm² (Figure 1.5).

Stern has recently studied the chick embryo epiblast at an earlier stage, just before primitive streak formation (Stern, 1982). The epiblast usually has a potential difference of 15mV across the sheet. The cells rest on a basal lamina on the positive or internal side. Stern has shown, by autoradiographic labelling of sodium pumps, that these pumps are initially more densely distributed on the internally facing cell membrane, throughout the zona pellucida. Just before primitive streak formation, sodium pumps become more numerous on the outer facing cell membranes in the region where the primitive streak will form. This would explain the flow of current out of the primitive streak and back into the rest of the epiblast. This is a clear demonstration of the redistribution of membrane pumps and the flow of current prior to developmental change, and it would seem likely that this redistribution of ionic flow is important in the
Figure 1.6. The pattern of current flow around the stump of the newt forelimb, twenty hours after amputation. After Borgens, Vanable, and Jaffe (1979).
formation of the primitive streak.

1.5 Currents through regenerating vertebrate limbs

It has long been a mystery why some amphibians, such as newts, can regenerate amputated limbs completely, whereas others, such as frogs, cannot. In 1941 Monroy showed that, in the newt, the skin around the stump of an amputated limb was negative to the skin around the shoulder. Borgens et al. (1977) used Jaffe's vibrating electrode to show that, in newts in normal pond water, currents of 30-100μA/cm² flow out of the stump of an amputated forelimb and back into the proximal skin (Figure 1.6). Similar measurements on frogs showed no such potential difference after limb amputation. In response to the voltage measurements described, Smith (1967 and 1974) artificially induced current flows of about 0.1μA out of the stumps of amputated frog forelimbs. In most frogs partial regeneration occurred and, in a few, almost complete regeneration of the forelimb and digits occurred. This work has been broadly confirmed by Borgens et al. (1977b), who tried to exclude the electrolytic effects by using a saline wick cathode. This sequence of experiments provide a good example of experiments involving passive measurement only, which then inspired experiments in which the normal system was perturbed. These latter experiments strongly suggest a causal relationship between the flow of current and limb regeneration.
Becker (1972) tried a similar experiment designed to induce regeneration in the stumps of rat forelimbs. He used a silver-platinum bimetallic rod to withdraw currents that he calculated to be 3-6nA. He described formation of new bone, muscle and nerve in several cases, including the formation of a complete supernumerary humerus. Regeneration of skin, and soft tissues was unknown in mammals at this time and these reports have been greeted with some scepticism. However, the possibility of such regeneration in mammals now seems more likely, following clinical reports that children, under the age of ten, can regenerate a finger tip, when at least half of the distal phalanx has been amputated (Illingworth 1974 and Douglas 1972). For regeneration to take place the wound must remain unsutured. Illingworth and Barker (1980) have used a modified vibrating electrode to show that, in such cases, currents of 10-15μA/cm² flowed from the amputated surface. This is obviously a fruitful area for further research.

1.6 Bone and Fracture healing

The research connecting bone growth and regeneration with electrical phenomena has followed a different path from that previously described, largely because of its therapeutic relevance to the healing of fractures in humans. Bone is one of the best healing tissues in the mammal, healing by new tissue formation rather than by fibrosis, so that the repair is afterwards indistinguishable from old bone. However, in
Figure 1.7. The potential difference caused by the mechanical stressing of a long bone.

Figure 1.8. Friedenberg, Dyer, and Brighton's measurement of the potential difference along growing and fractured bone. Voltages were measured using skin electrodes, with the reading at the proximal epiphysis of the unfractured bone as the arbitrary zero.
some cases, new bone forms in the fracture site slowly, or not at all. Instead, the bone at the fracture ends becomes dense, and the gap is bridged only by fibrous tissue. This condition is known as non-union. Occasionally, a false joint is formed, complete with synovium and synovial fluid. Non-union is classically treated by bone grafts and mechanical fixation. Each procedure cures about 80% of non-unions, but a small proportion are resistant to all treatment. If the non-union is in a long limb bone, amputation of the limb may be necessary.

Electricity was used in the nineteenth century to treat non-unifying fractures (Hartshorne, 1841, Lente, 1850), but so were many other esoteric techniques, and the technique did not become popular. Interest in electrically induced osteogenesis was re-awakened in 1953 when Yasuda demonstrated the appearance of new bone formation in the vicinity of the cathode when a current of a few microamps was applied continuously for three weeks to a rabbit femur. He also described (Fukada and Yasuda, 1957) stress generated potentials in bone, showing that, if a bone was bent slightly, then the side under mechanical compression became electronegative and the side under tension became electropositive (Figure 1.7). Many workers since have confirmed this charge separation in bone, though its origins remain uncertain. Friedenberg and Brighton (1966) reported yet another kind of electrical potential in bone, this time in the non stressed but growing bone. Areas
of active growth or repair were said to be electronegative by 2-15mV when compared with other less actively growing areas (Figure 1.8). Lokietek et al. have since questioned the source of these potential differences, suggesting they come from damaged muscles rather than the bone (Lokietek, Pawluk and Bassett, 1974).

Within a few years of these findings, electrical stimuli were (and are now) being used to treat non-union. In 1971, Friedenberg et al. reported the successful healing of a long established non-union, after treatment with direct current. The currents used were of magnitude 10-20μA and were supplied though stainless steel electrodes, the cathode being implanted in the fracture site and the anode placed on the skin some distance away. This work led to a full clinical trial of the method (Brighton et al., 1981), which proved successful in 80% of non-unions, many of which had already undergone bone grafting and internal fixation without apparent effect. It was established that currents of below 5μA fail to stimulate osteogenesis and that cellular necrosis occurs at the fracture site if currents above 20μA are used. If a silver cathode rather than a stainless steel cathode is used, optimum bone growth occurs in the 0.1-1.1μA range.

Attempts to show an effect of direct current on fresh fractures have been less conclusive. Direct current delivered to a fresh fracture site in the rabbit fibula for 18 days produces a callus that has twice the maximum resistance to
bending that control callus exhibits (Friedenberg et al., 1971). At 4 weeks, however, the experimental and control fracures have similar resistance to bending. It should be remembered that bone is one of the most efficiently healing tissues in the body. It would not be surprising if current stimulation could not improve upon the normal process of fracture healing, but did stimulate repair when the normal process is impaired.

It is not clear whether the important stimulus in this method of treating non-union is the steady electrical current or field, or the chemical changes occurring at the cathode. It has been shown (Brighton et al., 1975) that, at these current levels, oxygen is consumed at the cathode and hydroxyl radicals are formed, thus causing a rise in the pH. In vitro studies have shown that bone growth is greatest in low (5%) oxygen tensions. The biological effect of increased pH on osteogenesis has not been studied in detail, but in vivo micropuncture sampling of fluid around epiphyseal plates shows pH values of 7.7±0.05, and it may be that an alkaline environment is favourable to calcium ion deposition. Against this evidence for a purely electrolytic effect are experiments (e.g. Inoue et al., 1977) showing that bone callus is formed in the vicinity of an implanted electret film consisting of polarised teflon. The polarisation of the teflon strip was not permanent but leaked away over a period of a few days with a current of the order of picoamps. Control animals implanted with non-polarised teflon film
showed no callus formation. It is difficult to see how electrolytic effects could be significant in this case.

Direct current is not the only method being used to treat non-union. In 1974, Bassett reported on the use of pulsed electromagnetic fields (PEMF) for this condition. He applied a pair of Helmholtz coils medially and laterally to a dog hind limb with the osteotomised fibula centred between the coils. A pulsed, alternating current applied to the coils produced a time varying magnetic field. At the end of 28 days, the fibula was excised and mechanically tested to determine stiffness. Results indicated that the osteotomised fibula subjected to the varying magnetic field was significantly stiffer than control osteotomised fibulae. Bassett and his colleagues went on to treat human non-union by pulsed electromagnetic fields, with a success rate of 80% (Basset et al., 1979). However, preliminary reports (on twenty subjects) from a double-blind trial, comparing active coils with dummy coils in the treatment of non-union, show no advantage of pulsed electromagnetic stimulation over adequate fixation and rest (Barker et al., 1984).

In vitro studies suggest that PEMF does have some effects on metabolic processes in growing tissues, such as bone and cartilage. Different frequencies of stimulation have been shown to affect levels of cAMP production, calcium ion uptake and DNA activity (Barker and Lunt, 1983). However, many of these effects are marginal, and the use of different
waveforms, frequencies, and strengths of signal make results difficult to compare. The mechanism of PEMF induced regeneration is uncertain, though some effect on the charged proteins in the cell or on the cell membrane is generally assumed.

In earlier sections of this review I described how, in several different biological systems, initial reliable measurements of potential differences or currents paved the way for further experiments that began to elucidate their role in the processes of cell division and organisation. In the field of fracture healing no such reliable early measurements exist. There is as yet no clear evidence of any natural current flow around healing fractures. This is not surprising as the measurement of potential differences is made difficult by the small voltages involved, and by the inherent problem that microelectrodes must always cause additional tissue damage. The wealth of diverse experiments, in which the system is perturbed with various results, have produced confusion rather than systematic knowledge of the system. This confusion is likely to continue until we know whether there is current flow around fractures, and, if so, its magnitude and distribution. This fundamental information is needed before we can say why the injection of current into the fracture site of non-unions should have a therapeutic effect.

It was this problem that first stimulated us to look for non-
invasive methods of investigating ionic currents. Jaffe's vibrating electrode had produced reliable results for simple, aquatic organisms but its applications are limited. An equivalent non-invasive technique is needed to produce reliable measurement of ionic currents in large, complex, non-aquatic animals and plants, both to confirm or disprove the presence of currents in systems already under investigation, and to look for their presence in developing organisms or healing tissue that have not yet been investigated. In the next chapters, I shall describe such a non-invasive method of investigating small currents, and show how its use has revealed the presence of hitherto undetected electrical currents in the human leg and in the developing chick embryo.
Chapter 2: SQUIDS and the measurement of biomagnetic fields

In the previous chapter I have described the need for non-invasive current measurements in large and non-aquatic organisms. Theoretically, one way that this can be done is by measuring the magnetic fields outside the organism. It is then possible to map back from the fields to the currents that produce the fields. There are two tasks here which may produce problems - the measurement of such small magnetic fields, and the difficulties in calculating currents from the magnetic field data. In this chapter I shall deal with the measurement of small magnetic fields. The modelling of current sources from this data will be discussed in Chapter 4.

The magnetic fields involved are in the region of $10^{-12}$ Tesla (the field at a point 5 cm from an infinite line current of 1 microamp). This is below the sensitivity of conventional magnetometers but within the range of SQUID (Superconducting Quantum Interference Device) magnetometers. These have already been used extensively to measure varying magnetic fields of frequency 1-100 Hz, arising from currents in the brain and heart (see review by Williamson and Kaufman, 1980).

Figure 2.1 shows the levels of some of the magnetic fields produced by biological organisms, and the levels of
Figure 2.1. The sensitivities of various types of magnetometer, compared with the magnetic fields produced by biological sources. After Romani, Williamson, and Kaufman (1982).
sensitivity of various magnetometers.

It is clear that any measurement of steady magnetic fields around regenerating systems will need a superconducting magnetometer. I shall start the next section by giving a simplified explanation of how such a magnetometer works and then go on to describe the appearance and day to day operation of such a magnetometer. (For reviews of SQUID magnetometers and their applications, see Gallop and Petley, 1976, and Swithenby, 1980.)

2.1 How a SQUID works

A SQUID magnetometer is a device for converting the magnetic flux through a detection coil (\( \Phi \)) into a voltage output, such that

\[
\Delta V_{\text{out}} \propto \Delta \Phi_{\text{detection coil}}
\]

The SQUID itself consists of a superconducting ring. Superconducting rings have the property of quantising the magnetic flux through them, one flux quantum being equal to \( \hbar/2e \), where \( \hbar \) is Planck's constant and \( e \) is the charge on an electron. The flux through the ring is therefore given by the equation

\[
\Phi = n\hbar/2e \quad \text{where } n \text{ is an integer}
\]

* since the SQUID responds to change in flux, it has no quantifiable base-line.
Figure 2.2. The change in the screening current in a superconducting ring with one weak link as the external flux is increased.
If the flux imposed on a superconducting ring is increased then a screening current \((I_s)\) will flow in the ring so as to maintain flux quantisation.

\[ \Phi_{\text{ring}} = \Phi_{\text{external}} - LI_s \]

where \(L\) is the inductance of the ring.

Included in the ring is a so-called weak link, which usually consists of a point junction or a thin layer of insulating material. Josephson showed that, when two superconductors are separated by a thin insulator, electrons can tunnel through the insulator so that a zero voltage current passes from one superconductor to the other. At a critical maximum current \((I_c)\) the junction ceases to be superconducting and a finite voltage appears across it. When the flux through a ring containing a weak link is gradually increased, the current \((I_s)\) increases up to the critical current. At this point the weak link momentarily becomes normally conducting and, with appropriate circuit design, a single flux quantum enters the ring. The link then reverts to the superconducting state since the ring no longer needs to support a high shielding current.

The relationship between an increasing external flux and the shielding current is shown in Figure 2.2. If this changing SQUID current can be monitored then the change in external flux through the SQUID can be deduced.
Figure 2.3. The relationship between the external ambient flux through the SQUID ($\Phi_{\text{ext}}$) and the total flux ($\Phi_i$) through the SQUID from the ambient flux and the flux produced by the screening current.

Figure 2.4. The resonant circuit in r.f. SQUIDS.
The relationship between the externally imposed flux through the ring and the resultant total flux through the ring (from the externally imposed flux and the screening current) is complicated. It is shown in graphical form in Figure 2.3.

2.2 Monitoring the SQUID behaviour

In radiofrequency (rf) SQUIDS the SQUID is monitored by measuring the rf voltage across an rf resonant circuit inductively coupled to the SQUID (Figure 2.4). An rf oscillating current \( I_{rf} \), driven through this circuit, induces an oscillating current in the SQUID. The amplitude of the rf voltage across the inductor \( V_{rf} \) is monitored. If the rf current is gradually increased from zero then the voltage, \( V_{rf} \), also increases linearly, with the oscillation covering an increasingly large fraction of the path FB, shown in Figure 2.3. This initial increase of \( V_{rf} \) with an increase in \( I_{rf} \) is shown in Figure 2.5. When the peak current in the SQUID just reaches \( I_C \) then a flux quantum will enter the SQUID and it will execute the flux path ABCDEA during one cycle of the radiofrequency input. Energy is dissipated when the weak link momentarily ceases to be superconducting, and this energy is absorbed from the resonant circuit, reducing its level of oscillation very slightly.

During the next rf cycle the level of the rf voltage will be too low to take the SQUID around the hysteresis loop, but it will build up over a number of cycles until the circulating
Figure 2.5. The variation in r.f. voltage with r.f. current, with and without an ambient 'dc' flux through the SQUID.

Figure 2.6. The variation in r.f. voltage with increasing ambient 'd.c.' flux at a fixed r.f. bias current.
current is again equal to the critical current of the junction. Once again, a flux quantum enters the ring and energy is lost from the inductor circuit. This process repeats, and, because of the energy losses, the average amplitude of the rf voltage across the resonant circuit remains constant even when the rf current is increasing — hence the plateau shown in Figure 2.5. Increasing the rf current does increase the frequency with which the hysteresis loop is executed by the SQUID. When the rf current is large enough to drive the SQUID around the hysteresis loop once in each rf cycle, then $V_{rf}$ will start to increase again.

A 'dc' magnetic field imposed on the SQUID results in a circulating current in the SQUID superimposed on the rf circulating current. The rf current oscillations are no longer centred on A (Figure 2.3) and the critical current is thus reached at a lower rf excitation current. It follows that the first plateau (see Figure 2.5) is reached at a lower value of $V_{rf}$.

If the rf voltage response to an increasing 'dc' external flux is plotted for a fixed amplitude of rf current ($I_{bias}$, marked on Figure 2.5), a characteristic triangular pattern is obtained with a periodicity of one cycle per flux quantum, $h/2e$ (Figure 2.6).

Although this response could of itself provide the basis of a magnetometer, in normal experimental systems a negative feed-
Figure 2.7. The input circuit of an r.f. SQUID, consisting of a pick-up coil connected in a superconducting circuit to a signal coil which is inductively coupled to the SQUID, such that the flux change at the SQUID, $\Delta \Phi_{in}$, is given by

$$\Delta \Phi_{in} \propto \frac{M_{in} \Delta \Phi_p}{(L_p + L_{in})}$$

where $M_{in}$ is the mutual inductance between the signal coil and the SQUID, $L_{in}$ is the inductance of the signal coil, $L_p$ is the inductance of the pick-up coil, and $\Delta \Phi_p$ is the change in flux at the pick-up coil.
back circuit is also introduced. This locks the flux at one of the extremes of rf voltage shown in Figure 2.6. A deviation from this voltage is sensed by imposing a square wave modulation field on the SQUID (at approximately 100 kHz) and using a phase sensitive detector to monitor changes in the SQUID signal at this frequency. The negative feed back circuit then applies a field to the SQUID that just cancels the external 'dc' flux. The feed-back current and hence the voltage across a resistor in the feed-back system is then proportional to the external flux. This flux-locking arrangement increases the range in which the change in external flux is accurately proportional to the voltage output. The characteristics of this feedback system are important, since its time constants impose a limitation on the maximum rate of change of applied flux that can be tracked by the system. This is known as the maximum slew-rate. If the maximum slew-rate is exceeded, then the flux through the SQUID will not be kept constant, the critical current may be reached, and another flux quantum will enter the SQUID. The voltage output will then jump to a new level.

2.3 Noise

Intrinsic noise in the system comes from the rf voltage amplifier (Figure 2.7), dissipative effects in the rf circuit, and intrinsic noise of the SQUID itself. The intrinsic noise is mainly due to thermal fluctuations, which produce variations in the exact current at which the weak
Figure 2.8. Three pick-up coils and the field terms to which they are sensitive. After Swthenby, 1980.

Figure 2.9. The relative response of the pick-up coils shown in Figure 2.8 to an axial magnetic dipole: straight line = magnetometer; A = first-order gradiometer; B = second-order gradiometer. After Swthenby, 1980.
link becomes normally conducting.

In practice the intrinsic noise of the SQUID magnetometer is much less than the ambient magnetic noise, and experiments are limited by the ability to discriminate between external noise and signal. This is a significant problem. For instance, the magnetic field produced by mains transmission lines one kilometre from the SQUID is typically $10^{-8} \text{T}$; the magnetic field due to a steel frame chair 10 metres from the SQUID would be approximately $10^{-7} \text{T}$. One way of decreasing noise is by magnetic screening, using multiple layers of highly conducting and high permeability material. However, shielded rooms are expensive, extremely heavy, experimentally inconvenient, and usually claustrophobic. An alternative approach is to minimise the signal to noise ratio by using appropriately designed superconducting detection coils to sense the magnetic flux. The detection coils form part of a closed circuit, which is then inductively coupled to the SQUID (Figure 2.7).

The simplest detection coil consists of a few turns of wire, as close to the 300K region as cryogenic constraints will allow (Figure 2.8a). In this case the system acts as a simple magnetometer, with no discrimination between distant or nearby sources of magnetic field. Two such coils, separated vertically and wound in the opposite sense (Figure 2.8b), will discriminate against distant sources in favour of nearby sources. The more uniform the field the smaller the net flux
Figure 2.10. The noise levels of different SQUID systems in different locations. The noise level of the system used in our experiments is approximately that of the second-order gradiometer in a suburban laboratory. After Romani, Williamson, and Kaufman (1982).
through the coils. This is known as a first order gradiometer, because, if the coils are identical, the coils are sensitive to the first order derivative of the $z$-component of the magnetic field, $dB_z/dz$. A second order gradiometer (Figure 2.8c) has three sets of coils, the central coil being wound in the opposite sense to the upper and lower coils and having twice the number of turns; this arrangement discriminates against distant sources and uniform field gradients, and is the conformation of detection coil in the SQUID magnetometer used in the experiments described in this thesis. Figure 2.9 gives the relative responses of different coil arrangements to an axial magnetic dipole.

The use of such gradiometers has meant that SQUID magnetometers can be used in unshielded laboratories in magnetically unfavourable environments. Figure 2.10 shows the noise levels of various SQUID systems in different laboratories around the world.

2.4 Maintenance of a SQUID magnetometer

Figure 2.11 shows the arrangement of a conventional SQUID magnetometer. The superconducting elements are immersed in liquid helium (at 4.2 K) contained in an insulating dewar. The detection coils are situated in the tail of the dewar with as little separation of the bottom turns from the exterior as is consistent with adequate thermal insulation. It is technically difficult to produce gradiometer coils of
Figure 2.11. A conventional SQUID magnetometer.
exactly similar area and orientation. To minimise the effects of such errors, small superconducting elements near the detection coils can be moved by external controls, so as to alter the magnetic field through the loops, thus altering their effective area. In this way the gradiometer can be 'balanced' so as to minimise the magnetometer response to distant noise sources. This balancing procedure has to be repeated each time the SQUID is cooled down from room temperature, a procedure taking a couple of hours. It is not usually necessary to rebalance as long as the dewar is maintained at 4.2 K.

The liquid helium within the dewar gradually evaporates. A six litre capacity dewar (such as ours), with average rates of evaporation, needs refilling about every five days. In Britain, at the time of writing (1984), it costs about £100 per week for the liquid helium necessary to run such a SQUID magnetometer continuously. Refilling with helium involves two people and takes 20 minutes for experienced people. Cooling the SQUID down from room temperature takes about 24 hrs of intermittent activity.

There are certain hazards associated with the use of liquid helium: for instance, cryogenic liquids can cause severe burns and helium storage dewars must be vented by one way valves to prevent the vents becoming plugged by frozen air, which would lead to a build up in pressure inside the dewar. However, the risks are minimal if normal safety precautions
2.5 SQUID biological measurements - a brief review

SQUID magnetometers have been used extensively, in the past ten years, to look at the magnetic signals from the heart (the magnetocardiogram) and brain (the magnetoencephalogram). In addition, they have been used to investigate ferromagnetic contamination in the lung, iron deposition in the liver, electrical activity in skeletal muscle, and to locate magnetite in bacteria and pigeons (reviewed in Williamson and Kaufman, 1981).

There have been few measurements of steady magnetic fields around biological organisms. Cohen and Kaufman (1975) described changes in the baseline of the magnetocardiogram after occlusion of the coronary artery in dogs. They ascribe these base line shifts to the injury currents produced by damage to heart muscle. Cohen et al. (1981) have briefly described the presence of steady magnetic field gradients of \(0.1 \mu \text{Gcm}^{-1} (10^{-9} \text{Tm}^{-1})\) over the forearm and legs. They also describe fields over the scalp, produced by 'touching the hairs of the head', and smaller variable fields over the torso. I will discuss Cohen's results and compare them with our own in a later chapter. These are the only published measurements of steady magnetic fields associated with biological systems, other than our own. Cohen uses a magnetically shielded room to make his measurements. We have
Figure 3.1. The drift in base-line of the SQUID output in our unshielded laboratory. The 1pT peak was due to a car moving in the road outside.
no such facility and have designed and built an experimental system that makes it possible to measure steady magnetic fields in the unshielded laboratory. The way in which this is done is described in the next chapter.
Chapter 3: Measurement of steady magnetic fields using a SQUID magnetometer

In the research described in this thesis, a SQUID magnetometer has been used to measure steady magnetic fields around the human leg and arm and over the developing chick embryo, in order to calculate the steady currents within the organism. The magnetometer has been incorporated into a specially designed dc field measurement system. Both are described in this chapter.

3.1 The magnetometer

The magnetometer used is a commercial second order gradiometer system with pick-up coils of diameter 23.6mm. The top coil is 63.5mm above the bottom coil, which is 12mm from the external surface of the bottom of the tail (Operating instructions - biomagnetic probe, SHE corporation instrument reference manual). Since the gradiometer coils are horizontal, the magnetometer is sensitive to the z-component of the magnetic field at the gradiometer.

3.2 Measuring steady fields

The magnetometer, with its second-order gradiometer, responds to changes in the ambient magnetic field, giving no output in a constant magnetic field of whatever level. This means that we cannot measure steady magnetic fields at the detection
Figure 3.2. The SQUID output for two passes over a 5 cm diameter cylinder of water (a) in the earth's magnetic field (b) after nulling the earth's field. Note the change in vertical scale between (a) and (b).
coils. Low frequency noise in the laboratory means that there is significant drift of the baseline (Figure 3.1). In order to measure the stable or slowly changing magnetic field produced by an organism it is necessary to move the organism with respect to the detector, so that the SQUID voltage output shows the change in magnetic flux through the detector coils as the object is moved.

3.3 Magnetic susceptibility

A problem arises if the measurements are made in the presence of an external magnetic field, since the magnetic susceptibility of the object will contribute to the change in magnetic flux, and the voltage output will reflect the magnetic susceptibility of the object as well as any currents within it.

Figure 3.2a illustrates the difficulty. It shows the SQUID output when a 5cm diameter cylinder of distilled water (water has a susceptibility of \(-9\times10^{-9}\) S.I/kg) is moved under the detector, with the surface of the water 1cm from the bottom of the tail (i.e. 2.2cm from the lowest pick-up coil). The signal produced is due to the magnetisation induced in the water by the earth's magnetic field (=50μT), and is of peak to peak amplitude 40pT, or 10 times the signal expected from regenerative currents. Figure 3.2b shows the same pass with the earth's magnetic field reduced to less then 0.2μT. Since most biological tissues are approximately 60% water this
Figure 3.3. The SQUID magnetometer in its wooden frame, the Helmoltz coils, and the moveable bed. The co-ordinate system used in our laboratory is shown.

Figure 3.4. The SQUID output for two scans over a human leg with the vertical component of the earth's magnetic field altered by $5 \mu T$ between scans.
effect must be eliminated by nulling the ambient magnetic field.

3.4 Nulling the ambient magnetic field

The earth's field can be nulled in the region of the detector using three orthogonal pairs of Helmholtz coils. Figure 3.3 shows the arrangement of such coils in our laboratory, with the SQUID at their centre. The coordinate system used throughout our experiments is marked beneath this photograph. The horizontal coils are of diameter 1.9 metres, and carry 80 turns of wire. A current of about 550mA through these coils is sufficient to null the vertical component of the earth's magnetic field. The large vertical coils are aligned so as to give a field in the N-S direction. They are of diameter 2.1 metres, and carry 30 turns of wire. A current of 750mA through these coils will null the horizontal component of the earth's field. The E-W coils are much smaller and have fewer turns since there is no component of the earth's field in this direction. They are needed to null magnetic fields from ferromagnetic materials in the surrounding laboratory and corridor.

The earth's field varies typically by 1% in 24 hours and the laboratory ambient field can be altered additionally by the movement of metal furniture or laboratory equipment. Therefore the ambient d.c field at the SQUID is measured daily, using a fluxgate magnetometer, and the currents
Figure 3.5. Schematic representation of the computer programme for the measurement of magnetic fields along the x-axis above an experimental subject (S. Switchenby, personal communication).
through the coils are altered to keep the field to less than 0.4μT in a region of diameter 50cm at the centre of the coils, just beneath the SQUID tail. Small alterations in the field do not seriously affect our results. Figure 3.4 shows a pass over a person's leg when the field is nulled to within 0.4μT and a second pass with the vertical component of the field altered by 0.5μT. There is an insignificant change in signal.

3.5 General method of measuring magnetic fields around an object

The SQUID dewar is supported in a wooden frame (see Figure 3.3) and can be raised or lowered over 20 cm. A padded bed underneath the SQUID runs on nylon ball bearings and can be moved in the x- and y-directions. The object whose magnetic field is being investigated, is placed on a table on the moveable bed, and the dewar tail lowered so that it nearly touches the object's surface. The bed is then moved through 60cm in the x-direction (in about 4 seconds), so that the object passes under the SQUID. The position of the bed in the x-direction with respect to fixed extreme positions is monitored potentiometrically during each pass.

The SQUID output is filtered with a band pass from 0-40 Hz (a lower cut-off frequency distorts the signal unless the bed is moved across more slowly). An additional notch filter at 50Hz reduces noise from mains electricity. During each pass the
Figure 3.6. (a) Noise level from a single pass  
(b) Noise level from a 3 pass average  
(c) 3 pass average over a horizontal wire carrying 1µA, 6cm from the lowest gradiometer coil.
SQUID output and x-coordinates are recorded and stored using a DEC 11-23 computer system (Figure 3.5). The computer takes readings every 6ms, or 5-6 readings per cm, and the squid output is first averaged over 0.5 or 1cm intervals in the x-direction. After visual inspection of the data to remove any passes affected by the movement of nearby magnetic objects (cars, etc.), a predetermined number of passes (usually 3 or 5) are averaged to give the final data set. For a three pass average this sequence of operations can be carried out in about one minute.

Figure 3.6 shows the nature of the traces obtained. Figure 3.6a shows the trace of a single pass with no experimental subject or object. The peak to peak noise is 1pT. Figure 3.6b shows three such passes averaged. The peak to peak noise level is 0.6pT. Figure 3.6c shows a three pass average over a copper wire carrying 1µA of current along the y-axis. The wire was 5cm from the tail. The peak to peak amplitude of the signal is 6.6pT.

Using this method the vertical component of the magnetic field in the x-y (horizontal) plane above an object can be mapped quite easily (though it takes several minutes to complete depending on size) and, if the object can be turned over, a field map of the magnetic field normal to the whole surface of the object can be obtained.

Before analysing this field, we must be sure that the field
Figure 3.7. SQUID output from scans over a contaminated perspex box (a) before magnetisation and (b) after magnetisation with the N-pole of a 0.1T permanent magnet.
arises from currents, rather than some other magnetic source. The effect of the magnetic susceptibilities of materials has been eliminated by nulling the ambient field. This leaves only the problem of materials with a permanent magnetic moment.

3.6 The problem of ferromagnetic contamination of signals

Ferromagnetic substances produce a magnetic field and may either mimic signals due to ionic currents or mask them. The achievement of consistent signals in different subjects suggests a non-ferromagnetic source, since ferromagnetic particles might be expected to give random results. Where the presence of ferromagnetic material is suspected, it can be detected by magnetising the object. This is most conveniently achieved by holding a permanent magnet within a centimetre of the object for 30 seconds. A second pass over the object will show a change in magnetic field pattern if ferromagnetic material is present. Figure 3.7 shows a scan over a perspex dish before and after magnetisation. Such a check is necessary whenever magnetic contamination is a possibility.

To minimise the effect of slight movements of the supporting structure during a field scan, and to reduce noise due to eddy currents, the Helmholtz coils and the frame are made of wood, with brass screws and aluminium supporting struts. The bed (which moves under the detector during a scan, but is always at least 50cm away) is made of plywood. Plywood does
contain small random magnetic contaminants, so the supporting table (which is nearer the detector) is made of solid wood, which is usually free from contaminants except at the knots.

It is not easy to make non-magnetic containers for biological specimens. Clean, new perspex is usually free from magnetic particles, but great care must be taken in cutting and milling it to prevent contamination. Rubbing it down with sand paper will remove the majority of contaminants, but it quickly picks up new particles if it is not kept in a clean, dust-free environment, presumably because of surface electrostatic charge. Most moulded polystyrene is free from contaminants, if clean. Nylon fabrics are non-magnetic if they are woven. Any material that is cut at the edges shows contamination along these edges.

Dust contains particles of magnetite so all containers must be kept in closed cupboards, and checked for contamination before use. It is important that the laboratory where magnetic measurements are made should not be used for technical procedures which may produce ferromagnetic dust.

We have found in our experiments that humans have few ferromagnetic contaminants in the skin, though their incidence is higher over the hands and feet, particularly in manual workers, and on the wrist, when a wrist watch is worn. Cleaned chick eggs are usually free from surface contaminants, though they may become contaminated by handling
during experiments. Where dissection of an organism is necessary for an experiment, it is better to use glass rather than steel cutters since a steel blade frequently leaves ferromagnetic particles in a preparation.

Once it is certain that the magnetic fields that have been measured arise from ionic currents, then the next task is to calculate the size and distribution of these currents from the magnetic field pattern obtained. The modelling of current sources from their magnetic fields is the subject of the next chapter.
Chapter 4: Modelling current sources from their magnetic fields

4.1 The inverse problem

It is one thing to measure the magnetic fields around organisms successfully but, if our interest is primarily in currents within the organism, it is useful only in so far as it is possible to deduce the presence, strength, or location of such currents from these fields. The problem of calculating a source from the fields it produces is known as the inverse problem. There are certain immediate limitations in all such inverse calculations, the most important being that there is no unique solution (Helmholtz, 1853). Thus, for instance, the field outside a conducting sphere may be exactly that produced by a small current element at a particular location in that sphere, but it is never possible to say that the actual source of the field is not some more complicated pattern of current sources producing the same magnetic field. This problem is important but not the major limitation it might be, since solutions can be chosen or rejected because of anatomical or physiological constraints.

It is possible to model magnetic field sources with two different types of objective:

(i) to localise and define the actual source of the fields measured, in which case all additional information about the
Figure 4.1. A current dipole in the brain. The dipole is represented by an arrow in the direction of current flow, with its length proportional to the dipole moment.

Figure 4.2. The magnetic field produced by the current dipole in Figure 4.1. The depth of the dipole can be deduced from the distance between the positions of maximum inward and outward field on the surface of the head. After Brenner et al. (1978).
currents must be used in the choice of solution.

(ii) to produce a parameterisation so as to facilitate comparison between different field data. If comparisons of data is all that is required then the source used to model data need not be physiologically likely.

An example of the first group is the modelling of certain evoked magnetic fields around the head. Work on evoked potentials on the surface of the head, and from electrode studies within the brain, suggests that the source of such fields are groups of parallel neurones depolarising simultaneously in response to the given stimulus, and that it is physiologically sound to model this pattern of neurone activity by a current dipole, i.e an element of line current of negligible length, having both direction and moment (defined as the product of the current amplitude and length), within the brain cortex (see Figure 4.1). If such a current element is used in modelling the evoked magnetic fields then the depth of the source can be located accurately using the distance between the positions of maximum inward and outward magnetic field on the surface of the head (Figure 4.2). The evoked signal can thus be localised to a specific area of the brain.

The second type of modelling, using an unrealistic source, has been used in the characterisation of magnetocardiograms. These have been modelled using a current
dipole, whose moment and orientation alters throughout the cardiac cycle. This is not considered to mimic the true electrical activity in the heart but has already been used successfully in the analysis and comparison of electrocardiograms. Magnetocardiograms have also been modelled using magnetic multipole expansions (see below for discussion of multipolar expansions) where the source is considered to be a combination of magnetic dipole, quadrupole, octupole and so on. This bears no simple relation to the physiological sources but again provides a way of parameterising and comparing data.

Theoretical work on the inverse problem has produced some general rules and simplifying circumstances which are of value in attempting to fit currents to known magnetic fields in experimental systems. The most useful of these are listed below (Tripp, 1983).

i) At the frequencies of interest, the effects of displacement currents may be neglected and the only significant magnetic field source is the current density, J. This can be expressed as the sum of two physically distinct contributions - the current source, \( J_i \) (a current dipole, or current dipoles), and the volume current, \( J_v \), where \( J = J_i + J_v \). In biological terms, \( J_i \) is the impressed current within the active biological tissue (the' battery'), and \( J_v \) is made up of the currents flowing passively in the volume conductor, as a result of the voltage produced by the battery.
Figure 4.3. A current element at position $r'$ contributes to the field at $r$.
$R = r - r'$. 
ii) In an infinite, homogeneous medium the contribution to the field from the volume currents can be shown to be everywhere zero (Tripp, 1983), and the source of the magnetic field, \( \mathbf{B} \), is the curl of \( \mathbf{J} \). \( \mathbf{B} \) is then defined by the following equation:

\[
\mathbf{B}(\mathbf{r}) = \left( \frac{\mu_0}{4\pi} \right) \mathbf{J}(\mathbf{r}') \times \mathbf{v}' \mathbf{R}^{-1} d\mathbf{v}
\]

where \( \mathbf{J} \) is the current element at \( \mathbf{r}' \) in a conducting volume, \( \mathbf{v} \), and \( \mathbf{B} \) the field at \( \mathbf{r} \) (see Figure 4.3).

iii) In an inhomogeneous medium the boundaries may act as secondary field sources additional to curl \( \mathbf{J} \), in which case the field is given by the following equation (Tripp, 1983).

\[
\mathbf{B}(\mathbf{r}) = \left( \frac{\mu_0}{4\pi} \right) \{ \int \mathbf{J}(\mathbf{r}') d\mathbf{v} - \sum (\sigma_1 - \sigma_j) \mathbf{v}(\mathbf{r}') d\mathbf{s} \times \mathbf{v}' \mathbf{R}^{-1} \}
\]

where the current source is within a volume, \( \mathbf{v} \), bounded by a surface which separates two volumes of conductivity \( \sigma_1 \) and \( \sigma_j \). In this equation the first part of the integral gives the field due to the source current, and the second part the field due to the effect of the boundary on the volume currents.

iv) If a current element is located on and oriented on the
axis of a closed volume conductor having axial symmetry, then it produces no external field - the field due to the dipole at any point outside the volume is equal and opposite to the field produced by the volume currents (Grynzpan and Geselowitz, 1973). Thus, a radially-oriented current source in a sphere or a cylinder will produce no external B field. It follows that any magnetic field measured outside a sphere must be due to tangentially-orientated current elements. It is worth noting here that the reverse is true in surface potential measurements, where the largest signal is obtained from radially-oriented current elements. Magnetic field measurements can therefore be used in conjunction with surface potential measurements, each giving different information about the current sources.

v) When current sources are located in a semi-infinite volume, an infinite slab, or a sphere, then the component of the magnetic field normal to the surface of the volume conductor is produced by the current sources alone; the volume currents generated by the sources do not contribute to this component of the magnetic field (Cuffin and Cohen, 1977). In the case of an infinite slab or semi-infinite volume, this can be easily seen from the equation in (iii), since the vector product form of the equation means that the volume currents produce no field component normal to the surface of the volume. In a sphere, the result follows from the integration.
vi) In an oblate or prolate spheroid, the contribution of the volume currents to the normal component of the field is small compared to that of the current source (Cuffin and Cohen, 1977).

4.2 Detection loop conformation

When modelling magnetic field data one must take into account the conformation of the detection loop or coils, and the fact that the magnetic field is measured over the area of the detection coils and not at a point. The gradiometer has the effect of smoothing the signal. These differences must be accommodated in any modelling calculations.

4.3 Modelling in practice

How does one start when attempting to analyse a magnetic field pattern? There is as yet no common or universal method for calculating currents from their magnetic fields, and each experimental situation has to be considered individually to find the best method for that particular geometry and magnetic field. In practice, this means looking at the field pattern and making a considered guess at the simplest current pattern which would produce such a field. The field of such a source is then calculated, and this modelled field is compared with the experimental field.

In the past, there has been concern that the measurement of
biomagnetic fields would prove useless, because of the difficulties of modelling current sources. In the next three chapters I shall show that a considerable amount of information about the currents within the human leg and the developing chick embryo can be obtained from the measurement of their magnetic fields, using the principles outlined in this chapter, in conjunction with anatomical and physiological considerations. Even where the exact current pattern cannot be established, magnetometry can be used to direct further research into ionic currents using more conventional electrophysiological methods.
Figure 5.1. Sections through the left leg (looking towards the foot)
(a) 10 cm below the knee.
(b) 6 cm above the ankle joint.
Note the asymmetrical position of the tibia, and the two main muscle compartments.
In this chapter I shall describe the magnetic fields and ionic currents associated with the human leg (that is that part of the lower limb between knee and ankle). These experiments form part of a longer study to investigate the currents associated with fractured bones. As I discussed earlier, small direct currents and steady electrical fields have been shown to stimulate bone growth and repair, but there is no clear evidence of currents or electrical fields being present in normally healing bone; the mechanism by which these techniques produce effects is therefore unclear.

When we first decided to investigate the magnetic fields around fracture sites and to deduce the ionic currents producing them, the leg was chosen as the most appropriate area of study because:

(i) it is approximately cylindrical, which makes modelling calculations mathematically tractable.

(ii) the main weight-bearing bone, the tibia, is asymmetrically placed in the leg (Figure 5.1), thus minimising the probability of cylindrically symmetrical current distributions, which (by Ampere's law) produce no net magnetic field outside the conducting volume.

(iii) there is no rotational movement between the tibia and
fibula, so anatomical relationships in the leg remain fairly constant throughout all movements.

(iv) it is a fairly common site of both undisplaced and displaced fractures, and tibial fractures form 50% of all non-unions in clinical trials of this condition.

(v) the measurement of magnetic fields around the leg involves little discomfort to the subject since the leg is peripherally situated and easy to position under the SQUID.

The experiments described in this chapter were intended as a quick series of controls, before looking at fractured limbs. We did not expect to find large, steady, reproducible magnetic fields around the normal leg. In this we were wrong. Once we had found such fields we investigated and characterised them for two reasons:

(i) a knowledge of currents in normal legs is necessary before we can assess the significance of currents around fracture sites.

(ii) the currents producing the magnetic fields are of the order of 10-20µA, similar in magnitude to the currents used in the stimulation of bone to produce osteogenesis. There have been no other reports of such currents in the normal leg (though Cohen, 1980, notes the existence of steady magnetic fields around all limbs). At present, clinicians and pure
scientists tend to consider their injected currents or the currents induced by changing electromagnetic fields as being the only macroscopic currents in the leg.

In characterising the naturally-occurring magnetic fields around the leg we have concentrated on the following variables:

(i) the variation of the signals with longitudinal position down the leg;
(ii) the variation of the signals with time, the leg being relaxed;
(iii) the day-to-day reproducibility of the signals within individuals (three subjects);
(iv) the range of signals in different individuals (twelve subjects);
(v) the effects of exercise, occlusion of blood flow and dilation of blood vessels.

The reported data is a subset of the total amount of data so chosen as to eliminate earlier noise-obscured work and later experiments in which the protocols were not established. There is still a considerable amount of data, making this a long chapter which is complicated by the introduction of a current source model. A list of section headings is given below so that the organisation is clear from the outset. The results presented in this chapter are discussed in Chapter 6.
5.1 **GENERAL METHOD**

5.2 **RESULTS**

5.2.1 Introduction to the general pattern

5.2.2 Modelling the magnetic field sources
   - a brief description

5.2.3 Variation of signal with longitudinal position
   for a typical subject

5.2.4 Day to day reproducibility of signals
   at position three in three individuals

5.2.5 Consistency of signals in twelve subjects

5.2.6 Variation of signals with time

5.2.7 Physiological experiments
   - effect of exercise
   - effect of bone stress
   - alteration of blood flow
   - miscellaneous results

5.3 **SUMMARY OF RESULTS**

5.1 **GENERAL METHOD**

In the majority of these experiments, the vertical magnetic field in a 'plane' above the anterior surface of the leg was measured. In order to standardise readings for comparison between people of different heights, the leg was divided longitudinally (i.e. along the experimental y-axis) into ten intervals, with position zero being at the level of the
Figure 5.2. The coordinate system of the leg.

Figure 5.3. The position of the subject on the bed during a scan over the anterior surface of the right leg.
popliteal crease, and position 10 being the base of the heel (Figure 5.2). With this coordinate system, position 3 is usually at the point of maximum muscle bulk and position 7 is just proximal to the lateral malleolus.

The subjects lay on their backs on the padded bed with their legs resting on a raised platform (see Figure 5.3), approximately at the centre of the Helmholts coils. The SQUID magnetometer was lowered to be as close to the surface of the leg as possible without actually touching it. In each case the distance between the surface of the leg and the bottom of the centre of the dewar was noted. Three or five passes under the SQUID were averaged for each scan and the sampling interval was always one cm. The position of each leg on the table (along the experimental x-axis) was recorded by noting the potentiometric postion of the bed when the anterior border of the tibia was directly under the lowest sensing coil of the SQUID.

Subjects lay in reasonable comfort in this position for up to one hour; after this they suffered from an overwhelming desire to move, and sometimes complained of cramps and paraesthesiae. The muscle bulk of the calf was soft and relaxed during the experiment but, after one hour of resting on the platform, it had been compressed by gravitational forces so that the upper surface of the leg dropped by up to 5mm. This was noted and allowed for in calculations. Because of anatomical variations, the muscle bulk was posteriorly
Figure 5.4. The position of the leg under the detector during scans over the posterior, anterior, lateral and medial surfaces.
<table>
<thead>
<tr>
<th>initials</th>
<th>height</th>
<th>age</th>
<th>sex</th>
<th>position of muscle bulk, (posterior, medial, or lateral)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC</td>
<td>5'11&quot;</td>
<td>53</td>
<td>M</td>
<td>P</td>
</tr>
<tr>
<td>SC</td>
<td>5'1&quot;</td>
<td>32</td>
<td>F</td>
<td>L</td>
</tr>
<tr>
<td>BD</td>
<td>5'10&quot;</td>
<td>34</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>AG</td>
<td>6'0&quot;</td>
<td>30</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>DG</td>
<td>6'4&quot;</td>
<td>31</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>ML</td>
<td>6'2&quot;</td>
<td>28</td>
<td>M</td>
<td>P</td>
</tr>
<tr>
<td>RL</td>
<td>5'2&quot;</td>
<td>29</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>JM</td>
<td>5'5&quot;</td>
<td>27</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>TO</td>
<td>5'9&quot;</td>
<td>25</td>
<td>M</td>
<td>P</td>
</tr>
<tr>
<td>CP</td>
<td>5'7&quot;</td>
<td>37</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>MS</td>
<td>6'1&quot;</td>
<td>24</td>
<td>M</td>
<td>P</td>
</tr>
<tr>
<td>SS</td>
<td>6'0&quot;</td>
<td>32</td>
<td>M</td>
<td>P</td>
</tr>
</tbody>
</table>

**Table 5.1** Details of the twelve subjects in the experimental group.
placed in some subjects, but could be more medially or laterally disposed in others. These differences tended to be accentuated when the legs were resting on the platform. A note was made of the muscle bulk orientation of each subject during the experiment. For individual subjects the orientation on different occasions was the same or similar. In some subjects there was a tendency for the foot to rotate externally during the experiment; this was prevented by tying the legs together loosely at the thighs.

In five subjects, we scanned over the posterior, lateral and medial surfaces of the leg, as shown in Figure 5.4. These orientations were far less comfortable to maintain, and could not be held for more than about 20 minutes. Nevertheless, the muscle bulk of the leg was soft and relaxed. The disposition of the muscle bulk obviously varied with the different orientations of the leg. For each orientation the shape of the leg was delineated using a specially constructed pin gauge.

The magnetic field pattern over the anterior surface of the leg was investigated in twelve adult subjects, whose relevant characteristics are given in Table 5.1. Each subject had the fields anterior to the leg measured for approximately one hour. Positions 3 and 7 were measured as soon as possible after the subject had started to relax. Positions 1-7 were then scanned sequentially three times, with the subject lying relaxed on the bed.
Figure 5.5. SQUID output from scans over positions 1-7 (anterior surface) in a typical subject, JM. The stars represent the positions of the anterior border of the tibia.
The consistency of signals from day to day was measured in three subjects, RL, DG, and SS, over two periods of one week separated by four months. The legs were scanned at positions 2-4, twice a day at 10am and 2pm, within five minutes of starting to relax in the first period, and within two minutes in the second period.

5.2 RESULTS

5.2.1 Introduction to the general pattern of magnetic fields around the leg

The magnetic fields around the leg soon after muscular exertion are substantially reproducible in any one subject, and there is considerable similarity of field pattern between subjects. Figure 5.5 shows the SQUID output from scans at y-positions 1-7 over both legs of a typical subject, immediately after she had climbed onto the bed and started to relax. The field pattern is broadly similar over both legs, and has a peak to peak magnitude of 9 pTesla, at position 3. The signals change gradually with y-position, the signals near the ankle being smaller and of opposite polarity when compared to those near the knee.

Figure 5.6 shows the traces of scans over the anterior, lateral, posterior and medial surfaces of the leg, at position 3. On first inspection of the anterior tracing, the
Figure 5.6. SQUID output from scans over anterior, posterior, lateral and medial surfaces of the left leg at position 3 (subject RL). The line-current model that best fits these scans is shown in the centre (explanation later in text). In all diagrams in this chapter, cross-sections of the leg are shown as if looking towards the feet. In this case the current shown is therefore down the front of the leg and up the back.
pattern of a positive then negative z-component of the magnetic field suggests that the data may be modelled either by a current dipole (as described previously) or, preferably, given the gradual nature of the change of signal with y-position, a line-current along the leg. As a first approximation the line current might be considered infinite. The posterior trace suggests a line current in the opposite direction from that of the anterior trace. The medial and lateral traces are consistent with scans across two horizontal, oppositely orientated line currents. Intuitively, it therefore seemed that the source of the magnetic field around the leg might be modelled by two oppositely orientated line currents.

It was not until we had obtained considerable data on the variation of magnetic fields with time and position that we attempted such modelling. However, I prefer to discuss the modelling of the current sources before presenting further experimental data, since it is considerably easier to compare data using a source current model than to compare the magnetic field scans themselves.

5.2.2 Modelling the magnetic field sources - a brief description

The first attempt at modelling the field data was by visual comparison with the field produced by two oppositely oriented, infinite line currents of equal magnitude within
Figure 5.7. The theoretical relationship (for two line currents within the leg) between the peak-to-peak separation in the trace and the depth below the lowest coil of the gradiometer of the mid-point between the two currents.

Figure 5.8. The relationship between the ratio of peak sizes in the trace and the angle between a line joining the two line-currents and the vertical.
the leg (see Figure 5.6). By infinite I mean that the current extends longitudinally to an effectively infinite distance from the line of scan. The position of the currents and their magnitude was varied until a good visual fit was obtained between the calculated field from the model current source and the actual signal. In these calculations we took into account both the finite area of the pick-up coils and the presence of three coils in the gradiometer.

In the analysis of the probable solution it was found that, for bipolar signals such as those shown in Figure 5.6, the depth of the mid-point between the line currents was indicated by the horizontal distance between the positive and negative going peaks (see Figure 5.7). The angle made between a line joining the two currents and the vertical was given by the relative heights of the positive and negative peaks (Figure 5.8). The position of the mid-point between the two currents along the experimental x-axis could be deduced from the positions of the two peaks on the tracing. Thus, these three parameters, depth, angle, and position, were easily determined by visual inspection.

We had more difficulty when we tried to deduce the actual depths of each of the two currents below the detector, and the magnitudes of these currents. Somewhat surprisingly, we found that only the product of the distance between the currents and the current magnitude could be established. Figure 5.9 shows the calculated SQUID output of scans across
Figure 5.9. Theoretical SQUID output for scans across two sets of line current pairs. Both have their mid-point 4 cm beneath the detector. Both have identical values for the current magnitude X current separation. The actual current separations and magnitudes differ. The peak to peak separations of the two traces are similar.

Figure 5.10. The coordinate system used in the multipolar analysis described in the text. P is in the transverse (x-z) plane containing the leg cross-section.
two pairs of line currents, both having the central point between the two currents 4 cm below the detector, both with the same value of 10μAcm for the quantity, current magnitude \( \times \) current separation, but having very different separations - 1cm and 6cm. It can be seen that these two extreme cases show only a minimal variation in peak separation and shape, though the amplitude of the signals are different. In identifying a source, the amplitude of signal, which scales with the current magnitude, is of little use unless the source magnitude is known. It follows that the actual depth of each line current cannot be defined unless the current magnitude is known.

This last finding suggested that the currents might also be modelled by the dipole term of a multipolar expansion* of the axial current flow within the leg. For this calculation, the current density, \( J_y(r) \), is considered to be everywhere parallel to the y-axis of the leg and to be constant along that axis. The distribution of current within the leg can be described by a series of terms relating the current flow to an arbitrary source, at a position \( 0 \), within the leg (Figure 5.10). The first term of the expansion, the monopole, describes the net flow along the longitudinal axis of the leg, and is given by \( \Sigma_{\lambda y}(\lambda) \Delta a \). Since the net current down the leg must be zero, the monopolar term must be zero. The second, or dipolar, term is given by \( \Sigma_{\lambda}J_y(\lambda) \Delta a \). The symmetry of the current distributions described by the monopole, dipole, and quadrupole terms of the expansion are shown in

*described more fully in the appendix to this thesis
Figure 5.11. The symmetry of current distribution described by the first three terms of the multipolar analysis of axial leg currents.
Figure 5.11. It should be noted that this dipole term is not the same as the infinitesimally short element of current flow that is commonly called a current dipole in literature on the magnetocardiogram and magneto-encephalogram, but is a vector joining the two centres of oppositely oriented current density within the leg. To differentiate it from the more usual current dipole, we will call it the 'line-dipole'.

The magnetic field produced at a point, P, outside the leg by each term of the current expansion can be calculated. Each successive term in this magnetic field expansion depends on one higher power of 1/R than its predecessor, so that higher order terms make a progressively smaller contribution to the magnetic field measured. Thus, the closer one is to the leg, the more terms might be expected to influence the signal significantly. We find that the field from the line-dipole term alone gives a good fit with our experimental data.

The line-dipole term is characterised by four parameters: two define its location in the x-z plane (the line-dipole depth and position on the x-axis), and one its orientation (the line-dipole angle, which in our calculations is measured anticlockwise from the vertical). The fourth parameter is the line-dipole strength. For two line currents, I and -I, separated by a distance s, the line-dipole strength would be given by the quantity Is. These four parameters can be chosen so as to minimise the difference between the experimental field data (S_{exp}) and the field calculated from the line-
Figure 5.12. Experimental data (crosses) and computed field of the best-fit line-dipole source (smooth line) for a typical scan across the left leg, position 3. The line-dipole angle is measured anticlockwise from the vertical. Errors are derived as described in the text.

Figure 5.13. The representation of the line-dipole source described in Figure 5.12, and the equivalent line current model. The centre of the arrow represents the position of the line-dipole. Its length is proportional to the line-dipole strength. The arrow points from the centre of current density down the leg to the centre of current density up the leg. In the right-hand diagram, the line-current separation is arbitrarily chosen.
dipole \((S_d)\). In our fitting procedure this is done by minimising the quantity

\[ R = \sum (S_{\text{exp}} - S_d)^2 \Delta x \]

where \(\Delta x\) is the sampling interval of lcm used in the experimental scan, and the sum is taken over all sampling intervals. The misfit between the modelled field and the data, using this formula, is often less than 5%. A typical trace with the magnetic field from the best fit line-dipole source is shown in Figure 5.12. Such a fit does not necessarily mean that higher order terms in the current expansion are insignificant (since their field drops off more rapidly with distance) but does strongly suggest that there are two areas in the leg with net current flow in opposite directions.

The line-dipole source can be represented by an arrow with its centre showing the position of the moment, its direction indicating the angle, and its length proportional to the line-dipole strength. Fig.5.13 shows such a representation with the equivalent line current model. Note that the arrow points from a current element going down the leg to a current element going up the leg. In the analysis of the results in the rest of this chapter, I shall use line-dipole current sources represented in this fashion. In using this method of analysis, I am indebted to Dr.T.Smith and Mr.D.Grimes for the mathematical analysis of this source model, and for the
Figure 5.14. SQUID output and best-fit line-dipoles for scans across the anterior surface of the left leg of subject BD at y-positions 1-7.

Figure 5.15. Schematic representation of the net current flow within the leg. Immediately after relaxation.
computerisation of the modelling procedure.

5.2.3 Variation of signals with longitudinal position for a typical subject

Figure 5.14 shows the SQUID output from scans across the anterior surface of the left leg of a typical subject at y-positions 1-7, i.e. at intervals from just below the knee to just above the ankle. The corresponding calculated line-dipole source is represented within a schematic leg beside the magnetic field scan. These traces were obtained sequentially within about 15 minutes of the subject climbing on the bed and beginning to relax. It can be seen that for positions 1-3 net current flow is down the front of the leg and up the back, whereas for positions 5 to 7 it is up the front and down the back, with the line-dipole placed more anteriorly and laterally within the leg. At position 4 the signal is small and complex; it cannot be modelled successfully using our usual line-dipole model.

Figure 5.15 shows a possible interpretation of the longitudinal current flow in the leg if the current flows at each y-position are considered together. This description is true of all subjects so far measured, though the depth, angle and strength of the line-dipole do vary from subject to subject and, to a lesser extent, in any one individual from day to day. These variations are discussed in the next two sections.
Figure 5.16. The line-dipole vectors in DG, SS, and RL, derived from twenty different scans across position 3 of both legs.
(b) RL - Only the line-dipole positions are shown for reasons of clarity. The line-dipole vectors are all vertically downwards (±20 degrees).
(c) SS – only 18 line-dipole vectors shown in each leg because in two traces for each leg the dipole-data misfit was greater than 10%.
Table 5.2. The average line-dipole source in subjects RL, DG, and SS. The line-dipole angle is measured anticlockwise from the vertical. The line-dipole depth is from the highest point on the anterior surface of the leg. For RL and DG, n=20. For SS, n=18 since two traces from each leg have been discounted because of large (>10%) errors in the fit of the model to the experimental data.

Figure 5.17. Line-dipole depth and strength in subject RL at 10am (dots) and 2pm (crosses).
5.2.4. Day to day reproducibility of signal at position 3 in three individuals

Individual variability was examined in the three subjects, RL, DG and SS (whose characteristics are shown in Table 5.1), twice a day over two periods of one week. Figure 5.16 shows the calculated line-dipole current source for each trace obtained in these three subjects. Two results from each of SS's legs have been discarded because of large (more than 10%) misfits between model and experimental data. It is clear that there is a strongly consistent current pattern in each individual.

Table 5.2 shows the average values and the variation between values of the line-dipole angle, depth, and strength. In subjects RL and DG the signals were fairly constant, the greatest variation occurring in the dipole moment. Subject SS showed greater variation in depth and angle, especially in the right leg. It should be noted that his signals were often smaller and showed a greater degree of misfit with the model than the others. A possible explanation for the variation in his signals is that they changed very rapidly with time, sometimes reversing in 10 minutes (see later section in this chapter on variation of signals with time). It may be, therefore, that at the time of measurement there was already considerable change in the signal, particularly in the first week's scans, which were done up to five minutes after starting to relax.
Figure 5.18. Line-dipole vectors at position 3 (12 subjects) and position 7 (10 subjects).
Table 5.3. The average line dipole angle, depth and strength of the experimental group (left leg only) for scans at position 3 \((n=12)\) and position 7 \((n=10)\)

<table>
<thead>
<tr>
<th>Position</th>
<th>Average Angle</th>
<th>Depth in cm</th>
<th>Strength in µA cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>-133</td>
<td>4.6</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>SD 29</td>
<td>0.9</td>
<td>13</td>
</tr>
<tr>
<td>7</td>
<td>-34</td>
<td>2.5</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td>SD 24</td>
<td>0.6</td>
<td>2.4</td>
</tr>
</tbody>
</table>
Subjects SS and DG showed no significant difference in their morning and afternoon scans. In subject RL, scans in the morning had line-dipole sources with smaller depth and strength. Figure 5.17 shows these diurnal variations.

5.2.5 Consistency of signals in twelve subjects

The twelve subjects studied in detail were described in the methods section. The magnetic fields anterior to the leg all fitted the general pattern described previously. Figure 5.18 shows the line-dipoles for all subjects for the initial scans at positions 3 and 7 (for two subjects the signals at position 7 were small and complicated: these have been left out of the diagram since the line-dipole model did not give a good fit). For simplicity of presentation the results are displayed on a schematic leg outline, since the size and shape of leg varied considerably. Table 5.3 shows the average values for depth, angle, moment and position in relation to the tibia of the current dipole. Given the anatomical variations, this data shows a consistent pattern. It is interesting to note that those subjects with the current dipole disposed more vertically in the leg tended to have legs with the muscle bulk arranged posteriorly, or posterolaterally in the leg, whereas those with more medial muscle bulk had more horizontal current dipoles.
Figure 5.20. The change in signal with time for position 3, the left leg, in two subjects. TO (rapid change in signal) and DG (slow change in signal).

Figure 5.21. The change in line-dipole strength ($m$) and angle ($\Delta\phi$) with time in 12 subjects.
Figure 5.19. The change in signal with time in RL's left leg, position 3.
5.2.6 Variation of signals with time

Twelve subjects had their signals measured while relaxing on the bed for about an hour. In all cases there was considerable change in the signals at the proximal end of the leg during this time.

The signals at the proximal end (positions 1 to 3 or 4) gradually decrease in magnitude in all subjects, though the rate at which this happens varies in different subjects. In those subjects where the decay of signal is rapid, the signals become small and/or complicated after about 30 minutes of relaxation. After this time, if these subjects continue to relax, new signals appear and gradually increase in magnitude. Figure 5.19 shows the change in signal at position 3 in one subject over an hour. The calculated source dipole is shown beside those signals where such modelling is appropriate. (For very small or complicated magnetic field signals, modelling by a dipole source gives a very large misfit and may be an inappropriate model.)

The wide variation in the decay rate of the initial signals in different subjects makes it difficult to compare signals. Figure 5.20 illustrates the change at position 3 for two subjects at either end of the range. It is tempting to speculate that DG follows the same basic pattern as TO but at a much slower rate.
Figure 5.22. The possible change with time of the net current flow within the leg. (from all data crudely combined)
It is possible to plot the changes at position 3 with time for all subjects, to show the range of change. Figure 5.21 shows such a plot. Since both the line-dipole strength and angle show gradual changes with time these parameters are combined as the quantity $m\sin\Delta\phi$, where $m$ is the line-dipole strength in $\mu$Acm, and $\Delta\phi$ is the change in line-dipole angle with time.

At the distal end of the leg, the signals are initially smaller than at the proximal y-positions. In most subjects (10 out of 12), the distal leg signals do not change significantly with time. Whereas the currents in the proximal part of the leg tend to decrease with relaxation then change in direction, the currents in the distal part of the leg remain constant. The change in current flow in the leg with time that these data suggest is shown in Figure 5.22. However, it must be stressed that there is much greater variation between different people in the time course of their signals than there is in the initial signal pattern. Any suggestions as to an overall pattern of change with time are put forward rather tentatively.

5.2.7 Physiological experiments

The effect of exercise

Since we found that the magnetic field around the leg changed with time if the leg was relaxed, it seemed logical to
investigate the results of muscular exertion. We had already found in the course of our experiments that, if a subject relaxed for half an hour until the usual changes in signal had occurred, then walked around for one minute prior to remeasurement, his or her signals reverted to their original shape and magnitude.

In order to investigate the effects of muscular exertion more systematically we studied the effects of slow continuous dorsiflexion and plantarflexion of the foot in three subjects. The subjects used the muscles of dorsiflexion or plantarflexion to maintain the position of the foot against a force of approximately 10 N, perpendicular to the foot at the ball of the foot. The muscle contraction was isometric, or nearly so. Results were not always consistent but some conclusions can be drawn.

i) During dorsiflexion the signals were reduced and complex

ii) After dorsiflexion the signals reverted to the pattern found on starting relaxation, and increased in size for about five minutes.

iii) Plantarflexion had little effect during or after muscle contraction.

The effect of stressing bone

In the experiments on muscular exertion the subjects did not weight-bear, but contraction of muscles must necessarily stress the bone, since the bone provides the stable point
against which the muscle pulls. We attempted to separate out the effects of bone stress and muscular contraction by stressing the bone externally. This was done by hanging 2kg weights over the leg, which was supported at the knee and ankle. Neither the presence of the weight nor its removal caused any alteration in the signal from scans across the leg.

**Alteration in blood flow**

The effect of blood flow on the magnetic signals was investigated by occluding the arterial blood flow in the thigh for ten minutes, using a sphygmomanometer cuff pumped to 200mm mercury pressure. During the ten minutes the leg became first red, then cyanosed, then pale and blotchy. The cyanosis indicates that there was at least 7gm% of reduced haemoglobin in the blood, i.e that it was at least 50% deoxygenated. For three out of the four times this experiment was performed, there was no change of the magnetic signal during the arterial occlusion or after release of the tourniquet. On one occasion the signal changed shape during the occlusion and remained abnormal for up to an hour after the release of the tourniquet, even after getting off the bed and walking around for ten minutes. The subject in this experiment complained of pain in that leg continuing after the release of the tourniquet and it was concluded that the change of signal on this occasion was due to tissue damage from the arterial occlusion.
The effect of blood flow on the signals was further tested in one subject by the administration of glyceryl trinitrate, a drug that dilates the blood vessels throughout the body. Two tablets were placed under the subject's tongue, and the effect of the drug was monitored by measurement of blood pressure. During the ten minutes after the tablets had dissolved the systolic blood pressure (which had previously been steady for ten minutes) fell by 10mm, suggesting that vasodilatation had occurred. During this time there was no change in the magnetic field over the leg. We conclude that the magnetic field around the leg is not significantly influenced by the flow of blood in the leg, nor is it sensitive to a 50% drop in oxygen concentration in the blood.

Miscellaneous observations

Miscellaneous experiments revealed that the magnetic fields around the limb are not significantly altered by: rubbing the skin; bruises on the anterior aspect of the leg incurred 24 hours before the magnetic field scan; small (less than 2 cm) full thickness cuts to the skin (though the spatial discrimination of the SQUID pick-up coil means that local variations could be missed in our experimental system). In addition they do not change when a subject falls asleep or wakes.

Measurements were made over the forearms of three subjects.
These revealed consistent magnetic field patterns over the dorsal and ventral surface of the arm. The signals appeared to diminish with time of muscular relaxation. However all three subjects showed ferromagnetic contamination at the wrist. This made the signals more difficult to analyse than those of the leg.

5.3 SUMMARY OF RESULTS

Sensitive magnetometry reveals the unexpected presence around the normal leg of steady reproducible magnetic fields, similar in 12 subjects. These magnetic fields change predictably during periods of muscle relaxation, but revert to their original form and magnitude after active dorsiflexion of the foot. The fields are unaffected by mechanical stress to the leg, and by alterations in blood flow. The magnetic field sources can be modelled using the line-dipole term of the multipolar expansion of a uniform axial current distribution within the leg.
Chapter 6: Discussion of ionic currents within the leg

I shall discuss the results described in Chapter 5 under five headings.

6.1 Comparisons with other magnetic field measurements over humans

6.2 Ionic currents as the source of the magnetic fields.

6.3 The validity of the line-dipole source model.

6.4 The location and magnitude of the currents within the leg.

6.5 The source of the currents.

6.6 The implications of these findings.

6.1 Comparison with other results

As I mentioned in chapter 2, Cohen et al. (1980) have also described steady magnetic fields over human limbs, though their measurements over the leg were very brief and they concentrated mainly on the magnetic fields over the head and forearm, taking no account of the effects of muscular exertion. They found no consistent magnetic field pattern over the limbs. Their gradiometer arrangement measured the tangential gradient, dB_z/dx, of the component of the magnetic field normal to the surface of the limb, and they found gradients of approximately 100nGcm^{-1}, or 10pTcm^{-1}. We can convert our data to give roughly equivalent units by calculating dB_z/dx along the SQUID output from our scans. A typical value of 5pTcm^{-1} is obtained. Our measurements seem
therefore to be consistent with those of Cohen et al.

6.2 Ionic currents as the source of the magnetic fields

The magnetic field scans across the leg do not change if a strong magnetic field is imposed on the leg and then removed (as described in Chapter 3 as the test for the presence of ferromagnetic materials). In addition, ferromagnetic contamination might be expected to give random results, rather than the consistent magnetic field patterns that we obtain. I conclude that the signals are not produced by ferromagnetic substances within the leg.

The ambient magnetic field at the SQUID is less than 0.4μT, and the magnetic susceptibilities of all types of tissue are similar to that of water. Calculations based on these observations, and the previously reported experiments show that the magnetic field measured over the leg cannot be due to the magnetic susceptibilities of its constituent elements. The only remaining possibility is that the magnetic field is produced by electrical currents within the leg.

6.3 The validity of the line-dipole source model

Our analysis of the location of the ionic currents in the leg depends largely on our use of the line-dipole term in a multipolar expansion as a model source. Before considering the model data in relation to the anatomy and physiology of
the leg, I shall consider possible faults in the model on theoretical grounds and faults that are suggested by the actual results.

In the modelling procedure, the currents in the leg are considered to be constant along the y-axis. It is clear that the currents are not constant down the leg since our traces change with y-position. This might be expected to distort the modelling process. However, measurements scanning across two current carrying wires suggest that only extreme non-uniformities (such as a sudden 180 degree twist) in the wires within five centimetres of the scan position will produce changes in the signal sufficient to alter the calculated source model significantly (that is, by more than 3mm for the dipole depth and x-position, 10 degrees for the dipole angle, and 30% for the dipole moment) (Grimes et al., 1984). This insensitivity is at least partially attributable to the insensitivity of the gradiometer arrangement to distant sources. Loss of accuracy was signified by a significant increase in the calculated error, R.

For the majority of our experimental scans the error is less than 5% but this does not necessarily indicate a purely dipolar current distribution. The higher the order of the term in the expansion, the more rapidly the magnetic field associated with the term falls away with distance. It follows that the dipole term alone will tend to fit the data well unless the higher order terms are large. With the distance
Figure 6.1. The relationship between line-dipole depth and strength for several scans at position 3 (DG). Deeper line-dipole sources have larger strengths.
between source and detector at only a few centimetres we might expect to see a large contribution from the quadrupole terms. Grimes et al. (submitted for publication) have shown that this is not so, because in a multipolar expansion of axial current flow in a cylinder, the fitting procedure which identifies the best fit line-dipole will tend to locate it at or near an origin at which the quadrupole term vanishes. There is such an origin for all uniform axial current distributions.

It follows that the good fit we obtain using a line-dipole source model for our data does not exclude the possibility of a more complicated current flow pattern within the leg. However, it is possible to say that the line-dipole term is a large component of the current flow, and that there is net current flow down one compartment of the leg and a return pathway up the other.

The fact that the currents may not have a purely line-dipolar distribution introduces a distortion in the calculation of the depth of the line-dipole. For more distant sources the signal will be more nearly line-dipolar. For close sources higher order terms will have a greater effect. This distorting effect is demonstrated in Figure 6.1 which shows the relationship between depth and strength of the line-dipole sources for a series of scans at position 3 on different occasions in the same individual. The correlation between depth and strength suggests that, for near sources,
Figure 6.2. The line-dipole source in the leg, calculated from scans across the anterior, lateral, posterior, and medial surfaces of the left leg at position 3 (RL). All leg outlines are shown with the anterior surface upwards. The outline of the leg shows how the distribution of the muscles bulk varies in the four different attitudes.
they are both slightly underestimated. Similar results have been obtained by the use of computer simulated data (Grimes, personal communication).

One way of testing the accuracy of the model is to scan the leg across all four aspects (anterior, posterior, medial and lateral), fit a line-dipole to each scan, and then compare the various positions, angle, and strengths of the four line-dipoles, and see how well they correspond. There are two obvious sources of error in such a comparison: the leg changes shape with the change in orientation for the different scans, and there may be a change in the actual currents within the leg during the course of the experiment. Figure 6.2 shows the results of such an experiment, in which the second source of error was minimised by the subject's walking around for two minutes before climbing onto the bed for each scan. Checks showed high levels of reproducibility in a given experiment with this procedure. The various conformations of the leg are shown along with the four modelled sources. It can be seen that there is considerable agreement in the line-dipole angles, but less similarity in the positions and strengths. Similar results were obtained in five subjects at position 3, and in three subjects at position 7. Overall, these results confirm that the use of the line-dipole term as a source current is reasonable. They suggest that any modelling discrepancies manifest themselves more in the calculation of line-dipole depth and strength than in the line-dipole angle.
Figure 6.3. The areas of net current flow within a left leg at position 3.

Figure 6.4. The change in line-dipole angle when the calf muscle is placed medially, posteriorly or laterally.
6.4 The location and magnitude of the currents

The model line-dipole source gives some information about the location and magnitude of the actual areas of current flow. The line-dipole angles in our experimental population suggest the location within the leg shown in Figure 6.3. If one compares these areas with the anatomical compartments in the leg (Figure 5.1), it is clear that, for position 3 at least, they do not correspond with the areas occupied by the two bones in the leg, and it is therefore unlikely that the currents lie within the tibia or fibula. The positions of the areas of current flow do correspond with the anterior and posterior muscle compartments in the leg, and it seems reasonable to suppose that the current flow is within the soft tissue. Further evidence for this is given by experiments when the posterior muscle bulk of the calf is moved passively to lie more medially, or more laterally than its usual position. Figure 6.4 demonstrates that the line-dipole angles of the modelled source currents change when the muscle is moved in this way. At the distal end of the leg the muscle compartments become much smaller and are more laterally disposed within the leg. It is not therefore surprising that the scans near the ankle can be modelled by current dipoles near the surface of the leg and lateral to the mid-line (Figure 5.18). There is no evidence from these anatomical considerations that the currents lie within the neurovascular bundles, although these would provide paths of low resistance within the leg.
Figure 6.5. A crude approximation of areas of current flow in the leg, as used in calculations in the text.
The strength of the line-dipole (m) gives information about the separation and magnitude of the axial currents. It is possible to some extent to separate out these two components. At position 3, the line-dipole strengths in different subjects lie between 10 and 54\(\mu\)Acm at the beginning of relaxation. If we start by considering the distance (d) between the centres of two areas of current flow (Figure 6.5), we can say immediately that d must be less than 10cm, since the diameter of the leg at position 3 is about 12cm and the line-dipole depth is less than 5cm below the surface of the leg. It follows that the minimum current magnitude is 1\(\mu\)A.

The minimum separation, and hence the maximum current magnitude, is less easy to establish. It seems reasonable to assume from electrophysiological considerations that the longitudinal voltage difference in the deep tissue within the leg is not more than 10mV. This is twice the potential difference that Friedenberg and Brighton (1966) found along mature long bones, and ten times the the potential difference between different regions of subcutaneous tissue found by Barker et al. (1982). If we assume that a maximum voltage drop of 10mV occurs in a distance of 20cm, and that the conductivity of soft tissue is in the region of 0.1 \(\Omega^{-1}m^{-1}\) (Piekarski, 1979), then the magnitude of the current density \(J\) will have a maximum value of \(5\times10^{-3}A/m^2\) (calculated from \(J=\sigma E\)). This can be used to calculate a minimum distance between the centres of the two areas of current flow in the following manner. For minimum axial current separation, the
two areas of current flow will be close to one another as in Figure 6.5. If we consider the two areas as squares of side length \( d \), the total current, \( I \), in each area will be given by the equation

\[
I = Jd^2
\]

and the magnitude of the line-dipole strength, \( m \), by the equation

\[
m = Id
\]

\[
= Jd^3
\]

The minimum value of \( d \) in a given experiment can be calculated from this equation using the experimental value for \( m \), and the maximum current density calculated above. If \( m = 50 \mu \text{Acm} \) (near our experimental maximum value) then

\[
d_{\text{minimum}}^3 = \frac{5 \times 10^{-7}}{5 \times 10^{-3}} \text{ m}^3
\]

and \( d_{\text{minimum}} \approx 4.5 \text{cm} \)

From the above calculations we can now say that the centres of current density must be separated by at least 4.5 cm, and the maximum total current that we have seen in the leg at position 3 cannot be larger than 12 \( \mu \text{A} \).
Using the above calculations it is also possible to argue that each of the areas through which the current flows is of area at least $20\text{cm}^2$ when the centres of current flow are separated by 4.5cm. If the separation is considered to be 10cm (the maximum possible given the dimensions of the leg) then the possible area reduces to $10\text{cm}^2$.

These calculations suggest that the currents within the leg are diffuse, rather than localised, providing we accept that the maximum voltage drop in deep tissues down 20cm of the leg is less than 10mV and that the longitudinal current flow in the leg is down a potential gradient. These assumptions seem reasonable. However, later in this chapter, I shall describe circumstances in which they are not valid, when charged molecules are moved by mechanical forces rather than down a potential gradient.

6.5 The source of the currents

Having found these currents within the leg, and analysed their positions and characteristics as far as is possible, the next step is to consider how they are produced. There is no immediately obvious answer. Any hypothesis must explain the macroscopic nature of the currents, their distribution within the leg, and their behaviour with time in the absence of muscular exertion.

I shall consider various possible sources: the movement of
charged molecules or ions in the blood; the potential difference across the skin; the potentials found in bone; the cell membrane of muscle cells; and the transport of charged molecules along nerve axons.

6.5.1 The movement of charged particles within the blood-vessels

This is unlikely to be the source of the currents we see in the leg, since stopping the blood flow with a tourniquet does not change the magnetic field around the leg.

6.5.2 The skin

The currents cannot be generated by the differences in potential difference across the skin at different regions, since the conducting pathway on the surface of the skin is of high resistance, and the potential differences that occur along the surface of the skin are too low to produce currents in the region of 1-10μA

6.5.3 Bone

Bone is known to produce macroscopic potential differences across an area that is mechanically stressed (Fukada and Yasuda, 1957). These macroscopic potential differences could produce macroscopic currents. However, this stress produced potential difference has a decay time of only about 0.5
Figure 6.6. The current loops around bone suggested by Brighton and Friedenberg's voltage measurements along intact bone.
seconds, once the initial movement of bending or compression of the bone has ended. It is difficult to see how the currents we measure could be produced by such a mechanism since they continue for half an hour or so after the cessation of weight bearing or muscular exertion.

The potential differences along the surface of bone found by Brighton and Friedenberg would be more likely to result in the current patterns we see. A potential difference of 9mV between epyphysis and diaphysis could produce current loops of the shape we see, reversing direction at the centre of a long bone (Figure 6.6). Given the conductance of soft tissues (approximately 0.1 Ω⁻¹m⁻¹), these potential differences would give current magnitudes in the region of 10μA. Although this value is consistent with our results, the supposed current distributions are not, since our modelling of currents within the leg suggest that they are not associated with the bones. In addition, as I mentioned in my review of Brighton and Friedenberg's work, there have been experiments suggesting that the potentials they measured over intact bone were actually due to injury currents resulting from their dissection of soft tissue to reveal the bone surface. Brighton and Friedenberg found that the voltage drop along bone was higher in growing bones. We have found experimentally that the currents found in a growing child (age eleven years) are similar in form and size to those found in adults. There is no evidence from Brighton and Friedenberg's work that potentials along their rabbit bones
Figure 6.7. The longitudinal current flow produced in a long thin cell by a localised difference in transmembrane potential difference.
diminished with time of muscular relaxation.

I conclude that the currents that might be produced by bone do not seem to correspond to the currents we measure, because of their location outside bone and their behaviour with time.

6.5.4 Muscle

In their paper first describing steady magnetic fields over the limbs in humans (1980), Cohen et al. found that subcutaneous injection of potassium chloride solution increased the magnetic signals from the limbs, though not from areas without long muscles under the skin. They suggest that macroscopic currents could be produced by long thin cells, such as muscle cells, if there is a localised variation in ion concentration. Figure 6.7 suggests how this might occur. Any steady gradient or localised variation of electrolyte concentration on one side of the membrane could produce a current loop with the dimensions of the cell (muscle cells may be as long as 20cm). Betz et al. (1980) have recorded such current flow out of the endplate-region of muscle cells. They used a vibrating electrode to measure current leaving whole muscles and individual muscle cells in the region of the neuromuscular junctions. The maximum current densities for whole muscles was about 1\mu A/cm^2, and for the single cells, 10\mu A/cm^2. The current was dependent on the activity of sodium-pumps. This experiment can be criticised as unphysiological, particularly in the case of
the whole muscle, which was placed in Krebs solution but was not perfused making it likely that only the outer cells of the muscle remained viable. However, the results do suggest that the activity or number of sodium pumps is greater at the end-plate region than over the rest of the muscle membrane.

It is tempting to think that such currents originating in the muscle endplate region form the current loops that we observe in the leg. The arrival of a nerve impulse at the leg, and the subsequent rapid passage of potassium and sodium ions through the membrane, causes changes in sodium and potassium concentrations, and these lead to an increase in activity of the sodium pump for a few minutes until the previous concentrations are restored. These changes could explain the current loops we observe after muscle contraction. Another hypothesis suggests that the passage of a nerve impulse and depolarisation of the muscle membrane causes a redistribution by electrophoresis of the ionic pumps or channels in the muscle membrane. (Jaffe, in 1977, suggested that membrane proteins will redistribute round a cell in response to electrical field changes).

These hypotheses 'explain' the change in current flow with time of muscular relaxation. It is harder to be sure whether such current loops around muscle could explain the pattern of current flow that we have observed within the leg. The end-plates of muscles are located approximately in the centre of muscle fibres, at about position 4 for a muscle whose fibres
extend for the length of the leg. In fact the different muscles extend over different portions of the length of the leg, and have their bulk at different levels. The posterior (flexor) compartment has its greatest bulk around position 3 and is much diminished by position 6, whereas the bulk of the anterior and peroneal compartments is more evenly distributed down the leg. These asymmetries in muscle arrangement could result in the asymmetry of current flow that we see, and it does seem that muscles could be a possible source of these currents.

6.5.5 Nerves

Early workers in the field of electrically-induced healing (e.g. Becker et al. 1962) suggested that regenerative fields were associated with nerves. It has been known since 1948 (Grafstein and Forman, 1980) that substances move from the nerve cell body along the axons. By the mid-1960's it had become clear that there are two different types of movement of substances along axons. The first is known as slow axonal transport and consists of the slow movement of the structural proteins that form a lattice within the axoplasm. The movement is slow, usually between 1-5mm/day, but the rate does vary between different nerves, being faster in nerves with longer axons. The major proteins concerned are actin and tubulin, both of which are negatively charged at the pH found in mammalian tissue. Together, these form 80% of substances moving in slow axonal transport. The mechanism of slow axonal
transport is not clear, but it seems that the movement down the axon is steady until it reaches the end when it stops abruptly. The movement is dependent on continued protein synthesis within the cell body. There is no evidence that it is affected by the passage of action potentials down the axon.

In fast axonal transport, diverse substances travel both away from and towards the cell body at rates of between 250 and 450 mm/day. Substances transported are mainly particulate, and include proteins and a large variety of membranous structures, such as smooth endoplasmic reticulum, plasma membrane, and vesicles of various kinds, including synaptic vesicles. There is also some evidence for fast transport of various small molecules, such as amino-acids and sugars. The mechanism of fast transport is not well understood, but is dependent on oxidative metabolism. The electrical activity of the nerve does not alter the velocity of fast transport, but there is some evidence that it may influence the amount of transported material.

In considering whether the movement of substances in nerves could constitute a significant current flow, it is easiest to concentrate on the slow transport system, since the bulk of this movement is of known proteins, and the flow is unidirectional. Actin, which is a major component of the axoplasmic skeleton, is a protein of approximate dimensions 10 by 5 by 5nm, and carries 5 negative charges. If we
consider actin to form 5% by volume of the axoplasm, moving at 5mm/day in nerves of cross-sectional area 1cm², then the current along that nerve bundle would be approximately 1μA. Although this is a rough estimate, it is clear that axonal transport of charged molecules could produce currents of the same order of magnitude that we measure in the leg. The movement of charges in axonal transport is by mechanical forces, rather than down a potential gradient. This means that the constraints on possible current volumes derived in Section 6.4 are not applicable to the movement of charges in axonal transport, and at least some of the currents we measure could be contained within the nerves of the leg. It is conceivable that the distribution of nerves within the leg could give the pattern of current flow we observe, and that the currents would vary with relaxation because the passage of action potentials alters the amount of material moving in the fast transport system. The sketchy knowledge of transport mechanisms, and the variety of substances transported, make it difficult to consider this system more precisely.

6.6 The implications of these findings

There are several points that may be made when considering the above results.

There has been very little reported work on small currents within intact mature tissues. Our chance finding of these currents within the leg as a result of control experiments
raise the possibility that macroscopic current loops of this magnitude may be far more ubiquitous than is generally assumed. The results also demonstrate the value of the SQUID magnetometer as a non-invasive screening device for such currents.

The presence of currents of about 10μA within the normal leg has clinical implications. Those who are engaged in the electrical stimulation of non-unions can no longer consider their imposed currents to be the only currents within the leg. The fact that our currents change when the leg is relaxed suggests that the pattern of current flow may be very abnormal when the leg is immobilised within a plaster of Paris splint. Unfortunately plaster of Paris contains many magnetic contaminants, and we have not yet been able to measure the currents within a leg that has been immobilised for hours or days.

Summary of this chapter

Measurement of the magnetic fields around the human leg indicate the presence of macroscopic current loops, with currents of magnitude 1-12μA, within the leg. The current patterns are broadly similar in all subjects and are affected by muscle activity, decaying and changing direction when the leg is relaxed. The origin of these currents is uncertain: the most likely sources are inhomogenous sodium pump activity in muscle cells, or the transport of charged particles along
nerve axons. It is uncertain whether these currents have any function, but their presence should be noted when considering the stimulation of healing by electrical techniques.
Chapter 7: DC Ionic Currents within Eggs

In this chapter I move on to describe a series of measurements on the developing chick embryo, a much smaller system than the human leg. This demonstrates that the SQUID magnetometer can be used to make non-invasive measurements on small organisms that produce currents within a larger closed system.

As I mentioned in Chapter 1, Jaffe and Stern (1979) have shown that currents flow out of the developing chick embryo in vitro at the primitive streak stage, that is between about 6 - 20 hours after incubation. It therefore seemed reasonable to measure the magnetic field around developing chicks in ovo at this and later stages.

Before describing the method and results we obtained, and the conclusions that can be drawn from them, it is necessary to provide some basic information about the structure of eggs and the way in which the embryo develops (Lillie, 1952).

7.1 The development of the embryo and its membranes

Figure 7.1 shows the structure of a fertilised egg when it is laid. The shell consists of minute crystals of calcium carbonate. It is lined by two membranes, which are separated at the blunt end to form the air-sac. These membranes enclose
Figure 7.1. The structure of an egg when it is laid.

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Table 7.1. The composition of yolk and albumen. After Lillie, 1957.
the albumen, which contains proteins, carbohydrates, a trace of fats, and salts in an aqueous medium (Table 7.1). A dense layer of albumen around the yolk is prolonged in the form of two spirally coiled opalescent cords towards the blunt and narrow ends of the egg. These restrict violent movements of the yolk. The vitelline membrane surrounds the yolk, which contains a considerable amount of fat (33%) as well as protein and a little carbohydrate. The yolk and the albumen provide the nutrients for the developing chick. The relatively high fat content of the yolk means that it tends to float to the upper surface of the egg, and can therefore be approximately located within the egg. The embryo develops on the upper surface of the yolk. Because the yolk rises to the upper surface of the egg, the developing embryo is in contact with the egg membrane and will stick to this membrane if the egg is not turned several times a day to move the yolk within the shell.

By the time that the egg is laid, the embryo consists of a whitish disc about 3.4mm in diameter, known as the blastoderm, which lies on the upper surface of the yolk. The peripheral zone of the blastoderm is an opaque single layered sheet (the area opaca). This area becomes two-layered and grows to cover the yolk, and eventually forms the extra-embryonic membranes - the yolk sac, the chorion, and the amnion (v.i). The central part of the blastoderm (known as the zona pellucida) will form the chick itself. It consists of two layers of cells, separated by a space. The upper
Figure 7.2. Stages in the development of the chick, after Lillie, 1957.

a) At twenty-six hours incubation. The embryo is viewed from above.

b) At fifty hours, seen from the side, to show the extent of overgrowth of the blastoderm around the yolk.

c) At three and a half days, similar view.

d) The arrangement of the membranes at four days.

e) The embryo and its membranes at seven days.
layer, known as the epiblast, consists of tall columnar epithelial cells. The lower layer, the hypoblast, is less well organised and consists of a loose array of rounded cells.

If the egg is incubated at above 22 degrees centigrade (optimum temperature 38 degrees) both cell division and cell movement take place as the embryo and its membranes develop. The embryo develops with its long axis approximately at right angles to the long axis of the egg, though there is some variation in the exact angle, 25% of embryos deviating by as much as 45 degrees.

Various stages in development are shown in Figure 7.2. At 24 hours of incubation the hypoblast of the embryo has organised into a continuous sheet of cells, the epiblast has thickened centrally to form the primitive streak, and cells have passed through this into the mesodermal area. The rudimentary head process is visible. Meanwhile the peripheral area of the blastoderm is extending over the yolk, and contains blood islands in the region near the embryo itself.

By 50 hours the blastoderm covers nearly all the yolk. Blood vessels have formed within it and connect with the rudimentary heart and vessels of the embryo. In the embryo the heart, brain and head are clearly demarcated, the primitive eye is visible, and about 30 somites are visible within the body. The head end of the embryo has begun to turn
towards the sharp end of the egg.

By three and a half days the blood vessels of the yolk sac have extended. The embryo is now lying completely on its left side. It has visible limb and wing buds. The embryo is surrounded by the amnion, which separates it from the extra embryonic tissues, except at the umbilical region. The allantois has begun to form from the hind-gut.

By day seven, all the major organs and systems have been laid down. The yolk is completely covered by the yolk sac (except in one small region) and the allantois is fully formed. In the next two days it grows to surround the embryo and yolk sac, providing a large surface area for the exchange of oxygen and carbon-dioxide.

For the rest of the incubation period, the embryo grows and matures, gradually filling the egg. It hatches after twenty-one days of incubation.

7.2 Method

For all experiments eggs were incubated at 38 degrees centigrade. They were turned at least three times a day during incubation. At intervals each egg was removed from the incubator and placed on a non-magnetic support under the SQUID magnetometer. In most experiments the egg was scanned
Figure 7.3. The direction of scans across the egg.
along 5 transverse axes (see Figure 7.3). The data collection interval was 0.5 or 1 cm and each scan was averaged five times. After the experiment the egg was replaced in the incubator. This procedure took about ten minutes for each egg. There was little alteration in scans over this time, so it was concluded that any effect from cooling was negligible.

After preliminary experiments to determine the presence of detectable magnetic fields around the egg, and to exclude the presence of ferromagnetic materials, the following measurements were made:

i) Ten eggs were scanned for the first five days of incubation, five daily and five at six-hourly intervals. Of these eight eggs were viable.

ii) Four of these eggs were scanned daily until twenty one days of incubation. Three hatched producing normal chicks. The fourth contained a large mass of autolysing tissue - evidence of late death in ovo.

iii) Three (additional) eggs were scanned at three days to investigate the effects of cooling.

iv) Three (additional) eggs were scanned at three and four days to investigate the effect of movement on the magnetic field pattern.
Figure 7.4. The effect of cooling on the magnetic field around the egg. Scans were across the centre of the eggs.
v) Ten (additional) eggs were opened or windowed during the first five days to check that the presence of magnetic fields correlated with the presence of a developing embryo.

7.3 Results

Signals from the embryos were first clearly visible above the noise level of the system after 40-48 hours of incubation and they continued to be present until hatching. Attempts to magnetise the eggs with a permanent magnet produced no change in their signal. Eggs with no discernible magnetic field did not contain a developing embryo (one in five of the eggs used in these experiments proved to be non-viable on incubation). Eggs that showed signals and were opened, or windowed, always showed signs of development, though in one case the embryo seemed rudimentary with normal extra-embryonic membranes.

The magnetic field around the egg decreased with cooling, and reappeared with rewarming. Figure 7.4 shows the trace obtained from field scans across two eggs before, during, and after being placed in a fridge at 4 degrees centigrade. In egg (a) the magnetic fields rapidly regained their former strength; in egg (b) the signals reappeared with their original shape, but remained smaller than before cooling.

The form and magnitude of the magnetic field around the egg changed in a characteristic fashion during the incubation
Figure 7.5. The traces obtained from scans across the centre of one egg throughout the incubation period.
Figure 7.6. Contour maps of the vertical component of the magnetic field in a horizontal plane one millimetre above the highest point of the shell, in three eggs between day two and day five of incubation. Numbers refer to field strength in pT.
period. Discernible signals appeared in all the viable eggs studied at between 40 and 48 hours of incubation. These signals were initially bipolar, and increased in magnitude during the first four days of incubation, reaching a peak-to-peak signal amplitude of between 10 and 30 pT. After the fifth day the signals tended to decrease in magnitude and to increase in complexity and variability. Figure 7.5 shows the scans across the centre of one egg throughout the incubation period.

Figure 7.6 shows magnetic field contour maps over three eggs from day two to day five of incubation. These contour maps have been constructed manually from points on the magnetic field scans across the egg. By day four the maps all show a simple field reversal over the egg, though the angle that the null line makes with the long axis of the egg varies between different eggs and in the same egg from day to day.

This variation in angle could be due to rotation and movement of the yolk within the egg. Rotation around the long axis of the egg produces a change in the size of the signal. Rotation in the x-y plane produces a change in the shape and size of the signal. It seems likely that the changes in the positions of the magnetic field maxima and minima that we see in the first five days of incubation are due to movement of the eggs, and the consequent movement of the yolk within the egg.
7.4 Discussion

7.4.1 The source of the magnetic field.

The magnetic signals observed seem to correlate with the presence of a developing embryo, though this does not prove that they arise from the embryo itself. The fact that the signals disappear with cooling and reappear on warming suggests some source that is dependent on metabolic processes. This source could lie within the embryo itself, or within the extra-embryonic membranes. If the source of the currents is within the embryo, it is perhaps surprising that the magnetic field pattern retains a simple field reversal after day 2 of incubation, since the changes occurring within the embryo are complex, and it is gradually turning to lie with the left side uppermost. However, complexities of the magnetic field would probably be obscured by the poor spatial resolution of the gradiometer, with its diameter of 2.4 cm, and their absence does not preclude the embryo as a source. The currents could originate within the yolk sac membranes, since these epithelial cells contain ionic and molecular pumps and channels for the absorption of nutrients from the yolk into the extra embryonic blood stream.

7.4.2 The location and distribution of the current source.

The simple field reversal of the magnetic field over the egg between days 2 and 5 is consistent with a source within the
embryo such as a dipole current element (that is an infinitesimally short element of line current), of magnitude at 4 days of about 10μA. If this were so, then it should be possible to calculate the depth of the current dipole beneath the detector from the distance between the magnetic field maxima and minima.

Cuffin and Cohen (1977) have described the equations used in the calculation of the magnetic field around a spherical conducting volume containing a current dipole. If we assume the egg to be such a conducting sphere, and convert their equations to give the field in a plane above the egg, then we find that the distance, Δ, between the maxima and minima of the magnetic field in the x-y plane and the depth, d, of the dipole element below that plane are related in the following way:

\[ Δ ≈ 1.4d \]  

(Swithenby, personal communication)

The constant in this equation allows for the smearing over the gradiometer coils, but depends on both the ratio of the depth of the dipole to the radius of the sphere, and the angle between the perpendicular to the scan and that radius of the sphere that passes through the dipole. The quoted value is accurate to ±20% for egg results showing approximately symmetric field patterns.

From Figure 7.6 the distance between the maxima and minima in
egg 2 day 4 was 4.0 cm. This would give a dipole depth of 2.9
±0.6 cm. As the egg itself was only 4.6 cm in diameter, and its
upper surface was only 12 mm from the lowest coil of the
gradiometer, it seems unlikely that the source of the
magnetic field could be a current dipole associated with the
developing embryo itself, since it is on the upper surface of
the yolk. The fact that the dipole current model gives
improbably large depths suggests that the currents are more
complicated, or that they are associated with the extra-
embryonic membranes, rather than the embryo itself. Without
more invasive experiments it is not possible to determine the
exact source of the currents.

7.4.2. Comparison with other work on currents around
developing chick embryos.

The only other published work on currents associated with
chick embryos is that of Jaffe and Stern (1979). The currents
they noted were at, and just before, the stage of primitive
streak formation, i.e. between 6 and 20 hours of incubation.
The distribution of current they found was shown in Figure
1.5, though it should be noted that the return current
pathway back into the epiblast may have been altered by the
in vitro arrangement of the embryo. The dimensions of the
current loop shown are of the order of a few millimetres. The
small dimensions of this current loop mean that we would be
unlikely to see its magnetic field outside the egg, using the
SQUID magnetometer.
Figure 7.7. The change in potential difference between the two surfaces of a fertilised egg during the first half of the incubation period.
There is no other published work showing later current patterns around more mature embryos with which to compare our results. However, Vorontsov and Emchenko (1947) reported a potential difference between the top and bottom surface of the fertilised egg. The potential difference is first apparent after two days of incubation, then increases attaining a maximum at four days, the top surface being negative to the bottom by about 10mV. The field decreases after four days (Figure 7.7) and reverses. After ten days there is no measurable potential difference across the egg. These results are consistent in their time course with the magnetic field signals we obtain.

7.5 Conclusions

These experiments on developing chick embryos clearly show the presence of currents, in the range of 10μA, associated with the developing embryo and its membranes, and dependent on metabolic processes. Although the magnetic fields normal to a plane over the surface of the egg show a simple field reversal at day four of incubation, it is not easy to determine the source or distribution of these currents mathematically from the magnetic field pattern without more information as to their approximate source, distribution or magnitude.

This type of experiment shows both the strengths and the
weaknesses of magnetometry as a means of investigating small ionic currents. The method clearly shows the presence (previously undetected) of macroscopic currents within the egg. SQUID magnetometry provides a useful method of screening for such currents. However, the amount of information that can be obtained using entirely non-invasive methods is limited. To find out more about the source of the currents we have seen, it will be necessary to open the egg and to scan different parts of the embryo. Once the current source has been identified, then a model of current flow can be constructed. With a reliable current model, further information could be obtained from non-invasive magnetic field scans of the developing chick in ovo.
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providing the dipole term is non-zero (Grimes et al. submitted for publication). Higher order terms, such as the octupole, will then have a larger contribution, but since their field falls off more rapidly with distance, they will contribute little to the measured signal.

The above description of the magnetic field from a line-dipole is in analytical form. For our experiments, it is adapted to allow for the geometry of the gradiometer. The equations can then be used to fit model line dipole currents to experimental data. Such a fitting procedure will define a line dipole current in terms of its position, magnitude and orientation. It therefore comprises a four-parameter fit.
APPENDIX: The multipole expansion

This appendix is a short introduction to the multipole expansion described by Grimes et al (submitted for publication). It develops a multipole expansion of the vector potential field associated with a current distribution that is constant along the y-axis and confined to a finite region in the x-z plane. This is the multipole expansion used in analysing the magnetic field scans across the human leg.

In developing this analysis, the Cartesian-axis system shown in Figure A.1 is the natural choice of coordinates. Figure A.2 shows these coordinates within a cylinder - the x-z plane is transverse to the current flow. The origin about which the expansion is developed is designated as O. At a point P outside the current-carrying region the magnetic field $B(R)$ is the sum of contributions from current elements $\Delta I(\xi) = J_y(\xi)\Delta^2r$ where $J_y(\xi)$ is the y-component of the current density at $\xi$, and $\Delta^2r$ is the element of area in the x-z plane. Under the assumptions of our model $J_x(\xi) = 0 = J_z(\xi)$ and therefore $J_y(\xi)$ will be denoted simply as $J(\xi)$. From the circuital rule, the magnetic induction at $\sim R$ due to the current element $\Delta I(\xi)$ is

$$AB(\sim R) = \left[\frac{\mu_0}{2\pi}\right] \frac{\Delta I(\xi) \times (\sim R - \sim \xi)}{|\sim R - \sim \xi|^2}$$

Since

$$\frac{(\sim R - \sim \xi)}{|\sim R - \sim \xi|^2} = \nabla_{\sim R} \log |\sim R - \sim \xi|$$

$$AB(\sim R) = \left[\frac{\mu_0}{2\pi}\right] \frac{\Delta I(\xi) \times \nabla_{\sim R} \log |\sim R - \sim \xi|}{|\sim R - \sim \xi|}$$
\[ \mathbf{B} = \mathbf{\nabla} \times \mathbf{A} \]

or, substituting

\[ \mathbf{A}(r) = \hat{j} \mathbf{\int} \mathbf{r} \mathbf{J}(r) \mathbf{d}^2 \mathbf{r} \]

Substituting over the region of current flow,

\[ \mathbf{A}(R) = \mathbf{\int} \mathbf{d}^2 \mathbf{r} \mathbf{J}(r) \mathbf{log}|R - \mathbf{r}| \]

The logarithm can be expanded in powers of \( \mathbf{r} \) using the expression

\[ \begin{aligned}
\log|\mathbf{R} - \mathbf{r}| &= \exp(-\mathbf{r} \cdot \mathbf{V}_R) \log \mathbf{R} \\
&= (1 - (\mathbf{r} \cdot \mathbf{V}_R) + \frac{1}{2}(\mathbf{r} \cdot \mathbf{V}_R)^2 - \ldots\ldots) \log \mathbf{R}
\end{aligned} \]  

This expansion generates a corresponding multipole expansion for \( \mathbf{A}(R) \), labelled, by convention, as follows:

\[ \mathbf{A}(R) = \mathbf{A}^{(0)}(R) + \mathbf{A}^{(1)}(R) + \mathbf{A}^{(2)}(R) + \ldots\ldots \]  

The monopole term is

\[ \mathbf{A}^{(0)}(R) = -\left[ \frac{\mu_0}{2\pi} \right] \mathbf{\int} \mathbf{d}^2 \mathbf{r} \mathbf{J}(r) \]  

In the leg experiments this term must be zero as it is electrophysiologically necessary for there to be zero net current along the leg.
The dipole term is

\[ A^{(1)}(\mathbf{r}) = \left[ \frac{\mu_0}{2\pi} \right] \mathbf{\hat{r}} \int d^2r J(\mathbf{r}) \left( \mathbf{r} \cdot \mathbf{\nabla}_R \right) \log R \]

\[ = \left[ \frac{\mu_0}{2\pi} \right] \mathbf{\hat{r}} \int d^2r J(\mathbf{r}) \mathbf{r} \cdot \frac{\mathbf{R}}{R^2} \]

If we define the line-dipole moment by

\[ \mathbf{m} = \int d^2r J(\mathbf{r}) \mathbf{r} \]

then

\[ A^{(1)}(\mathbf{r}) = \left[ \frac{\mu_0}{2\pi} \right] \mathbf{\hat{r}} \frac{\mathbf{m} \cdot \mathbf{R}}{R^2} \]

The magnetic induction associated with the dipole term is

\[ B^{(1)}(\mathbf{r}) = \left[ \frac{\mu_0}{2\pi} \right] \mathbf{\nabla}_R \times \left[ \frac{\mathbf{m} \cdot \mathbf{R}}{R^2} \right] \]

\[ = \left[ \frac{\mu_0}{2\pi} \right] \frac{1}{R^4} \left( \mathbf{R}^2 \mathbf{m} - 2(\mathbf{m} \cdot \mathbf{R}) \mathbf{R} \right) \times \mathbf{\hat{r}} \]

The sensing coils are only sensitive to the z-component of the field, which is

\[ B_{z}^{(1)}(\mathbf{r}) = \mathbf{\hat{z}} \cdot B^{(1)}(\mathbf{r}) \]

\[ = \left[ \frac{\mu_0}{2\pi} \right] \frac{1}{R^4} \mathbf{\hat{z}} \cdot \left( \mathbf{R}^2 \mathbf{m} - 2(\mathbf{m} \cdot \mathbf{R}) \mathbf{R} \right) \]

It is convenient to specify the dipole moment, \( \mathbf{m} \), by its magnitude, \( m \), by its position in the x-z plane, and by its angle \( \phi \) with respect to the z-axis (Figure A3). A representative point, \( \mathbf{R} \), along the line of sweep of the
lowest coil has Cartesian coordinates, \((x,z)\). From the figure

\[
\begin{align*}
\mathbf{R} \cdot \hat{1} &= x \\
\mathbf{m} \cdot \hat{1} &= m \sin \phi \\
\mathbf{m} \cdot \mathbf{R} &= m(z \cos \phi + x \sin \phi)
\end{align*}
\]

Using these relationships, the dipole contribution to the \(z\)-component of magnetic induction can be written

\[
B_z^{(1)}(x,z) = \left( \frac{\mu_0}{2\pi} \right) m \left[ \frac{(z^2-x^2) \sin \phi - 2zx \cos \phi}{(z^2 + x^2)^2} \right]
\]

Evaluation of the quadrupole contribution \(B_z^{(2)}\) is more complicated and will not be considered in this appendix.

Each successive term in the multipole expansion depends on one higher power of \(1/R\) than its predecessor. In making typical measurements, the lowest sensing coil has a distance of closest approach to the top surface of the leg of about 12 mm. Thus, at first thought, it might appear that the quadrupole contribution \(B_z^{(2)}\) would contribute significantly to the measured signal, unless the currents are either distributed in such a way as to produce a small quadrupole term or are localised within a part of the leg well separated from the line of sweep of the lowest coil.

In fact, the quadrupole contribution depends on the choice of origin relative to the current distribution; it can be shown that an origin can be chosen such that \(A^{(2)}(R)\) vanishes,