THE EFFICACY OF DIFFERENT METHODS OF PREPARATION OF ULTRA HIGH DILUTIONS OF GIBBERELLIC ACID ON THE SYNTHESIS OF α-AMYLASE IN DE-EMBRYONATED ENDOSPERM HALVES OF BARLEY SEED.

By

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Dissertation submitted in partial compliance with the requirements for the Master's Degree in Technology: Homoeopathy in the Department of Homoeopathy at the Technikon Natal, Durban.

I, Tamara Balding, do hereby declare that this dissertation represents my own work in both concept and execution.

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DEDICATION

I DEDICATE THIS RESEARCH TO MY FAMILY, WHO HAVE GIVEN ME LOVE SUPPORT AND UNDERSTANDING IN THE WRITING OF THIS DISSERTATION AS WELL AS THROUGHOUT MY ACADEMIC CAREER. TO THE LORD GOD WHO HAS GIVEN ME OPPORTUNITIES AND ANSWERED PRAYER IN MYSTERIOUS AND WONDERFUL WAYS I AM INDEBTED.

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ABSTRACT

The mode of action of homoeopathic remedies is not well understood and is questioned by the scientific community due to the high degree of dilution of such remedies. The method by which remedies are produced is thought to instill the efficacy into these remedies. This research examines whether effective homoeopathic remedies may be prepared by electromagnetic induction, by serial dilution alone, or by conventional Hahnemannian production, which includes succussion. These methods were investigated in terms of the ability of dilutions of gibberellic acid, prepared by the above methods, to induce production of α-amylase by de-embryonated endosperm halves of barley seed.

Dilutions of the plant hormone gibberellic acid were prepared in three ways: Hahnemannian dilutions (succussed), serial dilutions (unsuccussed) and water electromagnetically imprinted with Hahnemannian dilutions. The electromagnetic dilutions were prepared with a Nexus 21 machine, using the Hahnemannian dilutions as a template for required electromagnetic frequency. This frequency was played back onto a vial of plain distilled water. The 4^{th}, 9^{th}, 15^{th}, 30^{th} and 200^{th} dilutions were investigated.

An incubation buffer [$Ca(NO_3)_2$] was added to all the dilutions and to distilled water (control) resulting in a concentration of 20mM. The solutions were applied to de-embryonated barley seeds. Gibberellic acid is produced by the barley seed embryo, and is vital to germination due to its stimulation of α-amylase synthesis. The α-amylase synthesis in de-embryonated barley seeds is a sensitive indicator of the presence of gibberellic acid and is dose-dependent.

The endosperm halves of the seeds were divided into groups of 20 half seeds, weighed and placed into petri dishes, lined with filter papers, and inoculated with 5ml of the relevant dilution. These were incubated for 48 hours at 16°C in a dark incubation chamber. Incubation was terminated by freezing. The seeds were thawed, macerated and the enzyme extracted. The α-amylase activity was measured using a dye-labeled substrate, Phabedas® tablets. These tablets react with α-amylase releasing a dye. Absorbance was measured at 620nm and converted to enzyme units using the α-amylase standard curve (based on pure α-amylase – 1650u/mg). The amount of the enzyme, α-amylase, produced per gram of dry seed was utilized as data for statistical analysis.

Data were analysed for significance of differences among treatment groups by two-way analysis of variance (ANOVA). The level of significance was set at $\alpha = 0.05$. Statistical analysis showed no differences between interactions between the method of preparation of the dilution and the degree of dilution ($p = 0.4937$) or the degree of dilution ($p = 0.3322$). There was a significant difference in the different methods of dilution ($p = 0.0170$). Multiple comparisons (least significant difference and Duncan's test) were performed which showed that there were differences between the electromagnetic dilutions and the Hahnemannian dilutions and serial dilutions.

It is clear that the electromagnetically imprinted remedies were not effective in terms of the production of α-amylase by de-embryonated endosperms of barley seeds. The biological action of Hahnemannian dilutions and serial dilutions could not be confirmed.

The value of succussion in the preparation of homoeopathic remedies could not be determined. These two areas warrant further investigation.

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ABBREVIATIONS

GA_3 – Gibberellic acid

cH – Centesimal Hahnemannian

cS – Centesimal Serial

cE – Centesimal Electromagnetic

Pertaining to the methods of dilution:

Hahnemannian – according to the method of Dr Samuel Hahnemann, as recorded in the German Homoeopathic Pharmacopoeia (British Homoeopathic Association 1985) i.e. serial dilution with succussion.

DEFINITION OF TERMS

Centessimal
1:100 deconcentration scale originally introduced by Hahnemann.

Dilution
The method of mixing one liquid with another liquid. The more one liquid is added the weaker the other liquid becomes (Banerjee, 1987:19).

Heisenberg's Uncertainty Principle
The uncertainty of a simultaneous measurement concerning both the momentum and position of subatomic particle.

Magnetic Moment
The intrinsic magnetic dipole, which itself is generated by the overall spin of a charged nucleus along its spin axis.

Mother Tincture
The strongest liquid preparation used in homoeopathy and made by maceration of the drug or portion of it in alcohol or water (Banerjee, 1987:24). This is prepared directly from the crude drug and prepared according to homoeopathic technique so that all the medicinal properties of a drug are extracted (Banerjee, 1987:181).

Potency
A dilution which has been either succussed or triturated. It is the process by which, what Hahnemann in the 5th edition of Organon of Medicine (183), refers to as "the latent curative properties", of the drug are brought into action (Banerjee, 1987:51).

Pharmacopoeia
A reference book where the preparation, uses, and contents of medicines are described.

Succussion
Rhythmical violent agitations carried out, either by hand or machine (Banerjee, 1987:24).

CHAPTER 1

INTRODUCTION

1.1 Introduction

The action of homoeopathic dilutions is doubted by the scientific community due to their high dilution (Linde *et al.*,1997; Kleijnen *et al.*,1991). Today many homoeopathic researchers are grappling to make sense of these dilutions. There are two models into which current theories fit: the geometric model and the dynamic model (Schulte, 1999). These models rely on the geometric nature of water molecules and the dynamic properties of water and how it interacts with other substances. Both models consider water to be imperative to the storage of remedy information. These models and others will be discussed more fully in Chapter 2.

Succussion is thought to be involved in the transfer of information to highly diluted solutions (Kayne, 1997:49). It is also thought that electromagnetic induction may be able to transfer remedy information to water (Inglis, 1964:23). By examining these processes it is hoped that light may be shed on the nature of the dilutions and currently held theories may be tested.

Plant research provides an objective and ethical method of experimentation (Zacharias, 1997). Research using plants is vital to dispelling skepticism about the placebo effect and researcher bias, which may be present in clinical homoeopathic trials (Kleijnen *et al.*, 1991).

Much research has been conducted on barley seed and its response to gibberellic acid. The Phabedas® test method has been found to be an accurate way to test for the presence of α-amylase in barley endosperms. De-embryonated seeds are known to produce α-amylase in response to gibberellic acid. The test is sensitive and accurate in determining the presence of gibberellic acid (Barnes and Blakeney, 1974).

This research compared serial dilution, succussion and electromagnetic imprinting as methods of preparing homoeopathic dilutions of gibberellic acid. Biological efficacy was tested using a plant model. The Phabedas® test method was used to determine whether barley endosperm half seeds responded to the dilutions by producing α-amylase.

1.2 Aim of the Study

The precise mechanism of storage of information by ultra high dilutions remains unknown. The aim of this study was to investigate succussion and electromagnetic imprinting as methods for transferring information to ultra high dilutions. Dilutions produced by electromagnetic imprinting were compared with those produced by unsuccussed serial dilution and with those produced by Hahnemannian methods.

1.3 Statement of the Objectives

1.3.1 The First Objective

The first objective was to evaluate the efficacy of Hahnemannian dilutions of gibberellic acid in inducing α-amylase production in barley endosperm half seeds.

1.3.2 The Second Objective

The second objective was to evaluate the efficacy of unsuccussed serial dilutions of gibberellic acid in inducing α-amylase production in barley endosperm half seeds.

1.3.3 The Third Objective

The third objective was to evaluate the efficacy of water electromagnetically imprinted with Hahnemannian dilutions of gibberellic acid in inducing α-amylase production in barley endosperm half seeds.

1.3.4 The Fourth Objective

The fourth objective was to compare the effect of the method of preparation of dilutions on the induction of α-amylase in barley endosperm half seeds.

1.3.5 The Fifth Objective

The fifth objective was to compare the interaction effect between the method and dilution levels of each method on the induction of α-amylase in barley endosperm half seeds.

1.4 Statement of the Hypotheses

All hypotheses are stated in the null form.

1.4.4 The First Hypothesis

It is hypothesised that Hahnemannian dilutions of gibberellic acid will have no significant effect on the induction of α-amylase production in barley endosperm half-seeds.

1.4.5 The Second Hypothesis

It is hypothesised that unsuccussed serial dilutions of gibberellic acid will have no significant effect on the induction of α-amylase production in barley endosperm half-seeds.

1.4.6 The Third Hypothesis

It is hypothesised that water electromagnetically imprinted with Hahnemannian dilutions of gibberellic acid will have no significant effect on the induction of α-amylase production in barley endosperm half-seeds.

1.4.7 The Fourth Hypothesis

It is hypothesised that there will be no significant difference between the method of preparation of dilutions on the induction of α-amylase in barley endosperm halves.

1.4.8 The Fifth Hypothesis

It is hypothesised that there will be no significant interaction between the method of dilution and the dilution levels on the induction of α-amylase in barley endosperm half seeds.

1.5 Significance of the Study

This study contributes to the knowledge base of homoeopathic pharmacy by assessing different dilution preparation methods and their ability to transfer information from the mother tincture into dilutions.

The validity of preparation methods was tested, so that important factors in the preparation of homoeopathic remedies can be emphasised in standard methodologies, and the ineffective factors excluded.

1.6 Implications of the Study

The study provides an objective method of investigation of the biological effectiveness of homoeopathic remedies, using a scientifically acceptable methodology upon which further research may be based.

The study brings electromagnetically imprinted dilutions into question in terms of their validity and efficacy. The study challenges whether succussion is vital to the production of remedies.

1.7 Benefits of the Study

Banerjee (1987:285) suggests that finished preparations should undergo chemical and physical control. He notes that it is an unfortunate fact that until now there has been no standard laid down by any authority. This study presents a model in which homoeopathic remedies may be compared and analyzed according to a set standard.

CHAPTER 2

REVIEW OF THE RELATED LITERATURE

2.1 The Preparation of Hahnemannian Dilutions

The British Homoeopathic Association states that homoeopathic medicines may be made from any substance when a specific method of preparation is followed. A liquid which contains the greatest concentration of the drug to be used is prepared in accordance with the composition of the drug; this is called the mother tincture. When making up remedies with a drug substance which is soluble, water or alcohol is used as a diluent to prepare the mother tincture (Banerjee, 1987:47). The mother tincture is then used to make up decimal or centesimal potencies (The British Homoeopathic Association, 1981:16). When making up decimal potencies, 1 part of the mother tincture is added to 9 parts of an inert liquid, such as distilled water or alcohol. In centesimal potencies, 1 part of the mother tincture is added to 99 parts of an inert liquid. This is followed by succussion, which is a vigorous mixing or shaking. The remedies are then known as 1X and 1cH respectively. One part of each of these remedies is added to nine or ninety-nine parts to continue the process of decimal or centesimal dilution respectively (The British Homoeopathic Association, 1981:16-18). Hahnemann himself introduced the centesimal scale and according to the British Homoeopathic Association, it is the scale most commonly used (1981:18). Homoeopathic medicines are produced by a series of dilutions and succussions, aimed at endowing the solutions with a greater therapeutic effect (Bellavita and Signorini, 1995:10).

After noticing the unnecessary aggravations of symptoms caused by administering large doses of medicines, Hahnemann attempted to minimize these effects by dilution (Banerjee, 1987:54). Hahnemann used these very weak dilutions as he felt that remedies given in their natural form were too powerful. In the natural form there was an excessive initial reaction, followed by a marked aggravation as well as side effects (Koehler, 1989:33). Hahnemann says in his book *Chronic Diseases*, that high dilutions ought to be used in sensitive patients to prevent such violent reactions (1992:XX). Homoeopaths feel that the higher the dilution, the more powerful the remedy, despite the paradox that the higher the dilution, the less likely one is to find even one molecule of the original substance in the dilution (Gerber, 1996:81). Today, the use of ultra high dilutions has become an accepted and vital axiom in homoeopathy, known as the law of the minimal dose. This means that the most minimal amount needed to result in a reaction is given as treatment.

2.2 Highly Diluted Medicines

2.2.1 Skepticism about Homoeopathic Dilutions

Hahnemann himself poses the question of how extremely small doses of highly attenuated medicine still have great power, in Volume 2 of his *Materia Medica Pura*. He suggests that succussion and trituration result in a change in the medicine. They liberate the dynamic medicinal powers of the substance, forming an intimate mixture and distributing the medicine uniformly thoughout the entire mass of the diluent. (1992: 43-4.)

Some pharmacists feel that there is a lack of evidence explaining homoeopathic remedies (Kayne, 1993). Linde *et al.* (1997), writing in the medical journal, *The Lancet* questioned whether the clinical effects of homoeopathy were due to placebo effects. This type of question is commonly asked by allopathic health care practitioners and other scientists, who find the principles upon which homoeopathy is based to be implausible. Despite this, in a meta-analysis of placebo-controlled trials, Linde *et al.* (1997) found that the clinical effects of homoeopathy were not entirely due to placebo. Kleijnen *et al.* (1991) in their study on clinical trials in homoeopathy similarly found a positive trend pointing towards the efficacy of homoeopathy.

2.2.2 Avodadro's Number

Commonly prescribed homoeopathic dilutions surpass Avogadro's number. Avogadro deduced that equal volumes of gasses at the same temperature and pressure contain the same number of molecules. The number of molecules per mole (the molecular weight in grams) was measured by Loschmidt with high precision at $6{,}022045 \times 10^{23}$ molecules per mole, and is the same for all substances. This would imply that when a homoeopathic dilution had been diluted 24 times resulting in a 12cH or a 24X, with a deconcentration value of 1×10^{24}, there would not be even a single molecule of the original substance left in the solution. An atom is defined as "that which cannot be divided" (Schiff, 1994:7). In ultra-high homoeopathic dilutions it would therefore appear that the divisions have surpassed the number of atoms available. Despite this, clinical results (as well as veterinary and horticultural results) are claimed for potencies with a higher deconcentration than Avogadro's number, and are supported by research

studies (Smith, 2000; Linde *et al.*, 1997; Yakir *et al.*, 2001; Sandoval *et al.*, 1998; Searcy *et al.*, 1995; Betti *et al.*, 1994; Bornoroni, 1991; Saxena *et al.*, 1986; Him Lok, 2001).

2.3 Theories of Mechanism of Storage of Information by Homoeopathic Remedies

Theories regarding the 'memory' of water attempt to explain the capacity of water molecules to organize in a stable way so that they can store information (Schiff, 1994:9). Scientific research has been conducted on the ability of water to store structural information. This research is of great interest to homoeopaths as it provides a means of explaining how information may be stored in aqueous solutions. At lower levels of dilution, the interaction between solute and solvent atoms predominates, while order and structure of the solvent are less dominant. At higher levels of dilution (*i.e.* beyond Avogadro's number), the solvent alone controls the order formation. This order formation has given rise to many different theories. There are two important theories on how water stores information about homoeopathic remedies. The first theory proposes that order is brought about by geometric structures in water, and the second maintains that order is brought about by the dynamic properties of water (Schulte, 1999).

2.3.1 Geometric Models

Berezin (1994:169) proposes that isotopically different atoms form the basis of order. The sequence of isotopic diversity becomes the core order parameter. Isotopic coding may not be influenced by electromagnetic means; instead nuclear forces are needed to influence nuclear properties such as isotopic variation. An indirect influence is thought to

be possible with electromagnetic forces through the coupling of hydrogen bonds. A global phase transition would cause changes in temperature and energy due to the rearrangement process and though small changes in specific heat and temperature can be measured accurately, such an effect has not been reported (Schulte, 1999).

Anagnoststos (1994:128) proposed a replicating clathrate model. The clathrate is thought to be developed by an external influence or contamination, such as the introduction of a mother tincture to a solution. The structure in the form of a cluster is thought to give rise to a localized short-range order, which in turn gives rise to the formation of clathrates or expanded clusters, which contain smaller cluster clathrates inside them. The clathrates are thought to be the mould, responsible for the replication of order. The water molecules become organised so that the information is stored in an order which is maintained by the transfer of the clatherate pattern throughout the solvent. The information is stored in the particular clathrate bonds and the bond lengths. It should be possible to test this theory by standard spectroscopic techniques, but no experimental evidence has been reported thus far (Schulte, 1999).

Antonchenko and Ilyin (1992) propose that hydration shells form the basis for the 'memory' of water. All substances when dissolved in water result in an individual and specific hydration shell. These shells are regions near the dissolved substance in which there is an alteration in the statistical proportion of molecules as compared to pure water. These hydration shells may be intact shells or fragments of shells which seed the development of larger clusters. In homoeopathic remedies hydration shells are formed by

the solvent remaining present even when the solute, around which the hydration shells were formed, has been diluted out. (Antonchenko and Ilyin, 1992).

2.3.2 Dynamic Models

Dynamic models are based on the knowledge that matter is perpetual motion. Theorists who use this basis relate the properties of water to the physics of quantum mechanics. Molecules are not considered to be isolated, but united by a continuous charge density pattern which extends throughout the universe. Reducing substances to their smallest parts and understanding the properties of each part does not shed light on how the substances behave in a system or collectively.

Quantum mechanics is a theory of modern physics in which packed molecules are thought to form coherent domains because such organization results in lower energy levels. Molecules spontaneously go from a chaotic state into a more ordered state if this represents a lower energy state. This relies on the principle that the most stable state of a substance is that which requires the least energy (Schiff, 1994:15).

Quantum physics would point to the fact that human beings and other life forms are not simply composites of atoms or living machines. Instead, there is a connection to, and influence by, universal energy fields (Eden, 1993:32). Eden states that the origin of the concept that human beings have a field of vibrating energy that surrounds and interpenetrates the human body is lost in antiquity. It seems that almost every culture

studied by modern anthropologists has recognized the existence of a human energy field and incorporated manipulation of that field into their healing traditions. (Eden, 1993:35.)

The physicist Max Plank hypothesized that atoms do not emit or absorb energy continuously, but in the form of energy packets, which he called quanta. The energy propagates as electromagnetic waves and behaves as particle-photons when interacting with matter. This means that matter has a dual nature. Scientists attempting to examine matter at the subatomic level have found that it does not exist in any degree of certainty in any particular plane which is embodied in Heisenberg's uncertainty principle. Predictions can therefore only be made in terms of probability. Modern physicists have concluded that energetic or waveforms of matter are not similar to sound waves or waves propagated in water, but are actually waves of probability. The conclusion is that the universe is a huge inseparable dynamic and perhaps incomprehensible web of energy fields that continually flow, change and interact and are never static or disconnected. (Eden, 1993:52-54.)

Resch and Guttman (1991) propose a supermolecular system organization of water. The organization occurs in hierarchical levels. The first level is at the interface and is a defense and communication with the environment. Interactions occur between the phases, resulting in a high degree of tension in the molecules. The second hierarchical level of organization is due to hydrophobic molecules, such as gas molecules. The oscillations of these gas molecules must come into harmony with those of the water molecules. Since these oscillations are influenced by the oscillations of the water molecules, they take on

and store structural information from the solution, forming synchronization nodes. The third hierarchical level is formed by the hydrophilic solutes. These solutes are influenced by the liquid and in turn influence the liquid by the creation of hydration shells. Through organisation of the water molecules beyond the shells, the information from the solutes is spread over the whole solution and stored in the oscillating patterns of the hydrophobic gas molecules. During potentisation, information is not lost, but spread out over the more dilute liquid. Molecules of the lower hierarchical level decrease in number while the hydrophobic gas molecules increase in number. Dissolved gases in the spaces between water molecules are tuned to the vibrational pattern of the fluid to maintain information. The dilution thus becomes more dynamic with information recorded in the solution in the oscillating patterns of gas molecules. (Resch and Guttman 1991.)

Del Guidice and Preparata (In: Bellavite and Signorini, 1995:249) propose that in the liquid state, water molecules interact via the hydrogen bonds between the molecules as well as an electromagnetic radiation field which brings order to the vibratory motion of the molecules. Large numbers of these molecules interact by means of an electromagnetic field and begin to be kept in phase by the field itself (Bellavite and Signorini, 1995:249). Water molecules oscillate between a ground and an excited state. Instead of being spheres, they may be considered as many sets of ovoid shaped molecules which are arranged in an orderly manner within a coherent domain (Del Guidice, 1996:43). These molecules pulsate and rotate inside an electromagnetic field, in the coherent domain, shifting from a sphere to an ovoid shape with discontinuity. They are thus in a coherent domain influenced by electromagnetic fields. Molecules are clustered in these coherent

domains with a diameter equal to the wavelength of the electromagnetic oscillation of the molecules. The domains minimize energy, unifying the phases of the relevant electromagnetic fields into a common coherent system. The electron clouds in these domains can be in a low energy state or an excited state in which it is possible for an electron to jump onto a nearby molecule. This produces a collective rotation of the coherent domains. The electron cloud of the coherent domain rotates and this produces a magnetic field according to the angle of rotation, the angular rotational velocity and the rotational frequency (Del Guidice, 2000). This acquired magnetic moment is able to interact with an external magnetic field and in this way an electromagnetic field becomes imprinted in water (Del Guidice, 1996:43).

Del Guidice and Preparata showed that in condensed matter electromagnetic fields could be trapped in coherent regions. Matter containing coherent regions is more stable than matter without such regions. These regions can cause condensation through coordinated oscillation of molecules and emission of their electromagnetic fields. Del Guidice and Preparata showed that an external electric field could create metastable extended polarization fields in a coherently moving system of water molecules. It should therefore be possible to create low frequency polarization fields in water as well as modifying already existing coherent fields. This means that it is not necessary for a mother tincture to be added to a solvent for it to develop a coherent region. Instead, a specific electromagnetic field could influence the formation of such coherent regions (Schulte, 1999).

Delinick (1996:50-52) conducted research at the National Technical University of Athens to develop and refine a machine able to measure the electrical properties of homoeopathic remedies. He concluded that during the potentisation process remedies undergo electrochemical reactions which convey specific information to the solution from the homoeopathic substance. In testing remedies, each remedy exhibited a specific waveform or frequency. This was determined by measuring the resistance, electric field and conductivity of the remedies and using Fourier analysis as a mathematical technique to calculate the frequency of the electromagnetic wave.

Shulte and Endler hypothesize that a mesoscopic model of intramolecular interaction leads to distinct order parameters. Coherent stable excitations of groups of atoms form stable aggregations of rigid or loose bound molecules. Excitations can be treated as quasi-particles. Quasi-particles are used in nuclear physics to describe real, measurable physical entities and are similar to the phonons (units of sound) of solid state physics. The mechanism of quasiparticle formation can be predicted and measured as a 'fingerprint' in their respective energy spectrum, in this case the infrared region. (Shulte, 1999.)

2.4 Succussion

Kayne defines succussion as the action of vigorously shaking a homoeopathic medicine in its vial or bottle, where each stroke ends with a jolt against a leather-bound book or the heel of the hand (1997:49). Mechanical shakers are also used (Kayne, 1997:49).

Since no molecules remain in potencies above a certain level, it would seem that at some stage a change of state occurs. Succussion is thought to be involved in an energy development or release in the remedies. Modern methods of analysis seek to understand what properties develop in the liquid, rendering the medicament active (The British Homoeopathic Association, 1981:18). Potentisation, the process of dilution and succussion, is thought to bring remedies from a latent state into one in which they become curative (Banerjee, 1987:51). Banerjee proposes that potentisation has various functions in remedy production. It converts drug substances into a dynamic state. Since Hahnemann sees disease as an alteration of the vital force on the dynamic plane, a dynamic drug is required to effect a cure on this plane. Potentisation is reputed to increase the pharmacological and therapeutic qualities of the drug.

Vithoulkas (1980:102) suggests that succussion adds kinetic energy to solutions. He claims that a remedy may be raised by one potency by succussion alone, without diluting the remedy. Thus the combination of succussion and dilution increases the therapeutic power of a remedy, beyond the point of even one molecule of original substance remaining.

Another possible explanation is that solvent molecules may be electrochemically changed by the process of succussion, enabling the solvent to 'memorize' an imprint of the original remedy (Kayne, 1997:49). This would explain how the base drug becomes less important with potentisation and ceases to function biochemically, but the biophysical

action is influenced by the kinetic energy transferred during succussion and the re-alignment of the solvent solution.

Hahnemann himself varied the number of succussions used when making remedies. The extent to which vials are shaken seems to be dependant on the individual homoeopath (Kayne, 1997:49) or manufacturer (Keijnen, 1991) preparing the remedy. The German Homoeopathic Pharmacopoeia sets the number at 10 (British Homoeopathic Association, 1985). A homoeopathic remedy is generally agitated at a frequency of 2Hz, although the reason for the selection of this frequency remains unknown. In practice the frequency may be 1 - 4Hz, and agitation may be by hand or mechanical (Zacharias, 1997).

Sukal *et al.* (1996) conducted studies on the effect of succussion, using successive dilution and sonication. Sonication was used in a previous study by these authors and was found to be more effective than succussion. Catalepsy was induced in Swiss albino mice and the mice were then treated with Agaricus muscarius. Remedies produced without sonication no longer produced an effect at the 30cH level, while those produced with sonication did have an effect. The authors concluded that potentised Agaricus muscarius 30cH contains an active principle that can be attenuated by dilution and multiplied by mechanical agitation and sonication.

A further study by Sukal *et al.* (2001) compared the effect of succussed and unsuccussed *Nux Vomica* 30cH on toads dosed with ethanol. Both the succussed and unsuccussed *Nux Vomica* 30cH produced a significant regain of the righting reflex relative to controls,

tested by the γ^2 and Mann-Whitney *U*-test. The electronic, infrared and nuclear magnetic resonance spectra of the remedies showed marked differences between the *Nux Vomica* 30cH preparations, and their dilution media.

Studies by Harisch and Dittmann (1997) on the efficacy of potentised and non-potentised substances showed a difference in effect between these substances. Mercury hydrogen phosphate, arsenic trioxide and sodium chloride were given to rats in a potentised form for the treatments group and an unpotentised form for the control group. Twenty-four hours after the last dose of the dilution, the rat livers were removed and the activity of the liver enzymes was assayed. In rats treated with mercury hydrogen phosphate treated rats, in 56% of the dilutions tested, comparisons between the un-potentised and potentised dilutions differed significantly. For arsenic trioxide treated rats, the value was 52% and for sodium chloride treated rats, the value was 22%. The percentage of PMP 70, a protein found in the peroxisomal membranes, was measured using the total membrane fraction obtained from the hepatic homogenate as an index. They concluded that there was a difference in effect between the un-potentised and potentised dilutions.

In the same series of studies Harish and Dittmann (1997) conducted a study on an *in vitro* cell-free system. Potassium cyanide and arsenic trioxide were prepared as potentised and unpotentised dilutions. These were applied to urate-oxidase (obtained from rat liver), urate-oxidase (obtained from hog liver), acid phosphatase (obtained from rat liver), acid phosphatase (obtained from potato), Cytochrome P-450 reductase (CYP 2E1; microsomal system from rat liver), xanthine oxidase/ dehydrogenase (rat liver) and

glutathione s transferase (cytosolic systems from rat liver). Biochemical differences in the identical active substance content between the dilutions were demonstrated. Unfortunately the exact statistical methods used were not given.

Christie (1995) applied succussed and unsuccussed homoeopathic potencies of Pulsatilla; Kalium carbonicum, Natrum muriaticum and Psorinum at the 8cH level, to yeast cell cultures. The medicines were separately added to the *Saccharomyces cerevisiae* cultures and the growth of the yeast was determined by direct cell counting. The application of Pulsatilla showed no difference from the control. The cultures treated with Kalium carbonicum and Natrum muriaticum showed a significant difference from the control. The optimum number of mechanical succussions was 80. No optimal number of succussions was identified in the hand succussed potencies. The Psorinum showed a significant difference from the control with an optimum of 40 succussions in both the hand and mechanically succussed potencies. The experiment found that both hand and mechanical succussion do play a significant role in the preparation of homoeopathic medicine. The statistical method used was the analysis of variance.

Bruni (2001) compared serial dilutions to succussed dilutions. Abscisic acid in the 4^{th}, 9^{th}, 15^{th}, 30^{th} and 200^{th} dilutions and a control, of water with a buffer, were added to de-embryonated barley seeds. The seeds were incubated and α-amylase produced by the seeds was assayed. After data analysis by the univariate analysis of variance, the ultra-high serial dilutions were found to be biologically active as there were differences between the control groups and the treatment groups. There was a significant difference

between the unsuccussed dilutions and the succussed dilutions and control. There were however no differences between the succussed dilutions and control. Bruni concluded that although ultra-high dilutions were biologically active, succussion could not logically be linked to the biological activity of homoeopathic dilutions.

2.5 Electromagnetic Medicine and Radiesthesia

Another method of remedy preparation has emerged in recent years, namely electromagnetic imprinting. It is thought that some form of energy storage must occur within the molecules in biological systems. One of the ways in which energy may be stored is by vibrational energy. This vibrational energy is found in the infrared part of the spectrum, where heat is also found. It is thought that in conventional remedy production, succussion causes particles to collide and vibratory energy is passed from the drug source to the water. This leaves the water with a vibratory imprint of the drug. (Inglis, 1964:238.) In electromagnetic remedy production the electromagnetic frequency of the substance is measured and transferred to a computer via infrared. This stored frequency is then played back to a vial of water into which the frequency becomes imprinted (Middleton, 2000). The equipment used in radiesthesia is termed a "black box" or simply a "box".

This method is often denounced as being non-sensical and fraudulent (Inglis, 1964:238). Vithoulkas (2000), one of today's most respected homoepaths, in a letter to the editor of the *Homoeopathic Links* journal dubbed the use of machines such as this an absurdity.

He called on homoeopaths to steer clear of such obvious nonsense, saying that it should not be tolerated.

Albert Abrams, the founder of radiesthesia, was interested in radium in treatment and proposed that radiation is a universal property of matter. If matter and energy were so bound up that they were indistinguishable at the atomic level, he argued that the basis of disease must be electronic rather than cellular. Differences in body radiations in health and disease should therefore be detectable with the equivalent of a radio receiving set, which could be used as a diagnostic tool. Transmitting equipment of a similar nature could be used for treatment. Abrams produced a machine for this purpose, known as the Abrams's "box". The production of similar devices continued. In the United States, the Dr Ruth Drown and in Britain, the de la Warr instruments are the best known. (Inglis, 1964: 246-247.)

The Abrams box consists of 9 variable rheostats, which are calibrated in turn to a detector unit. The instrument is not electrically powered. A vibration rate is read by the machine and allotted to each organ and tissue in health. A drop in the reading indicates disease, including diseases caused by pathogens such as parasites or viruses. Diagnosis involves turning the 9^{th} dial and feeling for a sticky sensation on the rubber pad in the detector unit. The treatment boxes aim to put the patient in series with the machine to use the patient's own energy as a means of treatment (Inglis, 1964:246). Dr Kuo-Gen Chen explains that the sample that is put in series in the circuit (*i.e.* the patient) emits electron waves, which are phase modulated. These waves pass into the body of the patient and are

transmitted to the organs or tissues by resonant absorption. This is an application of quantum mechanical quasiphase matching. (Scott-Mumby, 1999:64-66.)

Dr Franz Morell (cited by Scott-Mumby, 1999:128) found that patients recovered if the correct frequency was played back like a recording into the tissues, via a bar electrode or acupuncture point. He found that although therapeutic responses were achieved, these were often short lived. To get around this he amplified the appropriate frequencies and then played them back. This led to the idea of imprinting which could extend the electronically derived treatment once the patient had returned home.

Dr Morell developed a specially designed amplifier, Mora III (Rasche, FRG and Nexus 21, operate in a similar way), with which electron transfer and amplification is claimed to be possible. The nature of the output appears to be noise if viewed using an oscilloscope; thus short wave energy is used. A signal analyzer or narrow band filter can retrieve a coherent signal. The antenna used to couple the ampoules may be a solid beaker or drilled block (matrix or honeycomb) of brass that may be plated with gold or aluminium (as in the Nexus 21). The output and input antennae are equivalent. A helical coil may be used but the direction of winding is significant. Water picks up bio-information slowly from a coil or antenna, but instantaneously if a magnetic field is applied or if a shock wave is induced (succussion) (Citro *et al.*, 1994:213).

Benveniste conducted some controversial experiments in 1992 and 1993 with 'black boxing'. This involved putting one dilution, made up of ovalbumin, in a sealed phial at

one end of a coil on the electric transmission machine and another sealed phial containing pure water on the other coil. The second phial was treated with the electromagnetic field of the first phial for 15 minutes. The tests were conducted on guinea pig hearts, which had been immunized with ovalbumin, and were then sensitive to the dilutions of this substance. The variation in coronary flow was observed after15 minutes and measured. The trial was randomised and blind. After statistical analysis using the maximum likelihood method and a linear model, the "treated" phial was found to be biologically active while control phials, containing pure water remained inactive. (Schiff, 1994:131.)

Benneviste conducted further research using human neutrophils. A dilution of phorbol-myristate-acetate (PMA) in a sealed phial was placed at one end of a coil on the electric transmission machine and another sealed phial containing pure water was placed on the other coil. The second phial was treated with the electromagnetic field of the first phial for 15 minutes. The production of oxygen radicals is stimulated by PMA, which results in a change in the optical density of the neutrophils in the presence of cytochrome c. This was measured with a spectrophotometer. Ten blood samples were used and each sample exposed to 4 phials, 2 active and 2 control which had not been exposed to the electromagnetic transmission. A second experiment was conducted in which tubes where put into the coil shielded by 4 layers of an alloy to stop the magnetic field transmission. A significantly higher optical density could be seen in the tubes containing the transmitted PMA, as compared to control. This effect disappeared in the shielded tubes. Unfortunately the statistical methods are not documented. (Schiff, 1994:135-136.)

Citro *et al.* (1994) conducted tests on lavae of the amphibian, *Rana temporaria.* The researchers used an electronic amplifier with one coil connected to an input with a sealed vial of molecular thyroxine and an output with a coil connected to a sealed vial of water. The exposed vials and control vials were put into water with amphibian larvae. Two metamorphoses of the larvae were investigated: the development from 2-legged to 4-legged tadpole and the development to juvenile frog. The larvae exposed to the treated vials showed a slowed development in both stages after an initial acceleration, relative to the control. Evaluation was a by 4-field table and by a chi-square test. Due to the small number of animals used in the trial, the results could not be considered statistically significant, but a clear trend was observed. (Citro *et al.* 1994.)

2.6 Plant Research in Homoeopathy

Hopkins (1998) states that the importance of plant-based trials as a method of testing efficacy of homoeopathic dilutions is due to their objectivity and lack of ethical implications. Zacharais (1997) also states their importance in their simplicity and reproducibility in comparison to clinical trials. Plant-based trials are less prone to the placebo effect and researcher bias, which may be present in clinical homoeopathic trials. Objective evidence is needed to clearly evaluate homoeopathy (Kleijnen *et al.*, 1991). Much homoeopathic research has been conducted on plants for these reasons.

Betti *et al.* (1994) conducted a pilot statistical study with homoeopathic potencies of Arsenic trioxide in wheat germ. The trial was blind and randomized. It assessed both the numbers of seeds that germinated and the germination time. The differences between the

treatment groups could not be shown to be an effect of intrinsic seed variability. Arsenicum album was shown, by parametric statistical methods, to have a significant effect in a wheat germination model.

A further study was conducted by Betti *et al.* (1997) on the effect of dilutions of Arsenic trioxide on wheat seedlings or seed poisoned with the same substance. The blind laboratory experiment was carried out with a 45X (1×10^{-45}) potency of Arsenic trioxide. Measurements of the length of the stem and the sum of the lengths of the primary and secondary roots were recorded each day. These measurements were statistically analyzed using Pearson's gamma index. The effect of homoeopathic treatment on seed growth was found to be significant.

In a study on wheat seedling growth, Pelikan and Unger (1971) exposed seeds to 12 different doses [ranging from 8X (1×10^{-8}) to 19X (1×10^{-19})] of Silver Nitrate. The series was repeated 240 times. Germination and sprouting were measured against control groups. Seedling length was found to vary with the degree of dilution. The length increased from 8X (1×10^{-8}) to 11X (1×10^{-11}), dropped for 12X (1×10^{-12}), rose for 13X (1×10^{-13}) and 14X (1×10^{-14}), dropped at 16X (1×10^{-16}) and rose again at 17X (1×10^{-17}) and 18X (1×10^{-18}), yielding a sinusoidal curve with increasing potency. Variance was analyzed using independent methods. The experiment provided evidence that potentised substances have an effect on plant growth.

Wannamaker (1966) tested Sulphur microdilutions on the growth of onion plants. The 12X (1×10^{-12}), 24X (1×10^{-24}), 30X (1×10^{-30}), 60X (1×10^{-60}) and 20M ($9{,}54 \times 10^{-93}$) microdilutions of Sulphur were tested against controls. Differences in the weight and dimensions of the onion bulbs and seedlings and differences in the calcium, magnesium, potassium and sodium content of the plants were recorded relative to controls. At 6 weeks the 24X (1×10^{-24}) dilutions prepared with 5 succussions and 10 succussions had weights of 95.10g and 91.00g respectively. The controls ranged between 9.90g and 19g and the placebo, containing lactose only, between 38.60g and 69.20g. At the 4 month period the unsuccussed 24X (1×10^{-24}), and the 24X (1×10^{-24}) prepared with 5,10, 100 and 1000 succussions and the 60X (1×10^{-60}) prepared with 5,10, 100 and 1000 succussions had values in the 40s. The controls had values of 29.6g and 30.5g. Unfortunately no statistical analysis was given.

Wannamaker (1968) also conducted experiments with Boron dilutions. The work was done on borage, curly cress, summer savory, pennyroyal, cucumber seed, nasturtiums, radishes, sunflowers, carrots and parsley. Plants treated with dilutions showed a better recovery from environmental damage and a longer life than those not treated with dilutions. Unfortunately no statistical analysis was done.

Bornoroni (1991) conducted a study on the effect of highly diluted solutions of $CaCO_3$ on the growth of oat coleoptiles stimulated by 100μM of indoleacetic acid. The coleoptiles were pretreated with homoeopathic dilutions of $CaCO_3$ 10 hours before the application of

the hormone. Dilution levels of 5cH (1×10^{-10}) 15cH (1×10^{-30}) and 30cH (1×10^{-60}) were used. Pretreatment with a 5cH (1×10^{-10}) potency resulted in a significant increase (8-9%) in growth as compared to coleoptiles treated with indoleacetic acid alone. Increase in length was measured and statistically evaluated. The statistical methods used are not given.

Saxena *et al.* (1986) studied the effect of certain homoeopathic drugs on incidence of seed-borne fungi and seed germination of *Abelmoschus esculentus*. The seeds were soaked in Thuja, Sulphur, Nitricum acidum, Calcarea carbonica and Teucrrium in various different potencies, and then incubated for 6 days. Analysis for mycoflora by agar plate and blotter methods revealed that the Thuja, Nitricum acidum and Sulphur in 200cH potencies checked the growth of all fungal species. Statistical analysis revealed a significant variation for total number of fungal species in relation to treatment (P=0.01), for the total number of fungal species in relation to treatment (P=0.05) and for seed germination(P=0.01). The statistical tests used are not given.

Him Lok (2001) investigated the effect of homoeopathic preparations of gibberellic acid on germination of barley seeds. The effects of 4cH, 15cH, 30cH and 200cH potencies of GA_3 were tested on high, medium and low vigor seeds. A germination index was calculated, including as measures of germination the rate at which the seeds germinated, shoot length, root length and seedling dry mass. The 15cH GA_3 preparation was found to be biologically active, stimulating root development in the medium vigor seeds when

analyzed by data factorial treatment classification. The 4cH, 30cH and 200cH GA_3 potencies resulted in a faster germination than the control seeds. The experiment provided evidence for the biological activity of homoeopathically prepared GA_3 in various potencies.

2.7 The Role of Gibberellic Acid and α-Amylase in Seed Germination

Barley is classified as an annual, which means that the plant germinates, develops and reproduces within one year (Brimbell, 1952:15). It is a hardy cereal and grows wild around the Caspian Sea. It may be cultivated in areas further north than the cereal wheat (Brimbell, 1975:78).

Cereal seeds consist of two major parts, the embryo and the endosperm. The embryo represents the future adult plant, while the endosperm is seen as starch-containing cells surrounded by a layer known as the aleurone layer which consists of two to four layers of living cells (Devlin, 1975:490).

Until 1958 it was thought that the endosperm was a passive part of germination, the embryo providing all the enzymes for the breakdown of starch. It was a Japanese scientist, Yomo, who discovered the enzyme activity in the endosperm. He conducted experiments in which endosperm halves of seeds and embryo halves of seeds were separated and then placed into flasks containing only embryo halves, only endosperm halves or a mixture of both. Since enzyme activity was only found in the latter he

concluded that amylase activity occurred in the endosperm but was controlled by an unknown factor from the embryo of the seed (Devlin, 1975:490).

The unknown factor was identified by Paleg as gibberellic acid. The production of reducing sugars from starch in an amylase assay, was found to be greatly increased when the endosperms were pretreated with gibberellic acid, indicating that gibberellic acid increases the water soluble amylolytic activity of the endosperm (Paleg, 1960). A later study by Moll and Jones (1983) on the secretion of α-amylase by single barley aleurone layers showed that the secretion of α-amylase begins 5 to 6 hours after exposure to gibberellic acid and reaches a maximum rate of release after 10 to 12 hours. Gibberellic acid when applied to seeds breaks the dormancy of dormant seeds and accelerates germination of non-dormant seeds. It can replace the need for environmental stimulus, such as light and low temperature, for the initiation of germination. (Karssen *et al.*, 1989.)

Gibberellins are chemicals related to terpinoids. Terpinoids are built from five carbon chains known as isoprene units, two units forming a monoterpene (C-10), three units forming a sesquiterpene (C-15) and four units forming a diterpene (C-20). The immediate precursor of the gibberellin is the diterpene kaurene (Devlin, 1975:480). The interlocking ring structures of the gibberellins contain at least one carboxyl group, which gives the molecule the properties of an acid. There are many different types of gibberellins. More than eighty having been isolated from various fungi and plants. Gibberellins are abbreviated as GA, with subscripts denoting the different types of gibberellin. GA_3 is

isolated from the *Gibberrella fujikuri* and is the most intensively studied gibberellin. (Moore, 1995:422.)

In the process of germination, starch from the endosperm is broken down into simple sugars that are translocated to the embryo for use as an energy source and for growth (Devlin, 1975:490). Various hydrolytic enzymes are produced, of which α-amylase is the most prominent. This enzyme is responsible for this mobilization of sugar from starch. (Atzorn and Weller 1983). There is no trace of α-amylase in ungerminated barley seed, but it appears quickly on germination (Thomas *et al.*, 1973:57). It is produced by the aleurone layer surrounding the endosperm. Gibberellin enhances the transcription of amylase mRNA in the aleurone layer. The release of gibberellins by the embryo is triggered by a process known as imbibition, which is the absorption of water by the seed (Moore, 1995:423).

The embryo, which is needed for the hydrolysis of starch, may be replaced by exogenous gibberellic acid (Atzorn and Weller, 1983). Addition of gibberellins to embryoless grains or isolated aleurone layers induces α-amylase synthesis in dose-dependent amounts (Schuurink *et al.*, 1992). Thus, the measure of α-amylase synthesis in endosperm half seeds is a specific and sensitive indicator for the presence of gibberellic acid (Chrispeels and Varner, 1967; Cairns and de Villiers, 1986; Nissen, 1988).

Barnes and Blakeney (1974) studied the determination of cereal α-amylase, using a commercially available dye-labeled substrate, the Phabedas® amylase test. They used a wheat α-amylase preparation on a soluble starch substrate. Phabedas® amylase test tablets were added and analysis was done by spectrophotometry. The cross-linked dye-labeled starch substrates were resistant to β-amylase, but readily hydrolysed by α-amylase. They found the method to be quantitative, sensitive, and accurate. It measured the α-amylase in milli International Enzyme Units and was simple and rapid. (1974:193). This method was chosen for use in the present study.

Phabedas® test tablets contain a substrate made by cross-linking partially hydrolyzed potato starch, using 1,4-butandioldiglycidether as the cross-linking agent. The soluble starch is converted into a three-dimensional lattice network which swells in water to a degree regulated by the number of cross-linking bridges formed. This also controls the degree of susceptibility of the substrate to attack. The substrate is labeled with Cibachron Blue by covalent bonds. Each tablet contains 45 mg of dry blue starch polymer and 25 mg of dry buffer salt. The tablets have been designed specifically for measurement of human α-amylase. (Barnes and Blakeney, 1974:193.) The enzyme hydrolyses the blue starch polymer into water soluble dye-labeled products (Barnes and Blakeney, 1974:193) which absorbs light at 620nm (Cairns and de Villiers, 1986:367).

Steele (1999) investigated the effect of ultra high dilutions of gibberellic acid on the synthesis of α-amylase in de-embryonated halves of barley seed. Activity of α-amylase was assessed using Phadebas® Test tablets (Pharmacia Diagnostics AB, Sweden). He

used the 4th, 9th, 15th, 30th and 200th centesimal dilutions prepared in two different ways - traditional Hahnemanian with succussion, and straight serial dilution without succussion. This study is based on Steele's (1999) methodology. It aimed to test the reproducibility of the results reported by Steele (1999). In the course of the study it was discovered that Steele had made an error regarding the proportion of incubation buffer in the control groups compared to the treatment groups, therefore rendering any comparison with the control groups unreliable. For this reason, the results obtained by Steele are unreliable, and his conclusions invalid. His research however laid the groundwork for further homeopathic research with barley seeds and gibberellic acid (Him Lok 2001, Bruni 2001, Couchman 2001, Pieterse 2001, Stubbs 2001).

CHAPTER 3

RESEARCH MATERIALS AND METHODS

3.1 Introduction

The study was carried out at the School of Life and Environmental Sciences, George Campbell Building, University of Natal, Durban Campus. The experimental protocol is that used by Steele (1999).

3.2 Summary of Protocol

- Homoeopathic dilutions (electromagnetically imprinted dilutions, unsuccussed serial dilutions and Hahnemannian dilutions) were prepared according to standard methods (see 3.4).
- The α-amylase standard curve was prepared by measuring the absorbance of α-amylase of known enzyme units.
- The seeds were prepared.
- Treatment dishes were prepared with different treatment solutions.

  Petri dishes were incubated in the dark in a growth chamber set at 16°C for 48 hours.

  The germination process was terminated by putting the petri dishes into a freezer set at -20°C.
- Enzyme extraction:

  The petri dishes were thawed (room temperature for 5 minutes);

  The contents of each petri dish were macerated with 10ml of extraction

buffer, decanted into numbered centrifuge tubes;

Samples were incubated in a shaker bath set at 30°C for 60 minutes; centrifuged and filtered.

- Enzyme assay:

  Samples were placed in a shaker bath set at 50°C then 1 Phadebas tablet was added per sample;

  These were incubated for 10 minutes then 1ml NaOH was added to each sample to terminate the reaction;

  They were then filtered;

  Using a spectrophotometer the absorbance of each sample was read at 620nm;

  The readings were recorded in table form.

- The data was analysed.

3.3 Barley

Seeds of variety *Hordeum vulgare* (Stirling, 1998 harvest) were obtained from the Caledon Farmers Cooperative, Western Cape, South Africa. The seeds were not treated with any chemicals.

3.4 Gibberellic Acid

Gibberellic acid was used as the potassium salt $C_{19}H_{21}O_6K$, for improved solubility. It has a molecular weight of 384.5. The source was Sigma-Aldrich. It was isolated from Fujikuroi fungus and contains not less than 90% GA_3.

3.5 α-Amylase

The α-amylase was obtained from Sigma-Aldrich. It had an activity of 1650u/mg. It was a type II-A obtained from *Bacillus species*, and was 4 times crystallised and lyophilised.

3.6 Phabedas® Amylase Test Tablets

The Phabedas® Amylase Test tablets were obtained from the Pharmacia Diagnostics AB, Uppsala, Sweden.

3.7 Preparation of the GA_3 Dilutions.

The Hahnemannian dilutions and unsuccussed serial dilutions were made up in the homoeopharmaceutic laboratory, Department of Homoeopathy, Technikon Natal, Durban. The remedies were prepared under lamina flow conditions, to prevent bacterial contamination. Measuring cylinders and pipettes were autoclaved before use. All other glassware was sterilized by rinsing with distilled water and then baking in an oven at 160°C for 2 hours. The stock solution was made up by combining 1 part KGA_3 (0,25g) with 99 parts (24,75ml) distilled water to yield a 1% solution of KGA_3 (26mM).

The Hahnemannian dilutions were made up from this solution in accordance with Method 5a ("Solutions") of the German Homoeopathic Pharmacopoeia (British Homoeopathic Association 1985:20-21). This is the pharmacopoeia most widely used by South African manufacturers. The 4^{th} (1×10^{-8}), 9^{th} (1×10^{-18}), 15^{th} (1×10^{-30}), 30^{th} (1×10^{-60}) and 200^{th} (1×10^{-400}) centessimal dilutions were made in 100ml volumes. These dilutions were labeled cH, thus 4cH, 9cH, 15cH, 30cH, 200cH.

The unsuccussed serial dilutions were made up from the stock solution using the same methodology, except that the bottles were not succussed. These dilutions were labeled cS, thus 4cS, 9cS, 15cS, 30cS, 200cS.

The electromagnetically imprinted dilutions were made up using a Nexus 21 Imprinter in the offices of The Best System in Hillcrest, Natal. They were made up by Wendy Middleton with the help of the researcher. Each of the method 1 (cH) potencies manufactured above was placed into the machine so that the short wave frequency was read and transferred to the computer and from there to a bottle of non-pre-treated distilled water via infrared radiation. The bottles of water were treated for 50 cycles, which took 2 minutes and 15 seconds per bottle. The glassware was sterilised as above in the laboratory at Technikon Natal and the distilled water was from the same source as that used to produce the cH and cS dilutions. The electromagnetically imprinted dilutions were labelled cE, thus, 4cE, 9cE, 15cE, 30cE, 200cE.

All dilutions were made with distilled water rather than alcohol to prevent any inhibition or stimulation of α-amylase production by alcohol (Cairns 1998 as cited by Steele 1999:39). Each dilution was made up to 20mM calcium nitrate. The proportion of incubation buffer remained constant throughout the study in order to maintain optimal conditions for the synthesis of α-amylase. Calcium is an important cofactor in germination. It confers stability to barley α-amylase and plays a role in the synthesis and release of hydrolytic enzymes (Eastwell and Spencer, 1982; Moll and Jones, 1983).

3.8 Preparation of the α-Amylase Activity Standard Curve

An α-amylase standard curve was prepared according to the method of Moore (1981:178-9), as adapted by Cairns in Steele (1999). One unit of enzyme activity is defined as the amount of enzyme catalysing the hydrolysis of 1μm glucosidic linkage at 50°C (Cairns and Devilliers, 1986).

3.9 Preparation of the Seed

For each series, 600 seeds were selected. Damaged or deformed seeds were excluded. The seeds were cut transversely, with a blade. The distal endosperm half was retained for the experiment. In each series, the half-seeds were divided into 30 groups of 20 half seeds each.

3.10 Preparation of the Treatment Groups

Each series consisted of 5 replicates. Each replicate consisted of 5 treatment groups and 1 control group (see Table 3.1). The control was water with the addition of the incubation buffer in the same concentration as the treatment groups. The incubation buffer was added to all the test solutions and the control resulting in the same concentration, 20mM Ca^{2+}. Each group of 20 half seeds was weighed, placed into a numbered 9cm-petri dish lined with two No.1 Whatman filter papers. These were moistened with 5ml of the various treatment solutions, placed in a drawstring plastic bag with 2ml of distilled water, and incubated for 48 hours in a dark incubation cabinet set at a constant temperature of 16°C. Incubation was terminated by freezing at -20°C.

TABLE 3.1 Layout of experiment.

METHOD 1 Hahnemannian Dilutions	METHOD 2 Serial Dilutions	METHOD 3 Electromagnetic Dilutions
4cH 20-half-seeds ×5 replicates	4cS 20-half-seeds ×5 replicates	4cE 20-half-seeds ×5 replicates
9cH 20-half-seeds ×5 replicates	9cS 20-half-seeds ×5 replicates	9cE 20-half-seeds ×5 replicates
15cH 20-half-seeds ×5 replicates	15cS 20-half-seeds ×5 replicates	15cE 20-half-seeds ×5 replicates
30cH 20-half-seeds ×5 replicates	30cS 20-half-seeds ×5 replicates	30cE 20-half-seeds ×5 replicates
200cH 20-half-seeds ×5 replicates	200cS 20-half-seeds ×5 replicates	200cE 20-half-seeds ×5 replicates
Control (water with 20Mm Ca^{2+}) 20-half-seeds ×5 replicates	Control (water with 20mM Ca^{2+}) 20-half-seeds ×5 replicates	Control (water with 20mM Ca^{2+}) 20-half-seeds ×5 replicates

3.11 Determination of α-Amylase Activity

3.11.1 Enzyme Extraction

The methodology used for enzyme extraction was based on studies by Nicholls (1979), Cairns and de Villiers (1986) and Steele (1999). Immediately prior to extraction the half seeds were thawed at room temperature for 5 minutes. The half-seeds were macerated with a mortar and pestle in 10ml of extraction buffer containing 58,4mM (5.0g) sodium chloride and 1.14mM (0.2g) calcium acetate. The slurry was decanted into numbered

plastic centrifuge tubes. The tubes were placed in a shaker-bath at 30°C for 60 min. The homogenate was centrifuged (temperature 4°C; speed 10000g; duration: 10 minutes). The homogenate was filtered though one thickness of Whatman No. 1 filter paper into 30ml test tubes.

3.11.2 Enzyme Assay

The enzyme assay methodology was based on studies by Barnes and Blakeney (1974), Cairns and de Villiers (1986) and Steele (1999). For reasons of practicality, samples were analysed one replicate at a time (*i.e.* 6 centrifuge tubes, containing the filtered, centrifuged slurry, and a 7^{th} tube containing extraction buffer [$(CH_3.COO)_2Ca$] alone, to be used as a blank). Tubes were placed in the shaker bath set at 50°C. Dye-labeled substrate in the form of one Phadebas tablet was added to each test tube. This was done sequentially every minute, to ensure that each test tube had a full 10 minutes incubation. Each tube was held on the mixer for 15 seconds to assist with dissolution of the tablet. After 10 minutes the reaction was terminated by adding 1ml of 0.5 M sodium hydroxide to each test tube. Each tube was again mixed thoroughly. The homogenate was filtered through one thickness of Whatman No.1 filter paper into 30ml test tubes. The absorbance of each sample was read at 620nm, against the blank which contained only extraction buffer and assay reagents. The α-amylase activity was expressed as enzyme units per gram fresh weight of endosperm halves.

3.12 Data Analysis

3.12.1 Calculation of Enzyme Units Synthesised

The α-amylase concentration corresponding to the measured absorbance for each sample was calculated by interpolation from the α-amylase standard curve. This result was divided by the fresh weight of each sample to arrive at the enzyme units synthesized by each sample per unit fresh weight.

3.12.2 Statistical Methods

The analysis of variance (ANOVA) was chosen to interpret the data, as it is particularly suited to agricultural data. The two-way analysis of variance was used to analyse the data in this study. A two-way analysis of variance considers factors both separately (main effects) and in combination (interaction effects). Data entry and analysis was performed using the statistical computer package SAS (SAS Institute Inc. SAS User's Guide for Personal Computers, Version 6 Edition. Cary NC: SAS Institute Inc., 1985).

There was only 1 dependent variable *viz.* α-amylase enzyme units produced.

The first factor was method at 3 levels, namely:

Method 1 – Hahnemannian dilutions

Method 2 – Serial dilutions

Method 3 – Electromagnetically imprinted dilutions

The second factor was dilution, at 6 dilution levels per method.

The dilutions are the 4^{th}, 9^{th}, 15^{th}, 30^{th} and 200^{th} dilutions and a control.

There were 5 observations per dilution level.

3.12.2.1 Statistical Model

The statistical model for the multifactoral ANOVA experiment is given as follows:

$Yijk = \mu + Ai + Bj + AiBj + \varepsilon ijk$

where:

μ is the overall or common effect

Ai is the effect of the methods

Bj is the effect of the dilution levels

$AiBj$ is the interaction effect between the methods and the dilution levels

εijk are random error terms

$i = 1, 2, 3$ = the number of methods

$j = 1, 2, 3, 4, 5, 6$ = the number of dilution levels per method

$k = 1, 2, 3, 4, 5$ = the number of observations per dilution level.

The total number of rows in the data spreadsheet = $3 \times 6 \times 5 = 90$

3.12.2.2 The Two Way Analysis of Variance to Test Main Effects for Significance

The two-way analysis of variance was used to test whether there were any interaction effects and any significant differences in the main effects.

Hypothesis testing

Before testing for significant differences in the methods and the dilutions, it was necessary to test whether there was any significant interaction effect. If no interaction effect was detected then the main effects could be examined.

The decision criterion in each case was to reject the null hypothesis if the P-value was less than the level of significance, α, of the test. It was accepted if the P-value was equal to or greater than the level of significance, α, of the test. In this study, the level of significance, α, was fixed at the 0.05 level.

Decision rule:

If $p < 0.05$, reject H_0

If $p \geq 0.05$ do not reject H_0

(accept H_0)

The interaction between the methods and the levels of dilution was tested. The null hypothesis, H_0, stated that there was no interaction between the level of dilution and the method. The alternative hypothesis, H_1, stated that there was an interaction between the level of dilution and the method.

H_0: There is no interaction between the level of dilution and the method.

H_1: There is an interaction between the level of dilution and the method.

$\alpha = 0.05$ (significance level).

In the case of the 3 methods, the null hypothesis, H_0, stated that there was no difference between the 3 methods. The alternative hypothesis, H_1, stated that at least one method was different from the rest.

H_0: There is no difference between the 3 different methods.

H_1: There is a difference between the 3 methods.

$\alpha = 0.05$ (significance level).

In dilutions, the null hypothesis, H_0, stated that there was no difference between the effect of the different levels of dilution. The alternative hypothesis, H_1, stated that there was a difference between the effect of the different levels of dilution.

H_0: There is no difference between the effect of the different levels of dilution.

H_1: There is a difference between the effect of different levels of dilution.

$\alpha = 0.05$ (significance level).

3.12.2.3 Multiple Comparisons

The least significance difference (LSD) and Duncan's multiple range tests were performed to determine where the possible differences between the methods lay Montgomery (1997).

CHAPTER 4

RESULTS

4.1 Criteria Governing the Admissibility of the Data

The data utilized were absorbance readings, from which α-amylase enzyme units produced in each sample was calculated by interpolation from the α-amylase standard curve (see Figure 4.1). This value was divided by the fresh weight of each sample to arrive at the enzyme units synthesised by each sample (as detailed in 3.11.1). No data obtained were excluded from statistical analysis.

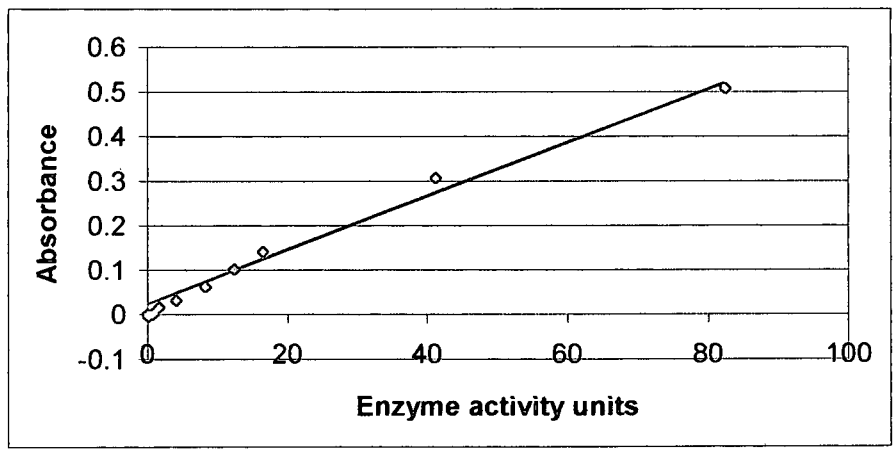

Figure 4.1 Standard curve of α-amylase activity.

4.2 Statistical Analysis of Results

4.2.1 Two Way Analysis of Variance

The ANOVA (Table 4.1) showed the following:

The first step in the interpretation of the ANOVA output was to investigate whether there was a significant interaction effect.

a) There was no interaction effect between method and level of dilution ($p=0.4937$).

b) There were no differences in α-amylase enzyme units induced for the different dilutions ($p=0.3322$). This means that there were no differences in α-amylase enzyme units produced at the different dilution levels.

c) However, there was a difference in the enzyme units induced by the different dilution methods ($p= 0.0170 < 0.05$). Multiple comparisons were therefore performed. The Least Significance Difference (LSD) as well as Duncan's Multiple Range test indicated that Methods 1 and 2 differed significantly from Method 3. Hahnemannian dilutions and serial dilutions therefore differed from the electromagnetically imprinted dilutions, in terms of the production of α-amylase in barley endosperm half seeds.

Table 4.1 ANOVA Table

Dependent Variable: ENZYME UNITS/GRAM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	17	3861.85316433	227.16783320	1.41	0.1573
Error	72	11596.0463876	161.05619983		
Corrected Total	89	15457.899552			

R-Square	C.V.	Root MSE	Enzyme Units Mean
0.249830	35.80205	12.69079193	35.44711136

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Dilution	5	942.45907959	188.49181592	1.17	0.3322
Method	2	1388.97155534	694.48577767	4.31	0.0170
Dilution*Method	10	1530.42252940	153.04225294	0.95	0.4937

R-Square = multiple correlation coefficient
C.V. = coefficient of variation
Root MSE = root of mean square error

Although the overall p-value of 0.1573 > 0.1 there is however a significant difference in the enzyme units produced for the different methods. This is indicated by the p value of 0.0170 obtained when comparing the methods to one another. For this reason multiple comparisons were done to identify the methods that are significantly different.

4.2.2 Multiple Comparisons

The Least significant difference test (LSD) (Table 4.2) and Duncan's multiple range test indicate that method 1 and 2 differ significantly from method 3.

Table 4.2 Least Significant Difference (LSD) Table
Means with the same letter are not significantly different.

T Grouping	Mean	N	Method
A	38.708	30	1
A			
A	37.712	30	2
B	29.921	30	3

LSD multiple comparison:

$|y_i - y_j| > \text{LSD}$

where $\text{LSD} = t_{\alpha/2,\, N-K} \sqrt{\frac{2MSE}{n}}$

where $t_{0.025,72} = 1.99$
MSE (mean square error) = 161.0562
LSD = 6.5321
$\alpha = 0.05$
y_i = sample mean for the ith mean
N = sample size
K = number of treatments

The ith mean differs from the jth mean if $|y_i - y_j| > \text{LSD}$
Using Fisher LSD method.

4.3 Physical Observations

The physical observations differed considerably from those of Steele (1999) due to the differences in the control used. Steele (1999) used the undiluted incubation buffer as control. The resultant high Ca^{2+} concentration inhibited expression of α-amylase activity in control endosperm in his study. In the present study, controls contained incubation buffer diluted to a final concentration of 20mM, as in the test samples. There were no observable differences among the seeds inoculated with the various test solutions. All the petri dishes contained seeds with clear-cut edges (see Plates 4.1 to 4.2). It is thought that the jelly-like protrusions seen in the control seeds in Steele's work are a result of the high osmotic activity of the control used by Steele.

There were visible differences between the test tubes after incubation with Phabedas® test tablets. The test tubes containing the extracts treated with the electromagnetically imprinted dilutions showed no colour variation between the different dilutions. Those treated with the serial dilutions and the Hahnemannian dilutions showed a variation in the colour depending on the degree of dilution (see Plates 4.3 to 4.5).

PLATE 4.1
Thawed barley endosperm half-seeds inoculated with the 200CH dilution. Appearance after the 48-hour incubation period, showing clear cut surfaces.

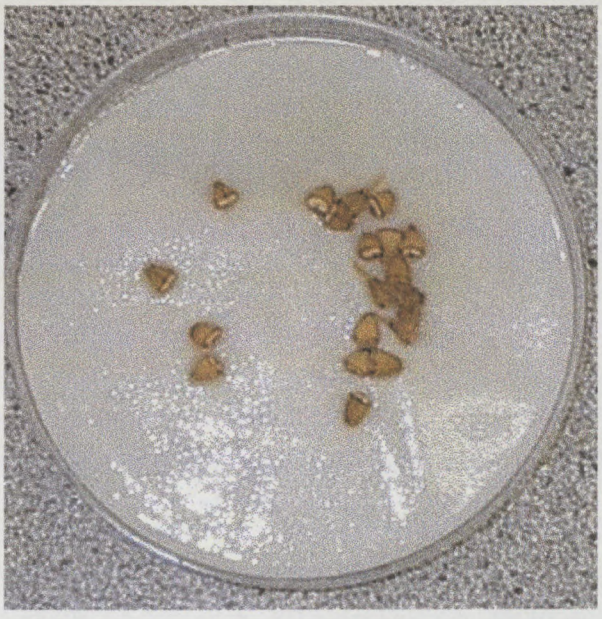

PLATE 4.2
Thawed barley endosperm half-seeds inoculated with the control. Appearance after the 48-hour incubation period, showing clear cut surfaces.

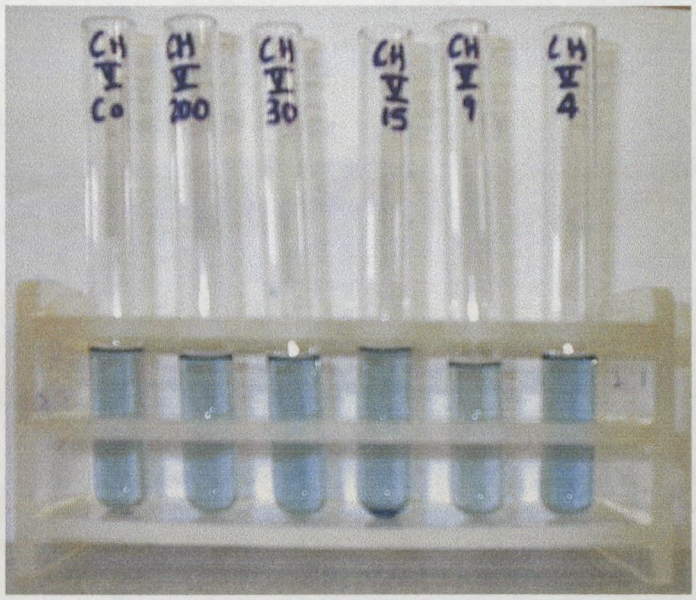

PLATE 4.3

The filtered extract of endosperm treated with Hahnemannian dilutions of gibberellic acid and addition of the Phabedas test tablets prior to determining absorbance. From left to right the tubes contain GA_3-free incubation buffer, 200cH, 30cH, 15cH, 9cH and 4cH dilutions.

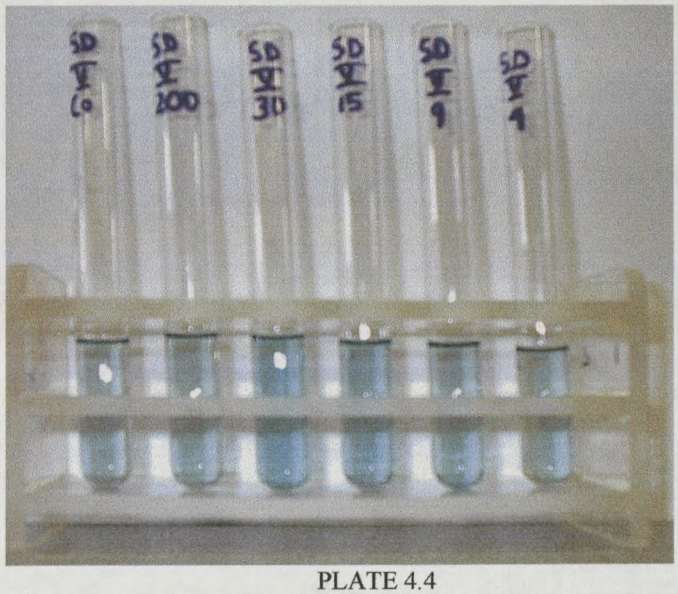

PLATE 4.4

The filtered extract of endosperm treated with serial dilutions of gibberellic acid and addition of the Phabedas test tablets prior to determining absorbance. From left to right the tubes contain GA_3-free incubation buffer, 200cS, 30cS, 15cS, 9cS and 4cS dilutions.

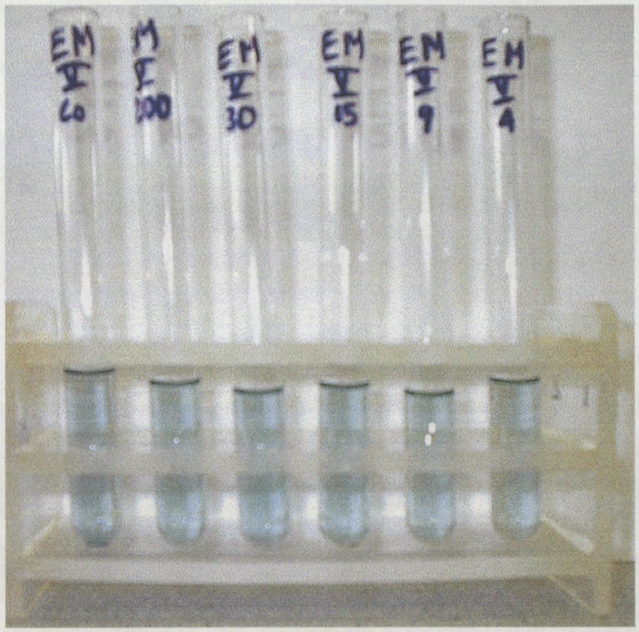

PLATE 4.5

The filtered extract of endosperm treated with dilutions electromagnetically imprinted with Hahnemannian dilutions of gibberellic acid and addition of the Phabedas test tablets prior to determining absorbance. From left to right the tubes contain GA_3-free incubation buffer, 200cE, 30cE, 15cE, 9cE and 4cE dilutions.

4.4 Enzyme Activity

4.4.1 Method 1 - Hahnemannian Dilutions

Hahnemannian dilutions of GA_3 did not result in a significant induction of α-amylase production by barley endosperm half-seeds when compared to control seeds treated with GA_3–free buffer dilutions. There was no interaction between the different dilutions and this method of enzyme induction. Although statistical significance was not achieved – probably as a result of high variability in the data - the activity of α-amylase appeared increased in endosperm treated with Hahnemannian dilutions of GA_3 in comparison to those treated with the control of incubation buffer alone. There was a consistent enzyme activity with all the dilutions rather than a trend showing decreasing enzyme activity with increasing dilution. The trends may have been more easily observed and more reliable had the sample size been larger. (see Figure 4.2).

4.4.2 Method 2 - Serial Dilutions

Serial dilutions of GA_3 did not result in a significant induction of α-amylase production by barley endosperm half-seeds when compared to control seeds treated with GA_3–free buffer dilutions. There was no interaction between the different dilutions and this method of enzyme induction. Although statistical significance was not achieved – probably as a result of high variability in the data - the activity of α-amylase appears higher than those treated with the control. There appeared to be a trend of decreasing enzyme activity with increasing serial dilution. The trends may have been more easily observed and more reliable had the sample size been larger. (see Figure 4.3)

4.4.3 Method 3 - Electromagnetically Imprinted Dilutions

Dilutions electromagnetically imprinted with Hahnemannian dilutions of GA_3 did not result in a significant induction of α-amylase production by barley endosperm half-seeds when compared to control seeds treated with GA_3–free buffer dilutions. There is no interaction between the different dilutions and this method of production. Mean activity of α-amylase also appeared similar to seeds treated with the control on visual comparison. (see Figure 4.4)

4.4.4 Comparison of Methods of Production

The effects of Hahnemannian dilutions and serial dilutions differed from that of dilutions electromagnetically imprinted with Hahnemannian dilutions of GA_3. Electromagnetically imprinted dilutions induced α-amylase activity in barley endosperm halves, similar to that in seed halves treated with GA_3–free buffer dilutions used as the control. Hahnemannian dilutions and serial dilutions induced α-amylase activity higher than that induced by electromagnetically imprinted dilutions. (see Figure 4.2 – 4.4)

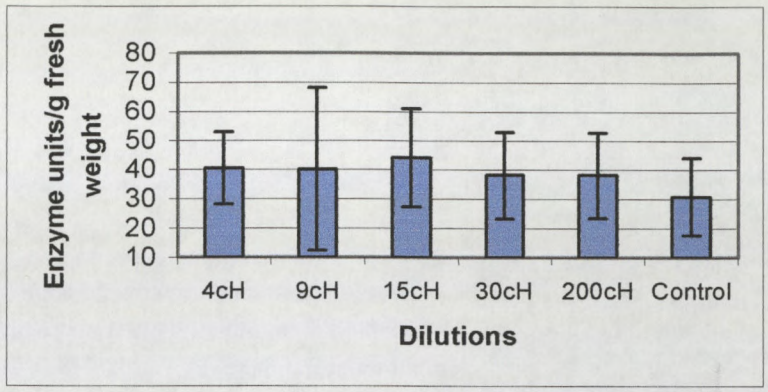

Figure 4.2 Mean α-amylase activity in endosperm half-seed treated with Hahnemannian dilutions of GA_3 (Method 1). Error bars show one standard deviation around the mean.

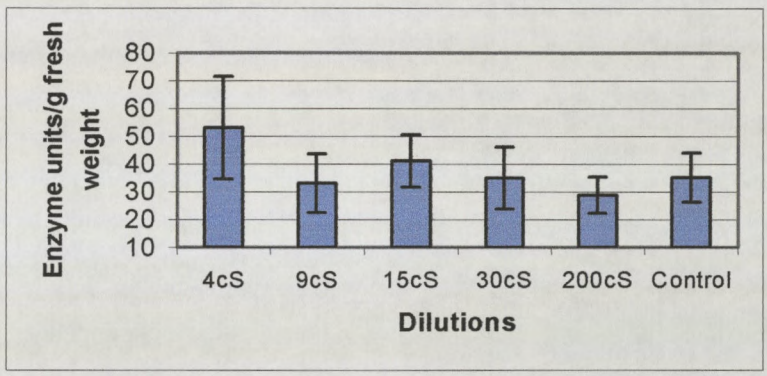

Figure 4.3 Mean α-amylase activity in endosperm half-seed treated with Serial dilutions of GA_3 (Method 2). Error bars show one standard deviation around the mean.

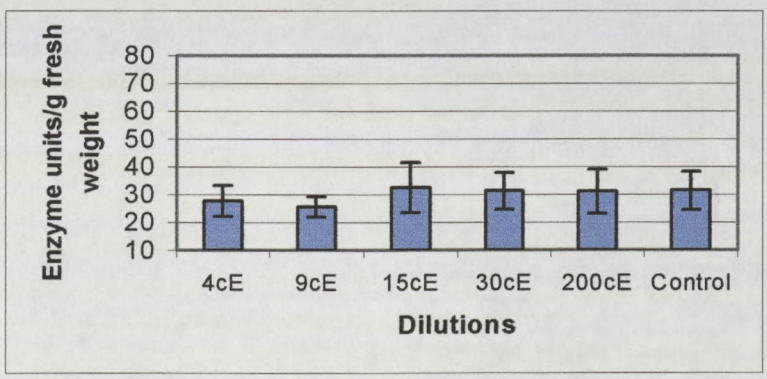

Figure 4.4 Mean α-amylase activity in endosperm half-seed treated with water electromagnetically imprinted with Hahnemannian dilutions of GA_3 (Method 3). Error bars show one standard deviation around the mean.

CHAPTER 5

DISCUSSION OF THE RESULTS

The results of the experiment did not support the biological activity of electromagnetially imprinted dilutions, serial dilutions and Hahnemannian dilutions of GA_3 in inducing α-amylase production by de-embryonated endosperm halves of barley seed. There were no differences between the different levels of dilution or the dilutions and the controls.

Studies by Couchmann (2001) on the response of barley seed to plant hormones support the findings of this research that homoeopathic dilutions of plant hormones do not evoke a biological response in barley seed. Studies by Him Lok (2001) and Bruni (2001) however, do show a response by barley seed to dilutions of plant hormones. Steele (1999) showed differences in the α-amylase produced by barley endosperm halves in response to different dilutions of GA_3. These cannot unfortunately be compared to the control used by Steele. It is certain that further research must be conducted on the efficacy of homoeopathic remedies in biological systems.

The barley seeds used for this experiment were stored in a freezer and were frozen and thawed a number of times which may have interfered with their ability to produce α-amylase. The vigor of the seeds used for this study was not tested before they were used. Pieterse (2001) and Stubbs (2001) found that seed vigor influenced the germination process. The quality of the base material GA_3 may have been a problem although this seems unlikely.

The optimum concentration of GA_3 is $10^{-4} - 10^{-5}$ mMol/l in the presence of approximately 20mMol/l Ca^{2+} (Wilkins 1984). The cH and cS with their concentrations of 2.6×10^{-10} mMol/l of GA_3 are far more dilute than $10^{-4} - 10^{-5}$ mMol/l. A positive control with a dilution of 2.6×10^{-5} mMol/l would therefore be expected to have some effect on the α-amylase produced due to the biological action of the GA_3 present in the solution. This would have determined the viability of the barley seed model and the response of the barley endosperm half seeds to GA_3 dilutions.

Response to highly diluted remedies is very subtle and small and may have been detected more reliably had the number of repetitions been increased. An increased number of repetitions may have allowed significant differences to be detected. It may have been better to decrease the number of dilutions tested and increase the repetitions done.

The effects of electromagnetically imprinted dilutions differed from those of serial and Hahnemannian dilutions, disproving the hypothesis that there would be no difference between the different methods of dilution. This indicates that the Hahnemannian and serial dilutions did have significantly more effect than the electromagnetically imprinted dilutions even though the biological activity of dilutions prepared by these methods could not be established conclusively.

In this study electromagnetic dilutions, which have been regarded with skepticism by classical homoeopaths, do not appear as effective as remedies prepared from a base substance. This supports opinions of homoeopaths such as Vithoulkas (2000), who write

about them as an "absurdity" and "obvious nonsense". It may, however, be advisable to test dilutions prepared with imprinting machines made by different manufacturers before dismissing this method totally.

The fact that remedies produced by serial dilution did not differ from those produced by Hahnemannian methods brings into question whether succussion is vital or at all necessary to the production of homoeopathic remedies. This suggests that dilution alone may be enough to render the remedies safe to use and therapeutically active. Theories which attempt to explain homoeopathy highlight the importance of the solute in the storage of information. The addition of mother tincture is proposed to result in some form of order in the solute. The mother tincture is shown by this research to be of importance as both dilution methods using the mother tincture as a starting point differed from the method which did not. In this regard it is interesting to note that Auerbach (1994) states that diffusion flow gives rise to the most active force in preparing ultra high dilutions. Other forms of flow (such as vortex flow, sheer flow *etc.*) serve diffusion and merely speed up bulk mixing.

In the geometric models, used to explain how information is transferred from the mother tincture to the dilution, succussion is of vital importance. Anastagnos proposed a replicating clathrate model in which succussion breaks open the clathrates which then form templates for the formation of further templates (Shulte, 1999). Antonchencko and Ilyin (1992) suggest that there are hydration shells in water which break into fragments and serve as embryos for the development of new shells. In their theory, succussion is

vital to the stabilisation and formation of these hydration shells. If succussed remedies act with the same efficacy as unsuccussed remedies, then these two theories do not hold. There could however be destruction of the templates, be they hydration shells or clathrates, by means other than succussion. The explanation of such means is outside the scope of this study and within the fields of physics and chemistry.

Dynamic theories, explaining the mechanism of action of homoeopathic remedies consider matter in motion. Molecules are considered both as particles and as waveforms. This means that all the molecules within a solution are considered to be connected rather than as isolated structures. Resch and Guttman (1991) and Del Guidice and Preparata's (Del Guidice, 2000) theories rely upon the fact that all molecules exert an influence upon one another. This influence occurs regardless of whether a solution is shaken or not. Thus dynamic theories allow for the possibility that succussion is not fundamental to remedy production. Rather, biological activity is imparted by the addition of one structured solution to another solution, which is changed and restructured according to the first.

Samuel Hahnemann talks of homoeopathic remedies as being dynamic because they have been succussed. Succussion is thought to be responsible for the release of energy in remedies. Vithoulkas suggests that the addition of kinetic energy brings into action the therapeutic power of homoeopathic remedies (1980:20-21). The energy contained in the remedies is thus a biophysical energy rather than biochemical energy. It may however be that this biophysical energy is transferred during dilution regardless of succussion. Biophysical energy is inherent in the nature of molecules. The dilutions are dynamic in

that they contain matter in a constant state of motion - waves and particles constantly interacting and influencing one another, transferring information to one another.

There is little research on the effect of succussion on dilutions. Studies by Harish and Ditman (1997), Sukal *et al.* (1996) and Christie (1995) show differences between succussed and unsuccussed dilutions. Studies by Sukal *et al.* (2001) show differences in succussed and unsuccussed dilutions in comparison to a control. Further research, utilising carefully conducted strict scientific methodology and with stringent controls, is needed to determine the importance of succussion. Experimental studies such as this one provide an accurate and sensitive test, not influenced by placebo effects or subject to ethical problems. Enzyme assays hold some advantage over germinability trials as they are subject to less external influence and measure a single parameter. Ideally a large number of replicates is needed to provide the statistical power to detect subtle changes in enzyme activity.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The results of this study show that homoeopathic dilutions of gibberellic acid are not effective in inducing the synthesis of α-amylase in barley seed endosperm halves. The biological activity of homoeopathic remedies is therefore questionable. This contradicts earlier studies which show a response by barley seed to homoeopathic dilutions of plant hormones (Bruni, 2001; Him Lok, 2001) as well as horticultural, veterinary and clinical trials (Smith, 2000; Linde *et al.*, 1997; Yakir *et al.*, 2001; Sandoval *et al.*, 1998; Searcy *et al.*, 1995; Betti *et al.*, 1994; Boroni *et al.*, 1991; Saxena *et al.*, 1986) showing homoeopathic dilutions to be active.

The fact that the methods which started from a mother tincture or base substance (Hahnemannian and serial dilutions), differed from the method which did not (electromagnetically imprinted dilutions), suggests that differences may have been more reliably determined with greater replications.

It is difficult to speculate how homoeopathic remedies carry the information which makes them therapeutically useful. It would seem that electromagnetic induction is not sufficient or effective in transferring the remedy information to the dilutions. It appears that the base substance undergoes some interaction with the water in which it is diluted, which results in the storage of remedy information. This study did not support the role of succussion in the transfer of information, but was limited in its statistical strength by the

sample size and data variability. Further studies must be conducted before firm conclusions regarding the validity of succussion and activity of homoeopathic remedies can be drawn.

6.2 Recommendations

It is recommended that:

1. The research is repeated to validate the results obtained.
2. A dilution of GA_3 which may be expected to yield a positive response is included in future studies using this model.
3. The experiment is repeated with a larger number of repetitions.
4. Electromagnetically imprinted dilutions are prepared by machines made by different manufacturers (*e.g.* Mora III, Rasche and FRG).
5. Clinical trials with electromagnetic dilutions are carried out.
6. Dilutions prepared by electromagnetic induction from the base substance as opposed to the homoeopathic dilution should be tested on the same model.
7. Future homoeopathic trials both clinical, *in vitro* and *in vivo* compare succussed and unsuccussed dilutions.
8. The experiment is carried out on seeds at various vigour levels.
9. The study is repeated in the form of a germinability trial on whole barley seed.
10. Varying methods of preparation of homoeopathic remedies (*e.g.* Korsakovian) remedies are studied
11. A varying number of succussions are investigated.
12. Decimal (1:10), centesimal (1:100) and quinquagenimillesimal (1:49 999) dilutions are compared.

13. Seeds of other species (*e.g.* wild oat) are used in the same experimental model to test whether any effect is noted.

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LIST OF APPENDICES

APPENDIX A

Results - METHOD 1 (Raw data)

HAHNEMANNIAN POTENCIES OF GIBBERELLIC ACID

Barley endosperm half-seeds (*Hordeum vulgare*: Stirling)

1. Mass of Endosperm half-seeds prior to treatment (grams)

Treatment	Replication				
	I	II	III	IV	V
1. 4ch	0.4488	0.4032	0.4132	0.4040	0.4420
2. 9cH	0.4444	0.4013	0.4396	0.3913	0.4455
3. 15cH	0.4452	0.4175	0.4515	0.4394	0.4195
4. 30cH	0.4359	0.4204	0.4668	0.4720	0.4439
5. 200cH	0.4031	0.3896	0.4258	0.4075	0.3949
6. control	0.4147	0.3899	0.4186	0.4697	0.4040

2. Absorbance (620nm) of extracts of treated half-seeds, incubated with PHADEBAS substrate

Treatment	Replication					
	I	II	III	IV	V	Mean
1. 4cH	0.0885	0.0941	0.1496	0.1310	0.1549	0.12362
2. 9cH	0.0209	0.2358	0.1991	0.0844	0.1898	0.1460
3. 15cH	0.1658	0.0590	0.2230	0.1384	0.1016	0.13756
4. 30cH	0.0677	0.0943	0.2070	0.1277	0.1525	0.12984
5. 200cH	0.0555	0.1606	0.1458	0.0870	0.1600	0.12178
6. Control	0.0468	0.0801	0.1152	0.0941	0.1377	0.09478

3. Amylase activity induced per gram of dry half-seeds

Formula: [optical density/ dry mass(g)] × enzyme units (0.104167 eu) as per α-amylase standard curve.

Treatment	Replication					
	I	II	III	IV	V	Mean
1. 4cH	25.87591	30.62488	47.50907	42.54957	56.34809	40.5815
2. 9cH	6.171312	77.10453	59.43179	28.30335	29.92618	40.18743
3. 15cH	48.86913	18.54389	64.81154	41.33151	47.70272	44.25176
4. 30cH	20.38013	29.43434	58.18952	35.50211	47.29771	38.16076
5. 200cH	18.06697	54.09185	44.93214	28.0154	45.75642	38.17256
6. Control	14.8087	26.9578	36.11258	26.28901	50.31243	30.89611

APPENDIX B

Results - METHOD 2 (Raw data)

UNSUCUSSED SERIAL DILUTIONS OF GIBBERELLIC ACID

Barley endosperm half-seeds (*Hordeum vulgare*: Stirling)

1. Mass of endosperm half-seeds prior to treatment (grams)

Treatment	Replication				
	I	II	III	IV	V
1. 4cS	0.4009	0.4422	0.4459	0.4508	0.3704
2. 9cS	0.4090	0.4306	0.4248	0.4557	0.3855
3. 15cS	0.4283	0.4240	0.4336	0.4459	0.4498
4. 30cS	0.4420	0.4404	0.4268	0.4787	0.4276
5. 200cS	0.4538	0.4208	0.4450	0.4477	0.4338
6. control	0.4542	0.4545	0.4098	0.4386	0.4691

2. Absorbance (620nm) of extracts of treated half-seeds, incubated with PHADEBAS substrate

Treatment	Replication					
	I	II	III	IV	V	Mean
1. 4cS	0.2360	0.1285	0.1581	0.2320	0.1008	0.17108
2. 9cS	0.1415	0.0820	0.1239	0.0682	0.1092	0.10496
3. 15cS	0.1064	0.1302	0.1857	0.1141	0.1473	0.13674
4. 30cS	0.0941	0.1172	0.1136	0.0884	0.1736	0.11738
5. 200cS	0.1054	0.1105	0.0925	0.0625	0.1118	0.09654
6. Control	0.1072	0.1240	0.1540	0.1126	0.0918	0.11792

3. Amylase activity induced per gram of dry half-seeds

Formula: [optical density/ dry mass(g)] × enzyme units (0.104167 eu) as per α-amylase standard curve.

Treatment	Replication					
	I	II	III	IV	V	Mean
1. 4cS	77.24692	38.132	46.52642	67.53195	35.71041	53.02954
2. 9cS	45.39817	24.98878	38.27296	19.6386	37.17094	33.09389
3. 15cS	32.59859	40.29493	56.19891	33.57789	42.97229	41.12852
4. 30cS	27.93654	34.92091	34.92683	24.23227	53.27424	35.05816
5. 200cS	30.47764	34.45814	27.27639	18.31885	33.81875	28.86995
6. control	30.97083	35.80083	49.31216	33.68798	25.67926	35.09021

APPENDIX C

Results - METHOD 3 (Raw data)

ELECROMAGNETICALLY IMPRINTED POTENCIES OF GIBBERELLIC ACID

Barley endosperm half-seeds (*Hordeum vulgare*: Stirling)

1. Mass of endosperm half-seed prior to treatment (grams)

Treatment	Replication				
	I	II	III	IV	V
1. 4cE	0.4131	0.4342	0.4116	0.3931	0.4000
2. 9cE	0.3986	0.4250	0.3953	0.4344	0.4331
3. 15cE	0.4599	0.4366	0.4508	0.4285	0.4794
4. 30cE	0.4191	0.4756	0.4069	0.3800	0.3835
5. 200cE	0.4493	0.4978	0.4871	0.4570	0.4855
6. Control	0.4633	0.4758	0.4649	0.4727	0.4830

2. Absorbance (620nm) of extracts of treated half-seeds, incubated with PHADEBAS substrate

Treatment	Replication					
	I	II	III	IV	V	Mean
1. 4cE	0.0663	0.1136	0.0712	0.0862	0.0966	0.08678
2. 9cE	0.0765	0.0731	0.0801	0.0725	0.1029	0.08102
3. 15cE	0.0673	0.1100	0.1227	0.1441	0.1112	0.11106
4. 30cE	0.1178	0.0918	0.0792	0.0840	0.1161	0.09778
5. 200cE	0.1468	0.0814	0.1006	0.1151	0.1150	0.11178
6. Control	0.0923	0.1006	0.1525	0.1029	0.1162	0. 1129

3. Amylase activity induced per gram of dry half-seeds

Formula: [optical density/ dry mass(g)] × enzyme units (0.104167 eu) as per α-amylase standard curve.

Treatment	Replication					
	I	II	III	IV	V	Mean
1. 4cE	21.06025	34.33158	22.69916	28.77461	31.69001	27.71112
2. 9cE	25.18427	22.57011	26.58955	21.90047	31.17686	25.48425
3. 15cE	19.20246	33.06086	35.71625	44.12842	30.43771	32.50914
4. 30cE	36.88356	25.3283	25.54128	29.00687	39.72575	31.29715
5. 200cE	42.87409	21.45728	27.10099	33.04946	31.08235	31.11283
6. Control	26.14235	27.74462	43.04429	28.56505	31.56925	31.41311

APPENDIX D

Descriptive Statistics

Dependent variable: ENZYME UNITS/GRAM
Mean enzyme activity in treated half-seeds at each dilution is shown for each of the three methods.

METHOD	DILUTION	MEAN	STD DEVIATION	MINUMIM	MAXIMUM	N
1	Control	30.8961040	13.2263511	14.8087000	50.3124300	5
1	4cH	40.5915040	12.4080472	25.8759100	56.3480900	5
1	9cH	40.1874324	28.0037433	6.1713120	77.1045300	5
1	15cH	44.2517580	16.7731648	18.5438900	64.8115400	5
1	30cH	38.1607620	14.8661084	20.3801300	58.1895200	5
1	200cH	38.1725560	14.6972216	18.0669700	54.0918500	5
2	Control	35.0902120	8.8072465	25.6792600	49.3121600	5
2	4cS	53.0295400	18.4457375	35.7104100	77.2469200	5
2	9cS	33.0938900	10.5068792	19.6386000	45.3981700	5
2	15cS	41.1285220	9.4997728	32.5985900	56.1989100	5
2	30cS	35.0581580	11.1778690	24.2322700	53.2742400	5
2	200cS	28.8699540	6.5606459	18.3188500	34.4581400	5
3	Control	31.4131120	6.7944629	26.1423500	43.0442900	5
3	4cE	27.7111220	5.7040968	21.0602500	34.3315800	5
3	9cE	25.4842520	3.7098914	21.9004700	31.1768600	5
3	15cE	32.5091400	9.0409647	19.2024600	44.1284200	5
3	30cE	31.2971520	6.6379942	25.3283000	39.7257500	5
3	200cE	31.1128340	7.9278498	21.4572800	42.8740900	5