

**THE EFFECT OF ULTRA HIGH DILUTIONS OF GIBBERELIC
ACID ON THE SYNTHESIS OF α -AMYLASE IN
DE-EMBRYONATED HALVES OF BARLEY SEED
(*HORDEUM VULGARE* STIRLING)**

by

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Degree in Technology: Homoeopathy in the Department of Homoeopathy at the
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Dedicated to my wife, Anita Kromberg,
and my parents, Dorothy and John Steele

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ABSTRACT

This study investigated the effect of ultra high dilutions of gibberellic acid (GA_3) on the synthesis of the enzyme α -amylase in de-embryonated endosperm halves of barley seed (*Hordeum vulgare* cv. Stirling, ex Caledon, Western Cape, South Africa, 1998 harvest), in order to determine whether such dilutions are capable of producing a biological effect or not. Homoeopathic theory and practice proposes that such dilutions are capable of such effects (Gaier 1991:445-447).

GA_3 is synthesised by the barley seed embryo, and stimulates *de novo* synthesis of α -amylase in the aleurone layer of the endosperm. This means that endosperm (embryoless) half-seeds contain very little or no GA_3 . Addition of GA_3 to endosperm half-seeds stimulates α -amylase synthesis in a dose-dependent manner. Therefore, the measurement of α -amylase synthesis in endosperm half-seeds is a specific and sensitive indicator for the presence of GA_3 .

Two series of experiments were conducted using different methods of dilution. The first series (Method 1) consisted of 5 Hahnemannian prepared dilutions (i.e. centesimal serial dilution with succussion at each stage), namely 4cH, 9cH, 15cH, 30cH and 200cH. The second series (Method 2) consisted of 5 non-Hahnemannian prepared dilutions (i.e. centesimal serial dilution without succussion), namely 4cs, 9cs, 15cs, 30cs and 200cs.

Each series consisted of 5 replications. Each replication consisted of 5 groups of 20 half-seeds treated with different dilutions of GA_3 , and a 6th (control) group of 20 half-seeds treated with the extraction buffer only. Thus, each series consisted of 30 groups comprising 600 half-seeds, resulting in 60 groups comprising a total of 1200 half-seeds.

An α -amylase activity standard curve was determined, based on pure α -amylase (1350 enzyme units/mg).

Each group of 20 half-seeds was weighed, placed in a numbered 9cm petri dish lined with two No 1 Whatman filter papers, moistened with 5ml of the various treatment solutions and incubated for 48 hours in a dark incubation cabinet set at a constant temperature of 15°C. α -Amylase enzyme was then extracted by means of seed maceration, incubation with 10ml of the extraction buffer (2M Ca (NO₃)₂) in a shaker-bath set at 30°C for 60 minutes, centrifuging and filtration. The enzyme assay was prepared by means of incubation of the solutions with a dye-labeled substrate (PHADEBAS Amylase Test tablets) in a shaker-bath set at 50°C for 10 minutes, followed by filtration. Finally, the absorbance at 620nm was determined spectrophotometrically as a measure of the enzyme activity of each sample.

There was an observable difference in appearance between the control groups and all the treatment groups at every stage of the process. These observable differences were due to the fact that starch was not hydrolysed in the control groups whereas it was in the treatment groups.

Data was analysed statistically by means of Univariate Analysis of Variance (SPSS for Windows version 9). The results indicate that α -amylase activity was found in all the treatment groups, but not in the control groups. There was no significant difference between the results of the two methodologies employed and no significant interaction between methodologies and dilution levels. All results were calculated at the $\alpha = 5\%$ level of significance.

The conclusion was reached that ultra high dilutions of GA₃ are biologically active so are capable of causing α -amylase synthesis, and that succussion as per the homoeopathic (Hahnemannian) method is not a significant factor underlying this capability.

This conclusion challenges the conventional homoeopathic perspective that succussion is necessary for ultra high dilutions to have an effect. It also challenges the conventional chemistry perspective that ultra-high dilutions cannot have an effect because of the presumed lack of molecules remaining from the original substance.

These experiments have demonstrated the suitability of the barley seed GA₃-α-amylase system as an experimental model for investigating ultra high dilutions.

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TABLE OF ABBREVIATIONS

GA₃ - Gibberellic acid

UHD - ultra high dilution

cH - centesimal Hahnemanian

cs - centesimal serial

Pertaining to 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;

Non-Hahnemannian - serial dilution with no succussion.

THE DEFINITION OF TERMS

Germination

The process whereby the embryo resumes the growth activities which were suspended during quiescence or dormancy, and during which new genetic programs are initiated (Jann and Amen 1977:8).

Plant hormone

Plant hormones are organic compounds made in one part of a plant and transported to another part, where they elicit a response (Moore *et al* 1995:411).

Homoeopathy

Homoeopathy is a therapeutic method which clinically applies the law of similars and which uses medicinal substances in weak or infinitesimal doses (Jouanny 1993:11).

The law of similars

The law of similars [is the] similarity between the toxicological action of a substance and its therapeutic actionThe same things which cause the disease cure it (Jouanny 1993:12).

Dilution

The volume of solvent in which a given amount of solute is dissolved (Anon 1990:97).

Ultra high dilution

Standardised aqueous or aqueous-alcoholic solutions where a substance has been diluted through a special dilution process in such a way that the concentration ratio of solute to solvent becomes of the order of Avagadro's number ($6.022 \times 10^{23} \text{mol}^{-1}$) or below (Endler and Schulte 1994:1).

Potency

1. The especially produced capability in a medicine to effect a dynamic stimulus in the appropriate patient [plant] (Gaier 1991:432).
2. The number appearing after the name of the homoeopathic substance, indicating the number of times the substance has been diluted and succussed or triturated. For example: *Causticum* 200cH, *Gibberellic acid* 200cH.

Potentisation

Dynamization; imparting (along serial dilutions) the pharmacological message of the original substance (i.e. creating a template of the active principle) by means of trituration or succussion (Gaier 1991:441).

Succussion

The action of shaking vigorously a liquid dilution of a homoeopathic medicine in its vial/bottle, where each stroke ends with a jolt. Usually effected by pounding the hand engaged in the shaking against the palm of the opposite hand (Kayne 1997:49).

CHAPTER 1

INTRODUCTION

1.1 Introduction

Seed germination is the most vital aspect of agriculture and horticulture and is therefore essential for life on earth in general. Without germination, there would be no plants.

Without plants, the planet would be bare and there would be no life.

This study investigated one aspect of seed germination - the formation of α -amylase in the presence of the plant hormone gibberellic acid (GA_3). The aim was to investigate the effect of high and ultra high dilutions (ranging from 10^{-8} to 10^{-400}) of GA_3 on the synthesis of α -amylase in barley seed endosperm halves, and in this way determine whether such dilutions are capable of producing a biological effect or not. Homoeopathic theory and practice proposes that such dilutions are capable of such effects (Gaier 1991:445-447).

GA_3 plays a crucial role in promoting germination in barley seed. GA_3 is released by the embryo and acts on cells of the endosperm aleurone layer, causing *de novo* synthesis of α -amylase. α -Amylase hydrolyses starch in the endosperm to produce sugar which is used as an energy source for the growing seedling (Moore 1995:424-423).

Barley endosperm halves contain very little or no GA_3 . This means that the amount of α -amylase formed in endosperm halves is directly proportional to the amount of GA_3 supplied (Nissen 1988). Thus, the measurement of α -amylase activity in endosperm half-seeds is a sensitive and highly specific indicator for the presence of GA_3 (Moore 1981:176).

Because of this direct relationship between treatment and effect, the barley seed endosperm GA_3 - α -amylase system was considered to be a suitable experimental model for investigating the effects of high and ultra high dilutions.

Ultra high dilutions (UHDs) are deemed to be standardised aqueous or aqueous-alcoholic solutions where a substance has been diluted through a special dilution process in such a way that the concentration ratio of solute to solvent becomes of the order of Avagadro's number ($6.022 \times 10^{23} \text{ mol}^{-1}$) or below (Endler and Schulte 1994:1).

One of the basic principles of homoeopathy is that of the "minimal dose" (Kayne 1997:26). Dilutions in common use amongst homoeopaths range from 1:9 up to $1:10^{20\,000}$ (representing a deconcentration of $10^{-20\,000}$). Considering this range, one can see that many homoeopathic medicines fall into the category of high and ultra high dilutions.

The dilution process involved in the manufacture of homoeopathic medicines is that of serial dilution and succussion, usually 1 part solute to 99 parts solvent, and is commonly known as the Hahnemannian method after the founder of homoeopathy, Dr Samuel Hahnemann. The solvent is purified water or alcohol unless the solute is initially not soluble, in which case lactose is the solvent, and serial dilution is by trituration until such time as the solvent becomes soluble (usually after 4th centesimal dilution). Succussion is a process of vigorous shaking with impact, and occurs at every step of the dilution process. (Kayne 1997:49-50.) Avagadro's dilution limit is reached in the process of homoeopathic centesimal serial dilution at 12cH (10^{-24}). In homoeopathic dilutions higher than this, therefore, not a single molecule of the original base substance or mother tincture is expected to remain (Gaier 1991:47-48).

This dilution process is known in homoeopathic terminology as "potentisation". This term is used because it conveys the sense that potentisation is an active process which is aimed at endowing the solution with a greater therapeutic effect (Gaier 1991:444). Most

homoeopathic theorists (starting with Hahnemann 1996 paragraph 269), propose that succussion is a crucial step in the preparation of homoeopathic medicines.

Five centesimal serial dilutions were utilised in this study: the 4th, 9th, 15th, 30th and 200th. This represented deconcentrations ranging from 10^{-8} to 10^{-400} . One series (Method 1) of dilutions was prepared according to Hahnemannian methodology (i.e. with succussion at every step) and another (Method 2) not (i.e. with no succussion). These dilutions were selected because they span Avagadro's dilution limit ($6.022 \times 10^{23} \text{ mol}^{-1}$), and the homoeopathic dilution levels are ones which have been commonly used in agricultural related homoeopathic research.

Several studies have been carried out in order to test the efficacy of homoeopathic medicine in the agricultural context, although no record of studies involving potentised GA_3 can be found.

Betti et al (1994) conducted a randomised laboratory trial where homoeopathic dilutions of *Arsenicum album* (decimal potencies 23^{-45}) were tested for their effect on seed germination. Three treatment groups showed a significance level of less than 1% and another was below 5% significance. The experimental results showed that differences between the treatment groups could not be explained as a mere effect of intrinsic seed variability.

Hopkins (1998) investigated the biological effects of homoeopathic medicine treatments (*Sulphur*, *Nitric acid* and *Camphor* in 3cH, 9cH, 15cH and 30cH potencies) on lettuce seed germination. A Germination Index was calculated, and results were analysed by means of a multifactorial analysis of variance. He concluded that biological effects (speeding up or slowing down of germination, depending on the treatment) are evident, represented by statistically significant results between treatments.

Another study which is close to the arena of the present study is that conducted by Bornoroni (1991). He conducted experiments using fragments of oat seedlings (coleoptiles) pretreated with highly diluted solutions of CaCO_3 (the homoeopathic medicine *Calcium carbonate*). During the rapid growth phase the coleoptiles were cultured in the presence of indoleacetic acid (IAA), a member of the auxin class of plant hormones. Pretreatment with homoeopathic dilutions of *Calcium carbonate* 5cH caused a statistically significant increase in growth as compared to those treated with IAA alone. He also noted an increase in growth in coleoptiles when he applied homoeopathically diluted IAA. However, Bornoroni does not specify the precise methodology used to prepare the homoeopathic solutions (or even the degree of dilution in the case of the homoeopathic IAA), and he does not specify the statistical methods used, and their power.

The effects of succussion were studied by Sukul and Ghosh *et al* (1996). They found that *Agaricus muscarius* 30cH, a potentised homoeopathic drug prepared by successive dilution with sonication in 90% ethanol, suppressed haloperidol-induced catalepsy in Swiss albino mice significantly. However, if they diluted the 30cH potency with 90% ethanol in proportions of 1:10 000, 1:20 000, 1:30 000, 1:40 000 and 1:50 000 without further sonication or mechanical agitation, these dilutions produced dose-dependent effects, with 1:30 000 and above producing no effect. However, the anticataleptic effect reappeared when the 1:50 000 dilution was sonicated. 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.

An example of low concentration (high dilution) effect in agriculture is contained in research by King and Oliver (1992) who studied the application rate and timing of various herbicides. They found that reduced rates may effectively control susceptible weeds if applied to young weeds when conditions are favorable for their growth. One of the

herbicides they studied was chlorimuron, where they found that 2 to 5 g ha⁻¹ (labeled rate = 9 g ha⁻¹) controlled common cocklebur (90%) when applied between 8 and 14 days after emergence. An application rate of 2 g ha⁻¹ is 2 000 mg per 10 000 m² of soil, or 0,2 mg per m².

Within the homoeopathic field, this study extends those mentioned above. In particular, it builds on recommendations by Hopkins (1998) that germinability trials be further developed as a way of testing the efficacy of homoeopathic medicines, considering that this methodology is not as complicated ethically as human trials are. He also recommended that the effects of homoeopathically prepared plant growth regulators such as auxins and gibberellins be explored, and that higher potencies be employed. The unique contribution of the present study was to compare the effect of homoeopathic dilutions of a substance not only to a control, but also to the effect of non-homoeopathic (i.e. unsuccussed) dilutions of the same substance.

Within the field of seed physiology and biochemistry, this study extends the many studies which have examined the effects of GA₃ on the synthesis of α -amylase in barley seeds (Paleg 1960, Chrispeels and Varner 1967, Takashi *et al* 1988, and see review by Ziegler 1995). The unique contribution of this study was to demonstrate the biological effects of GA₃ at ultra high dilutions (ultra low concentrations).

1.2 Aim of the study

The aim of this study was to investigate the effect of high and ultra high dilutions of GA₃ on the synthesis of α -amylase in barley endosperm half-seeds.

1.3 Statement of the objectives

1.3.1 The first objective

The first objective was to determine the effect of a range of Hahnemannian (i.e. succussed) serial dilutions of GA_3 on the production of α -amylase in barley endosperm half-seeds.

1.3.2 The second objective

The second objective was to determine the effect of a range of non-Hahnemannian (i.e. unsuccussed) serial dilutions of GA_3 on the production of α -amylase in barley endosperm half-seeds.

1.3.3 The third objective

The third objective was to compare the effect of the methods of dilution on the production of α -amylase in barley endosperm half-seeds.

1.3.4 The fourth objective

The fourth objective was to compare the interaction effect between the methods and dilution levels of each method.

1.4 Statement of the hypotheses

All hypotheses are stated in the null form.

1.4.1 The first hypothesis

It is hypothesised that Hahnemannian (i.e. succussed) serial dilutions of GA₃ will have no significant effect on the synthesis of α -amylase in barley endosperm half-seeds.

1.4.2 The second hypothesis

It is hypothesised that non-Hahnemannian (i.e. unsuccussed) serial dilutions of GA₃ will have no significant effect on the synthesis of α -amylase in barley endosperm half-seeds.

1.4.3 The third hypothesis

It is hypothesised that there will be no significant difference between the methods of dilution on the synthesis of α -amylase in barley endosperm half-seeds.

1.4.4 The fourth hypothesis

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

1.5 The significance of the study

The results have proved that ultra high dilutions (up to 10^{-400}) can have significant biological effects. This provides an answer to a fundamental question regarding the mechanism of action of UHD homoeopathic medicines: do they work through some intrinsic effect, or do they work by means of a placebo effect? (Reilly and Taylor 1986). The results from this study indicate that it may well be an intrinsic effect. This study, therefore, makes a substantial contribution to the scientific validation of homoeopathy.

At the same time, the results break new ground in chemistry and biochemistry by demonstrating biological activity of a solution at ultra low concentrations.

1.6 The implications of the study

Implications of the study are primarily theoretical. Considering that succussion is a central doctrine of homoeopathic practice and theory, the results of this study challenge researchers in homoeopathic theory to pay more attention to the role of dilution per se in the process of potentisation. At the same time, the results of this study challenge chemists and physicists to accept that dilutions greater than $6.022 \times 10^{23} \text{ mol}^{-1}$ can have an effect, and to develop theoretical models to explain the mode of action of very high dilutions (low concentrations).

1.7 The benefits of the study

Besides the scientific benefits, the main benefit of this study is the demonstration of a suitable experimental model for studying the effects of ultra high dilutions. The standardised preparation procedures and objective outcome measurements ensure reliability, and the direct relationship between treatment and effect ensure validity. In addition: the materials are easily obtainable and relatively inexpensive; the equipment is commonly available in scientific research laboratories, and is easily operated; the skills required are basic laboratory skills; the practical preparation of materials and execution of the experiment can be comfortably achieved within two weeks of full-time application.

The demonstration of GA_3 activity at ultra low concentrations points to potential economic benefits in the field of agriculture, where gibberellins are widely used for growth regulating purposes (Vermeulen *et al* 1997 and see Appendix 3), and in the brewing industry, where GA_3 is added to malt after steeping to ensure quicker and more even germination (Lewis and Young 1995:82).

CHAPTER 2

REVIEW OF THE RELATED LITERATURE

2.1 Seed Germination

Seed germination is the process whereby the embryo resumes the growth activities which were suspended during quiescence or dormancy, and during which new genetic programs are initiated. Many factors are involved in the germination process, including anatomical, genetic, metabolic and hormonal events, the last named being the primary agents of germination (Jann and Amen 1977:8-15).

2.2 Plant hormones

Plant growth and development are strongly influenced by plant growth-regulating substances, traditionally referred to as hormones. Plant hormones are organic compounds made in one part of a plant and transported to another part where they elicit specific biochemical, physiological, and/or morphological responses. (Moore *et al* 1995:411.) Hormones also modulate specific environmental cues into biochemical messages (Jann and Amen 1977:14). Plant hormones are active in concentrations as low as $<1\mu M$ (Moore 1979:28). The five major classes of plant hormones are gibberellins, auxin, cytokinins, ethylene, and abscisic acid, each of which have many effects (Moore *et al* 1995:411). Collectively these hormones are known as plant growth regulators (Plant Growth Regulator Working Group 1981) or bioregulators (Schott and Walter 1991:247).

2.3 Gibberellins and gibberellic acid

Gibberellins comprise the class of hormones most directly implicated in the control and promotion of seed germination, gibberellic acid (GA_3) being the most important hormone in this class (Hartmann *et al* 1990:119). Germination is absolutely dependent on the presence of endogenous or exogenously applied GA_3 (Karssen 1995:346).

Gibberellins were first discovered in 1926 by Japanese researchers studying a disease of rice plants caused by the fungus *Gibberella fujikuroi*. Plants infected with the fungus grew excessively and abnormally. Extracts from this fungus applied to noninfected plants stimulated the same abnormal growth. By 1939 the active ingredient was extracted from the fungus, crystallized, and named gibberellin. (Hartmann *et al* 1990:137.) However, natural gibberellins from higher plants were only isolated and chemically identified in 1958 (Moore 1979:92). Gibberellins occur in angiosperms, gymnosperms, mosses, ferns, algae, and fungi, but are unknown in bacteria (Moore *et al* 1995:423).

Gibberellins are synthesised in the seed embryo via the mevalonic acid pathway (Figure 2.1.). Each of the gibberellins has an interlocking ring structure and one or more carboxyl groups that impart acidic properties to the molecule. While there are many molecular variations of gibberellin, the most widely used experimentally and commercially is gibberellic acid (GA_3), whose chemical formulation is $C_{19}H_{22}O_6$. (Hartmann *et al* 1990:119.) (Figure 2.2.)

Besides their role in stimulating α -amylase synthesis during seed germination, gibberellins stimulate cellular elongation, and so are used commercially to stimulate shoot elongation in sugar cane, internode elongation in biennial plants, bolting and early production of seeds, and to increase the size of seedless grapes. By enhancing cellular

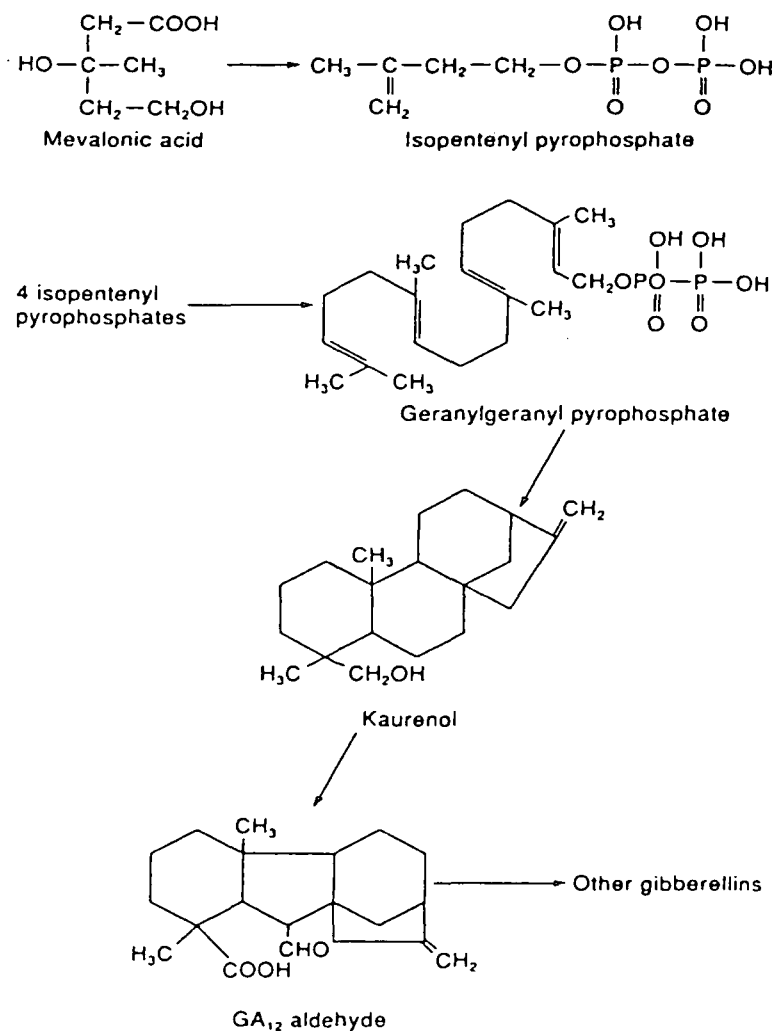


Figure 2.1 Gibberellin is made via the mevalonic acid pathway (Moore 1995:422)

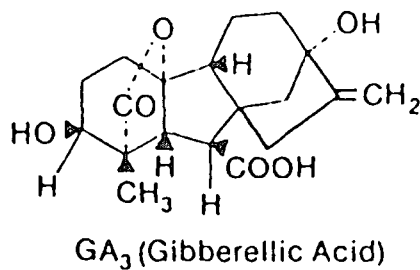


Figure 2.2 Structural formula of gibberellic acid (Hartmann 1990:135)

elongation in the embryo and so causing the embryo to rupture the seed coat, gibberellins will stimulate germination in seeds such as tobacco, lettuce and oats which

usually only germinate after exposure to cold or light. (Moore 1995:424-423.) Brewers add GA₃ to malt after steeping, to boost naturally produced gibberellins. This ensures quicker and more even germination and lowers malting losses by effectively advancing α -amylase synthesis in the aleurone layer (Lewis and Young 1995:82). (See Appendix 3 for a list of agricultural uses of GA₃.)

2.4 Barley

Barley seeds were selected for use in this study because they are highly sensitive to GA₃ (Foskett 1994:447, Karssen 1995:345). Many studies on the role of GA₃ in α -amylase synthesis and the germination of seeds have utilised barley seeds (for example: Paleg 1960, Chrispeels and Varner 1967, Takashi *et al* 1988, and see review by Ziegler 1995). The variety of barley seed utilised in this study was *Hordeum vulgare* Stirling (ex-Caledon, Western Cape, 1998 harvest).

Barley is a monocotyledon of the Graminales order, Gramineae family. It is an extensively grown cereal, with worldwide distribution and adaptation. Barley is mainly used as a feed for livestock and for malting. Malt is used in beer, distilled alcoholic products, malt syrup, breakfast foods, and coffee substitutes. (Metcalf and Elkins 1980:430.)

2.5 The role of GA₃ and α -amylase in barley seed germination

In barley seed, the sequence of events leading to germination begins with imbibition, which is the absorption of water by a seed. Imbibition causes the embryo to secrete GA₃ into the starchy endosperm. The hormone then diffuses to the aleurone layer (a specialised layer of the endosperm 2-4 cells thick located just inside the seed coat) where it triggers the synthesis and secretion of hydrolytic enzymes, including α -amylase, β -1,3-glucanase, protease and ribonuclease. These enzymes are secreted by dictyosomes into the seed's endosperm where they mobilize the stored food reserves.

action, α -amylase catalyses the conversion of starch to sugar thereby producing an energy source for the growing seedling. (Moore 1981:175, Moore 1995:423, Ziegler 1995:452, 461). (See Figure 2.3.)

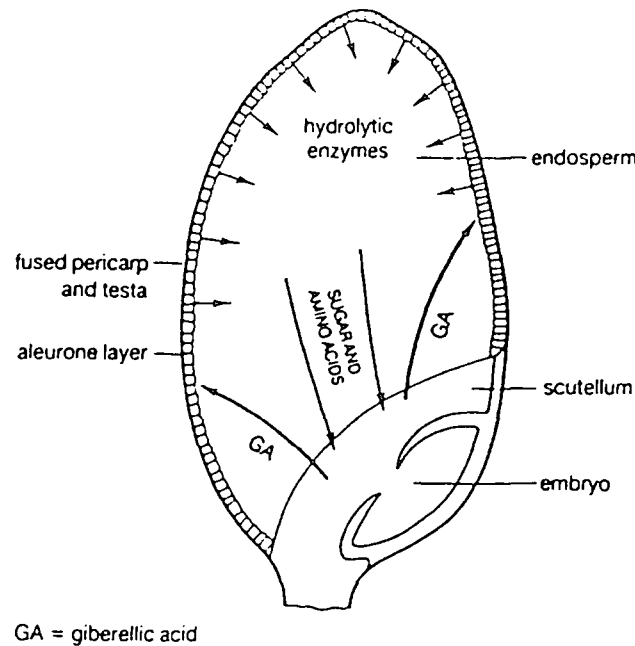


Figure 2.3 The mobilisation of food reserves in barley (Barnes and Poor 1994:8)

α -Amylase is synthesised *de novo* in the endoplasmic reticulum (ER) of the aleurone cells. GA₃ stimulates two key processes in the ER which facilitate the synthesis and secretion of α -amylase: the transcription of α -amylase mRNA, and the transport of Ca²⁺ from the cytosol into the ER. Elevated levels of ER Ca²⁺ are required to sustain the synthesis of active, stable α -amylase. GA₃ achieves this by stimulating an ATP-dependant pump responsible for transporting Ca²⁺ into the ER and so maintaining the necessary Ca²⁺ concentration. (Bush *et al* 1991.)

The primary role of α -amylase is mobilisation of the stored carbohydrate reserves found in the endosperm. Starch is the carbohydrate reserve most commonly found in seeds, including barley seeds. Starch is laid down in discrete subcellular bodies called starch grains. Barley starch grains are predominantly spherical in appearance, and are

separable into two main groups - large ones and small ones. Although the latter account for about 90% of the total number of grains, they comprise only 10% of the total starch by weight. (Bewley and Black 1994:12-13, 296-7.) A number of hydrolytic and phosphorolytic enzymes are responsible for starch degradation and mobilization in barley, but α -amylase appears to be the only species able to attack effectively the native polymer structure. Thus, α -amylase effects the initial depolymerization of the starch granule. The polysaccharides thereby released are further degraded by a combination of α -amylase, β -amylase, starch phosphorylase, and debranching enzyme (limit dextrinase) action. (Ziegler 1995:452.)

2.6 Barley endosperm half-seeds as vehicles for studying the relationship between GA_3 and α -amylase and the effect of ultra high dilutions

Barley endosperm half-seeds and isolated aleurone layers have proved to be useful vehicles for studying the relationship between GA_3 and α -amylase (see review by Ziegler 1995). Many studies have shown that the increase of α -amylase activity in barley endosperm half-seeds endosperm halves is dependent upon added GA_3 . Isolated embryos generally synthesise GA_3 whereas embryoless half-seeds or isolated aleurone layers do not. Addition of GA_3 to endosperm half-seeds or to isolated aleurone layers stimulates α -amylase synthesis. (Paleg 1960, Chrispeels and Varner 1967, Moll and Jones 1983, Takashi *et al* 1988.) Furthermore, the amount of α -amylase formed is directly proportional to the amount of GA_3 supplied (Nissen 1988). Thus, the measurement of α -amylase activity in endosperm half-seeds is a sensitive and highly specific indicator for the presence of GA_3 (Moore 1981:176).

The PHADEBAS Amylase Test, developed by Pharmacia offers a simple, yet sensitive and accurate method for quantitative assay of α -amylase activity (Anon 1994). Originally developed for the purpose of determining human α -amylase, it has been successfully

utilized to determine α -amylase in cereals and cereal products (Barnes and Blakeney 1974, Cairns and de Villiers 1986). PHADEBAS tablets consist of a polymer substrate made by cross-linking partially hydrolyzed potato starch. The substrate is labeled with Cibacron blue by covalent bonds. α -Amylase hydrolyzes this polymer into water-soluble blue dye which absorbs light at 620nm (Anon 1994).

Experimental studies testing the response of biological systems to a particular treatment are very difficult due to the complexity of the system itself and its interaction with the environment, but the barley endosperm GA₃- α -amylase model reduces that complexity considerably by relating directly any effect to the corresponding treatment. Therefore, it is a suitable experimental model for the investigation of high and ultra high dilutions (UHDs).

In addition, experiments involving seeds have certain strategic advantages which are relevant in homoeopathic (and medical) research: no ethical concerns (Hopkins 1998), and elimination of the placebo effect (Pelikan and Unger 1971). The placebo effect is one of the main criticisms leveled at homoeopathic practice (Reilly *et al* 1986)

2.7 High and ultra high dilutions

2.7.1 Introduction

The term "ultra high dilution" is used in this study, although the term "ultra low concentration" could have been used. The former term is chosen because it is more common in homoeopathic research circles. The popularity of this term amongst these researchers is interesting because it probably reveals their bias - "dilution" emphasises the solvent, while "concentration" emphasises the solute. Homoeopathic researchers are generally more interested in the solvent and so-called biophysical effects than they are in the solute and so-called biochemical effects (e.g. Towsey and Hasan 1995).

Endler and Schulte are eminent researchers on ultra high dilution effects and have edited a book of research papers on the topic. In the introduction to this book they define ultra high dilutions as being "standardised aqueous or aqueous-alcoholic solutions where a substance has been diluted through a special dilution process in such a way that the concentration ratio of solute to solvent becomes of the order of Avagadro's number ($6.022 \times 10^{23} \text{ mol}^{-1}$) or below" (1994:1).

2.7.2 Non-homoeopathic examples of high dilutions

An example of low concentration (high dilution) effect in agriculture is contained in research by King and Oliver (1992) who studied the application rate and timing of various herbicides. They found that reduced rates may effectively control susceptible weeds if applied to young weeds when conditions are favorable for their growth. One of the herbicides they studied was chlorimuron, where they found that 2 to 5 g ha⁻¹ (labeled rate = 9 g ha⁻¹) controlled common cocklebur (90%) when applied between 8 and 14 days after emergence. An application rate of 2 g ha⁻¹ is equivalent to 2 000 mg per 10 000 m² of soil, or 0,2 mg per m².

Corbett *et al* (1984:342-3) state that pesticides cause marked inhibition or other interference *in vitro* at concentrations of about 10 μ M and frequently much lower. They also estimate a possible lower limit for herbicide application rates based on the assumption that a weed will be killed when one molecule of a herbicide binds to a single site in each cell in the green part of the plant. Using *Avena sativa* with a leaf area of 11cm³ and a herbicide of molecular mass 250 g as an example, they calculate that 1.25×10^{-13} g herbicide must be applied to each plant.

Rate of degradation of herbicides in the soil differs according to the type of chemical used and the environmental conditions, but many herbicides are known to persist in the soil for periods greater than 6 months (Fryer and Makepeace 1977:72). In some cases, this

residual effect can still be toxic after many years when only an infinitesimal proportion of the active ingredient is expected to remain. Farmers in the Limpopo valley (Northern Province, South Africa) believe that negative biological effects of bromacil (Hyvar X), tebuthiron (Spike) and ethidimuron (Ustilan) are still present in the soil on their farms 15 years after the South African Defence Force applied these herbicides as part of a security operation. A study was conducted on behalf of these farmers by the Pesticide Dynamics division of the Plant Protection Research Institute (Pretoria, South Africa), in which they found photosynthetic inhibitor symptoms and decrease in dry mass with the application of concentrations as low as 0,005 mg/kg of soil. (Cairns, personal communication 1999.)

Within the field of horticulture, application of small quantities (0.1 ppm) of the tertiary amine bioregulators HTA (*N,N*-diethylaminoethyl hexanoate) has been found to accelerate the root development of chrysanthemum cuttings compared to controls (Keithly *et al* 1991:238).

The plant hormone ethylene is active at very low concentrations. Ethylene has nearly full biological activity at 1 μ l/liter, which corresponds to 6.5×10^{-9} M at 25°C (Abeles *et al* 1992:15). Unripe apples which have been stored in rooms with small amounts of O₂ and large amounts of CO₂ (conditions which inhibit ethylene synthesis) ripen when exposed to normal air which contains about 1 part per million of ethylene (Moore 1995:429). A laser-based photoacoustic method for ethylene analysis has been developed: a mechanically chopped CO₂ laser beam passes through an acoustic cell and the absorption of energy by ethylene is detected with a microphone. The lower detection limit using this method is 0.03 nl/liter (Abeles *et al* 1992:19). Ethylene promotes ripening of lemons at concentrations as low as 0.2 μ l/liter (Abeles *et al* 1992:6).

Researchers studying GA₃ have used dilutions up to 10^{-9} M (e.g. Paleg 1960, Cairns *et al* 1986; Schuurink *et al* 1992; Strydom *et al* 1996).

The following are examples of concentrations of various analytes which can be detected within human biological systems (in mol/litre): metabolites $> 10^{-6}$, therapeutic drugs $> 10^{-8}$, steroid and amino acid hormones 10^{-9} to 10^{-4} , protein polypeptide hormones 10^{-20} to 10^{-5} , antibodies 10^{-8} to 10^{-5} , cellular antigens 10^{-9} to 10^{-5} , viral antigens 10^{-12} to 10^{-9} , tumour antigens 10^{-11} to 10^{-9} (Hall 1990:14-16).

It is likely that chemical substances, particularly plant and mammalian hormones, are also active at much lower concentrations than those mentioned above, but technological methods of measurement are not sensitive enough to verify this. For example, smoke as a cue for breaking dormancy of seeds has been investigated since 1990 (De Lange and Boucher 1990, van Staden *et al* 1995, Strydom *et al* 1996, Jager *et al* 1996, Brown and van Staden 1997), but to date researchers have been unable to find the identity of the active molecules. This may well be because of the low concentration of these molecules.

A fascinating example of an UHD effect is described within one of the theories concerning the manner in which the green sea turtle (*Chelonia mydas*) navigates from its feeding grounds off the Brazilian coast to its mating and spawning grounds on Ascension Island 2 000km away. Researchers theorise that chemical substances originating from Ascension Island give rise to an odour plume within the South Atlantic equatorial current that turtles are able to sense and use as a navigational aid (Luschi *et al* 1998).




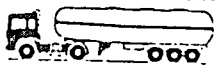



2.7.3 Homoeopathy and ultra high dilutions

One of the basic principles of homoeopathy is that of the "minimal dose" (Kayne 1997:26), so homoeopathic medicines are mostly high and ultra high dilutions. Dilutions in common use amongst homoeopaths range from 1:100 up to $1:10^{20\,000}$. (Table 2.1 and Table 2.2.)

Table 2.1 Homoeopathic centesimal potencies (Kayne 1997:50)

| Dilution | Concentration | Centesimal potency |
|------------------|-----------------|--------------------|
| 1:100 | 10^{-2} | 1cH |
| 1:10 000 | 10^{-4} | 2cH |
| 1:1 000 000 | 10^{-6} | 3cH |
| $1:10^{12}$ | 10^{-12} | 6cH |
| $1:10^{30}$ | 10^{-30} | 15cH |
| $1:10^{60}$ | 10^{-60} | 30cH |
| $1:10^{400}$ | 10^{-400} | 200cH |
| $1:10^{2\,000}$ | $10^{-2\,000}$ | 1000cH or M |
| $1:10^{20\,000}$ | $10^{-10\,000}$ | 10 000cH or 10M |

Table 2.2 Pictorial illustration of the process of dilution (Anonymous nd)

| | | | |
|---|--|---------|--|
|  1 sugar cube (6g) dissolved in |  0.6 Liter 1 % (percent) = 1 part per hundred | 10 g/kg | 10 mg/g |
| |  6 Liter 1‰ (permille) = 1 part per thousand | 1 g/kg | 1 mg/g 1 milligram = 10^{-3} g = 0.001 g |
| |  6000 Liter 1 ppm = 1 part per million | 1 mg/kg | 1 µg/g 1 microgram = 10^{-6} g = 0.000 001 g |
| |  6 million Liter 1 ppb = 1 part per billion | 1 µg/kg | 1 ng/g 1 nanogram = 10^{-9} g = 0.000 000 001 g |
| |  6 billion Liter 1 ppt = 1 part per trillion | 1 ng/kg | 1 pg/g 1 picogram = 10^{-12} g = 0.000 000 000 001 g |
| |  6 trillion Liter 1 ppq = 1 part per quadrillion | 1 pg/kg | 1 fg/g 1 femtogram = 10^{-15} g = 0.000 000 000 000 001 g |

The logic of the "minimal dose" is to be found in an explanation of the Law of Similarity which is central to homoeopathic practice. Originally expressed as *Similia similibus curentur* meaning "let Similars be treated (cured) by Similars", this Law means that when

a substance in a high dose is able to induce defined symptoms in a healthy living system, it is also able under certain circumstances to cure these symptoms when applied in a low or very low dose (Gaier 1991:264).

This dose-dependent reverse effect (also known as hormesis, and the biphasic effect) is recognised within conventional medicine, agriculture and horticulture as well. Examples from medicine include digitalis, which in large quantities causes a picture of arrhythmic tachycardia but in smaller doses is used to treat atrial fibrillation (Reynolds and Prasad 1982:541), and mercury salts which in large doses cause oliguria and anuria, but in smaller doses act as diuretics (e.g. the pharmaceutical drug mersalyl) (Reynolds and Prasad 1982:581). Examples from agriculture include all hormone related herbicides, which in high doses inhibit growth but in smaller doses stimulate growth. For example 2,4-D (2,4-dichlorophenoxyacetic acid) speeds up the rate of respiration and cell division in small quantities but has the opposite effect in large quantities (Klingman 1961:62). Within the field of horticulture, application of small quantities of tertiary amine bioregulators such as HTA (*N,N*-diethylaminoethyl hexanoate) have been found to accelerate the root development of chrysanthemum cuttings compared to controls (0,01 ppm HTA) while being highly toxic to root growth when applied in large quantities (1.0 ppm HTA) (Keithly *et al* 1991:238).

2.7.3.1 Homoeopathic method of preparing ultra high dilutions

The homoeopathic preparation of ultra high dilutions includes stepwise decimal (1:9) centesimal (1:99) or quinquagenimillesimal (1:50 000) serial dilution with succussion at every step. Succussion is a process of vigorous shaking with impact (Kayne 1997:49). The solvent used is distilled water or alcohol. Insoluble substances are first triturated with lactose powder up to 4cH when they become soluble. Quinquagenimillesimal dilutions use lactose as the vehicle to convey the representative proportion from the previous dilution to the next. (Kayne 1997:49-50). Avagadro's dilution limit is reached in the

process of homoeopathic centesimal serial dilution at 12cH (10^{-24}). In homoeopathic dilutions higher than this, therefore, not a single molecule of the original base substance or mother tincture is expected to remain (Gaier 1991:47-48).

The above preparation process is known in homoeopathic terminology as "potentisation". This term is used because it conveys the active nature of the process which is aimed at endowing the solution with a greater therapeutic effect (Gaier 1991:444). This process was developed by Dr Samuel Hahnemann, the founder of homoeopathy, in the late 18th century. He started by simply diluting medicinal substances because he saw the toxic effects of large doses of medicines. His aim was to reduce the toxic effects while retaining the therapeutic effects. At about the same time, he began to experiment on himself by taking material doses of medicinal substances (e.g. Cinchona bark) while he was healthy and then observing the symptoms which developed. He soon realised that symptoms caused by the substance on a healthy person were similar to those in a sick person the substance was meant to cure. This he formulated in 1796 as the Law of Similars. The term "homoeopathy", arising from the Greek words "*homoios*" (similar) and "*pathos*" (suffering), first appeared in writing in 1807. (Kayne 1997:22-25.) In due course, Hahnemann added succussion into the process of preparation. However, although he was a prolific writer, Hahnemann never explained the origin of his decision to introduce succussion, and historians have not been able to pinpoint exactly when the transition happened. Hahnemann only started using the term potentization in 1827. (Koehler 1986:33.) He came to regard succussion as the central feature of homoeopathic medicine preparation, seeing this as the refining force which converted a toxic (e.g. arsenic re: the homoeopathic medicine Arsenicum album) or inert (e.g. oyster shell re: the homoeopathic medicine Calcium carbonicum) substance into a therapeutic one (Organon paragraph 269, Hahnemann 1996). Succussion is still regarded today, by homoeopathic practitioners and researchers alike, as being the element which enables the retention and development of pharmacological information from the original substance throughout the process of dilution (see 2.7.4.1 below).

2.7.4 Scientific investigations and theories of ultra high dilutions, with special reference to homoeopathic medicines

The major questions regarding homoeopathic medicines which require scientific investigation are succinctly stated by Schulte and Endler (1994:245): what are the mechanisms of (A) the interaction between the molecular mother substance and the solvent water; (B) the storage of molecule-specific information in the solvent; (C) the interaction of the medicine with the organism. The following literature review will concentrate on (A) and (B), which are more pertinent to the scope of this study.

Several physical experimental methods have been applied in researching the mechanism of information storage in homoeopathic high dilutions. These are reviewed by Schulte and Endler (1994), and include electrical conductivity and dielectricity of aqueous solutions, surface tension, Raman laser spectroscopy, nuclear magnetic resonance spectroscopy (NMR), UV-spectroscopy, X-ray spectroscopy, and differential microcalorimetry. All of these methods have demonstrated various degrees of physicochemical changes, although the editors point out that more rigorous experimental methodology and analysis is warranted in some cases.

Demangeat and Poitevan *et al* (1992) conducted a detailed physical study into high dilutions of Silica using NMR. The aim of the study was to investigate any changes in relaxation times of water in dilutions of a medicinal substance. Statistical analysis of the results showed a highly statistically significant difference in T1 and T1/T2 between physiological saline (NaCl) and dilutions of lactose-based Silica. This was for dilutions higher than or equal to the 9cH dilution. On the basis of these results, the authors hypothesised that there is a destructuring of free water, with the breaking of hydrogen bonds resulting in greater mobility of water molecules.

Ross (1997) used NMR spectroscopy to examine samples of quinquagenimillesimal (1:50 000) potencies of homoeopathic lactose-based Sulphur compared to a lactose control, and found significant statistical differences between the integration values of the CH_2 signals of six of the treatment samples.

Berezin (1994) discusses the possible relationship between the homoeopathic effect as a physical phenomenon and the fact that most chemical elements are mixtures of several stable isotopes. Homoeopathic remedies are based on water or water-alcohol mixtures which are basically molecules built up of hydrogen (H), carbon (C) and oxygen (O) atoms. These all have degrees of isotopic diversity, so are an ideal matrix for information transmission and storage. Besides the different chemical and physical properties of atoms in a molecule, isotopic diversity provides two more characteristics: diversity in mass and in abundance. With a different mass, the vibrational interaction among molecules changes. From the natural abundance of the isotopes an average distance may be estimated, and vibrational modes and mode difference determined. This provides additional levels of high-density information storage.

Schulte (1994) bases his theory on the concept of clustering of solution molecules, the unique structure of which is formed by the interaction of the solute and the hydrogen bonds of the water molecules. The clustering which occurs around a solute molecule (reference molecule) influences other water molecules contiguous to and at a distance from the reference molecule by virtue of their interconnection. In this way solute clusters and water molecules react to each other and adjust their energy levels accordingly. This is called a 2-, 3-, 4-, ..., n-body collision, a many-body or collective interaction. This phenomenon can also be seen in collective charge motion in plasma physics. This collective interaction of clusters is a way in which information can be transferred. Schulte cites an experiment conducted by Berezin in 1991 which illustrated this type of information carrying property of water: gelatinized water is formed when only one molecule of pseudo-iso-cyanin pigment is mixed in about 2 000 molecules of water. The

information "presence of pseudo-iso-cyanin" is carried through by all hydrogen bonds, and it remains permanent i.e. the water becomes gelatinized. Schulte writes that hydrogen bonds give rise to an incredible number of meta stable structural states, where each state represents a certain structure. He illustrates this by considering hydrogen bonds as a system of logical switches, where the logical rules relate to the rules of the physical interaction. Stimulating those switches with suitable fields of suitable energy, the switches react according to the rules of the interaction, like the magnetic and electric stimulated rearranging of molecules in a liquid crystal. Combining his model of cybernetic switches with that of Berezin on isotopic diversity, Schulte visualizes the (rare) isotopes as characteristic nodes in a pattern of information, and the diversity of vibrational modes as a dynamic carrier (amplifier, multiplier) of information. Thus, a drug molecule may change the pattern of nodes, which causes a different coupling of vibrational modes. The pattern of isotopic nodes may be stabilised (amplified, multiplied) by the coupling of vibrational modes, even when the drug molecule is no longer present in the system.

Anagnostatos (1994) proposes that a particular form of small water clusters, namely clathrates, is a suitable model for proposing information transfer in the preparation process of homoeopathic medicines. A clathrate is a crystalline compound formed when the solvent encases the solute in a hydrogen-bonded water shell-like lattice structure. With the succussion process, the solute molecule separates from the clathrate due to the different inertial kinematics between the relatively large density (small volume) of the solute and the small density (large volume) of the clathrate. The solute molecule at its new, relocated position interacts with the surrounding water molecules leading to the formation of a new clathrate identical to the initial one. After the separation of the molecule of the solute from its clathrate, the clathrate, because of its symmetric structure, survives and in addition shrinks due to absence of the hydrophobic forces between the solute and water molecules of the clathrate. This shrinking increases the strength of hydrogen bonds, and causes the organisation of the layer of water contiguous to the clathrate shell which takes on the form of a pentagonal dodecahedron - the form of the

initial clathrate. In this way the original clathrate becomes a core clathrate with a mantle clathrate. The difference in kinematics between core and mantle clathrates again leads to separation during succussion, with the formation of new clathrates etc. Thus, once the basic structure of the original clathrate is formed, the replication process may go on without the solute molecules being present. Anagnostatos concludes that since the formation of a specific clathrate corresponds to a molecule of a specific substance, a specific remedy in homoeopathy resulting from the microdilution of a certain substance will have characteristic properties of that substance, even without its physical presence.

Del Guidice (1994) applies the superradiance model to water to explain the transfer of information from the molecule to the UHD. Superradiance is the phenomenon occurring in a dense set of particles interacting through the electromagnetic (e.m.) radiative field. Del Guidice shows that beyond a threshold of density, the particle and e.m. fluctuations couple, giving rise to a new "coherent" configuration in which particles and field oscillate with a constant phase relationship within regions ("coherence domains"), the size of which is the wavelength of the e.m. radiation field. With regard to information storage in a solvent, this model proposes that there is a coherent interaction between the electromagnetic or magnetic vector potential fields of molecules of the diluted mother substance and the dipoles of the solvent water, including the permanent polarisation of the water, which thus becomes coherent.

Drawing on the mechanism of action of gas lasers, bioluminescence, crystals and microwave resonance therapy, Towsey and Hasan (1995) extend cluster and clathrate theories of UHDs to propose a crystal lattice structure theory of UHDs. They propose that the layers of water molecules surrounding the solute molecule are held in semi-crystalline array, the shape of which is determined by the enclosed molecule. When a potentised medicine is prepared, the technique of succussion causes parts of the crystals to break off and form the seed for new crystals in the same pattern as the original crystals enclosing the solute molecule, but now not being dependent on the presence of those

molecules. Dilution has two effects; it progressively removes crystals containing the solute molecule and it supplies a fresh source of unstructured water. Each water envelope vibrates at a specific frequency as determined by its crystalline structure. Studies of biological luminescence suggest that the absorption and emission of coherent radiation inside cells are an integral part of cellular coordination and control. Because crystals can absorb and emit radiation at a specific frequency as determined by its structure, Towsey and Hasan postulate that each potentised water crystal acts like a radiation receiver and transmitter in the blood stream. Given the penetrating power of coherent radiation, it can pass into adjacent tissue and by resonance restore an inactive enzyme to its active conformation. Only proteins able to absorb or resonate with the transmitted frequency would be affected. Another researcher, Delinick (1991) also proposes that homoeopathic medicines, which oscillate at the frequencies imparted by the dissolved substance, impart a certain portion of free energy to the organism. Quoting the Principle of Superposition, Delinick states that for maximum effect the applied force (the correct homoeopathic medicine) should have the same frequency as the object (pathological symptoms of the organism). Due to resonance, there will be an initial aggravation phase. This mirrors homoeopathic theory and practice regarding the Law of Similars and the homoeopathic aggravation, or "healing crisis" (Gaier 1991:22).

Popp (1990, 1994) also proposes a resonance-like interaction between the dilution and the organism, except that he reverses the direction of energy flow. He uses a mechanical model consisting of two weakly coupled pendula to demonstrate how energy can be transferred from system 1 ("disregulatory" oscillation within the patient's body) to system 2 (the homoeopathic medicine). He points out that the basic effect of succussed highly diluted substances is always a delocalisation of the energy in a resonance-like interaction between emitter (organism) and absorber (dilution). In the case of stress, the organism would work as a boson (coherent photon) store. Its typical - stress-linked - oscillations give rise to perturbations of homeostasis. The high dilution acts as a resonance absorber

of the stress-linked oscillations as soon as the adequate substance in the appropriate dilution is used. Popp states that mechanical shaking of the homoeopathic medicines favours the creation of coherent states.

Research has been conducted on the possibility of transfer of molecular information from a substance to water without the material presence of the substance in the test liquid. This was accomplished by Citro and Smith *et al* (1994) who transferred information from a molecular thyroxine suspension ($1:10^3$) to non-pre-treated distilled water ("test liquid") by means of an electronic amplifier. Two transitions in the metamorphosis of *Rana temporaria* were investigated under the influence of this test liquid. In these preliminary experiments, the test liquid, after an initial acceleration period, slowed down both the development from the two-legged to the four-legged tadpoles as well as the juvenile frogs. The researchers concluded that a molecular signal can be transferred via an electronic device, and that electro-magnetic (or magnetic vector potential) fields play a decisive role in the information transfer from biomolecules to the organism.

2.7.4.1 Investigation of the role of succussion in preparing homoeopathic ultra high dilutions

The succussion phase in the preparation of UHDs is described within the framework of fluid dynamics by Auerbach (1994). The mechanical mixing process is discussed in terms of saddle flow, vortex flow, shear flow, and diffusion flow. The relevance of the different flows are discussed in relation to the drug dissolving process. By analyzing the flows Auerbach concludes that the diffusion flow gives rise to the most active force in preparing UHDs. It seems to be slow, but, theoretically (according to Fick's law), if a concentrated substance is put into water then a trace of this substance will be found arbitrarily far away immediately: a trace of the substance moves with high velocity from a region of high concentration to a region of low concentration. Auerbach states that the force of diffusion is a dissolving force, and is the force which really activates the all-penetrating quality of

water. Other forms of flow serve diffusion, and merely speed up bulk mixing.

Unfortunately Auerbach does not state what it is that "diffuses" in an UHD where there are presumed to be no molecules left of the original solute.

Antonchenko and Ilyin (1992) explain that water structures in hydration shells and similar microclusters of water are dissipative structures, and their stabilisation may depend on the movement of protons along spiral water molecule chains within these structures. The formation of such open co-operative systems in water is connected with the soliton mechanism of proton-transfer through hydrogen-bonded chains. Succession causes the formation of cavitation microbubbles in a liquid. The collapse of the bubbles results in water molecule dissociation and finally in the release of the protons necessary for stabilisation of the dissipative structures.

Torres and Ruiz (1996) interpret succession and the process of potentiation in terms of stochastic resonance, which is a non-linear response of certain systems when perturbed by noise and a weak periodic signal. In this system, if a weak periodic modulation (weak in the sense that acting alone it would be unable to produce transitions) is added to the noise input, the output drastically changes its character, and is now dominated by the frequency of the applied perturbation i.e. the periodic signal entrains (phase-locks) the output. Interpreting this scheme in terms of potentiation, the solute is identified with the source of applied noise, succession with the periodic perturbation, and the solvent with the non-linear system. The transition between two states of the solvent can be produced by noise alone, but the energy in white noise is evenly distributed among its many frequencies, so the power to produce transition at a specific frequency interval is small. Adding a periodic signal makes this transition process much more efficient, in that when a periodic signal is added, a substantial part of the noise energy becomes available to produce transitions in the solvent. Thus, the solute is a source of energy to be entrained

by succussion to produce transitions in the solvent. Different active agents (solutes) correspond to diverse degrees of colouring, and uniquely affect the nature of the transition states and the transfer of energy.

Resch and Gutmann (1987, 1991) propose a supermolecular system organisation of liquid water as their starting point for an examination of the role of dilution and succussion in the potentiation process. A solution in water shows greater similarities to "pure" water the smaller the concentration of the solutes. When a so-called "mother tincture" is diluted in pure solvent, two similar systems come into contact, namely the more differentiated solution with its better developed static structural aspects and the less differentiated and dynamically more active solvent. By mixing them - e.g. by the process of dilution - a new system with a system organisation which differs from those of the component liquids is produced. The greater "openness" and the better developed dynamic aspects of the pure liquid allow the integration of structural information from the mother tincture into the more diluted solution. In each step of dilution, the concentration of molecules of the mother tincture is decreased, but its information is not lost, and rather spread over the whole of the more dilute solution. At the same time, the solubility of the gases is somewhat increased, and hence the ratio of hydrophobic to hydrophilic solute molecules. Thus, the number of molecules is increased in the higher hierarchic levels and decreased in the lower hierarchic levels. The original structural information is not lost, but transformed and dynamically "inscribed" in the higher hierarchic levels. In each attenuation step the less differentiated system (pure solvent as the diluent) is modified by the structural aspects of the differentiated system (the solution) to a greater extent than vice versa. In this way, integral structural information from the solution is spread over the new system, and this process is facilitated by shaking the solution. Shaking the mixture adds energy and intensifies the "existential conflict" between the two systems of organisation. In the course of responding to this conflict, the energy is redistributed within the developing system organisation and the concentration of dissolved gas molecules is increased. Because the solute molecules of the mother tincture are subordinated to the dissolved gas molecules,

the latter will consume a greater amount of the additional energy provided by the shaking procedure, and the additional transfer of structural information from the original solute molecules to the gas molecules is accomplished. In this way, the structural information originally present in the solute is readily integrated and dynamically maintained in the more dilute solution. Besides adding kinetic energy, Resch and Gutman (1987:329-330) show that succussion improves the system organisation of the solution. They illustrate this with an example of the changes in properties of metal melts near their melting point as a result of strong mechanical agitation. When a solidifying alloy is vigorously agitated during the first fifty percent or so of solidification, a highly viscous slurry with thixotropic properties is produced. This speeds up the cooling process. The crystallinity of this system is less pronounced, but its wear resistance is improved and its strength is appreciably higher than that of an alloy cooled in the normal way. These casting techniques are known as Rheocasting and Thixocasting processes.

2.7.4.2 A clinical study of the effects of succussion

The effects of succussion were studied by Sukul and Ghosh *et al* (1996). They found that Agaricus muscarius 30cH, a potentised homoeopathic drug prepared by successive dilution with sonication (more effective than mechanical agitation, according to previous research by the authors(1992)) in 90% ethanol, suppressed haloperidol-induced catalepsy in Swiss albino mice significantly. However, if they diluted the 30cH potency with 90% ethanol in proportions of 1:10 000, 1:20 000, 1:30 000, 1:40 000 and 1:50 000 without further sonication or mechanical agitation, these dilutions produced dose-dependent effects, with 1:30 000 and above producing no effect. However, the anticataleptic effect reappeared when the 1:50 000 dilution was sonicated. 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.

2.7.5 Summary and critique of ultra high dilution theories

Most modern theories regarding the manufacture of homoeopathic medicines focus on the central role played by water (and water-alcohol mixtures) in this process. Water has a unique structure (hydrogen bonds etc), and is able to: rearrange its structure (be "informed") relative to an incoming solute; continue developing the new structure even when there are apparently no material molecules of the original solute remaining; maintain the new level of organisation; deliver the relevant information at the relevant location.

Not all theorists explain the role of succussion in this process, but they all presuppose it as a vital ingredient. No theorists dispute the necessity of succussion, while those theorists that specifically examine succussion all agree that succussion somehow facilitates or improves the organisation of the resulting solution. However, Auerbach's findings (1994) that diffusion (which is not dependent on agitation) is the most important element in activating the all-penetrating quality of water is an intriguing contribution to the discussion.

In a critical review of theories regarding the composition and action of ultra high dilutions, Poitevan (1995) criticises theories relating to structural changes in the solvent (e.g. those of Resch and Gutmann 1991), saying that he cannot see how groupings of some water molecules around the substrate could remain stable with time and survive shaking and the transfer of solutions from one receptacle to another. Setting aside the criticism regarding stability for the moment, the latter points of his criticism are astonishing, considering that later in the same article he says that "simply putting together substance and solvent is not enough to register a biological message. External energy in the form of vigorous shaking is vital to trigger any physiochemical reaction." Regarding stability, Resch and Gutmann (1991) and Antonchenko and Ilyin (1992), describe in great detail how shaking helps stabilise the resulting solution - through improving organisation (Resch

and Gutmann 1991) and through causing the release of protons which increase effective water molecule interaction energy (Antonchenko and Ilyin 1992). Poitevan also criticises theories relating to the electromagnetic effects within the solvent (e.g. those of Popp 1990, and Del Guidice 1994) saying that the two essential problems raised by the action of high dilution, namely, the specificity and permanence of information, are not sufficiently explained by these theories. However, all these theories explain specificity in terms of the specific characteristics of the original solute which are carried through in the process of succussion. Regarding permanence of information, Popp (1994), for instance, says that succussion favours the creation of coherent states due to a certain symmetry breaking that takes place as a result of the succussion action. He says that theoretically coherent states should not decay at all, but this ideal situation will never occur in reality. Rather, the high number of non-thermal bosons introduced by mechanical shaking provides relatively long decay times. In addition, with progressive relaxation, the decay will turn (by the change of its exponential decay law into a hyperbolic decay law) more and more into the stable subradiance regime. This explains the longevity of homoeopathic medicines.

A more telling critique of the theory of structural changes in water due to succussion is made by Silvio and Arnaldo (1990), who investigated the possibility of microstructural differences between "normal water" and "homoeopathic" water solutions by propagation of ultrasonic waves in diluted water-ethanol mixtures. The homoeopathic solutions consisted of 5cH, 9cH and 30cH solutions compared to sterilised double-distilled water. Spectroscopic results did not display any difference between the two liquid systems. However, the researchers did not examine solutions which were made up with a solute to start with, which is the case with all homoeopathic medicines. The results of such a study may well be different, due to the influence of the solute.

Most theories on the potentisation process imply a progressive "benification" of the original substance. The clearest example of this is in the work of Resch and Gutmann,

where they state that the precision of the remedy information is improved in the course of successive dilution and shaking procedures (1987:334), which implies a linear course of development. However, several studies have demonstrated a sinusoidal shape to the wave of remedy effectivity (Pelikan and Unger 1971) and of certain physical characteristics of remedies (e.g. infra-red absorption [Gibson 1968], surface tension and electrical conductivity [van Schalkwyk, 1998]). In addition, some potency levels are not statistically effective even though other potency levels in the same study are (e.g. Pongratz and Endler 1994). None of the above theories seem robust enough to explain these non-linear effects.

The obvious vulnerability of many of the UHD theories is the lack of experimental models to demonstrate and measure such sophisticated physical characteristics. As these develop, more light will be shed on the arrangement of UHDs.

2.8 Homoeopathic agricultural research

This researcher can find no record of GA_3 ever having been manufactured in homoeopathic dilutions or having been utilised in homoeopathic treatment or homoeopathic agricultural research. However, there are some examples of homoeopathic medicines which are manufactured from hormones. These medicines are known as "sarcodes". A sarcode is a homoeopathically prepared medicine made from healthy plant, animal or human tissue or secretions (Gaier 1991:292). Hormone related sarcodes used in homoeopathic practice include: *Corticotrophin*, *Folliculinum*, *Parathyroidinum*, *Thyroidinum*, *Thyreostimuline* (see Julian 1979).

Various studies have been carried out in order to test the efficacy of homoeopathic medicine in the agricultural context. Many of these are reviewed and discussed by Scofield (1984). Studies directly relevant to seeds and seed germination will now be discussed.

Boyd conducted a series of controlled experiments over a period of 14 years in which he measured the rate of hydrolysis of starch by diastase under the influence of microdoses (up to $1:10^{-61}$) of mercuric chloride, and found statistically significant inhibition of hydrolysis (Kollerstrom 1982). Diastase is a mixture of α -amylase and β -amylase and is produced in seed germination.

Netien *et al* (1966, in Scofield 1984) experimented with seeds obtained from dwarf pea plants which had been sprayed with copper sulphate solution, then at a later point half the plants were treated with 15cH of copper sulphate and the remaining half with double distilled water. Seeds from plants treated with *Copper sulphate* 15cH had better germination than those from plants which received double distilled water, although the statistical tests used are not mentioned.

Saxena *et al* (1986) investigated the effects of five homoeopathic medicines (*Thuja*, *Sulphur*, *Nitric acid*, *Clacarea carbonica*, *Teucrium*) in different potency levels (Q, 30cH, 200cH) on seed germination of *Abelmoshcus esculentus*. He found that the percentage of seed germination and root-shoot lengths had increased in all treatments in comparison to the control, although the statistical tests used are not mentioned.

Betti *et al* (1994) conducted a randomised laboratory trial where homoeopathic dilutions of *Arsenicum album* (decimal potencies 23 to 45) were tested for their effect on seed germination. The percentage of germinated seeds as a function of time was calculated and compared to the Poisson distribution of the distilled water group, which allowed for parametric statistical evaluation of the significance of the different treatment groups. Three treatment groups showed a significance level of less than 1% and another was below 5% significance. The experimental results showed that differences between the treatment groups could not be explained as a mere effect of intrinsic seed variability. Average germination time statistically analysed by means of a one-factor analysis of variance did not yield significant results.

Hopkins (1998) investigated the biological effects of homoeopathic medicine treatments (*Sulphur*, *Nitric acid* and *Camphor* in 3cH, 9cH, 15cH and 30cH potencies) on lettuce seed germination. A Germination Index was calculated, and results were analysed by means of a multifactorial analysis of variance (nested design model). He concluded that biological effects are evident, represented by statistically significant results (at the $\alpha = 0.05$ level of significance) between treatments. He states that his study supports the employment of germinability trials as a possible methodology for testing the efficacy of homoeopathic medicines without ethical implications. Amongst other things, he recommended an increase in the number of potency levels used (he went up to 30cH), and that the effects of plant growth substances or plant growth regulators prepared according to homoeopathic principles be investigated. The current study included the 200cH potency level, and used a homeopathically prepared plant regulator substance.

Another study which is close to the arena of the present study is that conducted by Bornoroni (1991). He conducted experiments using fragments of oat seedlings (coleoptiles) pretreated with highly diluted solutions of CaCO_3 (the homoeopathic medicine *Calcium carbonate*). During the rapid growth phase the coleoptiles were cultured in the presence of indoleacetic acid (IAA), a member of the auxin class of plant hormones. Pretreatment with homoeopathic dilutions of Calcium carbonate 5cH caused a statistically significant increase in growth as compared to those treated with IAA alone. He also noted an increase in growth in coleoptiles when he applied homoeopathically diluted IAA. However, Bornoroni does not specify the precise methodology used to prepare the homoeopathic solutions (or even the degree of dilution in the case of the homoeopathic IAA), and he does not specify the statistical methods used, and their power.

2.9 Summary

The measurement of α -amylase synthesis in barley endosperm half-seeds is a standard procedure for investigating the effect of GA_3 , (e.g. Chrispeels and Varner 1967, Ziegler 1995). This study extended previous studies of this nature by investigating the effect of highly diluted (homoeopathic and non-homoeopathic) applications of GA_3 on the synthesis of α -amylase in de-embryonated endosperm halves of barley seed (*Hordeum vulgare* cv. Stirling) in terms of enzyme units produced per gram dry weight of endosperm halves. The inclusion of non-homoeopathic (i.e. non-succussed) dilutions in this study extended previous studies on the action of homoeopathic ultra high dilutions.

CHAPTER 3

RESEARCH MATERIALS AND METHODS

3.1 Introduction

The study was carried out in the Seed Physiology Laboratory, Department of Agronomy, Faculty of Agriculture, University of Natal, Pietermaritzburg. See Figure 3.1 for a summary of the experimental protocol.

1. Prepare dilutions - homoeopathic and non-homoeopathic.
2. Prepare α -amylase standard curve.
3. Prepare seed.
4. Prepare petri dishes with the different treatment solutions.

Incubate petri dishes in the dark in a growth chamber set at 15°C for 48 hours.

Terminate the germination process by placing the petri dishes in a freezer set at -20°C.
1. Enzyme extraction:

Unfreeze the petri dishes (room temperature for 5 minutes).

Macerate the contents of each petri dish with 10ml of extraction buffer, decant into numbered centrifuge tubes;

Incubate samples in a shaker bath set at 30°C for 60 minutes.

Centrifuge and filter;

Make up to 10ml with the extraction buffer.
2. Enzyme assay:

Place samples in a shaker bath set at 50°C then add 1 Phadebas tablet per sample. Incubate for 10 minutes then add 1ml NaOH to each sample to terminate the reaction;

Filter;

Make up to 10ml with the extraction buffer;

Using a spectrophotometer, read the absorbance of each sample at 620nm. Record the readings in table form.
7. Data analysis

Figure 3.1 Summary of the experimental protocol

3.2 Barley

Variety: *Hordeum vulgare* cv. Stirling, 1998 harvest. Obtained directly from the Caledon Farmers Cooperative, (Western Cape, South Africa). The seed was not treated with any chemicals.

3.3 Gibberellic acid (GA₃)

The source of GA₃ was the potassium salt of GA₃ (C₁₉H₂₁O₆K, molecular weight 384.5), obtained from Sigma Chemical Co, St Louis, USA. This salt of GA₃ (KGA₃) is readily soluble in water.

3.4 Preparation of the GA₃ dilutions

All the GA₃ dilutions were prepared by the researcher under laminar flow conditions in the homoeopharmaceutical laboratory of the Department of Homoeopathy, Technikon Natal, Durban. All glassware was sterilised first by means of rinsing in distilled water then baking in an oven set at 160°C for 2 hours. The stock solution was made up by combining one part KGA₃ (0.25g) with 99 parts (24.75mls) of distilled water, forming a 1% solution of KGA₃ (26mM). From this stock solution two sets of dilutions were made: Method 1 (serial dilution with succussion) and Method 2 (serial dilution without succussion). Each dilution in Method 1 was succussed 10 times, in accordance with Method 5a ("Solutions") of the German Homoeopathic Pharmacopoeia (British Homoeopathic Association 1985:20-21). This is the Pharmacopoeia most commonly used by homoeopathic pharmaceutical manufacturers in South Africa. See Figure 3.2 for a model diagram of the homoeopathic dilution and succussion process.

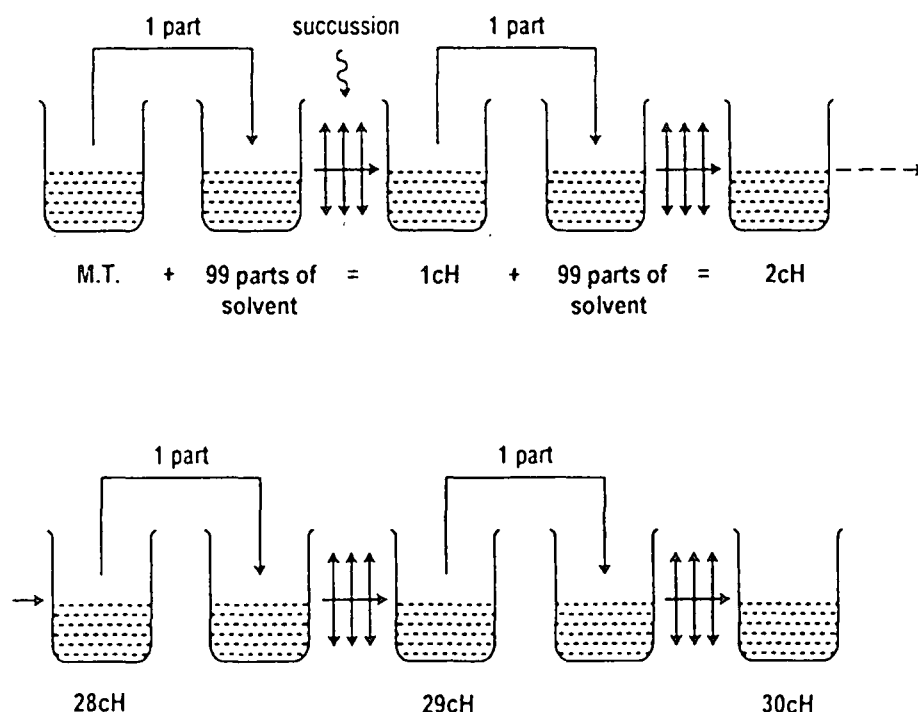


Figure 3.2 Homoeopathic centesimal dilution method

Dilutions were made up in distilled water so as to avoid any possible inhibition

(van der Burg and Bino, 1994) or stimulation (Cairns 1998, personal communication) of the germination process due to the alcohol. Dilutions required for experimental purposes (4th, 9th, 15th, 30th, 200th) were made up in 100ml volumes (1ml of the preceding dilution mixed with 99ml of distilled water). To each of these dilutions was added 1ml of the incubation buffer $\text{Ca}(\text{NO}_3)_2$, resulting in a 20mM solution of $\text{Ca}(\text{NO}_3)_2$. The proportion of the incubation buffer remained constant throughout the study in order to maintain optimal conditions for the synthesis of α -amylase. Calcium plays a role in the synthesis of active and stable α -amylase molecules (Bush *et al* 1991).

3.5 Preparation of α -amylase activity standard curve

Preparation of the α -amylase standard curve was based on the method of Moore (1981: 178-9) adapted by Cairns (1998, personal communication), using pure α -amylase (Boehringer Mannheim GmbH, Germany, 1350 enzyme units/mg) and PHADEBAS Amylase Test tablets (Pharmacia Diagnostics AB, Uppsala, Sweden). An enzyme unit is defined as the amount of enzyme catalyzing the hydrolysis of 1 μ m glucosidic linkage at 50°C (Cairns and de Villiers 1986). See Figure 3.3.

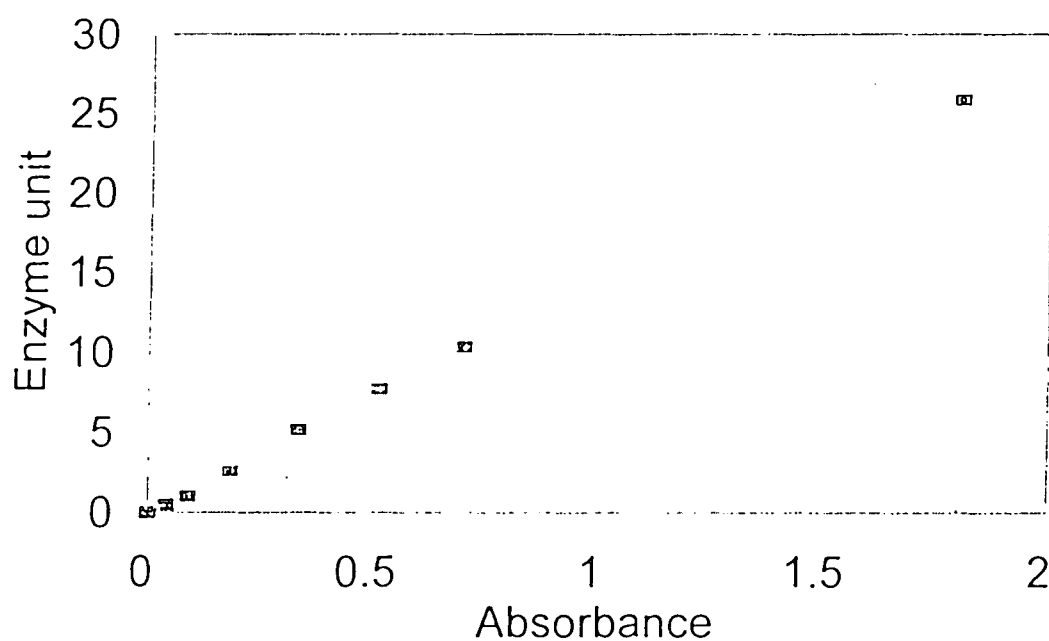


Figure 3.3 α -Amylase activity standard curve. One absorbance unit is equal to 15.34 enzyme units.

3.6 Preparation of the seed

For each series (i.e. methodology) 600 seeds were selected. Damaged or deformed seeds were excluded from selection. Each seed was cut transversely, with the distal endosperm half being retained for experimental purposes. (Figure 3.4). In each series, the half-seeds were divided into 30 groups of 20 half-seeds each. In total, 1200 half-seeds were used, divided into 60 groups.

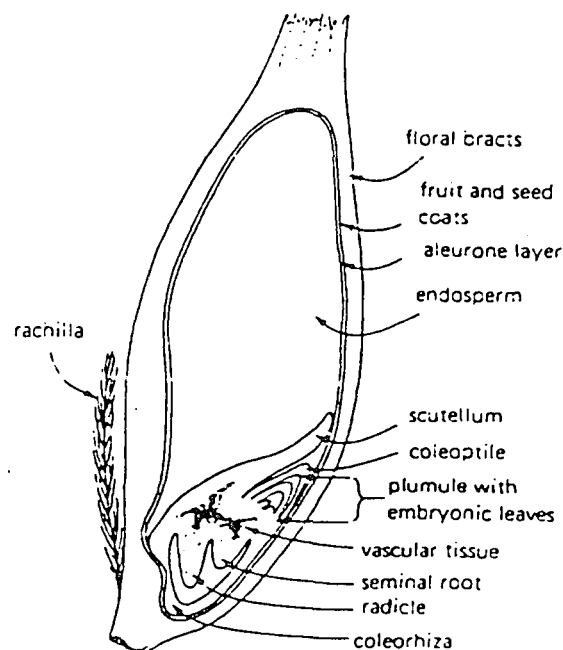


Figure 3.4 Barley seed sectioned to illustrate major tissues (Salisbury and Ross 1978:254)

3.7 Preparation and treatment of the groups

Each series (methodology) consisted of 5 replications. Each replication consisted of 5 treatment groups and 1 control group. Each group of 20 half-seeds was weighed, placed in a numbered 9cm petri dish lined with two No 1 Whatman filter papers, moistened with 5ml of the various treatment solutions, placed in draw-string plastic bags with 2ml of distilled water, and incubated for 48 hours in a dark incubation cabinet set at a constant temperature of 15°C. Incubation was terminated by freezing.

3.8 Determination of α -amylase synthesis

3.8.1 Enzyme extraction

Extraction of α -amylase was based on the method of Nichols (1979) and Cairns and de Villiers (1986), adapted by Cairns (1998, personal communication). Immediately prior to extraction, the contents of the petri dishes were thawed and macerated with a mortar and pestle with 10ml of extraction buffer containing 58.4 mM sodium chloride and 1.14 mM calcium acetate. The slurry was then decanted into plastic centrifuge tubes and extracted in a shaker-bath for 60 minutes at 30°C. The homogenate was then centrifuged at 10 000 *g* for 10 minutes at 4°C, then filtered through one thickness of No.1 Whatman filter paper into 30 ml test tubes. Test tube contents were made up to 10 ml with the extraction buffer.

3.8.2 Enzyme assay

α -Amylase activity was assayed according to the method of Barnes and Blakeney (1974) and Cairns and de Villiers (1986), adapted by Cairns (1998, personal communication). The extracts were incubated with PHADEBAS Amylase Test tablets in a shaker-bath set at 50°C for 10 minutes. The reaction was terminated by adding 1 ml of 0.5 M NaOH to each test tube after which the tubes were shaken vigorously. The samples were filtered through one thickness of No.1 Whatman filter paper into 30 ml test tubes. Test tube contents were made up to 10 ml with the extraction buffer. The absorbance of each sample was read at 620nm. α -Amylase activity was expressed as enzyme units per gram fresh weight of endosperm halves.

3.9 Data analysis

3.9.1 Calculation of enzyme units synthesised

The α -amylase concentration corresponding to the absorbance value measured for each sample was calculated by interpolation from the α -amylase standard curve prepared earlier. This result was divided by the dry mass of each sample to arrive at the enzyme units synthesized by each sample.

3.9.2 Statistical methods

The univariate multifactorial Analysis of Variance (MANOVA) method was used for data analysis in this study. There was only 1 dependent variable: reading.

This was a balanced multifactorial experimental design.

There were 2 methods.

There were 6 dilution levels per method.

There were 5 observations per dilution level.

3.9.2.1 Statistical model

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

$$Y_{ijk} = \mu + A_i + B_j + A_iB_j + \varepsilon_{ijk}$$

where:

μ is the overall or common effect;

A_i is the effect of methods;

B_j is the effect of dilution levels;

A_iB_j is the interaction effect between methods and dilution levels;

ε_{ijk} are random error terms;

$i = 1, 2$ = number of methods;

$j = 1, \dots, 6$ = number of dilution levels per method;

$k = 1, \dots, 5$ = number of observations per dilution level.

The total number of rows in the data spreadsheet is equal to $2 \times 6 \times 5 = 60$.

3.9.2.2 Procedure 1: The univariate MANOVA to test main effects for significance

The effects of methods and dilution levels were tested for significance. In each case, the null hypothesis states that there is no significant difference between the categories of the main effects. (Example: In the case of the 2 methods, the null hypothesis states that there is no significant difference between the 2 methods). The alternative hypothesis states that there is a significant difference between the 2 methods. The null hypothesis was rejected if the P-value was smaller 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.

3.9.2.3 Procedure 2: The univariate MANOVA method to test interactions of order two for significance

There is only 1 interaction of order two in the multifactorial model. The null hypothesis states that there is no significant interaction effect between methods and dilution levels. The alternative hypothesis states that there is a significant interaction effect. The null hypothesis was rejected if the P-value was smaller 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.

3.9.3 Statistical package

The statistical package SPSS for Windows (version 9) was used for data entry and analysis.

CHAPTER 4

RESULTS

4.1 The criteria governing the admissibility of the data

The data utilised was optical density, from which α -amylase enzyme units synthesised by each sample was calculated (as set out in 3.8.1)

No data obtained was excluded from statistical analysis.

4.2 Physical Observations

There was a noticeable difference in appearance of the incubated endosperm halves between the control groups and all the treatment groups at every stage of the process (See Plates 4.1 to 4.9).

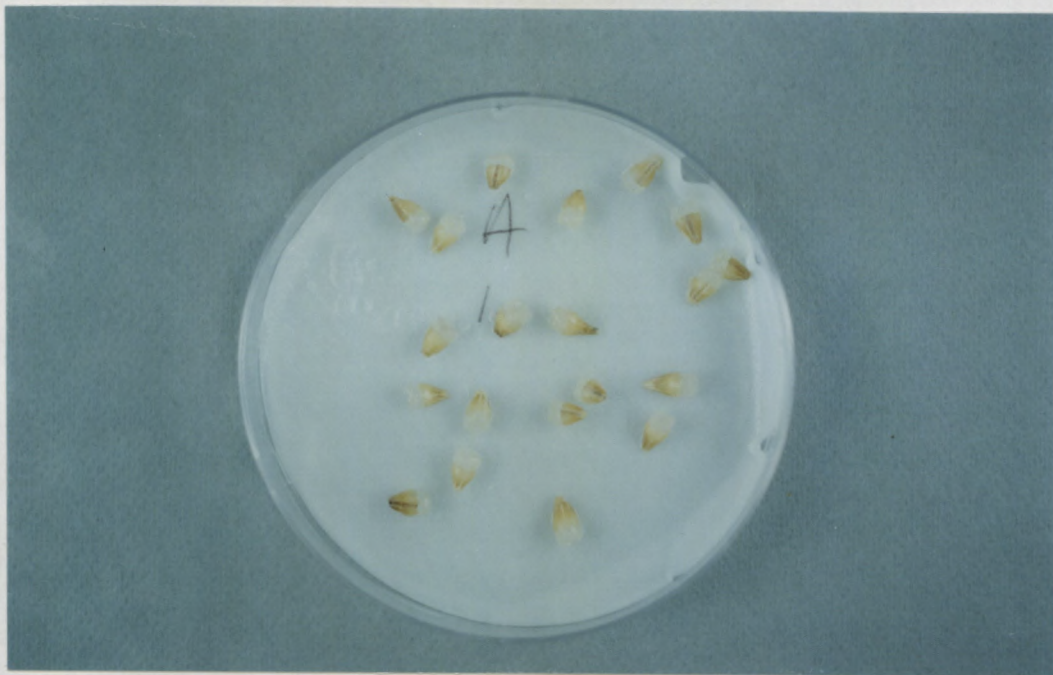


Plate 4.1 The control group of barley endosperm half-seeds at the conclusion of the initial 48 hour incubation in the incubation cabinet, showing jellylike contents protruding from the cut surfaces

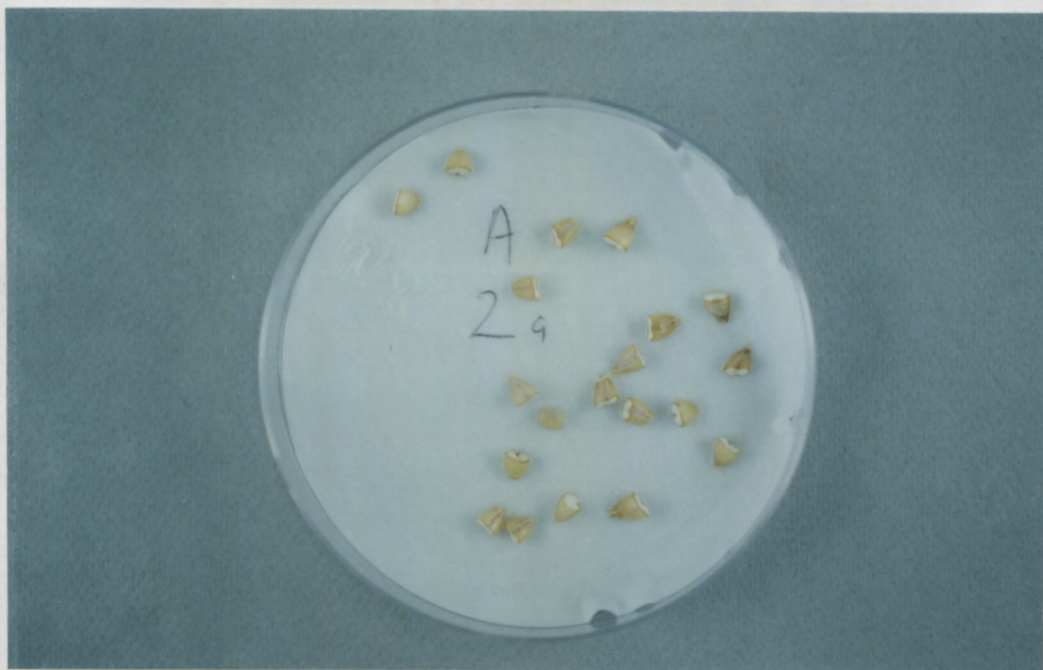


Plate 4.2 The 4cH treatment group of barley endosperm half-seeds at the conclusion of the initial 48 hour incubation in the incubation cabinet, showing clear cut surfaces

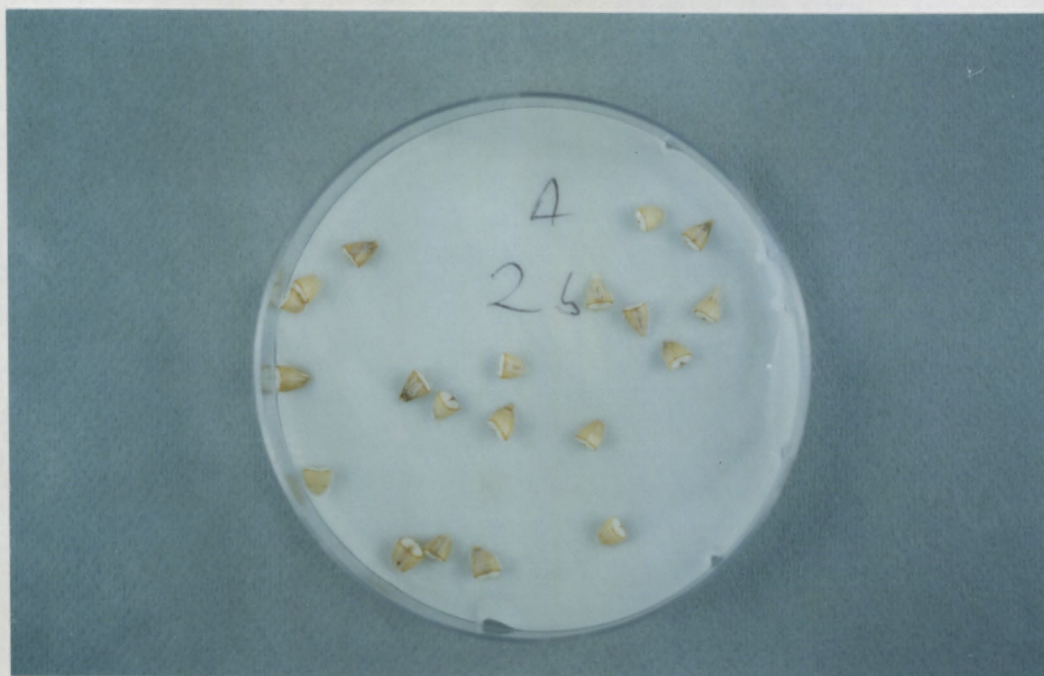


Plate 4.3 The 4cs treatment group of barley endosperm half-seeds at the conclusion of the initial 48 hour incubation in the incubation cabinet, showing clear cut surfaces

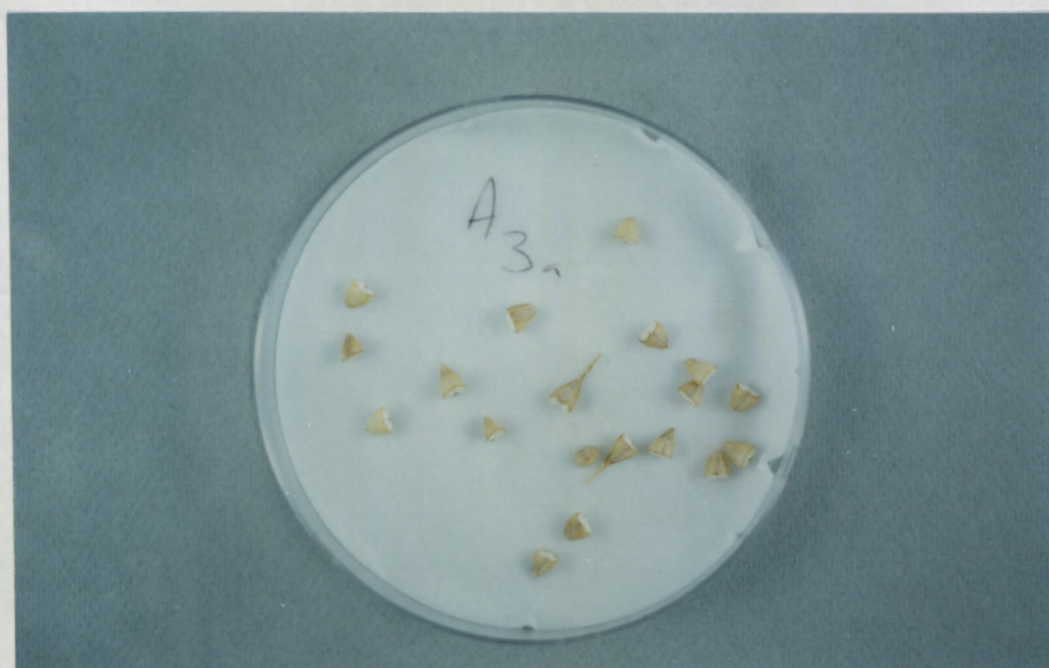


Plate 4.4 The 200cH treatment group of barley endosperm half-seeds at the conclusion of the initial 48 hour incubation in the incubation cabinet, showing clear cut surfaces

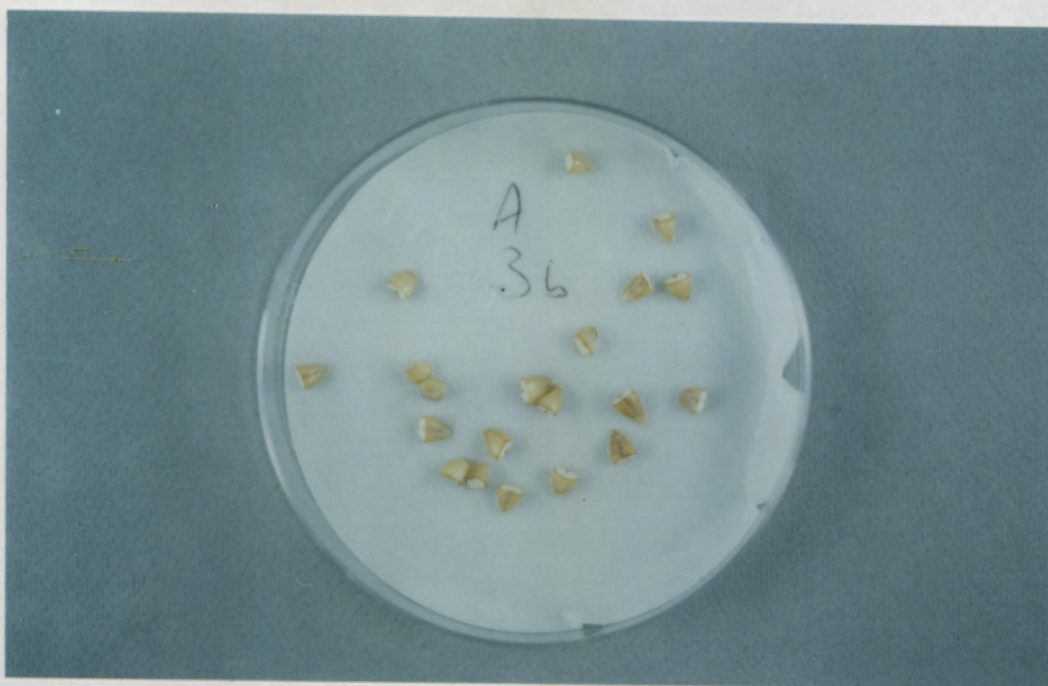


Plate 4.5 The 200cs treatment group of barley endosperm half-seeds at the conclusion of the initial 48 hour incubation in the incubation cabinet, showing clear cut surfaces



Plate 4.6 Extracts of the macerated barley endosperm half-seeds after incubation in the water-bath, centrifuging and filtration (refer to 3.7.1). Test tube 1 is the control solution, test tubes 2 and 3 are 4cH and 4cs respectively, test tubes 4 and 5 are 200cH and 200cs solutions respectively, and test tube 6 is the blank solution (extraction buffer only)

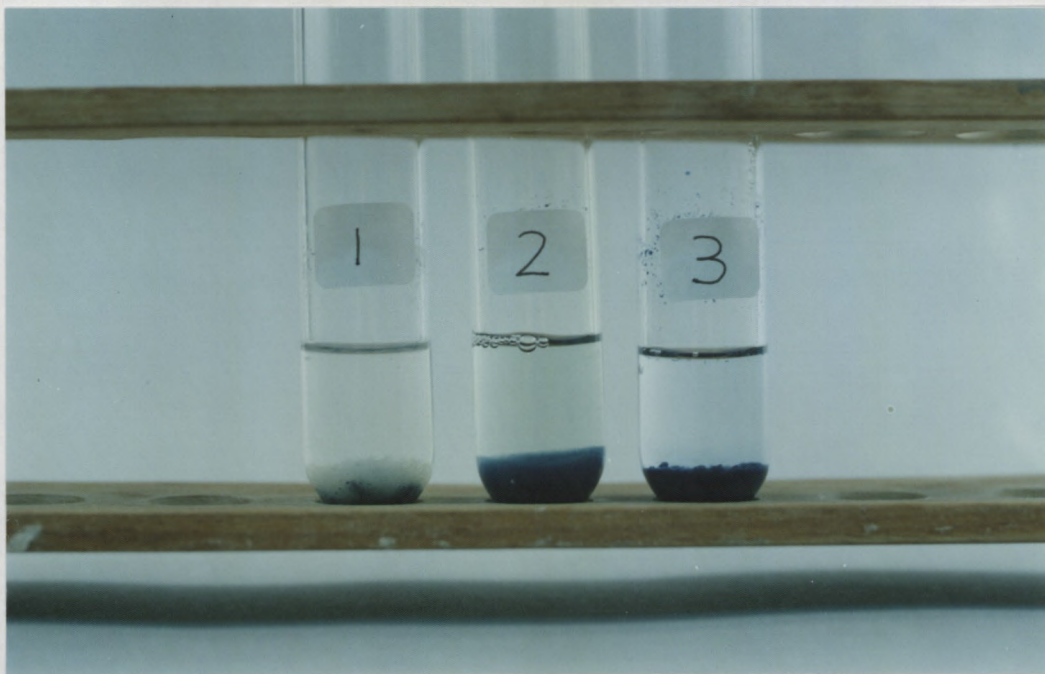


Plate 4.7 Unfiltered solutions after the enzyme extract had been incubated with the PHADEBAS tablets (refer to 3.7.2). Test tube 1 is the control solution, test tube 2 the 200cs solution, and test tube 3 the blank solution (extraction buffer only)



Plate 4.8 A close-up picture of test tube 1 from Plate 4.7



Plate 4.9 Filtered solutions after incubation with the PHADEBAS tablets (refer to 3.7.2), immediately prior to determination of the optical density. Test tube 1 is the control solution, test tubes 2 and 3 are 4cH and 4cs respectively, test tubes 4 and 5 are 200cH and 200cs solutions respectively, and test tube 6 is the blank solution (extraction buffer only)

4.3 Results

4.3.1 Method 1 - dilution with succussion

Table 4.1 shows that α -amylase was not produced in the control groups, whereas it was produced in all the treatment groups. Figure 4.1 is a graphical representation of the mean results from Table 4.1.

4.3.2 Method 2 - dilution with no succussion

Table 4.2 shows that α -amylase was not produced in the control groups, whereas it was produced in all the treatment groups. Figure 4.2 is a graphical representation of the mean results from Table 4.2.

Table 4.1 Results: Method 1. Dilution with succussion.
Enzyme units synthesized, per gram of dry barley endosperm half-seeds

| Treatment | Replication | | | | | |
|------------|-------------|--------|--------|-------|-------|--------|
| | I | II | III | IV | V | Mean |
| 1. Control | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 2. 4cH | 10.454 | 12.862 | 11.817 | 6.383 | 9.434 | 10.183 |
| 3. 9cH | 2.346 | 1.152 | 1.545 | 0.414 | 2.139 | 1.519 |
| 4. 15cH | 0.709 | 1.418 | 0.411 | 0.105 | 0.113 | 0.551 |
| 5. 30cH | 1.981 | 1.497 | 0.670 | 0.606 | 0.653 | 1.081 |
| 6. 200cH | 1.811 | 1.287 | 0.402 | 0.882 | 1.708 | 1.218 |

Table 4.2 Results: Method 2. Dilution with no succussion.
Enzyme units synthesized, per gram of dry barley endosperm half-seeds

| Treatment | Replication | | | | | |
|------------|-------------|--------|--------|-------|--------|-------|
| | I | II | III | IV | V | Mean |
| 1. Control | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 2. 4cs | 9.971 | 10.384 | 12.027 | 4.425 | 10.397 | 9.441 |
| 3. 9cs | 1.991 | 1.381 | 3.382 | 0.965 | 1.163 | 1.776 |
| 4. 15cs | 3.464 | 1.997 | 2.550 | 2.709 | 3.448 | 2.834 |
| 5. 30cs | 0.682 | 0.902 | 1.354 | 2.219 | 1.741 | 1.380 |
| 6. 200cs | 1.054 | 1.905 | 1.534 | 1.085 | 1.321 | 1.380 |

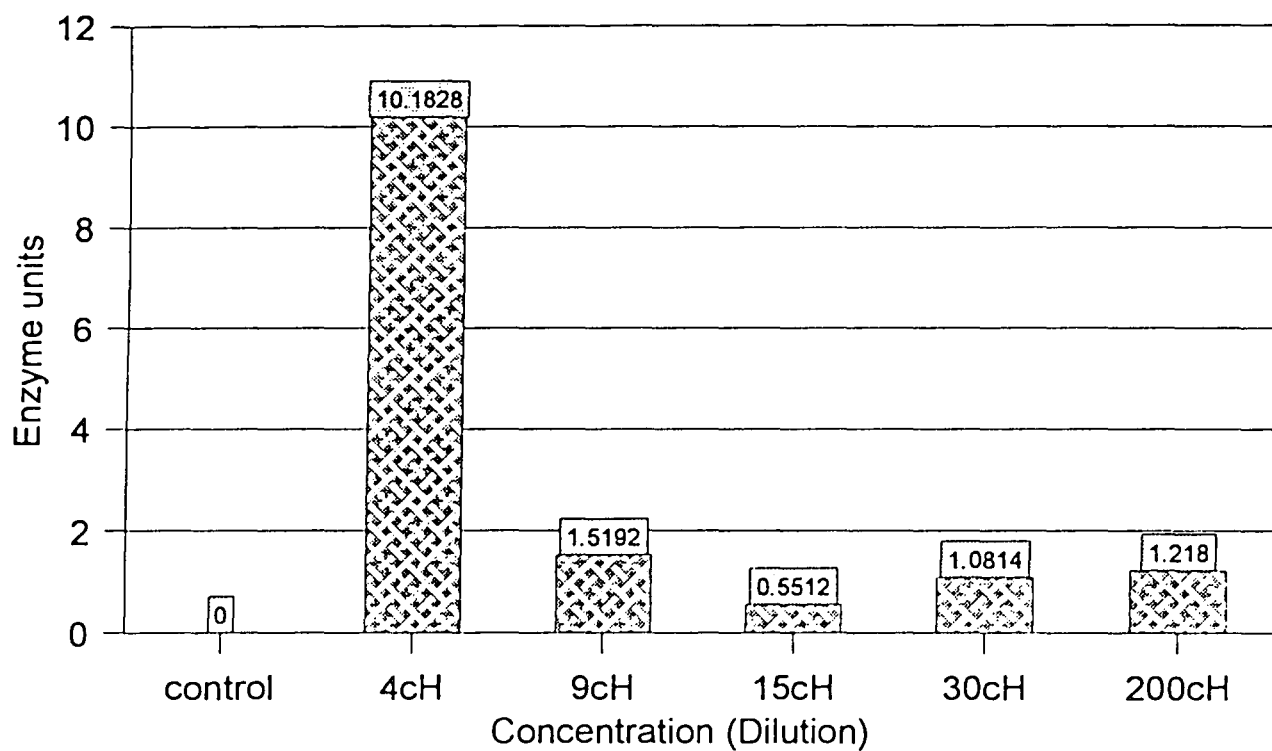


Figure 4.1 Bar chart for Method 1. Dilution with succussion.
Control and 4cH differ significantly from all other treatments. 9cH, 15cH, 30cH and 200cH do not differ significantly from each other. See Table 4.11.

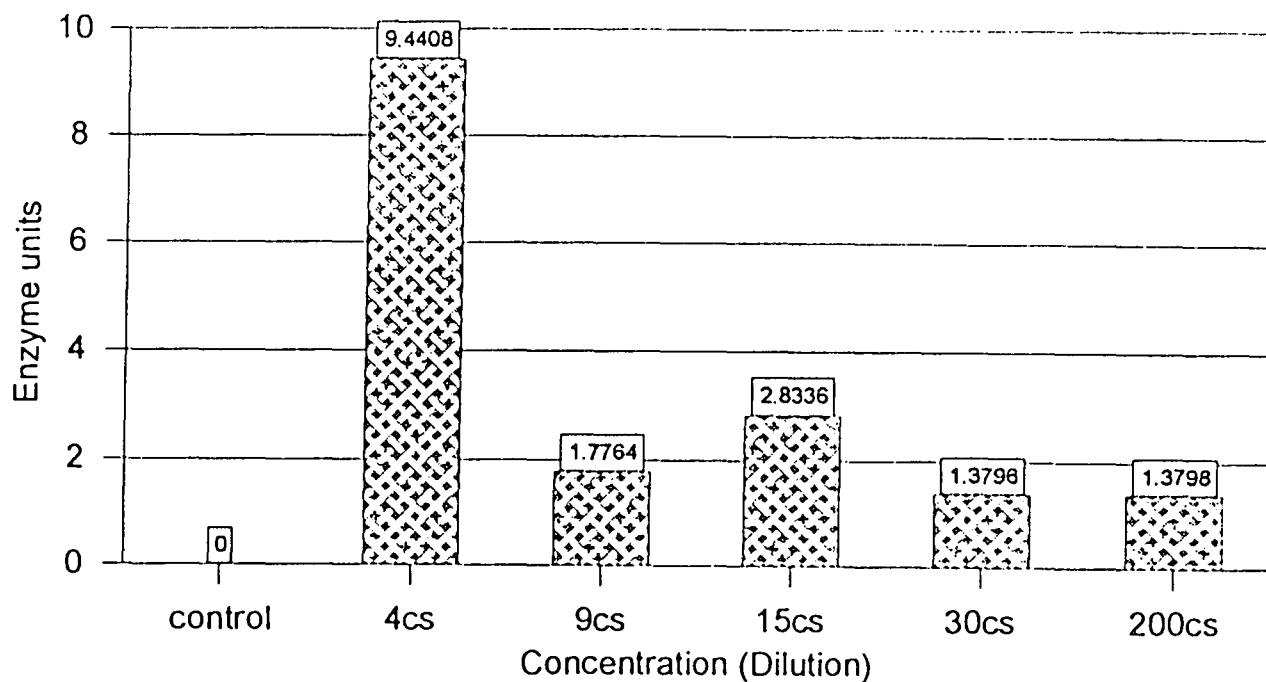


Figure 4.2 Bar chart for Method 2. Dilution with no succussion.
Control and 4cs differ significantly from all other treatments. 9cs, 15cs, 30cs and 200cs do not differ significantly from each other. See Table 4.11.

4.4 Descriptive statistics

As can be seen from Table 4.3, the standard deviations are fairly small, indicating that the estimated model is reliable.

Table 4.3 Descriptive statistics

Dependent variable: READINGS

| METHODS | DILUTION | Mean | Std Deviation | N |
|---------|----------|----------|---------------|----|
| 1.00 | 1.00 | .00000 | .00000 | 5 |
| | 2.00 | 10.18280 | 2.48646 | 5 |
| | 3.00 | 1.51920 | .77858 | 5 |
| | 4.00 | .55120 | .54475 | 5 |
| | 5.00 | 1.08140 | .62466 | 5 |
| | 6.00 | 1.21800 | .58636 | 5 |
| | Total | 2.42543 | 3.71189 | 30 |
| 2.00 | 1.00 | .00000 | .00000 | 5 |
| | 2.00 | 9.44080 | 2.91255 | 5 |
| | 3.00 | 1.77640 | .97660 | 5 |
| | 4.00 | 2.83360 | .62665 | 5 |
| | 5.00 | 1.37960 | .62262 | 5 |
| | 6.00 | 1.37980 | .35218 | 5 |
| | Total | 2.80170 | 3.35530 | 30 |
| Total | 1.00 | .00000 | .00000 | 10 |
| | 2.00 | 9.81180 | 2.58281 | 10 |
| | 3.00 | 1.64780 | .84361 | 10 |
| | 4.00 | 1.69240 | 1.32441 | 10 |
| | 5.00 | 1.23050 | .60861 | 10 |
| | 6.00 | 1.29890 | .46390 | 10 |
| | Total | 2.61357 | 3.51310 | 60 |

4.5 Statistical analysis of results

H_0 : null hypothesis

H_1 : alternative hypothesis

Decision rule:

at the $\alpha = 0.05$ level of significance,

1. Reject H_0 if $p < \alpha$
2. Accept H_1 if $p \geq \alpha$

Refer to 3.9.2.1 for the statistical model.

4.5.1 Levene's Test of Equality of Error Variances

This tests the null hypothesis that the error variance of the dependent variable is equal across groups.

Table 4.4 shows that $p = 0.001$, therefore $p < \alpha$ which means that the null hypothesis is rejected at the $\alpha = 5\%$ level.

Conclusion: the error variance of the readings varies significantly across groups.

Table 4.4 Levene's Test of Equality of Error Variances

Dependent variable: READINGS

| F | df1 | Df2 | p-value |
|-------|-----|-----|---------|
| 3.731 | 11 | 48 | .001 |

Design: Intercept+METHOD+DILUTION+METHODS*DILUTION

4.5.2 Tests of between-subjects effects (Analysis of Variance table)

The overall ANOVA table (Table 4.5) shows the following:

- a) The effect of methods of dilution: $p = 0.242$, therefore $p \geq \alpha$ which means that null hypothesis is accepted.

Conclusion: there is no significant difference between the two methods of dilution at the $\alpha = 5\%$ level. This means that the third hypothesis (see 1.3.3) is true i.e. there is no difference between the methods of dilution on the production of α -amylase in barley endosperm half-seeds.

- b) The effect of dilution levels: $p = 0.000$, therefore $p < \alpha$ which means that the null hypothesis is rejected.

Conclusion: the six dilution levels are significantly different from each other at the $\alpha = 5\%$ level. This means that the first hypothesis (see 1.3.1) is not true i.e. Hahnemannian serial dilutions of GA_3 do have an effect on the production of α -amylase in barley endosperm half-seeds. This also means that the second hypothesis (see 1.3.2) is not true i.e. non-Hahnemannian serial dilutions of GA_3 do have an effect on the production of α -amylase in barley endosperm half-seeds.

- c) The interaction effect between methods and dilution: $p = 0.157$, therefore $p \geq \alpha$ which means that the null hypothesis is accepted.

Conclusion: there is no significant interaction between the two methods and the six dilution levels. This means that the fourth hypothesis (see 1.3.4) is not true i.e. there is no interaction between the methods of dilution and the dilution levels in the production of α -amylase in barley endosperm half-seeds.

Table 4.5 Tests of between-subjects effects (Analysis of Variance table)

Dependent variable: READINGS

| Source | Type III Sum of Squares | df | Mean Square | F | p-value | Noncent. Parameter | Observed Power ^a |
|----------------------|-------------------------|----|-------------|---------|---------|--------------------|-----------------------------|
| Corrected model | 655.531 ^b | 11 | 59.594 | 39.378 | .000 | 433.159 | 1.000 |
| Intercept | 409.844 | 1 | 409.844 | 270.815 | .000 | 270.815 | 1.00 |
| METHODS | 2.124 | 1 | 2.124 | 1.403 | .242 | 1.403 | .213 |
| DILUTION | 640.678 | 5 | 128.136 | 84.669 | .000 | 423.345 | 1.000 |
| METHODS* DILUTION | 12.729 | 5 | 2.546 | 1.682 | .157 | 8.411 | .534 |
| Error | 72.642 | 48 | 1.513 | | | | |
| Total | 1138.016 | 60 | | | | | |
| Corrected total | 728.172 | 59 | | | | | |

a Computed using $\alpha = 0.05$

b R squared = .900 (adjusted R Squared = .877)

* Interaction effect

Observed power is a measure of how reliable (robust) the test is. In the case of methods of dilution it is 0.213, which is low. In the case of dilution levels it is 1.000 which is very high. In the case of the interaction between methods of dilution and dilution levels it is 0.534, which is fairly high.

R Squared = 0.900. This statistic shows that 90% of the total variability in the dependent variable (readings) is explained by the estimated model. This shows that the model is highly reliable.

4.5.3 Parameter estimates

This test (Table 4.6) reveals how significant is the influence of the method on the dilutions levels.

Table 4.6 Parameter estimates

Dependent Variable: READINGS

| Parameter | Estimated Regression Coefficient β | Std. Error | t-value | p-value | 95% Confidence Interval | | Noncent. Parameter | Observed Power ^a |
|--------------|--|------------|---------|---------|-------------------------|-------------|--------------------|-----------------------------|
| | | | | | Lower Bound | Upper Bound | | |
| Intercept | 1.389 | .550 | 2.508 | .016 | .274 | 2.486 | 2.508 | .691 |
| [METH=1.00] | -.162 | .778 | -.208 | .836 | -.726 | 1.403 | .208 | .055 |
| [METH=2.00] | 0 ^b | | | | | | | |
| [DILU=1.00] | -1.380 | .778 | -1.773 | .083 | -2.944 | .185 | 1.773 | .412 |
| [DILU=2.00] | 8.061 | .778 | 10.361 | .000 | 6.497 | 9.625 | 10.361 | 1.000 |
| [DILU=3.00] | .397 | .778 | .510 | .613 | -1.168 | 1.961 | .510 | .079 |
| [DILU=4.00] | 1.454 | .778 | 1.869 | .068 | -.111 | 3.018 | 1.869 | .449 |
| [DILU=5.00] | -2.00E-04 | .778 | .000 | 1.000 | -1.565 | 1.564 | .000 | .050 |
| [DILU=6.00] | 0 ^b | | | | | | | |
| [METH=1.00]* | | | | | | | | |
| [DILU=1.00] | .162 | 1.100 | .147 | .884 | -2.051 | 2.374 | .147 | .052 |
| [METH=1.00]* | | | | | | | | |
| [DILU=2.00] | .904 | 1.100 | .821 | .415 | -1.309 | 3.116 | .821 | .127 |
| [METH=1.00]* | | | | | | | | |
| [DILU=3.00] | -9.540E-02 | 1.100 | -.087 | .931 | -2.308 | 2.117 | .087 | .051 |
| [METH=1.00]* | | | | | | | | |
| [DILU=4.00] | -2.121 | 1.100 | -1.927 | .060 | -4.333 | 9.174E-02 | 1.927 | .472 |
| [METH=1.00]* | | | | | | | | |
| [DILU=5.00] | -.136 | 1.100 | -.124 | .902 | -2.349 | 2.076 | .124 | .052 |
| [METH=1.00]* | | | | | | | | |
| [DILU=6.00] | 0 ^b | | | | | | | |
| [METH=2.00]* | | | | | | | | |
| [DILU=1.00] | 0 ^b | | | | | | | |
| [METH=2.00]* | | | | | | | | |
| [DILU=2.00] | 0 ^b | | | | | | | |
| [METH=2.00]* | | | | | | | | |
| [DILU=3.00] | 0 ^b | | | | | | | |
| [METH=2.00]* | | | | | | | | |
| [DILU=4.00] | 0 ^b | | | | | | | |
| [METH=2.00]* | | | | | | | | |
| [DILU=5.00] | 0 ^b | | | | | | | |
| [METH=2.00]* | | | | | | | | |
| [DILU=6.00] | 0 ^b | | | | | | | |

METH Methods

DILU Dilutions

a. computed using alpha = 0.5

b. This parameter is set to zero because it is redundant.

4.5.4 Estimated marginal means

4.5.4.1 Grand mean

Table 4.7 gives the estimated grand mean, its standard error of estimation, and a 95% confidence interval for the true grand mean. The small standard error indicates the reliability of the model.

Table 4.7 Grand mean

Dependent Variable: READINGS

| | | 95% Confidence Interval | |
|-------|------------|-------------------------|-------------|
| Mean | Std. Error | Lower Bound | Upper Bound |
| 2.614 | .159 | 2.294 | 2.933 |

4.5.5 Methods of dilution

4.5.5.1 Estimates

Table 4.8 gives estimates for the two methods. The small standard errors indicate the reliability of the model.

Table 4.8 Estimates: methods of dilution

Dependent Variable: READINGS

| | | | 95% Confidence Interval | |
|---------|-------|------------|-------------------------|-------------|
| METHODS | Mean | Std. Error | Lower Bound | Upper Bound |
| 1.00 | 2.425 | .225 | 1.974 | 2.877 |
| 2.00 | 2.802 | .225 | 2.350 | 3.253 |

4.5.5.2 Pairwise comparison (least significant difference [LSD])

The pairwise comparison table (Table 4.9) shows a p value of 0.242, therefore $p \geq \alpha$ which means that the null hypothesis is accepted.

Conclusion: no significant difference exists between the pairs i.e. between the two methods of dilution.

Table 4.9 Pairwise comparison: methods of dilution (LSD)

Dependent Variable: READINGS

| | | | | | 95% Confidence Interval for Difference ^a | |
|------------|------------|-----------------------|------------|----------------------|---|-------------|
| (I)METHODS | (J)METHODS | Mean Difference (I-J) | Std. Error | p-value ^a | Lower Bound | Upper Bound |
| 1.00 | 2.00 | -.376 | .318 | .242 | -1.015 | .262 |
| 2.00 | 1.00 | .376 | .318 | .242 | -.262 | 1.015 |

Based on estimated marginal means.

a Adjustment for multiple comparisons: Least significant Difference (equivalent to no adjustments).

4.5.6 Dilution levels

4.5.6.1 Estimates

Table 4.10 gives estimates for the six dilution levels. The small standard errors indicate the reliability of the model.

Table 4.10 Estimates: dilution levels

Dependent Variable: READINGS

| | | | 95% Confidence Interval | |
|----------|------------|------------|-------------------------|-------------|
| DILUTION | Mean | Std. Error | Lower Bound | Upper Bound |
| 1.00 | -1.388E-17 | .389 | -.782 | .782 |
| 2.00 | 9.812 | .389 | 9.030 | 10.594 |
| 3.00 | 1.648 | .389 | .866 | 2.430 |
| 4.00 | 1.692 | .389 | .910 | 2.475 |
| 5.00 | 1.231 | .389 | .448 | 2.013 |
| 6.00 | 1.299 | .389 | .517 | 2.081 |

4.5.6.2 Pairwise comparisons (LSD)

In table 4.5 it was shown that there is a significant difference between the 6 dilution levels. Table 4.11 gives a pairwise comparison between all possible levels. Eighteen out of 30 pairs differed significantly from each other at the $\alpha = 5\%$ level. Note that dilution level 1, which is the control solution, differs from all treatment levels quite significantly. This shows that the biological activity demonstrated in the all the treatment groups was not due to chance.

Table 4.11 Pairwise comparisons: dilution levels (Least Significant Differences)

Dependent Variable: READINGS

| | | | | | 95% Confidence Interval for Difference ^a | |
|-------------|-------------|-----------------------|------------|----------------------|---|-------------|
| (I)DILUTION | (J)DILUTION | Mean Difference (I-J) | Std. Error | p-value ^a | Lower Bound | Upper Bound |
| 1.00 | 2.00 | -9.812* | .550 | .000 | -10.918 | -8.706 |
| | 3.00 | -1.648* | .550 | .004 | -2.754 | -.542 |
| | 4.00 | -1.692* | .550 | .003 | -2.799 | -.586 |
| | 5.00 | -1.231* | .550 | .030 | -2.337 | -.124 |
| | 6.00 | -1.299 | .550 | .022 | -2.405 | -.193 |
| 2.00 | 1.00 | 9.812* | .550 | .000 | 8.706 | 10.918 |
| | 3.00 | 8.164* | .550 | .000 | 7.058 | 9.270 |
| | 4.00 | 8.119* | .550 | .000 | 7.013 | 9.226 |
| | 5.00 | 8.581* | .550 | .000 | 7.475 | 9.687 |
| | 6.00 | 8.513* | .550 | .000 | 7.407 | 9.619 |
| 3.00 | 1.00 | 1.648* | .550 | .004 | .542 | 2.754 |
| | 2.00 | -8.164* | .550 | .000 | -9.270 | -7.058 |
| | 4.00 | -4.460E-02 | .550 | .936 | -1.151 | 1.062 |
| | 5.00 | .417 | .550 | .452 | -.689 | 1.523 |
| | 6.00 | .349 | .550 | .529 | -.757 | 1.455 |
| 4.00 | 1.00 | 1.692* | .550 | .003 | .586 | 2.799 |
| | 2.00 | -8.119* | .550 | .000 | -9.226 | -7.013 |
| | 3.00 | 4.460E-02 | .550 | .936 | -1.062 | 1.151 |
| | 5.00 | .462 | .550 | .405 | -.644 | 1.568 |
| | 6.00 | .393 | .550 | .478 | -.713 | 1.500 |
| 5.00 | 1.00 | 1.231* | .550 | .030 | .124 | 2.337 |
| | 2.00 | -8.581* | .550 | .000 | -9.687 | -7.475 |
| | 3.00 | -.417 | .550 | .452 | -1.523 | .689 |
| | 4.00 | -.462 | .550 | .405 | -1.568 | .644 |
| | 6.00 | -6.840E-02 | .550 | .902 | -1.175 | 1.038 |
| 6.00 | 1.00 | 1.299* | .550 | .022 | .193 | 2.405 |
| | 2.00 | -8.513* | .550 | .000 | -9.619 | -7.407 |
| | 3.00 | -.349 | .550 | .529 | -1.455 | .757 |
| | 4.00 | -.393 | .550 | .478 | -1.500 | .713 |
| | 5.00 | 6.840E-02 | .550 | .902 | -1.038 | 1.175 |

Based on estimated marginal means.

* The mean difference is significant at the 0.5 level.

^a Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

4.5.7 Methods interacting with dilution

4.5.7.1 Pairwise comparisons (LSD)

No p-values are given for the interaction effects in Table 4.12. This is because the interaction effects between methods and dilutions are not significant (at the $\alpha = 5\%$ level), as is shown in Table 4.5.

Table 4.12 Pairwise comparison: interaction effect between methods of dilution and levels of dilution (LSD)

Dependent Variable: READINGS

| | | | | 95% Confidence Interval for Difference ^a | |
|---------|----------|------------|------------|---|-------------|
| METHODS | DILUTION | Mean | Std. Error | Lower Bound | Upper Bound |
| 1.00 | 1.00 | -2.776E-17 | .550 | -1.106 | 1.106 |
| | 2.00 | 10.183 | .550 | 9.077 | 11.289 |
| | 3.00 | 1.519 | .550 | .413 | 2.625 |
| | 4.00 | .551 | .550 | -.555 | 1.657 |
| | 5.00 | 1.081 | .550 | 2.477E-02 | 2.188 |
| | 6.00 | 1.218 | .550 | .112 | 2.324 |
| 2.00 | 1.00 | .000 | .550 | -1.106 | 1.106 |
| | 2.00 | 9.441 | .550 | 8.335 | 10.547 |
| | 3.00 | 1.776 | .550 | .670 | 2.883 |
| | 4.00 | 2.834 | .550 | 1.727 | 3.940 |
| | 5.00 | 1.380 | .550 | .273 | 2.486 |
| | 6.00 | 1.380 | .550 | .274 | 2.486 |

4.5.8 Scattering of standardized error terms

Figure 4.4 shows that the standardized error terms are scattered randomly, and without any definite pattern or shape. This means that the estimated model fits the data well.

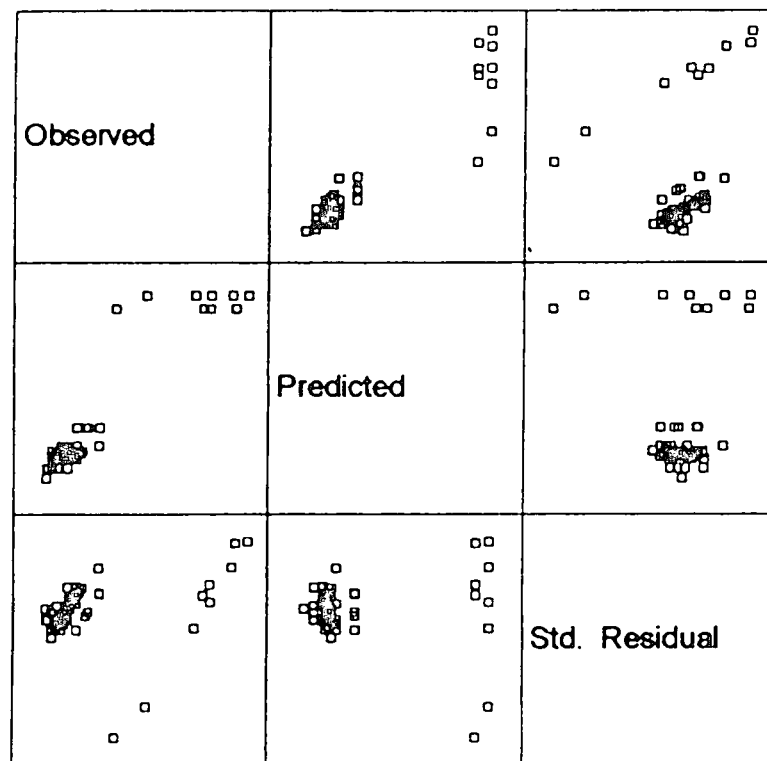


Figure 4.3 Scatter diagram of the dependent variable: readings

CHAPTER 5

DISCUSSION OF THE RESULTS

5.1 Introduction

The results of this investigation clearly indicate that ultra high dilutions of GA_3 (up to 10^{-400}) are biologically active. Furthermore, succussion as per the Hahanemannian method is not a significant factor underlying this activity.

On physical observation there was a noticeable difference in appearance between the control groups and all the treatment groups at every stage of the process (See Plates 4.1 to 4.9). After the initial incubation period, the control group half-seeds had a jellylike exudate protruding from their cut surfaces, whereas all the other groups did not (Plates 4.1 - 4.5). The jellylike exudate is a result of water adsorption by the starch colloids of the endosperm, indicating that α -amylase was not produced and so the starch was not hydrolysed in the control groups. The clean cut surfaces of all the other groups indicate that α -amylase was produced and that starch was hydrolysed at least to an extent in all the groups except the control groups. The turbidity evident in test tube 1 of Plate 4.1.6 was caused by the presence of fine starch granules indicating that hydrolysis did not occur in the control solution, whereas all the other treatment solutions were clear indicating hydrolysis did occur in those solutions. The white granules overlying the blue granules of the PHADEBAS tablet in test tube 1 of Plate 4.1.7 are starch granules. The solution in that test tube had no colour which is a further indication that α -amylase was not produced in the control groups. The slightly blue colour of the solution in test tube 2 in Plate 4.1.7 indicates that α -amylase was produced because the PHADEBAS dye has been hydrolysed by the enzyme. From Plate 4.1.9 it is evident that the control solution had no colour whereas the lowest and the highest treatment solutions did have colour, indicating that all the GA_3 dilutions did cause α -amylase to be synthesised.

Statistical analysis (univariate Multivariate Analysis of Variance) indicated that there was a significant difference between the control groups and all the treatment groups, but that there was no significant difference between the two methods of dilution in terms of their effectiveness in producing biological activity.

The results demonstrate that the effect of this particular series of UHDs is due to a factor intrinsic to the dilutions and not one extrinsic to them such as the power of suggestion or the placebo effect

These are extremely significant results from a homoeopathic as well as a biochemical point of view.

Succussion is the cornerstone of homoeopathic philosophy and pharmaceutical practice, and is regarded as a vital ingredient in the "potency" of the medicine so produced (Organon paragraph 269, Hahnemann 1996; Gaier 1991:432, 441). This point of view is well represented by Poitevan (1995), who states that the part played by succussion is of key importance to those interested in the way homoeopathy works. He says: "Simply putting together substance and solvent is not enough to register a biological message. External energy is vital to trigger any physicochemical reaction". The results of this investigation, then, fundamentally challenge this point of view.

Avagadro's constant is a fundamental principle in chemistry (Gillespie *et al* 1989:65-66). The logic of this principle is that if there are no molecules of the original solute remaining in a solution, that solution cannot be active. The results of this investigation, however, demonstrated that a solution many times more dilute than Avagadro's dilution limit was still biologically active. This challenges a central principle of chemistry, and brings into question Avagadro's theory.

The results of this investigation prove that there was storage of substance-specific information in all the selected dilution levels, which was recognised by the cells of the endosperm aleurone layer such that α -amylase was synthesised. The burning questions, then, are: (1) what is active? and (2) is there a particular procedure that facilitates the process of information storage?

5.2 What is active?

In a high and ultra high dilution, is it the molecules of the original substance, or is it the solvent, that is active? It could be that the two possibilities are not mutually exclusive. All possibilities need to be carefully considered.

5.2.1 The molecular approach

The molecular approach assumes that molecules of the original substance are the active agents in a solution which produce an effect consistent with the properties of that substance. This is the standard chemical approach. (Gillespie *et al* 1989:3.) This approach is relevant for succussed and non-succussed dilutions.

Avagadro's constant (6.022×10^{23}) is based on the number of molecules in a mole of any particular substance. Theoretically, therefore, the mirror image of that constant (6.022×10^{-23}) should contain no molecules. However, there is a possible objection to this logic. A substance can never itself be divided to a point that none of it exists anymore. Even if only one molecule remains, when that is divided, new sub-entities will emerge e.g. electrons, protons, quarks etc. These divide into other entities in turn. In other words, it seems to be impossible to not have any of the original substance remaining in a process of division.

The process of division of a substance which has been dissolved within a solvent which is then divided different from that of a substance which is itself divided. A substance dissolving within a solvent, such as water, becomes diluted. Its molecules disperse within the solution by diffusion, and so become interspersed with the molecules of the solvent. These solute molecules remain as discrete entities, and are not themselves divided in the process of dilution. When a portion of this solution is combined with a fresh portion of the solvent, the substance molecules are further diluted. As this process continues, there will be fewer and fewer of the original substance molecules remaining in the solution. From a logical point of view, there will come a point in time when it is a matter of chance whether any of the original molecules will be "in the right place at the right time" i.e. be in that portion of the solution which is extracted in order to be added to a fresh portion of the solvent. If at that point at least one molecule of the original substance is not extracted, the material presence of that substance is lost and will not be present in any subsequent dilutions.

One other aspect to be considered in the molecular theory, is that one would expect any influence by the molecules to diminish in a consistent fashion i.e. the less the number of molecules, the less the effects of the solution.

Regarding the present study, the chances of a molecule of the original substance remaining after 200 centesimal dilution steps (1:99) are very slim, especially considering that the starting solution was only 26mM. If some molecules were present, (deduced from the biological action of the final solution) this would probably only occur in a few of the samples. However, in this study, every sample produced a biological effect. The consistency of these results seem to indicate that chance was not the basis upon which biological activity was found in the treatment groups.

In addition, the results are not consistent with a "less molecules therefore less activity" point of view. True, the greatest activity is registered with the lowest dilution (i.e. highest

concentration), the 4cH and 4cs, but the results of the 9cH/cs, 15cH/cs, 30cH/cs and 200cH/cs dilutions are not statistically different from one another even though the dilution changes from 10^{-18} to 10^{-400} and span Avagadro's dilution limit.

However, one cannot rule out the possibility that it is original substance molecules which are the active agents of UHD. This possibility is supported by the hypothesis advanced by Carr and co-workers regarding chemical substances originating from Ascension Island which are sensed by green sea turtles and enable them to navigate an ocean journey of 2000km from their feeding grounds to breeding grounds on the island (Luschi *et al* 1998). This must surely be an UHD effect! One wonders though whether it is the molecules directly which stimulate the turtles, or whether the molecules act as "radiation emitters" which cause a resonance within the turtles, as per the ability of male cabbage moths to detect the pheromone "scent" of female cabbage moths (Towsey and Hasan 1995). Either way, original substance molecules are still required to be present in the ocean current.

5.2.2 The solvent approach

This approach was extensively outlined in Chapter 2, and is the approach most favoured by homoeopaths.

Explanations of how a solute and water as a solvent mix and react with each other (i.e. polarity, hydrogen bonding, clusters, clathrates, etc) apply to succussed and unsuccussed solutions alike. The basic tenet of this approach is that water molecules are able to rearrange themselves in relation to the solute molecules in such a way that they partake in the effect of the solute molecules, and can carry on that information and effect even when there are no longer any original solute molecules remaining remaining in the solution.

This approach seems more robust than the molecular approach, in that it does not ultimately depend on original solute molecules, although it does depend on them initially in order to set the unique orientation of the solution. There is a growing body of research contributing to the theoretical development of this approach, whereas there does not seem to be the same degree of research on the molecular approach.

5.3 Is there a particular procedure that facilitates the process of information storage?

Homoeopaths believe that succussion facilitates - if not actually causes - the process of information storage (see 2.7.4 and 5). However, the results of the present investigation reveal that the non-succussed dilutions were just as active as the succussed ones. In fact, although the difference is not statistically significant, it is interesting to note that the overall mean for the production of enzyme units by the non-succussed dilutions (2.80170), was greater than the mean of the succussed dilutions (2.42543). (See Table 4.3.)

From the literature it would appear that, at the least, succussion facilitates the mixing of a solution, and therefore may enhance the information exchange process between solute and solvent. The most attractive theory in this regard is that put forward by Resch and Gutmann (1991), who argue that succussion improves the structural organisation of liquids (see 2.7.5.1). But in terms of fluid dynamics, the most powerful force in the mixing process (even with succussion) is diffusion, according to Auerbach (1994). Diffusion is not dependent on succussion.

The results of this investigation indicate that the two methods employed had the same effect, therefore there must be a common underlying mechanism of action. What could this be? The only common denominator between the two methodologies was the process of serial centesimal dilution. Therefore, dilution *per se* must be a relevant factor.

5.4 Biological sensitivity

Whichever approach one adopts, at the end of the day one needs to be able to measure the effect of the dilution, which depends on the sensitivity of the measuring vehicle.

Biological systems are generally much more sensitive than mechanical systems. In this case, the barley aleurone layer was an extremely sensitive instrument, and played an important role in the success of the study.

5.5 Towards a unified theory of UHD activity

Perhaps the way forward is not to regard the molecular approach and the solvent approach as mutually exclusive. The theory of light is instructive in this regard. Light exhibits both wave and particle characteristics. In 1924 the French physicist Louis de Broglie went further and suggested that since light exhibits both wave and particle characteristics, particles (such as electrons, protons, and atoms) should show wavelike properties. When he put forward his hypothesis there were no experimental observations to support it, although not long thereafter (1927), this hypothesis was proven when Davisson in the United States and Thomson in Britain independently demonstrated that beams of electrons are diffracted by crystals. (Gillespie 1989:345-346.)

In the case of high and ultra high dilutions, the activity of the earlier dilutions may be more molecular based whereas the activity of the later dilutions may be more solvent based. This would be consistent with the theory of Resch and Gutmann (1991).

5.6 Economic implications of the results

If it were demonstrated that UHD versions of GA_3 are also effective in field trials, this would have significant economic implications. The use of plant growth regulators (bioregulators) has developed into a permanent feature of agricultural and horticultural

production techniques during the past 30 years. Their percentage of the current world agrochemical market is 4%, but are set to have the highest rate of increase in market share and to eventually overtake herbicides in the market. The major portion of the bioregulator market (60%) is made up by gibberellic acids, ethylene generators and onium compounds.(Schott and Wilder 1991:247.) (See Appendix 4 for a list of agricultural uses for GA₃.) The brewing industry could benefit economically as well, where GA₃ is added to malt after steeping to ensure quicker and more even germination (Lewis and Young 1995:82).

5.7 Summary

Considering that succussion is a central doctrine of homoeopathic practice and theory, the results of this study challenge researchers in homoeopathic theory to pay more attention to the role of dilution *per se* in the process of potentisation. At the same time, the results of this study challenge chemists and physicists to accept that dilutions greater than $6.022 \times 10^{23} \text{ mol}^{-1}$ can have an effect, and to develop theoretical models to explain the mode of action of very high dilutions (very low concentrations).

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

It is evident from the results of this evaluation of the effect of ultra high dilutions of GA_3 on the synthesis of α -amylase in barley endosperms, that ultra high dilutions of GA_3 are biologically active, whether they have been succussed or not. Statistically significant differences were noted between treatment groups and control groups, but not between the two methodologies employed to make up the dilutions.

By minimising the role played by succussion in producing the effect of UHDs, the results of this study may help to demystify an aspect of homoeopathy, thereby making a contribution to the scientific validation of homoeopathy.

Besides the significance of the results, the value of this study has been to demonstrate the feasibility of the barley endosperm half-seed GA_3 - α -amylase system as an experimental model for examining the action of ultra high dilutions.

While it is clear from this study that UHDs do have a biological effect, many questions remain unanswered with regard to the mechanism of information storage in the UHDs. Further research needs to be conducted with a view to clarification, and ultimately the explanation, of this phenomenon.

6.2 Recommendations

1. That all future homoeopathic clinical, *in vitro* or *in vivo* trials utilise the unsuccussed dilutions as an additional control.

2. Repeat this study in other laboratories.
3. Vary the initial incubation period.
4. Vary the initial incubation temperature.
5. Vary the time of the PHADEBAS incubation period.
6. Test the resulting solutions for the presence of sugar, using, for instance, the somogy nelson test.
7. Test smaller dilution steps e.g. every dilution between 4 and 30, in order to more closely track the activity (sinusoidal graph shape etc).
8. Compare the effects of different degrees of dilution e.g. decimal (1:9), centesimal (1:99) and quinquagenimillesimal 1:49 999).
9. Apply various external forces to the dilutions to evaluate how they influence the efficacy of the dilution e.g. heating, freezing, magnetism.
10. Repeat this study using the same dilutions after 1 month, 2 months, 3 months etc., to test the longevity of the active principle of the dilutions.
11. Make up the dilutions of GA_3 , with pure alcohol. Using dormant barley seeds, soak the seeds in the dilutions for long enough to drive the GA_3 into the seeds. Dry the seeds cut them in half and use the endosperm half for the remainder of the experiment.

12. Apply different dilutions of GA₃, succussed and unsuccussed, to the whole seed and measure germination via a Germination Index.
13. Use other seeds (e.g. wild oat) in the same experimental model.
14. Utilise other plant hormone substances in the same experimental model (α -amylase production in barley the endosperm), e.g. absissic acid.
15. Develop other *in vitro* models of enzyme modulation under the influence of UHDs, including mammalian tissue models.

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APPENDIX 3 Plant Growth Regulator Handbook. Gibberellic acid.

APPENDIX 1

Results: METHOD 1

SERIAL DILUTION WITH SUCCUSSION (cH = centesimal Hahnemanian)

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

1. Endosperm half-seed mass prior to treatment (grams)

| Treatment | Replication | | | | |
|------------|-------------|-------|-------|-------|-------|
| | I | II | III | IV | V |
| 1. Control | 0.436 | 0.356 | 0.443 | 0.410 | 0.459 |
| 2. 4cH | 0.405 | 0.415 | 0.418 | 0.447 | 0.426 |
| 3. 9cH | 0.425 | 0.426 | 0.427 | 0.444 | 0.459 |
| 4. 15cH | 0.433 | 0.422 | 0.410 | 0.439 | 0.406 |
| 5. 30cH | 0.426 | 0.410 | 0.412 | 0.430 | 0.423 |
| 6. 200cH | 0.432 | 0.417 | 0.458 | 0.452 | 0.431 |

2. PHADEBAS Optical Density (620nm) reading

| Treatment | Replication | | | | | Mean |
|------------|-------------|-------|-------|-------|-------|-------|
| | I | II | III | IV | V | |
| 1. Control | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 2. 4cH | 0.276 | 0.347 | 0.322 | 0.186 | 0.262 | 0.279 |
| 3. 9cH | 0.065 | 0.032 | 0.043 | 0.012 | 0.064 | 0.043 |
| 4. 15cH | 0.020 | 0.039 | 0.011 | 0.003 | 0.003 | 0.015 |
| 5. 30cH | 0.055 | 0.040 | 0.018 | 0.017 | 0.018 | 0.030 |
| 6. 200cH | 0.051 | 0.035 | 0.012 | 0.026 | 0.048 | 0.034 |

C. Enzyme units synthesized. per gram of dry half-seeds

Formula: optical density divided by dry mass multiplied by enzyme units (15.34 eu) as per α -amylase standard curve.

| Treatment | Replication | | | | | Mean |
|------------|-------------|--------|--------|-------|-------|--------|
| | I | II | III | IV | V | |
| 1. Control | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 2. 4cH | 10.454 | 12.826 | 11.817 | 6.383 | 9.434 | 10.183 |
| 3. 9cH | 2.346 | 1.152 | 1.545 | 0.414 | 2.139 | 1.519 |
| 4. 15cH | 0.709 | 1.418 | 0.411 | 0.105 | 0.113 | 0.551 |
| 5. 30cH | 1.981 | 1.497 | 0.670 | 0.606 | 0.653 | 1.081 |
| 6. 200cH | 1.811 | 1.287 | 0.402 | 0.882 | 1.708 | 1.218 |

APPENDIX 2

Results: METHOD 2

SERIAL DILUTION WITH NO SUCCUSSION (cs = centesimal serial)

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

1. Endosperm half-seed mass prior to treatment (grams)

| Treatment | Replication | | | | |
|------------|-------------|-------|-------|-------|-------|
| | I | II | III | IV | V |
| 1. Control | 0.471 | 0.408 | 0.446 | 0.389 | 0.425 |
| 2. 4cs | 0.440 | 0.421 | 0.389 | 0.416 | 0.319 |
| 3. 9cs | 0.416 | 0.422 | 0.440 | 0.429 | 0.409 |
| 4. 15cs | 0.434 | 0.407 | 0.403 | 0.436 | 0.423 |
| 5. 30cs | 0.405 | 0.425 | 0.453 | 0.394 | 0.423 |
| 6. 200cs | 0.524 | 0.443 | 0.500 | 0.495 | 0.511 |

2. PHADEBAS Optical Density (620nm) reading

| Treatment | Replication | | | | | |
|------------|-------------|-------|-------|-------|-------|-------|
| | I | II | III | IV | V | Mean |
| 1. Control | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 2. 4cs | 0.286 | 0.285 | 0.305 | 0.120 | 0.265 | 0.238 |
| 3. 9cs | 0.054 | 0.038 | 0.097 | 0.027 | 0.031 | 0.049 |
| 4. 15cs | 0.098 | 0.053 | 0.067 | 0.077 | 0.091 | 0.077 |
| 5. 30cs | 0.018 | 0.025 | 0.040 | 0.057 | 0.048 | 0.038 |
| 6. 200cs | 0.036 | 0.055 | 0.050 | 0.035 | 0.044 | 0.044 |

C. Enzyme units synthesized. per gram of dry half-seeds

Formula: optical density divided by dry mass multiplied by enzyme units (15.34 eu) as per α -amylase standard curve.

| Treatment | Replication | | | | | |
|------------|-------------|--------|--------|-------|--------|-------|
| | I | II | III | IV | V | Mean |
| 1. Control | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 2. 4cs | 9.971 | 10.384 | 12.027 | 4.425 | 10.397 | 9.441 |
| 3. 9cs | 1.991 | 1.381 | 3.382 | 0.965 | 1.163 | 1.777 |
| 4. 15cs | 3.464 | 1.997 | 2.550 | 2.709 | 3.448 | 2.834 |
| 5. 30cs | 0.682 | 0.902 | 1.354 | 2.219 | 1.741 | 1.380 |
| 6. 200cs | 1.054 | 1.905 | 1.534 | 1.085 | 1.321 | 1.380 |

APPENDIX 3

PLANT GROWTH REGULATOR HANDBOOK

of the

PLANT GROWTH REGULATOR WORKING GROUP

Second Edition
1981

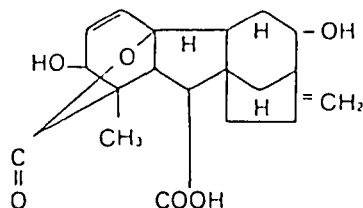
This book may be purchased from the:

Plant Growth Regulator Working Group
Edward F. Sullivan
Great Western Sugar Company
Agricultural Research Center
Sugar Mill Road
Longmont, CO 80501

GIBBERELIC ACID

A. NOMENCLATURE, CHEM. AND PHYS. PROPERTIES OF THE PURE CHEMICAL

1. Common name(s): gibberellic acid, GA₃
2. Trade name(s) and manufacturer(s): Berelex (ICI), Gib-Tabs (Elanco), Gib-Sol (Elanco), Pro-Gibb (Abbott), Pro-Gibb Plus (Abbott)
3. Structural formula:



Wiswesser Line Notation: TC5C6556/C—F/JP C—3ACJ P CX EY JXOV OUTJ BVQ EU1 FQ M:1 NO

4. Molecular formula: C₁₉H₂₂O₆
5. Molecular weight: 346.37
6. Physical state, color, odor, etc.: White, crystalline, free-flowing powder.
8. Melting point °C: 223-235°C
10. Decomposition temperature (°C): < 50°C.
12. Solubility at 20°C or temperature shown: Readily soluble in ethanol, methanol, isopropanol and in acetone. Moderately soluble in ethyl acetate and in petroleum ether and slightly soluble in water.

B. PGR USES

Blueberries: For improving fruit set on plants with insufficient natural honeybee pollination on varieties such as Coville, Jersey, Earlieblue Weymouth and others. Use at 200 ppm.

Sour Cherries: To counteract the effects of cherry yellows virus. Apply from 10-14 days after bloom to about the shuck-split stage at 10-20 ppm. Don't apply within 1 month of harvest. Perennial treatment is necessary to maintain satisfactory fruit production. An excessive concentration may increase the vegetative growth at the expense of fruit production.

Sweet Cherries: Used for better color, firmer fruit, larger fruit and to prolong the harvest period. Apply 10-20 ppm when fruit is light green to straw colored.

Grapes: For cluster loosening and elongation and berry size increase.

- a. Thompson Seedless Variety — apply appropriate rate at berry shatter or normal girdling time. This is 7-10 days after bloom, but before berries are 3/16" in diameter. For cluster loosening apply when shoots are 15-21" in length and cluster forms are 1-3" long, about 2-3 weeks before bloom.
- b. Black Corinth — apply at 1-7 ppm 3-4 days after bloom but before berry shatter begins.
- c. Seibel 5279 — apply at 50 ppm when the shoots are 3-5" long several weeks before bloom.
- d. Alicante, Carignane, Salvador, Zinfandel, Grenache, Tinta Madeira, Palomino, Valdepenas, Aleatico and Petite Sirah — apply at appropriate rate depending upon variety to loosen compact bunches, to reduce bunch rots. Apply when bunches average 3-4" in length and the shoots are normally 15-20" long.

Lemons: To control fruit maturity by delaying the development of yellow color and to increase the numbers of large fruit. Apply at approximately 10 ppm in November or December prior to the development of the yellow fruit. Don't apply in the spring or summer. The results are a larger percentage of fruit with a long storage life as well as a decrease in small tree-ripe fruit.

Oranges: Used to reduce rind stain, improve skin quality, retard aging of the rind, and improve storage properties. Apply in October on into December and January just after marketable color has been obtained at 5-20 ppm. Don't apply within 10 days of harvest.

Pears: For increasing fruit set of low cropping varieties spray at white bud or full bloom.

Italian Prunes: Reduces the internal browning and increases the yield. Apply 3-4 weeks prior to harvest.

Rhubarb: Application increases the yield of the forced crop. Apply to the crown within 24 hours after bringing into the forcing house.

Sugar Cane (Hawaii): For increase in sucrose yield. Use up to 2000 ppm in 7 gal/A.

Artichokes: Used to hasten maturing as an overall spray in the fall. Apply at 25 ppm.

Asparagus: Used to produce more spears and to stimulate dormant buds into normal growth.

Beans: Sprays or seed soaks with 100 ppm solutions will increase green weight and have advanced the opening of *Vicia faba* and *V. villosa*.

Celery: Used to increase stem length and to promote elongation of winter crop celery. Apply at 25-50 ppm, 1-4 weeks before harvest.

Cucumbers: Applied to produce staminate flowers on gynoeocious inbred pickle lines. Used for producing hybrid cucumbers. Apply when the first true leaf expands to 1" in diameter. Repeat twice at 5 day intervals.

Lettuce (for seed): Used to induce uniform bolting, increase seed production, and is effective in promoting vegetative growth of winter lettuce. Apply 3 sprays starting at the 4 leaf stage and repeating at the 8 and 12 leaf stage. Each application should be made at 10 ppm. This promotes the fast uniform bolting of varieties such as Great Lakes. Don't feed wastes to livestock.

Parsley: Used to increase crop yield. A spray application after the first cut will advance cropping up to two months.

Peas: Seed soaks and sprays applied to seedlings have often increased yields and advanced harvesting by a week. Sprays applied before bloom have proved better for increasing yields than those applied at bloom.

Seed Potatoes: Used to break dormancy and stimulate sprouting. Dip potatoes in 1/10-1 ppm solution prior to planting. This allows immediate planting of red varieties of seed potatoes which normally takes 2-6 months of storage to break the dormancy period.

Barley (Malting): Increase the enzymatic content of malt when used on barley. Distillers can then use less malt in the production of neutral grain spirits used in gin and vodka.

Oats, Peas, Cotton, Rye, Soybeans and Wheat: Used as a seed treatment to promote rapid emergence.

Hops: Increase yields and aid in harvest. Apply 3 weeks before bloom when vines are 5-8 feet long. Use at 10 ppm.

Ornamentals: Ornamental plants react differently with this compound depending upon the species. Earlier blooming is the result on such plants as Ajuga, Chrysanthemums, English Daisy, Larkspur, Pansies, Petunias, Stocks and others. More profuse flowering is characteristic of such plants as African Violets, Cyclamens, Jerusalem Cherry and Oxalis. Larger flowers develop on such plants as Geraniums and Hydrangeas. Other common ornamentals that have responded to treatment include Coleus, Forsythia, Fuschia, Holly, Ivy, Sedum, Yucca, and others. Plants that have shown no response for one reason or another include Lilies, Iris, Ferns, Palms, Glads, Tulips, Poppies, Boxwood, Spruce, Fir and Junipers. Most herbaceous annuals and perennials respond to applications made in the spring, as new growth starts at the 100 ppm rate. Herbaceous plants that don't respond at this concentration, as well as woody ornamentals and shrubs should be sprayed at the 100 ppm rate.

C. USE PRECAUTIONS

3. Corrosiveness: None
4. Cleaning glassware and spray equipment: Wash with detergent and rinse with water.
5. Storage stability: Prepared solutions are unstable and must be used immediately after preparation — store in dry place, tightly closed at temperature of less than 32°C. Protect wettable powder formulations from moisture.

D. PHYSIOLOGICAL AND BIOCHEMICAL BEHAVIOR

1. Mechanism(s) of action: GA₃ is a growth promotor — accelerates vegetative growth of shoot producing larger plants; mostly due to cell elongation but sometimes cell multiplication may be involved. Induces flowering by breaking dormancy.

F. TOXICOLOGICAL PROPERTIES

2. Acute toxicity: Oral administration of massive single doses of 1 g kg⁻¹ produced no toxic symptoms in rats. Mice — 15 g kg⁻¹ no toxic symptoms.
3. Subacute toxicity: Dogs and rats no ill effects from daily doses of 1 g kg⁻¹ six days per week for 90 days. One group of rats have been fed for 14 months, no toxic symptoms observed.
4. Chronic toxicity: No ill effects on rats fed 5-8 weeks with 5% GA₃ in diet. No changes found in organs or tissue. Guinea pigs — no toxic signs fed with kale for 3 months, treated with 250 ppm.

I. SOURCE

Abbott Laboratories
Chemical and Agricultural Products Division
North Chicago, Illinois 60064

ICI Plant Protection Division
Farnhurst
Haslemere
Surrey, England GU27 3JE

Lilly Research Laboratories
Division of Eli Lilly and Company
Greenfield Laboratories
P.O. Box 709
Greenfield, Indiana 46140