A study of the efficacy of homoeopathic treatment in controlling lactic acid accumulation and exercise fatigue.

By

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I Colin La Grange hereby declare that this dissertation representative of my own work.

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Abstract

The action of homoeopathic medicine on physiological processes is poorly understood. It is hypothesized that homoeopathic potencies of Sarcolactic acid could have an effect in increasing the metabolism of lactate in the body, lowering its concentration change due to exercise, and thus reduce exercise fatigue. There is a need to investigate whether a homoeopathic remedy made from a physiological chemical can influence the action of this chemical in the body. Much has been done on the influence of homoeopathy on toxins, and their detrimental effects, but little is known of its effects on metabolic by-products and the influence of homoeopathy on their regulation.

60 males, in good health, aged 18-25 years old, all involved in some form of physical activity, except cycling, were used to perform cycle ergometry trials. They were separated into groups of 20 per group. One group took a 9CH potency of Sarcolactic acid, the second took a placebo and the third took a 2CH potency of Sarcolactic acid. The medication was given 30 minutes prior to the exercise trial. The trial was a double blind study. Each volunteer was randomly placed in a group and asked to perform a cycle trial at 240 Watts, 80 revs/minute at 3kg resistance, until exhaustion. Blood was taken, from the median cubital vein, at the start of the exercise and at the point of exhaustion, when the exercise could no longer be maintained. The time was recorded from the start of the exercise to the exhaustion point. The blood was centrifuged and the supernatant was used as the sample for measuring the lactate levels. The Boehringer

fully enzymatic test kit, Cat. No. 256733 , together with photospectometry readings of the supernatant were used to measure the actual lactate levels of the blood.

The data was statistically analyzed using the ANOVA (Analysis of variance), Least Squares Means and the multiple range tests. All at the 95% significance level, $\alpha = 0.05$. The results showed a significant difference, $\alpha = 0.026$, with respect to lactate change per time taken to reach fatigue, between the 9CH and placebo groups. The 2CH and the placebo groups showed no significant differences. All the groups showed no significant differences with respect to time and lactate changes alone.

It can be concluded that the homoeopathic Sarcolactic acid 9CH potency has some influence on the lactate - fatigue system in the body. Although this affect was only marginal, the short time of exposure to the medication makes it a significant result.

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CHAPTER 1

Introduction

The role of lactic acid in exercise fatigue has been studied since 1907, initially by W. Fletcher and F. Hopkins (Fitts & Metger 1993). Subsequently, with more accurate methods of measurement, other factors have been linked to muscle fatigue, but none so strongly as H^+ protons formed during lactate production (Shephard & Astrand 1992). Studies done on muscle endurance and fatigue have noted the onset of fatigue to occur in the absence of oxygen, when the muscle is functioning anaerobically. During anaerobic exercise the primary fuel is converted into lactate, instead of carbon dioxide and water in the presence of oxygen. Anaerobic energy for muscle function is approximately 2.5 times as fast as oxidative mechanisms. (Guyton 1996.) Anaerobic mechanisms however are very expensive, and they cannot occur for too long before fatigue sets in. Due to the fuel inefficiency of the anaerobic system, another limiting factor is fuel availability. For this reason the exercise chosen to induce fatigue in this study was high intensity short-term cycle ergometry, so as to rule out fatigue due to fuel depletion (Fitts & Metzger 1993). Little is understood about the physiological mechanisms of homoeopathic remedies on the body. This study has been designed to demonstrate a direct effect of a homoeopathic remedy on lactate. Lactate was chosen as it is easy to measure, and the processes leading to its production are well understood.

The purpose of this investigation is to evaluate the efficacy of homoeopathic potencies of Sarcolactic acid on the accumulation of excess lactate in the body, in terms of change in blood lactate levels and time to fatigue, in order to determine the

efficacy of potentised Sarcolactic acid in reducing lactate build up and exercise fatigue.

The objectives of this investigation are twofold:

- To investigate the direct effect homoeopathic Sarcolactic acid potencies can have on blood lactate level changes due to exercise.
- To measure the effect homoeopathic Sarcolactic acid can have on exercise induced fatigue, measured as time taken to reach fatigue during short-term high intensity cycle ergometry.

This study may give further insight into the long debated relationship between fatigue and lactate as well as to demonstrate a direct physiological effect possible of homoeopathic remedies and the benefit of a safe, inexpensive way to reduce lactate build up and muscle fatigue.

CHAPTER 2

The Literature review

2.1. Introduction

A brief description of Homoeopathy is given, to emphasise why Sarcolactic acid (muscle lactic acid) was chosen for the treatment of lactic acidosis induced by exercise. The mechanisms of how lactate is proposed to bring about fatigue will also be briefly mentioned. The aim is not to describe this in detail but it is mentioned together with mechanisms of lactate control as a background, which will later be useful for proposing possible hypotheses of where homoeopathic Sarcolactic acid is able to influence in the body.

2.2. Homoeopathy

The principles of Homoeopathy are based upon the findings of Doctor Samuel Hahnemann at the beginning of the last century. The principle is best explained by the Latin phrase: "Similia similibus curentur", "Let like substances be used to cure like diseases." (Hahnemann 1962.) The term similimum remedy is used for the substance which, when given to a healthy individual in its natural or toxic dose, produces a pattern of symptoms similar to those symptoms that a diseased person would present with. It does not produce the same disease; rather it mimics the disease in symptom presentation. The remedy could not, for example, cause a viral disease, yet the symptoms produced in the healthy person taking the toxic dose of that remedy will have the same symptoms as the viral disease but without the virus being present. Prescribing the similimum remedy for a disease whose symptoms appear to be the

same as those produced by the similimum remedy will bring about a cure of that disease. To give an example of this, Belladonna, a common poison taken from the plant, Deadly Nightshade, taken in its natural form produces high fever, restlessness, thirst, irritability, a hot burning skin, sore throat and sore ears. The Belladonna in homoeopathic form is in turn used for a patient presenting with an acute illness, which has symptoms of restlessness, a high temperature with thirst and hot burning skin, irritability with painful infections of the throat and ear. (Boericke 1990.)

Thus the principle of "like cures like" will be used to bring about an effect on the build-up of excess lactate in the body. Lactate build-up is a natural consequence of exercise (Gollnick et al. 1974). The excessive increase of lactate in the muscles and body as a whole, leads to fatigue. In a study done by Haller et al. (1988), the investigation of a disease blocking normal aerobic cell respiration, showed the lactate path of respiration was the only alternative, with the result that the patient suffered from extreme exercise intolerance manifested by fatigue from lactic acidosis. Therefore according to the "Like cures Like" principle, Sarcolactic acid is the similimum for the effects produced by lactate build-up caused by exercise.

2.3. Homoeopathic dilution

The homoeopathic remedies are prepared by serial dilution of natural/original substances, which possess clinical/medicinal application. The serial dilution may follow either a decimal scale, 1 part original medicine plus 9 parts vehicle (i.e. one in 10) or a centicimal scale of 1 part original medicine plus 99 parts vehicle (i.e. one in 100). The diluent vehicle is lactose for solid dilutions /trituration, or water - alcohol mixture for liquid dilutions. This serial liquid dilution is coupled with a process called

succussion. Succussion is a vigorous agitation or shaking of the dilution at each dilution step. (Muntz 1987.) (Kayne 1997.)

The purpose of the succussion is to create an intimate mixture between the medicinal substance and the vehicle. This intimate mixture is thought to form a lattice structure of the water and alcohol to make a type of template of the medicinal molecules (Gaier 1991). This forms an energy pattern, which is carried throughout the mixture, but the physical molecular structure of the original product is lost with dilution and thus loses its toxic effect. The strength or potency of homoeopathic remedies is increased with each dilution and succussion. (Bellavite et al. 1995)

Dilutions with succussion were termed by Hahnemann as potencies or dynamisations. Part of this project is to determine which potency will be most effective in influencing lactic acid.

2.4. Lactic acid

2.4.1. Cellular Respiration

In order for a cell to function and sustain life it needs to produce a usable form of energy. This energy is in the form of adenosine triphosphate (ATP), a product formed from the breakdown of energy foods like fatty acids, amino acids, glycogen and glucose. The process by which this happens is a complex chain of enzyme-driven reactions. The first process is called Glycolysis. (See fig.1.) As the name suggests, it is the breakdown of glycogen and /or glucose and intermediate products eventually producing pyruvate. The pyruvate is taken into a cycle called the Tricarboxylic acid cycle, which is followed by the electron transfer system and oxidative phosphorylation step. The whole process from pyruvate to oxidative phosphorylation ending with

water and carbon dioxide is dependent on the availability of oxygen. The respiratory process takes place in an intracellular apparatus called a mitochondrion, the energy centre of the cell. (Guyton 1996.) The production of 1 Watt of metabolic power requires 3ml/min of oxygen. At rest the oxygen consumption is 300ml/min. thus producing 100 Watts. At a certain stage of muscular activity the body cannot maintain the oxygen consumption increase and it reaches a plateau: this is called maximal oxygen consumption or $VO_{2\ max.}$ A young, untrained man is capable of maintaining a power output of 200Watts, consuming approximately 3litres/min of oxygen, whereas a trained cyclist is capable of maintaining a 400Watts-power output. (Komi 1992.)

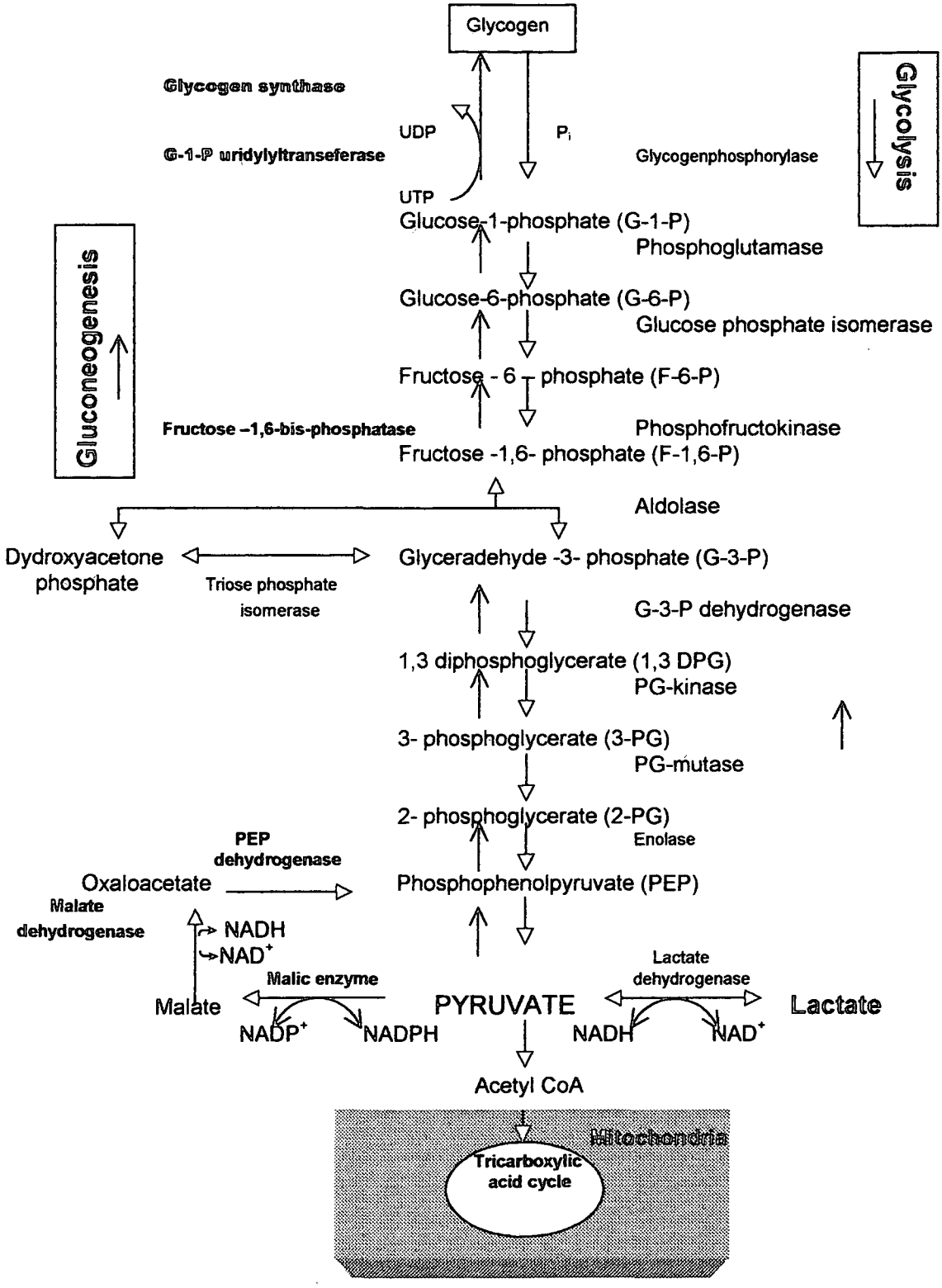

Figure.1 Glycolytic and gluconeogenic pathway in skeletal muscle.

2.4.2. Anaerobic Respiration

Anaerobic respiration is the alternate path of ATP-energy production in the absence of oxygen. This takes place outside the mitochondrion and involves the conversion of pyruvate to lactate. This alternate pathway can only be fuelled by glycogen or glucose. Glucose→ 2 lactate + $2H^+$ is called anaerobic glycolysis. (Guyton 1996) The problems inherent with this process is it produces only 10% of the ATP produced by the aerobic respiration, and it produces H^+(hydrogen protons) (Komi 1992). This has two implications: one being that large amounts of glycogen and glucose are needed to sustain anaerobic respiration, the second is that the hydrogen ions produced are the cause of acidosis, which is a drop in pH. It is believed that the hydrogen ions produced by lactate metabolism are the agents that interfere with muscle cell function and inhibit recovery, (Renaud 1989) and an increase in hydrogen ion concentration can lead to fatigue (Hogan et al. 1984).

2.4.3. Exercise and lactate

It has been known for over 60 years that both sub-maximal and maximal exercise results in an increase in lactic acid levels in the blood (Hagberg 1984). One can view exercise as an increase in energy demand. Continued exercise with increasing intensity can cause the supply of oxygen by the blood to be inadequate to meet the muscle's energy requirements, at VO_{2max}. The body must then call into play anaerobic respiration in order to meet energy demands. At the point in exercise where the oxygen supply is inadequate an upsurge in lactate production in the muscle and lactate levels in the blood is observed. This point is called the anaerobic or lactate threshold (Jacobs 1983). Heck et al. (1985) proposes this level to be at

4 mmols/l. This level can vary from person to person and is also influenced by factors other than oxygen debt. Ivy et al. (1981) showed that blood lactic acid level changes with substrate availability. There is an increase in lactic acid with an increase in blood glucose and decrease in lactic acid with an increase in fatty acid levels. Vokovich et al. (1992) demonstrated that protein supplementation decreased lactate accumulation, thereby marginally affecting performance. The influence of the substrate availability on lactic acid during work-rates below the anaerobic threshold means lactic acid can be produced even when oxygen is still available, but a significant rise only occurs during activity above the lactic acid threshold. In the trained athlete, the lactic acid threshold is higher, and sub-maximal levels of lactate are lower than the untrained person at the same work-rate (Henritze et al.1985). These two facts about training and substrate availability are important as far as the use of homoeopathic treatment is concerned. The mechanisms which allows the fit athlete to have a higher lactate threshold may be the mechanisms which can be influenced by the homoeopathic remedy, where the muscles are better able to cope with anaerobic stress, lactate increase and fatigue. It is the change in the blood lactate level, and the proposed influence that homoeopathic Sarcolactic acid has on that change, which is important in this study.

2.5. Blood lactate levels.

The increase in blood lactate is a good reflection of the increase in the muscle lactate, as lactate is a small molecule and is easily diffusible into the blood (Jorfeldt et al. 1978). Although blood lactate levels are never as high as those in muscle during maximal exercise, the increase of lactate in the blood is proportional to that in the muscle, so blood levels remain a good indicator of muscle anaerobic activity (Jacobs

et al. 1983). At the start of maximal or intense exercise there is a sharp increase in the lactic acid level due to a delay in increasing blood supply to the muscle, to meet the extra oxygen requirement (Jacobs et al. 1983).

2.6. Metabolism and control of lactate

Lactate can be used in two ways:

1. It can be oxidised in the presence of oxygen by: Lactate + $3O_2 \rightarrow CO_2 + H_2O$ via the Tricarboxylic acid cycle (Guyton 1996). This explains the maintenance of lactic acid levels below threshold while the oxygen demand is still being met.
2. By the process of gluconeogenesis lactate can be re-converted back into glucose in the liver. It is also believed that muscle tissue itself has the ability to perform gluconeogenesis (Pascoe & Gladden 1996). The body has mechanisms whereby it can use glycogen and glucose in the muscle to produce lactate and allow the formation of energy in this process, it also has processes that can re-convert the lactate in the body back into glucose and then into glycogen (See fig 1.). The process of gluconeogenesis provides a refuelling system, which allows anaerobic respiration to remain affective, as without a substrate to metabolise, the system ceases to function, which results in pronounced fatigue or exhaustion. The more efficient the metabolism of lactate, the greater the increase of glucose or glycogen which causes an increase in the lactic acid threshold. The metabolism of lactate means its levels decrease in the body. At the same time the removal of lactate by gluconeogenesis also increases the pH as the protons produced in the process of anaerobic respiration are taken up by re-conversion of lactate to pyruvate. (Guyton 1996).

2.7 Lactic Acid and Fatigue

Fatigue can be defined as the inability of muscle to maintain a given power output. It is a complex process involving both psychological and biochemical factors. The psychological factor can play a role in the perceived experience of fatigue, as power output can remain the same, but the experience of fatigue is different (Fitts & Metzger 1993). This study is, however, more concerned with the biochemical effect on fatigue. In fatigue there is a failure or interference with one or more of the processes involved in a motor unit/ muscle unit. The motor unit has a nervous component and motor component. Fatigue can be demonstrated in an isolated muscle preparation where the function of the nervous component is unimpaired. "It appears that the primary sites of fatigue are located within the muscle, and do not generally involve the central nervous system, peripheral nervous system or the neural-muscular junction." (Fitts & Metzger 1993). This means that there is a chemical effect on the muscle tissue fibres causing an effect on the performance. This chemical effect is demonstrated most powerfully in conditions of decreased oxygen supply to the muscle resulting in an increase in lactic acid, with the resultant drop in the pH, (Hogan & Welch 1984). Hermansen (1979), showed that a drop in pH below 7.0 caused a reduction in muscle power output. Possible fatigue sites are found in the excitation- contraction (EC) coupling, an impairment of the action potential (AP) of the sarcolemma and also the t-tubular AP occurs, which has been associated with reduced Ca^{2+} release, binding and re-uptake. All these factors interfere with actin- myosin contractile ability in the muscle fibre. The H^+ ion can produce fatigue at numerous sites. It is responsible for a direct inhibition of the actomyosin ATPase and ATP hydrolysis. An intracellular decrease in pH can also inhibit phosphofructokinase thus slowing the rate of glycolysis. Low pH can also produce competitive inhibition of Ca^{2+} binding to troponin

C, in so doing reducing cross-bridge activation and a direct inhibition of the cross-bridge transition from a low to a high force state. The inhibition of the Sarcoplasmic reticulum -ATPase reducing the Ca^{2+} re-uptake causing the reduction of Ca^{2+} .(Fitts & Metzger 1993.)

Increased lactate and Hydrogen ions have been shown to interfere with Sarcoplasmic reticulum Ca^{2+} release channels (Favero et al. 1995). In the muscle, calcium is stored in the Sarcoplasmic reticulum and released under nervous stimulation to cause binding of the muscle proteins, actin and myosin, which allows for muscle shortening, ie. contraction. The calcium binding which is affected is the binding to the sarcoplasmic reticulum, and thus when the muscle receives a nervous impulse calcium ions are not released and the muscle cannot contract properly (Hagberg 1984). Regardless of the process, the pH in the muscle is still the limiting factor in the fatigue process. Therefore, factors that reduce the cell pH are of paramount importance for the reduction of fatigue. Factors which can combat the accumulation of hydrogen ions are the intracellular buffers and the blood bicarbonate buffer system and obviously a reduced lactate production. The protons produced during anaerobic glycolysis bind to bases to form acids. Most of these bases are the enzymes found within the cell. (Shephard & Astrand 1993)

The enzymes do not function properly when bound to protons. Thus further cell function is inhibited by an increase in protons. The rate of fatigue of the muscle can also be seen as the capacity of the cell to buffer the increase in hydrogen ions (Shephard & Astrand 1993). Hydrogen ions are not only buffered within the cell but there is also a significant loss of the hydrogen ions into the blood, as lactic acid which diffuses into the blood where it dissociates to lactate + H^+. In the blood the much larger bicarbonate buffer system can deal with the hydrogen ions efficiently.

The bicarbonate buffer produces $CO_2 + H_2O$, by: $HCO_3^- + H^+ \rightarrow H_2CO_3 \rightarrow CO_2 + H_2O$

Some research has shown the highest rate of lactate efflux from the cell occurred in a muscle bathed in a solution with bicarbonate buffers in the solution, as compared with other buffering solutions (Nagesser et al.1994). The success of this system is dependent on the speed at which the lactic acid escapes from the muscle into the blood. In the blood, lactate together with potassium binds to the red blood cells where it can be metabolised. (Lindinger et al.1995)

This may be a potential area of action of the homoeopathic Sarcolactic acid.

2.8 Conclusion

The effect of lactic acid on muscle performance is variable in different people. Those who have high intensity training cope better with it than untrained people. (Komi 1992.)

There exists in the body a balance between lactate production and breakdown in the presence of oxygen. When the body is stressed, as in the form of strenuous exercise, the balance shifts and the process to re-establish the equilibrium of the whole system is accelerated. An increase in lactic acid has a direct effect of increasing enzymes favouring the conversion of lactate back to glucose in the liver. Products produced during gluconeogenesis cause the inhibition of enzymes favouring the glucose breakdown to lactate. With the use of homoeopathy, the aim is to influence the autoregulation with the aid of a drug that relates to the way the individual patient reacts, and produces symptoms. In this case the symptoms produced by the lactic acidosis are best influence by the remedy Sarcolactic acid. In this study the aim is to bring about an increase, as with trained subjects, in the efficiency with which the body deals with lactate.

CHAPTER 3

Materials and methods

3.1 Study design and protocol

The format of this study was a double-blind clinical trial.

Three groups of 20 individuals were chosen, one control group and two experimental groups. The control group received a placebo indistinguishable from the experimental medication in appearance and taste. The experimental groups received a homoeopathic potency of Sarcolactic acid in 2CH and 9CH respectively. Each group was designated a letter A, B or C, which correlated to the letter marking each of the three medicines. Each new subject was randomly assigned to a group and given the corresponding medicine. The subject was given the medication 30 minutes prior to doing the exercise.

3.2. Subjects

The subjects chosen for this research project were all males. Subjects had to be aged 18 to 25. Race was of no consequence for admissibility to this project. Each subject had to take part only once. All the participants were in good health at the time of their clinical trial, and all were participating in some form of physical activity, in their past - time. The statistically significant number of subjects per group is twenty so sixty subjects were chosen to participate.

3.3 Ethics

Ethical considerations taken into account for this project were addressed as follows:

3.3.1. Professional nursing staff did drawing of blood. All participants were made fully aware that they would be veno-punctured, to draw blood before and after their exercise trial, before they were invited to participate.

3.3.2. No incentives were offered to participate and participation was on a completely voluntary basis.

3.3.3. It was necessary for all subjects to cycle to exhaustion. For this reason only people in good health were used. To avoid potential health risks, a delimitation of age was chosen and subjects had to also be participating in some form of physical activity, so that a sudden high intensity exercise would not be a danger to their health, as could be the case if they were unfit. All vital signs were checked approximately half an hour before the exercise trial.

3.3.4. Considerations for testing medication on human subjects were taken into account. In this trial the medications used were homoeopathic potencies, which in this case were two separate dilutions. One was a 2CH potency of Sarcolactic acid of dilution 1×10^{-4} of Sarcolactic acid. The second was a 9 CH potency of $1X10^{-18}$ dilution of Sarcolactic acid. At such a low dilution there are no side effects of the medicine, so the subjects were at no risk taking the medicine.

3. 4. Interventions

The medication used in this clinical trial was homoeopathic Sarcolactic acid. Two potencies of the medication were used a 2CH potency and a 9CH potency. As explained earlier the homoeopathic medicine is a serial dilution of a base product in this case Sarcolactic acid, the lactic acid that is produced in the muscle during anaerobic respiration. The dilution was done in 70% alcohol and 30% water, as the carrier vehicle, in accordance with homoeopathic preparation methods, as was discussed earlier. The potencies were hand succussed 100 times per potency. The medicine was acquired from Natura laboratories. The potencies were placed on to lactose neutral pills in a laminar flow chamber; the same pills without medication were used as the placebo. This made the medicated pills indistinguishable from the placebo so neither the experimenter nor the subject was aware of the true identity of the medication.

3.4.1. Treatment of the subject

Each subject was examined prior to performing the exercise, as mentioned in 3.3. The subject was given a dose in the form of 5ml of pills according to the group he was allotted to, half an hour before he performed the exercise. In this half-hour period he was made to rest.

3.5. Measurement and observations

3.5.1. The exercise trial

The exercise trial was performed once by each of the subjects. Each trial was performed as identically as possible for each of the subjects. Room temperature was between 23 -28°C. The work rate that each subject performed was 240 watts on a cycle ergometer. The same cycle ergometer was used for all the subjects. Each subject had to pedal at a rate of 80 revs/minute at a weight of 3kg resistance, this being equal to 240 watts.

A one minute unloaded warm up was performed before the exercise trial began. During the exercise each subject was encouraged to pedal till they could no longer maintain the required work rate or for a maximum of ten minutes. If they pedalled for more than the ten-minute cut off they were stopped at this point and the final blood sample was taken, otherwise the sample was taken at the point they could no longer pedal. The work rate was chosen to fatigue most people within ten minutes.

3.5.2. Data collection

Two sets of data were collected during this trial:

- Lactic acid concentration change
- Time taken to reach fatigue

3.5.2.1. Lactic acid concentrations

3.5.2.1.1. Blood samples

Blood was taken from the median cubital vein of the forearm. Two separate quantities were taken, one at the start of the exercise trial and the second at the time the subject reached fatigue and was no longer able to cycle. The blood was taken in

Fluoride/ EDTA vacutainer tubes, as specified by the test kit protocol. The amount of blood was approximately two millilitres per time taken. The blood was then centrifuged at 3000 revolutions per minute for 5 minutes within 2 hours of taking it. This was to separate the cells from the supernatant plasma. The plasma was then drawn off and decanted into a sterile tube. The plasma was stable for six days at +4 °C and for three days at + 15 to 25 °C. All the samples for this experiment were stored in a fridge and all the samples were tested within 3 days after the blood was drawn.

3.5.2.1.2. Lactate measurement

The Test-Combination Lactate fully enzymatic kit, made by Boehringer Mannheim GmbH Diagnostica, was used to measure blood lactate levels. The UV-method, (Cat. No. 256733 for 3 x 100ml) as stipulated by the test kit protocol was performed on all the blood samples. See appendix 2 for detailed experiment sheet.

The lactate kit protocol was followed for the sample preparation and a photospectometry reading was taken at 340nm. A 1 centimetre-path length was used. To get the blood lactate levels in mmols/l, the sample blank photospectometry reading was subtracted from the sample photospectometry reading, this answer was multiplied by the number 16.3. The number is a conversion factor specific to the lactate kit, to give the results of the photospectometry readings in mmols/l.
The difference between the final blood sample and the initial blood sample gave the change in blood lactate concentration over the time taken to reach fatigue (see appendix 1.).

3.5.2.2. Time readings

The time readings taken were from the start of the exercise trial to the point of exhaustion of the subject or 10 minutes, whichever came first. The readings were in minutes and seconds. See appendix 1. Time column.

3.6. Statistical analysis

The statistical analyses performed were the one way analysis of variance, performed on the time change for each group, the lactate concentration change for each group and lactate versus time for each group. A table of Least Squares Means was performed for time, lactate concentration changes and lactate versus time at a 95% confidence interval. A multiple range test for time, lactate concentration changes and lactate versus time was also performed at 95% confidence interval. All tests presumed the null hypothesis to be: $H_0 \quad \mu_1 = \mu_2 = \mu_3$ and the alternate hypothesis to be: $H_1 \quad \mu_1 \neq \mu_2 \neq \mu_3$. At $\alpha = 0.05$

Chapter 4

Results

4.1. The criteria for governing the admissibility of the data

- Only subjects from the ages of 18 - 25 were used in this study.
- All were in good health, and taking part in some form of sporting activity other than cycling.
- All time measurements were done accurately under my supervision.
- Blood samples taken were done by a qualified person to ensure the correct amount needed for accuracy was taken.
- Subjects ill on the day of experimentation were not used until such time that they had fully recovered.
- None of the subjects were on any other medication which could influence their lactate levels.

4.2. Lactate concentration change results

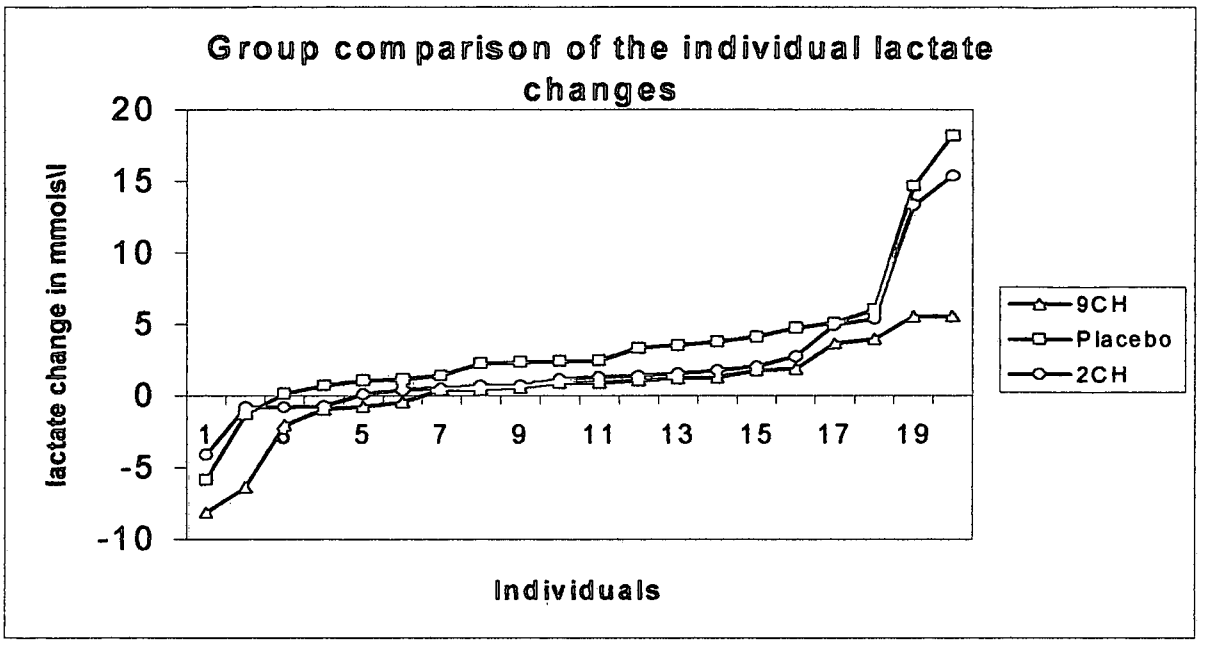

Figure 2. Comparison between the concentration changes for each individual in the separate groups.

The graph in figure 2 demonstrates the trend of lactate change per subject to as the lowest in the experimental group 9CH potency, followed by the 2CH group and the placebo group has the highest figures.

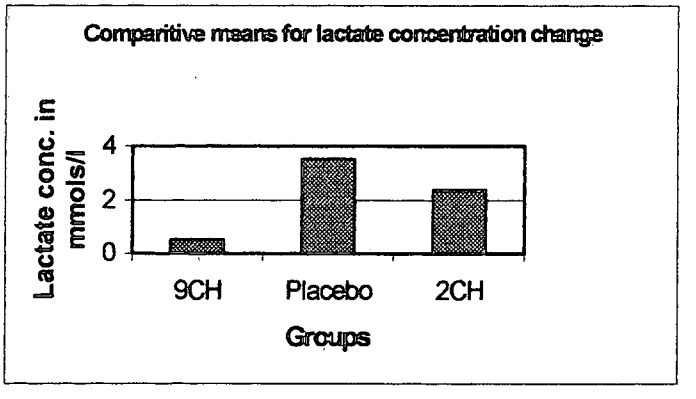

Figure 3. The comparison of the means of the three groups showing the average lactate level to be lowest in the 9CH and highest in the placebo group; the 2CH group is more similar to the placebo than it is to the 9CH group.

The statistical analysis with respect to lactate change according to the analysis of variance (ANOVA) showed the following:

Table 1. ANOVA for Lactate change - Type III Sums of the Squares

	Sum of Squares	d.f.	Mean square	F-ratio	Sig. Level
Main effects	90.159629	2	45.079814	2.317	0.1077
Residual	1108.8070	57	19.452754		
Total	1198.9666	59			

To find out if the three groups were similar with respect to lactate concentration change, where H_o , the null hypothesis, is accepted. If they are significantly different H_1 , the alternate hypothesis, is accepted, at α = 5% significance level.

The null hypothesis stated: $H_{o\ Conc.}: \mu_1 = \mu_2 = \mu_3$. $H_{1\ Conc.}: \mu_1 \neq \mu_2 \neq \mu_3$. At $\alpha = 0.05$

At the α = 5% significance level, according to the above shown table, the analysis of variance for lactate concentration change, the significance level: 0.1077 > 0.05 thus the null hypothesis is accepted.

It is thus concluded that there is no significant difference between the means of the three groups.

Table 2. Table of Least Squares Means for Lactate change

Level	count	Average	Stnd. Error	95% Confidence for mean	
Grand Mean	60	3.9216667	0.2700723	3.3807332	4.4626001
9CH	20	4.4145000	0.4677790	3.4775757	5.3514243
Placebo	20	3.7135000	0.4677790	2.7765757	4.6504243
2CH	20	3.6370000	0.4677790	2.7000757	4.5739243

Table 3. Multiple range analysis for lactate change

Method: 95% LSD

Group	Count	LS Mean	Homogenous groups	
9CH	20	0.5325210	☼	
2CH	20	2.3659450	☼	⌘
Placebo	20	3.5085750		⌘

Contrast	Difference	+/- limits	
9CH-Placebo	-2.97605	2.79354	∈
9CH- 2CH	-1.83342	2.79354	
Placebo- 2CH	1.14263	2.79354	

∈ denotes a statistically significant difference.

The multiple range test at the α = 5% level has revealed a significant difference between the 9CH and the placebo groups with respect to lactate change. It also shows there is a similarity between the 2CH and the placebo groups. However the difference is not significantly big enough to reject the null hypothesis in the ANOVA test.

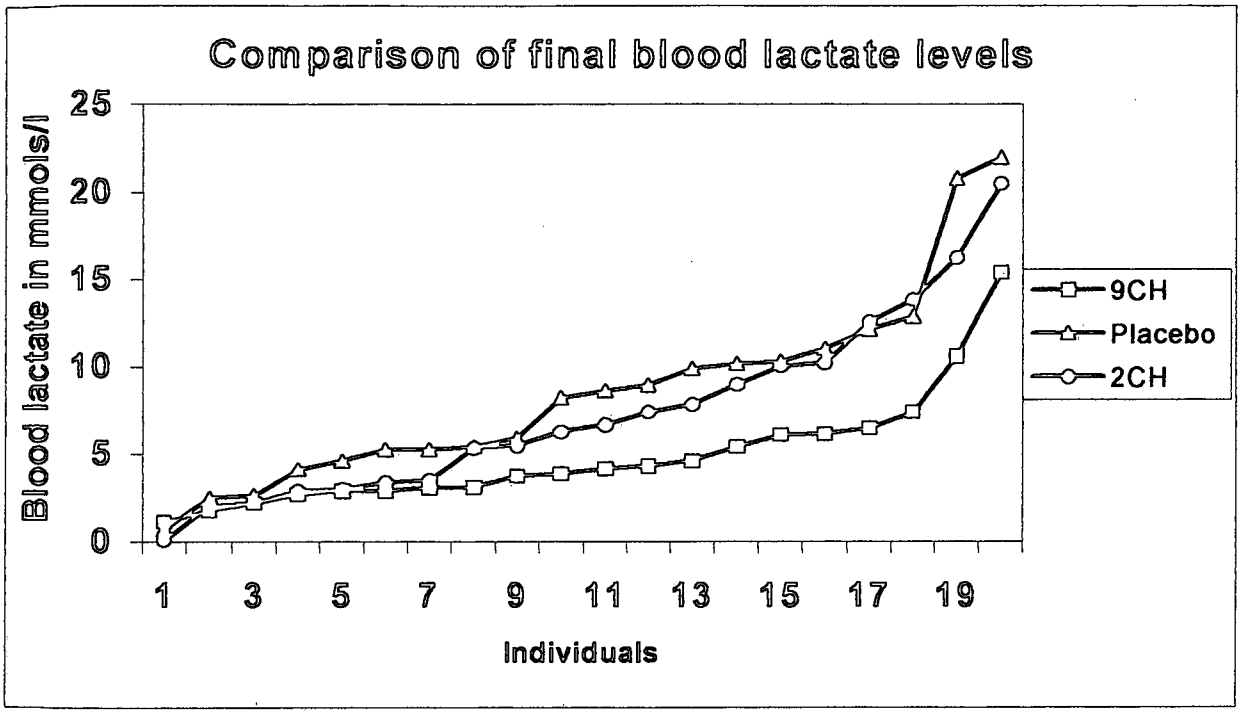

Figure 4. The Comparison of the individual lactate levels at fatigue of the three groups.

From figure 4 one can see the final lactate readings follow the same trend as the lactate change graph, with the 9CH having the lowest readings on average and the 2CH group and placebo having higher readings. This trend does point to the fact that

the 9CH on both the final lactate and lactate change has a greater effect than the other two groups on the lactate accumulation showing its ability to slow this process.

4.3. **Time change results**

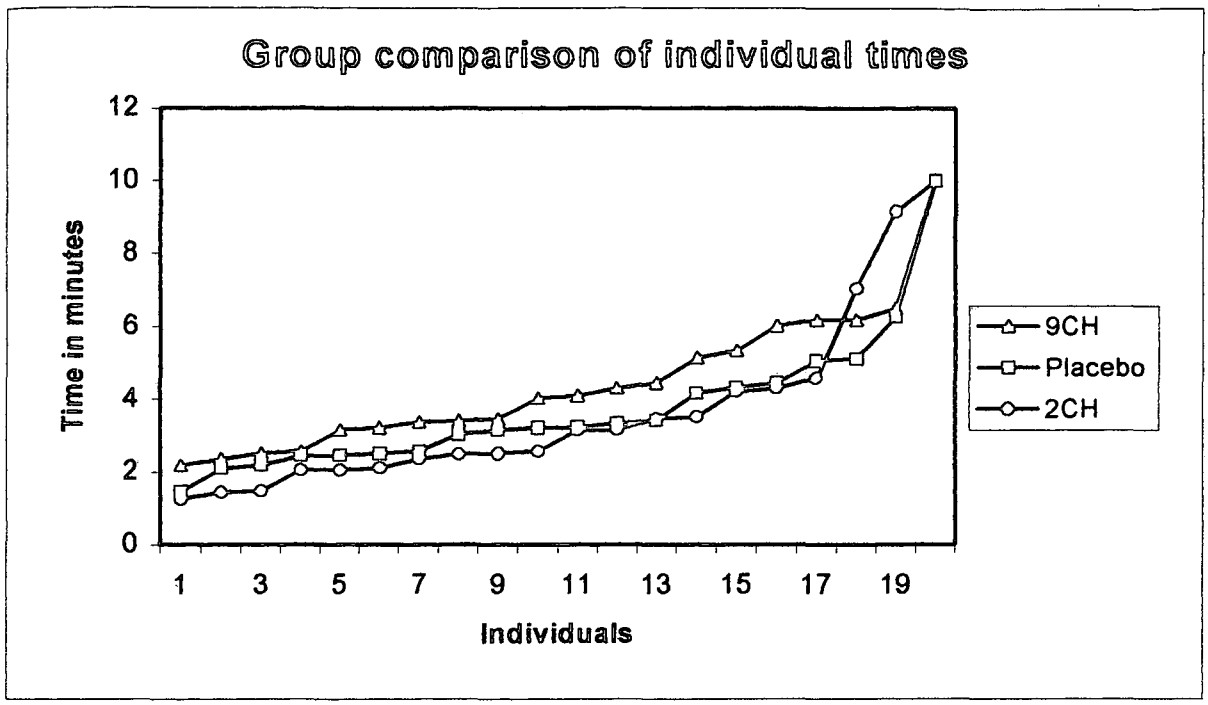

Figure 5. Comparison between the time taken to reach fatigue for each of the individuals in the three groups

Time changes from the graph figure 5 demonstrates a tendency for the individuals in the 9CH group to take longer to reach fatigue than individuals in the placebo and 2CH groups except in two instances.

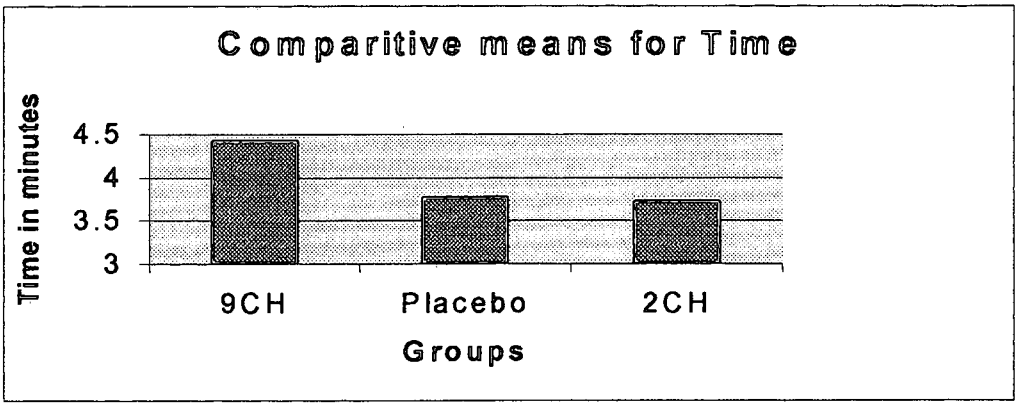

Figure 6. Bar graph showing the average times taken to reach fatigue for each of the three groups.

Although the trend for time shows a higher average time for the 9CH group than the placebo and 2CH, the difference is not statistically significant.

Table 4. ANOVA for Time - Type III Sums of squares

	Sum of Squares	d.f.	Mean square	F-ratio	Sig. Level
Main effects	7.3450633	2	3.6725317	.0839	0.4373
Residual	249.45157	57	4.3763433		
Total	256.79663	59			

The null hypothesis stated: $H_{o\ Time}: \mu_1 = \mu_2 = \mu_3$. $H_{1\ Time}: \mu_1 \neq \mu_2 \neq \mu_3$. At $\alpha = 0.05$

The null hypothesis at $\alpha = 0.05$ is accepted as $0.4373 > 0.05$. It is thus concluded that with respect to time taken to fatigue there is no significant difference between the means of the three groups.

Table 5. Least Squares means for time

Level	count	Average	Stnd. Error	95% Confidence for mean	
Grand Mean	60	3.9216667	0.2700723	3.3807332	4.4626001
9CH	20	4.4145000	0.4677790	3.4775757	5.3514243
Placebo	20	3.7135000	0.4677790	2.7765757	4.6504243
2CH	20	3.6370000	0.4677790	2.7000757	4.5739243

Table 6. Multiple range analysis for time

Method: 95% LSD

	Count	LS Mean	Homogenous groups
2CH	20	3.6370000	☼
Placebo	20	3.7135000	☼
9CH	20	4.4145000	☼

Contrast	Difference	+/- limits
9CH- placebo	0.70100	1.32501
9CH – 2CH	0.77750	1.32501
Placebo- 2CH	0.07650	1.32501

There is no significant difference in all tests for time taken to fatigue. Although there is a difference in the trend favouring the longer time for the 9CH group.

4.3 Lactate versus Time results

The lactate versus time comparison is an indicator of the effects lactate change will have on the performance of an individual. The measure of lactate change alone regardless of its amount varies according to the amount of time spent exercising. If one were to exercise for a long period in oxygen debt then it is probable that a greater change in lactate would be expected than if the exercise were performed over a short period of time. It is for this reason we have to put it into perspective by comparing the lactate changes that took place in relation to how much time was spent performing the exercise.

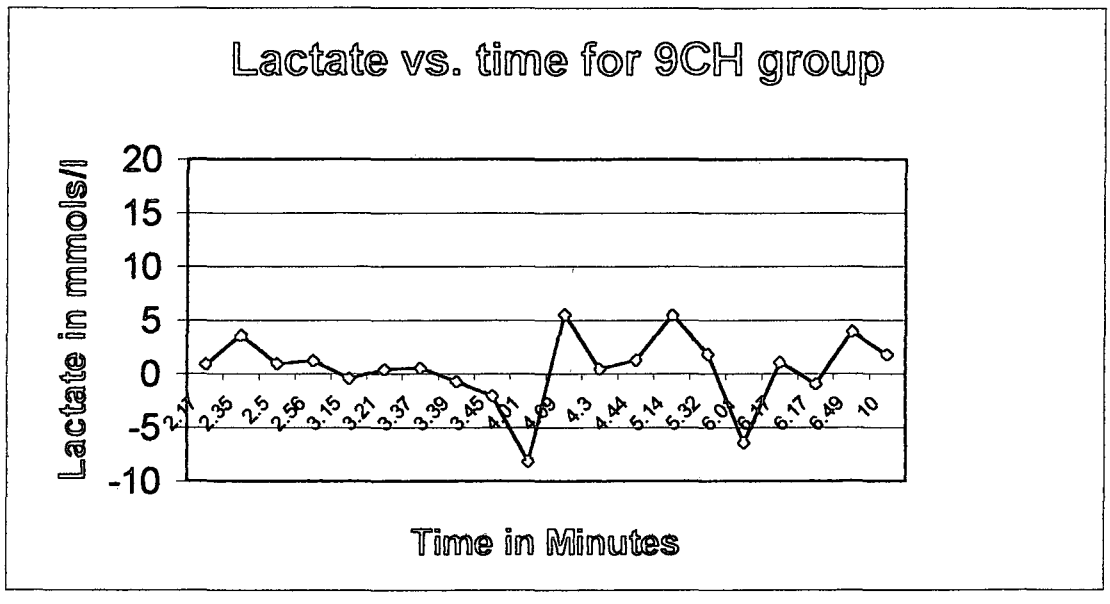

Figure 7. The graph for Lactate vs. time for the 9CH group, showing each participant's blood lactate concentration change reading at the time they fatigued.

The graph in figure 7. indicates the lactate reading per time taken to fatigue to be generally lower than the other group as would be expected from the trends seen in

the lactate change graph (fig. 2.) showing the 9Chto be the lowest and the time graph (fig. 5.) showing the 9CH to be the highest.

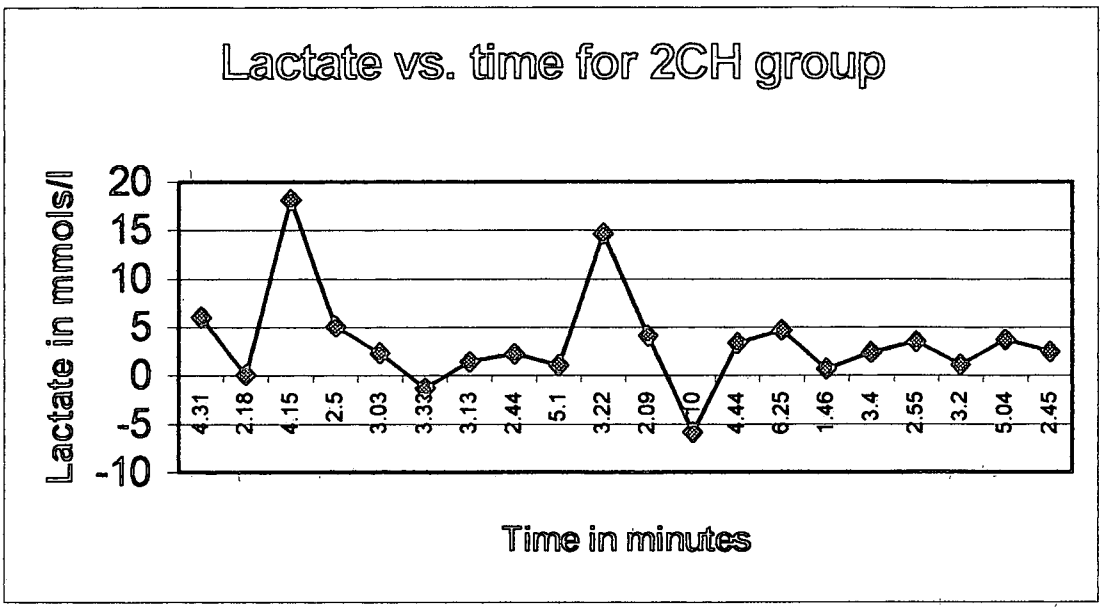

Figure 8. The graph for Lactate vs. time for the 2CH group, showing each participant's blood lactate concentration change reading at the time they fatigued.

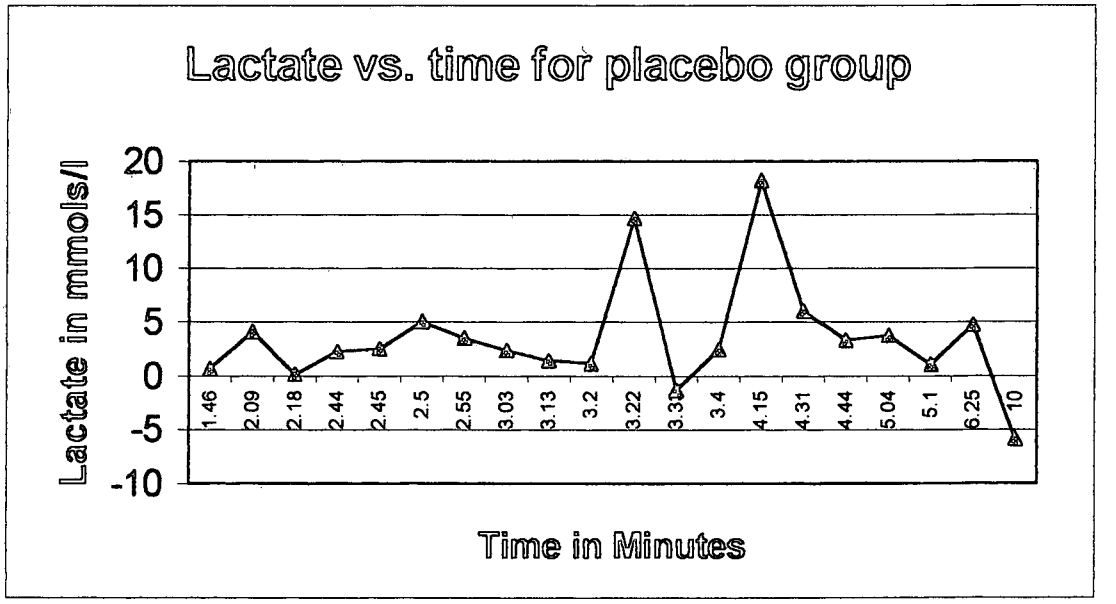

Figure 9. Graph of Lactate vs. time for the placebo group, showing each participant's blood lactate concentration change reading at the time they fatigued.

Table 7. Analysis of Variance for Lactate vs. time. –Type III Sums of Squares

	Sum of Squares	d.f.	Mean square	F-ratio	Sig. Level
Main effects	8.8967003	2	4.4483501	3.895	0.0260
Residual	65.098018	57	1.1420705		
Total	73.994719	59			

The null hypothesis stated with respect to Lactate vs. Time that $H_o: \mu_1 = \mu_2 = \mu_3$. And the alternate hypothesis with respect to Lactate vs. Time stated $H_1: \mu_1 \neq \mu_2 \neq \mu_3$. At $\alpha = 0.05$

The null hypothesis at $\alpha = 0.05$ is rejected, and the alternate hypothesis is accepted as $0.026 < 0.05$. It is thus concluded that with respect to lactate concentration change versus time taken to fatigue there is a significant difference between the means of the three groups.

Table 8. Least squares means for Lactate vs. time.

Level	Count	Average	Stnd. Error	95% Confidence for mean	
Grand Mean	60	0.6347767	0.1379656	0.35384425	0.9111109
9CH	20	0.1553087	0.2389634	-0.3233162	0.6339335
Placebo	20	1.0981182	0.2389634	0.6194934	1.5767431
2CH	20	0.6509032	0.2389634	0.1722783	1.1295280

Table 9. Multiple range analysis for lactate vs. time.

Method: 95% LSD

	Count	LS Mean	Homogenous groups
9CH	20	0.1553087	☼
2CH	20	0.6509032	☼ ⌘
Placebo	20	1.0981182	⌘

Contrast	Difference	+/- limits	
9CH – Placebo	-0.94281	0.67688	∈
9CH – 2CH	-0.49559	0.67688	
Placebo – 2CH	0.44722	0.67688	

∈ Denotes a significant difference

The multiple range analysis shows that the 9CH group and the placebo group differ significantly from one another. The placebo and the 9CH groups do not show a significant difference from the 2CH group.

CHAPTER 5

Discussion

The results of this trial have revealed that homoeopathic Sarcolactic acid has a significant effect on the lactic acid levels in relation to time taken to fatigue. The results reflect that the 9CH homoeopathic remedy was more effective than both the 2CH and the placebo groups, with respect to blood lactate change and time to fatigue in cycle ergometry trials.

The effects that the 9CH homoeopathic Sarcolactic acid had on the lactate levels alone were slight and only significantly different in the multiple range analysis. This change was not significantly large enough in the analysis of variants. The reason for this may be that the duration of the trial was insufficient. If the remedy was to have enough of an effect on the mechanisms involved in the lactate re-metabolism, perhaps a longer exposure to the treatment and follow -up measurements after a few days may show more significant results. However the protocol for this experiment was chosen to see if the remedy could produce an immediate response, which it did. The other factors not taken into account were the relatively small sample size and the fact that the homoeopathic principles of specificity and individuality were not able to be brought into a study of this kind. Homoeopathy in clinical practice is very reliant on each individuals specific presentation of symptoms and responses to stimuli and specific remedies are chosen according to those symptoms and responses. The study using a scientific model of randomised selection without the possibility of individualisation is not the most appropriate way to test remedies to their full potential. However, a significant result in a randomised group does show that homoeopathy potencies of physiological substances does have a potential for physiological action on that particular substance. Other factors that influence each individuals lactate metabolism capabilities may indicate the type of subjects who would benefit more from the homoeopathic Sarcolatic acid.

The effect on time to fatigue alone was also slight and not statistically significant. This possibly has to do with the other factors that play a role in fatigue. Perhaps the medication is able to effect a change in the lactate levels without changing the Hydrogen ion concentration significantly enough. From the fig.5.one can see a generally lower trend in the time taken to fatigue in the 9CH, as opposed to the placebo group, but as discussed above, the short duration of measurement may have influenced on the results. This trend may be stronger over a longer period of treatment with the Sarcolactic acid. The principle of the mechanisms of homoeopathic medicine, having a regulatory effect on the body's normal functions must be taken into account; it does not have a direct chemical inhibition or stimulation effect. The fact that the body's regulation can be affected even slightly in such a short period of time is significant. However, as noted in the figure 4.one could see that the trend for the people in the 9CH group to fatigue was at lower plasma concentration levels of lactate. This must suggest that the Sarcolactic acid potency is having an effect on lactate and not on the other mediators of fatigue. The slight difference in time to fatigue indicates that a decrease in lactate must also have some effect on the fatigue. However this occurrence may well point to the role that the medicine is playing, the influence on lactate is occurring after its production rather than before. The fact that fatigue occurred without a significant lengthening of the time and at a lower level of blood lactate must show that the H^+ ions were produced as a result of anaerobic glycolysis, and caused fatigue. The lactate may have been metabolised outside the cell thus producing a lower lactate level in the blood at the time of fatigue, although this lower level outside the cell does not have a marked effect inside the muscle cells.

If we now look at what was originally proposed: that the homoeopathic potency of Sarcolactic acid can have an effect on the lactate blood levels and time taken to fatigue, it is important to discuss the effects of the two in combination. Alone they did not produce significant differences between the groups. However, looking at the trends shown

graphically it is not surprising that the combination of lactate against time showed a significant difference. The differences were between the 9CH and the placebo and not between the 2CH and either of the other groups. This obviously means the potency aspect of the homoeopathic remedy plays a role in its effectiveness and not the remedy alone. The 2CH potency is far less diluted than the 9CH and in keeping with the homoeopathic principle of like cure like and infinitesimal dose, not only is the remedy important but, as Hahnemann stated, the smallest amount necessary to bring about cure is the correct amount. (Hahnemann 1962) The fact that the 9CH proved to be more effective bears witness to this statement.

The fact that alone neither the lactate change nor the time to fatigue showed significant differences but the analysis of the two combined shows the interdependence of the two on each other. The slightly lower lactate change was obviously enough to make the fatigue time increase slightly. The mechanisms acted on by the remedy may be the same as those involved in the fit person, whose mechanisms of keeping the lactate threshold down have adapted to the increased demand that comes with repeated exercising, such as training. If this is the case it may be worthwhile to investigate the effect the medicine could have if it was given regularly, it may stimulate these mechanisms repeatedly just as training does.

How these changes were brought about was not part of this trial, but in showing that potentised Sarcolactic acid has some effect on the fatigue process could open the way to investigating possible mechanisms of this action.

Its possible level of influence could be at the enzymatic level of re-conversion of lactate to pyruvate, or it could be a more efficient removal of lactate from the cells into the blood thus reducing intracellular pH, and improving the Calcium binding ability. If we were to look at the homoeopathic principle of like cures like, we must consider the direct effect that increased lactate levels have in the cell, in order to propose how, in potency, it can reduce

these effects. Lactate's strong association with fatigue provided a simple to measure system for determining whether the homoeopathic potency could affect lactate by affecting time to fatigue and objectively the blood lactate build up. In more advanced studies one could measure cellular changes due to homoeopathic remedies, that take place within the whole exercise fatigue-lactate model, one which is already well understood. Changes occurring with the use of homoeopathic Sarcolactic acid may be easy to determine in this physiological process. It may prove valuable, instead of trying to investigate the processes taking place using homoeopathy in complicated disease processes, which are themselves still poorly understood, rather to first study changes occurring in well known processes. Many critics claim homoeopathy has no scientific basis, but this is in light of a scientific model, which may not have the means available to objectively measure the mechanisms of action of homoeopathic medicine. Homoeopathy is a holistic medicine having an effect on the whole body, and to isolate a particular area to investigate has thus far proved impossible. The only real evidence is that of clinical trials where subjects report either an improvement or deterioration in their condition based on subjective and objective clinical findings. The question is not whether it works or not, but rather how. We cannot determine to what degree something is incorrect if we do not understand what correct is. So, by the same token, understanding how potentised products of a natural cellular process affect its operation, could give us a vital understanding into the mechanisms of homoeopathy and possibly its effect on disease processes.

CHAPTER 6

Conclusion and Recommendations

Sarcolactic acid in homoeopathic potency was effective on lactate change versus time taken to fatigue in short-term, high-intensity cycle ergometry. The higher potency, the 9CH, showed a significant difference to the placebo, but the 2CH showed no significant difference to the placebo. The effect on the lactate alone and time alone was not significant, but the effect was enough that the combination of lactate and time was significant.

I believe that the findings of this trial although encouraging, do not show the full potential of the effect of potentised Sarcolactic acid on the lactate/fatigue process. Further trials involving a longer treatment time before the exercise may show a different result. The measuring of the time taken to recover from fatigue, together with continuous measures of the lactate levels in the recovery period may also be a good indicator of the lactate re-metabolism process.

Another interesting option may be to measure over a long period of time the effect on one subject if exposed to both the potencies and the placebo, at different times, with sufficient time between trials to ensure a wash-out effect. This would be meaningful to see as all individuals deal with lactate differently so trials using the same individual could possibly give a more accurate evaluation, as it would rule out one more non-controlled variable.

CHAPTER 7

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Appendix 1

Group	Time	Initial blood sample		initial result	Final blood sample		final result	final - initial	lacchange mmol/l
		sample	blank		sample	blank			
A	4.09	0.72	0.117	0.603	1.114	0.17	0.944	0.341	5.5583
A	2.35	0.636	0.484	0.152	0.8	0.426	0.374	0.222	3.6186
A	6.49	0.676	0.466	0.21	0.925	0.472	0.453	0.243	3.9609
A	6.17	0.597	0.246	0.312	0.735	0.358	0.377	0.065	1.0595
A	2.56	0.436	0.324	0.112	0.811	0.624	0.187	0.075	1.2225
A	3.45	0.751	0.342	0.409	0.764	0.482	0.282	-0.127	-2.0701
A	4.01	1.29	0.55	0.74	0.864	0.626	0.238	-0.502	-8.1826
A	6.01	1.16	0.432	0.728	0.76	0.426	0.334	-0.394	-6.4222
A	3.21	0.356	0.214	0.142	0.385	0.219	0.166	0.024	0.3912
A	4.3	0.349	0.267	0.082	0.531	0.423	0.108	0.026	0.4238
A	2.17	0.276	0.255	0.021	0.382	0.252	0.068	0.047	0.7661
A	5.14	1.045	0.732	0.313	1.38	0.73	0.65	0.337	5.4931
A	5.32	0.373	0.255	0.118	0.536	0.306	0.23	0.112	1.8256
A	6.17	0.596	0.358	0.238	0.742	0.396	0.178	-0.06	-0.978
A	3.15	1.34	1.18	0.16	0.378	0.245	0.133	-0.027	-0.4401
A	3.37	0.621	0.478	0.143	0.573	0.396	0.177	0.034	0.5542
A	4.44	0.772	0.452	0.32	0.9	0.506	0.398	0.078	1.2714
A	3.39	0.756	0.446	0.31	0.68	0.416	0.264	-0.046	-0.7498
A	10	0.584	0.436	0.148	0.694	0.438	0.256	0.108	1.7604
A	2.5	0.723	0.589	0.134	0.819	0.629	0.19	0.056	0.9128
B	4.31	0.88	0.462	0.418	1.46	0.672	0.788	0.37	6.031
B	2.18	0.616	0.12	0.496	0.664	0.161	0.503	0.007	0.1141
B	4.15	0.83	0.6	0.23	1.953	0.618	1.345	1.115	18.1745
B	2.5	0.458	0.328	0.13	0.692	0.368	0.324	0.311	5.0693
B	3.03	0.55	0.363	0.187	0.714	0.382	0.332	0.145	2.3635
B	3.33	0.514	0.392	0.122	0.424	0.382	0.042	-0.08	-1.304
B	3.13	0.668	0.432	0.236	0.792	0.468	0.324	0.088	1.4344
B	2.44	0.218	0.205	0.013	0.39	0.238	0.152	0.139	2.2657
B	5.1	0.406	0.313	0.093	0.424	0.265	0.159	0.066	1.0758
B	3.22	0.885	0.51	0.375	1.974	0.7	1.274	0.899	14.6537
B	2.09	0.918	0.888	0.03	1.17	0.888	0.282	0.252	4.1076
B	10	0.988	0.376	0.612	0.674	0.422	0.252	-0.36	-5.868
B	4.44	0.965	0.494	0.471	1.18	0.504	0.676	0.205	3.3415
B	6.25	0.905	0.45	0.455	1.28	0.536	0.744	0.289	4.7107
B	1.46	0.759	0.438	0.321	0.792	0.428	0.364	0.043	0.7009
B	3.4	0.771	0.298	0.473	0.731	0.209	0.622	0.149	2.4287
B	2.55	0.915	0.526	0.389	0.97	0.365	0.605	0.216	3.5208
B	3.2	1.038	0.476	0.562	0.854	0.279	0.631	0.069	1.1247
B	5.04	0.818	0.521	0.297	0.785	0.361	0.527	0.23	3.749
B	2.45	0.83	0.435	0.395	0.966	0.478	0.547	0.152	2.4776
C	2.05	0.396	0.291	0.105	0.326	0.266	0.06	-0.045	-0.7335
C	2.48	0.654	0.326	0.328	0.935	0.483	0.452	0.124	2.0212
C	7.02	0.995	0.682	0.313	1.998	0.742	1.256	0.943	15.3709
C	4.57	0.925	0.464	0.461	0.652	0.444	0.208	-0.253	-4.1239
C	9.16	1.21	0.59	0.62	1.26	0.608	0.625	0.005	0.0815
C	2.55	0.588	0.361	0.227	0.522	0.344	0.178	-0.049	-0.7987
C	1.25	0.651	0.382	0.269	0.75	0.412	0.338	0.069	1.1247
C	1.43	0.664	0.414	0.25	0.752	0.424	0.328	0.078	1.2714
C	10	0.369	0.291	0.078	0.402	0.279	0.123	0.045	0.7335
C	4.3	0.88	0.698	0.182	1.934	0.516	0.997	0.815	13.2845
C	3.15	0.741	0.415	0.326	0.746	0.336	0.41	0.084	1.3692
C	3.5	0.3	0.245	0.055	0.352	0.218	0.134	0.079	1.2877
C	4.21	0.833	0.364	0.469	1.055	0.285	0.77	0.301	4.9063
C	2.35	0.838	0.159	0.679	1.083	0.235	0.848	0.169	2.7547
C	1.48	0.588	0.361	0.227	0.522	0.344	0.178	-0.049	-0.7987
C	2.1	0.292	0.11	0.182	1.22	1.005	0.215	0.033	0.5379
C	2.5	0.993	0.467	0.526	0.879	0.329	0.55	0.024	0.3912
C	2.05	2.052	1.9	0.152	1.869	1.389	0.48	0.328	5.3464
C	3.45	0.775	0.254	0.521	0.963	0.349	0.614	0.093	1.5159
C	3.14	0.732	0.456	0.276	0.835	0.45	0.385	0.109	1.7767

Test-Combination Lactate fully enzymatic

UV-method
Cat. No. **256 773** for 3 x 100 ml

Assay without deproteinization

Additional reagent:
Fluoride/EDTA (Cat. No. 243 710)

Method
mod. from Noll, F. (1974). L-(+)-Lactate. Determination with LDH, GPT and NAD. Page 1475 ff. *in* H. U. Bergmeyer, ed. *Methods of Enzymatic Analysis*, 2nd ed. (Transl. from 3rd German ed.) Verlag Chemie Weinheim *and* Academic Press, Inc., New York and London. 4 vols.

Test principle

L-lactate + $NAD^+ \xrightleftharpoons{LDH}$ pyruvate + NADH + H^+

pyruvate + L-glutamate $\xrightleftharpoons{GPT}$ L-alanine + α-oxoglutarate

Normal values
In plasma: 5.7–22 mg/100 ml (0.63–2.44 mmol/l)
Reference: Kühnle, H. F., et al. (1977). *J. Clin. Chem. Clin. Biochem.* **15**:171.

In C.S.F.: 10.8–18.9 mg/100 ml (1.2–2.1 mmol/l)
Reference: Kleine, T.O., et al. (1979) *Dtsch. Med. Wschr.* **104**:553.

Sample material
Plasma (see "Sample preparation", at right), C.S.F.

Reagents

1 **NAD**

2 **Buffer**
carbonate buffer
L-glutamate

3 **LDH/GPT**

4 $(NH_4)_2SO_4$
ammonium sulphate

Initial concentrations of solutions:

Reagent solution
carbonate buffer: 0.5 mol/l, pH 10.0; L-glutamate: 63 mmol/l; NAD: 4.6 mmol/l

Suspension 3
LDH ≧ 1632 U/ml; GPT ≧ 102 U/ml

Solution 4
ammonium sulphate: 3.2 mol/l

Quality control
In the normal range: Precinorm® S
In the pathologic range: Precipath® S

Preparation and stability of solutions

1 Dissolve contents of **one** bottle 1 in 25 ml redist. water.
Stable for three weeks at +2 to 8° C.

2 Use contents undiluted.
Stable up to expiry date specified when stored at +2 to 8° C.

Reagent solution

No. of assays	Solution 1, ml	Solution 2, ml
ca. 3	3	12
5	5	20
10	10	40
20	20	80

Use immediately, or keep refrigerated.
Stable for six hours at +2 to 8° C.

3 and 4 Contents are ready to use.
Stable up to the expiry date specified when stored at +2 to 8° C.

Sample preparation
The veins may be compressed only briefly (30 sec at most) before the blood specimen is obtained.
Obtain plasma by mixing ca. 2 ml of blood with 2 drops of fluoride/EDTA reagent (ca. 80 µl) and centrifuging for ca. 5 min at 3000 rpm within 2 hrs. The supernatant plasma can then be drawn off or decanted.
Stable for six days at +4° C
three days at +15 to 25° C.
C.S.F. is used as obtained.
Stable for 24 hours at +4° C
three hours at +15 to 25° C
at least one month at −20° C.

Procedure
Measurement at Hg 365 nm
Cuvette: 1 cm light path
Incubation temperature: 20–25° C
Measure against air (absorbance increase).

Pipette into test tubes:		
	sample blank (SB)	sample
reagent solution plasma or C.S.F.	5.0 ml 0.1 ml	– –
Mix well.		
Pipette off from sample blank*	–	2.50 ml
Add:		
suspension 3 solution 4	– 0.05 ml	0.05 ml –
Mix immediately, and after 10 - 15 min, read absorbances of sample blank (A_{SB}) and sample (A_S) in the same cuvette in immediate succession. $A_S - A_{SB} = \Delta A$.		

*Return remainder in pipette to sample blank.

After each measurement, flush suction or flow-through cuvette with the sample blank for the next sample.

If the lactate concentration exceeds
160 mg/100 ml (17.8 mmol/l)
as determined by measurement at Hg 365 nm, **dilute** 0.1 ml of sample with 0.5 ml redist. water and repeat the assay (result x 6).

Measurement at 340 nm and Hg 334 nm
Cuvette: 1 cm light path
Incubation temperature: 20–25° C
Measure against air (absorbance increase).

Pipette into test tubes:		
	sample blank (SB)	sample
reagent solution plasma or C.S.F.	5.0 ml 0.05 ml	– –
Mix well.		
Pipette off from sample blank*	–	2.50 ml
Add:		
suspension 3 solution 4	– 0.05 ml	0.05 ml –
Mix immediately, and after 10–15 min, read absorbances of sample blank (A_{SB}) and sample (A_S) in the same cuvette in immediate succession. $A_S - A_{SB} = \Delta A$.		

*Return remainder in pipette to sample blank.

After each measurement, flush suction or flow-through cuvette with the sample blank for the next sample.

If the lactate concentration exceeds 175 mg/100 ml (19.4 mmol/l) as determined by measurement at 340 nm/Hg 334 nm, **dilute** 0.1 ml of sample with 0.5 ml redist. water and repeat the assay (result x 6).

Calculation
Calculate the concentration (c) of lactate in the sample as follows:

Wavelength	c [mg/100 ml]	c [mmol/l]
Hg 365 nm	137.9 x ΔA	15.3 x ΔA
340 nm	147.3 x ΔA	16.3 x ΔA
Hg 334 nm	150.1 x ΔA	16.7 x ΔA

Please note
For single assays, pipette out 1.0 ml of solution 1 and 4.0 ml of solution 2 instead of 5.0 ml of reagent solution.
The dilution of the sample by the addition of 2 drops (ca. 80 µl) of fluoride/EDTA reagent has not been taken into account in the calculation factors and can be neglected in routine assays.
Pipette tips, plastic spatulas, and stoppers that come into contact with the specimen or any solution used in this test must not be touched with the fingers, since sweat can contain considerable amounts of lactate.
Solution 2 contains sodium azide as stabilizer. Do not swallow. Avoid contact with the skin or mucous membranes.

Boehringer Mannheim GmbH
Diagnostica

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Appendix 2

INFORMED CONSENT FORM

(To be completed in duplicate by patient/subject*) *Delete whichever is not applicable.

TITLE OF RESEARCH PROJECT

_____

_____

NAME OF SUPERVISOR

_____

NAME OF RESEARCH STUDENT

_____

DATE:

PLEASE CIRCLE THE APPROPRIATE ANSWER

1. Have you read the research information sheet? YES/NO

2. Have you had an opportunity to ask questions regarding this study? YES/NO
3. Have you received satisfactory answers to your questions? YES/NO

4. Have you had an opportunity to discuss this study? YES/NO
5. Have you received enough information about this study? YES/NO

6. Who have you spoken to? _____

7. Do you understand the implications of your involvement in this study? YES/NO

8. Do you understand that you are free to withdraw from this study? YES/NO
   a) at any time
   b) without having to give a reason for withdrawing, and
   c) without affecting your future health care.

9. Do you agree to voluntarily participate in this study? YES/NO

PATIENT/SUBJECT* Name_____ Signature_____
(in block letters)

PARENT/GUARDIAN* Name_____ Signature_____
(in block letters)

WITNESS Name_____ Signature_____
(in block letters)

RESEARCH STUDENT Name_____ Signature_____
(in block letters)