THE EFFECT OF CO-TRIMOXAZOLE, USED IN CONVENTIONAL AND HOMOEOPATHIC PREPARATIONS, UPON GROWTH OF *E. coli* AND *S. aureus*

ABSTRACT

The aim of this study was to establish the effect of co-trimoxazole prepared in conventional form , homoeopathic form and a mixture of both forms , on E. coli and S. aureus in terms of maximum specific growth rate, time taken to reach maximum specific growth rate, maximum cell population and metabolic activity for the purpose of evaluating the efficacy of co-trimoxazole when used in a homoeopathic form.

Co-trimoxazole used conventionally , homoeopathically (9CH or 15CH) and a mixture containing the conventional and homoeopathic (9CH or 15CH) forms were compared. The growth of E. coli and S. aureus in the presence of these medications (added separately) was monitored. Enumeration of colony forming units was conducted at 0 ; 6 ; 12 ; 24 and 48 hours , by the serial dilution - spread plate procedure. This was conducted in triplicate. Enzyme activity was used as an indicator of metabolic activity and was done at times 0 ; 12 ; 24 and 48 hours.

The maximum specific growth rate (MSGR) , time taken to reach maximum specific growth rate were calculated and compared for the various preparations. Enzyme reactions were semi-quantitively assessed.

The conventional antibiotic decreased cell growth of both E. coli and S. aureus when compared to the control. Inhibition of E. coli growth was most pronounced after 6 hours and that of S.aureus after times 6 and 48 hours. The MSGR for E. coli showed

a 78 % difference to the control whilst S. aureus displayed a 40 % difference. Enzyme activity of E. coli in the presence of the antibiotic also decreased.

The homoeopathic preparations 9CH and 15CH had minimal effect upon the MSGR. The MSGR for E. coli showed a difference of 14 % for the 9CH and 25 % to the control whilst the S. aureus showed a difference of 5 % and 14 % respectively. Little difference in enzyme activity was noted.

Mixtures containing homoeopathic (9CH or 15CH) and conventional co-trimoxazole were comparable to the conventional antibiotic with an exception that the 15CH mixture failed to retard growth of E. coli after 24 hours and S. aureus after 48 hours. The 9CH mixture suppressed growth of E. coli after 48 hours.

Integration of the above findings indicate that homoeopathic preparations influenced cell growth. These differences are however not significant. Graphically there is no significant difference . Since the serial dilution - spread plate technique is a relatively inaccurate method , a difference of two or more logarithms is required as an indicator of difference.

Homoeopathic co-trimoxazole is not effective as an antibiotic. The activity of the combination of conventional and homoeopathic co-trimoxazole remains unclear. Further research on co-trimoxazole and other antibiotics are required.

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I, Danny Pillay, do hereby declare that in respect of the following dissertation/thesis, "THE EFFECT OF CO-TRIMOXAZOLE , USED IN CONVENTIONAL AND HOMEOPATHIC PREPARATIONS, UPON GROWTH OF E. coli AND S. aureus"; as far as I know and ascertain no other dissertation/thesis exists, and all references detailed in the dissertation are complete in terms of all personal communications engaged in and published works consulted.

SIGNATURE OF CANDIDATE DATE

APPROVED FOR FINAL SUBMISSION

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SUPERVISOR

THIS DISSERTATION IS DEDICATED TO

MY PARENTS IN ADMIRATION

AND PETER & NOLA FRAZER

FOR THEIR CONTRIBUTION TO HOMOEOPATHY

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LIST OF ABBREVIATIONS

$CFUml^{-1}$	- colony forming units per millilitre
CH	- centesimal Hahnemanian
UTI	- urinary tract infections
MIC	- minimum inhibitory concentration
MSGR	- maximum specific growth rate
AIDS	- acquired immune deficiency syndrome
SD	- standard deviation

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CHAPTER ONE

1.0 **INTRODUCTION**

1.1 **IMPORTANCE OF THE STUDY**

Antibiotics are not only capable of destroying micro-organisms , they have a much broader and alterative effect on the human being. This has been demonstrated by pathogenic experimentation with drugs , for example on chloramphenicol [chloromycetin] and sodium salt of benzyl-penicillin [penicillin G] (Julian 1979). Their use and dosage are not only critical to ward off the offending micro-organism but impinge on the well-being of the human body.

This study endeavoured to establish the effects of co-trimoxazole in conventional form upon growth of E. coli and S. aureus. There were three main aspects that needed to be considered. Firstly , one needed to determine whether co-trimoxazole in homoeopathic form is capable of having a bactericidal effect in-vitro. This implies a cost benefit to our already ailing health care system. It also implies a decrease in patient toxicity as evidenced by the doses of antibiotics used today.

Secondly , a combination of conventional and homoeopathic forms may decrease cell growth to a greater extent than when

conventional co-trimoxazole is used alone. This potentiation renders the drug to be a more effective antibiotic. This may be applicable in cases where antibiotics are becoming less effective against previously susceptible micro-organisms. The converse may apply , where stimulation of growth of the micro-organism may occur. This would obviously contra-indicate the concomitant use of homoeopathic and conventional co-trimoxazole.

Thirdly , homoeopathic practitioners use conventional drugs in homoeopathic preparations to counter the side - effects of these conventional drugs. No research has been done on examining the effects of an antibiotic in conventional and homoeopathic preparations in-vitro. Co-trimoxazole was the drug of choice due to it's increased use and dosage against Pneumocystis carinni infections in patients with AIDS (Greenberg et.al., 1993). The homoeopathic preparations , if they do not interact with the therapeutic effect of the conventional co-trimoxazole may offer the patient the benefit of reduced side - effects when on conventional co-trimoxazole treatment.

1.2 **PROBLEM STATEMENT**

THE OBJECTIVE OF THIS STUDY WAS TO ESTABLISH THE EFFECT OF CO-TRIMOXAZOLE PREPARED IN CONVENTIONAL FORM , HOMOEOPATHIC FORM , AND IN A COMPLEX FORM CONTAINING A MIXTURE OF BOTH FORMS , ON E. coli AND S. aureus IN TERMS OF MAXIMUM SPECIFIC GROWTH RATE , MAXIMUM CELL POPULATION , TIME TAKEN TO REACH MAXIMUM SPECIFIC GROWTH RATE AND METABOLIC ACTIVITY FOR THE PURPOSE OF EVALUATING THE EFFICACY OF CO-TRIMOXAZOLE WHEN USED IN A HOMOEOPATHIC FORM.

1.3 **SUBPROBLEMS**

1.3.1 SUBPROBLEM ONE

To evaluate the effects of conventional co-trimoxazole on E. coli and S. aureus in terms of maximum specific growth rate , maximum cell population , time taken to reach the maximum specific growth rate and metabolic activity in order to confirm that co-trimoxazole does influence cell growth.

1.3.2 SUBPROBLEM TWO

To evaluate the effects of co-trimoxazole in homoeopathic form on E. coli and S. aureus in terms of maximum specific growth rate , maximum cell population , time taken to reach maximum specific growth rate and metabolic activity in order to observe the influence they have on cell growth.

1.3.3 SUBPROBLEM THREE

To evaluate the effects of a complex containing both conventional co-trimoxazole and the homoeopathic preparation on *E. coli* and *S. aureus* in terms of maximum specific growth rate , maximum cell population , time taken to reach maximum specific growth rate and metabolic activity in order to observe the influence they have on cell growth.

1.3.4 SUBPROBLEM FOUR

To integrate the knowledge of subproblems one , two and three , to identify to what extent the conventional co-trimoxazole , homoeopathic form of co-trimoxazole , and the conventional plus homoeopathic form of co-trimoxazole influence growth rates and metabolic activity for the purpose of evaluating the efficacy of co-trimoxazole in homoeopathic form.

1.4 **HYPOTHESES**

1.4.1 HYPOTHESIS ONE

Conventional co-trimoxazole will have a bactericidal effect on growth rates and will decrease the metabolic activity of *E. coli* and *S. aureus* .

1.4.2 HYPOTHESIS TWO

Co-trimoxazole in homoeopathic form will have no effect on growth rates and metabolic activity of E. coli and S. aureus .

1.4.3 HYPOTHESIS THREE

The complex containing conventional co-trimoxazole and the homoeopathic form of co-trimoxazole will decrease growth rates and metabolic activity of E. coli and S.aureus but to a lesser extent than in subproblem 1.

1.5 **THE DELIMITATIONS**

1.5.1 This study was limited to a specific strain of E. coli (strain ATCC 25922) and a specific strain of S. aureus (ATCC 25923).

1.5.2 Only the following growth media were used :

a) Nutrient Agar

b) Nutrient Broth

1.5.3 This study was limited to a specific antibiotic i.e. Co-trimoxazole. It is also known as Septrin or Bactrim

1.5.4 Only 9CH and 15CH homoeopathic co-trimoxazole were used because they are known to have an inhibitory effect. 9CH falls within Avogadro's constant and as such will contain some of the original substance (co-trimoxazole). 15CH exceeds Avogadro's constant and accordingly will have none of the original substance (co-trimoxazole) present. Avogadro's constant states that one mole of any substance contains $6,02 \times 10^{23}$ (Brown and Rogers , 1987) atoms but 15CH is 10^{-30} therefore none of the original substance exists in the solvent.

1.5.5 The incubation temperature for cell growth was 37 °C.

1.5.6 This was an in-vitro study.

1.6 **ASSUMPTIONS**

1.6.1 The first assumption

All cultures of E. coli and S. aureus used were grown under optimal conditions.

1.6.2 The second assumption

The preparation of the homoeopathic antibiotic was done according to homoeopathic principles.

1.7 **DEFINITIONS**

1.7.1 POTENCY - The especially produced capability in a medicine to effect a dynamic stimulus in the appropriate patient (Gaier, 1991).

1.7.2 HOMOEOPATHIC PREPARATION - For this research , homoeopathic preparations refer to the 9CH and 15CH potencies only.

1.7.3 DYNAMISATION - The process of serial dilution and sucussion

1.7.4 CH - Denotes Centismal Hahnemanian potencies and refers to a one in hundred dilution during the process of dynamisation..

CHAPTER TWO

2.0 **LITERATURE REVIEW**

2.1 INTRODUCTION

Homoeopathy is the therapeutic method based on the application of a law of pharmacology termed "Law of Similars" or "the analogy principle" as evidenced by the dictum "similia similibus curentur". This form of medicine and the therapeutic method used in its administration , was developed by Hahnemann in 1796 (Tetau, 1986). Homoeopathy is derived from the Greek words "homoios" , meaning "like" or "similar" , and "pathos" meaning "suffering" (Boyd, 1989). This therapeutic method is used to treat a wide variety of ailments.

Several trials have been performed which demonstrate that homoeopathic preparations exhibit the "similia similibus curentur" phenomenon (Netien et.al., 1966 ; Endler et.al., 1991 ; Gaier, 1991). No trial has used an antibiotic to demonstrate this.

Co-trimoxazole is a commonly used antibiotic and consists of two components viz., trimethoprim and sulphamethoxazole. They act on the microbial metabolic system (Gilman et.al., 1991).
E. coli is a commensal of the human gastrointestinal tract. It

becomes pathogenic when it invades other body tissues. More than 50 % of E. coli infections involve the urinary tract. Other infections include diarrhoea , bacteraemia , skin and lung infections (Wilson et.al., 1991).

S. aureus is an ubiquitous organism found in the nares of 30 % and the skin of 20% of healthy people. It commonly causes food poisoning , endocarditis , gastroenteritis , osteomyelitis , pneumonia , cellulitis and impetigo (Lennette et.al., 1985).

2.2 THE LAW OF SIMILARS

The Law of Similars states that a substance which is capable of producing symptoms in a healthy individual, when administered to a sick person showing the same symptoms, will bring about a cure (Roberts, 1942). Expressing this in a pharmacodynamic scheme it becomes : "Any product which imitates determined disorders when administered in a strong dose to a person in good health , can cause the disappearance of these same disorders when administered in a weak dose to a sick person" (Tetau, 1986). Hahnemann by experimentation noticed that Belladonna produces the signs and symptoms of scarlet fever. He used Belladonna to treat scarlet fever in an epidemic , which brought about cure (Blackie, 1976).

The law of "similia similibus curentur" or "let likes be cured by likes" is the cornerstone of the practice of homoeopathy. Therefore , it deserves rigorous testing by scientific means. Although empirical evidence for the efficacy of homoeopathy as observed by homoeopaths is available , very few well designed scientific trials have been conducted. This view is shared by Kleijnen et.al. (1991) when they concluded "at the moment the evidence of clinical trials is positive.....that there is a legitimate case for further evaluation of homoeopathy , but only by means of well performed trials".

Trials were conducted to demonstrate the effects of Cuprum Sulphuricum 15CH on the growth of dwarf peas. The plants were poisoned with copper sulphate and produced seeds that were sub-standard i.e. , reduced number and size of seeds. Some seeds were grown with and some without the presence of Cuprum Sulphuricum 15CH and both were compared to normal seeds (control). The growth of the seeds treated with Cuprum Sulphuricum 15CH was similar to that of the control. This trial indicated that the homoeopathic preparation facilitated elimination of copper from the seeds , thus confirming the action of homoeopathic preparations (Netien et. al., 1966) Folliculinum 9CH was used in treating premenstrual syndrome and 88% of patients showed an overall improvement of their symptoms. This study although showing a positive trend , used a small number (32), of patients (Martinez, 1990).

Bacillinum (made from tinea corporis prepared homoeopathically) was used for tinea infections in a study involving 36 patients , 22 of whom noted varying degrees of improvement in itching. Improvement in lesions and itching was recorded in eight patients whilst a complete cure was observed in only three patients. These three patients on follow up showed a relapse. This study was unable to show any promising results that could impact on the "law of similars" (Bhardwaj, 1990).

Benveniste (1988) examined the inhibitory effects on achromasia by the homoeopathic medicine Apis Mellifica . Basophils were incubated with substantial concentrations of anti-IgE serum after treatment with either Apis Mellifica or NaCl. Some dilutions of Apis Mellifica inhibited basophil achromasia. Apis showed peaks of activity at 30CH and 34CH with weaker effects at 32CH and 40CH and no significant change at 36CH and 38CH. The NaCl dilutions produced no effect (Fischer, 1991). Homoeopathic Apis Mellifica, which is the honey bee prepared homoeopathically, is able to reverse the crude effects of a bee sting i.e, basophil degranulation and other features of an acute inflammation (Fischer, 1991).

Arsenicum album solutions were injected into a batch of pigeons and they excreted 31.3% of this dose within 10 days whilst their vestibular chronaxia (measurement of muscular electrical excitability) increased and remained so for 90 days. A second batch of pigeons were subjected to these same injections but on

the 45th day (when they no longer excreted Arsenicum album) received Homoeopathic Arsenicum Album 7CH. They excreted a further 12-17% of Arsenicum album and their vestibular chronaxia returned to normal. A similar trial using alloxane on mice was conducted. The alloxane produced experimental diabetes in the mice. Mice that were first exposed to homoeopathic alloxane and then exposed to conventional alloxane did not have diabetes (Gaier, 1991).

Another study , on the climbing activity of frogs, was carried out. The presence of thyroxine (T4) is essential for the spontaneous tendency of juvenile frogs to leave the water and climb on land. Thyroxine was prepared homoeopathically (dilution T4 -30x) and added to the basins in which the frogs were placed. Climbing activity was suppressed by the dilution T4 30x . There was a statistical significance both in the comparison control observations as well as in the potentised solvent (H2O .30x). This also demonstrated the law of similars i.e., thyroxine caused spontaneous climbing of frogs whilst homoeopathic thyroxine suppressed this climbing activity (Endler et.al , 1991).

From the above , various studies indicate that the "law of similars" does have an influence . No trial involving antibiotics and bacterial cultures has been done. This study evaluates the effects of an antibiotic when used conventionally and in homoeopathic potencies. The homoeopathic arsenicum album

, mentioned above , was able to afford "protection" against the toxic effects of the conventional arsenic. Is the homoeopathic antibiotic able to provide similar "protection" ?

2.3 THE LAW OF MINIMUM DOSE AND DYNAMISATIONS

When Hahnemann formulated the law of similars , he realised that the crude sustances he was using had undesirable side-effects. He therefore attenuated the dose by dilution. He termed this process of attenuation , the "law of minimum dose" : "Minima Minimus" (Singh, 1991). Hahnemann also claimed that the medicine was to be serially diluted and vigorously shaken , and this process releases " the medicinal properties , which are latent in natural substances while in their crude state , become aroused , and then enabled to act" (Hahnemann 1896).

2.4 THE PREPARATION OF HOMOEOPATHIC MEDICINES.

Homoeopathic medicines are prepared predominantly from plants , animals and minerals. Excretions , secretions etc. are also used. A specified amount is macerated and dissolved in alcohol (or triturated with lactose if it is insoluble). This is called the mother tincture. One part of the mother tincture is added to 99 parts (1C) or 9 parts (1D) of 90% alcohol and is then vigorously shaken (dynamisation). This process is repeated by taking one part 1C , or one part 1D which is added to 99 parts

or 9 parts respectively of 90% alcohol and is then dynamised resulting in 2C and 2D respectively. This process is repeated resulting in very high dilutions. Potencies = dilution + dynamisations. Potencies equal to and above 12C or 24D are unlikely to contain any of the molecules of the mother tincture since these dilutions are beyond Avogadro's constant. The higher potencies are considered to have a stronger effect than the lower potencies. (Kleijnen et.al. , 1991 ; Singh , 1991).

2.5 CO-TRIMOXAZOLE

Trimethoprim was synthesized in the Burroughs Wellcome Laboratories , New York . Initially in 1969 , it was used in combination with sulphamethoxazole which is now called co - trimoxazole. Trimethoprim was used alone in 1979 (Garrod et. al., 1981). Domagk (1932) a research director at the I.G. Farbenindustrie is credited with Prontosil and its active metabolite sulfanilamide . This gave rise to the synthesis of sulphamethoxazole and the other sulfonamides (Gilman et.al., 1991). Sulphamethoxazole was used with trimethoprim because it has a similar half life , although the distribution of the two drugs in the body differs i.e., trimethoprim is present in a higher concentration in tissues than in blood (Rogers et.al, 1981).

2.5.1 Synergy

Trimethoprim and sulphamethoxazole are potent antibacterial agents alone but when combined , they markedly potentiate each other. When either is used alone , it is bacterio-static , when it is used in combination it is bacterio-cidal. (Barker and Prescot 1973) The maximum potentiation occurs when the drugs are present in the ratio of their minimum inhibitory concentrations (MICs). If an organism is sensitive to 1 $ugml^{-1}$ trimethoprim and 20 $ugml^{-1}$ sulphonamide , it will show maximum inhibition when exposed to a 1:20 mixture . This applies to most organisms but with a few exceptions e.g., Neisseria species where more trimethoprim is required for optimum synergy (Garrod et.al., 1981). For a typical isolate of E. coli the MIC for sulphamethoxazole alone is 3 $ugml^{-1}$ while that for trimethoprim is 0.3 $ugml^{-1}$. When the combination is used in the ratio 20:1 the inhibitory concentrations are 1.0 $ugml^{-1}$ and 0.05 $ugml^{-1}$ respectively (Gilman et.al., 1991).

2.5.2. Absorption and Distribution

Trimethoprim is absorbed more rapidly than sulphamethoxazole. They both have a half life of approximately 10 - 11 hours. When 800 mg sulphamethoxazole is given with 160 mg trimethoprim (conventional 5:1 ratio) two times daily , the peak concentration of the drugs in plasma are approximately 40 and 2 $ugml^{-1}$, the optimum ratio (Gilman et.al., 1991).

The volume of distribution of trimethoprim is greater than that of the total body water , implying a concentration at one or more sites. On the other hand the distribution of sulphamethoxazole is only about 20 % of body water , a volume corresponding to the extracellular fluid. This difference in distribution accounts for the fact that the ratio of sulphamethoxazole to trimethoprim is 20:1 in plasma while that of the administered dose mixture is 5:1 . If large volumes reached by trimethoprim but not by sulphamethoxazole also applies to sites of infection , then this has serious significance to the validity of synergic interaction (Garrod et.al., 1981).

Reid et.al. (1975) cited by Garrod et.al. (1981) found distribution of trimethoprim in maternal , fetal serum and amniotic fluid to be similar while those of sulphamethoxazole were lower in fetal than in maternal serum and lower in amniotic fluid.

2.5.3. Excretion

Excretion occurs almost wholly via the urine . About 8 % of this is in a conjugated inactive form. 70 % of the drug is excreted in the first twenty four hours , and remains detectable in the urine for 4-5 days by which time 90% of the dose can be recovered (Garrod et.al., 1981).

2.5.4. Toxicity and Side-effects

Nausea , vomiting and skin rashes have occurred in some patients. Amongst almost 30 000 patients reviewed by Lawson and Jick (1978) [cited by Garrod et.al. (1981)] eight percent experienced adverse drug reactions. About 75 % of the side - effects involve the skin and are known to be produced by the sulphonamide component (Gilman et.al., 1981).

The most foreseeable toxic effect of trimethoprim is the induction of folate deficiency . The low affinity of the drug for the mammalian enzyme and the possibility of feeding folate supplements (which cannot be utilized by the parasite) removes the likelihood of serious haematological disorders. In a compromised patient , there is a possibility that co - trimoxazole may precipitate megaloblastosis , leukopenia and thrombocytopenia (Gilman et.al., 1991).

Thrombocytopenia and leucopenia which could be due to the sulphamethoxazole component has been reported . This is usually reversible (Evans and Tell, 1969). Chanarin and England (1972) described four patients with megaloblastic anaemia who responded poorly on haematinics while receiving co - trimoxazole.

High dose co - trimoxazole therapy used for the treatment of P. carinii pneumonia in HIV infected patients leads to an increase in serum potassium and could result in life threatening

hyperkalemia (Greenberg et.al., 1993). This finding is supported by another study using trimethoprim alone. The trimethoprim blocks apical membrane sodium channels in mammalian distal nephrons resulting in the transepithelial voltage to be reduced and therefore potassium secretion is inhibited (Vela'zquez et.al., 1993).

2.5.5. Clinical Use

Co-trimoxazole can be used against all common pathogenic bacteria except Pseudomonas and M. tuberculosis . It has been successful in the treatment of disease such as acne , gonorrhoea , typhoid fever , toxoplasmosis , Q fever , brucellosis (Baker and Prescot 1973) , endocarditis , enteric fever , cholera , generalized infections of childhood and Pneumocystis carinii . It has also been successful in the treatment of plague , meningitis (both components penetrate well into cerebro-spinal fluid) and for prophylaxis and treatment of immunodeficient and immunosuppressed patients (Garrod et.al., 1981). Co - trimoxazole is used widely in the treatment of urinary tract infections as well as respiratory disorders. Treatment of uncomplicated lower urinary tract infections is very successful.

It is also efficacious for recurrent urinary tract infections (Gilman et.al., 1991).

Prophylaxis with co - trimoxazole which is well tolerated , significantly reduces the incidence of bacterial infection following renal transplant , especially infection of the urinary tract and blood stream , and can provide protection against Pneumocystis carinii pneumonia and is cost beneficial (Fox et.al., 1990).

2.5.6. Mechanism of Action

The synthesis of folate is a metabolic pathway found in bacteria but not in humans. Folate is necessary for DNA synthesis both in man and bacteria. But man has the necessary transport mechanisms to assimilate preformed folate whilst bacteria need to synthesize their own folate. This difference makes chemotherapeutic intervention possible (Rang and Dale, 1991).

Sulphamethoxazole acts as a typical sulphonamide and inhibits the process whereby bacteria form dihydrofolate from para - amino benzoic acid (PABA). The bacteria erroneously incorporates the sulphamethoxazole instead of PABA and therefore cannot form the dihydrofolate. (Gilman et.al., 1991)

Dihydrofolate reductase , which reduces dihydrofolate to tetrahydrofolate , is many times more sensitive to the folate antagonist trimethoprim in bacteria than in man. Trimethoprim binds 10 000 - 100 000 times more effectively to bacterial dihydrofolate than it does to the human enzyme (Barker and

Prescot, 1973). Thus inhibition occurs at two points in the organism 's metabolism of folate and consequently on its ability to synthesize DNA hence affecting it's reproductive capability (Bowman and Rand, 1982).

2.5.7. Bacterial Resistance

The frequency of development of bacterial resistance to co - trimoxazole is lower than either drug when used alone. Trimethoprim resistance may arise from mutation. Resistance in gram negative bacteria is often associated with the acquisition of a plasmid that codes for an altered dihydrofolate reductase. The development of resistance to co-trimoxazole also occurs in vivo . Resistance of S. aureus increased from 0,4 % to 12.6 % during one five year period of use (Gilman et.al., 1991).

2.6 ESCHERICHIA COLI

The species E. coli belongs to the genus Escherichia. This genus together with other genera such as Salmonella , Shigella , Klebsiella , Serratia , Enterobacter , Proteus , Morganella , Yersinia and Providencia belong to the family Enterobacteriaceae (Wilson et.al., 1991).

2.6.1 History

E. coli. was seen and described by Buchoerin (Zinsser and Bayne - Jones, 1939). Escherich made the first steps in their differentiation in 1885 (Christie, 1980).

2.6.2 Morphology and Other Characteristics

E. coli is a gram-negative motile or non-motile rod of varying size . It is usually between 2u and 3u long and 0.6u broad. It is unicellular , nonsporing and nonphotosynthetic (Skerman, 1973 ; Christie, 1980).

Most strains are killed at 55 °C in one hour. Outside the body E. coli may survive weeks or months in a water medium or it may survive up to a month in the dust of shady areas. It is readily killed by dyes (Christie, 1980). E. coli like other Enterobacteriaceae , are aerobic , but are capable of growing under anaerobic conditions. All Enterobacteriaceae are characterised biochemically by their ability to ferment glucose , to reduce nitrates to nitrites and are oxidase-negative (Wilson et.al., 1991). Escherichia is able to ferment lactose , and this criterion distinguishes them from salmonellae and shigellae (Christie, 1980).

2.6.3 Habitat

E. coli is a commensal in the gastrointestinal tract of warm blooded animals. The optimal temperature for growth is 37 °C but growth is possible at a range of 10 °C to 40 °C. The optimal ph for growth is 7.0 - 7.5 with a minimum of ph 4.5 and a maximum of ph 8.5 (Skerman, 1973).

2.6.4 Antigenic Structure

E. coli possesses somatic (O) ; flagellar (H) and capsular (K) antigens. At least 140 O antigens and 40 H antigens have been defined for the E. coli. species. The K antigens are of 3 types , viz. L , A and B antigens. L antigens are inactivated by heating at 100 °C for one hour , and the A antigens at 121 °C for two hours. B antigens bind to the antibody after heating at 121 °C for two hours. At least 22 B , 26 A and 31 L antigens have been identified. Thus a strain labelled O111:K58(B4):H12 indicates that it's somatic O antigen was 111 ; it's K antigen 58 of B type , numbered 4 and it's H antigen 12. This indepth analysis of antigenic properties not only helps identify various strains but also illustrates the permutations of strains possible (Christie, 1980 ; Schaeter et.al., 1989 ; Wilson et.al., 1991)

2.6.5 Escherichia coli Infections

2.6.5.1 Aetiology

E. coli is a commensal inhabiting the gastrointestinal tract. It may spread from there to infect contiguous structures if normal anatomical barriers are disrupted e.g. , an appendix perforation. Urinary tract infections occur via urethral contamination but may be blood borne. If an infection occurs in a primary focus , spread by haematogenous means may ensue. With bacteraemia , there is a potential for septic shock to occur. In more than 50 % of E. coli. infections , the urinary tract is the portal of entry. Infections that stem from the lung , skin , peritoneal cavity and hepatobilliary tree are frequent . Other defects in host resistance due to Diabetes mellitus , cirrhosis , sickle cell anaemia , recent irradiation , cytotoxic drugs , glucocorticoids or antibiotics may predispose to infection (Bondy et.al., 1987 ; Wilson et.al., 1991).

2.6.5.2. Gastroenteritis

Some strains of E. coli are able to produce gastroenteritis. They accomplish this by a variety of pathogenic mechanisms. Depending on their mechanisms , they are presently classified into four major classes , viz. , Enterotoxigenic E. coli [ETEC] , Enteroinvasive E. coli [EIEC] , Enterohaemorrhagic E. coli [EHEC] and Enteropathogenic E. coli [EPEC]

(Christie, 1980 ; Bondy et.al., 1987 ; Schaeter et.al., 1989 ; Wilson et.al., 1991)

a) ETEC

They can produce one of two toxins. One is heat labile (LT) and the other is heat stable (ST) . This is a toxin mediated process where LT causes no pathological changes in the intestines. There may be some oedema of the lamina propria of the small intestine , dilatation of mucosal capillaries and flattening of the epithelium. However electron microscopy does not indicate any ultrastructure changes. It is not clear whether the heat stable enterotoxin binds to a receptor on the mucosal wall (Christie, 1980 ; Schaeter et.al., 1989 ; Wilson et.al., 1991).

Both toxins cause diarrhoea by the activation of adenylate cyclase in mucosal epithelial cells. An increase in adenylate cyclase acts as a catalyst to transform adenosine triphosphate (ATP) to cyclic monophosphate (CAMP). The increased CAMP brings about secretion of chloride and bicarbonate from the mucosa into the lumen of the bowel. This results in the net movement of water into the lumen resulting in diarrhoea (Christie, 1980).

b) EIEC

These enteroinvasive strains are taken up into the epithelial cells of the colon by a phagocytosis - like mechanism. The E. coli divides within the cytoplasm of the epithelial cells and produce intracellular colonies which spread to adjacent host cells. They produce a toxin which inhibits protein synthesis in host cells , which results in death of host cells. The sloughing of the dead cells produce a micro-ulcer. An inflammatory response ensues. The goblet cells discharge mucus. A typical dysentric stool with a small bloody viscous glob is passed. The inflammatory process disrupts normal peristalsis and produces severe cramps (Schaeter et.al, 1989).

c) EHEC

The enterohaemorrhagic E. coli cause haemorrhagic colitis but without invading the cells of the colon. They produce large amounts of toxin that damage the intestinal cells and cause necrosis and sloughing. In one to two thirds of patients , a bloody diarrhoea is present (Schaeter et.al., 1989 ; Wilson et.al., 1991).

d) EPEC

Enteropathogenic E. coli cause a watery diarrhoea especially in infants under two years old. The infection is localised in

the ileum instead of the colon. The gastroenteritis is typified by nausea , vomiting and watery diarrhoea. These strains have a special adherence to mucosal cells. The major problem in infants is dehydration (Christie, 1980 ; Bondy et.al., 1987 ; Wilson et.al., 1991).

2.6.5.3 Urinary Tract Infections (UTIs)

E. coli accounts for over 75 % of urinary tract infections (Schaeter et.al., 1989 ; Edwards and Bouchier, 1991 ; Wilson et.al., 1991). These include cystitis (Bowman and Rand, 1982) , pyelitis , pyelonephritis and asymptomatic bacteriuria (Johnson , 1990). The urinary tract is the site most commonly affected and is usually colonized from without (Bondy et.al. 1987).

a) Clinical presentation

The patient usually exhibits some of the following signs and symptoms. Frequency , dysuria , haematuria , incontinence , urinary retention is common. The patient may complain of right iliac fossa pain , loin pain or suprapubic pain (Hope et.al., 1993).

b) Treatment of UTIs

Many antimicrobials have been employed thus far. These include ampicillin , cephalosporins (for severe infections) , gentamicin

, tobramycin and co-trimoxazole (Wilson et.al., 1991) , trimethoprim or amoxycillin (Hope et.al., 1993). Several studies support the use of co -trimoxazole (as single dose regimens) for the treatment of cystitis (Carlson and Mulley , 1985 ; Fihn et.al., 1988 ; Johnson, 1990).

2.6.5.4 Peritoneal and Billiary Infections

E. coli infections may occur from a perforated or inflamed appendix or from abscesses secondary to perforated diverticula , peptic ulcers , subphrenic or lesser sac abscesses or from a mesenteric infarction. Acute cholecystitis with gangrene and perforation is often associated with E. coli (Wilson et.al., 1991).

2.6.5.5 Bacteraemia

The invasion of the bloodstream by E. coli causes sudden fever and chills but sometimes mental confusion , dyspnoea and unexplained hypotension may be the presenting symptoms (Wilson et.al., 1991).

2.7 STAPHYLOCOCCUS AUREUS

2.7.1 Classification

Staphylococcus aureus belongs to the genus Staphylococcus and the family Micrococcaceae (Wilson et.al., 1991).

2.7.2. Epidemiology

Staphylococcus is a ubiquitous pathogenic microorganism. They are normally present in the nares of about 30 % and on the skin of about 20% of healthy adults. Hospital personnel and patients have a slightly higher rate of carriage. A wide range of illnesses predispose to staphylococcus infection. Staphylococcus food poisoning due to the production of enterotoxins is common. The organisms are present in cutlery , hands etc. It is also commonly found in cow's milk especially if the cow has mastitis but this is normally counteracted by pasteurizing the milk S. aureus spread may also be vector (e.g. flies) or air borne (Christie, 1980 ; Bondy et.al., 1987 ; Wilson et.al, 1991).

2.7.3. Morphology and Identification

A typical S. aureus cell is spherical , about 1 um in diameter and are arranged in irregular clusters. In a liquid medium , single cocci , pairs , tetrads and chains may be observed. Young

cocci stain strongly gram positive whilst many aging cells may stain gram negative. S. aureus is a non - motile , nonsporing , aerobic or facultatively anaerobic , catalase positive cocci (Jawetz et.al, 1976 ; Wilson et.al., 1991).

2.7.3.1 Culture

S. aureus grows readily on most media. They grow rapidly at 37 °C but form pigments when they are incubated at 20 C (room temperature). They do not form pigments in broth or when they grow anaerobically.

2.7.3.2 Growth Characteristics

S. aureus is able to slowly ferment many carbohydrates , thus producing lactic acid but without gas production. S. aureus also ferments mannitol and produces Dnase (Wilson et.al., 1991) Staphylococci are relatively resistant to drying ; to heat ; to 9 % sodium chloride but are readily inhibited by hexachlorophene (3%). They will withstand 50 °C for 30 minutes (Jawetz et.al, 1976) and are killed in 30 minutes at a temperature of 60 °C (Christie, 1980). Many strains are penicillin resistant because they produce penicillinase (beta - lactamase) (Garrod et.al., 1981 ; Schaeter et.al., 1989). The incubation period for gastroenteritis in man is approximately 1-6 hours (Hope et.al., 1993).

2.7.4 Antigenic Structure

Staphylococci contain antigenic polysaccharides and proteins . This allows for the grouping of strains , though to a limited extent. Teichoic acid , a cell wall constituent can be antigenic. It is thought to be involved in complement activation by means of adherence to mucosal cells of the host. Murein in the cell wall activates compliment by the alternate pathway , thereby triggering an inflammatory response. S. aureus is sometimes surrounded by a capsule which prevents it from being phagocytized by neutrophils. A fourth wall component , protein A binds non - specifically to the Fc portion of immunoglobulin G (Ig G). This incapacitates IgG since the active portion Fab is now "dangling" away from the surface of the microorganism. Although the protein A and Fc complex is non -specific , it is able to act as an antigen-antibody complex in that it activates complement through its classical pathway (Jawetz et.al., 1976 ; Schaeter et.al., 1989 ; Wilson et.al., 1991).

2.7.5 Toxins and Enzymes

S. aureus is capable of producing many extracellular substances. These substances mediate in the inflammatory process and in the production of disease.

2.7.5.1 Exotoxins : cause necrosis of the skin . It also contains several haemolysins which can damage platlets ,

erythrocytes and vascular smooth muscle (Jawetz et.al., 1976).

2.7.5.2 Leukocidin : makes pores in the membrane of neutrophils resulting in their destruction . It is heat labile and antigenic. S. aureus is also capable of being phagocytized and then multiply intracellularly. Antibodies to leukocidin may play a role when recurrent staphylococcal infections occur (Jawetz et.al., 1976 ; Schaeter et.al., 1989).

2.7.5.3 Enterotoxins : Enterotoxin is a soluble material when in the presence of high concentrations of carbon dioxide. It has four antigenic types [A - D]. Ingestion of 25 ug enterotoxin B results in diarrhoea and vomiting. Enterotoxin is resistant against intestinal enzymes as well as boiling for 30 minutes (Jawetz et.al., 1976).

2.7.5.4 Coagulase : converts fibrinogen to fibrin. This may prevent S. aureus from getting phagocytised since white blood cells penetrate fibrin poorly (Jawetz et.al., 1976 ; Wilson et.al., 1991).

2.7.5.5 Other substances : Several haemolysins are produced which may avail iron for the organism. Some S. aureus strains make hyaluronidase which hydrolyses the matrix of connective tissue and this probably facilitates the spread of infection. B -lactimase hydrolyses the classical penicillins. It also produces staphylokinase , proteinases and an exfoliative toxin

that produces the "scalded skin syndrome" (Jawetz et.al., 1976 ; Schaeter et.al., 1989 ; Wilson et.al., 1991).

2.7.6. Staphylococcus aureus Infections

The common presentations of S. aureus infections include , furuncles , carbuncles , abscesses , pneumonia , bacteraemia , endocarditis , osteomyelitis , enterocolitis and gastroenteritis (Bondy et. al., 1987 ; Wilson et.al., 1991).

Furuncles or boils occur when there is a sebaceous gland infection with some involvement of subcutaneous tissue. Pruritis , erythema and local oedema may follow. Carbuncles are infectious within thick , fibrous , inelastic skin of the back and posterior neck. The lesion progresses laterally , producing fever , leukocytosis and extreme pain. Bacteraemia is common (Wilson et.al. 1991).

Staphylococcal pneumonia may develop in patients with influenza and who develop dyspnoea , cyanosis or persistent or recurrent fever. It may also occur in patients hospitalised for broncho-pulmonary disease or other risk disease. Osteomyelitis is common in children and cause chills , fever and pain over the involved bone. Enterocolitis occurs in patients especially post - operatively (Wilson et.al., 1991).

The staphylococcal scalded skin syndrome (SSSS) is an acute widespread erythemous process in which an epidermolytic toxin (produced by S.aureus) splits the upper part of the epidermis , just below the granular cell layer (Bondy et.al., 1987).

Bacteraemia due to S. aureus may arise from any local infection , either extravascular (e.g. cutaneous infections , burns , osteomyelitis) or intravascular (e.g. intravenous catherters and intravenous drug abuse). A complication of bacteraemia is usually endocarditis. There is valvular destruction or insufficiency (Wilson et.al. 1991).

2.7.7. Resistance to Antibiotics

Certain strains of S. aureus have developed resistance to various antibiotics. Streptomycin , tetracycline and penicillin resistance have been common. Similar resistance to chloramphenicol and erythromycin has been observed. Some strains have exhibited multiple drug resistance. Approximately 5% of S. aureus strains are methicillin resistant S. aureus (MRSA). In the United Kingdom , MSRA incidence rose from 0,1 % in 1960 to 4-5 % in 1969. In 1970 , Denmark had a 40 % incidence (Jawetz et.al., 1976 ; Garrod et.al., 1980). MRSA usually responds to treatment with vancomycin (Wilson et.al 1991).

2.8 SUMMARY

The law of similars states that a substance which is capable of producing symptoms in a healthy individual , when administered to a sick person with these same symptoms , will bring about a cure. This law is the conerstone of the practice of homoeopathy and therefore , requires rigorous testing by scientific means.

Several experiments using copper sulphate , arsenicum album , alloxane etc., have been performed which demonstrated the law of similars.

E. coli is a common pathogen affecting primarily the urinary tract. Certain strains are able to induce diarrhoea viz. EPEC , ETEC , EIEC and EHEC. They also cause infections of the lungs , skin , peritoneal cavity and hepatobilliary tree.

S. aureus is an ubiquitous organism. It causes infections of the skin , gastroenteritis , osteomyelitis , pneumonia , endocarditis , abscesses , bacteraemia and impetigo. It contains many toxins and enzymes which cause lysis or prevent it's own destruction. It produces beta lactamase and is therefore resistant to penicillin. It is resistant to many other antibiotics.

Co-trimoxazole is a potent antimicrobial agent and is effective against many gram positive and negative organisms. It contains

two components viz., trimethoprim and sulphamethoxazole which act synergistically. Sulphamethoxazole is erroneously taken up by bacterial cells and this prevents the formation of dihydrofolic acid. Trimethoprim inhibits dihydrofolate reductase which prevents the reduction of dihyrofolic acid to folinic acid. Co-trimoxazole , is thus an effective antimicrobial agent.

Therefore, the objective of this study was to evaluate the efficacy of co-trimoxazole when used in homoeopathic form. This was an in-vitro study , on a unicellular level. Bacterial cultures were exposed to co-trimoxazole alone , secondly to co-trimoxazole combined with a homoeopathic preparation of co-trimoxazole and thirdly to a homoeopathic preparation of co-trimoxazole used alone. The growth rates and metabolic activity of the cells were monitored and a comparative evaluation conducted.

CHAPTER THREE

3.0 **METHODOLOGY**

3.1 **THE DATA , TREATMENT AND THEIR INTERPRETATION**

3.1.1 THE DATA

This research involves two types of data : primary and secondary. The nature of these data follows :

3.1.1.1 THE PRIMARY DATA

1. Results of the experiment determining the effects of conventional co-trimoxazole on the growth of E. coli.
2. Results of the experiment determining the effects of conventional co-trimoxazole on the growth of S. aureus.
3. Results of the experiments determining the effects of co-trimoxazole in homoeopathic potencies on the growth of E. coli.
4. Results of the experiments determining the effects of co-trimoxazole in homoeopathic potencies on the growth of S. aureus.
5. Results of the experiments determining the effects of conventional co-trimoxazole and the homoeopathic potencies

on the growth of E. coli.

6. Results of the experiments determining the effects of conventional co-trimoxazole and the homoeopathic form on the growth of S. aureus.
5. Results from API ZYM analyses

3.1.1.2 THE SECONDARY DATA

Research articles from journal publications , books and manuals were referred to.

3.2 CRITERIA GOVERNING THE ADMISSIBILITY OF DATA

Only data obtained from laboratory experiments carried out by the researcher at the Department of Biotechnology , Technikon Natal will be used.

3.3 MATERIALS AND METHODS

3.3.1 Preparation of Media

Nutrient Agar No.2 and Nutrient Broth were the media of choice. They were prepared as directed , by the manufacturer's specifications. (Appendix A)

3.3.2 Acquisition and Verification of E. coli

A single strain of E. coli , strain ATCC 25922 was obtained from the Department of Microbiology , Natal University - Medical School. The strain was maintained on Nutrient Agar. The E. coli was tested for purity. This included a Gram stain , streak plates on Nutrient Agar , EMB plate , as well as API 20E analysis was performed. (Appendix B)

3.3.3 Acquisition and Verification of S. aureus

A single strain of S. aureus , strain ATCC 25923 was obtained from the Department of Microbiology , Natal University - Medical School. The strain was maintained on Nutrient Agar. The S. aureus was tested for purity. This included a Gram stain , streak plates on Nutrient Agar , Baird Parker plate , as well as API Staph analysis was performed. (Appendix C)

3.3.4 Preparation of Inoculum

A single colony of E. coli from a Nutrient Agar plate was introduced into a universal bottle containing Nutrient Broth which was previously autoclaved at 121 °C for 15 minutes. This culture was incubated for 24 hours and constituted the inoculum.

S. aureus was also prepared similarly to the E. coli inoculum described above.

3.3.5 Preparation of Co-trimoxazole

The two components , sulphamethoxazole (Batch No. A04812) and trimethoprim (Batch No. A04836) were obtained from Wellcome Ltd.

The homoeopathic potencies of co-trimoxazole were prepared by a qualified pharmacist and homoeopath . The conventional co-trimoxazole was prepared aseptically by the researcher such that each millilitre contained 1000 $ugml^{-1}$ sulphamethoxazole and 50 $ugml^{-1}$ trimethoprim.

3.3.6 Effect of Co-trimoxazole (in it's various preparations) on E. coli

Nutrient broth was prepared and dispensed , in 100ml aliquots , into six conical flasks (250ml volume). Flasks were capped and autoclaved at 121 °C for 15 minutes. The flasks were labelled 1 to 6. A laboratory technician dispensed 1ml aliquots of E. coli inoculum into each flask , under aseptic conditions. She was given 5 x 4ml aliquots of distilled water , 3 x 4ml aliquots conventional antibiotic , 2 x 4ml aliquots 9CH homoeopathic co-

trimoxazole and 2 x 4ml aliquots 15CH homoeopathic co-trimoxazole. These were added randomly such that the flasks contained one of the following mixtures.

a) 8ml distilled water - this served as the control
b) 4ml distilled water and 4ml of the previously prepared conventional co-trimoxazole.
c) 4ml conventional co-trimoxazole and 4ml 9CH homoeopathic co-trimoxazole.
d) 4ml distilled water and 4ml 9CH homoeopathic co-trimoxazole
e) 4ml conventional antibiotic and 4ml 15CH homoeopathic co-trimoxazole.
f) 4ml distilled water and 4ml 15CH homoeopathic co-trimoxazole

Addition of 4ml of conventional co-trimoxazole to 100ml of Nutrient Broth and 4ml distilled water (or 4ml homoeopathic potency of co-trimoxazole) resulted in a final concentration of 37.04 $ugml^{-1}$ sulphamethoxazole and 1.85 $ugml^{-1}$ trimethoprim.

Homoeopathic 9CH was prepared by aseptically adding 2 drops of 8CH (homoeopathically prepared co-trimoxazole) to 4ml distilled water and succussed 100 times resulting in 4ml 9CH. Similarly 2 drops 14CH was used to prepare 4ml 15CH. These preparations were made 15 minutes prior to the

inoculation of flasks.

Samples were removed immediately from the six flasks (T0) , and introduced into test-tubes which were labelled. Test-tubes contained 0.85% w/v saline. All flasks were then incubated on an orbital shaker at 136 rpm and 37 °C. Further samples were taken at 6 , 12 , 24 and 48 hours after inoculation (T0 , T6 , T12 , T24 and T48).

The number of viable cells were enumerated in all samples using the serial-dilution-spread-plating procedure (Atlas, 1984). Samples at each time interval were serially diluted to 10^{-8}. Aliquots of 0.1 ml were removed in triplicate from each dilution and transferred to the centre of a 90mm Nutrient Agar plate. An L-shaped glass rod was dipped in 99% alcohol and then flamed. The glass rod was then cooled by placing on agar and the 0.1 ml suspension was then spread over the plate and a dilution gradient was created so that individual cells are separated. Plates were then incubated for 24 hours at 37 C. Colonies were enumerated on plates containing 30 - 300 colony forming units. The identity of flasks were revealed once the cell counts (Appendix D) were tabulated.

3.3.7 Effect of Co-trimoxazole (in it's various preparations) on S. aureus

The procedure is the same as 3.5. above with the following exceptions :

a) S.aureus was used instead of E. coli

b) 9ml aliquots of distilled water , conventional co-trimoxazole , 9CH homoeopathic co-trimoxazole and 15CH homoeopathic co-trimoxazole instead of 4ml aliquots were used. The final concentration of conventional co-trimoxazole was 76.27 $ugml^{-1}$ sulphamethoxazole and 3.81 $ugml^{-1}$ trimethoprim. This is in keeping with the 20:1 ratio found in-vivo.

3.3.8 Effect of Co-trimoxazole (in it's various preparations) on Metabolic Activity of E.coli

API ZYM (Appendix E) which constitutes 19 enzymes was used to detect enzyme activity as an indicator of metabolic function. Samples from the six flasks were taken at T0 , T12 , T24 and T48. Two drops (using a Pasteur pipette) of respective sample was introduced into each cupule on the enzyme strip. The enzyme strips were placed in moist trays and incubated for 4 hours. Thereafter one drop of Zym A and one drop Zym B were added. The strip was exposed to a bright light and the colour changes on the enzyme strip were give a numerical rating as provided by the

manufacturer of API ZYM. The ratings were recorded on data sheets provided (Appendix E).

3.3.9 Effect of Co-trimoxazole (in it's various preparations) on Metabolic Activity of S. aureus.

The same procedure as 3.3.7 above was used for S. aureus

3.4 THE SPECIFIC TREATMENT OF EACH SUBPROBLEM

3.4.1 **Subproblem One**

The first subproblem was to evaluate the effects of co-trimoxazole on E. coli and S. aureus in terms of maximum specific growth rate , maximum cell population , time taken to reach maximum specific growth rate and metabolic activity in order to confirm that co-trimoxazole does influence cell growth.

Data Needed

a. maximum specific growth rate
b. maximum cell population
c. time taken to reach maximum specific growth rate
d. metabolic activity
e. growth rate

Location of Data

The experiments were conducted at the Department of Biotechnology , Technikon Natal.

Means of obtaining the data

All data were collected by tabulating cell counts on agar plates (Appendix D - Tables 5 and 6) as well as API ZYM analysis (Tables 1 and 3).

Treatment and interpretation of data

The growth rate and the maximum specific growth rate were calculated by using the formulae below.

$$\text{GROWTH RATE} = \frac{CD2 - CD1}{T2 - T1}$$

$$\text{SPECIFIC GROWTH RATE} = \frac{\text{GROWTH RATE}}{1/2\ (CD1 + CD2)}$$

CD2 = The cell density at T2

CD1 = The cell density at T1

T1 = Time one

T2 = Time two

The Maximum Specific growth rate and the time taken to reach it can be obtained once the growth rates and specific growth rates have been calculated. The maximum cell population is the maximum number of colony forming units reached for that particular medication. The metabolic activity was semi- quantitatively assessed , and Tabulated (Tables 1 and 3).

3.4.2 **Subproblem Two**

The second subproblem was to evaluate the effects of co-trimoxazole in homoeopathic form on E. coli and S. aureus in terms of maximum specific growth rate , maximum cell population , time taken to reach maximum specific growth rate and metabolic activity in order to observe the influence they have on cell growth.

Data Needed

a. maximum specific growth rate

b. maximum cell population

c. time taken to reach maximum specific growth rate

d. metabolic activity

e. growth rate

Location of Data

The experiments were conducted at Department of Biotechnology , Technikon Natal.

Means of obtaining the data

All cell counts as obtained from colony forming units on petri dishes were be tabulated (Appendix D - Tables 7 and 8). All API ZYM results were tabulated (Tables 1 and 3).

Treatment of and Interpretation of the Data

The Growth Rate , the MSGR , the time taken to reach MSGR and maximum cell population were calculated as in subproblem one. Results were compared with the control and between themselves to establish if any significant difference exists. The API ZYM results were semi - quantitatively assessed for both E. coli and S. aureus.

3.4.3 **Subproblem Three**

The third subproblem was to evaluate the effects of a complex containing both conventional co-trimoxazole and the homoeopathic form on E.coli and S.aureus in terms of maximum specific growth

rate , maximum cell population , time taken to reach maximum specific growth rate and metabolic activity in order to observe the influence they have on cell growth.

Data needed

a. maximum specific growth rate

b. maximum cell population

c. time taken to reach maximum specific growth rate

d. metabolic activity

e. growth rate

Location of the data

The data was be obtained from the experiments carried out at the Department of Biotechnology , Technikon Natal

The means of obtaining the data

All cell counts as obtained from colony forming units on petri dishes were tabulated (Appendix D - Tables 9 and 10). All API ZYM results were tabulated (Tables 1 and 3).

The Treatment and Interpretation of the Data

The growth rate , MSGR , maximum cell population and the time taken to reach MSGR were calculated as in subproblem one. The results will be compared with the control and between themselves to establish if any significant difference exists.

The API ZYM results were semi-quantitatively assessed for both E.coli and S. aureus.

3.4.4 **Subproblem Four**

The fourth subproblem was to integrate the findings of subproblems one , two and three , to identify to what extent the conventional co-trimoxazole , the homoeopathic form of co-trimoxazole , and the conventional plus homoeopathic form of co-trimoxazole influenced growth rates and metabolic activity for the purpose of evaluating the efficacy of co-trimoxazole in homoeopathic form.

Data needed

a. growth rate

b. maximum specific growth rate

c. metabolic activity

Location of the data

Results from subproblems one , two and three were used. These were tabulated (Tables 2 and 4). The log of cfu ml^{-1} for various preparations was plotted against time for E. coli (Fig. 1) and S. aureus (Fig. 2). The API ZYM results were semi-quantitatively assessed (Tables 1 and 3)

CHAPTER FOUR

4.0 **RESULTS**

4.1 **Effect of co-trimoxazole on growth of E. coli**

The antibiotic , co-trimoxazole , at a concentration of 37.04 $ugml^{-1}$ sulphamethoxazole and 1.85 $ugml^{-1}$ trimethoprim inhibited growth of E. coli. This inhibition was most pronounced after six hours of growth (Fig. 1). The antibiotic administered homoeopathically at potencies of 9CH and 15CH resulted in little if any effect on E. coli growth (Fig. 1). The effect of the mixture of conventional co-trimoxazole and homoeopathic 9CH was similar to the effect of the conventional antibiotic , with the exception at 48 hours where the mixture resulted in a higher inhibitory effect (Fig. 1). The effect of the mixture of conventional co-trimoxazole and homoeopathic 15CH was also similar to the effect of the conventional antibiotic , with the exception at 24 hours where the mixture failed to retard growth (Fig. 1)

4.2 **Effect of co-trimoxazole on MSGR and time taken to reach MSGR of E. coli**

Co-trimoxazole at a concentration of 37.04 $ugml^{-1}$ sulphamethoxazole and 1.85 $ugml^{-1}$ trimethoprim resulted in the

lowest MSGR and the time taken to reach MSGR was 12 hours (Table 1) . The MSGR of the antibiotic , when administered in homoeopathic potencies of 9CH and 15CH were similar to that of the control. The time taken to reach the MSGR was 6 hours in all three instances (Table 1) . The MSGR for the mixture of conventional co-trimoxazole and homoeopathic 9CH was similar to that of the conventional antibiotic. The mixture containing 15CH was also similar to the MSGR of the conventional antibiotic. In both cases the time taken to reach MSGR was the same as the conventional antibiotic i.e., 12 hours (Table 1) .

4.3 **Effect of co-trimoxazole on maximum cell population of E. coli**

E. coli in the absence of any medication reached a maximum population at T48. E. coli when exposed to conventional co-trimoxazole reached a maximum population at T24. The homoeopathic potencies 9CH and 15CH were similar to the control in that they reached their maximum cell population at T48 and they also showed similar cell counts to the control (Table 1). The mixture containing conventional co-trimoxazole and homoeopathic 9CH reached a maximum population similar to that of the conventional co-trimoxazole at T24. The mixture containing conventional co-trimoxazole and homoeopathic 15CH showed the highest maximum cell population at time T24 (Table 1).

4.4 **Effect of co-trimoxazole on metabolic activity of E. coli**

Semi-quantitative assessment of enzyme activity was used as an indication of metabolic activity. E. coli both in the presence and absence of co-trimoxazole only showed activity of the following enzymes ; 2-naphtyl-phosphate ; L-leucyl-2-naphtylamide ; n-benzoyl-DL-arginine-2-naphtylamide ; 2-naphtyl-phosphate ; 2-naphtyl-Bd-galactopyranoside and 2-naphtyl-Ad-glucopyranoside. The activity of 2-naphtyl-phosphate and 2-naphtyl-butyrate were present only in the inoculum. The enzyme activity of conventional co-trimoxazole and that of the mixtures containing 9CH and 15CH respectively were similar, with the exception that the mixture containing conventional co-trimoxazole and homoeopathic 15CH indicated a higher enzyme activity of 2-naphtyl-phosphate at T24. The antibiotic when administered in homoeopathic potencies of 9CH and 15CH displayed enzyme activities similar to that of the control (Table 2).

4.5 **Effect of co-trimoxazole on growth of S. aureus**

The antibiotic , co-trimoxazole at a concentration of 76.27 $ugml^{-1}$ sulphamethoxazole and 3.81 $ugml^{-1}$ trimethoprim inhibited growth of S. aureus. This inhibition was marked after 6 hours and 48 hours (Fig. 2). The antibiotic administered homoeopathically at potencies of 9CH and 15CH result in little if any effect on S. aureus growth (Fig. 2).

The effect of the mixture of conventional co-trimoxazole and homoeopathic 9CH was similar to the effect of the conventional antibiotic , with the exception at 48 hours where the mixture failed to retard growth (Fig. 2). The effect of the mixture of conventional co-trimoxazole and homoeopathic 15CH was also similar to the effect of the conventional antibiotic and also , with the exception at 48 hours where the mixture failed to retard growth (fig. 2).

4.6 **Effect of co-trimoxazole on MSGR and time taken to reach MSGR of S. aureus**

The antibiotic , co-trimoxazole at a concentration of 76.27 $ugml^{-1}$ sulphamethoxazole and 3.81 $ugml^{-1}$ resulted in the lowest MSGR and the time taken to reach MSGR was 6 hours (Table 3) . The MSGR of the antibiotic when administered in homoeopathic potencies of 9CH and 15CH were similar to the MSGR of the control. The time taken to reach MSGR in all three instances was 6 hours (Table 3) . The MSGR for the mixture of conventional co-trimoxazole and homoeopathic 9CH was similar to that of the conventional antibiotic. The mixture containing the 15CH potency was also similar to the conventional antibiotic. In both cases the time taken to reach MSGR was 6 hours (Table 3).

4.7. **Effect of co-trimoxazole on maximum cell population of S. aureus**

S. aureus in the absence of any medication reached a maximum cell population at T48. S. aureus when exposed to conventional co-trimoxazole alone , reached a maximum population at T12. The homoeopathic potencies 9CH and 15CH were similar to the control in that they also reached maximum cell population at T48. The mixture containing conventional co-trimoxazole and 9CH homoeopathic co-trimoxazole reached maximum population at T48. The mixture containing conventional co-trimoxazole and 15CH homoeopathic co-trimoxazole reached maximum cell population at T24 (Table 3).

4.8 **Effect of co-trimoxazole on metabolic activity of S. aureus**

Semi-quantitative assessment of enzyme activity was used as an indication of metabolic activity. S. aureus both in the presence and absence of co-trimoxazole only showed activity of the following enzymes ; 2-naphtyl-phosphate ; 2-naphtyl-butyrate ; 2-naphtyl-caprylate ; 2-naphtyl-phosphate ; Naphtol-AS-BI-phosphate and 2-naphtyl-Ad-glucopyranoside. Enzyme activity of the various preparations were similar with exception that the homoeopathic 15CH potency and the mixture of conventional antibiotic and homoeopathic 15CH showed higher activity of the enzyme 2-naphtyl-phosphate (Table 4).

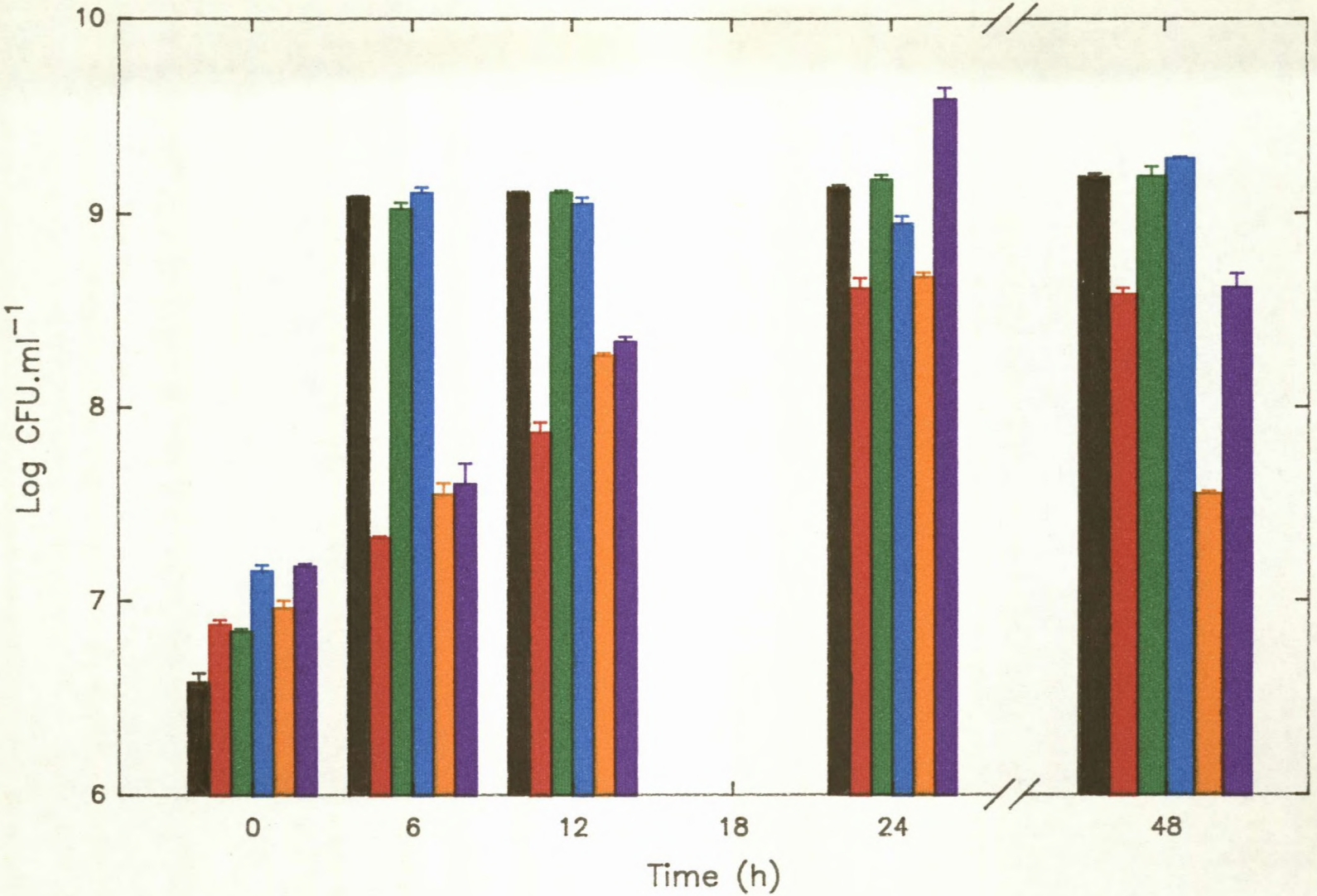
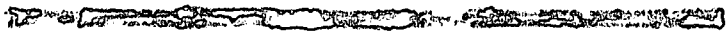


Fig. 1. Effect of Co-trimoxazole (37.04ug.ml^{-1} sulphamethoxazole and 1.85ug.ml^{-1} trimethoprim), in conventional preparation ( ), and Co-trimoxazole in homoeopathic potencies of 9CH ( ) and 15CH ( ) and mixtures of conventional Co-trimoxazole and homoeopathic potencies of 9CH ( ) and 15CH ( ) on the growth of Escherichia coli. Control ( ) denotes growth in the absence of any medication.



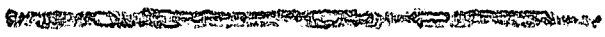


Table 1 : The MSGR , percentage difference to the control , time taken to reach MSGR, maximum cell population and time taken to reach maximum cell population of E.coli when exposed to various preparations of co-trimoxazole.

	M.S.G.R.	% DIFF. OF M.S.G.R.s TO CONTROL	TIME TAKEN TO REACH M.S.G.R. (HOURS)	LOG OF MAX. CELL POPULATION	TIME TAKEN TO REACH MAX. POP. (HOURS)
CONTROL	0.0533		6	9.1855	48
CO-TRIMOXAZOLE	0.0119	77.57	12	8.6177	24
9CH	0.0458	13.95	6	9.1913	48
15CH	0.0400	24.89	6	9.2833	48
CO-TRIM + 9CH	0.0151	71.73	12	8.6748	24
CO-TRIM + 15CH	0.0153	71.21	12	9.5885	24

Table 2 : Semiquantitive determination of 19 enzymes on *E. coli* in the presence of co-trimoxazole in a variety of preparations and in the absence of co-trimoxazole.

ENZYMES	TIME	2-naphtyl - phosphate	2-naphtyl - butyrate	2-naphtyl - caprylate	L - leucyl - 2 - naphtylamide	N - benzoyl DL-arginine-2-naphtylamide	2-naphtyl - phosphate	2-naphtyl - bD - galactopyranoside	2-naphtyl - aD-glucopyranoside	2 - naphtyl - myristate	L - valyl - 2 - naphtylamide	Naphtol-AS-BI-phosphate	L - cystyl - 2 - naphtylamide	N-glutaryl-phenylalanine-2-naphtylamide	6-Br-2-naphtyl- D-galactopyranoside	Naphtol-AS-BI- D-glucuronide	6-Br-2-naphtyl- bD -glucopyranoside	1-naphtyl-N-acetyl- D-glucosaminide	6-Br-2-naphtyl- D-mannopyranoside	2-naphtyl- aL-fucopyranoside
INOCULUM	0	3	1	1	0	0	5	0	1	0	0	0	0	0	0	0	0	0	0	0
CONTROL	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	12	3	0	0	2	0	5	3	1	0	0	0	0	0	0	0	0	0	0	0
	24	4	0	0	5	0	5	4	0	0	0	0	0	0	0	0	0	0	0	0
	48	2	0	0	3	4	5	3	0	0	0	0	0	0	0	0	0	0	0	0
CO-TRIMOXAZOLE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	12	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
	24	1	0	0	1	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0
	48	1	0	0	1	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0
9CH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	12	4	0	0	1	0	5	4	1	0	0	0	0	0	0	0	0	0	0	0
	24	5	0	0	4	0	4	4	0	0	0	0	0	0	0	0	0	0	0	0
	48	3	0	0	3	0	4	3	0	0	0	0	0	0	0	0	0	0	0	0
15CH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	12	1	0	0	3	0	5	3	1	0	0	0	0	0	0	0	0	0	0	0
	24	2	0	0	1	0	4	3	1	0	0	0	0	0	0	0	0	0	0	0
	48	2	0	0	2	0	5	3	0	0	0	0	0	0	0	0	0	0	0	0
CO-TRIMOXAZOLE + 9CH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	12	1	0	0	1	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0
	24	1	0	0	1	0	2	2	0	0	0	0	0	0	0	0	0	0	0	0
	48	1	0	0	2	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0
CO-TRIMOXAZOLE + 15CH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	12	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
	24	1	0	0	1	0	4	2	0	0	0	0	0	0	0	0	0	0	0	0
	48	1	0	0	1	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0

0 : 0 nanomoles of enzyme
1 : 5 nanomoles of enzyme
2 : 10 nanomoles of enzyme
3 : 20 nanomoles of enzyme
4 : 30 nanomoles of enzyme
5 : > 40 nanomoles of enzyme

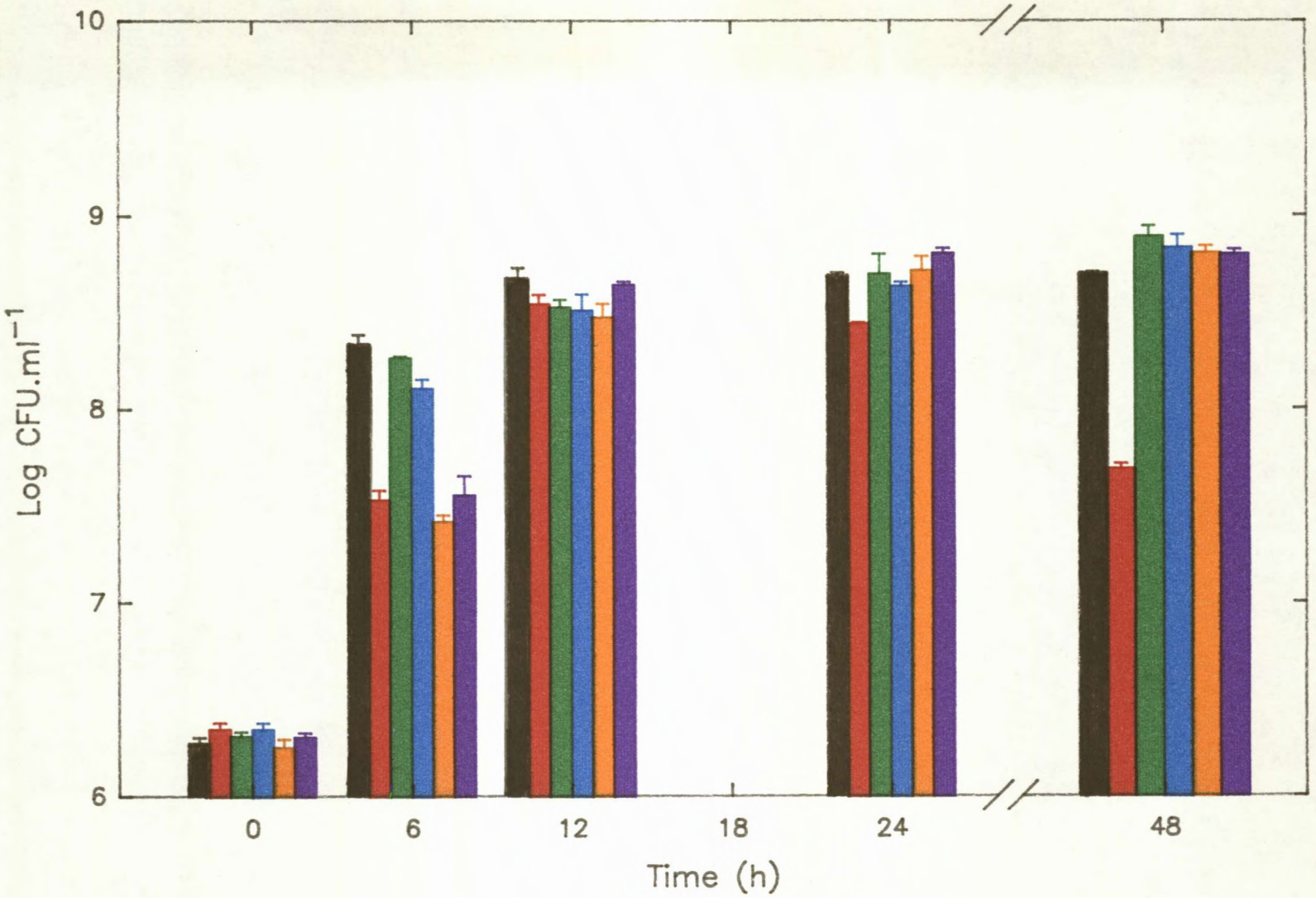
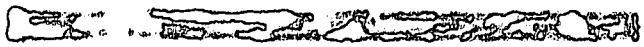
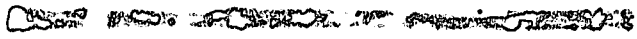
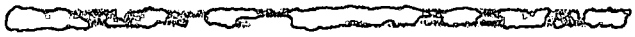


Fig. 2. Effect of Co–trimoxazole (37.04ug.ml^{-1} sulphamethoxazole and 1.85ug.ml^{-1} trimethoprim), in conventional preparation ( ), and Co–trimoxazole in homoeopathic potencies of 9CH ( ) and 15CH ( ) and mixtures of conventional Co–trimoxazole and homoeopathic potencies of 9CH ( ) and 15CH ( ) on the growth of Staphylococcus aureus. Control ( ) denotes growth in the absence of any medication.







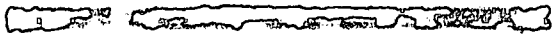


Table 3 : The MSGR , percentage difference to the control , time taken to reach MSGR, maximum cell population and time taken to reach maximum cell population of S. aureus when exposed to various preparations of co-trimoxazole.

	M.S.G.R.	% DIFF. OF M.S.G.R.s TO CONTROL	TIME TAKEN TO REACH M.S.G.R. (HOURS)	LOG OF MAX. CELL POPULATION	TIME TAKEN TO REACH MAX. POP. (HOURS)
CONTROL	0.048		6	8.705	48
CO-TRIMOXAZOLE	0.028	39.61	6	8.546	12
9CH	0.045	5.08	6	8.894	48
15CH	0.041	13.63	6	8.837	48
CO-TRIM + 9CH	0.029	37.84	6	8.810	24
CO-TRIM + 15CH	0.030	36.19	6	8.808	48

Table 4 : Semiquantitive determination of 19 enzymes on S. aureus in the presence of co-trimoxazole in a variety of preparations and in the absence of co-trimoxazole.

ENZYMES	TIME	2-naphtyl - phosphate	2-naphtyl - butyrate	2-naphtyl - caprylate	2-naphtyl - phosphate	Naphtol - AS - BI - phosphate	2-naphtyl - D - glucopyranoside	2-naphtyl - myristate	L - leucyl - 2 - naphtylamide	L - valyl - 2 - naphtylamide	L - cystyl - 2 - naphtylamide	N-benzoyl-DL-arginine-2-naphtylamide	N-glutaryl-phenylalanine-2-naphtylamide	6-Br-2-naphtyl- D-galactopyranoside	2-naphtyl- D-galactopyranoside	Naphtol-AS-BI- D-glucuronide	6-Br-2-naphtyl- D-glucopyranoside	1-naphtyl-N-acetyl- D-glucosaminide	6-Br-2-naphtyl- D-mannopyranoside	2-naphtyl- L-fucopyranoside
INOCULUM	0	3	1	1	5	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
CONTROL	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	12	5	1	1	3	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	24	3	1	2	4	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	48	2	1	1	4	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
CO-TRIMOXAZOLE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	12	4	1	1	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	24	3	1	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	48	3	1	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9CH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	12	4	1	1	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	24	3	1	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	48	3	1	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15CH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	12	4	1	1	5	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	24	3	1	2	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	48	3	1	2	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CO-TRIMOXAZOLE + 9CH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	12	2	1	1	3	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	24	2	1	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	48	2	1	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CO-TRIMOXAZOLE + 15CH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	12	4	1	2	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	24	3	1	2	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	48	4	1	2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

0 : 0 nanomoles of enzyme
1 : 5 nanomoles of enzyme
2 : 10 nanomoles of enzyme
3 : 20 nanomoles of enzyme
4 : 30 nanomoles of enzyme
5 : > 40 nanomoles of enzyme

CHAPTER FIVE

5.0 **DISCUSSION**

5.1 SUBPROBLEM ONE

E. coli in the presence of conventional co-trimoxazole resulted in both the lowest MSGR and maximum cell population (Table 1). This confirms that co-trimoxazole does retard cell growth. This is further supported by the API ZYM test activity (Table 2) which indicated the least enzyme activity displayed by the culture containing only conventional co-trimoxazole.

S. aureus when exposed to co-trimoxazole also showed the same trend (Tables 3 and 4) as E. coli above. However , co-trimoxazole was less effect as an antibiotic against S. aureus. The percentage difference of the MSGR between the control and the conventional co-trimoxazole for E. coli was 78 % but only 40 % for S. aureus. S. aureus culture took 6 hours to reach MSGR when compared to E. coli which took 12 hours (Tables 1 and 3). In both instances the antibiotic had optimal effect at the end of 6 hours. The conventional co-trimoxazole affected cell growth as hypothesised.

5.2 SUBPROBLEM TWO

The homoeopathic form of co-trimoxazole in both 9CH and 15CH potencies showed a difference in growth rates to the control. This occurred for both E. coli and S. aureus cultures (Table 1 and 3).

Whilst 9CH still contains some of the original substance (co-trimoxazole) , it is very dilute and is unable to act as a competitive antagonist in sufficient quantity to effect the formation of dihydrofolate from PABA. There is thus a minimal effect (14 % difference to MSGR of control - Table 1) of 9CH on E. coli.

The 15CH according to Avogadro's constant will contain none of the original substance but showed a 25 % difference in the MSGR (Table 1) when compared to the control in this experiment. The same trend was also observed for S. aureus where the 9CH showed 5 % and 15CH showed 14 % difference to the MSGR of the control (Table 3). This difference however is not confirmed by the API ZYM test which shows little if any difference to the control for both E. coli and S. aureus (Tables 2 and 4).

5.3 SUBPROBLEM THREE

The mixture containing conventional co-trimoxazole and homoeopathic 9CH paralleled the effects of conventional co-trimoxazole when used alone , upon growth of E. coli, with the exception that homoeopathic 9CH potentiates the effect of the conventional co-trimoxazole at T48. In this instance , cell growth was suppressed to a greater extent than when the conventional co-trimoxazole was used alone.

The mixture containing 9CH homoeopathic co-trimoxazole however did not demonstrate the same effect upon growth of S. aureus. The 9CH mixture stimulated S. aureus growth minimally at T24 and to a greater extent at T48. The homoeopathic potency was able to nullify to some extent , the effects of the conventional co-trimoxazole. This difference , though , is not significant.

The mixture containing conventional co-trimoxazole and homoeopathic 15CH had a stimulatory effect on E. coli growth at T24. Similar results were noted for S. aureus at T48 when in the presence of the same mixture. The API ZYM test also indicated higher activity of 2-naphtyl-phosphate at T24 for E. coli and at T48 for S. aureus for the mixture containing 15CH homoeopathic co-trimoxazole. A similar increase in 2-naphtyl-phosphate activity was observed when 15CH homoeopathic co-trimoxazole was used alone. This suggests that the 15CH homoeopathic potency may stimulate cell growth by effecting a change in enzyme activity.

It also demonstrates the Law of Similars.

5.4 SUBPROBLEM FOUR

Integration of the above three subproblems shows (graphically) that the homoeopathic co-trimoxazole when used alone showed little difference from the control.There was no difference in their enzyme activity. The mixture of 9CH homoeopathic co-trimoxazole and conventional co-trimoxazole showed no consistency of action. It caused suppression of E. coli growth at T48 (Fig 1) but pronounced the growth of S. aureus at T48 (Fig 2). The homoeopathic potency being the only variable was able to minimally influence cell growth. In the case of E. coli growth suppression , the enzyme 2-naphtyl-phosphate activity was low in comparison to it's activity in the other medications [excepting conventional co-trimoxazole] (Table 2).

The mixture containing conventional and 15CH homoeopathic co-trimoxazole was able to enhance growth of E. coli at T24 and S. aureus at T48. There was also higher activity of 2-naphtyl-phosphate at these two times and indicates that the medications indirectly effect growth rate via this enzyme.

There was a difference in the MSGR , time taken to reach MSGR and maximum cell population when the control , homoeopathic 9CH and homoeopathic 15CH were compared for both E. coli and S. aureus (Table 1 and 3). These differences were minimal when the

difference between the control and the conventional co-trimoxazole were compared. Also , little difference could be cited when the conventional ; conventional and homoeopathic 9CH ; and conventional and homoeopathic 15CH were compared for both organisms.

CHAPTER SIX

6.0 **CONCLUSIONS AND RECOMMENDATIONS**

6.1 CONCLUSIONS

The purpose of this study was to establish the effect of an antibiotic , co-trimoxazole expressed in conventional form , homoeopathic form and a mixture containing both forms on E. coli and S. aureus in terms of MSGR , time taken to reach MSGR , maximum cell population and metabolic activity for the purpose of evaluating the efficacy of co-trimoxazole when used in homoeopathic form.

The graphical results did not show any significant difference for both E. coli and S. aureus between the control and the homoeopathic potencies when used alone. A difference of two logarithms is required for a significant difference to be observed. No significant difference could be cited when the conventional , conventional and homoeopathic potencies were compared.

The MSGR showed a difference between the control and the homoeopathic potencies used alone. A similar difference in MSGR was also noted for the conventional co-trimoxazole and the

mixture containing conventional and homoeopathic potencies. This implies that the homoeopathic preparations are able to influence cell growth to some extent. However , homoeopathic forms of co-trimoxazole are not able to demonstrate any significant difference when compared to the control or to the conventional co-trimoxazole.

6.2 RECOMMENDATIONS

It is recommended that more research in this field be continued as the homoeopathic forms of co-trimoxazole have demonstrated that they are capable , though minimally to effect cell growth. This is especially important as the use of co-trimoxazole against respiratory infections in AIDS patients is becoming more prevalent. More organisms as well as more antibiotics should also be tested to establish the effect of these antibiotics when used homoeopathically. It is suggested that the organism also be exposed to the homoeopathic form of antibiotic prior to it encountering the conventional form .

The homoeopathic preparations of co-trimoxazole when used alone are not effective anti-microbials and should not be used as an antibiotic. This should also apply to other antibiotics unless demonstrated otherwise.

It remains unclear what the action of a combination of conventional co-trimoxazole and homoeopathic potencies of it have on cell growth. More studies are needed to confirm their action and they as such , should not be used together.

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APPENDICES

APPENDIX A

NUTRIENT BROTH No.2 : Supplied by BIOLAB DIAGNOSTICS (PTY) (LTD)

Lot 29 ART No. C22

Composition (g/l) : Meat extract 10.00

: Peptone 10.00

: NaCl 5.00

Ph = 7.5 (+/- 0.1)

Exp Date : 04/97

Prep : Suspend 25 g in 1 litre of distilled water. Mix well and distribute into final container. Sterilize autoclaving at 121 °C for 15 minutes.

NUTRIENT AGAR No.2 : Supplied by BIOLAB DIAGNOSTICS (PTY) (LTD)

Lot 19 ART No. C82

Composition (g/l) : Beef extract 10.00

: Peptone 10.00

: NaCl 5.00

: Agar 15.00

Ph = 7.5 (+/- 0.1)

Exp. Date : 04/97

Prep : Dissolve 40g in 1 litre of distilled water. Boil until completely dissolved. Autoclave for 15 minutes at 121 °C.

APPENDIX B

API 20E analysis was done for the E. coli inoculum.

API PROFILE RECOGNITION PROGRAM

STRAIN REFERENCE : Danny - E. coli

API 20E V2.0 PROFILE : 7 154 552

ONPG+ ADH + LDC + ODC + CIT - H2S - URE + TDA - IND +

VP - GEL - GLU + MAN + INO - SOR + RHA + SAC - MEL +

AMY - ARA + OX -

==========

GOOD IDENTIFICATION OF Escherichia coli

Escherichia coli 1 ..... 89.2 %

Escherichia coli 2 ..... 7.8 %

==========

ATYPICAL TEST RESULTS FOR Escherichia coli 1

ARGININE DIHYDROL..ADH 2% UREASE .....URE 1%

ATYPICAL TEST RESULTS FOR Escherichia coli 2

ARGININE DIHYDROL..ADH 8% UREASE .....URE 1%

NEXT TAXON

ATYPICAL TEST RESULTS FOR Salmonella arizonae

H2S PRODUCTION....H2S 96% CHECK! UREASE...URE 0%

CHECK! INDOLE.....IND 0%

APPENDIX C

API PROFILE RECOGNITION PROGRAM

STRAIN REFERENCE : 67

API STAPH V2.0 PROFILE : 6 734 153

O - GLU + FRU + MNE + MAL + LAC + TRE + MAN + XLT -

MEL - NIT - PAL + VP + RAF - XYL - SAC + MDG - NAG +

ADH + URE + LSTR-

==========

ACCEPTABLE IDENTIFICATION OF Staphylococcus aureus

Staphylococcus aureus ..... 89.7 %

CONFIRM BY COAGULASE TEST

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ATYPICAL TEST RESULTS FOR Staphylococcus aureus

NITRATE PRODUCTION.NIT 75%

NEXT TAXON

ATYPICAL TEST RESULTS FOR Staphylococcus simulans

NONE

APPENDIX D

TABLE 5 : Log of CFU/ml of E. coli in the presence of distilled water (control) and conventional co-trimoxazole (co-trim).

TIME	0	6	12	24	48
CONTROL (log CFU/ml)	S D 6.6 ± 0.047	S D 9.1± 0.002	S D 9.1± 0.009	S D 9.1± 0.012	S D 9.2± 0.016
CO-TRIM (log CFU/ml)	S D 6.9 ± 0.023	S D 7.3± 0.009	S D 7.9± 0.052	S D 8.6± 0.052	S D 8.6± 0.032

TABLE 6 : Log of CFU/ml of S. aureus in the presence of distilled water (control) and conventional co-trimoxazole (co-trim).

TIME	0	6	12	24	48
CONTROL (log CFU/ml)	S D 6.3 ± 0.029	S D 8.3± 0.046	S D 8.7± 0.051	S D 8.7± 0.010	S D 8.7± 0.005
CO-TRIM (log CFU/ml)	S D 6.4 ± 0.034	S D 7.5± 0.051	S D 8.6± 0.048	S D 8.5± 0.005	S D 7.7± 0.026

APPENDIX D

TABLE 7 : Log of CFU/ml of E. coli in the presence of 9CH homoeopathic co-trimoxazole and 15CH homoeopathic co-trimoxazole.

TIME	0	6	12	24	48
9CH (log CFU/ml)	S D 6.9 ± 0.012	S D 9.0± 0.032	S D 9.1± 0.009	S D 9.2± 0.022	S D 9.2 ± 0.048
15CH (log CFU/ml)	S D 7.2 ± 0.030	S D 9.1± 0.028	S D 9.1± 0.033	S D 9.0± 0.039	S D 9.3 ± 0.006

TABLE 8 : Log of CFU/ml of S. aureus in the presence of 9CH homoeopathic co-trimoxazole and 15CH homoeopathic co-trimoxazole.

TIME	0	6	12	24	48
9CH (log CFU/ml)	S D 6.3 ± 0.023	S D 8.3± 0.007	S D 8.5± 0.038	S D 8.7± 0.102	S D 8.9 ± 0.053
15CH (log CFU/ml)	S D 6.4 ± 0.034	S D 8.1± 0.046	S D 8.5± 0.083	S D 8.6± 0.021	S D 8.8 ± 0.066

APPENDIX D

TABLE 9 : Log of CFU/ml of E. coli in the presence of a mixture containing conventional and 9CH homoeopathic co-trimoxazole , and in a mixture containing conventional and 15CH homoeopathic co-trimoxazole.

TIME	0	6	12	24	48
CO-TRIM + 9CH (log CFU/ml)	SD 7.0 ± 0.038	SD 7.6± 0.057	SD 8.3± 0.013	SD 8.7± 0.023	SD 7.6± 0.012
CO-TRIM + 15CH (log CFU/ml)	SD 7.2 ± 0.013	SD 7.6± 0.106	SD 8.3± 0.025	SD 9.6± 0.057	SD 8.6± 0.072

TABLE 10 : Log of CFU/ml of S. aureus in the presence of a mixture containing conventional and 9CH homoeopathic co-trimoxazole , and in a mixture containing conventional and 15CH homoeopathic co-trimoxazole.

TIME	0	6	12	24	48
9CH (log CFU/ml)	SD 6.3 ± 0.042	SD 7.4± 0.033	SD 8.5± 0.071	SD 8.7± 0.072	SD 8.8± 0.034
15CH (log CFU/ml)	SD 6.3 ± 0.021	SD 7.6± 0.099	SD 8.7± 0.015	SD 8.8± 0.026	SD 8.8± 0.024

APPENDIX E

ENGLISH

2 520 0

API ZYM

FOR THE RESEARCH OF
ENZYMATIC ACTIVITIES

Instruction manual
Version A

API ZYM is a semi-quantitative micromethod designed for the research of enzymatic activities. The technique is applicable to all specimens (tissues, cells, biological fluids, microorganisms, washings, soil, oil, etc.). It allows the systematic and rapid study of 19 enzymatic reactions using very small sample quantities.

API ZYM has not been developed in view of obtaining the precision of spectrophotometric or electrophoretic procedures but has been mainly developed to permit the performance of enzymatic determinations from a complex sample which has not been purified. It can be used to screen specimens, thus providing a spectrum of enzymatic determinations which can be further tested by spectrophotometric and/ or electrophoretic procedures.

Principle

The **API ZYM** strip is composed of 20 microtubes, the bottom of which forms a support especially designed to contain the enzymatic substrate and buffer. This support allows contact between the enzyme and the generally insoluble substrate.

- The **API ZYM** kit enables the performance of 25 tests. It consists of :
  - 25 **API ZYM** strips
  - 25 incubation trays
  - 25 report sheets
  - 1 instruction manual

- In order to use **API ZYM**, it is necessary to have available :
  - **pipettes** (# 9 756 0) or **PSIpettes** (# 7 025 0)
  - **Ampoule rack** (# 7 020 0)
  - **Suspension Medium** (# 2 011 0) or sterile distilled water
  - **ZYM A** and **ZYM B** reagents (# 7 047 0 and 7 048 0)
  - **McFarland Standard** (# 7 090 0)

Plus standard laboratory equipment :

Incubator, refrigerator, Bunsen burner, marker pen

Storage

API ZYM strips should be stored at 2-8° C. The expiry date is printed on each pack.

The reagents should also be stored at 2-8° C. except for **ZYM A** which forms a precipitate at this temperature. The precipitate does not affect any of the properties of the reagent and dissolves again at 60° C. It is better to store the reagent at room temperature.

ZYM B is light-sensitive and its deterioration is marked by the development of a pink colour. The reagent should be kept in the dark ; it is best to wrap the ampoule in aluminium foil and keep it in the refrigerator.

Composition of reagents

- **ZYM A** :

Tri-hydroxymethyl-amino-methane	25	g
Hydrochloric acid (37 %)	11	ml
Lauryl sulphate	10	g
Distilled water	qsp 100	ml

- **ZYM B** :

Fast Blue BB	0.35	g
2-methoxyethanol	qsp 100	ml

Instructions for use

1. Preparation of the specimen

Dilute the specimen in a minimum volume of 2 ml of sterile distilled water or in another diluter such as normal saline without any buffer.

- **Microorganisms** :

  Prepare a suspension with a turbidity between a McFarland N° 5 and N° 6 standard. Pure growth from an agar slant or sediment from a centrifuged broth culture can be used to prepare the suspension. In order to obtain reproducible results, it is important that the organisms to be compared be initially grown on the same isolation medium, the diluter be the same and the suspension be of the same optical density. This technique assays for constitutive enzymes. Inductive enzymes can be detected by adding the corresponding inducer(s) to the culture medium. In view of the absence of growth during the test, no particular precaution concerning sterility or incubation need be taken - even for anaerobes.

- **Cell Suspensions** :
  (Cell cultures, spermatozoa) :

  With sterile distilled water or saline, the specimen is centrifuged and the sediment is adjusted to contain between 10^5-10^7 cells / ml. The concentration will depend on the enzymatic activity of the specimen.

- **Tissues** :

  The specimen should be ground with a Poter tube (few mg) and suspended in 2 ml of distilled water.

- **Biological fluids** :

  Undiluted fluids may be used directly or if the enzymatic activity of the raw liquid is too strong, it may be diluted. If the volume of the specimen is insufficient (less than 2 ml), bring it up to 2 ml with distilled water.

2. Preparation of strips

- Set up an incubation tray and lid.
- Record the specimen number on the elongated flap of the tray.
- Dispense approximately 5 ml of tap water into the incubation tray to provide a humid atmosphere during incubation. A plastic squeeze bottle may be used for this.
- Remove the API ZYM strip from the sealed envelope and place it in the incubation tray.

3. Inoculation of the strip

With a Pasteur pipette, inoculate two drops of specimen in each cupule of the strip (65 microliters).

4. Incubation of the strip

After inoculation, place the plastic lid on the tray and incubate generally for 4 hours at 37° C. The time of incubation and temperature may vary depending on the sample to be tested. However, when samples are being compared all test conditions (time, temperature, growth media, density of suspension) must be the same. The inoculated strip should not be placed in bright light.

5. Reading the strip

- After incubation, add one drop of ZYM A and one drop of ZYM B reagents.
- Let the colour develop for five minutes. If possible, put the strip under a powerful light source (1.000 watt bulb) for about 10 seconds with the bulb being placed about 4" above the cupules. The procedure will eliminate any yellow colour which may appear in the cupules due to any excess of Fast Blue which has not reacted. After light exposure, negative reactions become colourless. Placing the strip in daylight for a few minutes will produce comparable results.

6. Recording the reactions

A value ranging from 0-5 can be assigned corresponding to the colours developed as per the **colour chart** herewith enclosed. Zero corresponds to a negative reaction ; 5 to a reaction of maximum intensity. Values 1 through 4 are intermediate reactions depending on the level of intensity. The approximate number of free nanomoles may be known from the colour strength : 1 corresponds to the liberation of 5 nanomoles, 2 to 10 nanomoles, 3 to 20 nanomoles, 4 to 30 nanomoles and 5 to 40 or more nanomoles.

7. Disposal

The colours remain stable for several hours after the strip has been inoculated with the reagents. After reading, the strips can be dried at room temperature and stored in a dry atmosphere. All hazardous or pathogenic material (strip and incubation box) should autoclaved, incinerated or immersed in a disinfectant for decontamination prior to disposal.

Quality control

The media, strips and reagents are subject to systematic quality control checks at various stages of manufacture. A further bacteriological and biochemical check may be carried out in the laboratory by using the following strains or enzymes:

1. Bacteroides thetaiotaomicron, ATCC 8492
2. β-Glucosidase, Sigma 8625
3. α-Chymotrypsin, Sigma C 4129

which give the following results :

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1.	–	+	V	+	–	V	–	–	–	–	+	V	+	+	+	+	V	+	–	+
2.	–	V	V	–	–	+	V	–	V	–	+	+	V	+	–	–	+	+	V	–
3.	–	–	+	+	–	V	–	–	V	+	–	–	–	–	–	–	–	–	–	–

SUMMARY OF RESULTS

No	ENZYME ASSAYED FOR	SUBSTRATE	pH	RESULT	
				Positive	Negative
1	Control			No colour or colour of the sample if it has an intense coloration	
2	Phosphatase alcaline	2-naphthyl phosphate	8.5	Violet	
3	Esterase (C 4)	2-naphthyl butyrate	8.5	Violet	
4	Esterase Lipase (C 8)	2-naphthyl caprylate	7.5	Violet	
5	Lipase (C 14)	2-naphthyl myristate	"	Violet	
6	Leucine arylamidase	L-leucyl-2-naphthylamide	"	Orange	
7	Valine arylamidase	L-valyl-2-naphthylamide	"	Orange	
8	Cystine arylamidase	L-cystyl-2-naphthylamide	"	Orange	
9	Trypsin	N-benzoyl-DL-arginine-2-naphthylamide	8.5	Orange	
10	Chymotrypsin	N-glutaryl-phenylalanine-2-naphthylamide	7.5	Orange	
11	Phosphatase acid	2-naphthyl phosphate	5.4	Violet	
12	Naphthol-AS-BI-phosphohydrolase	Naphthol-AS-BI-phosphate	"	Blue	
13	α galactosidase	6-Br-2-naphthyl-αD-galactopyranoside	"	Violet	
14	β galactosidase	2-naphthyl-βD-galactopyranoside	"	Violet	
15	β glucuronidase	Naphthol-AS-BI-βD-glucuronide	"	Blue	
16	α glucosidase	2-naphthyl-αD-glucopyranoside	"	Violet	
17	β glucosidase	6-Br-2-naphthyl-βD-glucopyranoside	"	Violet	
18	N-acetyl-β glucosaminidase	1-naphthyl-N-acetyl-βD-glucosaminide	"	Brown	
19	α mannosidase	6-Br-2-naphthyl-αD-mannopyranoside	"	Violet	
20	α fucosidase	2-naphthyl-αL-fucopyranoside	"	Violet	

– No colour or colour of the control if the strip has been exposed to an intense light source after addition of the reagents.
– Very pale yellow if the strip has not been exposed to an intense light.

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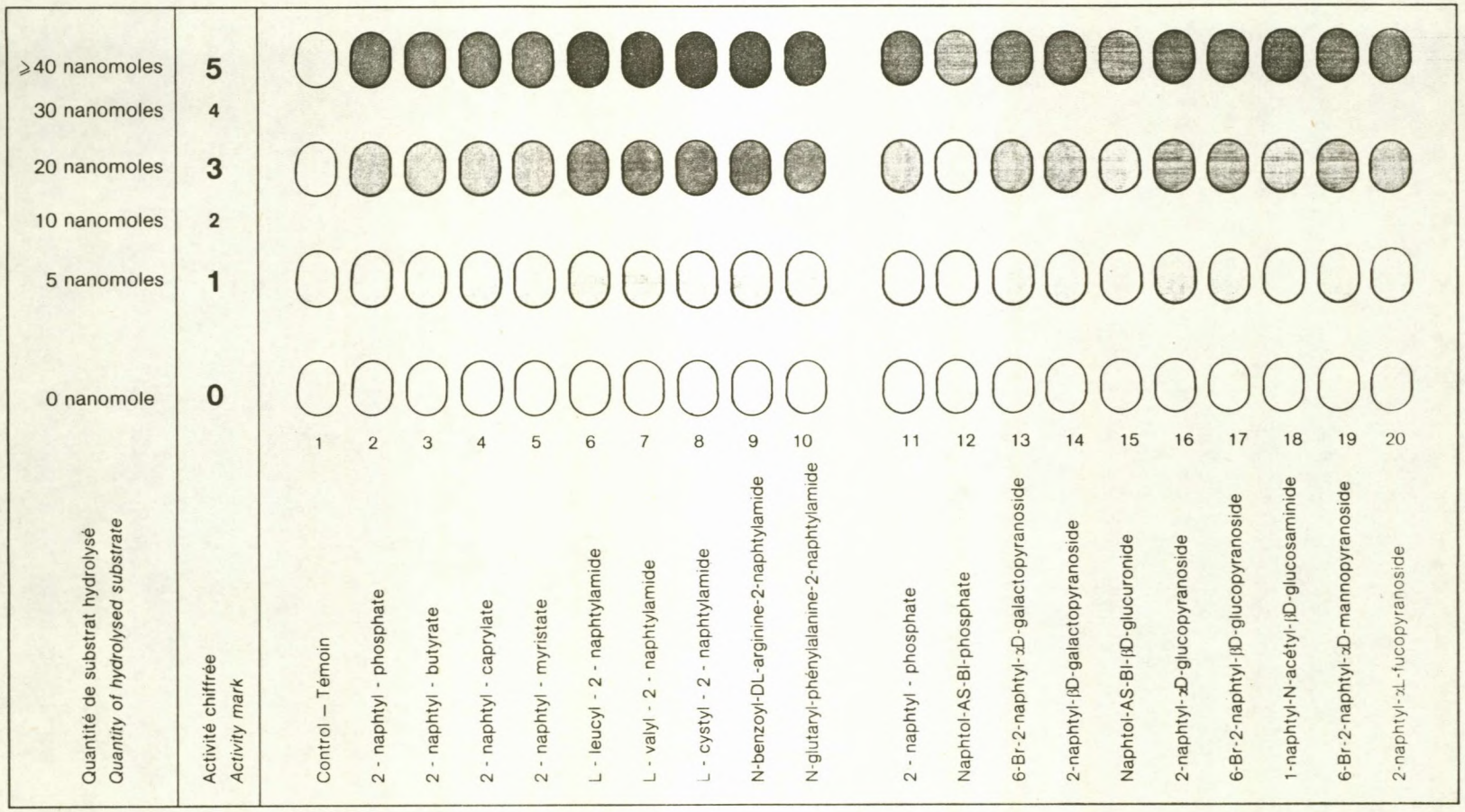
API ZYM

ECHELLE DE LECTURE - *READING SCALE*



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API ZYM REPORT SHEET